# Molecular changes in dissolved organic matter during photodegradation

Shafi M TAREQ[1,2]*, Masahiro MARUO[1], Osamu NAGAHUCHI[1] and Keiichi OHTA[1]

[1]School of Environmental Science, The University of Shiga Prefecture, Japan

[2]Department of Environmental Sciences, Jahangirnagar University, Bangladesh.

Email: smtareq@yahoo.com

**Abstract:** The high-performance-size exclusion chromatograph (HPSEC) separated dissolved organic matter (DOM) of lake Biwa and river water into three major peaks of apparent molecular weight of 1200 (peak1) 800 (peak2) and 300 Da (peak3). Mimic photodegradation experiments on DOM in Lake Biwa showed that solar irradiation caused a decrease in the both UV and fluorescence intensity. The decreasing trends of the ratio between peak area of peak2 and peak3 with photodegradtion process indicated that large photo sensitive molecules are fragmented into small molecules during photodegradation. The first-order reaction kinetics was applied to analyze the photodegradation of DOM. Two-step photodegradation was suggested for the fluorescent DOM with different rate constant values. Results obtained in the present study suggested that fluorescent DOM is susceptible to photodegradation and fragmented to relatively smaller size molecules with the progress of photodegradation.

**Keywords:** Humic substance, HPSEC, Lake Biwa, Biogeochemistry

## Introduction

Dissolved organic matter (DOM) affects the character and function of aquatic ecosystems and is a major reservoir of organic carbon. It plays a significant role on the biogeochemistry of metals, nutrients and organic pollutants in the aquatic and terrestrial environments (Kattner et al., 1999; Lou et al., 2006; Hirose, 2007). DOM is a complex heterogeneous mixture of mostly unidentifiable compounds, consisting of aromatic, aliphatic, phenolic and quinolic functional groups with varying molecular sizes and structure. Separation and characterization of DOM are extremely difficult, and proper separation and characterization of DOM are the key to further understanding its role in

natural environments.

There is a continuous supply of DOM to freshwaters, both from terrestrial input (allochthonous material) as well as from indigenous primary production (autochthonous material). These organic material is not conservative in freshwater systems, rather undergoes various biological and photochemical transformations (Zuo and Jones 1997; Saadi et al., 2006; Dalzell et al., 2009). Major portions of the DOM are in the form of humic substances (HS), often considered to be biologically refractory. It is well established that photochemical degradation of HS by sunlight takes place, and may be an important source of substrates for microbial activity (Kieber et al., 1989). Absorption of sunlight by the chromophores in DOM leads to structural modification and gradual decompositions with direct impact on DOM ecological functions in the biogeochemical processes. Recently there has been increased interest in the photodegradation of these biologically refractory materials to find out the degradation pathway and chemical changes in the naturally occurred macromolecules.

The aim of this work was to separate and quantify different molecular size after photodegradation of DOM in lake Biwa using high-performance-size exclusion chromatograph (HPSEC) and to estimate the degradation rate based on the florescence detection. We also analyzed the molecular size distribution in DOM of lake Biwa, sewerage treatment plants water and outlet river water samples. Additional evaluation was conducted considering the variation of depths and it influence exerted on the photodegradtion reactivity.

## Materials and methods

Lake water samples were collected from both surface (0 m depth) and 30 m depth of the northern part of lake Biwa and river water samples colleted from an out flow river (Seta river) of the lake Biwa (Fig. 1) using polyethylene bucket or Van-Dorn sampler. Water samples from sewerage treatment plants were supplied by Shiga prefectural
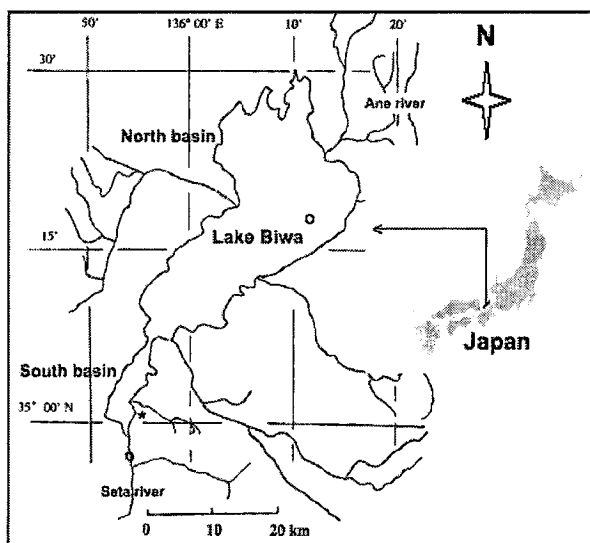


Fig.1. Location of lake Biwa in Japan and sampling site in lake Biwa and outflowing Seta river river (o) and waste water treatment plants (*).

sewerage treatment plant. Water samples were filtered through a Whatman GF/F filter during sampling and preserved in cool box during transportation to laboratory for analysis.

