

Seasonal variations and distributions of dissolved free and total carbohydrates at the İzmir Bay, Aegean Sea

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Abstract

Seasonal variations and distributions of dissolved carbohydrate concentrations at the İzmir Bay were investigated with salinity, chlorophyll *a* (Chl *a*), and dissolved organic carbon (DOC) levels to understand their relationships. Samples were collected from surface, subsurface and bottom depths at seven stations. DOC concentrations ranged from 32.2 to 244.2 $\mu\text{mol/L}$, and in general, DOC levels increased from winter to summer, then slightly decreased in autumn. Monosaccharide (MCHO), polysaccharide (PCHO) and total dissolved carbohydrate (TDCHO) levels were found between 0.7–8.3, 0.7–19.5, and 2.6–24.6 $\mu\text{mol/L}$. DOC, MCHO, PCHO and TDCHO levels were found higher in middle-inner bays, under the influence of anthropogenic inputs, compared to outer bay. Seasonal changes of MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios were statistically significant ($p < 0.05$) and the ratios showed decrease trends from winter to summer-autumn seasons. Distributions of TDCHO/DOC ratios at wide ranges (2.5%–42.3%) indicated the presence of newly forming and degrading fractions of DOM. According to results of factor analysis, Chl *a*, MCHO and TDCHO were explained in the same factor groups. In conclusion, the results showed that dissolved carbohydrate levels in the İzmir Bay might be influenced by biological processes and terrestrial/anthropogenic inputs.

Key words: monosaccharides, polysaccharides, carbohydrates, DOC, Chl *a*, İzmir Bay

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1 Introduction

Dissolved organic matter (DOM) is one of the most important components in seawater and it is under constant circulation and conversion within the marine environment. DOM could be originated from terrestrial processes (called as allochthonous) or *in situ* marine (called as autochthonous) sources (Hedges, 2002; Libes, 2009). Terrestrial organic matter including degradation products of plants and other organisms could be transported to the marine environments by rivers, rain runoffs or winds. On the other hand, a great amount of DOM in marine environments is produced *in situ* by primary producers like phytoplankton, macroalgae and bacteria found in seawater. Chemical and physical characterization of DOM pool is difficult since it includes vast amounts of biomolecules at different chemical structures and molecular sizes. Among these biomolecules, most abundant ones found in DOM are amino acids, carbohydrates, lipids, fatty acids, sterols, humic acids, fulvic acids, and lignins. Carbohydrates are one of the major groups that are commonly utilized by the microorganisms (Rich et al., 1997; Kirchman et al., 2001; Khodse et al., 2010). They are also the products of photosynthesis process that takes place within phytoplankton and marine algae. Qualitative and quantitative studies have been performed for understanding the biogeochemical cycling of dissolved carbohydrates including its molecular and polymeric forms. Dissolved carbohydrates could be divided into groups as monosaccharides (MCHO) and polysaccharides (PCHO) or neutral and acidic sugars based on their chemical structures (Hedges et al., 1994; Myklestad et al., 1997; Benner and Opsahl, 2001; Chanudet and

Filella, 2006; Lin and Guo, 2015). Glucose, fucose, galactose, mannose, and xylose are reported as dominant monosaccharides in DOM from different regions (McCarthy et al., 1996; Borch and Kirchman, 1997). Polysaccharides, such as starch and cellulose, are polymeric forms of monosaccharides linked to each other with glycosidic bonds. Total dissolved carbohydrates (TDCHO) are one of the well identified components of DOM that constitutes up to 3%–30% of bulk DOM (Pakulski and Benner, 1994; Benner, 2002; Hung et al., 2003; Wang et al., 2006). This fraction contains mono-, oligo- and polysaccharides, the latter one is used as storage material, cell wall components and extracellular exudates (Myklestad and Børsheim, 2007; He et al., 2015). Polysaccharides have been studied extensively due to their tendency for aggregation and colloidal properties. It has been reported that they were responsible from biofilm production, mucilage events (Baldi et al., 1997; Leppard, 1997; Pettine et al., 1999; Penna et al., 2003, 2009), complexation with trace metals (Jang et al., 1990, 1995), and marine snow formation (Alldredge et al., 1993; Passow et al., 1994; Skoog et al., 2008; He et al., 2015).

In the water column, carbohydrate concentrations are changed both vertically and horizontally based on their production and uptake rates by the organisms, and they are also influenced by terrestrial inputs (Zhang, 2010; He et al., 2015). In estuaries, gulfs and bays, carbohydrate concentrations were reported at high levels, whereas, they were observed at intermediate and low levels in coastal waters and in oligotrophic ocean waters, respectively (Handa, 1966, 1967; Pakulski and Benner, 1994; Amon and Benner, 2003; Hung et al., 2003; Wang et al., 2006; Khodse et

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al., 2010; Yang et al., 2010; He et al., 2015).

The study area in this study, the İzmir Bay, is located at the western coast of Anatolia. Its structure is L-shape which is oriented to north with its longer part and connected to the Aegean Sea. According to Sayin (2003), the hydrography of the İzmir Bay is influenced by several factors such as freshwater inputs that carry anthropogenic loads to the bay, atmospheric transport of low molecular weight molecules, exchange of water between the Aegean Sea and the bay, topography of the bay, the sea level changes, movement of waters directed by wind-driven circulation and winter convection. Under the influences of these factors, the İzmir Bay could be divided into three parts as outer, middle and inner bays since each part have different water mass characteristics. While inner bay water has been anthropogenically polluted, outer bay water is influenced by the Gediz River and Aegean Sea, the upwelling water at the Gülbahçe Bay, and the water mass located at salt production area (Sayin, 2003). The third water mass at middle bay connects outer bay to inner bay. Due to the different physical and chemical characteristics of the water masses, remarkable differences have been reported for dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*), dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) levels at outer, middle and inner bay stations in the previous studies (Kontas et al., 2004; Kucuksezgin et al., 2005; Sunlu et al., 2012). Also, eutrophication has been reported for inner part of the İzmir Bay (Kontas et al., 2004). On the other hand, outer bay has oligotrophic character. Studies on the fractions of DOM in the İzmir Bay are very limited and this will be the first study on the dissolved carbohydrates (MCHO, PCHO and TDCHO). The aim of this study was to investigate seasonal variations and distributions of dissolved carbohydrates in the bay and their relationships with salinity, Chl *a* and DOC levels.

