

Interactive comment on “Transparent exopolymer particle binding of organic and inorganic particles in the Red Sea: Implications for downward transport of biogenic materials” by Abdullah H. A. Dehwah et al.

Abdullah H. A. Dehwah et al.

tmissimer@fgcu.edu

Received and published: 25 July 2019

eTTransparent exopolymer particle occurrence and interaction with algae, bacteria, and the fractions of organic carbonbinding of organic and inorganic particles in the Red Sea: Implications for downward transport of biogenic materials

Abdullah H. A. Dehwah^{1,6}, Donald M. Anderson², Sheng Li^{1,4}, Francis L. Malion³, Zenon Batang³, Abdullah H. Alshahri¹, Seneshaw Tsegaye⁵, Michael Hegy⁵⁴, Thomas M. Missimer⁵ 1 King Abdullah University of Science and Technology (KAUST),

C1

Water Desalination and Reuse Center (WDRC), Biological and Environmental Science and Engineering (BESE), Thuwal 23955-6900, Saudi Arabia 2Woods Hole Oceanographic Institution, Biology Department, Woods Hole, MA 02543, USA 3Coastal and Marine Resources Core Laboratory, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia 4Guangzhou Institute of Advanced Technology, CAS, Haibin Road #1121, Nansha district, Guangzhou 511458, China 5U. A. Whitaker College of Engineering, Emergent Technologies Institute, Florida Gulf Coast University, 16301 Innovation Lane, Fort Myers, Florida 33965-6565 6Desalination Technologies Research Institute (DTRI), Saline Water Conversion Corporation (SWCC), P.O. Box 8328, Al-Jubail 31951, Saudi Arabia

Abstract: Binding of particulate and dissolved organic matter in the water column by marine gels allows sinking and cycling of organic matter into deeper water of the Red Sea and other marine water bodies. A series of four offshore profiles were made at which concentrations of bacteria, algae, particulate transparent exopolymer particles (p-TEP), colloidal transparent exopolymer particles (c-TEP), and the fractions of natural organic matter (NOM), including biopolymers, humic substances, low molecular weight neutrals, and low molecular weight acids were measured to depths ranging from 90 to 300 m. It was found that a statistically-significant relationship occurs between the concentrations of p-TEP with bacteria and algae, but not with TOC in the offshore profiles, but not the nearshore samples.while a minimal, non-significant relationship between p-TEP and algae occurs. This likely reflects the low abundance of larger algal species in the study region. Variation in the biopolymer fraction of NOM in relationship to TEP and bacteria suggests that extracellular discharges of polysaccharides and proteins from the bacteria and algae are occurring without immediate abiotic assembly into p-TEP. In the water column below the photic zone, TOC, bacteria, and biopolymers show a generally common rate of reduction in concentration, but p-TEP decreases at a diminished rate, showing that it persists in moving organic carbon deeper into the water column despite consumption by bacteria.

C2

1 Introduction

Mechanisms that control the biogeochemical cycles influenced by microorganisms in the world 's oceans are complex and poorly understood (Azam and Malfatti, 2007). The relationships between microalgal and bacterial abundance, total organic carbon (TOC), fractions of natural organic matter (NOM), polysaccharides, and transparent exopolymer particles (TEP) in seawater with depth play important roles in the transport and cycling of nutrients and particulate organic matter (POM) in general sediments (Aldredge and Crocker, 1995; Passow, 2002; Azam and Malfatti, 2007). In particular, the binding of suspended sediments and particulate organic matter by TEP and other acidic polysaccharides, in addition to general aggradation, tends to increase particle size and weight, thus increasing settling rates in the water column (Passow et al., 2001; Wurl et al., 2011). It has been demonstrated that gel-type particles link particulate and dissolved organic matter in the ocean (Verdugo et al., 2004). The sinking of biogenic particles drives elemental cycling, which in turn controls primary and secondary productivity through the water column (Wurl et al., 2011). Particulate organic material is commonly occupied or influenced by bacteria which can reduce the biomass by consumption of some organic matter over various timeframes from days to weeks (Bižić-Ionescu et al., 2018). TEP are ubiquitous in the oceans (Passow, 2002), likely formed caused by abiotic coagulation and aggradation of dissolved carbohydrates or primarily acidic polysaccharides, but also by biotic formation as extracellular secretions by algae or bacteria (Chin et al., 1998; Stoderegger and Herndl, 2001; Passow et al., 2001; Berman and Viner-Mozzini, 2001; Passow et al., 1994). Particulate TEP (p-TEP) is in the size range 0.4–200 μm , with a number of forms, including amorphous blobs, disseminated clouds, sheets, filaments or clumps (Zhou et al., 1998; Passow, 2002; Mari et al., 2004). Colloidal TEP (c-TEP) consist of particles that are stained by Alcian blue with a diameter range of 0.05 to 0.4 μm (Villacorte et al., 2009). However, c-TEP is defined based solely on staining with Alcian blue, which is known to also stain

C3

other substances in seawater, including sulfated and carboxylated polysaccharides, glycoproteins, polyanions in general, and acidic polysaccharides not associated with TEP (Winters et al., 2016). TEP are composed of acidic polysaccharides enriched with fucose and rhamnose, thus serving as a food source in the water column and commonly associated with layers of intense microbial and biochemical activity (Azam and Long, 2001). TEP generally decrease in concentration with depth in the sea (Engel et al., 2004), with a tendency to float to the sea surface if unballasted to contribute a gelatinous layer to the sea surface microlayer (Azetsu-Scott and Passow, 2004; Wurl and Holmes, 2008; Wurl et al., 2009). Bar-Zeev et al. (2015) have documented that p-TEP is mainly composed of polysaccharides, which can be dispersed in the presence of different types of chelators, be fractured to form colloids or reassemble abiotically. The trends in TEP concentrations in the seawater column have been previously examined (Jennings et al., 2017), and the relationship of TEP with TOC, DOC, and bacteria have also been investigated in many areas of the ocean (Engel, 2004; Simon et al., 2002; Ortega-Retuerta et al., 2009; Ortega-Retuerta et al., 2011; Bar-Zeev et al., 2011). However, these relationships have not been studied in the Red Sea. Studies on TEP distribution in relation to other forms of organic matter in the Red Sea have focused mainly on assessing the links between TEP and phytoplankton and bacterial production (Bar-Zeev et al., 2009b) and the impacts of TEP and dissolved forms of NOM on biofouling in seawater desalination plants (Bar-Zeev et al., 2009a; Dehwah et al., 2015a; Dehwah et al., 2015b; Dehwah et al., 2015c; Dehwah and Missimer, 2016; Rachman et al., 2014; Rachman et al., 2015). The intakes for reverse osmosis seawater desalination plants are located in shallow, nearshore areas of the Red Sea, so little consideration has been given to changes in TEP concentration with depth until it was suggested that deep-water intake systems may produce seawater quality with lower concentrations of algae, bacteria, and organic compounds, such as TEP, thus possibly lessening rates of membrane biofouling (Dehwah et al., 2015c). The relationships between TEP concentrations and abundance of microalgae, bacteria, TOC, and dissolved fractions of NOM, including biopolymers, humic substances, building blocks,

C4

low molecular weight (LMW) acids, and LMW neutrals from the sea surface to 300 m depth are herein presented. The present study provides the first data from the Red Sea, with initial insights into the vertical transport of organic carbon, including the fractions of natural organic matter from the surface to depths near or below the photic zone. The authors are keenly aware that the data presented herein have not been collected in a systematic manner with spatial and temporal comparisons to assess the biogeochemical cycles within the Red Sea comprehensively. However, the compiled data can be used to better characterize the biogeochemical cycles of the Red Sea as other researchers add new data. The reported datasets represent the first measured in the Red Sea wherein the fractions of organic matter, including biopolymers, humic substances, building blocks, low molecular weight neutrals, and low molecular weight acids (very expensive to measure), are linked with measurements of algae, bacteria, TOC, and TEP.

