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Chemical Properties of Sediment in Nanakita Estuarine Tidal Flat: Estimation of Sedimentary Organic Matter Origin by Stable Isotope and Fatty Acid

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Abstract

The spatial variation of organic matter sources in tidal flat sediment of the Nanakita River estuary, involving Gamo lagoon on the north-east coast of Honshu Island, Japan, was examined using carbon stable isotopes and fatty acid biomarkers. The spatial variation of total organic carbon (TOC) contents and $\delta^{13}C$ values were highly variable in between the stations, such as sandy flat (1.3 mg/g, -21.0‰), sand-muddy flat (2.6 mg/g, -21.9‰), and muddy flat (24.9 mg/g, -25.9‰), respectively. Particularly, at the muddy flat, high TOC content and low $\delta^{13}C$ value of the sediments indicated that the surface sediment was composed largely of terrestrial organic matter. Whereas, at the sandy flat and sand-muddy flat, the high ratios of diatom and bacteria biomarkers indicated the high contribution of abundant microorganism along with marine organic matter in sediment composition. From these results, it considered that the amount and origin of transported sedimentary organic matter indicated different characteristics in this study stations.

Keywords: Estuary, Intertidal flat, Microorganism, Sediment, Spatial variation

1. Introduction

Estuarine tidal flats are important sites for production and re-mineralization of organic matter (OM) and recycling of nutrients [1, 2]. These sites are the transitional zone from land to ocean system, and receive materials, such as OM and nutrients transported from rivers or oceans. Especially, sedimentary organic matter (SOM) is an important food source of macrobenthos in estuary systems. However, an overabundance may lead to reductions in species and biomass of macrobenthos, due to oxygen depletion and buildup of toxic by-products (e.g., ammonia and sulphide), which is associated with the break-down of these materials [3, 4]. Therefore, to accomplish sustainable estuary management consistent with conserving such valuable estuarine ecosystems, better understanding of the sediment environment in estuarine systems is needed. Although the dynamics of OM in estuaries have been studied, it still remains about the sources, fate and role of OM. The reasons for these uncertainties include the complex interactions among the various physical, geological and biochemical factors that define each estuarine ecosystem and control OM in these environments [5, 6].

In the last decade, more attention has been paid to the SOM composition in estuary ecosystem [7, 8]. However, many of these investigations focused on the influential relationship between the role of terrestrial and marine OM. Because of the terrestrial-derived OM with high C/N tends to be less, and chemical properties of OM can affect its consumption and abundance in sediment of estuarine tidal flats. Whereas, according to the some studies, it indicated that the dynamic of OM is influenced by the benthic microorganisms (e.g., bacteria and benthic microalgae) in estuarine sediment, due to high productivity and re-mineralization of OM [9, 10]. For instance, according to Lee et al. [9] in 1998, the degraded OM amount by bacteria in Hiroshima Bay tidal flat indicated about 5-14 times more than macrobenthos. Thus, benthic microorganisms play important roles in the sediment environments, but little is known concerning its role and fate of microorganism in sediment OM dynamic [11, 12].

Isotopic ratios of C can also be helpful in distinguishing between the marine and terrestrial OM. However, if more than two sources are suspected of being present, carbon isotope studies alone cannot detect the contribution of the other sources [13]. Whereas, fatty acid biomarker have also been used as trophic markers to determine the transfer of OM, within food webs, since these fatty acids are fundamental components of cellular material with high biological specificity, which are generally incorporated by higher trophic levels with little modification in its structure [14]. Therefore, we used a combination of fatty acid biomarkers and stable isotope ($\delta^{13}C$) to identify the SOM origins

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in a Nanakita estuary.

The currently studied tidal flats are located on the north and south banks, within 500 m, and showed diverse sediment properties from sandy to muddy. The fact is thought that sediments have the same OM origin because the tidal flats are located on the same location. However, Sediments are shown obviously with different properties. The purpose of this study is to determine the relative contribution of OM to the sediment of a Nanakita River estuary, with a different sediment characteristic, Nanakita River. We estimated the contribution of organic matters in the sediment by carbon stable isotopes and fatty acids.

