



Short Communication

Contributions of humic substances to the dissolved organic carbon pool in wetlands from different climates

Akira Watanabe^{a,*}, Kunio Moroi^a, Hiromu Sato^a, Kiyoshi Tsutsuki^b, Nagamitsu Maie^c, Lulie Melling^d, Rudolf Jaffé^e^a Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan^b Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan^c Faculty of Environment and Veterinary Sciences, Kitasato University, Towada, Aomori 034-8628, Japan^d Tropical Peat Research Laboratory Unit, Chief Minister's Department, Jalan Badruddin 93400, Kuching, Sarawak, Malaysia^e Southeast Environmental Research Center and Department of Chemistry & Biochemistry, Florida International University, 3000 NE 151 Str., Marine Sciences Building, North Miami, FL 33181, USA

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ABSTRACT

Wetlands are an important source of DOM. However, the quantity and quality of wetlands' DOM from various climatic regions have not been studied comprehensively. The relationship between the concentrations of DOM (DOC), humic substances (HS) and non-humic substances (NHS) in wetland associated sloughs, streams and rivers, in cool temperate (Hokkaido, Japan), sub-tropical (Florida, USA), and tropical (Sarawak, Malaysia) regions was investigated. The DOC ranged from 1.0 to 15.6 mg C L⁻¹ in Hokkaido, 6.0–24.4 mg C L⁻¹ in Florida, and 18.9–75.3 mg C L⁻¹ in Sarawak, respectively. The relationship between DOC and HS concentrations for the whole sample set was regressed to a primary function with y-intercept of zero ($P < 0.005$) and a slope value of 0.841. A similar correlation was observed between DOC and NHS concentrations, with a smaller slope value of 0.159. However, the correlation coefficient of the latter was much larger when the data was regressed to a logarithmic curve. These observations suggest the presence of a general tendency that the increased DOC in the river waters was mainly due to the increased supply of HS from wetland soils, whereas the rate of the increase in the NHS supply has an upper limit which may be controlled by primary productivity.

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1. Introduction

Dissolved organic matter (DOM) in the hydrosphere is imported from terrestrial environments (soils and higher plants), leached from sediments, deposited from the atmosphere, and generated through primary productivity. Watershed soils are generally considered as the most important source of the fluvial DOM (Otero et al., 2003; Bengtsson and Törneman, 2004). Recently, increase in the dissolved organic carbon (DOC) concentration in river or lake waters has been observed throughout North America and Europe (Reynolds and Fenner, 2001; Evans et al., 2005; Eimers et al., 2008). This phenomenon is considered to be related to global climate change, which has the potential not only to increase the decomposition rate of soil organic matter and enhance its transfer to the dissolved phase, but also to change the precipitation patterns and associated soil leaching rates (Freeman et al., 2004; Eimers et al., 2008; Fenner and Freeman, 2011). Besides, the declining sulfur deposition is reported to reduce the solubility of DOM by

decreasing pH (Evans et al., 2006). While a variety of biogeochemical drivers control C cycling in rivers (Battin et al., 2008), much of the recycling of C is dependent on its composition and thus, its quality. DOM quality and quantity also serve as controls of various ecological functions. As such, DOM provides energy and C sources to microorganisms, transports and supplies nutrients to aquatic biota, and influences the solubility and bioavailability of organic pollutants and heavy metals (Findlay and Sinsabaugh, 2003).

Similarly to soil organic matter, DOM can be grouped into humic substances (HS) and non-humic substances (NHS). HS in soils are recognized as brown to black colored amorphous organic materials having different structures from the NHS that are considered compounds with known molecular structures of biochemical importance. NHS that experience major or minor structural alterations during biological degradation and chemical, photochemical, or pyrolytic reactions, become incorporated into the geopolymeric materials that make up the HS pool. Since the 1980s, hydrophobic Amberlite XAD-8 resin has been widely used to fractionate DOM into HS and NHS (Leenheer, 1981; Peuravuori et al., 2005). The proportion of HS in DOM estimated by the fractionation using XAD-8 was variable across different aquatic systems, such as 20–80% in

* Corresponding author. Tel./fax: +81 52 789 4137.

