Introduction

Rivers have an essential role in the transport of trace metals by rivers to coastal areas and the characteristics of organic matter in the sediment, including the effect of humic substances on the transport of such metals. The structural features of humic acids (HAs) derived from surface sediments collected from the mouths of four rivers in northwest Hokkaido, Japan, were evaluated from the perspective of land use in their basins. The sediments in two rivers (referred to as NS and SK) were lacking in HAs, but the sediments of the others (referred to as SM and PO) contained relatively high levels of HAs. UV-vis spectroscopic characteristics suggested that the HAs in SM and PO contained components derived from humified materials. The PO and SM basins contain rice paddy fields, and TMAH-py-GC/MS analysis showed that the pyrograms of paddy soil HA had a similar character to those of PO-HA and SM-HA samples. Thus, the differences of the structural features can be attributed to the land use in river basins. The findings indicate that the properties of organic river sediments are heavily influenced by the type of land use in the river basins.

Keywords Humic acid, structural features, river sediment, land use, rice paddy field

Colloidal organic matter is an important factor in our understanding of the transport of trace metals by rivers to coastal areas and the characteristics of organic matter in the sediment, including the effect of humic substances on the transport of such metals. The structural features of humic acids (HAs) derived from surface sediments collected from the mouths of four rivers in northwest Hokkaido, Japan, were evaluated from the perspective of land use in their basins. The sediments in two rivers (referred to as NS and SK) were lacking in HAs, but the sediments of the others (referred to as SM and PO) contained relatively high levels of HAs. UV-vis spectroscopic characteristics suggested that the HAs in SM and PO contained components derived from humified materials. The PO and SM basins contain rice paddy fields, and TMAH-py-GC/MS analysis showed that the pyrograms of paddy soil HA had a similar character to those of PO-HA and SM-HA samples. Thus, the differences of the structural features can be attributed to the land use in river basins. The findings indicate that the properties of organic river sediments are heavily influenced by the type of land use in the river basins.

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Influence of Land Use on the Structural Feature of Sedimentary Humic Acids in Rivers in Northwest Hokkaido, Japan

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Materials and Methods

**Sampling sites and methods**

Surface sediments were collected in December 2016 near the mouths of the Nobusya River (NS, 43° 87′ 57″ N, 141° 58′ 56″ E), the Shumonbetsu River (SM, 43° 86′ 21″ N, 141° 56′ 45″ E), the Shokanbetsu River (SK, 43° 85′ 34″ N, 141° 51′ 29″ E) and the Ponotarumanai River (PO, 43° 84′ 88″ N, 141° 50′ 65″ E), as shown in Fig. 1. Although there is no formal name of the last river, we refer to it as the “Ponotarumanai River” based on the name of the bridge located at the river mouth. Meanwhile, the NS and SK rivers are rivers designated as second-grade. The stream lengths of NS, SM, SK and PO rivers were estimated to be approximately 20.1, 3.5, 24.6 and 3.9 km, respectively, by a measurement function of map issued by the Geospatial Information Authority of Japan (GSI). The surface sediment samples were collected (sampling depth: <3 cm) into nylon zipped bags that were first rinsed with the corresponding river water, and were transported to the laboratory in the frozen state. The PO river, which is a branch stream from the SK river, contributes to supply and drain agricultural water. Thus, to refer to it as the “Ponotarumanai River” based on the name of the bridge located at the river mouth. Meanwhile, the NS and SK rivers are rivers designated as second-grade. The stream lengths of NS, SM, SK and PO rivers were estimated to be approximately 20.1, 3.5, 24.6 and 3.9 km, respectively, by a measurement function of map issued by the Geospatial Information Authority of Japan (GSI). The surface sediment samples were collected (sampling depth: <3 cm) into nylon zipped bags that were first rinsed with the corresponding river water, and were transported to the laboratory in the frozen state. The PO river, which is a branch stream from the SK river, contributes to supply and drain agricultural water. Thus, to investigate the influence of the land use on the feature of the river sediment, surface soil of the rice paddy field located around the PO river (43° 50′ 40″ N, 141° 30′ 23″ E) was also collected in October 2018 into nylon zipped bags and stored in a frozen state until used in analysis.