2. Materials and Methods

2.1. Study Area

This study was conducted in a tidal flat system of the Nanakita River estuary, involving Gamo lagoon on the north-east coast of Honshu Island, Japan (38˚15'N, 141˚00'E) (Fig.1). Nanakita River discharges to the Pacific Ocean at ~15 m^3/sec (annual mean). Moreover, the studied tidal flats are located on the north and south banks, within a 500 m distance, and showed diverse sediment properties from sandy to muddy. Whereas, Gamo lagoon extends over 0.22 km^2 with 5 ha of tidal flats, at low tides. Reed (*Phragmites australis*) marshes have developed along the shore. Three sampling stations were established in the intertidal zone of the tidal flat system. Sediments were collected in August 2007. Three sediment cores of 10 cm diameter were collected at low tides in each sampling station (sediment depths between 10 and 15 cm). In the laboratory, the core sediment samples were sliced into 0-1 cm (upper sediment) and analyzed for total organic carbon (TOC) content, silt-clay content and chlorophyll *a*. All samples were immediately dried in a freeze-dryer, and then ground into powder to analyze for carbon stable isotopes and fatty acids.

2.2. Chemical Analyses

Sediment samples (about 1 g) were acid-treated with 1 N HCl, for 24 hr in pre-combusted glass test-tubes to remove carbonate [8]. Then, samples for carbon stable isotope analysis were combusted in a DELTA^{plus} elemental analysis (Thermo Fisher Scientific, Waltham, MA, USA), and were passed online to an isotope ratio mass spectrometer (Delta plus MAT Finnigan; Thermo Fisher Scientific) to determine $^{13}C/^{12}C$. The results are expressed as per mill deviations, relative to the conventional standards, i.e., Pee Dee Belemnite carbonate (PDB) for carbon, as follow:

$$\delta^{13}C\ (‰) = \{(R_{Sample} / R_{Standard}) - 1\} \times 10^3 \qquad (1)$$

Where R = $^{13}C/^{12}C$. Reproducibility in the analyses was ± 0.2‰ for $\delta^{13}C$.

In this study, terrestrial plant (TP), marine particulate organic matter (POM), estuarine POM, and benthic microalgae (BMA) were assumed as a potential OM of SOM. In case of terrestrial plant, reed (*Phragmites australis*) is the most common plant present abundantly in the studied area. Reed was collected, washed with deionized water and dried in an electric oven at 60°C for 1 day. The dried reed was ground, using a grinder (T-351; Rong Tsong Iron Co., Taichung, Taiwan), to a powder and was passed through the 250-μm sieve (JIS Z 8801; Nonaka Rikaki Co., Tokyo, Japan). Estuarine POM (nearby water of station A) and Marine POM (surface seawater of Sendai Bay) samples were collected with a bucket (1-2 L). The water samples were then filtered through precombusted (2 hr, 450°C) Whatman (Clifton, NJ, USA) GF/F filters to collect POM. The filters were dried in an electric oven (50°C) and the dried filter was washed with 6 N HCl before being kept in a desiccator for half a day, to allow for decarbonization. Benthic microalgae on the surface sediment of the large sand flat as sampled at the stations A, and the benthic microalgae extraction was conducted, following the method described by Riera et al. [11].

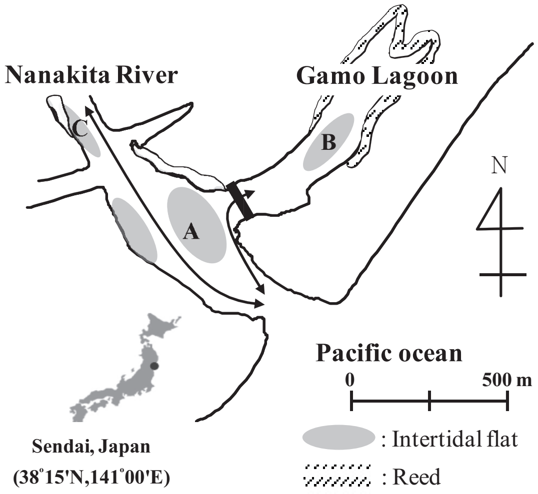

Fig. 1. The study site and sampling points in tidal flats of the Nanakita River estuary, Japan.

Lipids of samples, 10 g dry weights (wt) of sediments were extracted, following the slightly modified version of method of Bligh and Dyer [15] and Meziane and Tsuchiya [16].

Fatty acids methyl esters (FAMEs) were separated and quantified by gas chromatography (GC-17A; Shimadzu Inc., Kyoto, Japan), equipped with a flame ionization detector. Separation was performed with an free fatty acid phase (FFAP)-polar capillary column (100 m × 0.25 mm internal diameter) with He as a carrier gas (flow rate, 1.4 mL/min). After injection at 150°C, the oven temperature was raised to 150°C at a rate of 4°C per minute, then to 230°C at 4°C per minute, and finally held constant for 50 min. The flame ionization was held at 250°C. Most FAMEs peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc., Bellefonte, PA, USA). For some samples, peaks of FA were identified with a GC-mass spectrometry (Table 1).