2 Methods

2.1 Compilation and comparison of available data

There have been several investigations on organic matter, including TEP, collected at depths near the sea surface along the Red Sea coast of Saudi Arabia, with the main focus to establish the relationships between seawater organic matter content and the potential for membrane biofouling in seawater desalination facilities (Rachman et al., 2015; Dehwah et al., 2015; Dehwah and Missimer, 2016; Alsahri et al., 2017; Dehwah et al 2017; Dehwah and Missimer 2017; Dehwah and Missimer, 2015d; Figure 1, blue dot locations). These shallow nearshore data were compiled and assessed to compare to the newly collected offshore data and to assess statistical relationships between various organic parameters. Note that these data have been collected at many different times of the year and were not used to attempt the characterization of the natural seasonal variations and the overall biochemical activity in the nearshore area of the Red Sea.

C5

2.2 Seawater vertical profiles in the Red Sea

Seawater properties of the water column were measured at four sites (A–D) north of Jeddah, along the Saudi coast of the Red Sea in deep water areas (> 1000 m) (Fig 1). In situ vertical profiles of temperature, salinity, dissolved oxygen (DO), pH, turbidity, chlorophyll-a (fluorescence), and photosynthetically active radiation (PAR) were determined with a multi-sensor assembly fitted to a Rosette carousel holding a set of Niskin water sampling bottles (General Oceanics, USA). Continuous vertical profiling was conducted from sea surface to 90 m depth at sites A–C, with seawater samples obtained at 10 m depth intervals for the analysis of organic parameters. At site D, continuous vertical profiles of physicochemical parameters were taken from 7 m below sea surface to 300 m depth, with seawater samples for organic parameters obtained at 10 m intervals from the surface to 100 m depth and at 20 m intervals thereafter to 300 m depth. Sampling at sites A–C was conducted in April 2014, whereas at site D in February 2015. The sample timing was based on ship availability and the data collected cannot be used to fully characterize the Red Sea in deep water located far from the coast. Note that the water depth drops almost vertically to greater than 1000 m beginning in the nearshore at the 20 m contour (Dehwah et al., 2015c). The multi-sensor assembly included the SBE 43 CTD device (Sea-Bird 911 plus CTDScientific) for salinity, temperature and depth profiling, with a DO add-on sensor; Wet Labs ECO AFL/FL (Sea-Bird Scientific) was used for turbidity and fluorescence detection; and a biospherical light sensor (LI-COR) was used for PAR measurement. All sensors were pre-calibrated according to manufacturer specifications before actual use in field sampling and was normalized.

2.3 Quantification and characterization of microalgae and bacteria

Microalgal abundances in water samples were determined by flow cytometry, using a BD FACSVerse flow cytometer for counting and characterizing algal cells, as described by van der Merwe et al. (2014).. An Accuri flow cytometer was used to measure bacterial abundance. Flow cytometry enables a rapid and accurate counting of mi-

C6

croorganisms (VivesRego et al., 2000). Light scattering properties and/or fluorescence intensity was determined by the flow cytometer to distinguish between different algal types, as described by van der Merwe et al. (2014). Lasers were used to excite both unstained autofluorescent organisms (algae) and stained bacterial cells with. The red laser wavelength was set at 640 nm and the blue laser at 488 nm. for the Accuri flow cytometer. Algal cell counting was performed by combining 500 μ L of each sample with a 1 μ L volume of a standard containing 1 μ m beads to calibrate size in a 10 mL tube. The tube was then vortexed and measured at high flow rate with a 200 μ L injection volume for 2 min. The counting procedure was repeated three times to assess the precision of measurements. There different types of algae, cyanobacteria, Prochlorococcus, and pico/nanoplankton and cyanobacteria, were distinguished based on their autofluorescence as well as by the cell side-angle scatter, which was used to identify them by size (Radíc et al., 2009). A comparative protocol employing SYBR®Green stain was used for bacteria counting. A volume of 500 μ L from each sample was transferred to a 10 mL tube, incubated in 35°C water bath for 10 min. SYBR® Green dye was added at a 5 μ L into a 500 μ L aliquot to stain the cells. The sample was vortexed and incubated for 10 min. The prepared samples were then analyzed at a medium flow setting with a 50 μ L injection volume for 1 min. For validation, 8-Peak calibration beads were used. Triplicate measurements were made on each sample to assess measurement precision.

2.4 Measurement of TOC and NOM fractions

TOC concentration was measured with a Shimadzu TOC-VCSH. Fractions of dissolved organic carbon, including biopolymers, humic substances, building blocks, low molecular weight (LMW) neutrals, and low molecular weight (LMW) acids, were determined by Liquid Chromatography Organic Carbon Detector (LCOCD, DOC-Labor), using a size exclusion chromatography column Toyopearl HW-50S (TOSOH), following the methods by Huber et al. (2011). A calibration curve was established for both molecular masses of humic substances and detector sensitivity before sample measurements. Humic

C7

acid and fulvic acid standards (Suwannee River Standard II) were used for the molecular mass calibration, whereas potassium hydrogen phthalate and potassium nitrate (KNO₃) for sensitivity calibration based on Huber et al. (2011). All seawater samples for LCOCD were manually pre-filtered using a 0.45 μ m syringe filter to exclude the undissolved particulate organics. Before sample analysis, a system cleaning was performed by injection of 4,000 μ L of 0.1mol/L NaOH through the column for 260 min. After cleaning, 2,000 μ L of the sample was injected for analysis at 180 min retention time and 1.5 mL/min flow rate. A mobile phase of phosphate buffer, with 28 mmol STD and 6.58 pH, was used to carry the sample through the system. The resulting chromatogram showed a plot of signal response of different organic fractions against retention time. Manual integration of the data, also following Huber et al. (2011), was performed to determine the concentrations of the different organic fractions, including biopolymers, humic substances, building blocks, LMW acids and LMW neutrals.

2.5 TEP measurement

Both p-TEP and c-TEP were simultaneously determined in each collected sample. The size range of p-TEP is between 0.4 and 200 μ m, whereas c-TEP between 0.05 and 0.40 μ m (Villacorte et al., 2009). TEP analysis was based on the method developed by Passow and Alldredge (1995), which involves sample filtration, membrane staining with Alcian blue, and then UV spectrometry. A staining solution was prepared from 0.06% (m/v) Alcian blue 8GX (Fluka) in acetate buffer solution (pH 4) and freshly pre-filtered through a 0.2 μ m polycarbonate filter before usage. A 300 mL volume of seawater from each water sample was filtered through a 0.4 μ m pore size polycarbonate membrane using an adjustable vacuum pump at low constant vacuum. After filtration, the membrane was rinsed with 10 mL of Milli-Q water to prevent the Alcian blue from coagulating, as salts may remain on the filter after seawater filtration, thus avoiding the likelihood of overestimating the TEP concentration. The retained TEP particles on the membrane surface were then stained with the Alcian blue dye for 10 seconds. After staining, the membrane was flushed with 10 mL of Milli-Q water to remove any excess

C8

dye. The flushed membrane was then placed into a small beaker, where it was soaked in 80% sulfuric acid for 6 hours to extract the dye that was bound to the p-TEP. Finally, the absorbance of the acid solution was measured by a UV spectrometer at 752 nm wavelength to determine the TEP concentration. The same methodology was applied to determine the colloidal TEP, except that a 250 ml volume of water sample from 0.4 μm polycarbonate membrane permeate was filtered through a 0.1 μm pore size to allow deposition of the c-TEP on the membrane surface. To relate the measured UV absorbance values to TEP concentrations, a calibration curve was established. Xanthan gum solutions with different volumes (0, 0.5, 1, 2, 3 mL) were used to obtain the calibration curve (Fig. 2). Note that the calibration curve for samples collected at sites A, B, and C are shown in Figure 2a and the curve for site D is shown as Figure 2b. The TOC concentrations of xanthan gum before and after 0.4 μm filtration were analyzed, and the TOC concentration difference was used to calculate the gum mass on each filter and the TEP concentration was estimated using the calibration curve. The same procedures were used for the 0.1 μm membrane to establish the calibration curve for colloidal particles. Afterwards, the TEP concentration was expressed in terms of Xanthan Gum equivalent in $\mu\text{g Xeq./L}$ by dividing the TEP mass by the corresponding volume of TEP samples. Because particulate and colloidal TEP is determined indirectly, these values must be considered to be semi-quantitative. The new method developed by Villacorte et al. (2009) for TEP measurement was not used, as it would limit the comparability of the measured data with previous results.

