Organic matter degradation characteristics of coastal marine sediments collected from the Seto Inland Sea, Japan

Asaoka, Satoshi ; Jadoon, Waqar Azeem ; Umehara, Akira ; Takeda, Kazuhiko ; Otani, Sosuke ; Ohno, Masaki ; Fujitake, Nobuhide ;…

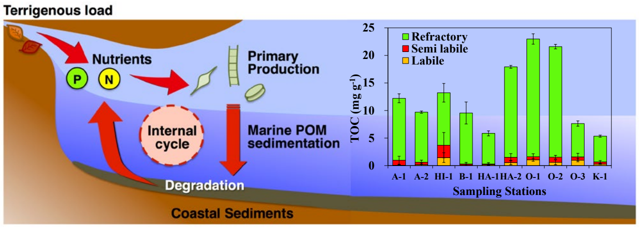
(Citation)
Marine Chemistry,225:103854

(Issue Date)
2020-09-10

(Resource Type)
journal article

(Version)
Accepted Manuscript

Abstract Art



Highlights

The degradability of coastal sediments from the Seto Inland Sea was investigated.

Percent composition of refractory organic matter in the sediments was 72–97% of TOC.

Approximately 93% of refractory organic matter was categorized into humin.

The source of refractory organic matter derives from dead phytoplankton cells.

Organic Matter Degradation Characteristics of Coastal Marine Sediments Collected from the Seto Inland Sea, Japan

Satoshi ASAOKA[a]*, Waqar Azeem JADOON[b], Akira UMEHARA[c],
Kazuhiko TAKEDA[d], Sosuke OTANI[e], Masaki OHNO[f] , Nobuhide FUJITAKE[g],
Hiroshi SAKUGAWA[d], Hideo OKAMURA[a]

a Research Center for Inland Seas, Kobe University
5-1-1 Fukaeminami, Higashinada, Kobe, 658-0022 JAPAN
b Department of Environmental Sciences, Faculty of Sciences, Hazera University.
Mansehra, Khyber, Pakhtunkhwa, PAKISTAN
c Environmental Research and Management Center, Hiroshima University
1-5-3, Kagamiyama, Higashihiroshima, Hiroshima, 739-8513 JAPAN
d Graduate School of Integrated Sciences for Life, Hiroshima University
1-7-1 Kagamiyama, Higashi-Hiroshima, 739-8521 JAPAN
e Osaka Prefecture University College of Technology
26-12 Saiwaicho, Neyagawa, Osaka, 572-8572 JAPAN
f Niigata University of Pharmacy and Applied Life Sciences

265-1 Higashijima, Akiha, Niigata, 956-8603 JAPAN

g Graduate School of Agricultural Science, Kobe University

1-1 Rokkodai-cho, Nada, Kobe, 657-8501 JAPAN

*Corresponding author:

Tel & Fax: +81-78-431-6357, E-mail: s-asaoka@maritime.kobe-u.ac.jp

Address: Research Center for Inland Seas, Kobe University, 5-1-1 Fukaeminami, Higashinada, Kobe, 658-0022 JAPAN

Abstract

Organic matter in marine sediment is mainly categorized into three fractions depending on degradability: labile, semi labile and refractory. The degradability of coastal marine sediments depends on the properties of the organic matter contained in the sediments. The purpose of this study was to quantify labile and refractory organic matter in coastal marine sediments with different characteristics collected from the Seto Inland Sea, and to discover the factors controlling the degradability of organic matter. Refractory organic matter content in sediments ranged from 4.7–21.4 mg g^{-1}, a percent composition equivalent to 72–97% of TOC. In contrast, labile and semi-labile content were 0.1–1.4 mg g^{-1} and 0.1–2.3 mg g^{-1}, respectively, a percent composition equivalent to 0.5–12.9% and 1.8–17.1% of TOC, respectively. Approximately 93% of refractory organic matter was categorized into humin. Organic matter originated from marine phytoplankton settled on the surface of sediments and changed to humin or a refractory organic matter. This study contributes to a better understanding of the organic matter degradation characteristics of coastal marine sediments and provides important parameters for estimating carbon budget and carbon cycling in coastal sea systems.

Key words

biodegradation, fulvic acid, humic acid, refractory organic matter

1. Introduction

Organic matter in marine sediment is mainly categorized into three fractions depending on degradability: labile, semi labile and refractory. Sediments settled in enclosed water bodies are affected by significant terrigenous organic matter loads, and their oxidative decomposition consumes dissolved oxygen in the water column. The degradation of labile organic matter is chiefly mediated by aerobic and anaerobic microbial processes in the dynamic interface, with a concurrent release of inorganic nutrients (Canfield, et al., 1993; Kristensen and Holmer, 2001). In addition, sulfate-reducing bacteria utilize sulfate as a terminal electron accepter for the degradation of organic matter, and generate toxic hydrogen sulfide under anoxic conditions (Muyzer and Stams, 2008; Hargrave, et al., 2008). Therefore, the decomposition of labile organic matter contributes to the formation of hypoxia and accelerates eutrophication, especially in enclosed water bodies, resulting in a decrease in benthic macro fauna diversity (Hargrave, et al., 2008).

Persistent organic pollutants (POPs) that are ubiquitous contaminants with a negative impact on top predator species (Kelly and Gobas, 2001; Voutsas, et al., 2002; Fisk, et al, 2001). Therefore, on a local scale, it is very important to understand the degradation characteristics of organic matter in coastal marine sediment in order to estimate hypoxia and establish environmental remediation strategies. On a global scale, because carbon cycling in coastal sea systems is one of the major components of global carbon cycles and budgets (Cole, et al, 2007; Bauer, et al., 2013; Middelburg, et al., 1999), it is necessary to understand the degradation characteristics of organic matter in coastal marine sediment so as to accurately estimate global carbon cycles. Organic matter degradation experiments have been eagerly pursued in recent years (Kristensen, 1995; Kristensen and Holmer, 2001; Martinez-Garcia, et al., 2015; Arndt, et al., 2013). However, the degradability of the organic matter is not an inherent or absolute property of the organic matter itself (Arndt, et al., 2013). Therefore, it is necessary to characterize the degradability of organic matters using sediments with different properties to discover the factors that control degradability of organic matter in coastal marine sediments.

The purpose of this study is to quantify labile and refractory organic matter in coastal marine sediments with different characteristics from the Seto Inland Sea. This is the largest semi-enclosed sea in Japan, with an eutrophic area affected by terrigenous loads facing one of the most industrialized and populated areas, and an oligotrophic area and facing a less populated area. Few studies have sought to measure the fraction of labile and refractory organic matter in coastal marine sediment collected from different parts of the Inland Sea under unified decomposition experiment conditions. This study will therefore contribute to a better understanding of the organic matter degradation characteristics of coastal marine sediments, and provide important parameters to estimate carbon budget and carbon cycling in coastal sea systems.

2. Experimental

2.1 Study site

Sediment samples were collected from ten stations in the Inland Sea, July 4–8th or November 14–17th 2016 and July 3–7th 2017 by the training and research vessel, Toyoshio Maru, of Hiroshima University (**Fig. 1)**. During the July 3–7th 2017 collection, particulate organic matter (POM) samples were also gathered. **Table S1** presents the coordinates of the sampling sites. The Inland Sea is a semi-enclosed body of water, about 450 km from east to west, and 15–55 km from north to south, with an average depth of 38 m. The sea is surrounded by Honshu Island, Shikoku Island and Kyushu Island, and contains more than 700 islands. The sea has a total area of 23,203 km^2 and the catchment is one of the most industrialized and populated areas (watershed population: 30 millions) in Japan.

2.2 Sampling procedures

Vertical profiles of temperature and salinity were measured using a CTD

(SBE9/11plus; Sea-Bird Scientific). Sediment core samples were collected using an undisturbed core sampler (ø11 cm, 50-cm long: HR type; Rigo) except at Sts. B-1 and HI-1 in 2016. A Smith-Mcintyre bottom sampler (5144-BS; Rigo) was used to collect surface sediments at those two stations. The collected cores were cut every 5 cm onboard. The surface sediments collected by the Smith-Mcintyre bottom sampler were taken from the top layer (5 cm). Collected sediments were immediately transferred into polypropylene container for decomposition experiments or stainless containers for extracting humic acid, fulvic acid and humin, respectively, and stored in a refrigerator at 4 ºC for transfer to the laboratory. The polypropylene and stainless containers were pre-cleaned by a detergent (Clean Ace; As one) and methanol, respectively. Finally, these containers were rinsed by ultra-pure water prior to use.

Particulate organic matter (POM) in surface seawater (0–2 m depth) for carbon and nitrogen isotope analyses was collected with pre-combusted (600 ℃, 2 h) glass-fiber filters (GF/F; Whatman), with filtering of 1.5–2.0 L of surface seawater.