E-mail address: akiraw@agr.nagoya-u.ac.jp (A. Watanabe).

stream water (Day et al., 1991), 60–80% in lake and swamp waters (Curtis and Adams, 1995; Bano et al., 1997; Mattsson et al., 1998), and 2–20% in estuarine and sea water (Druffel et al., 1992; Otero et al., 2003). Day et al. (1991) observed an increase in the proportion of XAD-8 adsorbed fraction in DOM with increasing DOC concentration for Australian stream and lake waters. As such, the relative abundance of HS and NHS in DOM can provide critical information on DOM dynamics in aquatic environments.

As NHS are rich in carbohydrates and proteinaceous materials while HS are more aromatic (Dilling and Kaiser, 2002), NHS are believed to be more bio-degradable while HS may be prone to photo-degradation. In fact, DOC bioavailability has been shown to link directly to the abundance of NHS such as proteinaceous materials (Balcarczyk et al., 2009) while DOC photo-reactivity in wetlands has been found to be mainly controlled by HS (Cawley et al., 2012). Although these reactivity characteristics have not been fully constrained (Bertilsson and Bergh, 1999; Labanowski and Feuilade, 2009), it is rational to recognize the reactivity and functionality of bulk DOM as the sum of different reactivity based on the difference in functionality between HS and NHS. In fact, Lu et al. (2003) reported an enrichment in NHS in a canal-influenced wetland due to the incorporation of plant exudates, including potentially bio-available carbohydrates, to the DOC pool in the wetland. In this context, the ratio of HS/NHS is an important characteristic of DOM. The DOC concentration in river water is generally $<10 \text{ mg L}^{-1}$ for watersheds consists primarily of mineral soils (Hope et al., 1994; Hood et al., 2005), whereas the presence of wetlands and particularly associated peat soils, increases these values up to $40\text{--}60 \text{ mg L}^{-1}$ (Laudon et al., 2004; Billett et al., 2006; Moore et al., 2011). Wetlands are therefore critical in the supply of DOC to adjacent aquatic systems such as rivers, lakes and estuaries, and thus may also affect the DOM quality as they commonly supply DOM with a higher degree of aromaticity (Williams et al., 2010; Maie et al., 2012).

The objective of this study was to determine general relationships between DOC, HS and NHS in waters associated with climatically different wetland systems. For this purpose, wetlands from three different climatic regions, characterized by cool temperate (Hokkaido, Japan), subtropical (Florida, USA), and tropical (Sarawak, Malaysia) climates were studied. Recently, the application of direct optical properties measurements, of non-fractionated water samples, has become common in the characterization of DOM in aquatic ecosystems (Jaffé et al., 2008; Fellman et al., 2010). However, considering that important fractions of the NHS, particularly the carbohydrates, are difficult to determine using UV or fluorescence techniques, a classical DOM fractionation method was adopted in this study.

2. Materials and methods

2.1. Sampling locations

A suite of surface water samples (100 mL) were collected in northern Japan, south Florida and Malaysia between July 2007 and May 2010. A total of 74 samples were collected from 16 wetland associated streams and rivers in Hokkaido, Japan ($n = 31$), 5 river, canals, and freshwater sloughs in the Everglades, Florida, USA ($n = 20$), and 13 streams and rivers in the central-west section of Sarawak, Malaysia ($n = 23$). A few samples from each region included estuarine samples. The Hokkaido sites were located in the Bekanbeushi (8300 ha), Kiritappu (3168 ha), Kimonto (46 ha), and Oikamanaito (176 ha) wetlands and distributed from $N42^{\circ}34'$ to $N43^{\circ}11'$ and from $E143^{\circ}10'$ to $E145^{\circ}07'$. Average meteorological data through the four investigated wetlands from 2001 to 2010 were as follows: annual precipitation, $1076 \pm 105 \text{ mm}$; daily mean