2.6 Statistical methods used for data comparison

It is essential to perform a multidimensional regression analysis at a certain meaningful abstraction level to find interesting patterns and to determine whether the result of a data set is statistically significant (Chen et al., 2002). Multiple regression has been used by several researchers and practitioners for theory testing or explanation purposes. The question of interest becomes understanding the significance of the variables, and the variation and interaction between them (Tonidandel and LeBreton,

C9

2011). It is herein desired to highlight the correlation between TEP concentrations and abundance of microalgae, bacteria, TOC, and dissolved fractions of NOM, including biopolymers. Thus, a multi-dimensional regression analysis, a correlation matrix, and two-way analysis of variance (ANOVA) with replication were performed to test the interaction and statistical significance between the various organic properties. In order to perform multiple regression analysis, there must be a relationship between the outcome variable and the independent variables the residuals are normally distributed, and the independent variables should not be highly correlated with each other (Osborne and Waters, 2002; Williams et al., 2013). The interdependency was tested using Pearson's bivariate correlations matrix. The spatial/temporal variation of the data is not the focus of this paper and the intricacies of dominance or relative weight between variables were not considered. The correlation coefficients, R² and p-values were calculated to assess the relationship, statistical significance and interaction between various organic properties. The correlation matrix is an identity matrix, which would indicate that variables are related or unrelated. Multi regression analysis is used to understand the significance of the two or more organic properties in predicting the value of a criterion variables (TEP and Biopolymers). The two-way ANOVA analyses were used to determine the significant difference and interaction among the sites and organic properties. When the p value was below 0.05, the null hypothesis was void and the relationship was deemed to be significant. A series of scatter plots were constructed to test the statistical significance between various organic parameters. The R² and p-values were calculated to assess the degree of fit to a curve and the statistical significance. When the p value was below 0.05, the null hypothesis was void and the relationship was deemed to be significant.

3 Results

3.1 Variations in salinity, temperature, fluorescence, pH, dissolved oxygen, PAR/irradiance, biospherical/licor, and turbidity

The thermocline in the three profiles (sites A-C) collected to a 90 m depth showed a

C10

slight decrease in temperature from near 29 oC to between 24 and 25 oC 90 m below surface (Fig. 3). The decline in temperature was relatively gradual at all three sites. In the deep profile, the temperature declined from about 26.5 oC at the surface to about 22 oC at 300 m. An inflection point occurred at about 115 m and the change in temperature below this depth to 300 m was only about 2.5 oC (Fig. 4). The difference in the temperature at the sea surface between profiles was likely caused by the time of year of measurements, with the 90 m profiles occurring in April versus the 300 m profile in February which is the peak of winter in the study area. The halocline showed similar salinity variations in the 90 m profiles with a slight, rather uniform increase from about 39 ppt at surface to 40 ppt at 90 m (Fig. 3). A slightly lower salinity gradient coinciding with a slightly higher temperature gradient occurred at site B. The salinity change in the 300 m profile showed a similar pattern from about 39 to 40 ppt in the upper 115 m, but an inflection occurred at about 115 m wherein the rate of increase declined to a few tenths of a ppt over the lower 185 m. The inflection point showing a slope change for both temperature and salinity occurred at about the same depth which may indicate the presence of two water masses (Figs. 3 and 4). The vertical trends in pH also exhibited minimal variations down to 90 m at sites A–C (Fig. 3), but with slightly lower pH values at site A (7.9–8.0) than at sites B and C (8.0–8.1). In the deep profile, pH was nearly stable at about 8.3 until 115 m and then steadily decreased to 8.1 at 300 m (Fig. 4). Dissolved oxygen (DO) concentrations in the shallow profiles at all three sites showed high variability (6–12.5 mg/L) in the top layer (unknown reason for variation), but with relative stability at about 5 mg/L from 20 to 90 m (Fig. 3). DO in the deep profile was at lower concentrations (0.8–1.5 mg/L) near the surface, increasing to around 2 mg/L at 115 m and then steadily declined to about 0.6 mg/L at 300 m with a saturation of only 10%. The vertical pattern in chlorophyll a (chl-a) concentrations markedly differed between shallow and deep profiles (Figs. 3 and 4). At sites A–C, chl-a was slightly detected at the surface but abruptly increased from 0.3 to 1.2 mg/m³ within 50–75 m and thereafter declined to near 0.2 mg/m³ at sites A and C and to about 0.5 mg/m³ at site B. Chl-a concentrations were relatively low in the deep profile,

C11

decreasing from about 0.45 mg/m³ at the surface to about 0.06 mg/m³ at 100 m, from which it remained unchanged until 300 m. Note that these chl-a concentrations were based on in situ fluorescence detection using a sensor that was pre-calibrated with a chlorophyll standard from the manufacturer (Wet Labs). As chlorophyll fluorescence may vary with cell physiological condition, time of day, light regime, and other factors, and since the sensor was not field-validated after calibration, the present chl-a values should thus be considered semi-quantitative. PAR levels at sites B and C were initially recorded at 600–700 μ mol/m²/s at the surface and then steeply decreased to 120–160 μ mol/m²/s at 20 m depth, from where it further decreased gradually until 90 m depth (Fig. 3). At site A, where the measurement was done at an earlier time, PAR varied between 220–300 μ mol/m²/s within the top 10 m layer and then coincided with the same values at sites B and C. PAR in the deep profile steeply declined from about 240 μ mol/m²/s near the surface to about 20 μ mol/m²/s at 40 m depth, after which it gradually decreased to near zero at about 75 m depth, which is generally similar to the trend in the shallow profiles (Figs. 3 and 4). The depths at which the PAR levels were at 1% of the surface values were in range of 38–54 m for all sites. Turbidity was generally low in the vertical profiles at all sites. Turbidity varied in the narrow range of 0.2–0.3 NTU, with only a few spikes up to 0.4 NTU, in all three shallow profiles (Fig. 3). In the deep profile (site D), most turbidity values were within 0.1–0.15 NTU, with intermittent spikes up to 0.2 NTU below 75 m depth (Fig. 4).

3.2 Algae and cyanobacteria concentrations

Total concentrations of algae and cyanobacteria (summed) with depth at the shallow and deep sampling stations are shown in Figs. 5 and 6, respectively. Previous results on total algal and cyanobacterial abundances, all collected from surface layers close to shore in the same study area, are compiled in Table 1, with a range of 1,677–137,363 cells/mL (mean 44,383 cells/mL out of 38 samples). Total algal and cyanobacterial concentrations from the surface at the shallow stations (A–C) during the present study were comparable to the mean of the previous data, while the surface concentration at

C12

the deep station (D) was close to the reported maximum (Figs. 5 and 6, Table 1). The vertical profiles of algal and cyanobacteria concentrations by group (cyanobacteria, Prochlorococcus and pico/nanoplankton) are shown in Figs. 5 and 6 for the shallow (A-C) and deep sites, respectively. At sites A-C, cyanobacteria were more abundant near the surface (top 10 m layer), below that Prochlorococcus was more predominant, with peak concentrations at about 50 m (Fig. 5). In general, algal and cyanobacterial concentrations showed a substantial decline below 80 m at all sites. The same compositional and abundance trends were exhibited in the deep profile, except that cyanobacteria had higher concentrations near the surface in the deep profile while Prochlorococcus was relatively denser at subsurface depths in the shallow profiles (Fig. 6). In addition, the concentrations of pico/nanoplankton in the upper layers were relatively higher at the deep site compared to the shallow sites (A-C) (Figs. 5 and 6).

3.3 Bacteria concentrations

The vertical trends in bacterial concentrations during the present study are shown in Figs. 7 and 8, indicating higher cell densities in the upper 50 m layer at the deep site compared to sites A-C. Previous results on nearshore bacterial concentrations from the same study area ranged from 1.13×10^5 to 2.18×10^6 cells mL⁻¹ (mean 5.26×10^5 cells mL⁻¹; 40 samples) (Table 1). The new data on offshore surface concentrations of bacteria are comparable to the average of the nearshore results (Table 1, Figs. 7 and 8). Bacterial abundance generally declined with depth, with a decrement of about 4.00×10^5 to 9.00×10^4 cells mL⁻¹ from the surface to 90 m depth at sites A-C (Fig. 7) and from about 5.00×10^5 cells mL⁻¹ at the surface to 1.60×10^5 cells mL⁻¹ at 160 m and to 1.00×10^5 cells mL⁻¹ at 300 m (Fig. 8).

