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## Changes in organic carbon properties during intense plankton blooms and macroaggregate formation in the coastal Adriatic Sea, Croatia (case studies in 2020-2022)

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### Abstract

Organic matter (OM) properties, assessed with measurements of dissolved and particulate organic carbon (DOC and POC), surface-active DOC (SAS), and DOC normalized surfactant activity (NSA), have been under scrutiny during several recent and notable phenomena along the Adriatic Sea coast in Croatia. These phenomena were (1) massive invasive ctenophore blooms during the summer of 2020 and red tide occurrences in spring 2021 in the northern Adriatic, (2) the formation of the gelatinous macroaggregates in the coastal waters of the central Adriatic during the summer of 2021, and (3) intense autotrophic activity within the water column of the highly stratified and eutrophic marine system of Rogoznica Lake. All these events encompassed single-species plankton blooms characterized by a remarkable increase of OM (up to 320 mg L<sup>-1</sup> of DOC and POC) with different surface-active properties (up to 15 mg L<sup>-1</sup> of SAS) appearing in the otherwise oligotrophic Adriatic Sea.

The NSA, in addition to voltammetric curves with well-defined desorption waves, is a good indicator of the presence of a different type of OM, which can be considered a possible tracer for certain processes in the marine environment. Based on our measurements, a rough threshold value for the OM hydrophobicity-reactivity of NSA = 0.083 is proposed. OM with a value above this threshold is more hydrophobic, as in the case of red tides and gelatinous zooplankton blooms with an average NSA between 0.13 and 0.16. OM with a value below this threshold indicates the presence of more hydrophilic substances such as pollen and OM produced by purple sulfur bacteria.

**Keywords:** surface-active DOC; DOC normalized surfactant activity; gelatinous zooplankton bloom; red tide; pollen; Rogoznica Lake.

### Introduction

Organic carbon (OC) plays a pivotal role in numerous marine biogeochemical processes and can function as a valuable indicator of shifts within the marine environment (Ciglencčki *et al.*, 2020a; Lønborg *et al.*, 2020; Paliaga *et al.*, 2020; Simonović *et al.*, 2023). The production and degradation processes of organic matter (OM), alongside their properties and concentrations in the sea, are subject to influences from environmental conditions directly and indirectly regulated by climate variability (Shen & Benner, 2022). In recent decades, extreme weather events such as droughts, floods, tropical cyclones, heatwaves, heavy rainfall, alterations in temperature, salinity, circulation, mixing, strong winds, and the transport of Saha-

ran dust have become more frequent and severe. These events may be linked to enigmatic marine phenomena, including harmful or undesirable algal accumulations, the proliferation of gelatinous macrozooplankton, or hypoxia/anoxia events (Ciglencčki *et al.*, 2015; Lejeusne *et al.*, 2010; Tian *et al.*, 2003; Wernberg *et al.*, 2013).

Such phenomena may contribute to the degradation of the quality of the marine environment and subsequently promote a pronounced accumulation of OC (Thornton *et al.*, 1999; Fonda Umani *et al.*, 2004; Ursella *et al.*, 2018; Ciglencčki *et al.*, 2021). The main sources of autochthonous OC in seawater are primary production, zooplankton grazing, and the release of dissolved organic carbon (DOC) by zooplankton (Ciglencčki *et al.*, 2020b; Lønborg *et al.*, 2020). Terrestrial and atmospheric inputs are considered the

predominant allochthonous sources of OC in the coastal marine environment (Galletti, 2020; Painter *et al.*, 2018).

Surface-active substances (SAS) represent a crucial portion of DOC in marine ecosystems (Ciglencečki *et al.*, 2020a; Čosović *et al.*, 2000; Simonović *et al.*, 2023). Due to their different origins (allochthonous or autochthonous) and complex composition, the different types of DOC have significantly different physicochemical properties, including surface activity (Ciglencečki *et al.*, 2023; Čosović & Ciglencečki, 1997; Karavoltos *et al.*, 2015; Plavšić *et al.*, 2007; Scoullou *et al.*, 2006).

SAS in seawater are mainly formed by biological activities, especially by phytoplankton production (Ciglencečki *et al.*, 2018, 2020; Čosović *et al.*, 2000; Žutić *et al.*, 1981), bacteria (Kurata *et al.*, 2016), and feeding of zooplankton (Croot *et al.*, 2007; Kujawinski *et al.*, 2002). SAS belong to the group of biosurfactants and represent the most reactive fraction of DOC with amphiphilic character, whereby they adsorb at various natural phase boundaries such as the sea surface microlayer (Čosović & Vojvodić, 1987; Karavoltos *et al.*, 2015; Plavšić *et al.*, 2007; Sabbaghzadeh *et al.*, 2017; Wurl *et al.*, 2011), the halocline in stratified estuaries (Čosović & Vojvodić, 1989; Žutić & Legović, 1988), the chemocline of a marine lake (Ciglencečki *et al.*, 2017; Marguš *et al.*, 2015; Simonović *et al.*, 2023), and in seawater eddies (Ciglencečki *et al.*, 2021).

SAS are a key indicator of alterations in ecosystem productivity, eutrophication, and broader alterations in marine biogeochemical cycles (Ciglencečki *et al.*, 2020a, 2021; Simonović *et al.*, 2023). These substances also play a critical role in converting DOC into POC through aggregation and flocculation, which may involve the formation of “mucus” or gel-like particles (Santschi *et al.*, 2020).

The most severe outbreaks of mucus macroaggregates to date have been documented in the northern Adriatic (NAd) during the summer of the early 2000s (Ciglencečki *et al.* 2003; Giani *et al.* 2005). This outbreak was characterized by the dominance of the epipelagic diatom *Cylindrotheca closterium*, which is specific to the occurrence of gel-like extracellular polymeric substances, mainly polysaccharides and glycoproteins (Bhaskar & Bhosle, 2005; Hoagland *et al.*, 1993). Other phenomena observed in the NAd that lead to rapid qualitative and quantitative changes in OC due to the production of highly reactive DOC are the massive blooms of the invasive ctenophore *Mnemiopsis leidyi* (Budiša *et al.*, 2021; Ciglencečki *et al.*, 2021) and outbreaks of the red tide-forming heterotrophic dinoflagellate *Noctiluca scintillans* (Fonda Umani *et al.*, 2004). The mentioned type of algae is neritic and non-toxic; however, it is classified as a harmful algal bloom (HAB) species because of the damage its blooms inflict on fish populations (Rameshkumar *et al.*, 2023).

The frequency of these two phenomena and their implications for the NAd ecosystems differ. *N. scintillans* blooms occur at irregular intervals and were first recorded in 1977 (Fonda Umani *et al.*, 2004), while *M. leidyi* blooms have occurred regularly in the second half of the year since the first records in 2016 along the Istrian pen-

insula (Budiša *et al.*, 2021). Due to its rapid proliferation, *Noctiluca* has the capacity to increase the concentration of OM up to several hundred times in a short time (Kitatsuji *et al.*, 2019), as will be shown in this study. The occurrence and duration of red tides are generally associated with higher temperatures, nutrient availability, eddies, and the absence of strong winds, ensuring the stability of eutrophic conditions favorable for the growth of *N. scintillans* and its prey (Fonda Umani *et al.*, 2004; Marini *et al.*, 2008). Red tides also occur because of the replacement of primary grazers on phytoplankton with copepods, which is one of their HAB aspects (Harrison *et al.*, 2011). Similarly, the abundance of copepods – a base of fish diet – is threatened because they are common prey for non-selective feeding *M. leidyi* (Budiša *et al.*, 2021). Both phenomena profoundly affect the marine carbon cycle and, ultimately, the NAd trophic system.

The electroanalytical method of phase-sensitive alternating current (a.c.) voltammetry is a reliable and practical method for the qualitative and quantitative analysis of surface-active DOC in natural waters (Ciglencečki *et al.*, 2023 and references therein; Orlović-Leko *et al.*, 2016, 2020; Pereira *et al.*, 2018; Sabbaghzadeh *et al.*, 2017).

Natural samples can generally be considered a mixture of OM with different electrochemical properties (i.e., the adsorption effects at the mercury (Hg) electrode). In a.c. voltammetry, materials with greater hydrophobicity are typically adsorbed first onto the hydrophobic surface of Hg, exerting a dominant influence on the voltammetric signal (Čosović & Vojvodić, 1998; Plavšić *et al.*, 1990;). Consequently, the parameter that standardizes all a.c. voltammetric readings to the common denominator of DOC content is deemed significant in studies concerning OM properties (Ciglencečki *et al.*, 2023 and references therein).

The normalization of SAS to DOC provides so-called DOC normalized surfactant activity (NSA = SAS/DOC) as a practical parameter for assessing and monitoring DOC reactivity-hydrophobicity in different water environments (Ciglencečki *et al.*, 2023; Karavoltos *et al.*, 2015; Simonović *et al.*, 2023 and references therein).

The comparison between the measured NSA values and those of model substances that are common or expected in the studied environment allows a rough estimation of the hydrophobic/hydrophilic properties of OM. Furthermore, to measure the concentration of SAS, additional insight into the type of DOC can be gained by analyzing the voltammetric curves, as shown in this study. The recorded voltammetric curves with characteristic shapes, with or without sharp and well-defined desorption peaks, may be characteristic of certain OM types (Ciglencečki *et al.*, 2018; Čosović *et al.*, 2010a; Orlović-Leko *et al.*, 2016).