TOC content of sediment samples was determined for dried (105°C, 24 hr), homogenized and HCl-treated sediments, using a

Table 1. Fatty acids used as biomarkers for different food sources

Trophic marker [2,14,16]	Source
16:2ω4, 16:3ω4, 20:5ω3	Diatoms
15:0 iso, 15:0 anteiso, 18:1ω7	Bacteria
18:2ω6, 18:3ω3	Macroalgae
22:6ω3	Dinoflagellates
Long chain fatty acids (fatty acids with more than 24 carbons)	Terrestrial plant

Heraeus Vario-el CHN-analyzer (Vario-eL III; Elementar Analysensysteme GmbH, Hanau, Germany). Chlorophyll *a* of the surface sediment samples were extracted by a 90% acetone and their concentrations were determined by a spectrophotometric measurement, following the method of Lorenzen and Jeffrey [17]. Sediment types were determined, according to a classification scheme based on sediment mud content (i.e., the percentage of the sediment fraction <63 μm to total sediment dry weight) [18].

2.3. Statistical Analyses

To determine whether the sediment properties differed between the stations, one-way analysis of variance (ANOVA) was calculated once with all data. All data were checked for normality and equality of variance and transformed, if necessary. Statistical analyses were conducted with SPSS ver. 17.0 software (SPSS Inc., Chicago, IL, USA).

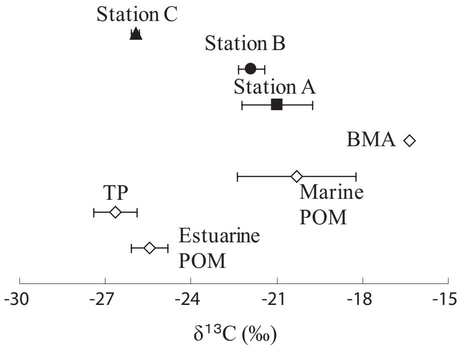

Fig. 2. δ^{13}C is sediments in relation to potential organic matters. Values are mean ± SD, n = 3. TP: terrestrial plant, POM: particulate organic matter, BMA: benthic microalgae.

3. Results and Discussion

3.1. Chemical Properties of Sediment

Table 2 shows sediment characteristics from the intertidal flats along the Nanakita estuarine and Gamo lagoon. Concentrations of TOC in sediments from the Nanakita estuary were relatively low, 1.3-24.9 mg/g. It is observed in Table 1 that TOC has the remarkable distribution differences along the Nanakita estuary (ANOVA, $p = 0.001$). These data for the spatial variation in sediment properties showed that TOC was related positively to the sediment mud content ($r^2 = 0.884$, $p < 0.001$). The results of TOC and sediment mud contents indicated a clear spatial trend, along the ocean-upstream direction; the fine fraction (<63 μm) and TOC content, which increased toward the upstream side in Nanakita estuary [5]. It is well known that the organic carbon of muddy sediment is generally higher than the sandy sediment [19].

Chlorophyll *a* concentrations, in the surface sediments, showed stations A (4.7 μg/g), B (6.6 μg/g), and C (11.4 μg/g) (ANOVA, $p = 0.000$; Table 2). The spatial distributions of chlorophyll *a* indicated that chlorophyll α concentration were high relatively with the sediment mud contents ($r^2 = 0.754$, $p < 0.05$). de Jong and Jonge [20] were apparent as a positive relation between silt-clay contents of the sediment and microphytobenthic chlorophyll *a*. Moreover, the large differences in chlorophyll *a* concentrations between the stations in the Nanakita estuary may be ascribed to the differences in hydrodynamic characteristic with nutrient ratio. In this study stations, muddy tidal flats represent more quiet and sandy tidal flats more turbulent hydrodynamic conditions (e.g., Sakamaki and Nishimura [5]). In addition, muddy sediments generally showed high C:N and N:P ratios, compared with sandy sediments [21].

From these results, station A as sand flat showed a low amount algae-derived OM, but station C indicated a high amount OM with benthic microalgae and terrestrial plant, derived OM as muddy flat. Whereas, station B as sandy-muddy flat showed intermediate characteristic of station A and C.