**Preparation and structural analyses of humic acids**

The collected sediments and soil were freeze-dried and passed through a 2-mm mesh sieve to remove large particles. HAs were extracted from the sediment with aqueous 0.05 M NaOH (solid/liquid = 1:10, w/w) by shaking under the N₂ atmosphere for 24 h. An alkaline extract was centrifuged (10 000 rpm, 10 min) and the supernatant was then filtered (5A, ADVANTEC). The filtrate was acidified with concentrated HCl to pH 1.2 – 1.5. HAs were precipitated from the acidified extract by centrifugation (10 000 rpm, 10 min), and rinsed three times with 0.1 M aqueous HCl. To replace HCl with pure water, HAs were subsequently dialyzed using SpectroPor® 6 dialysis tubes (MWCO: 1000 Da) against pure water. After dialysis, powdered HAs were prepared by freeze-drying. The total acidity and carboxyl group content were determined by the Ba(OH)₂ and Ca(CH₃COO)₂ methods, respectively. UV-vis spectra of the HAs were recorded using a UV-vis spectrophotometer (JASCO V-630, Japan). The absorbivity at 600 nm for humic substances appears to be related to the development of conjugation in both aromatic and aliphatic structures, the conjugating which could be associated with the degree of humification. In this study, the degree of humification of the HAs were estimated by the absorbivities (L cm⁻¹ g⁻¹-HA) at 600 nm (\(E_{600}\)).

**Results**

The macroscopic structural characteristics of HAs

The yields, acidic functional group content and \(E_{600}\) values of the HAs are summarized in Table 1, where the HAs derived from the sediment collected at NS, SM, SK and PO are denoted as NS-HA, SM-HA, SK-HA and PO-HA, respectively. The yields of the NS-HA and SK-HA were low, while those of SM-HA and PO-HA were higher. The total acidic functional group content in the PO-HA (6.18 mmol g⁻¹) was higher than the corresponding values for the other HAs (3.76 – 4.21 mmol g⁻¹). In general, acidic functional group content of humic substances effects to metal binding capacity directly. The dominant component of the acidic functional groups for all of the HAs was the phenolic hydroxyl group content (ca. 67 – 76%). PO-HA contained considerably higher values in both the carboxyl and phenolic hydroxyl groups than the others. Figure 2 shows UV-vis spectra for the HAs. As shown in Fig. 2, the NS-HA and SK-HA samples were lighter in color than the other HAs.
The $E_{600}$ values for the SM-HA and PO-HA samples were considerably higher than those for the NS-HA and SK-HA samples (Table 1).

**Detail structural character of HAs**

The pyrograms of the HAs are shown in Fig. 3, and the 48 pyrolysat compounds that were identified are summarized in Table 2. Fatty acid methyl esters were designated to C$_x$y-Me, where $x$, $y$ and -Me indicate the number of carbon atoms, the number of double bonds and -methyl ester, respectively. Nitrogen-containing pyrolysat compounds are derived from proteins, nucleic acids and amino acids via decarboxylation, homolysis, cyclization and fragmentation. Peaks of Nos. 40, 41, 43 and 45, which are common in plants, animals and fungi, were detected in all HAs in this study. Aromatic moieties detected in pyrolysates of humic substances are mainly derived from lignin. Hexadecan-1-ol (No. 42, Fig. 3) and phytol (No. 44, Fig. 3) are likely the fragments of chlorophylls. Phosphoric acid trimethyl ester (No. 10, Fig. 3) is known as a pyrolysat compound of phospholipids. Methanesulfonic acid methyl ester (No. 6, Fig. 3) was detected only in the SM-HA and PO-HA samples (Table 2). There is no difference in the relative area of toluene (No. 4), which is regarded as a pyrolysat product of polyphenol or proteins. The conspicuous peaks of 3,4-dimethoxy-benzoic acid (No. 32) and 3,4,5-trimethoxy-benzoic acid methyl esters (No. 35) appeared in the SM-HA and PO-HA samples. These compounds are known as lignin derivatives. In the SM-HA and PO-HA, 4-methoxyphenyl- and 3,4-methoxyphenyl-3-(2-propenoic acid) methyl esters (No. 33 and 39), which are cinnamic acid derivatives, also appeared as remarkable peaks.