This study studied the changes in the properties of OM by measuring DOC, POC, SAS, and NSA, including a detailed analysis of voltammetric curves (with or without the presence of characteristic desorption peaks) during the following occurrences: (1) invasive gelatinous zooplankton (GZ) blooms caused by the ctenophore *M. leidyi* in the summer of 2020 in Valdibora Bay (NAd) and

the red tide caused by *N. scintillans* bloom in spring 2021 in the bays of Valdibora and Lone (NAd), (2) the formation of gelatinous diatoms macroaggregates, detected for the first time in summer 2021 in the coastal waters of the central Adriatic (CAd), and (3) the highly eutrophic waters of the marine system of Rogoznica Lake (CAd) characterized by a strong pycnocline-chemocline enriched by phototrophic sulfur bacteria.

## Material and Methods

### Study locations

The samples were collected from 2020 to 2022 in several coastal areas of the eastern Adriatic, Croatia (Fig. 1): Valdibora Bay (site 1) and Lone Bay (site 2) in the NAd, the karst marine system of Rogoznica Lake (RL; site 3), the coastal waters of Šolta (site 4), and Kaštela Bay (site 5) in the central Adriatic (CAd). Samples from sites 1–5 (Fig. 1A–D) were collected only at the surface as organisms causing those phenomena were concentrated in the top layer (Budiša *et al.*, 2021; Sarma *et al.*, 2019), while samples from the RL (Fig. 1E–F) were collected throughout the water column.

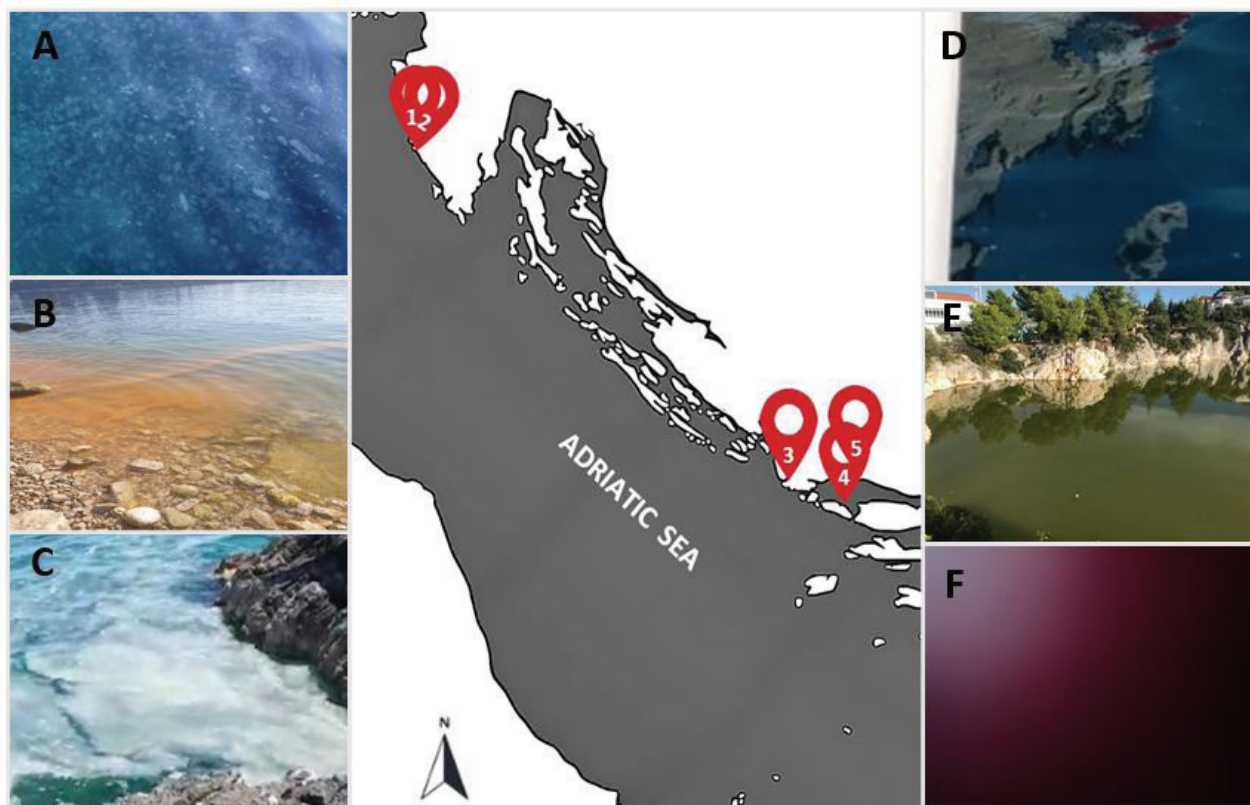
Additional samples of the red tide and zooplankton blooms were collected along the coastal waters of

Rovinj-Rovigno (Supplementary Material Table S1). In this particular case, additional long-term data sets (for years 1998 to 2022) from the RL investigation will be considered (Simonović *et al.*, 2023). All these samples were evaluated in the interpretation and discussion of the results.

### Valdibora and Lone Bay (northern Adriatic)

Valdibora Bay (45.09021°N, 13.63339°E; Fig. 1) and Lone Bay (45.06988°N, 13.63295°E; Fig. 1) are small water bodies located along the western coast of the Istrian peninsula in the NAd region. Valdibora Bay is located in the northern part of the town Rovinj-Rovigno and has an area of 1.3 km<sup>2</sup>, an average depth of 16 m, and a maximum depth of 22 m (Paliaga *et al.*, 2021). Terrestrial inputs from storm events, drainage, and discharge from a fish cannery are significant for this bay (Paliaga *et al.*, 2021). In contrast, Lone Bay, in the south of the town, is surrounded by a large forest park with flourishing Mediterranean vegetation consisting mainly of pines (*Pinus halepensis*). In this case, the abundant seasonal release of pollen provides a significant airborne terrestrial OM input (Pini *et al.*, 2009) that spreads over several km<sup>2</sup> of surrounding coastal waters.

Both sites exhibit comparable yearly variations in phy-



**Fig. 1:** Location of sampling sites on the eastern Adriatic coast, Croatia: (1) Valdibora Bay (45.09021°N, 13.63339°E) and (2) Lone Bay, site 2 (45.06988°N, 13.63295°E) in Rovinj, northern Adriatic (NAd); (3) Rogoznica Lake (43.53091°N, 15.95876°E), (4) coastal waters of Šolta (43.40172°N, 16.29976°E), and (5) Kaštela Bay (43.49438°N, 16.34777°E) in the central Adriatic (CAd). The photographs show (A) a zooplankton bloom (NAd); (B) a red tide bloom (NAd); (C) gelatinous aggregates in the coastal waters of Šolta (CAd); (D) mucilaginous accumulations in Kaštela Bay (CAd); (E) the surface of Rogoznica Lake on 20.10.2020 during the bloom of *Picochlorum* sp.; and (F) the chemocline layer of Rogoznica Lake (CAd) enriched with purple sulphur bacteria, (Photo credits: diver D. Petricioli, June 2021).

toplankton activity influenced by factors such as light intensity, ambient temperature, and nutrient concentration (Zavodnik, 1973) and display a surge in phytoplankton abundance succeeded by an increase in zooplankton population. Such a trend was also observed for the GZ blooms, which were detected and sampled in the summer (2020), and the red tide in the spring of the following year.

Both sampling locations are part of the same large ecosystem of the NAd, the shallowest Adriatic sub-basin, which is strongly influenced by the freshwater discharge of the Po River from the northwest and the cyclonic thermohaline circulation of water masses moving northwards along the east coast of the Adriatic (Degobbi *et al.*, 2000; Supić *et al.*, 2024). The inflow of freshwater from the Po River ( $1496 \text{ m}^3 \text{ s}^{-1}$ ; Cozzi & Gianni, 2011) is the main source of nutrients (up to  $30 \text{ } \mu\text{M}$  for  $\text{NO}_3^-$  and over  $3 \text{ } \mu\text{M}$  for  $\text{PO}_4^{3-}$ ) in the NAd during spring and autumn and the primary initiator for the formation of eddies (Piontkovski *et al.*, 2021), which influence the dynamics of OM (Dautović *et al.*, 2017; Marini *et al.*, 2008). Such circulation systems can retain nutrient-rich riverine water and trigger blooms. For instance, nutrient uptake by phytoplankton provides sufficient food for the proliferation of the heterotrophic dinoflagellate *N. scintillans* (Fonda Umani *et al.*, 2004) but can also accumulate sufficient amounts of smaller zooplankton as prey for the invasive ctenophore *M. leidyi* and ensure its spread across the region (Budiša *et al.*, 2021; Ciglencčki *et al.*, 2021).

#### *Coastal waters of Šolta Island and Kaštela Bay (central Adriatic)*

Šolta ( $43.40172^\circ\text{N}$ ,  $16.29976^\circ\text{E}$ ; Fig. 1) is an island in the CAd region, not far from the city of Split and Brač Island, with an area of  $58.98 \text{ km}^2$ . The semi-enclosed, oligotrophic marine coastal environment of Šolta Island is under the influence of strong marine currents caused by bora (NE wind) and sirocco (SE wind) wind episodes (Klaić *et al.*, 2011), with terrestrial input of OM changing the quality of OC (Bajt *et al.*, 2019; Lovrenčić Mikelić *et al.*, 2017).