2.3 Sample treatments and analyses

Extraction of humic acid, fulvic acid and humin from marine sediments was conducted using the IHSS method, with minor modifications (Kuwatsuka et al., 1992). Freeze-dried sediments (100-mg carbon) were passed through a 2-mm mesh sieve, placed in a 50-mL centrifugation tube, and acidified to pH 1-2 by the addition of 30-mL 1 mol L^{-1} HCl, and agitated for 1 h at a constant temperature of 25 °C in an oven equipped with a multi shaker (MMC-1000, EYELA). The suspended solution was then centrifuged at 10,000 rpm for 15 min. The supernatant was designated as 'FA-1'. The sediment residue was neutralized by successive addition of 5-mL 1 mol L^{-1} NaOH, and 25-mL 0.1 mol L^{-1} NaOH. The suspension was agitated for 1 day under a N_2 gas atmosphere and the extract was separated by centrifugation at 10,000 rpm for 15 min; the residue was designated as 'Humin'. Meanwhile, 20 mL of the supernatant was transferred to another centrifugation tube and acidified to pH 1.0 by the addition of 2-mL 3 mol L^{-1} HCl, allowed to stand for 12-16 h, and then centrifuged at 10,000 rpm for 15 min. The supernatant solution was designated as 'FA-2'. A 10-mL sample of 1 mol L^{-1} NaOH solution was added to the precipitated HA fraction, and the HA fraction was dissolved in 30 mL of 0.1 L^{-1} NaOH solution and centrifuged at 15,000 rpm for 15 min. Total organic carbon (TOC) concentration in the HA fraction FA fraction (sum of FA-1 and FA-2) was measured by TOC analyzer (Sievers InnovOx, General Electric Company). For TOC analyses of HA fractions, fractions were diluted with 1/15 mol L^{-1} KH_2PO_4 solution and 0.1 mol L^{-1} NaOH solution to keep solution pH basic. The FA fraction was diluted by 5 to 10 times with ultrapure water before TOC analyses. TOC of humin fraction was measured using an elemental CN analyzer (JM1000CN, J-Science Lab).

Carbon and nitrogen content and isotopes ($\delta^{13}C$ and $\delta^{15}N$) for POM in seawater and sediments were measured using the following procedure. These samples were treated with HCl vapor to remove inorganic carbonates (Yamamuro and Kayanne, 1995), dried in a vacuum, and desiccated over silica gel and NaOH to neutralize for 7 days. Carbon and nitrogen content, and $\delta^{13}C$ and $\delta^{15}N$ of samples, were analyzed by using an elemental analyzer (Flash Elemental Analyzer 1112 Series, Thermo Electron) and continuous flow isotope ratio mass spectrometer (Delta Plus, Thermo Electron). The isotope ratios are expressed in delta notation (‰) in Equation 1.

$$\delta\ {}^{13}\mathrm{C}\ \text{or}\ \delta\ {}^{15}\mathrm{N}\ (‰) = \left({R_{sample}}/{R_{standard}} - 1 \right) \cdot 10^3 \cdot \cdot \cdot (1)$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. Pee Dee Belemnite (PDB) and air N_2 were used as references for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standard deviation of the $\delta^{13}C$ and $\delta^{15}N$ values of the standards were 0.20‰ and 0.26‰, respectively.

2.4 Decomposition experiments

Sediment decomposition experiments were carried out using a polypropylene (PP) bottle (approximately 44-cm^2 surface area, 500-mL volume) in triplicate. The bottle lid had two apertures, connecting the polyethylene (PE) air pipe with an air stone at the tip of the pipe to supply air and attached to a 0.2-μm pore size nylon membrane filter (Millex; Merck) for gas exchange.

For the experiment, 200 mL of homogenized wet sediment was placed in the PP bottle, and 29 psu of 200-mL artificial seawater (MARINE ART SF-1; Osakayakken) was dispensed into the bottle. The depth of the air stone was adjusted to the boundary between sediments and overlying water, and air was supplied at a rate of 0.2 L min^{-1} with an air pump. The sediments were incubated at 25 ^{o}C in a constant temperature oven for 90 days under a dark condition. Twenty mL of overlying water and the surface layer of the sediments (0-0.5 cm depth) were collected at days 5 and 90. If the volume of overlying water decreased due to evaporation and sampling, artificial seawater was added to the bottle.

Collected overlying water was filtered through a 0.45-μm pore nylon syringe filter (Millex; Merck) and the concentration of dissolved organic carbon was measured with a TOC analyzer (Sievers InnovOx, General Electric Company) and Emission-Excitation Matrix (EEM) using a fluorescence spectrophotometer (FR-6200; JASCO). EEM fluorescence spectra were obtained by collecting emission scans (λEm 300-550 nm, 1-nm intervals) at 5-nm excitation wavelength intervals between λEx 240 and 450 nm. UV–Vis absorbance spectra were collected prior to measuring EEM fluorescence spectra using a double-beam spectrophotometer (UV-2600; Shimadzu) in the wavelength range of 240–550 nm. Primary and secondary inner filter effects, Raman scattering, and Rayleigh-Tyndall scattering of obtained EEM fluorescence spectra were corrected (Larsson, et al., 2007). The EEM was characterized by parallel factor analysis (Stedmon et al., 2003; Stedmon and Bro, 2008). The humification index (HIX) was calculated using Equation 2 (Ohno, 2002).

$$\mathrm{HIX} = \frac{\sum I_{435-480}}{\sum I_{300-345} + \sum I_{435-480}} \quad \cdot\ \cdot\ \cdot (2)$$

where *I* is the fluorescence intensity at each wavelength.

PARAFAC analysis was carried out to identify fluorescent components obtained by EEM. DOM Fluor v.1.7 Toolbox (University of Copenhagen) for MATLAB (R2013a, MathWorks, lnc., MA, US) was applied to fit the PARAFAC model over a data set comprising all overlying water samples obtained by the sediment decomposition experiments. The components were verified by using random initialization and split half analysis (Stedmon et al., 2008).

3. Results and discussion

3.1 Characteristics of sediments

The characteristics of sediments analyzed in this study are shown in **Table 1**. TOC in sediments ranged from 5.3–22.9 mg g^{-1}, and concentration at Sts. O-1 and O-2 (22.9 and 21.6 mg g^{-1}, respectively) was high compared to other stations. This was because Sts. O-1 and O-2 are located at the innermost area of Osaka Bay, adjacent to big metropolitan and industrial areas (catchment area: 11,200 km^2, population 17 million), and are significantly affected by terrigenous loads from the Yodo River (catchment area: 8,240 km^2, yearly averaged discharge: 267.51 m^3 s^{-1}). The TOC at Sts. HI-1 and HA-2 were also high (13.2 and 17.9 mg g^{-1}, respectively), which was attributed to the stations being located at the center of Hiuchi-Nada or Harima-Nada, which has one of the highest sedimentation fluxes of the observed areas (Hoshika, et al., 1989; Hoshika, et al., 1983). The mud content of these stations therefore exceeded 96%. TOC at St.A-1 was 12.2 mg g^{-1}, because this station is located in Kure Bay, near the industrial zone, and is affected by industrial wastewater. In contrast, TOC at Sts K-1 and HA-1 was low (5.4 and 5.3 mg g^{-1}, respectively) compared to other stations, because these stations are located on the border of the Kii Channel and Akahi Strait, respectively, where the high flow rate prevents sediment from accumulating. The water content of the sediments collected from these stations was also low (38.1-44.1%) compared to other stations (52.3-75.0%).

Carbon and nitrogen isotopes corresponding to the δ^{13}C and δ^{15}N of the sediment before degradation ranged from -21.41 to -20.30‰ and 5.28 to 8.79‰, respectively (**Table S2**). The average isotope ratios (δ^{13}C and δ^{15}N) for marine POM collected from each station were -23.0 to -17.4‰ and 5.59 to 10.0‰, respectively. The isotope ratios of δ^{13}C and δ^{15}N of the sediment accorded well with those of marine POM. The ratio of carbon to nitrogen in the sediments was 6.4–8.3, which also fell within the range of marine phytoplankton (Burkhardt, et al., 1990). Hence, the organic matter in sediments collected in this study originated from marine POM.

3.2 Composition of organic matter in the sediments characterized by degradability

We divided the organic matter defined for degradability here into 3 fractions: labile, semi labile, and refractory; these decomposed within 5 days, between 5–90 days, and remained for 90 days, respectively (**Fig. 2**). Most of the organic matter in the marine sediment was refractory, with content ranging from 4.7–21.4 mg g^{-1}, a percent composition equivalent to 72–97% of TOC. In particular, the sediment from the coastal area of Osaka Bay (Sts. O-1 and O-2) affected by significant terrigenous load from the

Yodo River was rich in refractory organic matter. In contrast, labile and semi labile were minor components, with contents ranging from 0.1–1.4 mg g^{-1} and 0.1–2.3 mg g^{-1}, respectively, a percent composition equivalent to 0.5–12.9% and 1.8–17.1% of TOC, respectively.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio changes were associated with organic matter degradation in the sediments (**Table S2**). The $\delta^{13}C$ of sediments other than at St. A-1 and St. B-1 decreased from -0.43 to -0.95‰ after 5 days (Fisher's least significant difference method; $p<0.01$). This was due to the loss of isotopically heavy components such as amino acids and sugars through biodegradation, so that the remaining POC was enriched in the isotopically light lipid fraction (Eadie and Jeffrey, 1973; Jeffrey, et al., 1983). However, the decrease of $\delta^{13}C$ of the sediments was small, within range of same source. The $\delta^{13}C$ of sediments between 5 days and 90 days did not show statistical difference except St. HA-1.