**Discussion**

The sediments obtained from the SM and PO rivers contained higher amounts of HAs in comparison with those from the NS and SK rivers (Table 1). This difference in HA yields can be attributed to the degree of humification in the sediments. The degree of humification can be estimated from UV-vis spectral data (Fig. 2). Fukushima et al. reported that the $E_{600}$ values of HA in compost derived from bark tips and conifer tips increased from 0.461 to 0.789 and from 0.256 to 0.963 (L cm$^{-1}$ g$^{-1}$), respectively, during 12 stages of composting. The values of the HAs derived from mature composts and natural peat soil were reported to be 0.933 - 1.32 and 0.707 (L cm$^{-1}$ g$^{-1}$), respectively.

The $E_{600}$ values of the HAs in the present study were quite small (0.135 - 0.554), suggesting that HAs in the surface sediments of the rivers were composed of relatively low humified compounds. Hence, comparatively more humified materials could be transferred into the SM and PO rivers.

Since the PO river originates from the SK river, the difference in structural features could be closely related to the land use difference in the PO river basin. Fruit farms and a relatively large park including a camping area are in the downstream basin of the SK river, whereas, the PO river was branched artificially from the SK river for use as agricultural water in rice paddies and flows into the sea via the rice paddies. Since the agricultural effluent from rice paddy fields flows directly into the river mouth in the basins of the PO river, not only dissolved organic matter, but also colloidal organic matter would be transported from the paddy areas and accumulated in the surface sediments. Organic matter derived from the paddies would influence the HA content and the structural features of these HAs and this would account for the higher value of HA content found in the PO river (Table 1). As rice paddies are also located in the basin of the SM river, the structural features of HAs from the SM river would be influenced by this type of land use as well. Rice paddies are also distributed around the basins of the NS river. The area of rice paddies around the NS river basin seems to be larger than those areas around SM and PO estimated by a GSI map. However, the flow rate of the NS river is considerably higher than those of the SM and PO, and many branches enter the NS upstream of the sampling point (Fig. 1). Two large rivers are located close to the sampling site of the NS river, and the type of land use around the two rivers are different. In addition, because the flow length for the NS river (ca. 20 km) is markedly longer than those for the SM and PO rivers (ca. 3.5 - 4.0 km), and the sediment of the NS river is sandy, the structural features of the SM-HA sample would be expected to be different from those of the SM-HA and PO-HA samples. Hence, the type of land use represents one of the reasons for why the characteristics of the SM-HA and PO-HA samples were different from those of the NS-HA and SK-HA samples.

The detailed structural information of HAs provides evidence in support of this conclusion. 2-Ethyl-hexanoic acid ester (No. 13) was detected mainly in NS-HA. This compound would be a component of an emollient or plasticizing agent, which are widely used in our daily life. As many rivers flow together to the NS river, it has a larger area of river basin (Fig. 1).
In addition, a residential area is distributed around this river. Meanwhile, the area around the SK river is less populated and the PO river mainly serves as an agricultural water resource. Thereby, the influence of organic matter in domestic water could appear in NS-HA. A large number of lignin derivatives were detected in the SM-HA and PO-HA samples (Table 2). The chemical structures of lignin units and their composition influence the mechanical properties of plant type. For example, sinapyl, coniferyl and p-coumaryl type lignin units dominate in lignins from hardwood, softwood and grasses/bamboo type plants, respectively. Lignin derivatives would be detected as tri-, di- or mono-methoxy benzoic acid methyl esters from humic substances by TMAH-py-GC/MS method. Cinnamic acid derivatives, such as Nos. 33 and 39 in Table 2, could appear in NS-HA.