Kaštela Bay ( $43.49438^\circ\text{N}$ ,  $16.34777^\circ\text{E}$ ; Fig. 1) is a semi-enclosed embayment in the CAd region north of the city of Split with an area of  $61 \text{ km}^2$  and an average depth of 23 m. The terrestrial anthropogenic input is significant due to the dense urban, industrial, and agricultural areas surrounding the northern coast (Anđelić *et al.*, 2020). The Jadro River, with an average annual inflow of about  $8\text{--}10 \text{ m}^3 \text{ s}^{-1}$ , is the main freshwater input into the bay (Anđelić *et al.*, 2020). Vertical stratification of the water column with a pronounced thermocline occurs in the warmer part of the year at a depth of 10–25 m (Lovrenčić Mikelić *et al.*, 2017). Bora, sirocco, and mistral (NW wind) influence the general circulation in Kaštela Bay, which is predominantly cyclonic in the surface layer. In June 2021, samples from the surface layer with certain gel-like aggregates were taken in the coastal waters of Šolta in the Split Channel and Kaštela Bay.

#### *RL (central Adriatic)*

RL is a unique eutrophic and stratified euxinic marine lake on the eastern coast of the Adriatic Sea ( $43.53091^\circ\text{N}$ ,  $15.95876^\circ\text{E}$ ; Fig. 1; Ciglencčki *et al.*, 2017; Simonović *et al.*, 2023). It is an oval sunken karst depression (with a depth of 14 m) filled with seawater, with an area of  $10.276 \text{ m}^2$ . RL is connected to the sea through porous karst and thus reflects the changes in the marine dynamics of the Adriatic Sea. The main feature of RL is its strong water column stratification with euxinic conditions below the chemocline and anoxic holomixis during the mixing of the lake in autumn, which is the main source of nutrient supply from the bottom to the surface (Ciglencčki *et al.*, 2015; Žic *et al.*, 2013). The lake also fluctuates between stratified and holomictic euxinic conditions under certain meteorological situations (Ciglencčki *et al.*, 2017). The depth of the chemocline changes seasonally (Simonović *et al.*, 2023), reflecting the strong changes in physicochemical parameters, and in the summer months, it is enriched by a dense population of green and purple sulfur bacteria (Čanković *et al.*, 2017, 2020; Pjevac *et al.*, 2015). OM in the water column of RL is mainly of autochthonous origin, and eutrophication is associated with the recycling of nutrients in the anoxic hypolimnion (Ciglencčki *et al.*, 2005; Čosović *et al.*, 2000). Samples were taken at the central, deepest point (14 m deep) of the lake at every meter from the surface to the bottom layer in summer and autumn 2020 and in spring 2022.

#### *Sampling and sample preparation*

Surface samples of seawater in the NAd and CAd were collected directly in 1.3 L dark glass bottles, while RL water column samples were collected using a 5 L Niskin sampler. Physicochemical parameters such as temperature (T), salinity (S), and dissolved oxygen (O) of the lake water column samples studied were measured *in situ* at the surface and from the surface to a depth of 12 m using the HQ40d Portable Meter (Hach Lange, Germany). The relative accuracies for T determination, pH, S, and oxygen content were  $\pm 0.3^\circ\text{C}$ ,  $\pm 0.002$ ,  $\pm 0.5\%$ , and  $\pm 1\%$ , respectively. In the NAd and CAd samples, temperature and salinity were measured using a pIONeer 65 probe (Radiometer Analytical, Copenhagen).

After sampling, samples were filtered through Whatman<sup>TM</sup> glass fiber filters (GF/F) with a pore size of  $0.7 \text{ } \mu\text{m}$ , which were previously pre-combusted at  $450^\circ\text{C}$  for 4 h for DOC and SAS measurements. Filtrate triplicates for DOC analysis were collected in 20 mL glass vials, preserved with mercury (II) chloride ( $c(\text{HgCl}_2)=10 \text{ mg L}^{-1}$ ), sealed with Teflon screw caps, and stored in a dark place until analysis, as previously described (Marguš *et al.*, 2015). For POC analysis, filters (after filtration of a known sample volume for DOC and SAS) were carefully stored in cryotubes and preserved by freezing at  $-50^\circ\text{C}$  until further analysis. Samples for SAS determination were stored at  $4^\circ\text{C}$  until measurement within 24 h of sampling.

GZ abundance and composition were determined according to Paliaga *et al.* (2021). Samples for phytoplankton composition determination were fixed in 2% formaldehyde immediately after sampling, and cells were counted within one day. *N. scintillans* cells were counted using a Sedgewick–Rafter counting chamber (Kopuz *et al.*, 2014) under a Nikon SMZ 745 T dissection microscopy.

### DOC and POC measurements

The concentration of DOC was determined using the high-temperature catalytic oxidation (HTCO) method at 680 °C with Pt/Si catalyst (Elemental Microanalysis, UK) and non-dispersive infrared (NDIR) CO<sub>2</sub> detection using the TOC-VCPH instrument (Dautović *et al.*, 2017; Shimadzu, Japan). POC was analyzed with an SSM-5000A solid sample module (Shimadzu, Japan) with a TOC-VCPH carbon analyzer. Inorganic carbon was removed from the samples by adding 2 mol dm<sup>-3</sup> HCl before POC measurement. Oxidation of OC in the stream of pure O<sub>2</sub> at 900 °C produced CO<sub>2</sub>, which was detected using the NDIR detector. The limit of quantification (LoQ) was 0.228 mg L<sup>-1</sup> for DOC and 10 µg L<sup>-1</sup> for POC. The precision of DOC measurements reported as relative standard deviations were based on the analysis of selected samples and the reference materials. Relative standard deviation (RSD) never exceeded 6% and 5%, respectively. The method is suitable for testing waters and sediment (POC) according to HRN EN ISO/IEC 17025:2017 (accreditation certificate number: 1577).

### SAS and transparent exopolymer particle (TEP) measurements

SAS were analyzed with the electrochemical method of a.c. voltammetry (out-of-phase mode; Ciglencečki *et al.*, 2023; Čosović & Vojvodić, 1998). The method

is based on the measurement of the adsorption effect of SAS on the surface of a hanging mercury drop electrode as a working electrode at a potential of -0.6 V (vs. Ag|AgCl, 3 M KCl; Čosović & Vojvodić, 1998) at initial seawater (pH around 8). Usually, SAS measurements are performed under initial and acidic conditions (by adding HCl, at pH values, pH = 2–3) when the negative charges of SAS polyelectrolytes are neutralized and more neutral and adsorbable organic substances are formed, giving a higher amount of SAS (Ciglencečki *et al.*, 2018; Čosović & Vojvodić, 1998).

The SAS measurements were conducted directly in the seawater samples, whereby ionic strength was adjusted to 0.55 M with 5.5 M NaCl if necessary (depending on the salinity). Due to the adsorption of surface-active organic material, the decrease in the capacitive current below the value for pure electrolyte was recorded (see voltammograms in the Results and Discussion sections), which was then expressed as the equivalent adsorption effect (mg L<sup>-1</sup>) of the non-ionic model substance Triton-X-100 (octyl phenol ethylene oxide condensate; Supplementary Material Fig. S1A; Ciglencečki *et al.*, 2023). Triton-X-100 has been shown to be a good representative of the majority of the reactive fraction of SAS in natural waters. The LoD for voltammetric SAS determination is 0.02 mg L<sup>-1</sup> eq. Triton-X-100, with a LoQ of 0.05 mg L<sup>-1</sup> and RSD around 7%. The external calibration of Triton-X-100 was checked under the following experimental conditions: accumulation potential E<sub>a</sub> = -0.6 V, accumulation time under stirring t<sub>a</sub> = 30 s, frequency f = 75 Hz, amplitude a = 10 mV, and a.c. phase angle = 90°.

SAS were then normalized to DOC content to obtain the value of NSA = [SAS (eq. T-X-100)/DOC], the graphical representation of which provides a more accurate visual characterization of the nature of DOC (Ciglencečki *et al.*, 2023; Čosović *et al.*, 2007). NSA can be compared with NSA of different model SAS (Table 1), which are representative of the surfactants present in the OM pool of natural samples (Ciglencečki *et al.*, 2023; Čosović & Vojvodić, 1998; Čosović *et al.*, 2000).

**Table 1.** Organic carbon and adsorption properties (NSA) of selected model surface active substances (SAS), as determined by SAS measurements on the Hg electrode in 0.55 M NaCl.

Model surface active substances	Compound concentration (mg L <sup>-1</sup> )	DOC (mg L <sup>-1</sup> )	SAS (eq. Triton-X-100) (mg L <sup>-1</sup> )	NSA
Triton-X-100 <sup>1</sup>	0.108	0.07	0.108	1.54
Protein <sup>1</sup>	1.10	0.54	0.108	0.20
Bovine serum albumin (BSA) <sup>3</sup>	0.38	0.204	0.108	0.53
Fluvic acid <sup>1</sup>	2.00	0.64	0.108	0.17
Humic acid <sup>2</sup>	5.00	2.50	0.095	0.04
Dextran-X-500 <sup>1</sup>	1.12	0.53	0.108	0.20
Xanthan <sup>1</sup>	9.60	3.07	0.108	0.04
κ-Carrageenan <sup>3</sup>	10.06	0.735	0.108	0.15
Model Pollen <sup>3</sup>	9.99	1.66	0.108	0.07

<sup>1</sup>Čosović & Vojvodić, 1998; <sup>2</sup>Čosović *et al.*, 2007; <sup>3</sup>this study.

In this study, the NSA parameter was determined for the first time for the protein model substances of Bovine Serum Albumin (BSA; 0.435), the polysaccharide  $\kappa$ -Carrageenan (0.096) and pollen (0.065) as representatives of OM that may be present in the organic-rich natural samples analyzed (Table 1). The NSA was calculated based on the voltammetric behavior and calibration curves of the apparent adsorption isotherm of these OM substances (Ciglenečki *et al.*, 2023).