The $\delta^{15}N$ of the sediments at Sts. A-1, O-3, and HA-2 was significantly decreased at 5 days ($p<0.01$–0.05). In contrast, in sediments at Sts. A-2, K-1, O-1, O-2, and HA-1, $\delta^{15}N$ decreased at 5 days, but began to increase after 5 days ($p<0.01$-0.05). The decrease of $\delta^{15}N$ within 5 days might have been caused by the degradation of organic nitrogen to inorganic nitrogen (Brandes and Devol, 1997) meanwhile, the increase after 5 days was attributed to denitrification (Mariotti et al., 1981).

We also investigated humic acid, fulvic acid, and humin concentration and composition, both initially and after 90 days (**Fig. 3**). Initially, humic and fulvic acid and humin concentrations in the sediments ranged from 0.05–0.56 mg g^{-1}, 0.56–3.3 mg g^{-1}, and 3.5–20.6 mg g^{-1}, respectively; their percent composition was 0.4–2.6%, 6.7–16.6% and 81.6–92.9%, respectively. After 90 days, humic and fulvic acid and humin concentrations were 0.08–0.52 mg g^{-1}, 0.63–2.4 mg g^{-1} and 3.7–19.5 mg g^{-1}, respectively; their percent composition was 1.0–2.9%, 8.5–19.7% and 78.1–89.7%, respectively.

Fulvic acid concentrations at Sts. A-1, K-1, HA-2 and B-1 were up 0.1–0.63 mg g^{-1} (statistically significant: $p<0.01$–0.05) after the 90-day incubation. On the other hand, fulvic acid concentrations at Sts. O-3, HA-1 and O-2 decreased 0.33–1.4 mg g^{-1} ($p<0.01$). Humic acid concentrations initially and after 90 days' incubation changed in the range of -0.18–0.23 mg g^{-1}, a relatively small difference compared to those of fulvic acid and humin. In the case of humin, concentration at Sts. A-1, HA-2 and HI-1 decreased from 1.8 to 4.2 mg g^{-1} ($p<0.01$ to 0.05). Therefore, 1.8–4.2 mg g^{-1} of humin, including coarse particulate organic matter (CPOM), was mainly biodegraded into fulvic acid after 90 days' incubation, which corresponded to fulvic acid concentration increases of 0.23–0.63 mg g^{-1}. The rest was biodegraded into DOC and CO_2 at Sts. A-1, HA-2 and HI-1. At Sts. O-3, HA-1 and O-2, fulvic acid was biodegraded into DOC and CO_2.

A strong positive correlation was observed in TOC concentration between refractory fraction and the sum of humic and fulvic acid and humin after 90 days, with a correlation coefficient of 0.991 (**Fig. S1**). The slope of the linear regression was 1.08, i.e., close to 1, indicating that almost all refractory organic matter was composed of humic acid, fulvic acid and humin. In particular, the slope of the linear regression in TOC concentration between refractory organic matter and humin was 0.93, with a correlation coefficient of 0.988 (**Fig. S1**). Hence, approximately 93% of refractory organic matter was categorized as humin.

As already mentioned, the main source of organic matter in the sediments was marine POM. We calculated the 5-year (2010–2014) average chlorophyll concentration in surface seawater near each sampling station in summer from the seasonal monitoring data of the Ministry of Environment and the Ministry of Land, Infrastructure, Transport and Tourism (Nishijima, et al, 2016). The concentration of refractory organic matter increased with increasing chlorophyll a concentration in surface seawater at the higher chlorophyll concentrations, indicating that the sedimentary organic material was derived for planktonic sources at high biogenic loadings (**Fig. 4**). Therefore, in the case of high biogenic loadings, it was believed that the organic matter mainly originated from marine phytoplankton changed to humin, or refractory organic matter. In contrast, the refractory organic matter ranged from 4.7 to 16.5 mg g^{-1} at lower chlorophyll a concentrations (0.3 to 3.5 μg L^{-1}). This means the contribution of terrigenous loads may become relatively more important at low biogenic loadings.

3.3 Changes in the overlying water during sediment decomposition experiments

The concentration of DOC in overlying water was significantly increased at 5 days ($p<0.01$; **Fig. S2**). The increase of DOC was small compared to that of organic carbon derived from the decomposition of labile organic matter in sediments for 5days. One of the reasons for this was considered that DOC in overlying water was further decomposed to inorganic carbon. Other possibilities were a slow dark release of organic material from the sediments and exuding the cellular materials from dead cell of organisms during these 5 days. The concentration of DOC in overlying water after 90 days at Sts. A-1, A-2, HI-1, HA-2 and O-3 was decreased ($p<0.01$-0.05). The decrease of DOC is considered due to the decomposition of DOC to inorganic carbon, biological utilization and conversion back to particulate matter. In the case of Sts. B-1, HA-1, O-1, O-2 and K-1, DOC concentration of overlying water did not change significantly.

PARAFAC analyses were performed to identify fluorescent components obtained by

EEM. Characteristics of fluorescent components identified and the EEM of overlying water at both 5 and 90 days are shown in **Table S3 and Fig. S3**, respectively. Components 1, 2, 3 and 5 represent UV humic acid (Stedmon, et al., 2003; Coble, et al., 1998); components 4 and 6 are derived from protein contained in Tyrosine and Tryptophan, respectively (Yamashita and Tanoue, 2003). The initial artificial seawater did not show any peaks. All fluorescence intensities of UV humic acid peaks at 90 days were higher than those at 5 days (**Fig. 5**). SUVA (UV absorbance at 254 nm divided by DOC concentration) was a good surrogate of the aromatic carbon content of natural organic matter (Lu, et al., 2009). The SUVA of overlying water at each station ranged from 0.75–1.6 after 5 days, and from 1.7–2.7 after 90 days (**Fig. S4**). That SUVA were higher after 90 days indicated that the aromatic carbon content in DOC increased. This increasing SUVA was in good accord with the UV humic acid (**Fig. 5**) and humification index (**Fig. S5**) increases at 90 days. What can be assumed here is the proceeding of humification of DOC and the dissolution of humic organic matter in sediments.

Fluorescence intensity of protein between 5 and 90 days differed depending on the sample (**Fig. 5**). In the case of Component 4, fluorescence intensity of Sts. A-1 and O-1 at 90 days was 20% that of 5 days. In contrast, the intensity of Sts. B-1 and HI-1 at 90 days increased by 80-90% of the 5-day level. In the case of Component 6, fluorescence intensity of St. B-1 at 90 days was more than double that at 5 days. In contrast, the intensity of St. O-1 at 90 days increased by only a half that of 5 days.

DOC concentration of overlying water was weakly correlated with fluorescence intensity of UV humic acid (correlation efficient; r= 0.455-0.760) at 5 days. The correlation between DOC concentration and UV humic acid became strong (r=0.683-0.807) at 90 days. In the case of protein, although there was no correlation between DOC concentration and protein (r=0.243-0.288) at 5 days, a negative correlation was observed between DOC concentration and Component 4 from protein at 90 days (r=0.639). **Fig. 6** shows the correlation between DOC concentration and UV humic acid (Component 5) or protein (Component 4) at 90 days. When St. HA-2 was eliminated from UV humic acid and Sts. A-1 and O-1from protein, the correlation coefficients were improved to 0.876 and 0.867, respectively. DOC concentration increased with increasing UV humic acid and decreasing protein, indicating that the DOC at 90 days originated from humin production such as CPOM from protein. Because Sts. A-1, O-1 and HA-2 were out of the linear regressions, protein decomposition at Sts. A-1, O-1, and CPOM production at St. HA-2 might have been higher compared to other stations.

4. Conclusion

Refractory organic matter content in sediments collected from the Seto Inland Sea ranged from 4.7–21.4 mg g^{-1}, a percent composition equivalent to 72–97% of TOC. In contrast, labile and semi-labile content were 0.1–1.4 mg g^{-1} and 0.1–2.3 mg g^{-1}, respectively, a percent composition equivalent to 0.5–12.9% and 1.8–17.1% of TOC, respectively. Approximately 93% of refractory organic matter was categorized into humin. It is generally believed that refractory organic matter in coastal marine sediments derives from terrigenous load. However, this study revealed that refractory organic matter in coastal marine sediments originated from marine particulate organic matter, phytoplankton, rather than terrigeneous organic matter at high biogenic loadings. The source of refractory organic matter might derive from the transformation of dead phytoplankton cells into humin.

Acknowledgements

This study was supported by the Japan Society for the Promotion of Science JSPS KAKENHI, Grant-in-Aid for Young Scientists A, Grant number 16H05892. The authors would like to thank Dr. Hiroaki Tsutsumi and Tomohiro Komorita, Prefectural University of Kumamoto, for providing the facility for isotope analysis. We are grateful to the captain and crew of the research & training vessel, Toyoshio-Maru, Hiroshima University, for

their help with sampling.