temperature, $5.61 \pm 0.1 \text{ }^{\circ}\text{C}$; highest temperature, $30.4 \pm 1.6 \text{ }^{\circ}\text{C}$; and lowest temperature, $-21.4 \pm 3.4 \text{ }^{\circ}\text{C}$ (Japan Meteorological Agency, 2011). These wetlands belong to low-moor or transient moor peatlands. Major vegetations were *Alnus japonica* Steud, *Phragmites communis* Trin, *Eriophorum vaginatum* Linn, *Moliniopsis japonica* (Hack.) Hayata, *Myrica gale* Linn., and *Calamagrostis purpurea* subsp. *langsдорffii*, and *Sphagnum* community was also observed. Samples from the Everglades were collected in the area from $N25^{\circ}15'$ to $N25^{\circ}50'$ and from $W80^{\circ}29'$ to $W80^{\circ}56'$. Those include pre-established, freshwater marsh study stations from the Florida Coastal Everglades long term ecological research program (FCE-LTER), namely the Shark River Slough and Taylor Slough, representing long hydroperiod (deep water), peat based and short hydroperiod (shallow water), marl based freshwater marsh environments respectively. Average annual precipitation in Everglades between 1971 and 2000 was 1323 mm, and mean temperature, mean maximum temperature and mean minimum temperature during the same period were 23.4, 28.5, and 18.2 $^{\circ}\text{C}$, respectively (Southeast Regional Climate Center, 2007). Vegetation is dominated by emergent wetland plants such as *Cladium* and *Eleocharis* and high abundance of calcareous periphyton mats. The Sarawak sampling area was located in Mukah Division distributed from $N1^{\circ}24'$ to $N2^{\circ}57'$ and from $E110^{\circ}09'$ to $E112^{\circ}21'$. Average annual precipitation, mean temperature, mean maximum temperature, and mean minimum temperature from 2007 to 2010 were 3854 mm, 26.4, 34.5, and 22.0 $^{\circ}\text{C}$, respectively. Along the streams and rivers, mixed swamp forests are developed on peat soil. The dominate species are Ramin (*Gonystylus bancanus*), Jongkong (*Dactylocladus stenostachys*), Kapur (*Dryobalanops rappa*), and Alan (*Shorea albida*). Recently, some areas associated with a wetland were converted to oil palm plantations.

2.2. Sample preparation and analysis

Samples were filtered using combusted glass fiber filters with nominal pore size of $0.40 \mu\text{m}$ (GB140, Advantec, Tokyo). The DOC concentration in the water samples was measured using a dissolved carbon analyzer (TOC- V_{CPH} , Shimadzu, Kyoto, Japan) after the pH was adjusted to around 3 with 6 M H_2SO_4 and CO_2 was excluded by sparging with N_2 .

For HS and NHS determination, an aliquot (40 mL) of water sample was adjusted to a pH of 1.5 with 6 M H_2SO_4 and passed through a column ($\phi 10 \text{ mm}$) packed with 3 mL of prewashed Supelrite DAX-8 (Supelco, Bellefonte, PA, USA), alternative of XAD-8 that is no longer manufactured. Then the resin was washed with 3-column volumes of water (pH 1.5). The eluate and washings were combined to make up the NHS fraction. The DOC concentration in the NHS fraction was measured using TOC- V_{CPH} after sparging with N_2 . The same procedures were also conducted using ultra pure water ($\text{DOC} < 0.05 \text{ mg L}^{-1}$) to obtain a blank value. The HS concentration was estimated from the difference between total DOC and the NHS C concentration taking the rate of dilution into consideration.