The model substances (BSA A9085-5G, Sigma-Aldrich; Xanthan gum from *Xanthomonas campestris* G1253, Sigma-Aldrich; Dextran 200000MW D7139, Invitogen;  $\kappa$ -Carrageenan C1263, Sigma-Aldrich; and model pollen as a nutrition supplement) were prepared by dissolving 0.55 M NaCl and then analyzed using a.c. voltammetry and HTCO. The solutions of different concentrations were prepared from the initial aliquots in a 250 mL volumetric flask. The natural pollen sample was collected from the tree *Betula pendula* and prepared by

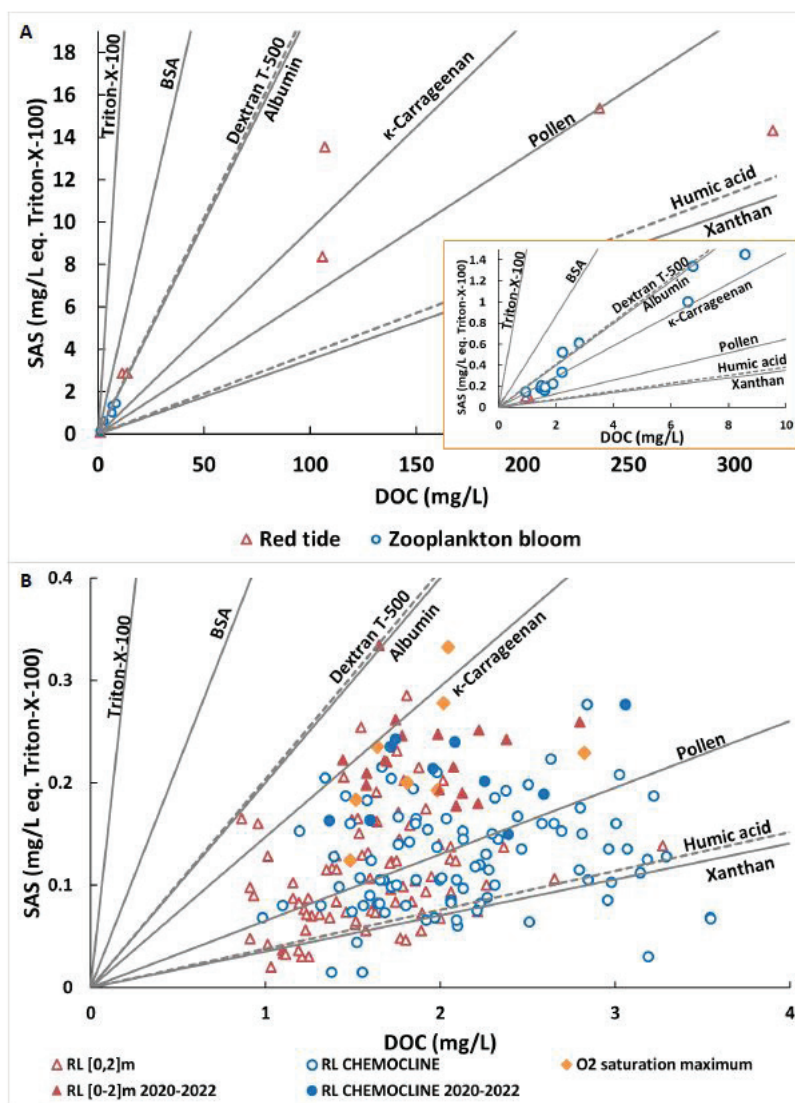
immersing seven flower spikes in 0.55 M NaCl (250 mL) to mimic the natural conditions in the marine environment. After 24 h, the sample was filtered and analyzed using the a.c. voltammetry and HTCO methods.

All glassware used in OM analysis, including sampling, filtration, and preservation, was washed with chromium sulfuric acid and thoroughly rinsed with organic-free Milli-Q water (Millipore).

TEPs were preliminarily measured in RL samples collected in April and May 2022 using the method described in Passow & Alldredge (1995) and Villacorte *et al.* (2015).

## Results

The OM properties represented by the concentration of DOC, POC, and SAS, including NSA and voltammetric curves recorded in samples of intense plankton



**Fig. 2:** SAS and DOC values for: (A) zooplankton bloom in summer of 2020 and red tide event in spring of 2021, both in the NAD (Table 2 and Supplementary Material, Table S1). Insertion displays samples with lower SAS-DOC values; (B) RL water column, i.e. surface oxia layer (0-2 m depth), chemocline as boundary between oxia-anoxia and samples from depths with maximum oxygen saturation (>100%), during 1998-2022 (data from 1998 to 2020 are from Simonović *et al.*, 2023, while data since 2020 are given in Supplementary Material, Table S2).

**Table 2.** Organic carbon (DOC, SAS, POC, NSA) and electrochemical (peak potential,  $\Delta I$  in original and acidic conditions) properties of the representative coastal seawater samples, including environmental parameters of temperature and salinity.

	SAMPLE	SAS (mg L <sup>-1</sup> )	DOC (mg L <sup>-1</sup> )	POC (mg L <sup>-1</sup> )	NSA	T (°C)	S (‰)	Desorption peak poten- tial (V)	$\Delta I$ 30s original ( $\mu A$ )	$\Delta I$ 30s HCl ( $\mu A$ )
VALDIBORA BAY	Zooplankton bloom* (27.8.2020)	1.45	8.58	n.m.	0.169	22.90	37.65	-1.64 V	0.133	0.149
	<i>M. leidy</i> 100 units m <sup>-3</sup>									
	Red tide* (31.3.2021)	14.3	318.1	13.65	0.045	14.20	37.90	-1.35 V	0.094	0.095
	<i>N. scintillans</i> 72010 cells L <sup>-1</sup>									
LONE BAY	Red tide* (16.4.2021)	2.88	11.39	16.87	0.253	16.10	37.40	-1.65 V	0.022	0.032
	<i>N. scintillans</i> 46800 cells L <sup>-1</sup>									
ŠOLTA coastal waters	Ambient water (12.6.2021)	0.317	3.18	1.95	0.100			-1.76 V	0.155	0.062
	confirmed diatoms									
	Surface** (12.6.2021)					22.09	37.5			
	confirmed diatoms	0.589	n.m.	326.6	n.m.			-1.63 V	0.213	0.239
KAŠTELA BAY	Mucilage (19.6.2021)	0.192	1.48	0.692	0.130	23.56	36.60	-1.56 V -1.16V	0.089	0.133
	confirmed diatoms									
ROGOZNICA LAKE	Surface oxic layer, 1 m (20.10.2020)	0.240	2.08	10.59	0.115	20.30	22.90	-1.58 V -1.41 V	0.159	0.164
	confirmed <i>Picochlorum</i> sp.									
	Chemocline, 7 m (28.6.2021)	0.189	2.59	18.45	0.073	22.00	35.00	-1.71 V -1.16 V	0.189	0.292
	enriched with purple sulfur bacteria									
	Surface oxic layer, 0 m (6.4.2022)	0.235	1.64	0.314	0.143	15.10	26.70	-1.57V	0.161	0.240
	enriched with TEP									

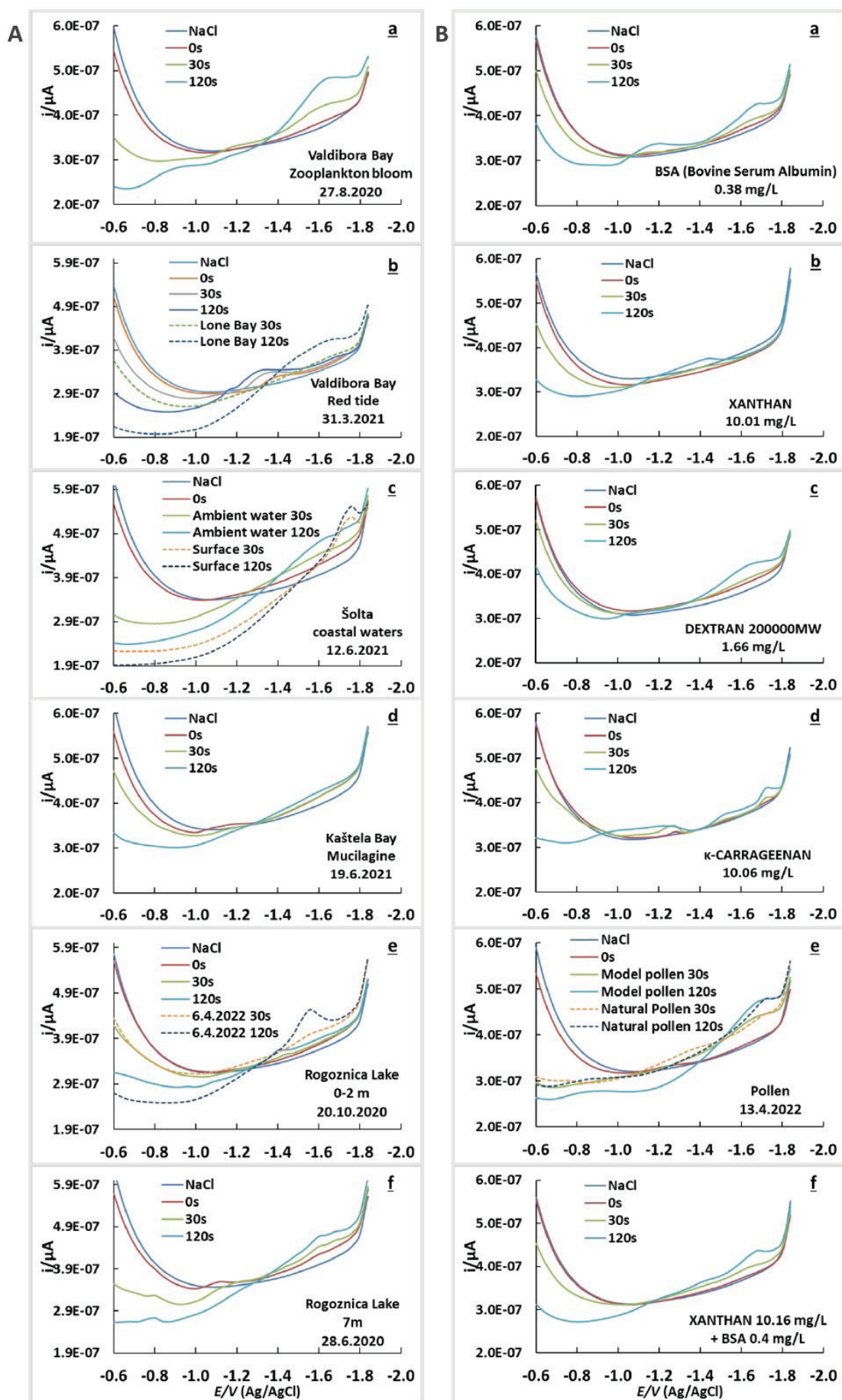
\*measured in dilution; \*\* it was not possible to take an aliquot for DOC analysis due to the presence of dense gel-like mucus.