References

Arndt, S., Jørgensen, B. B., LaRowed, D. E., Middelburg, J. J., Pancost, R. D., Regnier, P., 2013. Quantifying the degradation of organic matter in marine sediments: A review and synthesis. Earth Sci. Rev. 123, 53-86., DOI 10.1016/j.earscirev.2013.02.008

Bauer, J. E., Cai, W. J., Raymond, P. A., Bianchi, T. S., Hopkinson, C. S., Regnier, P. A. G., 2013. The changing carbon cycle of the coastal ocean. Nature 504, 61-70., DOI 10.1038/nature12857

Burkhardt, S., Zondervan, I., Riebesell, U., 1990. Effect of CO_2 concentration on C:N:P ratio in marine phytoplankton: A species comparison. Limnol. Oceanogr. 448(3), 683-690., DOI 10.4319/lo.1999.44.3.0683

Brandes, J.A., Devol, A.H., 1997. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. Geochim. Cosmochim. Acta. 61(9), 1793-1801., DOI 10.1016/S0016-7037(97)00041-0

Canfield, D. E., Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., Thamdrup, B., Hansen, J. W., Nielsen, L. P., Hall, P. O. J., 1993. Pathways of organic carbon oxidation in three continental margin sediments. Mar. Geol. 113(1-

2), 27-40., DOI 10.1016/0025-3227(93)90147-N

Coble P.G, Del Castillo C.E, Avril B., 1998. Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon. Deep Sea Res. II. 45(10-11), 2195-2223., DOI 10.1016/S0967-0645(98)00068-X

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., Melack, J., 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosys., 10, 171-184., DOI 10.1007/s10021-006-9013-8

Eadie, B.J., Jeffrey, L. M., 1973. $\delta^{13}C$ analyses of oceanic particulate organic matter. Mar. Chem. 1(3), 199-209., DOI 10.1016/0304-4203(73)90004-2

Fisk, A. T., Hobson, K. A., Norstrom, R. J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environ. Sci. Technol. 35(4), 732-738., DOI 10.1021/es001459w

Hargrave, B. T., Holmer, M, Newcombe, C. P.,2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Mar. Pollut. Bull. 56(5), 810-824., DOI 10.1016/j.marpolbul.2008.02.006

Hoshika, A., Tanimoto, T., Kawana, K., 1989. Oxygen uptake rate at benthic layer in Hiuchi-Nada. Jpn. J. Water Pollut. Res. 12(7), 423-430., DOI

10.2965/jswe1978.12.423 (Japanese with English abstract)

Hoshika, A., Shiozawa, T., Matsumoto, E., 1983. Sedimentation Rate and Heavy Metal Pollution in Sediments in Harima Nada (Harima Sound), Seto Inland Sea. J. Oceanogr. Soc. Jpn. 39, 82-87., DOI 10.1007/BF02210762

Jeffrey, A.W.A., Pflaum, R.C., Brooks, J.M., Sackett, W.M., 1983. Vertical trends in particulate organic carbon ^{13}C: ^{12}C ratios in the upper water column. Deep Sea Res. 30(9), 971-983., DOI 10.1016/0198-0149(83)90052-3

Kelly, B. C., Gobas, F. A. P. C., 2001. Bioaccumulation of persistent organic pollutants in lichen-caribou-wolf food chains of Canada's central and western arctic. Environ. Sci. Technol. 35(2), 325-334., DOI 10.1021/es0011966

Kuwatsuka, S., Watanabe, A., Itoh, K., Arai, S., 1992. Comparision of two methods of preparation of humic and fulvic acids, IHSS method and NAGOYA method. Soil Sci. Plant Nutr. 38(1), 23-30., DOI 10.1080/00380768.1992.10416948

Kristensen, E., Ahmed, S. I., Devol, A. H., 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: which is fastest?. Limnol. Oceanograph., 40(8), 1430-1437., DOI https://doi.org/10.4319/lo.1995.40.8.1430

Kristensen, E., Holmer, M., 2001. Decomposition of plant materials in marine sediment exposed to different electron acceptors (O_2, NO_3^-, and SO_4^{2-}), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. Geochim. Cosmochim. Acta, 65(3), 419-433., DOI 10.1016/S0016-7037(00)00532-9

Larsson, T., Wedborg, M., Turner, D., 2007. Correction of inner-filter effect in fluorescence excitation-emission matrix spectrometry using Raman scatter. Anal. Chim. Acta 583(2), 357-363., DOI 10.1016/j.aca.2006.09.067

Lu J., Zhang T., Ma J., Chen Z., 2009. Evaluation of disinfection by-products formation during chlorination and chloramination of dissolved natural organic matter fractions isolated from a filtered river water. J. Hazard. Mater. 162(1), 140-145., DOI 10.1016/j.jhazmat.2008.05.058

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: Some principles, illustration for the denitrification and nitrification processes. Plant Soil, 62(3), 413-430., DOI 10.1007/BF02374138

Martinez-Garcia, E., Carlsson, M. S., Sanchez-Jerez, P., Sánchez-Lizaso, J. L., Sanz-Lazaro, C., Holmer, M., 2015. Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture. Biogeochem. 125(1), 133-148., DOI 10.1007/s10533-015-0119-y

Middelburg, J. J., Nieuwenhuize, J., van Breugel, P., 1999. Black carbon in marine sediments. Mar. Chem., 65(3-4), 245-252., DOI 10.1016/S0304-4203(99)00005-5

Muyzer, G., Stams, A. J. M., 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nature Rev. Microbiol. 6(6), 441-454., DOI 10.1038/nrmicro1892

Nishijima, W., Umehara, A., Sekito, S., Okuda, T., Nakai, S., 2016. Spatial and temporal distribution of Secchi depths and chlorophyll a concentrations in the Suo Nada of the Seto inland Sea, Japan, exposed to anthoropogenic nutrient loadsing. Sci. Total Environ. 571, 543-550., DOI 10.1016/j.scitotenv.2016.07.020

Ohno, T., 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environ. Sci. Technol., 36(4), 742-746., DOI 10.1021/es0155276

Stedmon, C.A, Markager S., Bro R., 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem. 82(3-4), 239-254., DOI 10.1016/S0304-4203(03)00072-0

Stedmon, C. A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnol. Oceanograph.: Methods, 6(11), 572-579. DOI 10.4319/lom.2008.6.572

Voutsas, E., Magoulas, K., Tassios, D., 2002. Prediction of the bioaccumulation of persistent organic pollutants in aquatic food webs. Chemosphere 48(7), 645-651., DOI 10.1016/S0045-6535(02)00144-3

Yamamuro M., Kayanne, H., 1995. Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediments with a Yanaco MT-5 CHN analyzer. Limnol. Oceanogr. 40(5), 1001-1005., DOI 10.4319/lo.1995.40.5.1001

Yamashita Y., Tanoue E., 2003. Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. Mar. Chem. 82(3-4), 255-271., DOI 1 0.1016/S0304-4203(03)00073-2

Figures

Figure Captions

Fig. 1 Sampling stations in Seto Inland Sea, Japan

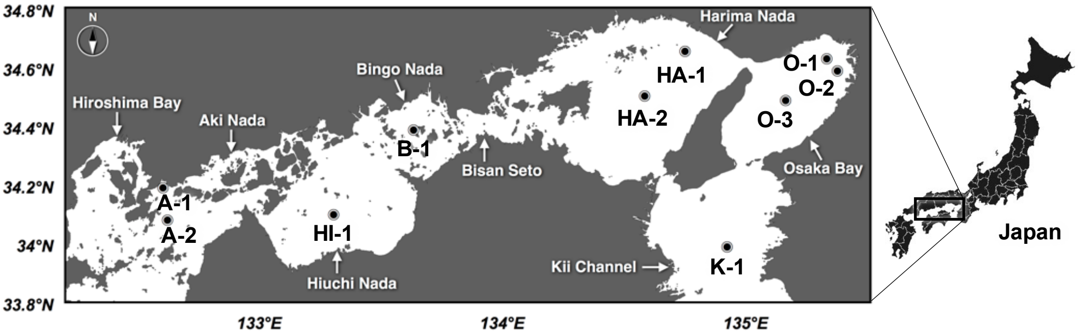
Fig. 2 Concentrations of labile, semi-labile and refractory fractions
Error bars are standard deviation (n=3)

Fig. 3 Concentrations of humic acid, fulvic acid and humin in the sediments
Error bars are standard deviation (n=3)

Fig. 4 Relationship between refractory organic matter and average concentration of summer chlorophyll a in surface seawater
Error bars are standard deviation of refractory organic matter (n=3)

Fig. 5 Fluorescence intensities of each component obtained by PARAFC analyses

Fig. 6 Correlation between DOC concentration and fluorescence intensity of UV humic acid or protein after 90 days



Map of sampling stations were drawn using software for the analysis and visualization of oceanographic and meteorological data sets, Ocean Data View ver. 4.7.10.