3.4 Total organic carbon (TOC)

TOC concentrations exhibited only minor differences between the sites, with fluctuations within a narrow range in the upper 50 m layer at both the shallow and deep sites (Figs. 7 and 8). Nearshore data on TOC ranged from 0.83 to 1.42 mg/L, with an aver-

C13

age of 1.0 mg/L from 42 measurements (Table 1). In the offshore near-surface profiles, the TOC ranged from 0.99 to 1.35 mg/L. TOC generally declined with depth at all sites, although only within a narrow range at sites A-C between 1.2 mg/L at surface and 0.9 mg/L at 90 m depth. The decline in the deeper profile was from 1.1 mg/L at surface to 1.0 mg/L at 120 m and then to 0.75 mg/L at 300 m.

3.5 Particulate and colloidal TEP concentrations

Nearshore p-TEP and c-TEP showed considerable variation in concentrations with ranges of 53–347 (mean 191) and 36–287 (125) $\mu\text{g Xeq./L}$, respectively (Table 1). Comparable ranges of concentration for both parameters were found offshore, except for the markedly higher c-TEP concentrations in the vertical profile at site A (Figs. 7 and 8). Both p-TEP and c-TEP generally declined with depth, although with fluctuations between 50–100 m depth and an elevated value at 200 m depth in the deep profile. The difference in concentrations was more pronounced for c-TEP in the deep profile, from $265 \mu\text{g Xeq./L}$ at 10 m to about $70 \mu\text{g Xeq./L}$ at 300 m. The change in concentration of p-TEP with depth in the deep profile was relatively slight, from about $285 \mu\text{g Xeq./L}$ at 40 m to $170 \mu\text{g Xeq./L}$ at 300 m. At the shallow sites, both p-TEP and c-TEP trends with depth showed similar patterns between sites B and C, except that c-TEP was unusually low in the surface layer at site C (Fig. 7).

3.6 NOM fractions

There was considerable variability in concentrations of the NOM fractions in nearshore seawater (Table 1). The range in concentration, number of samples, and average of the concentrations are the following: biopolymers (28–164 $\mu\text{g/L}$, 42, 62 $\mu\text{g/L}$), humic substances (159–442 $\mu\text{g/L}$, 42, 248 $\mu\text{g/L}$), building blocks (81–260 $\mu\text{g/L}$, 42, 118 $\mu\text{g/L}$), LMW neutrals (16–477 $\mu\text{g/L}$, 42, 271 $\mu\text{g/L}$), and LMW acids (10–130 $\mu\text{g/L}$, 42, 40 $\mu\text{g/L}$). The range in biopolymer concentrations in the surface offshore samples are similar to the nearshore samples. All of the NOM fractions have higher concentrations at the A, B, and C profiles compared to the deep profile. The biopolymer fraction of NOM shows

C14

a general reduction with depth in all offshore profiles. At sites A and B there is a spike in biopolymers at 10 m with minor variation between 10 m to 90 m. In the deeper profile, there is considerable variation in the photic zone with the surface having the highest value and subsequent spikes occurring at 30 and 60 m. Beginning at about 90 m, there is a constant downward trend in concentration beginning at about 90 m. Humic acid concentrations showed only minor variations with depth in the shallow profiles, but the deep profile showed a reduction by about 29% from 90 to 300 m depth. There is a general decreasing trend in concentration of building blocks with depth at the deep site and only minimal differences throughout the depth profiles at sites A–C (Figs. 7 and 8). The concentrations of LMW neutrals at the shallow sites were the highest amongst NOM fractions, although with a wide range of variation. In contrast, LMW acids had the lowest concentrations without marked discrepancies in concentration in the vertical profiles between sites A–C, but a general reduction occurred below 120 m in the deep profile (Figs. 7 and 8).

4 Discussion

4.1 Algal and cyanobacterial concentrations

The flow cytometry approach used in this study was used highly effective in characterizing and enumerating the small size classes of phytoplankton and cyanobacteria that are readily distinguishable on the basis of cell size and autofluorescence. Thus, cyanobacteria (presumably *Synechococcus* spp), *Prochlorococcus*, and the general class of pico/nanoplankton were numerically dominant, with very few larger eukaryotic algal species detected. This is consistent with prior studies that reported that phytoplankton in the oligotrophic northern Red Sea and Gulf of Aqaba are dominated (>95%) by cells <5 μm in size (Lindell and Post 1995; Yahel et al. 1998). Only during the summer does the large macroalgae *Trichodesmium* sp. also become prominent. As reported here, algae ranging from 5 to several hundred μm are extremely scarce, although not totally absent (Sommer 2000; Kimor and Goldanski 1992).

C15

4.2 Statistical significance and dependency of p-TEP, c-TEP and biopolymers

The two-way ANOVA was employed to provide an important insight into the pattern of the data and its interdependency. Each organic parameter (sample) has been drawn independently of the other parameters and is normally distributed. The analysis shows that there is a significant difference ($p<0.05$) in the mean of the concentrations of TEP, bacteria, algae, TOC and biopolymer, and the mean of the sites. Also, it shows that there is no interdependency between the sites where samples are measured (Table 2). In order to validate the appropriateness of the multiple regression analysis, multicollinearity of the concentration of Bacteria, Algae, Biopolymer, TOC, p-TEP, and c-TEP were checked using a bivariate correlation matrix (Table 3). The matrix of Pearson's bivariate correlations among all independent variables shows that the magnitude of the correlation coefficients are less than 0.8. A series of multi regression statistical analyses were performed to test if there are significant relationships between dependent and independent variables/ organic properties. A summary of results of this analysis is presented and shown in Table 3.

4.2.1 p-TEP Multiple regression analysis between the p-TEP and the concentrations of bacteria, and algae, shows a significant statistical correlation for all offshore profiles while the concentrations of TOC is not significant parameter for the p-TEP. The overall regression is significant when the three variables are considered as a group. The individual variables and their significance are shown in Tables 3 and 4. However, the analysis shows that there was no statistically-significant relationship between p-TEP and the three parameters in the shallow, nearshore samples (Tables 3 and 4).

4.2.2 c-TEP The overall regression between the c-TEP and the concentrations of bacteria, algae, and TOC shows no significant statistical correlation for the shallow, nearshore (sites A, B, C) and offshore (Site D) profiles. However, the individual linear regression analysis shows statistically-significant relationships between c-TEP and TOC, and c-TEP and bacteria. There is no statistical relationship of significance between p-TEP and c-TEP with an adjusted $R^2=0.12$. Also, the nearshore samples show

C16

that there was no statistically-significant relationship between c-TEP and TOC and Bacteria (Tables 3 and 4).

4.2.3 Biopolymers Multiple regression analysis between the biopolymers and the concentrations of bacteria, p-TEP and c-TEP shows a significant statistical correlation for all the offshore profiles while the concentration of algal and cyanobacteria is not a significant predictor of the biopolymers. The overall regression is significant when the four variables are considered as a group. The individual variables and their significance are shown in Table 3. The relationship between the biopolymers and all the four independent variables shows no significant statistical correlation in the shallow, nearshore samples.

4.32 Correlations between TEP, bacteria, algae, the biopolymer fraction of NOM, and TOC

TEP is composed of acidic polysaccharides and some large proteins that occur mostly in the biopolymer fraction of NOM and some of the proteins within the humic acid part of NOM (Bar-Zeev et al. 2015; Winters et al. 2016). TEP can be produced both abiotically and as extracellular discharges from bacteria and algae (Zhou et al. 1998; Passow et al. 2001; Passow 2002; Engel et al. 2004; Iculano et al. 2017). Therefore, there should be some statistical relationship between TEP, the biopolymer fraction of NOM, bacterial concentration or algal concentration. A series of statistical analyses were preformed to test if there are significant relationships between the various organic properties (Tables 2, 3 and 4). There is a significant statistical relationship between p-TEP with algae (grouped with cyanobacteria) and bacteria but this does not occur with c-TEP. There is a significant statistical relationship between the biopolymer fraction of NOM with bacteria, p-TEP, and c-TEP in all of the offshore profiles. Since c-TEP is considered to be the precursor to formation of p-TEP, the association with biopolymers is logical and could indicate potential for abiotic assembly in the water column. In all cases there was no statistically-significant relationship between any of these parameters in the shallow, nearshore samples with the exception of bacteria