blooms and macroaggregate formations along the coast of the Adriatic Sea (Croatia) studied here are presented in Table 2 and Figures 2–4, as well as in Tables S1–S2 in the Supplementary Material.

#### OM properties during GZ bloom and red tide in the NAd

GZ blooms formed by the invasive ctenophore *M. leidy* (Fig. 1A) and the red tide formed by the heterotrophic dinoflagellate *N. scintillans* (Fig. 1B) occurred in the coastal waters of Rovinj-Rovigno (NAd) in summer 2020 and spring 2021, respectively. Both phenomena were characterized by a significant production of DOC with different surface-active properties. The SAS and DOC data for two different ecological contexts in Valdib-

ora Bay are shown in Figure 2A. It is evident that during the red tide events (depicted by red triangles), significantly elevated concentrations of DOC were observed, ranging from 11.39 to 318.1 mg L<sup>-1</sup>, with corresponding SAS concentrations ranging from 2.58 to 15.4 mg L<sup>-1</sup>. The corresponding NSA values ranged from 0.045 to 0.253 (average value of 0.130); that is, between the lines of Dextran-T-500/Albumin for lower concentrations of DOC and around the lines for  $\kappa$ -Carrageenan, pollen and Xanthan/Humic acid for higher ( $\geq 100$  mg L<sup>-1</sup>) DOC concentrations (Fig. 2A; Tables 1–2), indicating the presence of a similar OM type compared to the model substances. For comparison, the control sample of “clean” seawater sampled in the coastal waters of Rovinj-Rovigno (3.3.2021) showed lower values of OM parameters (DOC = 0.829 mg L<sup>-1</sup>, SAS = 0.072 mg L<sup>-1</sup>), with an NSA value of 0.087.



**Fig. 3:** A.c. voltammograms of the studied coastal seawater samples (A), and the corresponding model substances (B). Measurements were performed in original samples, and 0.55 mol L<sup>-1</sup> NaCl. Experimental conditions: accumulation time,  $t_a = 0, 30, 120$  s at  $-0.6$  V vs. Ag/AgCl.

Extremely high DOC concentrations (318.1 mg L<sup>-1</sup>) with high surface-active properties (SAS = 14.3 mg L<sup>-1</sup>; Table 2) were measured in the Valdibora Bay red tide sample (Table 2; Fig. 3Aa; Supplementary Material Table S1), followed by an elevated concentration of POC (13.65 mg L<sup>-1</sup>).

In comparison, during the GZ bloom of *M. leidy* in Valdibora Bay (blue circles, Fig. 2A; Supplementary Material Table S1B), the surface activity of DOC was several times lower (0.959–8.58 mg L<sup>-1</sup>), with most of NSA lying between the reference values for Dextran-T-500/Albumin and  $\kappa$ -Carrageenan (Fig. 2A), which ranged from 0.101 to 0.217 (average value of 0.160), as previously reported for the east coast of the NAd during the summer months of 2016–2019 (Budiša *et al.*, 2021; Paliaga *et al.*, 2021; Ciglenečki *et al.*, 2021).

Additional insight into the nature of the DOC in the samples studied was obtained by analyzing the recorded voltammetric curves, from which the SAS were determined by external calibration with Triton-X-100. The occurrence of specific desorption peaks in the recorded voltammograms and their comparison with different model substances can be a good indication of a particular OM type, as shown in Figure 3A, B and in Tables 2 and 3, where the electrochemical properties of the studied marine samples (Table 2) and the corresponding model substances (Table 3) are given. In this case, the stronger the adsorption effect (i.e., the more hydrophobic the organic substance), the more desorption waves are recorded at the more negative potentials (Table 3), as shown previously (Ćosović *et al.*, 2010b).

Figure 3Aa shows the voltammograms of a sample of a GZ (*M. leidy*) bloom of 100 individuals (ind.) per m<sup>3</sup> (Supplementary Material Table S1), in which high concentrations of SAS (1.45 mg L<sup>-1</sup>) and DOC (8.58 mg L<sup>-1</sup>) were measured, resulting in a relatively high value of NSA (0.169; Table 2). This voltammogram is characterized by a characteristic broad desorption peak at a

potential of approximately -1.64 V (Table 2), which was attributed to the presence of protein type OM, as a similar desorption peak at -1.68 V was recorded in the model protein solution BSA (Fig. 3Ba; Table 3).

The sample taken from the red tide had an *N. scintillans* bloom that numbered ~72 000 cells L<sup>-1</sup> (along with some pine pollen that was detected microscopically) and had a low NSA of 0.045 (Table 2), indicating a more hydrophilic, less reactive character of the OM, similar to the NSA of the model polysaccharide Xanthan (Table 1). Further electrochemical characterization of the same sample revealed a pronounced desorption peak at a potential of -1.35 V (Fig. 3Ab; Table 2), again most likely due to the presence of the polysaccharide Xanthan in whose model solution a similar desorption peak was recorded at about -1.41 V (Fig. 3Bb; Table 3).

In addition, a red tide sample collected two weeks later in Lone Bay, which contained lower counts of *N. scintillans* (46 800 cells L<sup>-1</sup>; Supplementary Material Table S1A), had lower concentrations of DOC (11.39 mg L<sup>-1</sup>) and SAS (2.88 mg L<sup>-1</sup>; Table 2), corresponding to an NSA of 0.253, which is close to the NSA of Dextran (0.20; Table 1). The voltammetric curves for this sample with desorption peak at a potential of about -1.65 V (Fig. 3Ab; Table 3A) indicate the predominance of the polysaccharide Dextran type (Fig. 3Bc; Table 3). Notably, both *N. scintillans* bloom samples (from Valdibora and Lone Bays) contained a proliferation of a single species.

Seasonal pollen loading is considered a significant terrestrial input of OM in the NAd coastal zone (Pini *et al.*, 2009). Since pollen was detected microscopically in the sample from Valdibora Bay (data not shown), the OM properties, including voltammetric behavior and NSA of pollen are additionally reported in Tables 1–3 and Figures 2 and 3Be.

To study the anionic properties of SAS as additional parameter of OM characterization, all voltammet-

**Table 3.** Electrochemical properties of the corresponding model substances.

	Model surface active substances	Concentration* (mg L <sup>-1</sup> )	Desorption peak potential (V)	$\Delta I$ 30s original	$\Delta I$ 30s HCl
				( $\mu A$ )	( $\mu A$ )
MODEL SUBSTANCES	Xanthan	10.01	-1.46 V	0.082	0.083
	Pollen	5.00	-1.77 V	0.098	0.100
	$\kappa$ -Carrageenan	10.06	-1.72 V -1.27 V	0.091	0.098
	Dextran 2000000MW	1.66	-1.65 V	0.057	0.053
	Bovine serum albumin (BSA)	0.038	-1.68 V -1.18 V	0.052	0.065
	Mixture of Xanthan/Bovine serum albumin (BSA)	10.16/0.40	-1.73 V -1.44 V	0.099	0.102

\*concentrations of the organic model substances were chosen so that the same effect ( $\Delta I$ ) on the Hg electrode is obtained under the given experimental conditions.

ric curves were plotted at initial and acidic conditions (pH = 2–3; Tables 2, 3). In all samples except the one of the red tide (Lone Bay, April 16, 2021) and of gel-like aggregates (June 12, 2021; Table 2), SAS increased upon acidification to pH 2, indicating the presence of more negatively charged polymeric SAS.

Considering that the natural samples as mixtures of organic substances with different surface-active properties have different effects on the Hg electrode, the electrochemical behavior of the mixture prepared from a more hydrophobic and a more hydrophilic type of model substances, represented by BSA (protein) and Xanthan (polysaccharide), respectively, is shown in Figure 3Bf and Table 3. Evidently, the 25.5 times lower concentration of BSA still had the dominant effect on the Hg electrode. Analysis of the same mixture under acidic conditions, where no or minimal increase in SAS was observed, indicates the predominance of the polysaccharide type of OM, considering the similar electrochemical behavior of the model polysaccharides studied after acidification (Tables 2, 3).

