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## COMPARISON OF CHEMICAL CHARACTERISTICS OF DISSOLVED ORGANIC MATTER IN RIVER WATER FLOWING THROUGH PEATLANDS IN SARAWAK, MALAYSIA AND EASTERN HOKKAIDO, JAPAN

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### SUMMARY

Composition and structural characteristics of dissolved organic matter in river water were compared between tropical (Sarawak, Malaysia) and cool temperate (eastern Hokkaido, Japan) climates. DOM samples were concentrated and purified by using a tangential flow filtration system, Millipore Pellicon Mini2 modules. Molecular size distribution, elementary composition, sugar composition, and fluorescence characteristics were compared between the samples. According to the size exclusion chromatography, the average molecular weight was larger and the molecular weight distribution was narrower in the Sarawak samples than those in Hokkaido samples. Protein like and sugar components were more depleted in Sarawak samples. Fluorescence characteristics suggested the larger contribution of peat humic substance in Sarawak samples than in Hokkaido samples.

**Keywords:** *dissolved organic matter, Sarawak, Hokkaido, size exclusion chromatography, PARAFAC analysis*

### INTRODUCTION

Dissolved organic matter (DOM) in river water has diverse functions related to the dissolution and transportation of trace elements by complex formation, the supply of nutrients to aquatic biota and microbes, and the transportation and storage media in the carbon cycle and contributes to the biogeochemical processes in the local ecosystem. However, these functions of DOM differ significantly according to its composition and structure, which vary according to the natural environment where it is formed. Therefore, it is important to investigate the DOM formed under different environments. In this study, we aimed at the comparison of DOM formed in the tropic wetland and in the cool temperate wetland, selecting Sarawak in Malaysia and the eastern estuarial area, Hokkaido, Japan. This is a part of a series of studies aimed to clarify the dynamics of DOM in wetlands from different climates (Watanabe *et al.*, 2012).

### MATERIALS AND METHODS

#### *Sites of sample collection*

Tropical river samples were collected at two sites along Mukah river in Sarawak, Malaysia. One site is a branch river Lebang (N2° 51.118', E112° 09.990') flowing through a relatively unaffected wetland swamp forest. Another site was a branch river Bakong (N2° 46.200', E112° 08.139') which was flowing through a newly cleared wetland swamp forest. We visited these places two times, December in 2007 and August in 2008, and collected the winter and summer samples. River water samples under cool temperate climate were collected at three wetland sites in eastern Hokkaido, Japan. These wetlands were Kiritappu (N43° 05.092', E145° 03.565') in Hamanaka town, Chiraikaribetsu (N43° 06.458', E144° 53.526') in Akkeshi town, and Kimontou (N42° 37.173', E143° 28.698') in Taiki town. These sites were visited several times in 2007 – 2009 and samples were collected in different seasons.

#### *Method of DOM sample preparation*

River water samples were passed through a 0.4 µm glass-fiber filter (Advantec GB-140), then the fraction with particle size < 0.22 µm and molecular weight > 1 kD was concentrated using a tangential flow filtration system, Millipore Pellicon Mini2 modules. DOM sample was purified by mixing with ultrapure water and treating

with > 1 kD module. The concentrated sample was passed through a 0.2 µm Millipore membrane filter and freeze-dried.

#### *Elementary composition*

Elementary compositions (C, H, N, O, S) of the DOM samples were determined by dry combustion method at the element analysis laboratory of Hokkaido University.

#### *Sugar composition*

Sugar composition of the DOM samples were determined by capillary gas chromatography following the alditol-acetate method.

#### *Size exclusion chromatography*

DOM sample (10 mg) was solubilized into 10 mL of 0.033 M borate buffer (pH 9.2, ionic strength 0.1). This solution was passed through a short column of Chelex 100 resin (Bio-Rad). One mL of the eluate was loaded on Toyopearl HW65S (i.d. 10 mm x 450 mm length) or Toyopearl HW50F column (i.d. 10 mm x 480 mm length), and eluted with the 0.033 M borate buffer. The eluate was collected in 5 mL fractions using a fraction collector. The absorbance of the eluate was also detected continuously at 250 nm using a flow cell UV detector unit (Amersham Biosciences UV-1). Calibration of molecular weight was done with SIGMA MW-GF-70 kit composed of blue dextran (> MW  $2.0 \times 10^6$ ), albumin (MW 66,000), carbonic anhydrase (MW 29,000), cytochrome (MW 12,400), and vitamin B12 (MW 1,355). Folic acid (MW 441) and tryptophan (MW 204) were also analyzed to cover the low molecular weight range.

#### *Analysis of the eluted solution*

Various constituents in the eluates fractionated by the fraction collector were determined by colorimetry. Carbon contents: after dichromate oxidation, sugars: with phenol sulfuric acid method and anthron method, phenol: with Folin-Ciocalteu's reagent. Protein like compounds were determined by Bio-Rad protein assay (Bradford method), and the absorbance at 250, 280 and 400 nm were determined in a quartz cell using a UV-Vis spectrophotometer (Shimadzu UV1240).

#### *EEM-PARAFAC analysis*

Excitation-emission matrix (EEM) fluorescence spectra of the DOM samples and their SEC fractions were determined using a spectrophotometer (FluoroMax-3, Horiba Jobin Yvon) according to Maie *et al.* (2014). Data of fluorescence spectra were statistically analyzed further using parallel factor analysis (PARAFAC) to obtain five representative components from the spectra as shown by Maie *et al.* (2014).