C17

and TOC. However, some important and statistically-significant relations were found between p-TEP and bacteria in the profiles measured at sites A, B, and C and in the 300 m profile. All of the offshore profiles showed a statistically significant relationship between c-TEP and bacteria with the exception of the site C profile. In comparison, there was only one statistically-significant relationship between p-TEP and algae at site B and for c-TEP the only profile showing statistical-significance was the deep profile. Based on these relationships, it appears that p-TEP may be produced by bacteria in greater amounts compared to algae at these locations. Consumption of the TEP by bacteria does not seem to be occurring in the water column at sites A, B, and C based on the p-TEP relationship, unless abiotically-generated p-TEP is replacing what is consumed. In the deep profile, the c-TEP concentration shows a statistically-significant relationship with bacteria which could indicate a breakdown of the p-TEP, particularly outside of the photic zone. The relationship between the biopolymers and bacterial concentrations shows a significant statistical correlation in all offshore profiles. while the correlation between biopolymers and algal concentrations is statistically significant only at site A and in the deep profile. These relationships suggest that extracellular discharges of polysaccharides and proteins from the bacteria and algae are occurring without immediate abiotic assembly into p-TEP. This suggestion is further supported by the statistical relationships between biopolymers and p-TEP and c-TEP which are statistically significant in several, but not all of the offshore profiles. The offshore profiles show statistically-significant relationships between both p-TEP and c-TEP and TOC at sites A and the deep profile. Therefore, TEP in general is a significant part of TOC in the Red Sea at these locations, particularly below the photic zone. There is usually no statistical relationship of significance between p-TEP and c-TEP. However, at site A the data produced an r^2 value of 1 and with a corresponding p-value of 0 (Table 2). This unusual relationship has no explanation but is noted. A considerable amount of additional research will be required to better establish the processes occurring within the Red Sea water column that relate to NOM production and transport and how these processes relate to the measured TEP and NOM fraction concentrations. Since there

C18

are few data available in the literature that relate these parameters within the water column at other geographic locations, it is difficult to provide definitive conclusions. The data provided here appear to be the first published that relate the biopolymer fraction of NOM to TEP and provide all of the five fractions of NOM in the offshore marine environment throughout the water column. The carbon compounds that occur in p-TEP are largely contained within the biopolymer fraction of NOM with the exception of some proteins which occur in the size range found in the humic substances. An assessment of the other fractions of NOM, humic substances, building blocks, LWM neutrals, and LMW acids, did not show any significant statistical relationships between these parameters, nor did it reveal potential relationships between them and the bacteria or algae.

4.43 Comparison of the offshore and onshore TEP data in the Red Sea

All of the onshore measurements of p-TEP and c-TEP were collected between the sea surface and a depth of 10 m (Rachman et al., 2015; Dehwah et al., 2015; Dehwah and Missimer, 2016; Alsahri et al., 2017; Dehwah et al 2017; Dehwah and Missimer 2017; Dehwah and Missimer, 2015d).. Therefore, only the data in this depth range can be compared to the offshore data. The full range of p-TEP in the nearshore measurements is from 53 to 347 $\mu\text{g Xeq./L}$ and the c-TEP range is between 36 and 287 $\mu\text{g Xeq./L}$. The ranges in the offshore profiles in the same depth range for p-TEP and c-TEP are 135.4 to 279.4 and 0 to 340.7 respectively. In both locations there was considerable variation between sites and in different times of the year which is expected based on production variations of TEP by algae and bacteria in the upper photic zone as well as the ability of TEP to have either negative or positive buoyancy at shallow depths (Zhou et al., 1998; Passow, 2002; Mari et al., 2004; Schuster and Herndl 1995; Ortega-Retuerta et al. 2017).

4.54 Comparison of TEP profiles in the Red Sea with other marine environments

Most TEP data profiles collected in the marine environment show an irregular variation

C19

in the upper 100 m of the water column (Schuster and Herndl 1995; Ortega-Retuerta et al. 2017), a general reduction of TEP with depth over 200 m (Busch et al. 2017; Jennings et al. 2017), but in some cases an increase at greater depths (Ramaiah et al., 2000). Also, the reported changes in TEP with depth are based mostly on p-TEP data and not both types of TEP which show differing trends in the water column. The TEP data collected from the profiles in this investigation within the photic zone (<100m) show differing concentrations with depth (Figs. 7 and 8). Within the upper 90, p-TEP declines between 31 and 39% at sites A, B, and C and shows no decline in the deep profile. The c-TEP concentration declines between 38 and 70% at sites A, B, and the deep profile, but increases by 150% at site C. For comparison, the TOC concentration reduction in the photic zone ranges between 10 and 32%. In the deep profile the difference between the surface and the 300 m depth showed a reduction in p-TEP of 20% and c-TEP of 69%. This may indicate that some abiotic assembly of p-TEP is occurring below the photic zone, particularly in the presence of bacteria which may feed upon the p-TEP. The TOC in the deep profile declines by about 32% comparing the surface to the 300 m depth.

4.65 Relationships between NOM fractions and other parameters

The primary fraction of NOM that shows a trend with depth is the biopolymers which track well to bacteria. Since the biopolymer fraction of NOM contains most of the polysaccharides, which can be food for bacteria, the relationship with the bacteria is to be expected. In the upper 100 m of the water column, the humic substances show a restricted range in concentrations with a small downward trend (Fig. 7), but below 100 m there is a lowering concentration following the same pattern as the biopolymers. The building blocks have a larger range in concentration changes in the upper 100 m of the water column compared to the humic substances (Fig. 7) and a similar downward trend in concentration similar to the humic substances below 100 m (Fig. 8). The LWM neutrals and acids show considerable variation in concentration in the upper 100 m and a slight downward change in concentration below 100 m. There are some general

C20

suggestions made by these data related to the concentration changes. In the photic zone, the biochemical activity of algae and bacteria affect the NOM fraction concentrations. The LMW fractions are likely affected by the biochemical breakdown of large molecular weight organics and by selective, abiotic aggradation of larger organic particles suggested by the larger concentration of the neutrals over the acids. The reduction in concentrations in biopolymers, humic substances and building blocks below 100 m follows the reduction in bacteria below the photic zone. As bacteria feed on p-TEP, they may leave behind the LMW neutrals which could be compounds that cannot be used by the bacteria as food. The LMW acids may tend to occur within the context of c-TEP and may be subject to abiotic aggradation during settling. Future research will be required to understand the complex relations between the NOM organic fractions and the biochemistry of the bacteria in the deep-water column.

5 Conclusions

Vertical changes in concentrations of TEP in the Red Sea tend to follow trends found in other locations of the world ocean in that there is a general reduction with depth. The changes in the photic zone tend to be quite irregular, as expected, because of variations in primary productivity and differing biochemical conditions. Although it was observed that no clear relationship between TEP and algae occurs in the Red Sea, this unusual result may be explained by the dominance of small algae and cyanobacteria. The measurement of the five fractions of NOM allows some preliminary conclusions to be made concerning the relationships between specific organic parameters and TEP variation with depth. These relationships suggest that extracellular discharges of polysaccharides and proteins from the bacteria and algae are occurring without immediate abiotic assembly into p-TEP in the photic zone of the water column. In the water column below the photic zone, TOC, bacteria, and biopolymers show a generally common rate of reduction in concentration, but p-TEP concentration changes at a reduced rate showing that it persists in moving organic carbon deeper into the water column despite consumption by bacteria. There may be some abiotic assembly

C21

of c-TEP into p-TEP to maintain the concentration without full bacterial removal. The multiple regression analysis showed that p-TEP is correlated with bacteria and algae offshore, but no statistical correlation occurs between these variable in the nearshore data. No statistical correlation occurs between c-TEP and bacteria, algae, and TOC in offshore and nearshore samples. Also, p-TEP and c-TEP are not statistically correlated. The concentrations of biopolymers is correlated to bacteria, p-TEP, and c-TEP in the offshore samples, but the onshore samples. The relationships between p-TEP and c-TEP and other organic parameters, especially the biopolymer fraction of NOM, is different when comparing the offshore water column to the nearshore area. Seasonal differences during sampling could have also impacted the results. The only statistically-significant relationship in the measured parameters in the nearshore was that between bacteria and TOC. Differences Irregularity in local conditions, such as circulation and anthropogenic influences, in the nearshore zone can causecauses large variations in the organic parameters measured, not allowing statistically-significant relationships to be established.

