Role of Microgel Formation in Scavenging of Chromophoric Dissolved Organic Matter and Heavy Metals in a River-Sea System

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Abstract

We use riverine and marine DOC polymers to examine their aggregation behavior, and to evaluate the roles of microgel formation in scavenging of chromophoric dissolved organic matter (CDOM) and heavy metals in a river-sea system. Our results indicate that riverine and marine microgels did not exhibit very much difference in size and self-assembly curve; however, the assembly effectiveness ($[\text{microgel}]/\text{DOC}$) of marine samples was much higher than riverine. Instead of concentration of DOC, other factors such as types and sources of DOC polymers may control the microgel abundance in aquatic environments. After filtering water samples (microgels removed), the CDOM and selected metals (Cu, Ni, Mn) in the filtrate were quantified. CDOM and metals were concurrently removed to an extent via DOC polymer re-aggregation, which also suggested that the microgels had sequestering capability in CDOM and metals. This finding provides an alternative route for CDOM and heavy metals removal from the water column. As such the process of re-aggregation into microgels should then be considered besides traditional phase partitioning in the assessment of the ecological risk and fate of hazardous materials.

Keywords: Microgel, Aggregation, Chromophoric dissolved organic matter, Heavy metals, Scavenging.

1. Introduction

Aquatic dissolved organic carbon is one of the largest pools of reduced carbon on Earth and also one of the most complex organic mixtures in the environment system. Organic materials can be derived from various natural and anthropogenic sources including photosynthetic production, bacterial activity, terrestrial input, and industrial effluents. The impacts of these materials, including colloid or DOC biopolymers, on the ecosystem, could be vast, for example, in their participation in various biogeochemical reactions such as microbial loop, element exchange, light absorption and food webs [1, 2]. Some studies also indicated that DOC alters the mobility of pollutants which in turn affects their bioavailability, and acts as a critical regulator for pollutant toxicity [3-5]. The capability of these materials comes from some functional groups present within the natural organic matter structure, such as polysaccharides, proteins, lipids, nucleic acids and various functional group types (carboxylate, sulfate, and phosphate) [6-8]. These functional groups have the potential to provide highly stable binding and adsorbing sites with metal ions and organic compounds [9-11]. It is therefore generally acknowledged that DOC can serve as natural organic ligands to sequester organic and inorganic materials and thus influence their availability to organisms [4, 12].

Marine DOC polymers were first shown to form self-assembly aggregates as an ionic-bond-stabilized tangle network, with size ranging from molecules to an approximate size of 3–5 μm microgels [13]. This DOC-particulate organic carbon (POC) conversion is also believed to play a crucial role in marine snow, micro-aggregates and biofilms formation [14] and in the vertical transport of carbon, nutrients and elements [15-17]. Recent observations confirmed unambiguously that, besides in the ocean, these self-assembly aggregates of DOC polymers are also present in natural environments such as coastal, lake and river water [13, 18, 19]. The process of self-assembly aggregation into larger sinking particles is not well studied in the river-sea system, although it should be an important process in the fate and transport of organic matter. This process should also have significant implications on contaminant or surrounding material transport, which has not been well studied either [20-23]. In addition, the scavenging of particulate pollutants is well known in the estuarine region and is a significant process in quantifying land-derived natural and anthropogenic chemicals into shelf seas [24, 25]. It is believed that the microgel formation should have a contribution to the process through self-assembly and sinking particle processes [26, 27]. Besides, anthropogenic activities such as wastewater discharge are believed to alter DOC composition, chemical characteristic, concentration, and reactivity, especially in densely populated or industrial areas. Therefore, further study of re-aggregation of aquatic DOC in river-sea systems is needed to understand the role of microgel formation in scavenging surrounding materials via vertical transport.