The relationship between DOC and HS concentrations was investigated by regressing them to $y = ax$. No intercept was considered since DOM without HS has never been reported. A trial regression of the data to $y = ax + b$ did not increase the correlation coefficients. The relationship between DOC and NHS concentrations was regressed to a logarithmic curve as well as $y = ax$, as best fit of the data. The significance level at $P < 0.005$ was expressed using ***.

3. Results and discussion

Fig. 1 shows the relationships between DOC and HS and NHS concentrations for the entire sample set. The DOC concentration

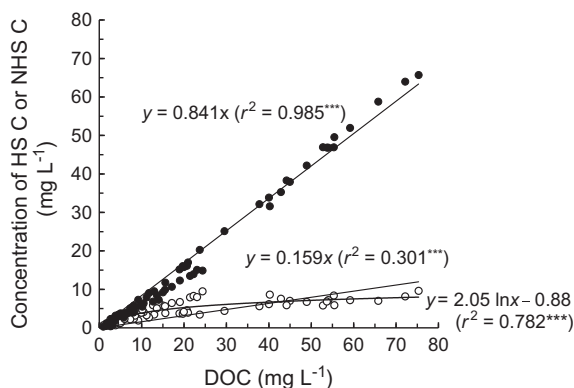


Fig. 1. Relationship between DOC and HS (closed circles) or NHS (open circles) concentrations in wetland waters.

ranged between 1.0–15.6 mg L⁻¹ in Hokkaido, 6.0–24.4 mg L⁻¹ in Florida, and 18.9–75.3 mg L⁻¹ in Sarawak, respectively. The HS and NHS concentrations ranged between 0.3–12 and 0.4–3.8 mg L⁻¹ in Hokkaido, 3.6–15.1 and 2.2–9.5 mg L⁻¹ in Florida, and 16–65 and 3.4–9.6 mg L⁻¹ in Sarawak, respectively. Regardless of the variations among the regions, a strong positive, linear correlation between DOC and HS concentrations was observed throughout the dataset, as expressed by the following equation:

$$y = 0.841x \quad r^2 = 0.985^{***} \quad (1)$$

This suggests that in fact HS drives the DOC concentration in wetland-associated waters regardless of the climatic regions. Since those data include both fresh and brackish water samples, no notable influence from seawater derived DOM on the relative abundance of HS was observed. Although a statistically significant linear correlation was also observed between DOC and NHS concentrations (Eq. (2)), correlation coefficients were much improved when the data were regressed on a logarithmic scale (Eq. (3)) as shown in Fig. 1.

$$y = 0.159x \quad r^2 = 0.301^{***} \quad (2)$$

$$y = 2.05 \ln x - 0.88 \quad r^2 = 0.782^{***} \quad (3)$$

This observation suggests that there is a significant but consistent difference between the supply and consumption of HS and NHS in wetland associated waters. The strong linear correlation between the soil derived HS and DOC suggests that their supply (soil leaching) seems to outweigh their loss through consumption (e.g. photo-degradation). In contrast, the rate of NHS supply in wetlands seems controlled by the pseudo-equilibrium between soils and biomass leaching/exudation contributions (Maie et al., 2006) and

the consumption through bio- and photo-degradation processes (Scully et al., 2004). However, in the case of the NHS, the lack of a strong linear correlation with DOC suggests that other ecological drivers such as primary productivity may play a much more important role in the NHS generation process compared to soil leaching.

Fig. 2 shows the relationships between DOC and HS and NHS concentrations in each region. When regression analysis between DOC and HS concentrations was conducted separately for each region, the slope of regression lines was 0.865 ($r^2 = 0.993^{***}$) in Sarawak samples, 0.619 ($r^2 = 0.974^{***}$) in Florida samples, and 0.709 ($r^2 = 0.946^{***}$) in Hokkaido samples, respectively (Fig. 2). No consistent trend in slopes along the climatic gradient was observed. The proportion of HS in total DOM on a C basis was in the range of 78–89% in Sarawak, 52–71% in Florida, and 15–83% in Hokkaido, respectively. Hence, a trend of increasing relative proportions of NHS from the tropical and subtropical to cool temperate wetland systems was observed. While the significance of this trend is unclear and requires further research, it suggests that source strengths of NHS may be driven in part by climatic factor. Thus, DOC, HS and NHS sources and fate are somewhat different for each wetland and driven by local conditions which likely include climate and associated vegetation cover, hydrological conditions and site specific biogeochemistry. However, the generalized correlation between DOC and HS or NHS was significant regardless of these regional differences (Fig. 1).

