Mechanism of the Elution of Iron from a Slag-Compost Fertilizer for Restoring Seaweed Beds in Coastal Areas—Characteristic Changes of Steelmaking Slag and Humic Acids Derived from the Fertilizer during the Elution Process

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The characteristic changes in steelmaking slag and humic acids (HAs) derived from a slag-compost fertilizer and their relation to the elution of Fe were evaluated in tank tests in Mashike, Hokkaido and Tsushima, Nagasaki. Analyses of iron, nitrogen and phosphate in the eluate, changes in the chemical states on the surface of the steelmaking slag, and the macro-structural features of the isolated HAs were investigated during the test. Temporal changes in Fe concentrations in the tanks were consistent with data collected in previous studies. Analyses of the surface by ⁵⁷Fe Mössbauer spectroscopy showed that the concentration of Fe²⁺ in the fertilizer decreased and the ratio of Fe³⁺ increased, indicating that Fe³⁺ was preferentially eluted from the slag surface. The yields of HAs were significantly decreased during the test when steelmaking slag was mixed with compost. Changes in the UV-vis absorptivities indicated that de-aromatization had occurred. These results indicate that microorganisms that were present under these experimental conditions became activated by the mixing of compost with steelmaking slag, and are closely related to the continuous elution of Fe. The residual Fe in the fertilizer after the tests was investigated by analyzing seawater and the levels of extractable Fe. The trends for extractable Fe concentrations were consistent with the results obtained by ⁵⁷Fe Mössbauer spectroscopy and UV-vis spectra.

Keywords Iron elution, steelmaking slag, compost, humic acids, seawater, ⁵⁷Fe Mössbauer spectroscopy, UV-vis parameter

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Introduction

The depletion of seaweed beds (referred to as “barren ground”) has been a serious problem in coastal areas. One of the main causes of barren ground is a shortage of iron (Fe), nitrogen, and phosphorus, which are transported from terrestrial areas through rivers and also through submarine groundwater discharge.¹² In addition to the elimination of herbivorous sea creatures in order to restore seaweed beds, the installation of adhesive substrates, the production of seed and saplings,³ nitrogen and phosphate fertilization,⁴ supplementation with iron⁵,⁶ have been tested as possible strategies for solving this problem.

Our research has focused on the shortage of dissolved Fe (D-Fe) and we have developed a method for restoring seaweed beds by using a mixture of steelmaking slag (hereafter referred to as slag) and a compost containing humic substances (HSs).⁶,⁸ Fundamental studies for understanding the relationship between the Fe emitted from the mixture (hereafter referred to as slag-compost fertilizer) and the restoration of seaweed beds have been conducted.¹⁵ Regarding field tests, the effectiveness of Fe fertilization in Mashike-cho, Hokkaido, Japan was confirmed by evaluating seaweed bed distribution, water analyses including D-Fe, and calculations of the distribution of Fe in an experimental area.⁶,⁸ In the fundamental studies, the trends for the elution of Fe from a slag-compost fertilizer into seawater,⁷,¹⁰ the characteristics and bioavailability of Fe complexed with HSs,¹¹,¹⁴ and the contribution of bacteria to the elution of Fe,¹⁵ have been investigated. These results indicate that organic matter including HSs strongly accelerate the elution of Fe from steelmaking slag and also increase the bioavailability of Fe, and that both iron-
reducing microorganisms and humic acids (HAs) also contribute to the continuous elution of Fe from the slag.

However, the mechanisms at play for the elution of Fe from slag-compost fertilizer are not well understood yet. In particular, temporal changes in chemical states on the slag surface and the structures of HSs during Fe elution from the fertilizer have not been investigated in detail. The objective of this study was to examine the characteristic changes in steelmaking slag and HAs derived from the fertilizer, and their relation to the mechanism responsible for the elution of Fe by conducting tank tests using slag and compost over periods of six months so as to simulate actual field test conditions. During the tests, analyses of Fe, nitrogen (N) and phosphate (P) in the water were conducted. In the analyses of the slag-compost fertilizer, $^{57}$Fe Mössbauer spectroscopy was utilized in analyses of the slag surface. This method is a useful approach for characterizing the chemical states of Fe in a wide variety of environmental samples. Because Fe can exist in various chemical states, this may serve as a probe for monitoring the chemical changes that occur in the environment and elucidating their mechanisms.16 This technique has been employed in various investigations of lake sediments,17,18 marine sediments,19,20 sliding mud21 and pelagic mudstone.22 However, applications of this method to examine the chemical state of the surface of steelmaking slag have been limited in previous studies. Regarding analyses of HA, the macrostructural features of isolated HAs were evaluated by UV-vis spectra. In addition, residual Fe in the samples, including slag-compost fertilizer, was investigated by analyzing seawater extractable Fe concentrations in the samples.