### Table 2 Pyrolysate compounds in HAs detected by TMAH-py-GC/MS

| Peak No. | Identified compound                                      | Group | NS-HA | SM-HA | SK-HA | PO-HA |
|---------|---------------------------------------------------------|-------|-------|-------|-------|-------|
| 1       | Dimethylamino-acetonitrile                               | N     | n.d.  | n.d.  | 76.8  | n.d.  |
| 2       | 1H-Pyrrole                                              | N     | 15.5  | n.d.  | 31.9  |       |
| 3       | Methoxy acetic acid methyl ester                        | O     | 58.4  | 47.7  | 88.0  | 110   |
| 4       | Toluene                                                 | L     | 64.1  | 35.7  | 47.0  | 54.5  |
| 5       | N,N-Dimethyl-1,2-ethanediamine                          | N     | 44.9  | 23.4  | 25.6  | 34.0  |
| 6       | Methanesulfonic acid methyl ester                       | O     | n.d.  | 51.0  | n.d.  | 30.1  |
| 7       | 2,5-Dimethyl-1H-pyrrole                                 | N     | 23.9  | 14.3  | 24.0  | 27.8  |
| 8       | 1,2-Dimethylenzene                                      | O     | n.d.  | 2.47  | 16.7  | n.d.  |
| 9       | Methoxy-benzene                                         | L     | 29.8  | 7.01  | 9.59  | 10.4  |
| 10      | Phosphoric acid trimethyl ester                         | O     | 21.1  | 32.5  | 10.3  | 67.5  |
| 11      | Hexahydro-1,3,5-trimethyltriazine                        | N     | 7.18  | n.d.  | 17.6  | n.d.  |
| 12      | 1-Methoxy-4-methylbenzene                               | L     | 14.2  | 26.1  | 16.4  | 36.1  |
| 13      | 2-Ethyl-hexanoic acid methyl ester                      | F     | 70.8  | 7.31  | 2.15  | n.d.  |
| 14      | 4-Methoxyphenol                                         | L     | 10.0  | n.d.  | 10.4  | 6.41  |
| 15      | 2,3,4,5-Tetramethyl-1H-pyrrole                          | N     | 4.73  | n.d.  | 11.4  | n.d.  |
| 16      | 1-Ethyl-4-methoxybenzene                                | L     | n.d.  | 12.8  | 12.3  | 16.7  |
| 17      | 1,4-Dimethoxybenzene                                    | L     | n.d.  | 22.3  | 2.58  | 44.5  |
| 18      | Diethylhydrzone-2-butane                                | N     | 100   | 21.3  | 29.1  | 45.2  |
| 19      | Cycropentanpyrrolidine                                  | N     | n.d.  | 23.4  | 9.63  | 25.5  |
| 20      | 1,2-Dimethoxy-4-methylbenzene                           | L     | n.d.  | 8.98  | 7.51  | 8.39  |
| 21      | 1-Methyl-1H-indole                                      | N     | 12.4  | 20.0  | 3.86  | 13.4  |
| 22      | Diethyl-1-methyl-2,4-pyrimidinedione                    | N     | 23.8  | 23.0  | 12.5  | 14.4  |
| 23      | 1-Methyl-5-oxo-proline                                  | N     | 23.1  | 24.8  | 8.50  | 22.4  |
| 24      | 2-Methoxy-benzoic acid methyl ester                     | L     | 5.77  | n.d.  | n.d.  | 32.1  |
| 25      | 1,3-Dimethyl-1H-indole                                  | N     | n.d.  | 12.2  | 2.34  | 1.79  |
| 26      | 1,24-Trimethoxybenzene                                  | L     | n.d.  | 23.4  | 9.63  | 25.5  |
| 27      | 4-Methoxy-benzoic acid methyl ester                     | L     | n.d.  | 14.6  | 0.858 | 15.6  |
| 28      | 1,2,3-Trimethoxy-5-methylbenzene                        | L     | n.d.  | 14.5  | n.d.  | 12.1  |
| 29      | 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone      | N     | 23.5  | 19.9  | 10.3  | 10.9  |
| 30      | 1,3,5-Trimethyl-2,4-pyrimidinedione                     | N     | 40.3  | 8.79  | 17.0  | n.d.  |
| 31      | C12:0-Me                                                | F     | 6.22  | 13.4  | 3.44  | 6.31  |
| 32      | 3,4-Dimethoxy-benzoyl methyl ester                      | L     | 11.1  | 31.9  | 23.0  | 53.7  |
| 33      | 4-Methoxyphenyl-1-(2-propenoyl) acid methyl ester       | L     | n.d.  | 63.2  | 3.56  | 47.1  |
| 34      | 3,4,5-Trimethoxyacetophenone                            | L     | n.d.  | 18.3  | n.d.  | 15.7  |
| 35      | 3,4,5-Trimethoxy-benzoic acid methyl ester              | L     | 12.0  | 47.0  | 16.4  | 28.9  |
| 36      | C14:0-Me                                                | F     | 22.0  | 40.7  | 12.7  | 8.52  |
| 37      | Anteosin-C15:0-Me                                       | F     | 38.1  | 48.2  | 20.9  | 10.3  |
| 38      | C15:0-Me                                                | F     | 22.8  | 47.0  | 13.2  | 9.21  |
| 39      | 3,4-Methoxyphenyl-3-(2-propenoyl) acid methyl ester     | L     | n.d.  | 43.0  | 3.51  | 47.8  |
| 40      | C16:1-Me                                                | F     | 83.6  | 75.5  | 13.8  | 14.8  |
| 41      | C16:0-Me                                                | F     | 138   | 152   | 66.9  | 51.6  |
| 42      | Hexadecen-1-ol                                          | O     | 30.8  | n.d.  | 2.90  | 23.7  |
| 43      | C18:1-Me                                                | F     | 66.4  | 67.6  | 54.7  | 12.0  |
| 44      | Phytool                                                 | O     | n.d.  | 27.0  | n.d.  | 5.79  |
| 45      | C18:0-Me                                                | F     | 19.5  | 33.9  | 12.4  | 16.9  |
| 46      | C20:0-Me                                                | F     | n.d.  | 10.4  | n.d.  | 5.35  |
| 47      | C22:0-Me                                                | F     | n.d.  | 18.4  | 3.94  | 9.83  |
| 48      | C24:0-Me                                                | F     | n.d.  | 25.5  | 4.39  | 16.4  |
| ISTD    | C19:0-Me                                                | ISTD  | 100   | 100   | 100   | 100   |