Fig. 1 Sampling stations in Seto Inland Sea, Japan

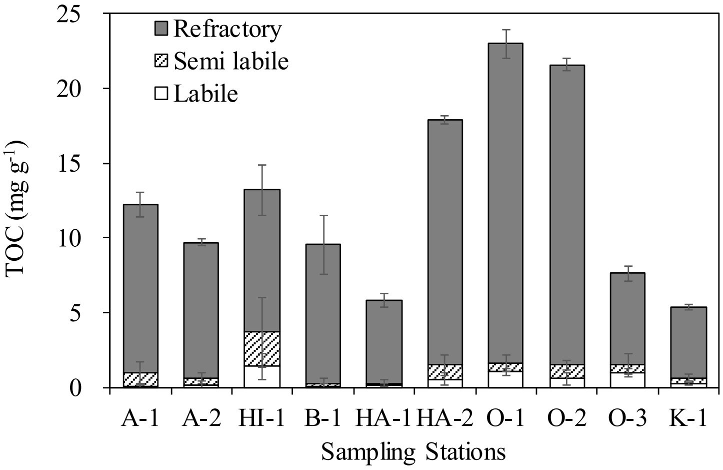


Fig. 2 Concentrations of labile, semi-labile and refractory fractions

Error bars are standard deviation (n=3)

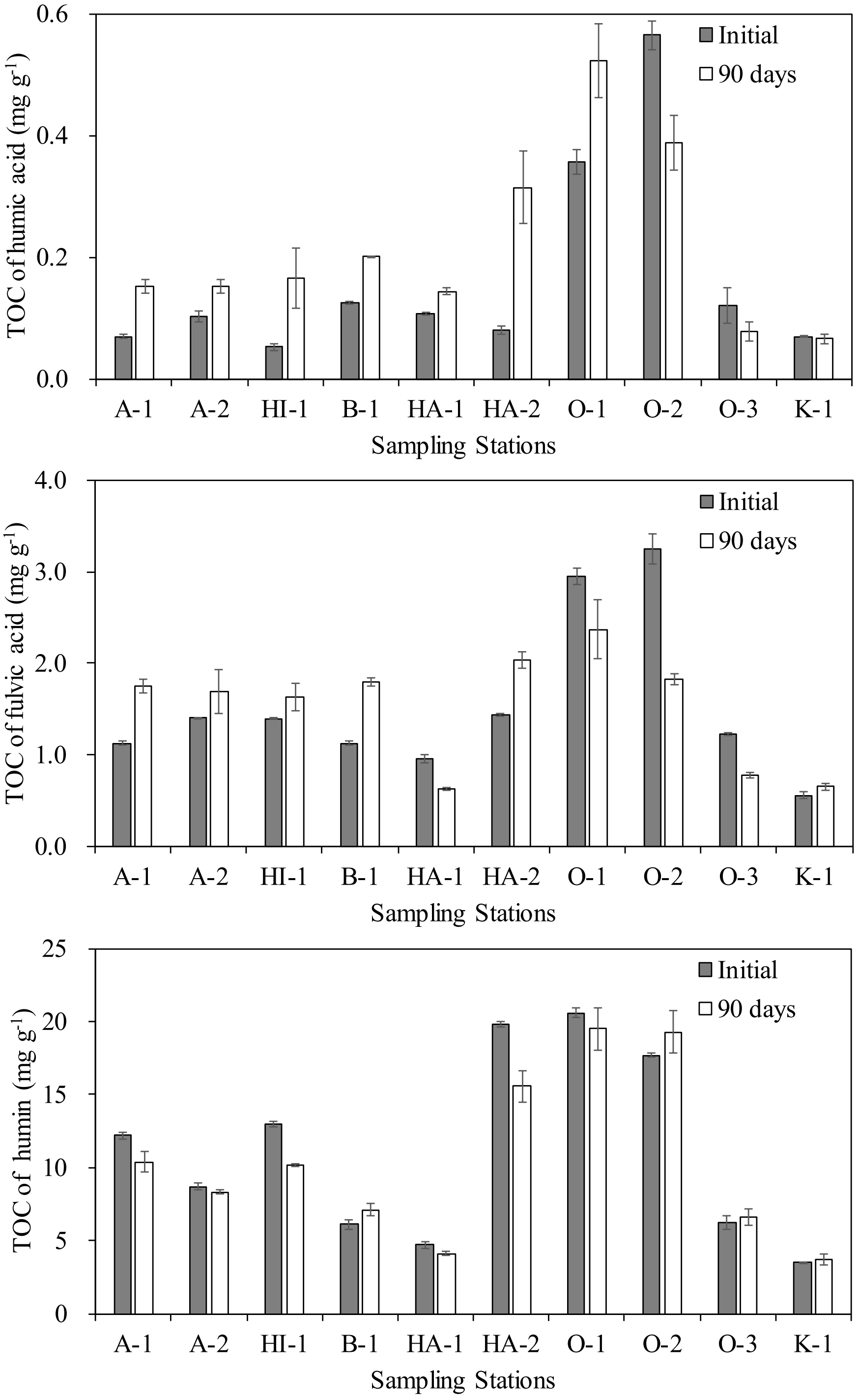


Fig. 3 Concentrations of humic acid, fulvic acid and humin in the sediments

Error bars are standard deviation (n=3)

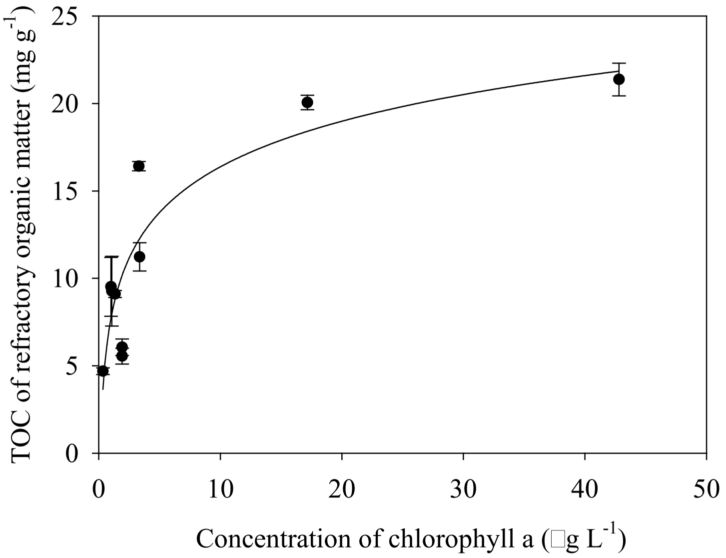


Fig. 4 Relationship between refractory organic matter and average concentration of summer chlorophyll a in surface seawater

Error bars are standard deviation of refractory organic matter (n=3)