**Experimental**

**Tank test**

Tank tests were conducted using 300 L tanks in Mashike-cho, Hokkaido, Japan (N43° 8′36″, E141° 60′46″) and at the Tsushima Aquaculture Center in Nagasaki Prefecture, Japan, respectively under the same conditions. The following three samples were used in the experiments.

- S-sample: 3000 g slag (the volume is defined as $V_0$)
- C-sample: Compost (= $V_0$)
- SC-sample: Mixture of slag (= $V_0/2$) and compost (= $V_0/2$)

The C-sample contained compost in a volume equal to that of the slag in the S-sample (i.e., 3000g; $V_0$), whereas the SC-sample ($V_0$) contained the equivalent of equal volumes of half of the S-sample and C-sample. The surface of the slag was covered with CaCO$_3$ by carbonation to reduce alkaline elution.3,11 The compost was produced from thinnings, as described in a previous report.11 The particle sizes of the slag and compost were <0.45 μm seawater collected from the area near the experimental locations. Although six tanks were used at each site, we divided them into two groups so as to understand the temporal changes in the chemical states in the slag and the compositions in the composts. In one group, the duration of the test was for three months for three samples and in the second group, the duration was for six months for three samples. The collected samples were packed in plastic bags in which the inner space contained nitrogen gas so as to not alter the conditions of slags and composts. These samples were transported to our laboratories in frozen states. Water samples were collected once per month from all the tanks. Raw seawater and filtered (φ <0.45 μm) seawater with nitric acid added were used for the analyses of total Fe (T-Fe) and D-Fe, respectively. Filtered (φ <0.45 μm) seawater samples were also transported to the laboratory in the frozen state for N and P analyses.

**Analytical methods**

T-Fe and D-Fe were analyzed by spectrophotometry using an absorption photometer (UV-2700, SHIMADZU) and nutrient salts, including NH$_4$-N, NO$_2$-N, NO$_3$-N and PO$_4$-P, were determined with an autoanalyzer (Bran + Luebbe II). The pH, electric conductivity (EC) and dissolved oxygen (DO) concentration were measured using pH, EC and DO meters, respectively.

$^{57}$Fe Mössbauer spectra were obtained by a TOPOLOGICAL SYSTEMS MFD-110D spectrometer using a 1.11 GBq $^57$Co/Rh source at room temperature. Curve fitting of the obtained spectra was performed using the least-squares method, assuming that the spectra were composed of peaks with Lorentzian line shapes. The intensity and half width of the peak within each quadrupole doublet were constrained to be equal. By comparing the Mössbauer parameters to standard data, we were able to identify the constituent iron species. The relative content of the individual Fe species in an analyzed sample was calculated from the peak area in the Mössbauer spectrum, assuming that all species have the same recoil-free fractions. Isomer shifts were expressed with respect to the centroid of the spectrum of a metallic iron foil.

HAs were extracted from the freeze-dried samples based on the protocols of the International Humic Substances Society (IHSS), details of which are described elsewhere.11,12,23 The extracted and purified HAs were freeze-dried to obtain powdered samples. The yields of the HAs were calculated from the dry-weight of powdered HA divided by that of the initial sample taken for extraction. The UV-vis absorances of the HAs, which are prepared to 0.020 g-HA in 0.1 M borate buffer (pH 8.00 ± 0.02), were measured by means of a U3010 spectrophotometer (Hitachi, Japan). The value of $E_i$ means the absorptivity (L cm$^{-1}$ g$^{-1}$ C) at arbitrary wavelength $i$ (nm) calculated by the following equation:

$$E_i = \frac{\text{Absorbance} \ (\text{cm}^{-1}) \ at \ i \ (\text{nm})}{[\text{HA}] \ (\text{g} \ L^{-1}) \times \%C/100}$$

where %C represents the total carbon percentage in corresponding HA, values which were calculated from the TOC values of 0.1 g-HA L$^{-1}$ in the borate buffer by a TOC-V CNS analyzer (Shimadzu, Japan).