2 Materials and methods

2.1 Seawater sampling

Seawater samples were collected from seven stations located in the İzmir Bay (Fig. 1). Sampling stations were selected according to physical and chemical properties of water masses in the bay, shortly explained at the introduction, based on the previous studies (Sayin, 2003; Kontas et al., 2004; Kucuksezgin et al., 2005). Samples were collected from surface (0–0.5 m), subsurface (5 m) and bottom depths. Water depths at sampling stations from 1 to 7 were as follows: 9, 22, 52, 36, 27, 66, and 44 m, respectively. The samplings were performed by seasonal cruises (February, April,

September and December 2015) with the R/V *K. Piri Reis*. Seawater samples were collected with 10 L Go Flo Rosette bottles (General Oceanic) and a CTD system (SBE911plus, Sea-Bird). DOC and carbohydrate samples were immediately filtered from 47 mm Whatman GF/F (0.7 µm) glass fiber filters (precombusted at 450°C for 4 h) and stored at –20°C until the analyses in the laboratory. Chl *a* samples were pre-filtered with 210 µm nylon mesh in order to remove the larger particles. Then, the samples collected on GF/F filters and fixed with saturated MgCO₃ just before the end of filtration. The filters stored at –20°C within 15 mL screw capped centrifuge tubes with Teflon liners.

2.2 Chlorophyll *a* analysis

Chl *a* was extracted with 10 mL of 90:10 acetone:water (v/v) solution for 24 h using a vortex mixer at 6 h of intervals and kept within refrigerator at 4°C. Following the extraction, the samples were centrifuged at 3 000 r/min for 10 min. Absorbances of the samples were recorded at 630, 647, 664 and 750 nm on a spectrophotometer (DR5000, Hach). Chl *a* concentrations (µg/L) were calculated according to APHA (1998).

2.3 Dissolved organic carbon analysis

DOC analysis is based on the discoloration of buffered phenolphthalein solution proportional to the CO₂ concentration. The analysis was performed on a continuous flow nutrient analyzer (San Plus, Skalar) according to instructions of the manufacturer (Cat.No: 311–412). First, 0.06 mol/L sulfuric acid was added to the sample. Then, the digestion reagent (12 g K₂S₂O₈ and 34 g Na₂B₄O₇·10H₂O per liter of distilled water) was added and UV digestion was applied. Following the digestion, hydroxylammonium chloride solution (includes 10% Triton X-100 and sulfuric acid) was added and CO₂ was separated from reaction mixture with a gas dialysis membrane. Then, CO₂ was reacted with the 1% phenolphthalein buffer solution (prepared in ethanol and sodium carbonate buffer). The colorimetric reading performed at 550 nm. Potassium hydrogen phthalate was used as organic carbon standard. Accuracy of the method was checked using potassium hydrogen phthalate at every 10 sample readings. Synthetic seawater including NaCl, MgSO₄ and Milli-Q water was used as blank. The system was washed with Milli-Q water until the low and stable instrumental blank.

2.4 Carbohydrate analysis

The carbohydrate analyses were performed according to the method proposed by Myklestad et al. (1997). TDCHO were ini-

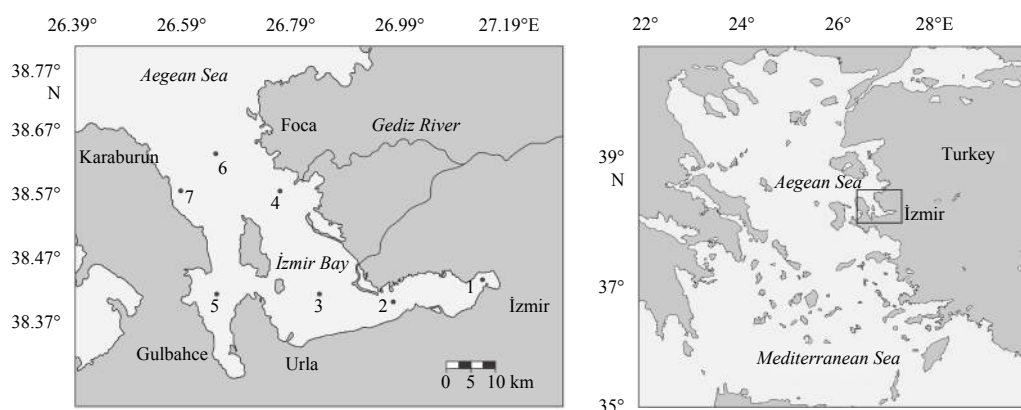


Fig. 1. Sampling stations at the İzmir Bay, Aegean Sea.

tially hydrolyzed and analyzed according to the standard procedure given below after neutralization. For hydrolysis, 4 mL of sample and 0.4 mL of 1 mol/L HCl were added into 20 mL amber hydrolysis vials and the vials were tightly closed with Teflon lined screw caps. The vials were kept at 150°C for 1 h on a block heater. Then, the vials allowed to cool at room temperature and neutralized with 0.4 mL of 1 mol/L NaOH. For analysis of dissolved free carbohydrates (MCHO), 1 mL of sample was mixed in 20 mL amber vial. A total of 1 mL of 0.7 mmol/L potassium ferricyanide solution was added to sample and kept on the block heater at 100°C for 10 min. Then, 1 mL of 2 mmol/L ferric chloride and 2 mL of 2.5 mmol/L TPTZ solutions were added and thoroughly mixed on a vortex mixer. The absorbance was read at 595 nm after 30 min in a 50 mm cuvette against distilled water. The absorbance of a reagent blank prepared in Milli-Q water subtracted before further calculations. The working standards were prepared at 3.3, 8.3, 16.7, 33.3, 66.7, and 133.3 $\mu\text{mol/L}$ (glucose-C) concentrations by dissolving neat *D*-glucose (47829, Supelco) analytical standard in Milli-Q water. Dissolved polysaccharide (PCHO) concentrations were calculated by subtracting the concentrations of MCHO from TDCHO. Precision of the method was between 3%–10% for high to low concentration samples and the detection limit was 0.4 $\mu\text{mol/L}$ (Myklestad et al., 1997; Engel and Händel, 2011).