3.2. Origin of Sediment

The Fig. 2 shows that δ^{13}C values of potential OM sources and SOM in this study. The δ^{13}C isotopic ratio for TP (δ^{13}C = -26.3‰) was significantly lower than Marine POM (δ^{13}C = -20.3‰). This large difference showed ease of the δ^{13}C stable isotope analysis for the estimates of sediment's potential OM. The δ^{13}C value for Estuarine POM (δ^{13}C = -25.5‰) were between TP and Marine POM, showing a mixture of material originating from terrestrial and marine OM [22]. Thus, phytoplankton production in rivers of Japan is usually small because water retention time is short, due to the steep slope [23]. Moreover, Kanaya [24] showed an average δ^{13}C values (-26.0‰ ± 0.1) of riverine particulate organic matter in Nanakita River (a tidal river as same as our study area). Therefore, the freshwater phytoplankton would not be the major contributor to Estuarine POM in our study area.

The δ^{13}C values of SOM in the study area showed stations A (-21.0‰), B (-21.9‰), and C (-25.9‰) (ANOVA, $p = 0.000$; Table 2). δ^{13}C values differed between Nanakita estuarine study

Table 2. Sediment characteristics in Nanakita River estuary

	Stn. A	Stn. B	Stn. C	*p*	n
TOC (mg/g)	0.13 ± 0.03^{a}	0.26 ± 0.13^{a}	2.49 ± 0.77^{b}	0.001	3
Silt-clay content (%)	0.10 ± 0.07^{a}	0.90 ± 0.26^{a}	34.9 ± 2.26^{b}	0.035	3
Salinity (psu)	10	23.3	6.1	-	1
Chl-a (ug/g dw)	4.7 ± 0.46^{a}	6.6 ± 3.55^{a}	11.7 ± 2.47^{b}	0.000	3
δ^{13}C (‰)	-21.0 ± 1.24^{a}	-21.9 ± 0.46^{a}	-25.9 ± 0.15^{b}	0.000	3

Values are mean ± SD, -: non-detected. ANOVA results are also shown significant difference between station. Adjacent letter codes indicate significant differences (ANOVA, $p < 0.05$) between sediments.

stations, which suggest that the inputs of terrestrial and Marine OM vary with distance down the estuary, and muddy sediments were more depleted in δ^{13}C, indicating that terrestrial OM content increased with increasing silt-clay content. This is supported by the results of a correlation analysis between the TOC contents and δ^{13}C values of the sediment, showing a highly significant negative relationship (r = -0.912, p = 0.001). In addition, terrestrial OM with high C/N tends to be less degradable than marine OM with low C/N (for example, algae) [25]. Therefore, if such low-degradable terrestrial OM enters tidal flat sediments, and if the hydrodynamic conditions are sufficiently calm to retain it, within the sediment, it can remain for a long time. That is, these results indicated that the terrestrial-derived POM, through the Nanakita River, accumulated at the station C, and contributed to the change in the sediment environment, such as the increase in OM amount and muddy contents.

The most ratio fatty acids were 16:0 and 16:1ω7 in surface sediments (Table 2). The fatty acid ratio for diatoms appeared with no significant difference in spatial variation (p = 0.128; Fig. 3). Although diatom ratios were not significantly different in the total fatty acids, chlorophyll *a* concentrations in sediments were the highest in station C (Table 2). Moreover, the benthic microalgae in the surface sediments of estuary are derived mainly from benthic diatoms [26]. According to Tables 2 and 3, the concentration of diatom may be the highest in station C, but the ratio of algal-originated carbon to total sediment carbon may be higher in station A. This is in an agreement with the findings of Ogawa et al. [27] in this study station. In the previous studies, sand sediments showed relatively low C:N and N:P molar ratios which may indicated easily degradable OM of a high nutritional value relatively rich in nitrogen and phosphorus [21]. Hence, the close correlation between benthic microalgae and bacteria fatty acids

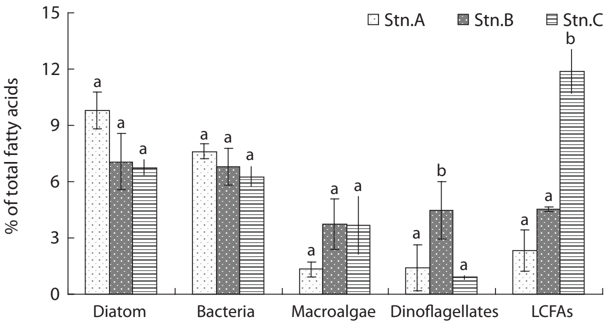

Fig. 3. Comparison of the contribution of fatty acid markers of organic sources in the estuarine surface sediments. Values are mean ± SD, n = 3. Adjacent letter codes indicate significant difference (ANOVA, $p < 0.05$) between sediments. LCFA: long chain fatty acid.