**Determination of seawater extractable Fe concentration**

A portion of a 1-mL volume of a dried sample was mixed with 40 mL of artificial seawater (Supporting Information: Table S1) (pH 8.0 ± 0.02) in a 50-mL poly-centrifuge tube, after which
the mixture was shaken for 24 h in the dark at room temperature (20 ± 3°C). The sample was centrifuged (10000 rpm, 10 min and 5°C) after the incubation and the supernatant was then passed through a 0.45-μm cartridge filter (ADVANTEC). Then, 10 mL portions of the filtrate were transferred to each of three 15-mL centrifuge tubes, which were used for measuring the background, the concentration of dissolved Fe²⁺ and total Fe species. A 100-μL aliquot of 10 mM aqueous 1,10-phenanthroline (o-Phen) was added to the samples for dissolved ferrous and total Fe, after which 88 mg of ascorbic acid was dissolved in the sample for the total dissolved Fe. To fully react o-Phen with Fe²⁺, these samples were incubated overnight in the dark. A 1 mL aliquot of 1 M aqueous NaClO₄ was added to the samples, after which the Fe(II)-(o-Phen)₃ complex was isolated by 3 – 4 extractions with 1 mL portions of chloroform. To concentrate and adjust the volume of the sample, the solvent was removed under a stream of N₂ gas at 75°C and the dried sample was then dissolved in 1 mL of 50% EtOH. After centrifugation (3000 rpm, 5 min), the Fe(II)-(o-Phen) concentration in the supernatant was determined based on the absorbance at 510 nm using a V-630 spectrophotometer (JASCO, Japan) with a 1-cm pass length quartz micro cuvette.

Results and Discussion

Trend of Fe, N and P elution

Figure 1 shows the temporal changes in concentrations of T-Fe, D-Fe, dissolved inorganic nitrogen (DIN: NH₄-N, NO₂-N, NO₃-N) and PO₄-P during the experiments in Mashike and Tsushima. The T-Fe concentrations in Mashike were found to be approximately more than twice as high as those in Tsushima in all samples. This is not surprising, since the tanks in Mashike were located outdoors and those in Tsushima were located indoors, and the seawater was not filtered and flowed directly into the tanks in Mashike. Other factors including particles and some external contaminants could influence the temporal changes of Fe, N and P concentrations. These are the reasons why the T-Fe concentrations in the tank for the SC-sample were the same as or lower than those in the other tanks. However, the D-Fe concentration in the SC-sample was higher than those in the S-sample and the control, except in a few sampling cases in Mashike. This indicates that the amount of Fe eluted from the fertilizer is increased when it is mixed with slag. Regarding the test in Tsushima, the T-Fe concentrations in the SC-sample were

Fig. 1 Temporal changes in concentrations of T-Fe, D-Fe, DIN, NO₂ + NO₃ and PO₄-P in (a) Mashike and (b) Tsushima.
constantly the highest for four months, except for August 2016, and the D-Fe concentrations in the SC-sample were higher than those in the S-sample and the control except in September 2016. These results are consistent with data reported in previous studies.\textsuperscript{7,8} Fe elution rates (μg-Fe/g-sample/d) of three samples were estimated by the Fe elution test on a small scale using 100 mL actual seawater and 0.37 slag for the S-sample. Fe was analyzed with no filtration and nitric acid addition. Estimated Fe elution rates of the S-sample and SC-sample were 0.14 and 0.76 μg-Fe/g-sample/d, respectively. Fe concentrations in the SC-sample condition had been over three times larger than those in the control, and more than twice as large as those in the S-sample after 3 months. This indicates that the difference of Fe concentrations between SC-sample and control or S-sample in this study was small. The reason was that Fe contaminations were considered to be larger than in the previous study since all the tanks had no lids and compositions of seawater were not constant through the seasons.