References

Alldredge, A. L., and Croker, K. M.: Why do sinking mucilage aggregates accumulate in the water column?, *Sci. Total Environ.* 165, 15-22, 1995. Azam, F., and Long, R. A.: Sea snow microcosms, *Nature* 414, 495-497, 1995. Azam, F. and Malfatti, F.: Microbial structuring of marine ecosystems. *Nature Reviews Microbiology*, 5(10), 782-791, 2007. Azetsu-Scott, K. and Passow, U.: Ascending marine particles: significance of transparent exopolymer particles (TEP) in the upper ocean, *Limnol. Oceanogr.* 49, 741-748, 2004. Bar-Zeev, E., Berman, T., Rahav, E., Dishon, G., Herut, B., Kress, N. and Berman-Frank, I., 2011. Transparent exopolymer particle (TEP) dynamics in the eastern Mediterranean Sea. *Marine Ecology Progress Series*, 431, pp.107-118. Bar-Zeev, E., Berman-Frank, I., Stambler, N., Domínguez, E. V., Zohary, T., Capuzzo, E. Meeder, E., Suggett, D. J., Iluz, D., Dishon, G., and Berman, T.: Transparent exopolymer particles (TEP) link phytoplankton and bacterial production in the Gulf of Aqaba,

C22

Aquat. Microbial Ecol. 56, 217-226, 2009a. Bar-Zeev, E. I., Berman-Frank, I., Liberman, B., Rahav, E., Passow, U., and Berman, T.: Transparent exopolymer particles: Potential agents for organic fouling and biofilm formation in desalination and water treatment plants, Desalination and Water Treatment 3, 136-142, 2009b. Bar-Zeev, E., Passow, U., Romero-Vargas, C. S., and Elimelech, M: Transparent exopolymer particles (TEP): from aquatic environments and engineered systems to membrane biofouling, Environ. Sci. Technol. 49(2), 691-707, 2015. Berman, T., Viner-Mozzini, Y.: Abundance and characteristics of polysaccharide and proteinaceous particles in Lake Kinneret, Aquat. Microb. Ecol. 24, 255-264, 2001. Bižić-Ionescu, M., Ionescu, D. and Grossart, H.P.: Organic particles: heterogeneous hubs for microbial interactions in aquatic ecosystems, Frontiers in Microbiology, 9, 2018. Busch, K., Endreas, S., Iversen, M. H., Michels, J., Nöthig, E.-M., and Engel, A: Bacterial colonization and vertical distribution of marine gel particles (TEP and CSP) in the Arctic Fram Strait, Frontiers Mar. Sci 4, Article 166, doi:10.2289/fmars.2017.00166, 2017. Chen, Y., Dong, G., Han, J., Wah, B.W., Wang, J.: Multi-dimensional regression analysis of time-series data streams. In VLDB'02: Proceedings of the 28th International Conference on Very Large Databases, 323-334, 2002. Chin, W.-C., Orellana, M. V., and Werdugo, P.: Spontaneous assembly of marine dissolved organic matter in polymer gels, Nature 391, 568-572, 1998. Dehwah, A. H. A., Al-Mashharawi, S., Kammourie, N., and Missimer, T. M.: Impact of well intake systems on bacterial, algae and organic carbon reduction in SWRO desalination systems, SAWACO, Jeddah, Saudi Arabia, Desalination and Water Treatment 55(10), 2594-2600, 2015a. Dehwah, A. H. A, Li, S., Al-Mashhaarwi, S., Winters, H., T. M. Missimer, T. M.: Changes in feedwater organic matter concentrations based on intake type and pretreatment processes at SWRO facilities, Red Sea, Saudi Arabia, Desalination 360, 19-27, 2015b, <http://dx.doi.org/10.1016/j.desal.2015.01.008>. Dehwah, A. H. A., Li, S., Al-Mashharawi, S., Mallon, F. L., Batang, Z., and Missimer, T. M.: Effects of intake depth on raw seawater quality in the Red Sea, Chapter 6, In: Missimer, T. M., Jones, B., and Maliva R. G. [eds], Intakes and Outfalls for Seawater Reverse Osmosis Desalination Facilities: Innovations and Environmental Impacts,

C23

Springer, Berlin, p.105-124, 2015c. Dehwah, A. H. A., and Missimer, T. M.: Sub-surface intake systems: green choice for improving feed Seawater quality at SWRO desalination plants, Jeddah, Saudi Arabia, Water Research 88, 216-224, 2016. Engel, A., 2004. Distribution of transparent exopolymer particles (TEP) in the northeast Atlantic Ocean and their potential significance for aggregation processes. Deep Sea Research Part I: Oceanographic Research Papers, 51(1), pp.83-92. Engel, A., Thoms, S., Rießell, U., Rochelle-Newall, E., and Zondervan, I.: Polysaccharide aggregation as a potential sink of marine dissolved organic carbon, Nature 428, 929-932, 2004. Iuculano, F., Mazuecos, I. P., Reche, I., and Agustí, S.: Prochlorococcus as a possible source for transparent exopolymer particles (TEP). Front. Microbiol. 8: Article 709, doi:10.3389/fmicro.2017.00709, 2017. Lindell, D. and Post, A. F.: Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea, Limnol. Oceanogr. 40(6), 1130-1141, 1995. Mari, X., Rassoulzadegan, F., and Brusseauard, C. P. D.: Role of TEP in the microbial food web structure. II. Influence of the ciliate community structure, Mar. Ecol. Prog. Ser. 279, 23-32, 2004. Ortega-Retuerta, E., Duarte, C.M. and Reche, I., 2010. Significance of bacterial activity for the distribution and dynamics of transparent exopolymer particles in the Mediterranean Sea. Microbial ecology, 59(4), pp.808-818. Ortega-Retuerta, E., Reche, I., Pulido-Villena, E., Agustí, S. and Duarte, C.M., 2009. Uncoupled distributions of transparent exopolymer particles (TEP) and dissolved carbohydrates in the Southern Ocean. Marine chemistry, 115(1-2), pp.59-65. Ortega-Retuerta, E., Sala, M. M., Borrull, E., Mestre, M., Aparicio, F. L., Gallisai, R., Antequera, C., Marrasé, C., Peters, F., Simó, R., and Gasol, J. M.: Horizontal and vertical distributions of transparent exopolymer particles (TEP) in the NW Mediterranean Sea are linked to chlorophyll a and O₂ variability, Front. Microbiol. 7, article 2159, doi:10.3389/fmicb.2016.02169, 2017. Osborne, J.: Waters E. Four assumptions of multiple regression that researchers should always test. Practical assessment, research & evaluation 8(2), 1-9, 2002. Passow, U.: Transparent exopolymer particles (TEP) in aquatic environments, Prog. Oceanogr. 55, 287-333, 2002. Passow, U., and Alldredge, A. L.: A dye-binding assay for spectrophotometric measurement of