Table 3. Percentage composition of individual fatty acids in sediments of study stations

	Sediment		
Fatty acids	**Stn. A**	**Stn. B**	**Stn. C**
14:0	6.2 ± 1.75	5.9 ± 1.09	4.5 ± 0.34
i-15:0	1.1 ± 0.33	0.9 ± 0.42	2.6 ± 0.57
a-15:0	0.9 ± 0.29	0.8 ± 0.31	1.6 ± 1.05
15:0	2.8 ± 1.79	9.1 ± 1.22	2.0 ± 0.42
15:1	0.4 ± 0.20	0.2 ± 0.37	0.8 ± 0.34
16:0	31.4 ± 1.64	27.5 ± 3.71	27.3 ± 1.61
16:1ω7	22.3 ± 1.45	12.8 ± 1.75	6.8 ± 0.78
16:2ω4	2.5 ± 0.52	1.4 ± 0.49	0.3 ± 0.44
16:3ω4	2.7 ± 0.95	1.2 ± 0.41	-
17:0	0.6 ± 0.37	2.0 ± 0.14	0.6 ± 0.54
17:1	1.1 ± 0.15	-	0.5 ± 0.42
18:0	2.9 ± 0.24	4.3 ± 0.53	4.9 ± 0.57
18:1ω9	3.3 ± 1.46	5.2 ± 0.49	4.2 ± 0.72
18:1ω7	5.6 ± 0.96	5.1 ± 0.49	5.0 ± 0.83
18:2ω6	1.2 ± 0.10	1.9 ± 0.89	2.0 ± 1.09
18:3ω3	0.2 ± 0.28	1.8 ± 0.50	1.7 ± 0.49
18:3ω6	0.7 ± 0.02	0.3 ± 0.21	0.7 ± 0.23
18:4ω3	-	-	0.8 ± 0.56
20:0	1.3 ± 1.12	1.1 ± 0.26	4.4 ± 0.37
20:1ω9	-	-	1.2 ± 0.54
20:4ω6	2.1 ± 1.34	1.1 ± 0.34	0.8 ± 0.23
22:0	-	1.5 ± 0.98	3.1 ± 2.03
20:5ω3	4.7 ± 0.53	4.5 ± 0.88	8.7 ± 2.15
22:5ω3	-	1.3 ± 0.27	1.5 ± 0.48
22:6ω3	1.4 ± 1.24	4.5 ± 0.88	0.9 ± 0.11
LCFAs	2.3 ± 1.12	4.5 ± 0.11	11.9 ± 1.18
Total FA	97.6 ± 3.10	98.8 ± 3.03	98.8 ± 1.10

Values are mean ± SD (%), n = 3. -: non-detected, LCFA: long chain fatty acid.

indicates that bacteria are largely dependent on organic matter from a benthic microalgae source (r = 0.867, $p = 0.002$). When compared with other OM in station C, fatty acids of microorganisms (e.g., diatoms and bacteria) and terrestrial plant showed higher ratio in the sediments (Fig. 3). The high microorganism and terrestrial plant fatty acid biomarkers implied that these OM contributions to SOM were elevated. Especially, the long chain fatty acids (LCFAs) were highly correlated with the $\delta^{13}C$ stable isotope in this study (r = -0.913, $p = 0.001$), and increased toward station C. It was suggested that the high contribution of terrestrial plant in station C sediment, and LCFAs detected at station A, were transported from station C (upstream). The most biomarker for dinoflagellates and macroalgae were indicated the highest percentages in station B, compared with stations A and C (Fig. 3). It is likely that the high ratio of this biomarker in station B would have come entirely from marine-derived OM.

4. Conclusions

This study was to determine the spatial variation of OM origin to sediment of estuarine tidal flats, with different sediment properties by carbon stable isotope and fatty acid biomarkers. Sediments of stations A and B were enriched in marine OM, diatom, and bacteria. Whereas, the OM at station C is dominated by terrestrial sources, along with diatom and bacteria, including high contents of OM. Moreover, microorganism-derived OM indicated high contribution to SOM composition, in all the study stations. Overall, the amount and origin of SOM showed obviously different relative properties in Nanakita estuary tidal flats, suggesting that the different transport characteristic of OM.

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