The N and P concentrations in the SC-sample were higher than those in the S-sample and the control in Tsushima. The highest concentrations of N and P were observed in October 2016. The DIN concentration of the SC-sample was twice as high as that of the control, while the PO\textsubscript{4}-P concentration of the SC-sample was almost 5 times greater than that of the control. These results indicate that DIN and PO\textsubscript{4}-P were mainly eluted from the composts. However, the trends for temporal changes appear to be different from those in T-Fe and D-Fe. Meanwhile, the trends for N and P in Mashike were not consistent with the corresponding values for Tsushima. The N and P concentrations after three months for the S-sample and the control were higher than those in the SC-sample. A possible reason is that the tanks in Mashike were located outdoors and may have become contaminated with fish and sea urchins that were transported there by birds in the autumn season.

Although these trends were approximately consistent with previous studies, the findings failed to provide new information for understanding the mechanism responsible for the continuous elution of Fe from the slag-compost fertilizer. This indicates that the analyses of temporal changes in the slag surface and structural changes in the HAs are important issues.

**Chemical states of iron in steelmaking slags**

Figure 2 shows a representative \textsuperscript{57}Fe Mössbauer spectrum obtained by the analysis of the SC-sample in Mashike after 6 months. The dots imply raw data and the colored lines indicate the fitted spectra for α-Fe, Magnetite (a), Magnetite (b), Fe\textsuperscript{2+} and Fe\textsuperscript{3+}. Magnetite appears to overlap with two sextets because magnetite contains two iron chemical states. These four iron species were confirmed to be present in both the S-sample and SC-sample in Mashike and Tsushima. The relative contents of each iron species in the slags are listed in Table 1. The symbol of the sample name is represented by the location, material and installation period. The first characters “M” and “T” denote “Mashike” and “Tsushima”, respectively. “S” and “SC” denote “slag (= S-sample)” and “slag + compost (= SC-sample)”, respectively. The numbers “3” and “6” indicate 3 and 6 months after the start of the experiments, respectively. For example, “MSC6” corresponds to the SC-sample after 6 months in Mashike.

In Mashike, the ratios of Fe\textsuperscript{2+} in MS and MSC were decreased and the Fe\textsuperscript{3+} ratios were increased from 3 to 6 months. The changes in the Fe\textsuperscript{2+} and Fe\textsuperscript{3+} ratios at the Tsushima site from 3 to 6 months showed the same trends as were observed at the Mashike site. These results indicate that Fe\textsuperscript{2+} was preferentially eluted from the slag surface. The increase in the relative ratio of Fe\textsuperscript{3+} is considered to be mainly due to the elution of Fe\textsuperscript{2+}, but the possibility of oxidation by dissolved oxygen cannot be excluded. No significant difference was found between the trends for the S-sample and SC-sample, but when focusing on the value of Fe\textsuperscript{3+}, the relative ratio of Fe\textsuperscript{3+} increased in both MSC and TSC compared to MS and TS, respectively for both 3 and 6 months. In addition, the tendency for α-Fe and magnetite was also different between the S-sample and SC-sample, and the

**Table 1 Relative contents (%) of each iron species in the steelmaking slag**

| Sample | α-Fe (%) | Magnetite (a) (%) | Magnetite (b) (%) | Magnetite (Total) (%) | Fe\textsuperscript{2+} (%) | Fe\textsuperscript{3+} (%) |
|--------|---------|------------------|------------------|-----------------------|----------------|----------------|
| MS3    | 11.2    | 9.4              | 8.7              | 18.1                  | 46.1           | 24.7           |
| MS6    | 12.8    | 10.3             | 12.3             | 22.6                  | 35.3           | 29.3           |
| MSC3   | 9.7     | 7.0              | 10.2             | 17.2                  | 46.8           | 24.6           |
| MSC6   | 11.6    | 9.5              | 10.5             | 20.0                  | 35.6           | 32.8           |
| TS3    | 14.7    | 10.4             | 15.2             | 25.6                  | 36.6           | 23.1           |
| TS6    | 14.8    | 13.2             | 8.8              | 22.0                  | 30.8           | 32.4           |
| TSC3   | 11.7    | 9.1              | 11.0             | 20.1                  | 38.6           | 29.7           |
| TSC6   | 11.6    | 9.8              | 7.9              | 17.7                  | 33.1           | 37.7           |
between microbes and slag. This is also another main factor. Furthermore, a previous study found that the microorganism, E. oxidotolerans, is capable of eluting Fe from the slag and humic substances and would function as an electron shuttle. This method for applying fertilizer is thought to be one of the main reasons for continuous elution of Fe. Additionally, the fact that Fe was continuously eluted for several months after the fertilizing experiment (SC0). These results indicate that HA fractions were clearly decreased by fertilization with slag.