In the hydrolysis step of carbohydrates, 1 mol/L HCl was used. According to Myklestad et al. (1997), the hydrolysis works sufficiently but, in some studies, equal or two to four fold higher hydrolysis yields were reported by using two step hydrolysis (12 mol/L H_2SO_4 at prehydrolysis and 1.2 mol/L H_2SO_4 at post hydrolysis) (Pakulski and Benner, 1992; Myklestad et al., 1997). However, it is not almost possible to achieve 100% yield during hydrolysis of polysaccharides (Myklestad et al., 1997).

2.5 Statistical analyses

Statistical analyses were performed with R Statistical Computing Software, v3.2.5 (R Core Team, 2016). In all statistical tests, significance level was $\alpha=0.05$. Seasonal changes of salinity, Chl *a*, DOC, MCHO, PCHO and TDCHO were evaluated with One Way ANOVA tests. Prior to ANOVA, test assumptions were checked with Shapiro-Wilk Normality test and Fligner-Killeen's test for homogeneity of variances. Spearman's rank correlation test used for correlation analyses between salinity, Chl *a*, DOC, MCHO,

and TDCHO. Linear relationships between MCHO, PCHO, Chl *a* and TDCHO/DOC levels were investigated with linear regression analyses. Factor analysis was used to determine how the independent variables (salinity, Chl *a*, DOC, MCHO and TDCHO) were effective on the variation of data. Factor analysis was performed by using psych package in R (Revelle, 2016). Both Horn's parallel analysis (Horn, 1965) and Very Simple Structure (VSS) (Revelle and Rocklin, 1979) methods were used to determine the correct number of factors. Prior to factor analysis, the data were divided into two subsets as middle-inner and outer bay. Factors were estimated with minimum residual (minres) method (Harman and Jones, 1966) and the data were not rotated.

3 Results

3.1 Seasonal variations

Seasonal and vertical salinity, Chl *a*, DOC, MCHO, TDCHO and PCHO levels were given in Table 1. According to One Way ANOVA test results, there were no significant vertical changes for salinity, Chl *a*, DOC, MCHO, TDCHO and PCHO ($p<0.05$). Sea-water salinity was found between 36.9 and 40.8 in all seasons. Salinity was remarkably higher in summer at middle-inner and outer bays ($p<0.05$, Table 2). Salinity was nearly constant with increasing depth.

Chl *a* concentrations were between 0.1–2.6 $\mu\text{g/L}$ and 1.0–25.4 $\mu\text{g/L}$ at outer and middle-inner bays, respectively. In middle-inner bays, maximum Chl *a* levels were found at surface waters and decreased at 5 m and bottom depths. Chl *a* levels were observed higher at Stas 1 and 2 in summer and autumn. Minimum Chl *a* levels were observed in autumn at middle-inner bays. Seasonal changes of Chl *a* levels were significant at middle-inner and outer bays ($p<0.05$, Table 2).

DOC concentrations ranged between 32.2–96.3 and 41.4–244.2 $\mu\text{mol/L}$ at outer and middle-inner bays, respectively. Highest DOC concentration was observed in summer at Sta. 1. DOC levels increased from winter to summer and slightly decreased in autumn. Vertical variations of DOC levels were not remarkable, but highest concentrations were observed at surface waters. Seasonal changes of DOC concentrations were significant at middle-inner and outer bays ($p<0.05$, Table 2).

MCHO levels were found between 0.7–8.3 $\mu\text{mol/L}$ (1.3–8.3 $\mu\text{mol/L}$ for middle-inner bay, 0.7–5.6 $\mu\text{mol/L}$ for outer bay) and

Table 1. Seasonal variations of physical and chemical parameters in the water column of the İzmir Bay ($n=7$ for each range)

Season/Depth	Salinity	Chl <i>a</i> / $\mu\text{g}\cdot\text{L}^{-1}$	DOC/ $\mu\text{mol}\cdot\text{L}^{-1}$	MCHO/ $\mu\text{mol}\cdot\text{L}^{-1}$	PCHO/ $\mu\text{mol}\cdot\text{L}^{-1}$	TDCHO/ $\mu\text{mol}\cdot\text{L}^{-1}$
Winter						
Surface	37.3–39.0	0.7–21.8	33–69	2.6–6.2	5.2–15.3	8.1–21.5
Subsurface	38.1–39.0	0.6–9.8	33–83	2.1–5.1	5.2–19.5	7.9–24.6
Bottom	38.2–39.1	0.3–13.6	32–85	1.5–5.6	6.1–13.0	8.6–18.6
Spring						
Surface	37.8–39.1	0.3–21.4	61–102	2.2–7.0	5.0–12.6	7.3–19.5
Subsurface	37.8–39.1	0.3–17.2	64–96	2.1–8.3	4.7–11.5	6.9–19.8
Bottom	37.8–39.0	0.5–16.0	59–96	1.9–4.8	4.5–12.8	6.5–17.6
Summer						
Surface	37.7–40.6	0.1–25.4	39–244	0.7–5.4	2.4–13.3	3.4–18.7
Subsurface	39.2–40.8	0.1–18.6	35–207	0.7–5.4	1.9–8.9	3.0–14.3
Bottom	36.9–39.5	0.3–18.5	39–208	0.7–4.9	1.8–9.2	2.6–14.2
Autumn						
Surface	37.4–39.0	0.3–2.2	47–160	1.0–2.7	1.3–3.0	3.2–4.2
Subsurface	38.4–38.8	0.2–1.9	47–137	0.7–3.4	0.7–2.8	2.8–4.0
Bottom	38.5–39.0	0.2–1.9	55–152	0.7–3.6	0.7–3.7	3.5–4.8