The photodegradation experiment of DOM was evaluated by natural sunlight irradiation experiment using both surface and deep water collected from lake Biwa. The experiment was conducted during maximum solar irradiation time (10 am - 16 pm) of clement days. A 330 ml quartz bottle containing 310 ml of the water sample was irradiated under natural solar light. As a control experiment under the dark condition, another bottle was wrapped with aluminum foil and placed in the same bath. The data and other meteorological parameters were obtained from Hikone city local meteorological observatory located 5 km north of the University of Shiga prefecture. After a certain interval small amount of (10 ml) water samples were collected from the quartz bottles for characterization of DOM by HPSEC.

The HPSEC analysis followed the method of Yamada et al., (2000) and Ohta and Kozawa (2009) using HPLC system (Shimadzu LC-10AD) equipped with a column of Superose 12 10/300 GL (Pharmacia Biotech, 10 mm ID × 300 mm) and 0.01 M NaOH solution was used as an eluent at a flow rate of 0.40 ml/min. Separated humic molecules are detected by both UV (280 nm) and fluorescence (Fl, Excitation: 340, Emission: 435) detectors. Dando fulvic acid purchased from Japan Humic Substance Society was used as a standard to quantify humic substance carbon. Sodium polystyrene sulfonates were used as apparent molecular weight (MW) calibration standards.

## Result and discussion

The HPSEC separated DOM of lake Biwa and river water into three major peaks with different intensities for both UV and Fl detector (Fig.2). The sewerage treatment plants water also showed similar spectra except one additional unresolved peak.
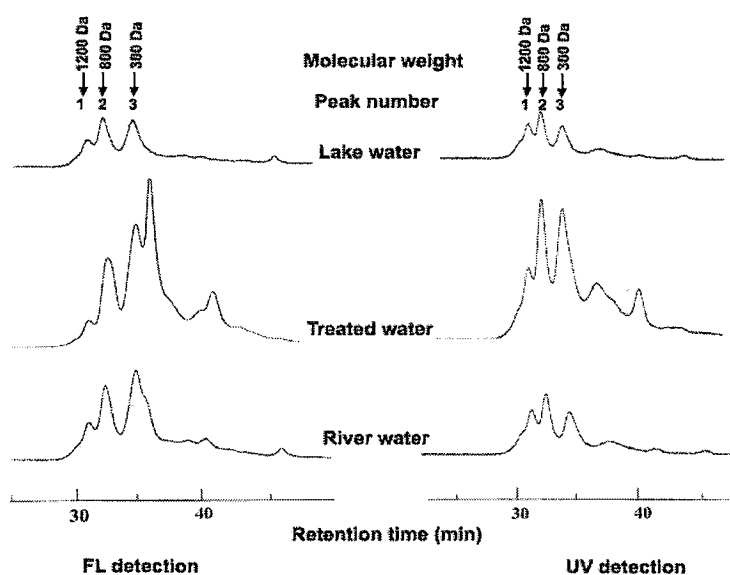


Fig.2. Florescence (Fl) and UV-detected high-performance-size exclusion chromatograms for lake water, river water and effluent of sewerage treatment plant

The apparent molecular weights of these three peaks are 1200, 800 and 300 Da (Ohta and Kozawa, 2009). The Fl-chromatogram showed a sharp difference in peak size/chromatographic pattern between lake water and river water. The 300 Da peak is a major peak in river water sample compare to that of lake water. This indicated that DOM is fragmented into smaller molecular size during transportation from lake to outlet River due to bio- or/and photo-degradation.

Result of the photodegradation experiment showed that the Fl peak intensity is sharply decreases after two hours solar irradiation (Fig.3) and photo reactivity of the DOM of the surface and deep water are different. The Fl intensity of the three peaks of the surface water sample is not
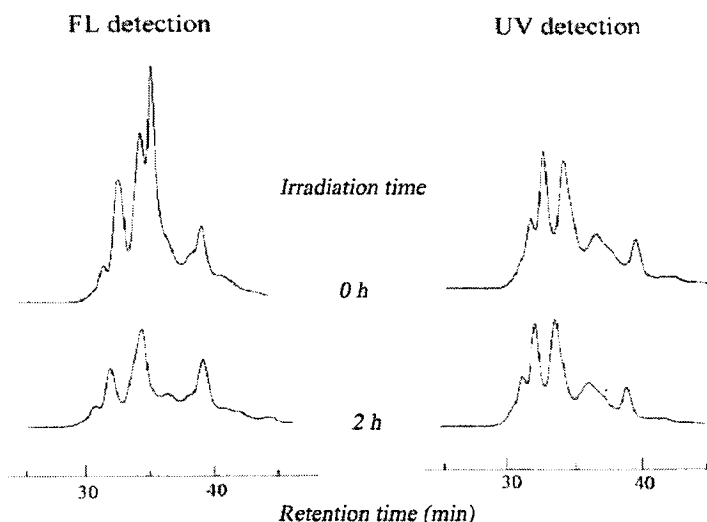


Fig.3. Changes in florescence (Fl) and UV-detected high-performance-size exclusion chromatograms peaks during photodegradation experiments using solar energy.

significantly changed with solar irradiation. While the Fl intensity of the three peaks of the deep water sample are quickly decreases with solar irradiation. Results of the control experiments under dark condition for both surface and deep water showed no changes or negligible amount of changes in florescence intensity.