Structural changes in HAs during Fe fertilization

Weight density, yields and UV-vis parameters for the materials are summarized in Table 2. No HA was extracted from the S-samples, regardless of the fertilizing. Weight densities and yields of HAs for the C-samples, such as MC and TC, were slightly decreased with the fertilization, suggesting fine compost particle and humic fraction flown out from the initial material. The values for weight densities and HA yields for C0 and SC0 per volume of compost did not change in view of the compost content. In both cases of MSC and TSC, however, the values of densities and yields were slightly increased and significantly decreased, respectively, in comparison to those values before the fertilizing experiment (SC0). These results indicate that HA fractions were clearly decreased by fertilization with slag.

UV-vis spectra for the HAs are shown in Fig. S2. The shapes of UV-spectra are similar among the samples, and those for the initial HAs were not significantly changed even if it was extracted from MSC-samples (Fig. S2(A)). However, the absorbivities of the MSC samples were decreased slightly compared to that of the initial sample (SC0), and those for the MC samples (Fig. S2(B)). The change was also observed in the case of the Tsushima samples (Fig. S2(C)). These results support the above-mentioned changes. The log $E_{250}$ value and $E_{280}/E_{220}$ ratio have been positively correlated with the aromatic contents and the degree of substitution in the aromatic rings by O-containing functional groups, respectively. The value of log $E_{280}/E_{600}$ has been inversely correlated with molecular size, and been correlated positively with the aromatic contents and the degree of substitution in the aromatic rings by O-containing functional groups, respectively. The aromatic compounds in HAs appear to have undergone degradation by fertilizing the compost with slag, in particular, the aromatic rings substituted by O-containing functional groups were decomposed. The value of log $E_{280}/E_{600}$ for SC-HAs, in contrast to C-samples, showed a trend toward decreasing with increasing fertilization period. Some studies demonstrated the bio-degradation of aromatic compounds, such as lignin and polycatechol, under anaerobic conditions. In their study of anaerobic decomposition of HAs provided an interactive result that carboxylic groups in HAs were decomposed by an anaerobic bacterium. Considering the UV parameters and the result of previous reports, it can be assumed that the decrease in the log $E_{280}/E_{600}$ value of SC-HAs after the fertilization was caused by the removal of O-containing functional groups as a result of bacterial decomposition, rather than an increase in molecular size. And also, a significant decrease in HA yields supported the hypothesis that the bio-degradation had occurred in the SC-samples during the 6-month tank test. Thus, changes in the structural properties of SC-HAs by fertilization can be attributed to the activation of anaerobic bacteria, and these findings support the results of Fe analysis, indicating that Fe was eluted from the slag-compost fertilizer by Fe reductive microorganisms.

The possibility that microorganisms were present and contributed to this process under these experimental conditions cannot be excluded and such organisms could have been activated by the mixing of the compost with slag, and also could contribute to the decomposition of the HAs. Such microorganisms are thought to utilize Fe oxide compounds for their anaerobic respiration. It is thought that HAs in the compost are capable of activating a microorganism and this could be an important factor for reductive elution of Fe from the slag in seawater.

Determination of seawater extractable Fe concentration

The Fe concentrations including T-Fe and Fe$^{2+}$ extracted from the samples are shown in Fig. 3. The trend in temporal changes for the extracted Fe concentrations from the S-sample indicates that the amount of soluble Fe appears to be gradually increased during the tank tests. This can be attributed to the fact that the characteristics of the surface of the slag were changed. Regarding the C-sample, no significant differences were found before and after the experiment. However, soluble Fe, especially T-Fe, was found to be increased. This result corresponds to the fact that Fe was continuously eluted for several months after the start of the experiment, even though the amount of Fe contained.
in the compost is much smaller than that in the slag. The concentrations of extractable T-Fe and Fe$^{2+}$ after 3 and 6 months were less than that in the initial conditions for SC-sample. A possible reason is that the amount of Fe eluted from the SC-sample was larger than that in S-sample and the residual Fe in the slag of the SC-sample was less than that in the S-sample.