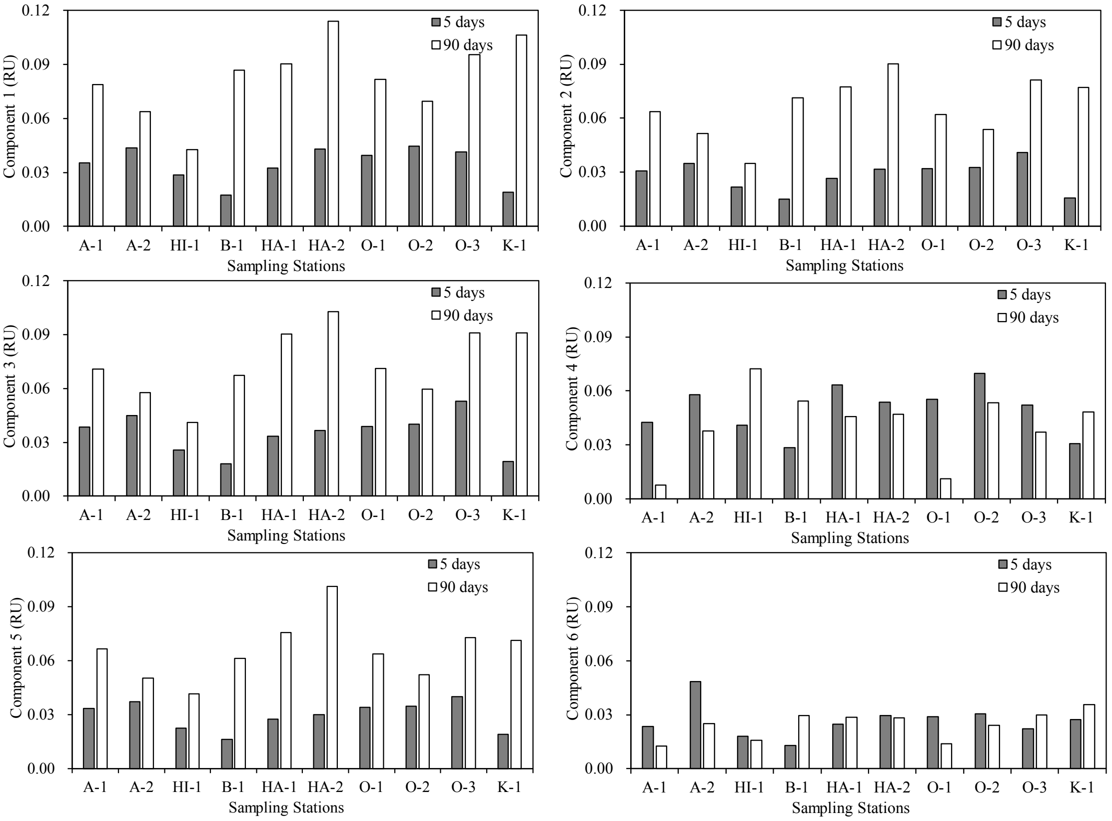


Fig. 5 Fluorescence intensities of each component obtained by PARAFC analyses

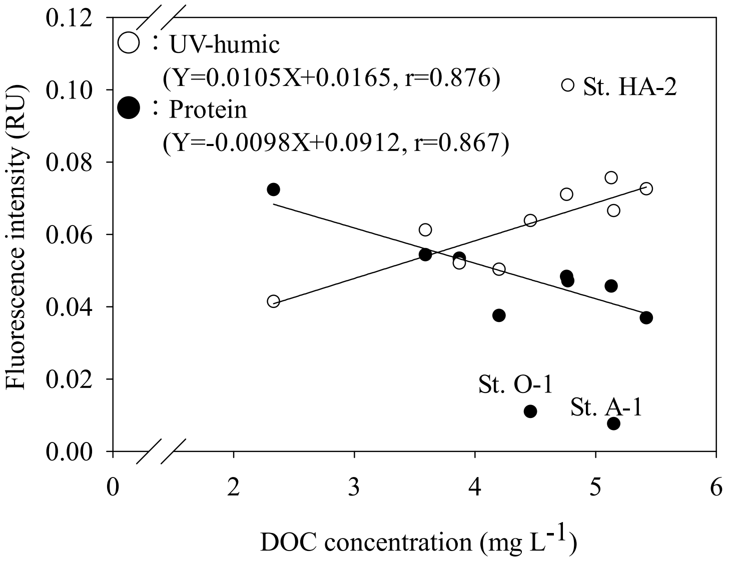


Fig. 6 Correlation between DOC concentration and fluorescence intensity of UV humic acid or protein after 90 days

Table

Table 1 The characteristics of sediments used in this study

Station No.	**TOC (mg g^{-1})**	**TN (mg g^{-1})**	**C/N ratio**	**δ^{13}C（‰）**	**δ^{15}N（‰）**	**Water Content (%)**	**Mud Content (%)**
A-1	12.2	1.6	7.7	-20.54	7.13	65.3	10.3
A-2	9.7	1.5	6.4	-20.31	6.92	55.8	44.2
HI-1	13.2	1.7	7.7	-20.83	7.50	68.8	96.2
B-1	8.0	1.1	7.3	-21.30	7.83	52.3	15.2
HA-1	5.3	0.8	6.4	-20.96	6.82	44.1	7.8
HA-2	17.9	2.4	7.5	-20.46	8.79	74.2	99.2
O-1	22.9	2.9	7.9	-20.49	8.06	75.0	97.3
O-2	21.6	2.6	8.3	-21.04	7.24	72.5	99.0
O-3	7.6	1.2	6.6	-20.54	7.74	42.8	51.7
K-1	5.4	0.8	6.7	-21.41	5.28	38.1	31.5

Average mud content was obtained from the Ministry of Land, Infrastructure, Transport and Tourism seasonal monitoring data (Nishijima et al., 2016)

Supplemental materials

Organic Matter Degradation Characteristics of Coastal Marine Sediments Collected from the Seto Inland Sea, Japan

Satoshi ASAOKA[a*], Waqar Azeem JADOON[b], Akira UMEHARA[c],
Kazuhiko TAKEDA[d], Sosuke OTANI[e], Masaki OHNOf , Nobuhide FUJITAKE[g],
Hiroshi SAKUGAWA[d], Hideo OKAMURA[a]

a Research Center for Inland Seas, Kobe University
5-1-1 Fukaeminami, Higashinada, Kobe, 658-0022 JAPAN
b Department of Environmental Sciences, Faculty of Sciences, Hazera University.
Mansehra, Khyber, Pakhtunkhwa, PAKISTAN
c Environmental Research and Management Center, Hiroshima University
1-5-3, Kagamiyama, Higashihiroshima, Hiroshima, 739-8513 JAPAN
d Graduate School of Integrated Sciences for Life, Hiroshima University
1-7-1 Kagamiyama, Higashi-Hiroshima, 739-8521 JAPAN
e Osaka Prefecture University College of Technology
26-12 Saiwaicho, Neyagawa, Osaka, 572-8572 JAPAN
f Niigata University of Pharmacy and Applied Life Sciences
265-1 Higashijima, Akiha, Niigata, 956-8603 JAPAN
g Graduate School of Agricultural Science, Kobe University
1-1 Rokkodai-cho, Nada, Kobe, 657-8501 JAPAN

*Corresponding author:
Tel & Fax: +81-78-431-6357, E-mail: s-asaoka@maritime.kobe-u.ac.jp
Address: Research Center for Inland Seas, Kobe University, 5-1-1 Fukaeminami, Higashinada,
Kobe, 658-0022 JAPAN

Table S1 Sampling stations in this study

Station No.	Latitude	Longtitude
A-1	34°-12.0'N	132°-36.0'E
A-2	34°-06.0'N	132°-38.0'E
HI-1	34°-06.7'N	133°-18.2'E
B-1	34°-23.7'N	133°-38.1'E
K-1	34°-00.0'N	134°-55.0'E
O-1	34°-40.0'N	135°-20.0'E
O-2	34°-36.5'N	135°-22.5'E
O-3	34°-30.0'N	135°-10.0'E
HA-1	34°-40.0'N	134°-45.0'E
HA-2	34°-30.0'N	134°-35.0'E

Table S2 The carbon and nitrogen isotope ratios of the sediments

	δ^{15}N（‰) (SD n=3)			δ^{13}C（‰）(SD n=3)		
Station No.	0 day	5 days	90 days	0 day	5 days	90 days
A-1	7.13	6.15 (0.12)	6.12 (0.34)	-20.54	-20.84 (0.24)	-20.75 (0.01)
A-2	6.92	4.45 (0.07)	6.38 (0.47)	-20.31	-21.05 (0.13)	-20.88 (0.24)
HI-1	7.50	7.85 (0.21)	7.76 (0.55)	-20.83	-21.26 (0.07)	-21.51 (0.13)
B-1	7.83	8.06 (0.21)	8.59 (0.60)	-21.30	-21.51 (0.22)	-21.47 (0.26)
HA-1	6.82	1.85 (0.36)	4.52 (0.39)	-20.96	-21.91 (0.23)	-21.29 (0.04)
HA-2	8.79	4.49 (0.64)	7.15 (2.51)	-20.46	-21.20 (0.10)	-21.09 (0.04)
O-1	8.06	4.87 (0.09)	7.30 (0.43)	-20.49	-21.13 (0.06)	-21.12 (0.12)
O-2	7.24	3.33 (0.13)	4.63 (0.45)	-21.04	-21.64 (0.05)	-21.78 (0.19)
O-3	7.74	3.80 (2.49)	7.15 (0.65)	-20.54	-21.48 (0.15)	-21.31 (0.10)
K-1	5.28	2.66 (0.70)	4.05 (0.16)	-21.41	-21.86 (0.13)	-21.67 (0.08)

Table S3 Major fluorescence components in overlying water obtained by PARAFAC analyses

Component	Type	Ex (nm)	Em (nm)	References
1	UV-Humic like	<240	406	Stedmon 2003
2	UV-Humic	265	495	Coble 1998
3	UV-Humic	255	400-460	Coble 1998
4	Tyrosine-like, protein-like	250	307	Yamashita 2003
5	UV-Humic	245	467	Coble 1998
6	Tryptophan-like, protein-like	275	337	Yamashita 2003

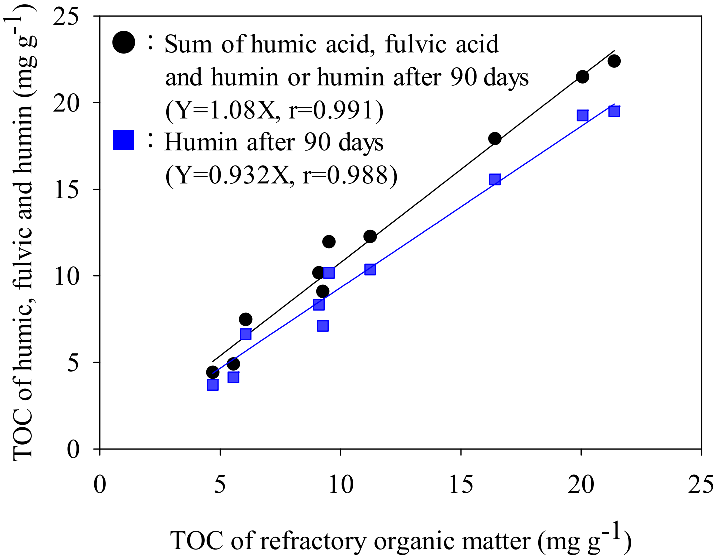


Fig. S1 Correlations of between sum of humic acid, fulvic acid and humin after 90 days and refractory organic matter.