## **RESULTS AND DISCUSSION**

#### *Elementary composition*

River water DOM samples collected at two sites in Mukah, Sarawak, compared with those from the three wetland areas in north-eastern Hokkaido, Japan, showed lower H, N, S %, lower H/C, and higher C/N and N/S ratios, which suggested that tropical DOM was in more oxidized state and depleted of protein-like components.

#### *Sugar composition*

Sugar contents in DOM as well as Xylose/Mannose ratio were lower in Sarawak samples than in Hokkaido samples, which suggested that sugar components derived from plants were more decomposed in tropical DOM samples. The Xyl/Man, Ara/Glc, Gal/Glc and Fuc/Glc ratios tended to be high in early summer in Hokkaido samples, which may reflect the vigorous plant growth in summer in Hokkaido. In the Mukah river DOM samples, little change was observed in sugar composition between wet season (December, 2007) and dry season (August, 2008) samples.

#### *Molecular size distribution by GPC*

According to the size exclusion chromatography, the average molecular weight was larger and the

molecular weight distribution was narrower in the Sarawak samples than those in Hokkaido samples. Mukah river DOM samples had maximum peaks at MW 26,400– 27,900, while DOM samples from eastern Hokkaido at MW 14,900– 19,100 when eluted on Toyopearl HW-50F column. Mukah DOM samples showed no excluded fraction on Toyopearl HW-50F column which cover the MW to 80,000, while Hokkaido samples showed large excluded fraction. On Toyopearl HW-50S column which cover the MW to 5000,000, excluded fractions did not appear even for most of Hokkaido samples.

#### *Characterization of size fractions by various colorimetric methods*

Fractions eluted from the Toyopearl HW-50F column were subjected to various colorimetric methods for the ratio of absorbance at 400 nm and 250 nm (degree of humification), phenolic substance, protein-like substance, and saccharides.

Ratio of the absorbance at 400 and 250 nm (OD 400/250) increases when dark brown color of the DOM becomes stronger, suggesting the larger contribution of longer conjugated double bond system. OD 400/250 of Mukah samples were larger than Hokkaido samples in the whole range of molecular size distribution, reflecting the darker color of the river water in Mukah samples. Both in Mukah and Hokkaido samples, OD 400/250 was highest in the largest MW fractions, but decreased when MW became smaller and attained to the lowest value at the MW lower than the peak MW. After attaining to the lowest value, OD 400/250 increased again with decrease in molecular weight. From this result, it is suggested that the fraction with high degree of humification are contained in the very large and very small size fractions and not in the dominant fraction.

Phenolic substance determined by Folin-Ciocalteu's reagent showed the same peak as the elution curve by the absorbance at 250 nm. Concentration of phenolic substance was higher in the Mukah samples than in Hokkaido samples. When the elution curves by 250 nm and phenolic substances were compared, phenolic substance had larger contribution in the permeated fraction than in the excluded fraction, suggesting that phenolic substance contributed to the smaller MW fractions of DOM.

Protein-like substance showed a broad molecular size distribution and a relatively larger contribution in the macro size components of the DOM. The peak of the elution curve was shifted to larger molecular size compared with that by 250 nm absorbance. The shapes of the elution curve for protein-like substance were more complex than those by 250 nm absorbance or by phenolic substance, and it showed also shoulders and small peaks in the elution curves. This suggests that protein-like substances are contained in various components with different MW sizes in the DOM. Mukah river DOM samples showed a single peak mode and stronger response to protein-like substance compared with those of Hokkaido DOM samples.

As Bradford reagent for protein is also reactive to phenolic substances, its interpretation should be made carefully and we expressed it as "protein like substance". However, the shapes of the elution curves for protein-like substance and phenolic substance were very different, and we may affirm the larger contribution of protein in the color reaction by the Bradford reagent.

Sugars were determined by phenol sulfuric acid method and anthron method, where phenol sulfuric acid method showed stronger response in the DOM. Molecular size of sugars was shifted to larger MW compared with those by 250 nm absorbance or by phenolic substances. Sometimes, the intensity in the excluded fraction was much larger than that in the permeated fraction for Hokkaido DOM samples. The Mukah river DOM samples showed a single peak molecular size distribution, with larger molecular size compared with the permeated fraction of the Hokkaido samples. Significant seasonal change in the molecular size distribution was observed both in Mukah and Hokkaido samples.

#### *PARAFAC analysis*

Based on the PARAFAC analysis of three dimensional fluorescence spectra, five components (fulvic acid like, humic acid like, microbial origin, photo-degradation products, and protein like) were characterized. "Humic acid like" component was higher in Sarawak samples, while "fulvic acid like", "microbial origin", "photo-degradation products" and "protein like" components were higher in Hokkaido samples. Fluorescence Index, the ratio of emission intensity (470 nm/ 520 nm) at 370 nm excitation, was lower in Mukah river DOM samples than in Hokkaido samples. This index decreases with the contribution of organic matter derived from soil or peat, while increases with the contribution of organic matter from microbial origin. Due to high temperature, microbial intermediate products are decomposed faster and the contribution of organic matter from peat is larger in the tropical river DOM samples and the opposite tendency may be applied to the DOM formed under cool temperate climate.

#### *Comparison of the river DOM in wetlands under tropical and cool temperate climates*

Compared with cool temperate river DOM, tropical river DOM was characterized by more oxidized state

from the elementary composition, and depleted of protein-like and sugar components. The average molecular weight was larger and the molecular weight distribution was narrower. It was richer in humic components and not susceptible to seasonal change in the tropical river DOM.

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