Figure 4 shows the ratios of Fe$^{2+}$ content in the corresponding total Fe derived from the results shown in Fig. 3. No characteristic results were obtained in terms of the S-sample and C-sample. However, the ratios of Fe$^{2+}$ content in the SC-sample after 3 and 6 months were higher than that in the initial conditions compared to those in the S-sample and C-sample. This result suggests that the conditions for the SC-sample readily promoted the reduction of Fe on the surface of the slag. This consideration is consistent with the results of the analyses of the chemical states on the slag surface and HAs structures.

Conclusions

The characteristic changes of steelmaking slag and HAs derived from a slag-compost fertilizer and their relation to the mechanism for the elution of Fe were investigated by conducting tank tests in Mashike and Tsushima. Water analyses including for Fe, nitrogen and phosphate were conducted during the tests. The change in chemical states on the surface of the slag was investigated by SSFe Mössbauer spectroscopy. The macro-structural features of the isolated HAs were evaluated by their UV-vis absorptivities. In addition, residual Fe in the fertilizer after the tests was investigated by analyzing the concentrations of seawater extractable Fe in the samples.

Temporal changes in Fe concentrations were reasonably consistent with findings reported in previous studies even though the characteristics of T-Fe concentrations at the Mashike site was slightly different because the tanks were located outdoors. The ratios of Fe$^{2+}$ in the S-sample and SC-sample were decreased and the Fe$^{3+}$ ratios were increased from 3 to 6 months. These results indicate that Fe$^{3+}$ was preferentially eluted from the slag surface and the reduced state is thought to be an advantage for the elution of Fe from the fertilizer. Analyses of HA structures show that HAs both at the Mashike and Tsushima sites were decomposed by mixing slag with compost. Based on these results, it appears that microorganisms were present and played a role in these experimental conditions by becoming activated when the compost was mixed with the slag, and are closely related to the decomposition and structural alteration of the HAs during the period of the experiments. The trends for seawater extractable Fe concentrations are consistent with and support the results for the speciation of Fe and the structural alterations of HAs.

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Supporting Information

Table S1 Composition of the artificial seawater. Figure S1 Experimental apparatus. Figure S2 UV-vis spectra for HAs

Table 2 Weight densities of dried fertilizer, yields and UV-vis parameters of HAs derived from the fertilizer

| Types of fertilizer | g mL$^{-1}$ | Yields/ g L$^{-1}$ | UV-vis parameters$^a$ |
|---------------------|------------|-------------------|-----------------------|
|                     |            |                   | log $E_{254}$/ $E_{220}$ | log $E_{400}$/ $E_{600}$ |
| S0                  | 1.69       | —                 | —                     |
| C0                  | 0.672      | 28.6              | 1.69                  | 0.527                  | 0.656                  |
| SC0                 | 1.27       | 13.4              | 1.68                  | 0.530                  | 0.647                  |
| MS3                 | 1.69       | —                 | —                     |
| MS6                 | 1.71       | —                 | —                     |
| MC3                 | 0.637      | 21.2              | 1.68                  | 0.536                  | 0.647                  |
| MC6                 | 0.655      | 21.7              | 1.68                  | 0.536                  | 0.648                  |
| MSC3                | 1.45       | 6.83              | 1.63                  | 0.494                  | 0.613                  |
| MSC6                | 1.50       | 3.58              | 1.60                  | 0.463                  | 0.594                  |
| TS3                 | 1.68       | —                 | —                     |
| TS6                 | 1.71       | —                 | —                     |
| TC3                 | 0.651      | 24.2              | 1.71                  | 0.550                  | 0.651                  |
| TC6                 | 0.639      | 23.2              | 1.70                  | 0.544                  | 0.659                  |
| TSC3                | 1.52       | 2.94              | 1.62                  | 0.463                  | 0.602                  |
| TSC6                | 1.51       | 1.84              | 1.59                  | 0.443                  | 0.584                  |

$^a$ The value was measured by means of a 50-mL volumetric flask.
$^b$ The values were estimated from the absorbances (cm$^{-1}$) of 0.02 g-HA L$^{-1}$ in 0.1 M borate buffer (pH 8.0).
—, Not extracted.
(20 mg L⁻¹ in 0.1 M borate buffer, pH 8.00). (A), (B) and (C) show the spectra of the initial HAs of Mashike samples and Tsushima samples, respectively.

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