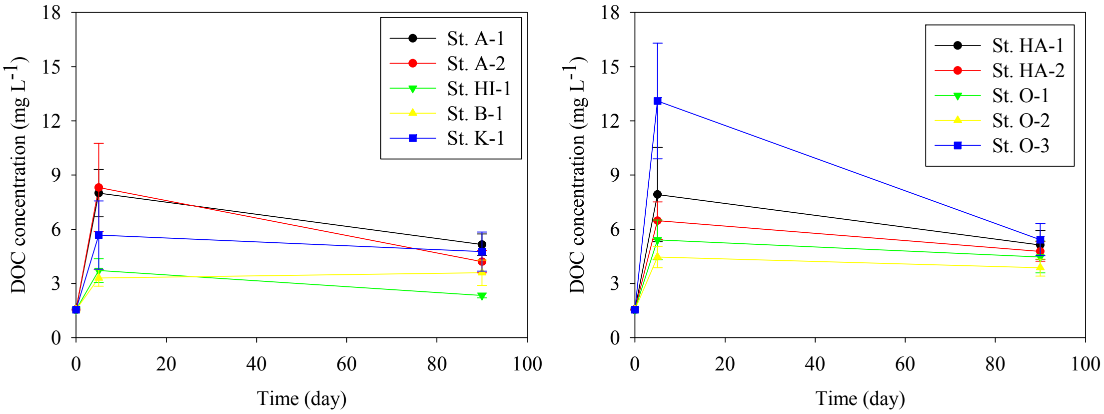


Fig. S2 Time course of DOC concentration in overlying water

Error bars are standard deviation (n=3)