The carbon content of HS (normalized to dando fulvic acid standard) versus integrated solar energy of the photodegradation experiments for surface and deep water samples are showed a figure 4.
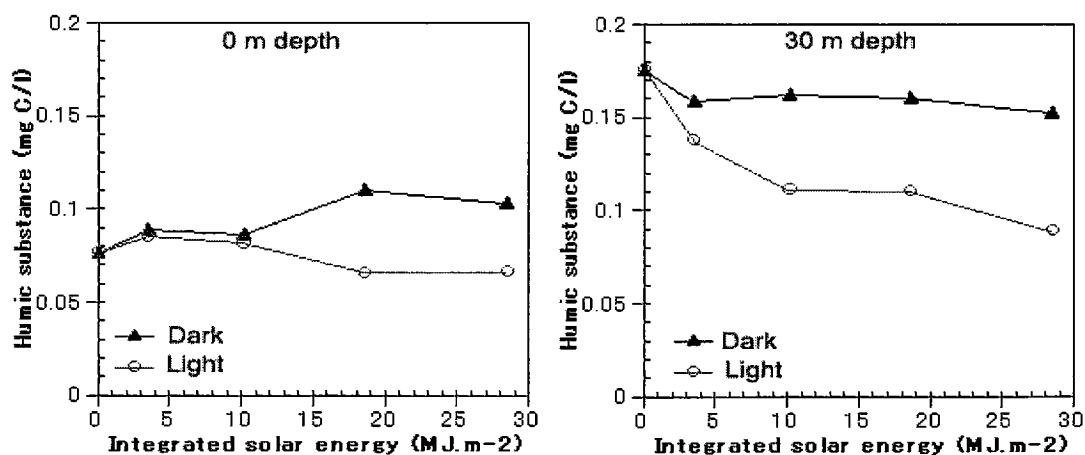


Fig.4. Variations in florescence humic substances carbon content with integrated solar energy of the surface (0 m) and deep water (30 m).

Deep water sample showed very sharp decreases of HS carbon content with solar irradiation

compare to that of surface water. There is no changes in HS carbon content of the surface water

during 10 MJ.m$^{-2}$ irradiation time while in deep water 37% of the HS carbon is lost due to

photodegradation at the same energy level. The differences in degradation rate of surface and deep

water might be attributed to the fact that the HS of the surface water already exposed to solar

irradiation and labile molecule degradated before sampling whereas deep water samples are not

exposed to solar irradiation and contain more photo sensitive fragile molecules.

Assuming a first-order kinetics for the photodegradation of DOM, the changes in HS carbon

content due to solar irradiation can be expressed as follows.

$$\mathrm{Log}(C_t/C_0) = -kS$$

Where, $k$ and $S$ is the reaction rate constants for photodegradation of DOM and integrated solar

energy (MJ m$^{-2}$) respectively and $C_0$ and $C_t$ are the concentration of HS at time 0 and t respectively.

The degradation of DOM is clearly understood from the relations between the $S$ and $\mathrm{Log}(C_t/C_0)$ (Fig. 5). The degradation rate of the HS was higher at the initial irradiation time (10 MJ m$^{-2}$) compared to that of the latter part for deep water samples. The temporal change in the degradation rate might be explained by the existence of the two types of fluorophores in the HS component
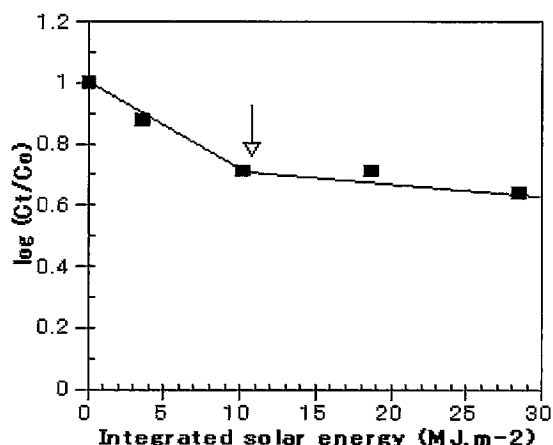


Fig.5. First-order kinetics plot of photodegradation of humic substance of the deep water sample (30 m) collected from lake Biwa.

(McKnight *et al.*, 2001). One type is highly sensitive to irradiation, and another type is relatively

less sensitive. Del Vecchio and Blough (2002) suggested that the initial high losses in FI were

caused by a discrete class of (fluorescent) chromophores, which are particularly sensitive to

photodegradation. The increasing trends of the ratio between peak size of peak2 and peak3 with

photodegradtion process (Fig.3) indicated that large photo sensitive molecules are fragmented into

small molecules during photodegradation. However, a careful further examination is needed to know

detail of the changes in molecular size of DOM during photodegradation in lake water.

## Acknowledgement

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