C24

transparent exopolymer particles (TEP), Limnol. Oceanogr. 40(7), 1326-1335, 1995. Passow, U., Alldredge, A. L., and Logan, B. F.: The role of particulate carbohydrates in the flocculation of diatom blooms, Deep-Sea Res. I 41, 335-357, 1994. Passow, U., Shipe, R. F., Muarry, A., Pak, D. K., Brzezinski, M. A., and Alldredge, A. L.: The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter, Cont. Shelf Res. 21, 327-346, 2001. Rachman, R. M., Li, S., and Missimer, T. M.: SWRO feed water quality improvement using subsurface intakes in Oman, Spain, Turks and Caicos Islands, and Saudi Arabia, Desalination 351, 88-100, 2014. Rachman, R., Dehwah, A. H. A., Li, S., Winters, H., Al-Mashharawi, S., and Missimer, T. M.: 2015. Effects of well intake systems on removal of algae, bacteria, and natural organic matter, Chapter 9, In: Missimer, T. M., Jones, B., and R. G. Maliva, R. G. (Eds.), Intakes and Outfalls for Seawater Reverse Osmosis Desalination Facilities: Innovations and Environmental Impacts, Springer, Berlin, pp. 163-193, 2015. Radíč, T., Šilovic, T., Šantíč, D., Fuks, D., and Micić, M.: Preliminary flow cytometric analysis of phototrophic pico- and nanoplankton communities in the Northern Adriatic Fresenius, Environ. Bull. 18, 715-724, 2009. Ramaiah, N., Sarma, V. V. S. S., Gauns, M., Dileep Kumar, M., and Madhupratap, M.: Abundance and relationship of bacteria with transparent exopolymers during the 1996 summer monsoon in the Arabian Sea, Proc. Indian Acad. Sci. (Earth Planet. Sci.) 109(4), 443-451, 2000. Schuster, S., and Herndl, G. J.: Formation and significance of transparent exopolymer particles in the northern Adriatic Sea, Mar. Ecol. Progr. Ser. 124, 227-236, 1995. Simon, M., Grossart, H.P., Schweitzer, B. and Ploug, H., 2002: Microbial ecology of organic aggregates in aquatic ecosystems. Aquatic microbial ecology, 28(2), pp.175- 211, 2002. Stoderegger, K. E., and Herndl, G. J.: Production of exopolymer particles of marine bacterioplankton under contrasting turbulence conditions, Mar. Ecol. Prog. Ser. 189, 9-16, 1999. Tonidandel, S., LeBretton, J.M.: Relative importance analysis: A useful supplement to regression analysis. Journal of Business and Psychology 26(1), 1-9, 2011. Verdugo, P., Alldredge, A. L., Azam, F., Kirchman, D. L., Passow, U., and Santschi, P. H.: The oceanic gel phase: a bridge in the DOM-POM continuum, Mar. Chem. 92, 67-85, 2004. Van der Merwe,

C25

R., Hammes, F., Lattemann, S., and Amy, G.: Flow cytometric assessment of microbial abundance in the near-field area of seawater reverse osmosis concentrate discharge, Desalination 343, 208-216, 2014. Villacorte, L. O., M. D. Kennedy, G. L. Amy, and J. C. Schippers: Measuring transparent exopolymer particles (TEP) as indicator of the (bio) fouling potential of RO feed water, Desalination and Water Treatment 5, 207-212 2009. Vives-Rego, Lebaron, P., and Nebe-von Caron, G.: 2000. Current and future applications of flow cytometry in aquatic microbiology, FEMS Microbiol. Rev. 24(4), 429-448, 2000. Williams, M.N., Grajales, C.A., Kurkiewicz, D.: Assumptions of multiple regression: Correcting two misconceptions, 2013. Winters, H., Chong, T. H., Fane, A. G., Krantz, W., Rzechorwsc, M., and Saeidi, N.: The involvement of lectins and lectin-like substances in biofilm formation of RO membranes – is TEP important? Desalination 399: 61-68, 2016. Wurl, O. L., and Holmes, M.: The gelatinous nature of the sea-surface microlayer, Mar. Chem. 100, 89-97, 2008. Wurl, O., Miller, L., Röttgers, R., and Vagle, S.: The distribution and fate of surface-active substances in the sea-surface microlayer and water column, Mar. Chem. 115, 1-9, 2009. Wurl, O., Miller, L., and Vagle, S.: Production and fate of transparent exopolymer particles in the ocean, J. Geophys. Res. 116, C00H13, 1-16, doi:10.1029/2011JC007342, 2011. Yahel, G., Post, A. F., Fabricius, K., Marie, D., Vaulot, D., and Genin, A.: Phytoplankton distribution and grazing near coral reefs, Limnol. Oceanogr. 43(4), 551-563, 1998. Zhou, J., Mopper, K., and Passow, U.: The role of surface-active carbohydrates in the formation of transparent exopolymer particulates by bubble adsorption of seawater, Limnol. Oceanogr. 43, 1860-1871, 1998.

Table 1. Compilation of related data from previous studies

Location	Date	Depth (m)	Total Algae (cells/mL)	Bacteria (cells/mL)	TOC (mg/L)	NOM (μ g/L)	TEP μ g Xeq./L	Biopoly. Humic substances	Building Blocks	LMWN	LWMA	p-TEP	c-TEP	1N. Obhor	1/8/2014	Surface	30,524	112,790	0.89	76	345	103	168	88	162	1Corniche	1/11/2014	Surface	3,603	196,377	0.94	90	360	91	192	94	121	73	1S. Jeddah	1/9/2014	Surface	1,677	264,728	1.02	116	351	139	197	103	157	122	1Buhayrat - Sur-
----------	------	-----------	------------------------	---------------------	------------	------------------	--------------------	---------------------------	-----------------	------	------	-------	-------	-----------	----------	---------	--------	---------	------	----	-----	-----	-----	----	-----	-----------	-----------	---------	-------	---------	------	----	-----	----	-----	----	-----	----	------------	----------	---------	-------	---------	------	-----	-----	-----	-----	-----	-----	-----	------------------

C26

face 30,395 320,870 1.053 47 343 82 16 85 58 - 2Site A (Buhayrat) 1/7/2014 Surface 14,956 179,837 0.88 63 367 131 230 130 123 130 2Site B (Saudia) 5/25/2013 Surface 23,773 317,174 0.83 84 289 101 45 101 53 56 3N. Obhor 10/25/2014 Surface 129,738 520,350 1.1 57 205 95 163 18 318 90 3Corniche 11/6/2014 Surface 89,033 254,450 1.0 44 201 86 249 16 249 120 3S. Jeddah 12/24/2014 Surface 42,923 216,400 0.9 32 196 95 276 24 255 115 4N. Obhor 6/7/2015 Surface - 707,100 1.262 40 194 85 466 19 111 223 4N. Obhor 6/17/2015 Surface - - 1.034 42 185 99 231 18 - - 4N. Obhor 7/1/2015 Surface 108,740 282,450 1.162 49 192 105 313 21 142 189 4N. Obhor 7/12/2015 Surface 87,615 252,233 1.036 50 188 105 477 31 - - 4N. Obhor 8/3/2015 Surface 135,603 908,100 1.104 80 209 122 269 31 231 216 4N. Obhor 8/16/2015 Surface 49,770 1,764,850 1.118 71 184 111 284 16 - - 4Saudia 6/7/2015 Surface - 317,567 1.055 29 172 100 369 21 215 242 4Saudia 6/17/2015 Surface - - 1.233 46 189 84 183 14 - - 4Saudia 7/1/2015 Surface 61,925 583,400 1.287 44 190 93 152 14 146 213 4Saudia 7/12/2015 Surface 137,363 1,070,400 1.294 40 159 82 188 16 - - 4Saudia 8/3/2015 Surface 53,810 1,736,450 1.164 93 180 111 238 19 347 287 4Saudia 8/16/2015 Surface 43,060 2,182,550 1.181 83 208 103 276 15 5N. Ohbor 2015/4/2 Surface 91,870 1,356,600 1.10 55 214 98 387 14 261 132 6KAUST SW 5/3/2014 Surface 4,766 273,400 1.42 29 217 119 315 24 278 100 6KAUST SW 5/22/2014 Surface 9,350 236,000 1.037 55 197 121 252 17 346 97 6KAUST SW 6/11/2014 Surface 3,140 287,850 0.992 36 246 81 319 18 229 170 6KAUST SW 7/3/2014 Surface 4,958 324,600 1.085 43 212 151 227 10 85 127 6KAUST SW 7/19/2014 Surface 11,080 389,450 0.97 53 212 91 233 16 99 117 6KAUST SW 8/18/2014 Surface 6,057 316,450 1.112 40 201 88 225 14 82 112 6KAUST SW 9/18/2014 Surface 52,453 321,250 0.923 35 193 93 171 13 97 69 6KAUST SW 10/21/2014 Surface 12,228 630,600 0.831 39 193 108 256 16 213 50 6KAUST SW 12/3/2-14 Surface 10,673 347,133 1.004 33 189 101 288 23 138 43 6KAUST SW 2/11/2015 Surface 12,890 292,500 1.275 36 200 102 343 31 182 87 6KAUST SW 5/21/2015 Surface 28,009 450,800 0.93 31 177 93 236 25 143 50 6KAUST SW 8/6/2015 Surface 44,153 336,900 1.041 42 184 86 229 18 186 36 6KAUST SW 9/17/2015 Surface 52,453 297,867 1.084 28 188 86 230