Table 2. Results of One Way ANOVA and Tukey's HSD tests for seasonal changes of salinity, Chl *a*, DOC, MCHO, TDCHO and PCHO

	Winter	Spring	Summer	Autumn
Middle-inner bays				
Salinity	38.4±0.4 ^b	38.0±0.4 ^b	40.0±0.6 ^a	38.3±0.5 ^b
Chl <i>a</i> /μg·L ⁻¹	8.5±8.1 ^{ab}	11.0±8.2 ^{ab}	15.8±8.1 ^a	1.7±0.4 ^b
DOC/μmol·L ⁻¹	52.5±8.2 ^c	84.7±13.8 ^{bc}	163.0±67.1 ^a	104.5±50.0 ^b
MCHO/μmol·L ⁻¹	4.5±1.2 ^{ab}	5.1±2.3 ^a	4.0±1.6 ^{ab}	2.5±0.9 ^b
PCHO/μmol·L ⁻¹	12.4±4.4 ^a	10.4±2.3 ^a	8.6±3.1 ^a	1.7±1.0 ^b
TDCHO/μmol·L ⁻¹	17.0±5.4 ^a	15.4±4.3 ^a	12.6±4.5 ^a	4.0±0.4 ^b
(MCHO/DOC)/%	8.6±1.2 ^a	5.8±1.8 ^b	2.4±0.4 ^c	2.5±0.5 ^c
(PCHO/DOC)/%	23.4±6.0 ^a	12.2±1.2 ^b	5.5±1.0 ^c	2.2±1.9 ^c
(TDCHO/DOC)/%	31.9±6.6 ^a	17.9±2.4 ^b	7.9±1.2 ^c	4.8±2.2 ^c
(MCHO/TDCHO)/%	27.4±4.4 ^a	31.7±6.7 ^b	30.9±4.9 ^b	60.3±21.0 ^b
(PCHO/TDCHO)/%	72.6±4.4 ^a	68.3±6.7 ^a	69.1±4.9 ^a	39.7±21.0 ^b
(PCHO/MCHO)/%	2.7±0.6 ^a	2.3±0.7 ^a	2.3±0.6 ^a	0.9±0.7 ^b
Outer bay				
Salinity	38.7±0.5 ^b	38.9±0.3 ^b	39.3±1.0 ^a	38.7±0.3 ^b
Chl <i>a</i> /μg·L ⁻¹	1.1±0.7 ^a	0.7±0.4 ^b	0.4±0.3 ^b	0.4±0.2 ^b
DOC/μmol·L ⁻¹	48.1±18.0 ^c	75.1±12.6 ^a	46.0±9.0 ^c	62.3±9.6 ^b
MCHO/μmol·L ⁻¹	2.9±0.6 ^a	3.2±1.2 ^a	0.9±0.2 ^b	1.2±0.4 ^b
PCHO/μmol·L ⁻¹	7.5±1.5 ^a	7.0±2.1 ^a	2.7±0.7 ^b	2.6±0.6 ^b
TDCHO/μmol·L ⁻¹	10.4±1.7 ^a	10.2±2.7 ^a	3.6±0.8 ^b	3.8±0.5 ^b
(MCHO/DOC)/%	6.6±1.9 ^a	4.4±2.0 ^b	2.0±0.5 ^c	2.0±0.8 ^c
(PCHO/DOC)/%	17.5±6.8 ^a	9.7±3.6 ^b	6.0±1.5 ^c	4.3±0.6 ^c
(TDCHO/DOC)/%	24.1±8.2 ^a	14.1±5.0 ^b	8.0±1.8 ^c	6.3±1.2 ^c
(MCHO/TDCHO)/%	28.7±6.1 ^{ab}	31.2±7.2 ^{ab}	25.3±5.1 ^b	31.6±7.3 ^a
(PCHO/TDCHO)/%	71.3±6.1 ^{ab}	68.8±7.2 ^{ab}	74.7±5.1 ^a	68.4±7.3 ^b
(PCHO/MCHO)/%	2.7±1.0	2.4±1.0	3.1±0.8	2.4±0.9

Note: Values represent mean±SD; *n*=6 for inner bay; *n*=15 for outer bay; *p*<0.05; Tukey's HSD test results are given with superscript letters.

maximum MCHO levels were observed in spring. MCHO levels were increased from winter to spring and decreased to minimum levels in autumn. Seasonal changes of MCHO levels were significant at middle-inner and outer bays (*p*<0.05, Table 2).

PCHO levels were found between 0.7–19.5 μmol/L (0.7–19.5 μmol/L for middle-inner bay, 1.8–10.8 μmol/L for outer bay). PCHO levels were decreased from winter to autumn and highest PCHO concentrations were observed at surface waters. Seasonal variations of PCHO levels were significant at middle-inner and outer bays (*p*<0.05, Table 2).

TDCHO concentrations were ranged between 2.6–14.4 μmol/L and 3.6–24.6 μmol/L at outer and middle-inner bays, respectively. Similar to PCHO, TDCHO levels were decreased from winter to autumn. Seasonal changes of TDCHO levels were significant at middle-inner and outer bays (*p*<0.05, Table 2).