### ***OM properties in gelatinous macroaggregates of the coastal waters of the central Adriatic***

In the summer of 2021, gelatinous aggregates and mucilaginous accumulations were detected and characterized for the first time in the coastal waters of the CAd around the island of Šolta (Fig. 1C) and in Kaštela Bay (Fig. 1D; Table 2). Samples from the coastal waters of Šolta Island contained a thick surface layer of gelatinous aggregates in the OM-rich ambient water (Fig. 1C), in which elevated concentrations of DOC (3.18 mg L<sup>-1</sup>, compared to seawater without gelatinous aggregates, which had a concentration of 1.49 mg L<sup>-1</sup>), SAS (0.317 mg L<sup>-1</sup>), and POC (1.95 mg L<sup>-1</sup>) were measured (Table 2). Extremely high concentrations of POC (326.6 mg L<sup>-1</sup>) and SAS (0.589 mg L<sup>-1</sup>) were found in the dense gelatinous layer of OM (Table 2). Although it was not possible to determine the DOC and, thus, the NSA parameter in this OM-rich gelatinous sample, the electrochemical analysis revealed a sharp desorption peak at a potential of -1.76 V (Fig. 3Ac surface sample; Table 2), which can be identified as the polysaccharides  $\kappa$ -Carrageenan and pollen based on its position and shape (Figs 3Bd and e; Table 3). The gelatinous samples from Kaštela Bay collected one week later were characterized by lower concentrations of DOC (1.48 mg L<sup>-1</sup>), SAS (0.192 mg L<sup>-1</sup>), and POC (0.692 mg L<sup>-1</sup>; Table 2). The NSA value of this sample (0.130; Table 2) indicates that the OM type is similar to the model polysaccharide  $\kappa$ -Carrageenan whose NSA value is 0.15 (Table 1). The voltammetric curves of the samples from Kaštela Bay, characterized by less pronounced desorption peaks at -1.16 V and -1.56 V (Fig. 3Ad; Table 2), resembled both samples from the coastal waters of Šolta Island and the voltammetric behavior of the model polysaccharide  $\kappa$ -Carrageenan (Fig. 3Bd; Table 3).

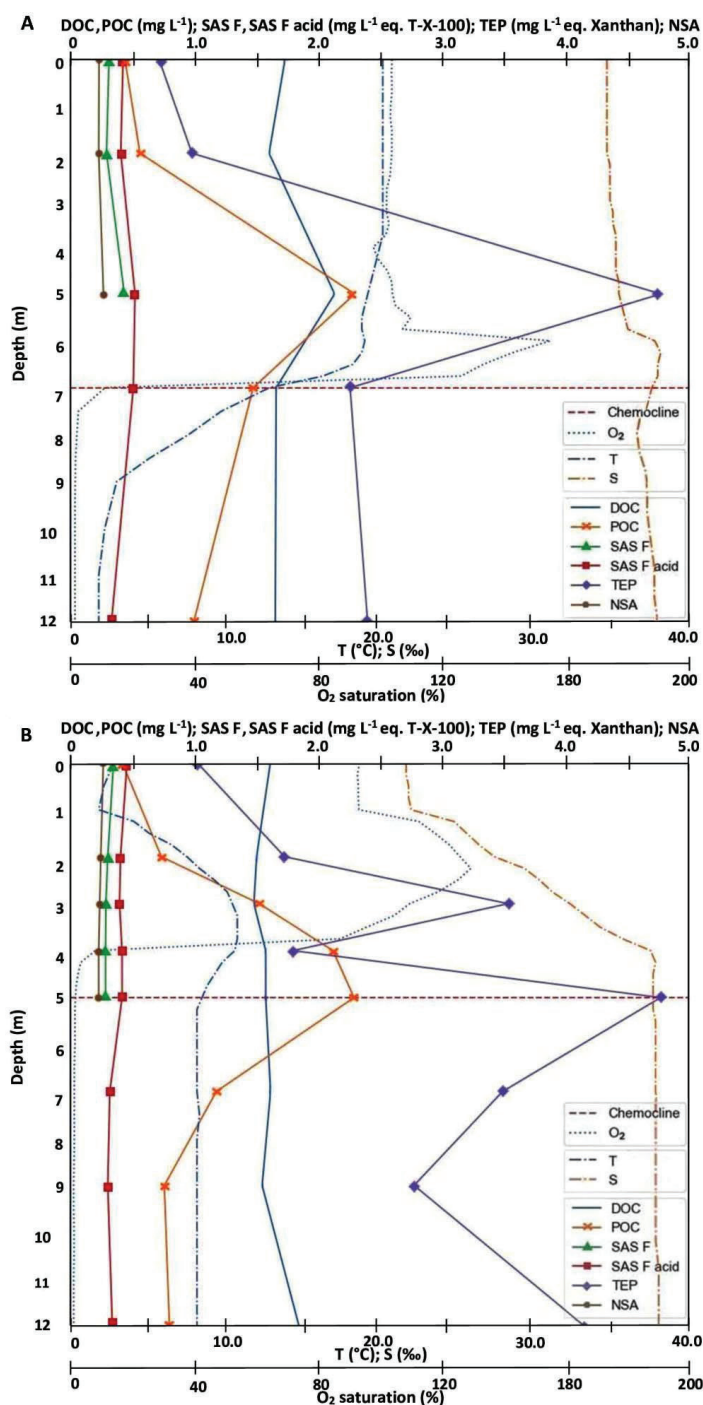
### ***OM properties of a eutrophic marine lake (RL)***

The DOC and SAS data for the two typical water layers in RL (surface and chemocline at the boundary between oxic and anoxic conditions; Fig. 1E, F) are shown in Figure 2B for the period 1996 to 2022. The samples from the period 2020 to 2021 (Table S2), which are studied in detail in this paper, are specially marked. The same diagram also shows the data for the samples collected at the depths with the highest biological activity, as indicated by the maximum oxygen saturation (> 100%). The NSA values for the surface layer ranged from 0.019 to 0.191 (average NSA value = 0.070) and were between the lines for Xanthan and Dextran-T-500 as representative model substances for the OM type in the marine environment. The NSA of the chemocline samples, which have nearly two orders of magnitude higher DOC concentrations, ranged from 0.010 to 0.542 (average NSA = 0.066). These values, which lie between the lines for Xanthan/Humic acid and  $\kappa$ -Carrageenan (Fig. 2B), indicate a slightly more hydrophilic and less reactive OM type compared with the surface layer of RL (Fig. 2B). Compared to the surface layer and chemocline, the samples from the depths with the highest biological activity are grouped between the lines for  $\kappa$ -Carrageenan and pollen (NSA ranged from 0.062 to 0.163 with an average of 0.110).

The voltammetric curves for the sample collected at depth with the highest biological activity were characterized by a sharp and pronounced desorption peak at a potential of -1.57 V (Fig. 3Ae shows the curves for the sample collected on April 6, 2022; Table 3A). In the same sample, the DOC content (1.64 mg L<sup>-1</sup>), the POC content (0.314 mg L<sup>-1</sup>), and the SAS content (0.235 mg L<sup>-1</sup>) were measured (Supplementary Material Table S2C), resulting in a relatively high NSA (0.143) between Albumin and  $\kappa$ -Carrageenan (Fig. 2; Tables 1–2). The same desorption peak was evident in the filtered (pore sizes 0.45 and 0.22  $\mu$ m) RL sample from 5 m depth (May 12, 2022; Supplementary Materials Table S2 and Fig. S2), in which desorption wave was even more pronounced. These two samples were enriched in TEP (Fig. 4), and the voltammetric results most likely indicate the conditions of intense primary production of more colloidal, highly surface-active, and labile OM.

A different type of OM was characterized in the RL sample from the surface layer (1 m depth; Fig. 1E, Table 2), collected in October 2020, when a bloom of the green algae *Picochlorum* sp. (Mucko *et al.*, 2020) was detected in the postholomictic period of the lake (M. Čanković, pers. comm.). The sample was enriched in POC (10.59 mg L<sup>-1</sup>), DOC (2.08 mg L<sup>-1</sup>), and SAS (0.240 mg L<sup>-1</sup>) with the NSA value of 0.115 (Table 2), which is between the lines of  $\kappa$ -Carrageenan and pollen (Table 1).

The voltammetric curves for the October sample with desorption peaks at potentials of about -1.41 V and -1.58 V (Fig. 3Ae; Table 2) indicate the behavior of a mixture ( $\kappa$ -Carrageenan, pollen), with the polysaccharide component predominating (Fig. 3Bf, Table 3). It is important to mention that all studied RL samples showed higher SAS after the addition of acid, especially the samples from the



**Fig. 4:** Organic matter parameters and physico-chemical properties of the marine Rogoznica Lake water column on April 6, 2022 (A) and May, 12 2022 (B). NSA parameter (SAS/DOC ratio) was determined up to the chemocline layer, considering that SAS cannot be measured in an anoxic sample due to reduced sulphur species interference (Simonović *et al.*, 2023).

chemocline layer (Table 2), indicating the presence of more anionic (negatively charged) polymeric surface-active DOC.

The light pink colored chemocline sample (collected at 7 m depth on June 28, 2021; Table 2, Fig. 2) was enriched in POC (18.45 mg L<sup>-1</sup>), DOC (2.59 mg L<sup>-1</sup>), and SAS (0.189 mg L<sup>-1</sup>) and had a lower NSA (0.073). At the same time, maximum oxygen saturation was measured as an indication of high biological activity at depths between 5 and 5.5 m, and decreasing NSA was measured from the surface to the chemocline as an indication of

changes in the quality and quantity of OM (Supplementary Material Fig. S3). The voltammetric curves for this chemocline sample with desorption peaks at a potential of -1.16 V and -1.71 V (Fig. 3Af; Table 2) most closely resemble the voltammetric behavior of the sulfated model polysaccharide  $\kappa$ -Carrageenan (Fig. 3Bd; Table 3). A small peak recorded with longer accumulation times ( $t_a = 30$  and 120 s) at about -0.75 V (vs. Ag|AgCl) could be an indication of incorporated S-groups within the OM molecule (Ciglenečki *et al.*, 2003).