C27

24 300 105 7KAUST SW 9/30/2016 Surface 11,955 369,300 1.073 112 429 213 385 86 - - 7KAUST SW 10/2/2014 Surface 10,600 367,000 0.993 105 363 193 353 92 307 - 7KAUST SW 10/9/2014 Surface 17,777 368,463 0.944 140 373 218 335 79 333 - 7KAUST SW 10/16/2014 Surface 22,030 319,950 0.961 88 340 216 346 80 206 - 7KAUST SW 10/27/2014 Surface 42,550 297,700 0.917 164 348 260 468 49 318 - 7KAUST SW 11/6/2014 Surface 86,033 587,200 0.864 73 442 93 470 73 124 - 7KAUST SW 11/17/2014 Surface 107,030 673,700 0.897 71 374 221 352 81 83 - No. Samples 38 40 42 42 42 42 42 35 27 Range in values 1,677-137,363 112,790-2,182,550 0.830-1.420 28-164 159-442 81-260 16-477 10-130 53-347 36-287 Average 44,383 525,820 1 62 248 118 271 40 191 125 1 R. Rachman et al. 2015; 2 Dehwah et al. 2015; 3Dehwah and Missimer 2016, 4Alsahri et al. 2017, 5Dehwah et al 2017; 6Dehwah and Missimer 2017; 7Dehwah and Missimer 2015d Table 2. Regression analysis of selected organic parameters at the 0.05 significance level 0.05 level Organic Parameters Location R2 p-value Significant (?) p-TEP v. Bacteria Site A 0.6677 0.0039 Y Site B 0.7295 0.001656 Y Site C 0.6691 0.00383 Y Deep Profile (300 m) 0.3034 0.009661 Y Nearshore 0.0593 0.158757 N p-TEP v. Algae Site A 0.1011 0.37063 N Site B 0.5363 0.016017 Y Site C 0.2463 0.144607 N Deep Profile (300 m) 0.1495 0.083384 N Nearshore 0.0169 0.471436 N c-TEP v. Bacteria Site A 0.6677 0.0039 Y Site B 0.6430 0.005265 Y Site C 0.2474 0.143485 N Deep Profile (300 m) 0.5512 0.000116 N Nearshore 0.2622 0.006329 N c-TEP v. Algae Site A 0.1011 0.37063 N Site B 0.2900 0.108267 N Site C 0.0141 0.743804 N Deep Profile (300 m) 0.5713 7.4E-05 Y Nearshore 0.1476 0.057986 N Biopolymers v. Bacteria Site A 0.8166 0.000335 Y Site B 0.6726 0.003663 Y Site C 0.6868 0.003043 Y Deep Profile (300 m) 0.7814 1.08E-07 Y Nearshore 0.0123 0.495799 N Biopolymers v. Algae Site A 0.5801 0.010465273 Y Site B 0.2918 0.10701 N Site C 0.2996 0.101512 N Deep Profile (300 m) 0.7078 1.77E-06 Y Nearshore 0.0107 0.537011 N Biopolymers v. p-TEP Site A 0.4890 0.024407 Y Site B 0.4824 0.0258132 Y Site C 0.4020 0.049006 Y Deep Profile (300 m) 0.1551 0.077318 N Nearshore 0.0808 0.09790 N Biopolymers v. c-TEP Site A 0.4890 0.024407 Y Site B 0.3696 0.062253 N Site C 0.2590 0.13302

C28

N Deep Profile (300 m) 0.5883 4.97E-05 Y Nearshore 0.0331 0.364097 N p-TEP v. c-TEP Site A 1 0 Y Site B 0.362578 0.065466 N Site C 0.3798 0.057758 N Deep Profile (300 m) 0.1660 0.066765 N Nearshore 0.0491 0.266597 N p-TEP v. TOC Site A 0.6591 0.00434 Y Site B 0.2760 0.118919 N Site C 0.0979 0.378796 N Deep Profile (300 m) 0.3156 0.008046 Y Nearshore 0.0284 0.332963 N c-TEP vs. TOC Site A 0.6591 0.0043396 Y Site B 0.0431 0.565154 N Site C 0.0165 0.723942 N Deep Profile (300 m) 0.6698 5.79E-06 Y Nearshore 0.1995 0.019513 N Bacteria v. TOC Site A 0.7717 0.000822 Y Site B 0.2994 0.101653 N Site C 0.1294 0.307187 N Deep Profile (300 m) 0.7812 1.08E-07 Y Nearshore 0.1144 0.032827 Y Algae v. TOC Site A 0.0928 0.3922134 N Site B 0.4907 0.024064 N Site C 0.3188 0.089015 N Deep Profile (300 m) 0.6220 2.16E-05 Y Nearshore 0.0388 0.236167 N

Table 2. Two-way ANOVA p-value for interdependency of site and its attributes

Source of Variation P-value Sample (offshore and nearshore site) 0.00702 Attributes (bacteria, algae, TOC, biopolymer and TEP) 0.00000 Interaction 0.00011

Table 3. Correlation matrix

P-TEP ($\mu\text{g Xeq./L}$)	C-TEP ($\mu\text{g Xeq./L}$)	Bacterial count (cells/ml)	Total algae (events/ml)
TOC (mg/L)	Biopolymers	P-TEP ($\mu\text{g Xeq./L}$) 1.000	C-TEP ($\mu\text{g Xeq./L}$) 0.327
Bacteria (cells/ml)	0.152	0.075	1.000
TOC (mg/L)	0.259	0.422	0.374
	-0.169	1.000	Biopolymers -0.263
			0.207
			-0.006
			-0.304
			0.209
			1.000

Table 4. Multiple Regression analysis of selected organic parameters at the 0.05 significance level

Dependent variable	Location	Attributes	P-value	R Square	Adjusted R Square	Overall Significance
p-TEP	Offshore	Bacterial count (cells/ml)	0.00106	0.51941	0.48874	0.0000001
Total algae (events/ml)		TOC (mg/L)	0.76006	Nearshore	Bacterial count (cells/ml)	0.48082
			0.13834	0.01525	0.36195	Total algae (events/ml) 0.15212

C29

TOC (mg/L) 0.37855

c-TEP Offshore Bacterial count (cells/ml) 0.91115 0.29356 0.24847 0.00090 Total algae (events/ml) 0.93401 TOC (mg/L) 0.00540 Nearshore Bacterial count (cells/ml) 0.88360 0.13412 0.01042 0.37736 Total algae (events/ml) 0.37529 TOC (mg/L) 0.18425

c-TEP Offshore p-TEP ($\mu\text{g Xeq./L}$) 0.00750 0.13704 0.11943 0.00750 Nearshore p-TEP ($\mu\text{g Xeq./L}$) 0.06876 0.14271 0.10374 0.06876

Biopolymers offshore Bacterial count (cells/ml) 0.00296 0.36179 0.32106 0.00009 P-TEP ($\mu\text{g Xeq./L}$) 0.00001 C-TEP ($\mu\text{g Xeq./L}$) 0.03707 Nearshore Bacterial count (cells/ml) 0.07978 0.21256 0.05507 0.28648 Total algae (events/ml) 0.51524 P-TEP ($\mu\text{g Xeq./L}$) 0.38547 C-TEP ($\mu\text{g Xeq./L}$) 0.34150

Acknowledgments

Funding for the offshore sample collection was provided by the King Abdullah University of Science and Technology Coastal and Marine Resources Core Laboratory. Analytical work was funded by the Water Desalination and Reuse Center, King Abdullah University of Science and Technology. Support for DMA was provided by the National Science Foundation (Grants OCE-0850421 OCE-0430724, OCE-0911031, and OCE-1314642) and National Institutes of Health (NIEHS-1P50-ES021923-01) through the Woods Hole Center for Oceans and Human Health.

Conflicts of Interest None declared

Figure captions

Fig. 1. Map showing the sampling profile locations in the Red Sea

Fig. 2. Xanthan gum standard calibration curves for determination of p-TEP and c-TEP. Curve a is for sites A, B, and C and curve b is for site D.

Fig. 3. Physical data from the three 90 m profiles

C30

Fig. 4. Physical data from the 300 m profile

Fig. 5. Algal and cyanobacteria composition and concentration data from the three 90 m profiles

Fig. 6. Algal and cyanobacteria composition and concentration data from the 300 m profile

Fig. 7. Organic carbon concentrations for the three 90 m profiles

Fig. 8. Organic carbon concentrations from the 300 m profile

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-59>, 2019.