Seasonal and vertical MCHO/TDCHO, PCHO/TDCHO, MCHO/DOC, PCHO/DOC, TDCHO/DOC and PCHO/MCHO ratios were given in Table 3. MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios showed significant decreasing trends from winter to summer-autumn seasons (*p*<0.05, Table 2). Maximum MCHO/DOC ratio was found as 11% at outer bay. Maximum PCHO/DOC (34%) and TDCHO/DOC (42%) ratios were observed at middle-inner bays. TDCHO/DOC ratios for middle-inner and outer bays were found in the range of 2.5%–42.3% (\bar{x} =15.6, s^2 =129.2) and 4.4%–37.1% (\bar{x} =13.1, s^2 =72.9), respectively. MCHO/TDCHO ratios were increased from winter to autumn, especially at middle-inner bays, and MCHO comprised up to 85% of TDCHO in autumn. MCHO/TDCHO ratios were observed between 21%–42% in winter, spring and summer. PCHO/TDCHO ratios were found lower at middle-inner bays compared to outer

bay in autumn. PCHO/MCHO ratio was significantly lower at autumn compared to other seasons at middle-inner bays (*p*<0.05). PCHO/MCHO ratios were not changed significantly at outer bay (*p*<0.05).

3.2 Spatial distribution

Spatial and vertical distributions of MCHO, PCHO, TDCHO and DOC were given in Fig. 2. Carbohydrate concentrations were not changed significantly with depth. In summer, all carbohydrate species at Stas 1 and 2 (especially at surface and subsurface) were found remarkably higher than outer bay stations. MCHO, PCHO and TDCHO levels at Sta. 1 were higher than other stations at all seasons, except for autumn.

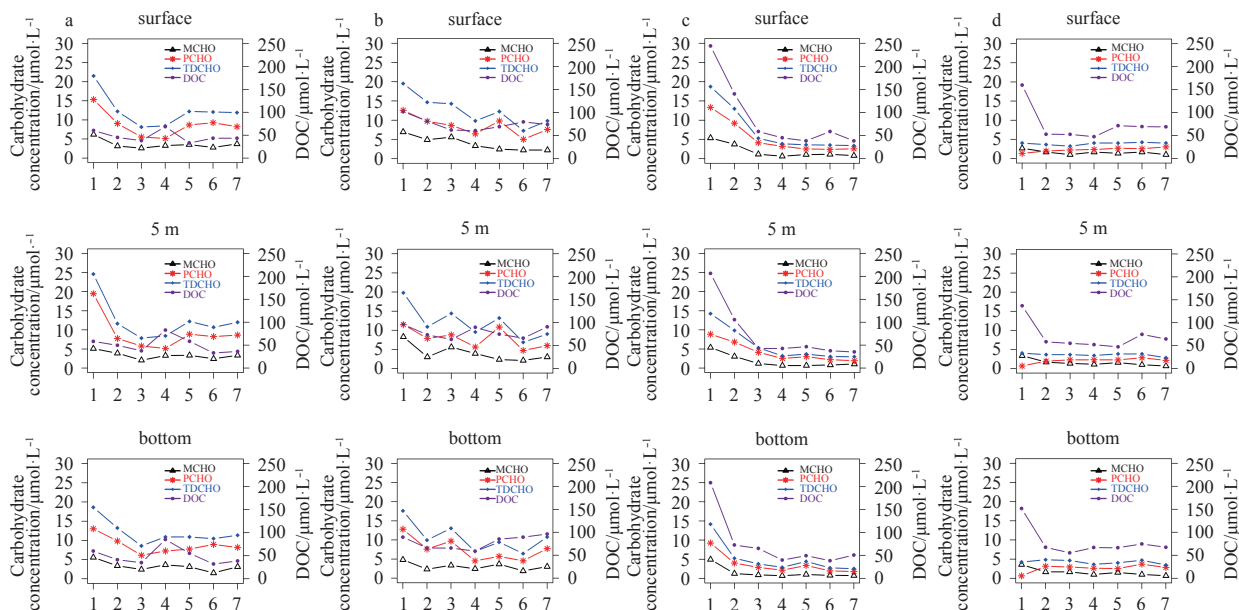
DOC concentrations were changed consistently with carbohydrate concentrations. In winter and spring, DOC levels were generally below 100 μmol/L except for Sta. 1. Maximum DOC concentration was found as 244.2 μmol/L at surface waters of Sta. 1 in summer. DOC levels were also found higher at 5 m and bottom depths of Stas 1 and 2 compared to the other stations in summer. DOC was slightly decreased from summer to autumn at all depths of Sta. 1, but DOC levels in autumn were higher than winter and spring seasons.

3.3 Correlation analysis

Correlations between MCHO, TDCHO, DOC, Chl *a*, and salinity were investigated with Spearman's rank correlation test (Table 4). MCHO and TDCHO were positively correlated in all seasons (*p*<0.05) and a strong positive correlation was observed in summer (ρ =0.822, *p*<0.001). DOC was positively correlated with MCHO and Chl *a* in winter and summer (*p*<0.01). There was

Table 3. Seasonal variations for MCHO/TDCHO, PCHO/TDCHO, MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios in the water column of the İzmir Bay ($n=7$ for each range)