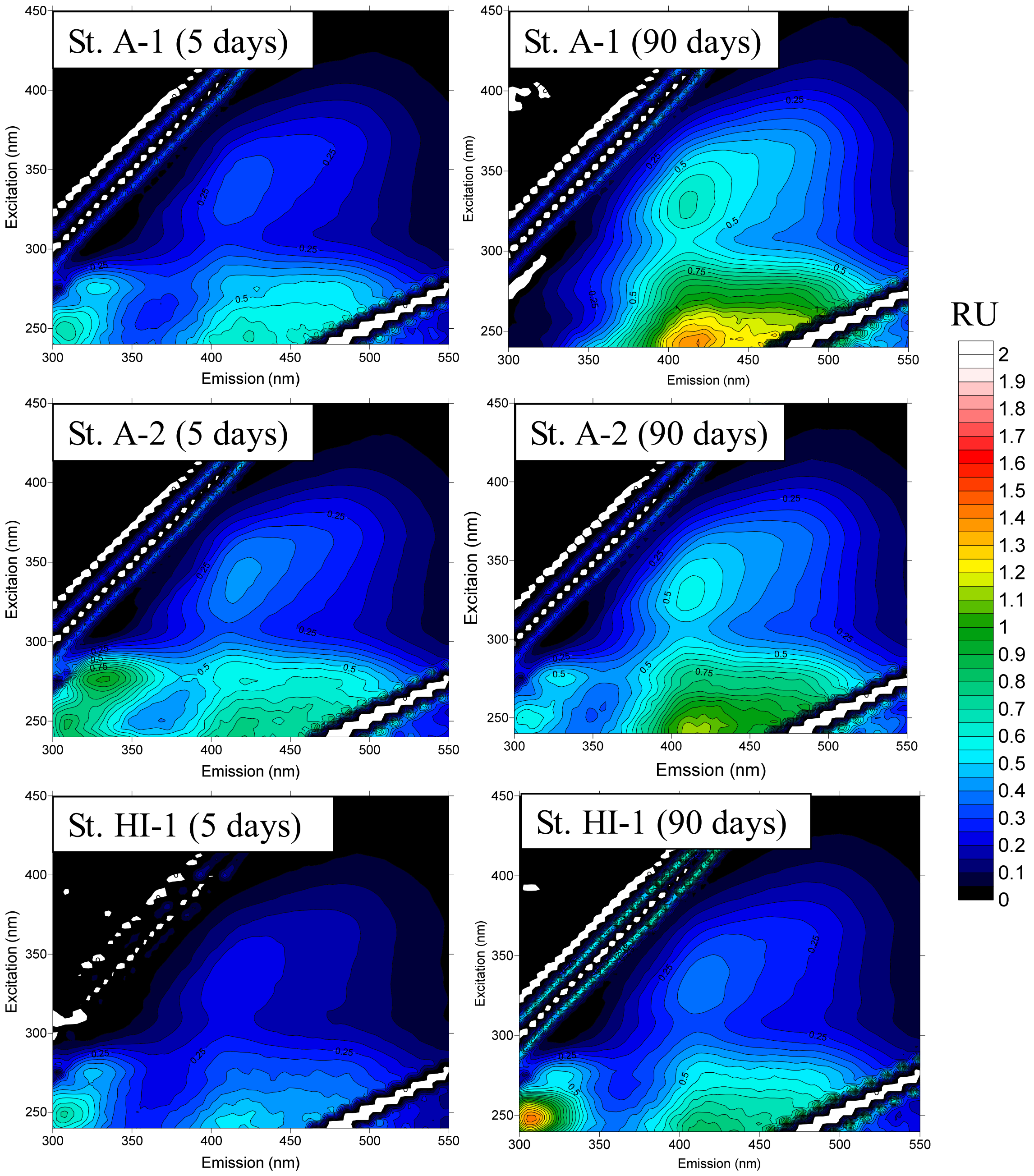


Fig. S3 EEM of overlying water at 5 and 90 days at Sts. A-1, A-2 and HI-1

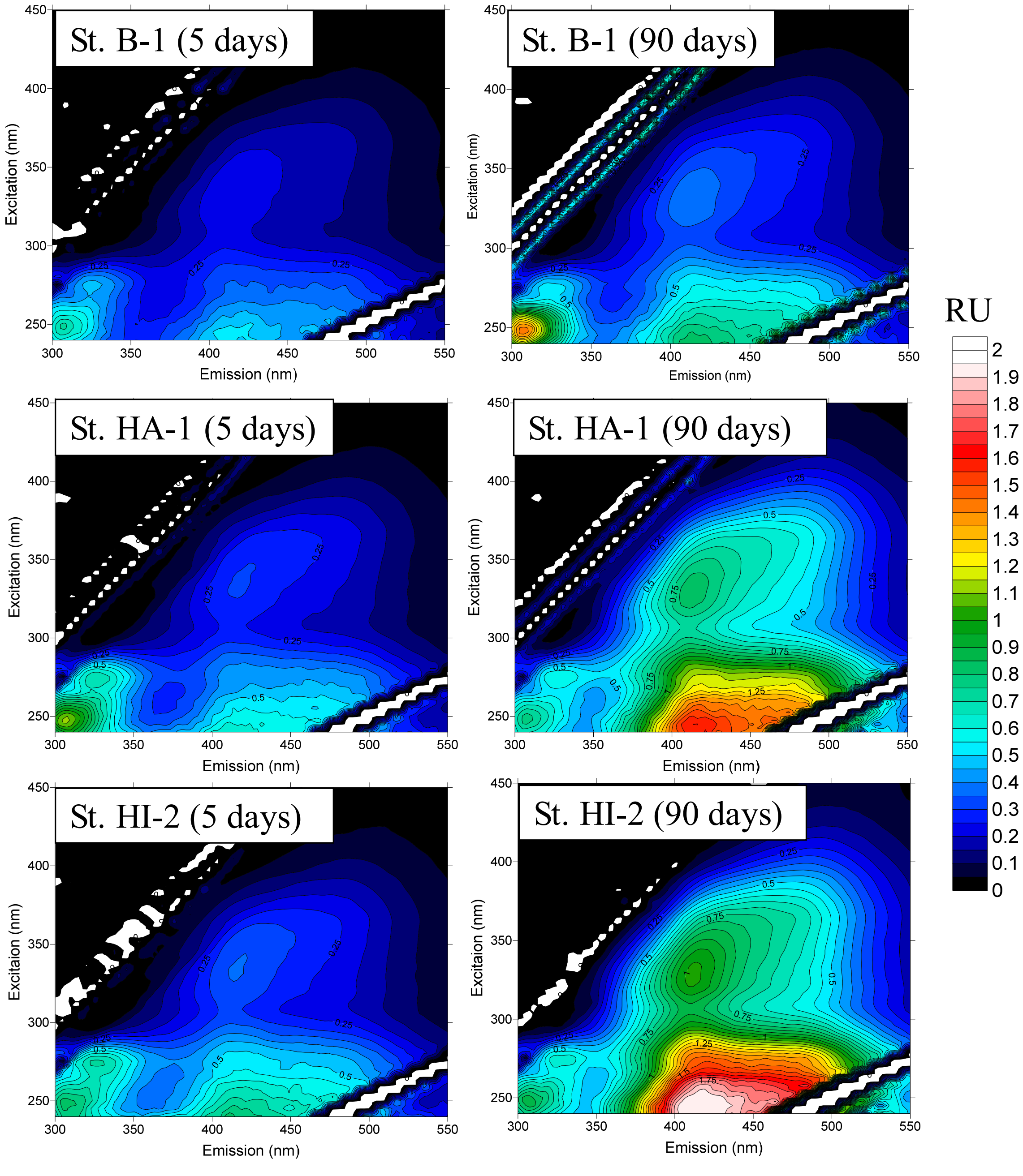


Fig. S3 EEM of overlying water at 5 and 90 days at Sts. B-1, HA-1 and HA-2

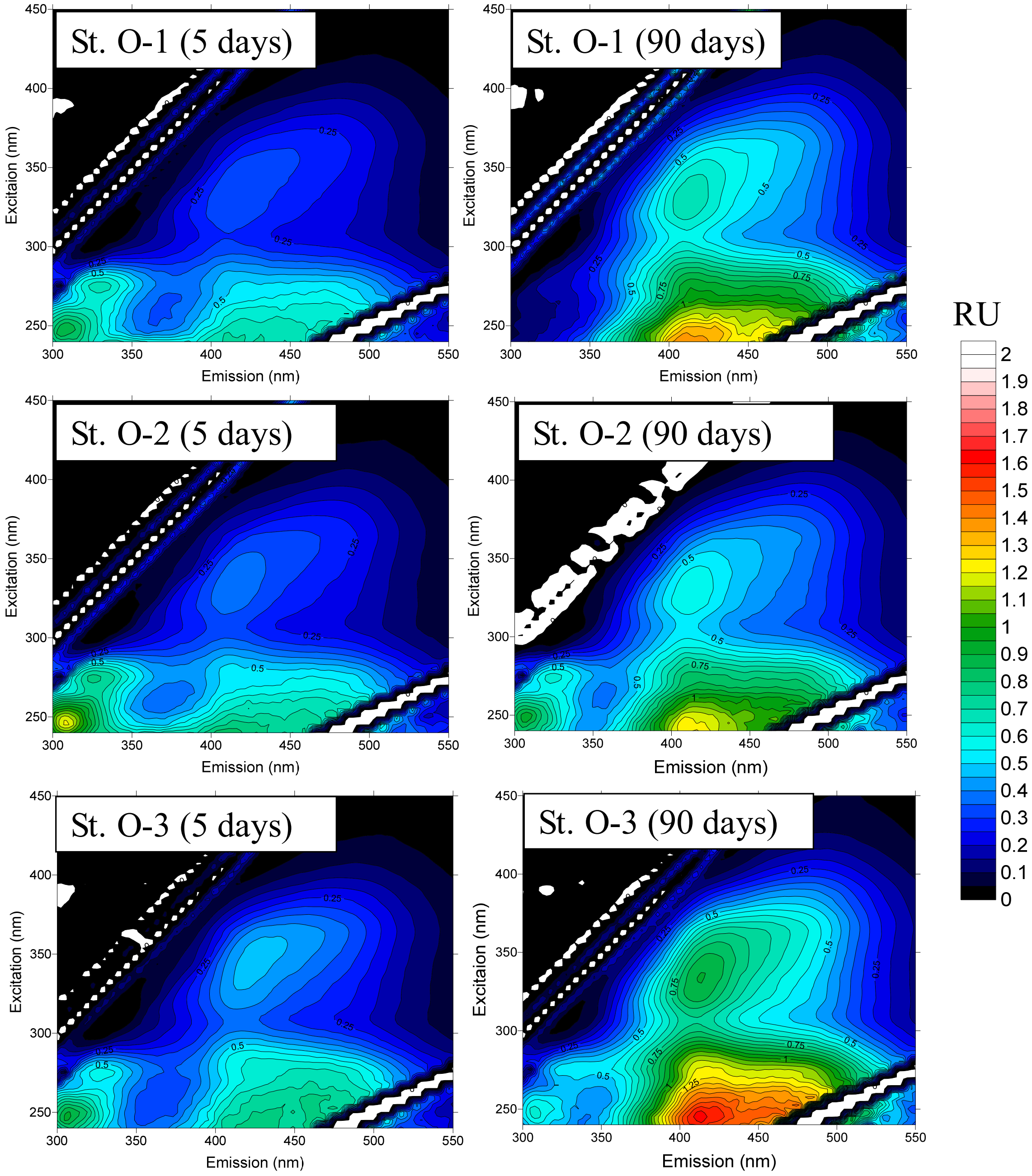


Fig. S3 EEM of overlying water at 5 and 90 days at Sts. O-1, O-2 and O-3

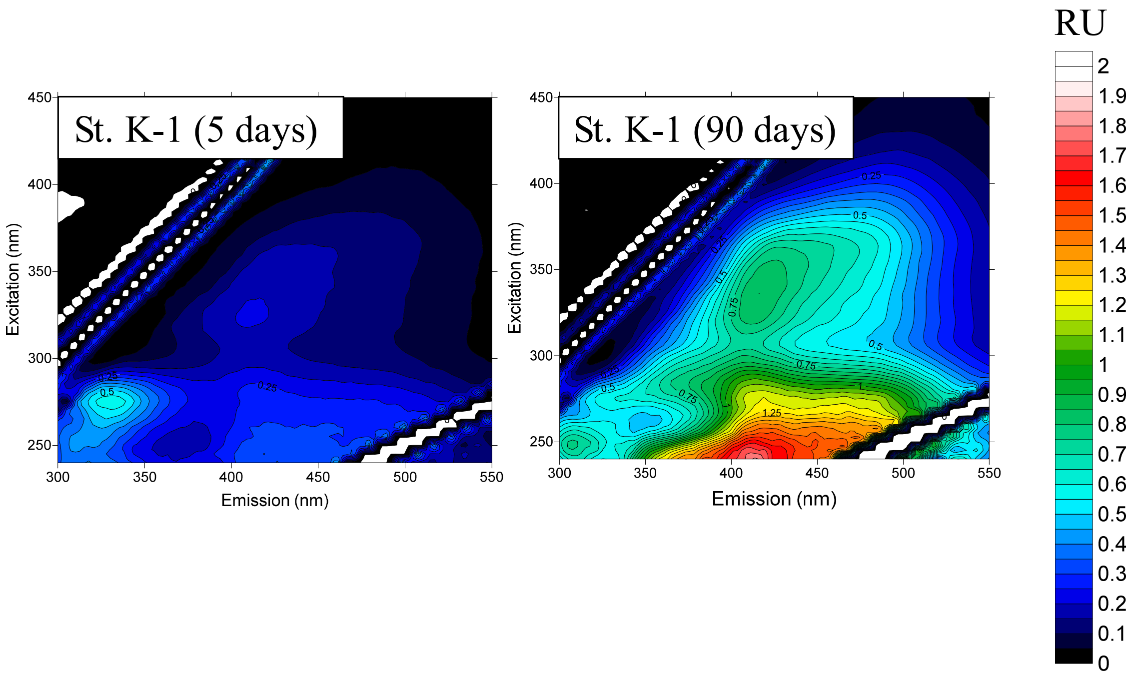


Fig. S3 EEM of overlying water both 5 and 90 days at Sts. K-1

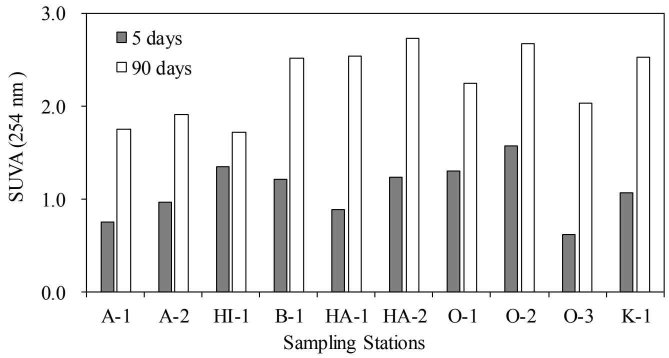


Fig. S4 SUVA of overlying water

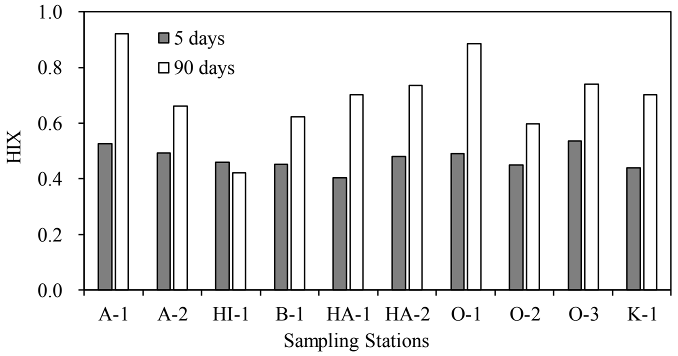


Fig. S5 The humification index of overlying water