| Total relative areas | NS-HA | SM-HA | SK-HA | PO-HA |
|----------------------|-------|-------|-------|-------|
| N                    | 319   | 168   | 260   | 202   |
| F                    | 464   | 540   | 208   | 162   |
| L                    | 147   | 369   | 163   | 455   |
| O                    | 110   | 161   | 118   | 237   |

a. N: Nitrogen-containing compounds, F: fatty acid methyl esters, L: lignin derivatives, O: others. b. Not detected.
recognized as characteristic lignin derivatives from cell walls of grasses. In PO-HA and SM-HA, the grass type lignin derivatives (Nos. 27, 33 and 39) were detected as characteristic pyrolysate compounds in lignin derivatives. To clarify the influence of the paddy fields, TMAH-py-GC/MS analysis of HA extracted from the paddy soil located around the PO river was carried out, as shown in Fig. 4. To compare the pyrogram of paddy soil HA with those of PO-HA and SM-HA samples, their pyrograms reversed upside down were also added (Fig. 4). The peaks of grass type lignin derivatives (Nos. 33 and 39) appeared clearly in paddy soil HA. In addition, the peaks of Nos. 46, 47 and 48 also appeared as characteristic peaks in the pyrogram of paddy soil HA. Even-numbered saturated fatty acid composed of 20–30 carbon atoms are found in plant lipids. The plant vegetation would be reflected in the components of soil lipids. C22:0 and C24:0 tend to be more abundant in soil under grassland than those of soil under trees. Rice cultivation would be largely reflected in the organic components in paddy soil, thus, the above pyrolysate compounds of grass lignin and fatty acids are regarded as the characteristics of paddy soil. The pyrolysate peaks in paddy soil HA also appeared in PO-HA and SM-HA samples; moreover, the shape of pyrograms of PO-HA and SM-HA was similar to that of paddy soil HA (Fig. 4). Thus, the detailed structural features provided that the pyrolysate compounds of grass derivatives in SM-HA and PO-HA could be the result of the influence of the drainage of rice paddies areas. The findings also showed that colloidal or dissolved organic matter derived from rice paddies would be easily deposited in the shorter rivers.

Conclusions

The structural features of humic acids derived from surface sediment in four rivers were evaluated by UV-vis and TMAH-py-GC/MS analyses. UV-vis spectroscopic characteristics could be associated with the degree of humification of the sediments, consistent with the yields of HAs. Molecular fragments produced by the pyrolysis of the HAs revealed their origin, provided information regarding the influence of land use and revealed the difference in the detailed structural features of the HAs. The high amount of grass derivatives would be expected to be enriched in the sediments by effluents from rice paddies. The findings reported herein show that the organic properties of the HAs are strongly influenced by the land use properties around the river basins in the Mashike, Hokkaido, Japan.

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