Season/Depth	(MCHO/TDCHO)/%		(PCHO/TDCHO)/%		(MCHO/DOC)/%		(PCHO/DOC)/%		(TDCHO/DOC)/%	
	Outer bay	Middle-inner bay	Outer bay	Middle-inner bay	Outer bay	Middle-inner bay	Outer bay	Middle-inner bay	Outer bay	Middle-inner bay
Winter										
Surface	23–39	26–29	61–77	71–74	5–11	7–10	8–26	20–26	12–37	27–36
Subsurface	23–39	21–33	61–77	67–79	4–9	8–9	6–25	16–34	10–33	23–42
Bottom	15–33	26–30	67–85	70–74	4–8	8–9	9–28	22–24	13–32	31–32
Spring										
Surface	20–39	34–36	61–80	64–66	3–9	6–7	6–14	12.0–12.3	9–23	18–19
Subsurface	18–41	28–42	59–82	58–72	3–9	4–9	6–14	11–12	10–23	15–21
Bottom	26–39	24–27	61–74	73–76	2–5	4–5	5–15	12–14	7–20	15–20
Summer										
Surface	17–32	28.8–28.9	68–83	71.1–71.2	1–3	2–3	4–7	6–7	6–9	8–9
Subsurface	19–35	31–38	65–81	62–69	2–3	2.6–2.9	6–10	4–7	8–13	7–9
Bottom	24–29	24–35	71–76	65–76	1–2	1.7–2.4	4–7	4–6	5–9	6.8–7.2
Autumn										
Surface	25–42	46–67	58–75	33–54	2–4	2–3	3.7–5.0	0.8–3.7	6–9	3–7
Subsurface	24–41	46–83	59–76	17–54	1–3	2.5–2.9	3.3–4.8	0.5–3.3	4–8	3–6
Bottom	19–38	35–85	63–81	15–65	1–3	2.4–2.5	3.8–5.3	0.4–4.7	5–8	3–7

**Fig. 2.** Spatial and vertical distributions of dissolved carbohydrate and DOC concentrations in winter (a), spring (b), summer (c), and autumn (d).

a strong positive correlation between DOC and TDCHO in summer ($\rho=0.798$, $p<0.001$). Chl *a* and MCHO was positively correlated in all seasons ($p<0.01$). Chl *a* was positively correlated with TDCHO in spring and summer ($p<0.01$). There were strong negative correlations between salinity and other variables (MCHO, TDCHO and Chl *a*) in spring ($p<0.001$).

As shown in Fig. 3, linear relationships were observed between MCHO and TDCHO/DOC ratio in middle-inner ($p<0.05$) and outer ($p<0.001$) bays, respectively. Strong linear relationships observed between PCHO and TDCHO/DOC ratio at both parts of the bay (middle-inner bays: $R^2=0.621$, $p<0.001$, outer bay: $R^2=0.684$, $p<0.001$). The ratio of TDCHO/DOC was linearly related with Chl *a* at outer bay ($p<0.05$). However, linear relationship between Chl *a* and TDCHO/DOC ratio was not signi-

ficant at middle-inner bays ($p=0.426$).

4 Discussion

In the literature, it has been reported that PCHO levels were changed on a seasonal basis and high PCHO values were observed in winter and summer (Mykkestad and Børsheim, 2007). Nutrient transport from seabed to surface waters might also support phytoplankton activity in winter following the breakdown of stratification at water column (Scoullou et al., 2006). In some studies, higher dissolved carbohydrate levels have been reported in spring and summer than in winter and autumn (Mykkestad and Børsheim, 2007; Hung et al., 2009; He et al., 2015). However, high carbohydrate levels have also been reported in winter (Lee et al., 2017). In this study, maximum MCHO levels were found in

Table 4. Correlations between MCHO, PCHO, TDCHO, DOC, Chl *a* and salinity

		PCHO		TDCHO		DOC		Chl <i>a</i>		Salinity	
		ρ	<i>p</i>	ρ	<i>p</i>	ρ	<i>p</i>	ρ	<i>p</i>	ρ	<i>p</i>
MCHO	winter	0.391	0.079	0.683	<0.001***	0.613	0.003**	0.578	0.006**	-0.285	0.211
	spring	0.588	0.005**	0.752	<0.001***	0.203	0.376	0.697	<0.001***	-0.815	<0.001***
	summer	0.754	<0.001***	0.822	<0.001***	0.661	0.001**	0.722	<0.001***	0.400	0.072
	autumn	-0.467	0.033*	0.533	0.013*	0.173	0.453	0.793	<0.001***	-0.328	0.147
PCHO	winter			0.900	<0.001***	-0.021	0.929	0.181	0.430	-0.380	0.089
	spring			0.951	<0.001***	0.219	0.340	0.507	0.019*	-0.595	0.004**
	summer			0.981	<0.001***	0.777	<0.001***	0.745	<0.001***	0.516	0.017*
	autumn			0.392	0.079	0.061	0.794	-0.552	0.010*	0.640	0.002**
TDCHO	winter					0.214	0.350	0.303	0.181	-0.343	0.127
	spring					0.165	0.475	0.660	0.001**	-0.743	<0.001***
	summer					0.798	<0.001***	0.749	<0.001***	0.546	0.010*
	autumn					0.487	0.025*	0.241	0.293	0.256	0.263
DOC	winter							0.643	0.002**	-0.270	0.237
	spring							0.050	0.830	-0.049	0.831
	summer							0.742	<0.001***	0.357	0.111
	autumn							0.159	0.492	0.019	0.936
Chl <i>a</i>	winter									-0.670	<0.001***
	spring									-0.950	<0.001***
	summer									0.246	0.282
	autumn									-0.503	0.020*

Note: *n*=21 for all tests; ρ is the Spearman's rank correlation coefficient and *p* the significance level; * significance level of 0.05; ** significance level of 0.01; *** significance level of 0.001.