As in the analysis of the total dataset (Fig. 1), the significance of the relationship between DOC and NHS concentrations for each region was highest by using a logarithmic function, particularly for Sarawak and Hokkaido (Fig. 2). Correlation coefficients in regression to primary and logarithmic functions were 0.302 versus 0.712 in Hokkaido, 0.919 versus 0.914 in Florida, and 0.464 versus 0.742 in Sarawak, respectively. Thus, as for the case presented in Fig. 1, the increase in the supply of NHS from wetland soils relative to that of total DOC was likely to become smaller with increasing supply of DOC. Most commonly, for high DOM environments, the DOC is more significantly controlled by HS than by NHS. The trend of increasing wetlands DOC (and associated HS) along a cool-temperate to tropical climate gradient suggests the possibility of an incremental supply or generation of HS with increasing temperature and/or precipitation.

In conclusion, the composition of DOM in wetland-associated waters with regard to the amount of HS or NHS was evaluated throughout a climatic gradient. DOC concentrations linearly correlated with HS concentrations and thus are directly associated with soil derived organic matter inputs. In contrast a log-linear correlation between DOC and NHS suggested a lower degree of coupling between these two parameters, as a result from different source strength, where NHS are likely predominantly derived from plant leachates and exudates and associated with local primary

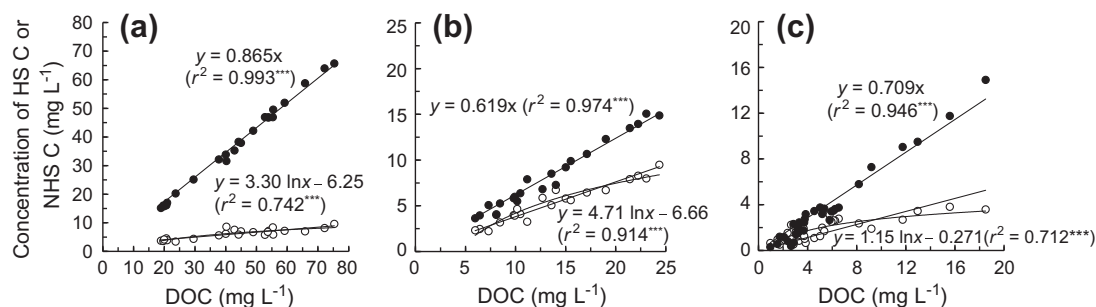


Fig. 2. Relationship between DOC and HS (closed circles) or NHS (open circles) concentrations in wetland waters in Sarawak, Malaysia (a), Florida, USA (b), and Hokkaido, Japan (c). Primary function of regression line for the relationship between DOC and NHS concentrations was: (a) $y = 0.135x$ ($r^2 = 0.464^{***}$), (b) $y = 0.381x$ ($r^2 = 0.919^{***}$), and (c) $y = 0.284x$ ($r^2 = 0.302^{***}$), respectively.

productivity while DOC is mainly soil derived. While slope differences in these correlations point to site-specific differences between the studied wetlands, a general trend of increasing relative abundance in HS (and concomitant decrease in NHS abundance) along the cool-temperate to tropical gradient was also observed, suggesting that climate related drivers could affect DOM composition in wetlands. This study clearly suggested the difference in the source/formation process of NHS and HS in wetland. As such, while more detailed research is needed to address such climatic issues, if confirmed, climate change may become an important driver not only in DOM export but also composition.

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