## NSA as an OM fingerprint

The analysis of the frequency distribution of the calculated NSA (i.e., SAS/DOC values for the studied samples; Fig. 5) shows significant differences among them, similar to the graphical representation of the relationship between the SAS and DOC for the same samples shown in Figure 2 where the difference in the concentrations and the type of OM can be clearly seen. The frequency distribution of the calculated NSA for the studied samples (Fig. 5; Supplementary Material Tables S1–S2) shows that the surface water layer (0–2 m) of RL and red tide in Valdibora Bay had relatively similar characteristics in terms of the OM type, with a dominant range of lower NSA values (0.041–0.080). These values indicate a more hydrophilic, less reactive type of OM previously characterized as polysaccharide material, mainly of Xanthan and  $\kappa$ -Carrageenan type (Figs 2–3; Tables 1–3).

## Discussion

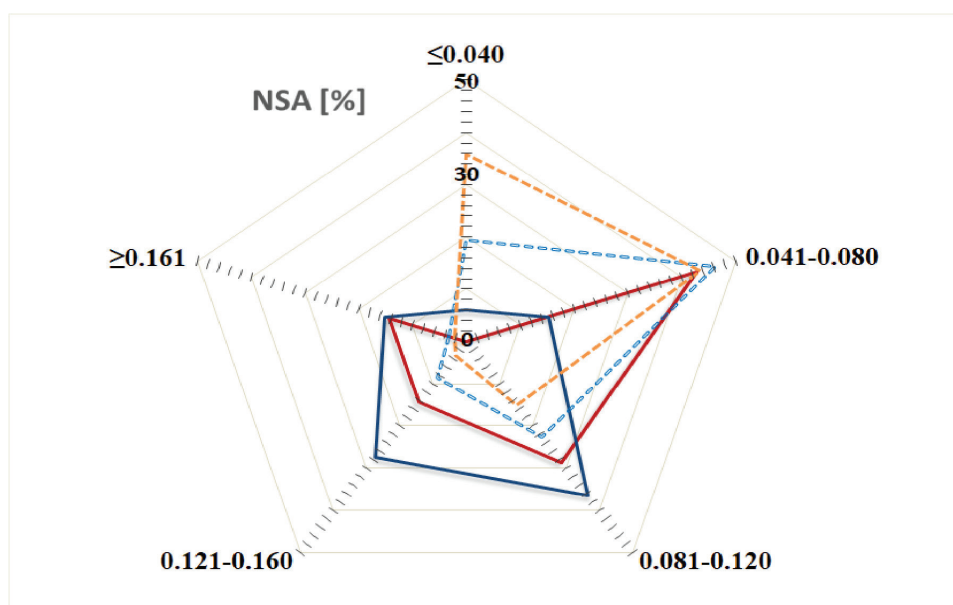
### OM properties of zooplankton bloom and red tide in the northern Adriatic

The NSA frequency distribution of the GZ bloom was in a higher range of values (Fig. 5), indicating a more hydrophobic, reactive protein type of OM, in agreement with the voltammetric curves that best correspond to the behavior of BSA (Figs 2–3; Tables 1–3). Indeed, *M. leidy* is reported to release significant amounts of labile surface-active DOC, which tends to adsorb at various phase boundaries and further support microbial activity (Paliaga *et al.*, 2020). An increased occurrence of nitrogen-containing polymeric OM (N-POM) was also measured in the NAd samples during the occurrence of *M. leidy* in the spring and summer of 2017 (Penezić *et al.*, 2022). The presence of labile carbohydrates and proteins ensures

higher OM reactivity (Nguyen *et al.*, 2005).

The release of OM associated with *N. scintillans* and *M. leidy* blooms differed significantly both quantitatively and qualitatively. The DOC concentrations during the *N. scintillans* bloom were one to two orders of magnitude higher than the concentrations reported during the *M. leidy* swarms (Fig. 2). This could be mainly due to the overall difference in carbon content in these two blooms. According to Tada *et al.* (2000, 2020), *N. scintillans* cells have an average of 0.35  $\mu\text{g C}$  per cell. This means that a bloom with abundances  $\sim 72\,000$  cells  $\text{L}^{-1}$  in Valdibora Bay resulted in concentrations of 25.4  $\text{g C m}^{-3}$ . On the other hand, the carbon content in *M. leidy* from swarms in the same area contained 28  $\mu\text{g C ind.}^{-1}$  (Budiša *et al.*, 2022), which sums to 2.8  $\text{g C m}^{-3}$  in reported abundances of 100 ind.  $\text{m}^{-3}$ . It might appear that swarms of macrozooplankton, such as *M. leidy*, contain more carbon than microplankton blooms. However, due to high water content in GZ biomass ( $\sim 96\%$ ), *M. leidy* swarms yield about an order of magnitude less C than *N. scintillans* blooms in the same volume of water.

Although *M. leidy* and *N. scintillans* are both non-selective heterotrophs, there are significant differences in their food intake. Therefore, the available OM in bloom or swarms inevitably differs and could affect the typology and quantity of the released DOC. For instance, it is reported that the optimal food fatty acid profile for *N. scintillans* comprises of  $\alpha$ -linolenic and eicosapentanoic acid (Zhang *et al.*, 2015), while most of the food consumed by *M. leidy* is abundant in docosahexaenoic acid (Vansteenbrugge *et al.*, 2016) otherwise absent in *Noctiluca*'s diet. In addition, the biochemical composition of the two species differs. Whereas *Noctiluca*'s lipid content is 0.5% of its wet weight (Sen Ozdemir *et al.*, 2017), in gelatinous microzooplankton, this is only achieved when the whole diet consists of fat-rich food (Nelson *et al.*, 2000). In contrast, proteins in *M. leidy* represent a major organic fraction in their biomass (especially in starvation)



**Fig. 5:** Frequency distribution of NSA for the zooplankton bloom phenomenon (dark blue line) and red tide (red line) in the NAd as well as the surface water layer 0–2 m (light blue dashed line) and chemocline (orange dashed line) of the stratified marine lake (Rogoznica Lake, CAd), for all samples studied and presented in the Fig. 2, Table 2, and in Supplementary Material, Table S1–S2.

and are ~ 30% higher than lipid or carbohydrate content (Yousefian & Erkan Kideys, 2003). However, the mechanisms of DOC release in *N. scintillans* and *M. leidy* are comparable. Most of the available DOC in both cases is released in the terminal phase of the bloom when the individuals start to die and decay (Treible *et al.*, 2014; Zhang *et al.*, 2015), and the same works for the release of nutrients (N and P; McNamara *et al.*, 2013; Zhang *et al.*, 2017).

A minor part of the DOC is released by sloppy feeding and egestion. Moreover, both *Noctiluca* (Zhang *et al.*, 2017) and *M. leidy* (Leonardi, 2020) excrete sticky mucus to trap food, while the surface epithelium of *M. leidy* is also covered in mucus (Hammann, 2012). All that material was reported to be readily taken up by heterotrophic marine bacteria (Paliaga *et al.*, 2021; Zhang *et al.*, 2017), indicating a pool of labile OM. On the other hand, when we consider the whole bloom phenomena, for some dinoflagellates, a shift in labile OM was noted only early in the blooming phase and became more refractory toward the final stage (Park *et al.*, 2022), whereas for *M. leidy*, the final stage is only reported to add more labile material (Budiša *et al.*, 2022; Condon *et al.*, 2010).

### OM properties of RL water column

The NSA frequency distribution of the samples from the chemocline of RL was in the lowest range (Fig. 5), corresponding to the NSA that lies between the Xanthan/Humic acid and  $\kappa$ -Carrageenan polysaccharide OM types (Figs 2B, 3Af; Tables 2, 3), making this OM a slightly more hydrophilic and less reactive compared to the OM in the surface water layer (0–2 m) of RL. The chemocline zone is the area with the highest microbiological activities of phototrophic sulfur bacteria (Pjevac *et al.*, 2015; Čanković *et al.*, 2019). The interaction of OM and microbiologically produced sulfide in the chemocline zone leads to the formation of less surface-active organosulfur species such as sulfopolysaccharides (Ciglencečki *et al.*, 2003) and polysulfide-linked macromolecules (Bura-Nakić *et al.*, 2009), while the primary production of labile DOC containing carbohydrates, amino acids, and amino sugars, are the main processes in the surface water layers (Nguyen *et al.*, 2005).

The chemocline layer is enriched with POC (Simonović *et al.*, 2023). Higher POC can also be found during the water column mixing in the post-holomictic period (Marguš *et al.*, 2015; Simonović *et al.*, 2023) when the lake is enriched with regenerated suspended nutrients, which are the main trigger for new phytoplankton blooms (Ciglencečki *et al.*, 2015). This situation triggered a bloom of *Picochlorum* sp. in October 2020 as it is known to be a high biomass producer efficient in nutrient removal, especially nitrates and phosphates (Couboulès *et al.*, 2021; Goswami *et al.*, 2021).