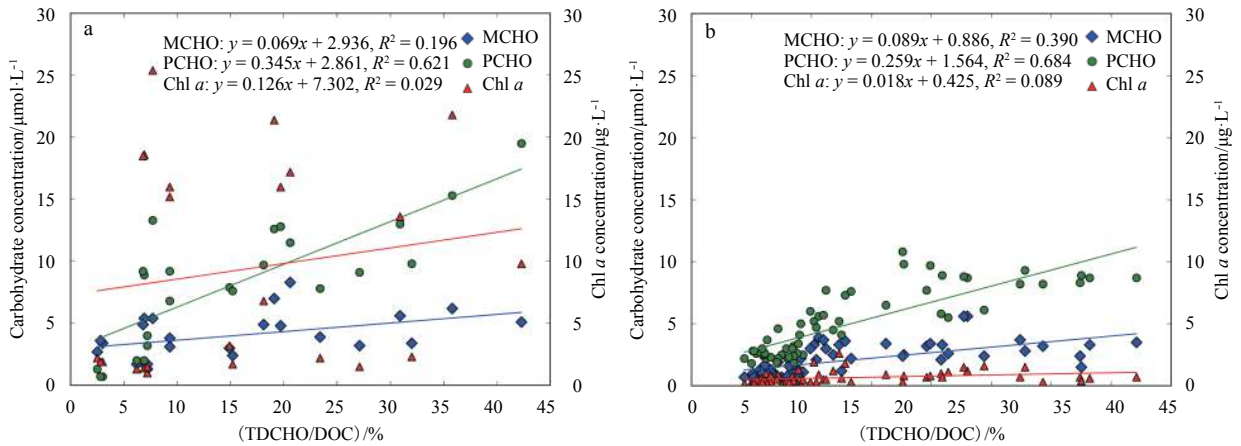


Fig. 3. Linear relationships between MCHO, PCHO, Chl *a*, and TDCHO/DOC (%) for middle-inner bay (a) and outer bay (b).

spring. The highest PCHO and TDCHO levels were observed in winter and spring.

Seasonal changes of PCHO and TDCHO were showed similar trends with Chl *a* levels. According to Table 4, PCHO and TDCHO were highly correlated with Chl *a* levels at spring and summer. PCHO, TDCHO and Chl *a* concentrations were decreased in autumn. These results indicated that PCHO and TDCHO levels might be influenced by biological processes especially in spring, summer and autumn. Similar significant correlations between carbohydrate and Chl *a* concentrations were reported in the literature (Hung et al., 2001, 2003; Khodse et al., 2007). However, car-

bohydrate concentrations are also influenced by other factors like bacterial utilization and grazing activities (Strom et al., 1997; Hopkinson et al., 2002; Guo et al., 2004; Wang et al., 2006). In winter, PCHO and TDCHO levels might be affected by rain run-offs, weathering and terrestrial inputs (Wang et al., 2003; Shin et al., 2003; He et al., 2015). DOC and carbohydrate levels (MCHO, PCHO and TDCHO) were found higher in middle-inner bays, under the influence of anthropogenic inputs, compared to outer bay.

Vertical variations of Chl *a*, DOC and dissolved carbohydrate concentrations were not significant in this study ($p < 0.05$). This

might be resulted from shallow water depths in the İzmir Bay. Depths of stations at middle-inner and outer bays range between 9–22 m and 27–66 m, respectively. In the literature, significant vertical variations in Chl *a*, DOC and dissolved carbohydrate concentrations were observed between upper water column (euphotic zone) and deep waters where light penetration is very low (Hung et al., 2003; Wang et al., 2006; Lin and Guo, 2015).

The ratio of MCHO/TDCHO increased from winter to autumn, especially at middle-inner bays, and similar results were reported in the literature (Wang et al., 2006; He et al., 2015; Lin and Guo, 2015). The ratios of MCHO/DOC and TDCHO/DOC decreased from winter to autumn and similar ratios were also observed in the literatures (Wang et al., 2006; Mykkestad and Børsheim, 2007; He et al., 2015; Lin and Guo, 2015). Increasing MCHO/TDCHO and decreasing TDCHO/DOC ratios indicated production of significant MCHO fraction or breaking down of significant PCHO fraction from winter to autumn in the bay. A similar case was also reported by Wang et al. (2006).

Carbohydrate concentrations in bulk DOC pool (i.e., TDCHO/DOC ratio) were used as a tool to investigate the degradation and diagenetic status of bulk DOM and its conversion rate in aquatic environments (Skoog and Benner, 1997; Hung et al., 2009; Kaiser and Benner, 2009; Khodse et al., 2010; Lin and Guo, 2015). Lin and Guo (2015) have reported TDCHO/DOC ratios in the ranges of 11%–71% and 14%–52% at surface waters of shelf and basin parts of Gulf of Mexico, respectively. Similar TDCHO/DOC ratios (2.5%–42.3% at middle-inner and 4.4%–37.1% at outer bays) were

found in the present study. According to Fig. 3, TDCHO/DOC ratios were distributed at wide ranges and it might be related to the presence of newly forming and degrading fractions of DOM in the bay. Higher carbohydrate fractions were observed in freshly produced DOM and lower TDCHO/DOC ratios were found during the degradation process of DOM (Opsahl and Benner, 1999; Lin and Guo, 2015).

According to factor analysis between salinity, Chl *a*, DOC, MCHO and TDCHO (Fig. 4a), two factors were extracted and the factors were statistically sufficient to model the variations in the data of middle-inner bays. Factors 1 and 2 sufficiently explained 68% and 32% of the variability in the data, respectively. While the MCHO, TDCHO, DOC and Chl *a* explained by Factor 1, salinity and also DOC identified by Factor 2 for middle-inner bays. Factor analysis showed that variability of the data could be explained by at least two factors at outer bay (Fig. 4b). Factors 1 and 2 were accounted for 83% and 17% of the variability in the data, respectively. DOC, MCHO, TDCHO and Chl *a* were explained by Factor 1. Salinity and Chl *a* were explained by Factor 2 at outer bay. According to results of factor analyses, Chl *a* and dissolved carbohydrate species (MCHO and TDCHO) were explained in the same factor groups and phytoplankton activities could have an important role on the dissolved carbohydrate concentrations in the İzmir Bay.

DOC, MCHO and TDCHO levels determined in this study were similar to levels reported from other bays and gulfs (Table 5). DOC concentrations in the present study were very close to DOC

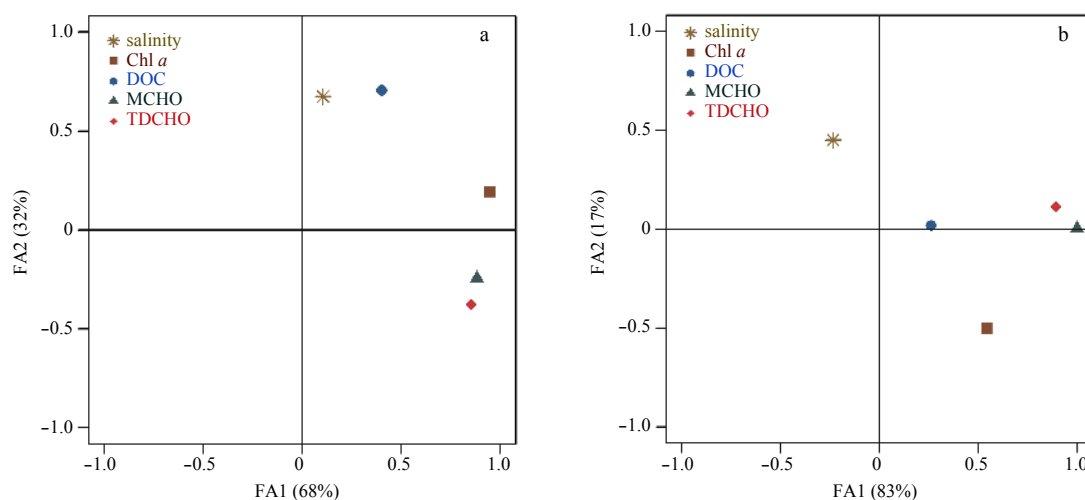


Fig. 4. Biplots of factor loadings indicating the explained proportions of variances for salinity, Chl *a*, DOC, MCHO, and TDCHO in the bay: middle-inner bay (a) and outer bay (b).

Table 5. Comparison of DOC, MCHO and TDCHO levels of the İzmir Bay with different parts of the world

Location	DOC/ $\mu\text{mol}\cdot\text{L}^{-1}$	MCHO/ $\mu\text{mol}\cdot\text{L}^{-1}$	TDCHO/ $\mu\text{mol}\cdot\text{L}^{-1}$	Reference
Atlantic Ocean and Pacific Ocean	47–119	2.4–6.2	7–33	Pakulski and Benner (1994)
Black Sea	148–270	–	12–20	Cauwet et al. (2002)
Northern Adriatic Sea	–	5–54	5–95	Ahel et al. (2005)
Beaufort Sea	30–202	–	0.6–1.3	Panagiotopoulos et al. (2014)
Bay of Bengal	–	0.9–2.9	4.5–7.9	Bhosle et al. (1998)
Trieste Gulf	108–200	2–13	11–126	Terzić et al. (1998)
San Francisco Bay	52–172	0.2–1.3	1–4	Murrell and Hollibaugh (2000)
Gulf of Mexico	205	–	28.8	Hung and Santschi (2001)
Galveston Bay	300–363	–	27.1–83.3	Hung and Santschi (2001)
İzmir Bay	32–244	0.7–8.3	2.6–24.6	this study

levels observed at the Beaufort Sea (Panagiotopoulos et al., 2014), Black Sea (Cauwet et al., 2002) and Gulf of Mexico (Hung and Santschi, 2001). DOC levels at the Galveston Bay (Hung and Santschi, 2001) were greatly higher than the DOC levels in this study. MCHO levels in this study were only lower than those reported from the northern Adriatic Sea (Ahel et al., 2005). Maximum MCHO concentration in this study was higher than the maximum levels in the Atlantic Ocean, Pacific Ocean, Beaufort Sea, Trieste Gulf, San Francisco Bay and Gulf of Mexico (Pakulski and Benner, 1994; Terzić et al., 1998; Murrell and Hollibaugh, 2000; Hung and Santschi, 2001; Panagiotopoulos et al., 2014). TDCHO levels in this study were close to the TDCHO levels at the Black Sea, Bay of Bengal, Gulf of Mexico and San Francisco Bay (Bhosle et al., 1998; Murrell and Hollibaugh, 2000; Hung and Santschi, 2001; Cauwet et al., 2002). On the other hand, maximum TDCHO levels at the northern Adriatic Sea and Trieste Gulf were much higher than the TDCHO levels in this study (Terzić et al., 1998; Ahel et al., 2005).

5 Conclusions

Seasonal variations and distributions of dissolved carbohydrates, salinity, Chl *a* and DOC levels were studied in the İzmir Bay. According to the results, the highest MCHO levels were found in spring and the MCHO levels decreased from summer to autumn. Maximum PCHO and TDCHO levels were observed in winter and the PCHO and TDCHO levels decreased from winter to autumn. Mean dissolved carbohydrate, DOC and Chl *a* levels were decreased from middle-inner to outer bay. Increasing MCHO/ TDCHO and decreasing TDCHO/DOC ratios indicated production of significant MCHO fraction or breaking down of significant PCHO fraction from winter to autumn. TDCHO/DOC ratio distributed at a wide range that might be linked with the presence of newly forming and degrading fractions of DOM. According to results of factor analysis, Chl *a* and dissolved carbohydrate species (MCHO and TDCHO) were explained in the same factor group and phytoplankton activities could have an important role on the dissolved carbohydrate concentrations in the İzmir Bay. As indicators of biological and physical processes in seawater, seasonal and vertical variations of DOC, Chl *a*, MCHO, PCHO and TDCHO were useful in investigation of organic matter distribution. The effects of other environmental factors (i.e., primary production, bacterial abundance, extracellular enzyme activities) on dissolved carbohydrate levels should be investigated in further studies.

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