TEPs, which play an important role in marine particle aggregation and floc formation (Passow, 2002), are enriched in adhesive, surface-active sulfate polysaccharides (Zhou *et al.*, 1998). Preliminary results on TEPs

in RL show that TEPs mainly follow the distribution of POC within the oxic water column (Fig. 4). However, the TEP–SAS relationship may be proportional or inversely proportional, likely due to a combination of physical and biological factors (wind-induced turbulence, salinity, production of dissolved polysaccharide precursors by phytoplankton and bacteria; Jennings *et al.*, 2017), which requires further investigation.

### OM properties in gelatinous macroaggregates of the coastal waters of the central Adriatic

The mucilaginous macroaggregates are considered the product of DOM agglomeration into macrogels and POM, which are mainly associated with diatom blooms (Kovač *et al.*, 2004). Diatoms were identified as the major phytoplankton community taxa in the studied CAD gelatinous samples (Z. Ljubešić, pers. comm.), similar to earlier reports for mucilage accumulations in the NAd in the late 1990s and early 2000s (Najdek *et al.*, 2002; Precali *et al.*, 2005), and eastern Mediterranean (Scoullou *et al.*, 2006). The similarity with NAd mucilage events is also evident from the voltammetric curves showing a desorption peak at negative electrode potentials (about -1.7 V vs. Ag|AgCl; Ciglencečki *et al.*, 2003), which was attributed to the presence of polysaccharides ( $\kappa$ -Carrageenan; Fig. 3; Table 3). Water-soluble heteropolysaccharides as the main components of the mucus aggregates formed by diatom activities (Degobbi *et al.*, 1995; Kovač *et al.*, 2002) were also confirmed by other independent analyses (Kovač *et al.*, 2002). Carbohydrates accounted for up to 33% of the DOC in the mucilaginous material in the Aegean Sea (Scoullou *et al.*, 2006), and the NSA of these samples lies around pollen and Xanthan in relation to Figure 2 in this paper.

OM properties, including voltammetric curves of RL samples enriched in TEPs (April to May 2022; Fig. 4), most likely indicate conditions of intense primary production of colloidal, highly surface-active, and labile OM (Nguyen *et al.*, 2005; Tranvik, 1994). The labile biodegradable material likely includes carbohydrates, amino acids, and amino sugars (Nguyen *et al.*, 2005), while the ratio of more hydrophobic proteins to more hydrophilic carbohydrates (P/C ratio) may change depending on biological conditions (Santschi *et al.*, 2020). Processes and properties that influence the P/C ratio in the marine environment include hydrophobicity, surface activity, surface tension, light-induced chemical cross-linking, and the sedimentation efficiency of marine snow (Santschi *et al.*, 2020). According to our results, the NSA parameter, which reflects the properties and reactivity of OM, appears to be sensitive to these changing environmental conditions.

Polysaccharides and mucopolysaccharides, as the main exudates of algae and bacteria, are highly adhesive by nature (Alldredge *et al.*, 1993; Passow *et al.*, 1994; Percival & McDowell, 1981). Mucopolysaccharides are preferentially produced toward the end of the algal bloom, when particle aggregation is pronounced, and are

a response to environmental stress (Mopper *et al.*, 1995). Released exopolymer material has a high molecular weight ( $> 5000\text{Da}$ ) and significantly increases the viscosity of the medium, which promotes particle aggregation (Jenkinson, 1986) and the conversion of DOC to POC as well as TEP formation (Mopper *et al.*, 1995). The appearance of the mucilaginous material in the Aegean Sea is reported to start from the sea bottom initially in the form of whitish small flocs becoming progressively darker during their vertical transport toward the sea surface, leading to a consequent increase of POC (Scoullou *et al.*, 2006).

### NSA as an OM fingerprint

Voltammetric measurements of initial seawater and in acidic conditions ( $\text{pH} = 2\text{--}3$ ; Tables 2, 3) indicate the presence of more negatively charged polymeric SAS, which transform from more to less anionic (negatively charged) and/or almost neutral polymeric SAS due to protonation under acidic conditions (Vojvodić & Čosović, 1996). These measurements can additionally help in the characterization of OM type. For example, different stages of growth of the Adriatic diatom *Cylindrotheca closterium*, upon addition of acid during measurements, indicate the transformation of SAS from more anionic (negatively charged) in the exponential phase to almost neutral and less anionic OM in the stationary phase of growth (Ciglenečki *et al.*, 2018).

The strongest increase in SAS under acidic conditions is characteristic of fulvic (Vojvodić *et al.*, 1994) and the protein OM type, as demonstrated here for BSA (Table 3). This effect can be explained by conformational changes in the polyelectrolytic properties of OM caused by the protonation of the negatively charged carboxyl groups (Buffle & Chalmers, 1988). There was no effect of pH on the SAS properties of Triton-X-100 and Dextran-T-500 (Vojvodić *et al.*, 1994) and for Dextran 2000000, Xanthan, pollen, and  $\kappa$ -Carrageenan (Table 3). Based on all voltammetric measurements at the hydrophobic Hg electrode in the model and natural samples from this study, we propose a rough limit for the threshold value of OM hydrophobicity-reactivity, which is an NSA value of 0.083. The proposed threshold is calculated by ordering all data points of all surface samples discussed in this paper (Table 2; Supplementary Material Table S2; Fig. 5), including the data published for RL in Simonović *et al.* (2023). When considering the NSA data of the RL chemocline layer, the threshold decreases to 0.068, as the chemocline layer is characterized by the retention and degradation of OM, including a dense population of phototrophic sulfur bacteria (Čanković *et al.*, 2019; Pjevac *et al.*, 2015; Simonović *et al.*, 2023) which produce OM with similar characteristic to model substances of microbiologically produced Xanthan and the sulfated model polysaccharide  $\kappa$ -Carrageenan (Fig. 3Bd; Table 3). Xanthan could be considered an indicator of certain microbiological activities (Ciglenečki *et al.*, 2023).

OM with a value above this threshold of 0.083 can be considered more hydrophobic (reactive), while OM with

values below this threshold is more hydrophobic and less reactive.

### Conclusions

The findings of this research significantly contribute to previous investigations by highlighting that the NSA (SAS/DOC ratio) can serve as a rough indicator for assessing the quality of DOC (TOC) within a marine ecosystem during diverse physico-chemical and biogeochemical processes. This insight aids in deciphering the occurrence of ecological changes within the marine environment. The analysis of voltammetric curves, which exhibit distinct desorption peaks, may signal the presence of specific types of OM, serving as tracers for particular processes.

The examined samples, including massive invasive ctenophore blooms, red tide formations, gelatinous macroaggregates, and autotrophic activities in a coastal marine system of RL, were marked by intense single-species plankton blooms followed by macroaggregate formation, leading to a significant increase in OM levels (DOC/POC up to  $320\text{ mg L}^{-1}$  and SAS up to  $15\text{ mg L}^{-1}$ ). Similar conditions were previously observed in the open and coastal waters of the NAd (Ciglenečki *et al.*, 2003) and the eastern Mediterranean (Scoullou *et al.*, 2006) in the late 1990s. However, the discovery of gelatinous macroaggregates with high POC content ( $326.6\text{ mg L}^{-1}$ ) and SAS ( $0.589\text{ mg L}^{-1}$ ) in the coastal waters of the central Adriatic in summer 2021 represents a novel observation.

The NSA parameters ranged between 0.04 and 0.25, with the highest values recorded for GZ blooms. A more hydrophilic and less reactive type of OM, resembling polysaccharide material primarily of Xanthan and  $\kappa$ -Carrageenan type, was identified for red tide and phototrophic activities of sulfur bacteria.

Based on the comprehensive voltammetric measurements conducted on the hydrophobic mercury electrode across the model and natural samples in this study, we propose a tentative threshold value for the OM hydrophobicity-reactivity, set at an NSA value of 0.083. OM with values exceeding this threshold of 0.083 can be categorized as more hydrophobic and reactive, whereas OM with values below this threshold are deemed more hydrophilic and less reactive.

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## Supplementary Material

The following supplementary material is available for this article:

**Fig. S1:** Calibration plot (linear scale) of  $\Delta i$  versus Triton-X-100 in 0.55 M NaCl. ( $\Delta i$  - adsorption effect measured as the difference between capacity current for base electrolyte and aqueous solutions of surfactant (A)).

**Fig. S2:** A.c. voltammograms of the water column sample of Rogoznica Lake sampled at 5 m depth, where intense biological activity was recorded. Voltammetric curves revealed the typical desorption wave at around -1.6 V vs. Ag/AgCl in the filtered fractions smaller than 0.45 and 0.22  $\mu\text{m}$ . In a fraction smaller than 0.22  $\mu\text{m}$ , due to the presence of colloidal, highly surface-active OM, the desorption wave was even more expressed and for about 50 mV more negatively positioned. Experimental conditions: accumulation time,  $t^a = 120$  s at -0.6 V vs. Ag/AgCl.

**Fig. S3:** Physico-chemical properties of the Rogoznica Lake water column on October 20, 2020 (A) and June 28, 2021 (B). NSA parameter (SAS/DOC ratio) was determined up to the chemocline layer, considering that SAS cannot be measured in an anoxic sample due to reduced sulphur species interference (Simonović *et al.*, 2023).

**Table S1.** Organic carbon properties (DOC, SAS, NSA) and plankton abundance in surface samples during: the red tide

caused by dinoflagellate *Noctiluca scintillans* (A) and zooplankton bloom of invasive ctenophore *Mnemiopsis leidyi* (B). **Table S2.** Organic carbon properties (DOC, SAS, NSA) of RL (from 2020 to 2022) surface oxic layer (A), chemocline (B) and samples with maximal oxygen saturation (C). Display C also represent samples where specific desorption peak (represented in Fig. 3Ae) was measured by a.c. voltammetry method.