In this study, we collected water samples from the Houjin River basin to the Taiwan Strait. This river flows through a metropolitan area and receives domestic and industrial discharges, with heavy metals and organic pollutants [28]. As the adsorptive and adhesive behaviors of aquatic organic materials by micro- and macrogels in an aquatic system are currently unclear [29], our principal aim was to examine the mechanism of aquatic microgel formation and to investigate the interaction of microgels with CDOM and metals in samples with different environmental parameters (river to sea). We tested the capacity of different types of DOC polymers forming microgels and their sequestering inorganic/organic materials in a river-sea system. The results will facilitate progress in the understanding of the behavior of different types of DOC polymers forming microgels and provide new insights into the interaction between aquatic surrounding materials and microgels.

2. Material and Methods

2.1 Sampling and pre-treatment

Three water samples were collected from the Houjin River to the Taiwan Strait in

December 2013. The Houjin River originates from two upstream branches, passing through four major Kaohsiung industrial parks and Yuanjhonggang Creek water intake stations and extending all the way down to the Taiwan Strait. Water samples were collected in precleaned glass bottles at each sampling site. Polyethylene bottles holding water samples for metal analysis were pre-cleaned by 10% HNO₃ and rinsed with Milli-Q water. After collection, these samples were returned to the laboratory and kept in the dark until further processing.

The analysis of chemical parameters (pH, salinity, temperature, suspended solid, Ca²⁺ and Mg²⁺) was based on the standard methods of the National Institute of Environment Analysis (NIEA, Taiwan EPA). The DOC concentration (passed through a 0.22 µm PVDF filter, Merck Millipore Ltd.) was measured as total carbon by catalytic high-temperature oxidation using a total organic carbon analyzer (OI Analytical, Aurora Model 1030 W). An overview of all chemical characteristics of the water samples is provided in Table 1. Salinity as an indicator of water types was ranged between 0.2 psu for fresh water (upstream), followed by brackish water (downstream, 22.6 psu), and sea water (Taiwan Strait, 33.7 psu). A similar trend was observed for the concentration of Ca²⁺ and Mg²⁺. The upstream sample had the highest concentration of DOC and suspended solid (SS).

2.2 Self-assembly of DOC polymer and microgel concentration measurement

All DOC samples were passed through a 0.22 µm filter which was pre-washed (0.1 N HCl) and rinsed with Milli-Q water (Millipore 18.2 MΩ·cm). To inhibit microbial activity, 3 mM sodium azide was added to water samples, which were then stored in the dark at 4°C. The aggregate size of DOC was monitored by MoFloTM XDP Flow Cytometry (FC, Beckman Coulter, Indianapolis, IN). This instrument was set at the 488 nm laser and emission at 170 mW [13, 30]. The size calibration of the FC was evaluated using a standard suspension of polystyrene latex microspheres (Spherotech, Inc., Libertyville, IL). Each measurement was replicated (≥5) in a 1 mL water sample at room temperature. The microgel concentration was determined by weight method. In brief, samples of the microgel were collected at 240 hrs and filtered through a 0.22 µm membrane. Then the membrane was washed with three portions of Milli-Q water (150 mL) to avoid salt interference, and was weighed after drying the membrane (Mettler-Toledo XS105DU analytical balance).

2.3 Scanning electron microscopy (SEM)

SEM images were used to confirm aquatic DOC polymer networks. Samples of microgel collected at 240 hrs were taken and the assembled microgels retained on filters were observed using FEI Quanta 200 SEM (North America NanoPort, Portland,

OR). The sample preparation was adopted from our previous study [31]. Microgel aggregation was filtered through a 0.22 μm Millipore IsoporeTM membrane (Fisher Scientific, Los Angeles, CA). The fixed microgels were dehydrated by soaking them in serially diluted ethanol (35%, 50%, 70%, 95%, and 100% ethanol) for 5 min and then coated in gold in preparation for viewing with SEM.

2.4 Fluorescence enhancement measurement

Chlortetracycline hydrochloride (CTC) was used as a bound Ca^{2+} indicator as in previous studies that reveal bound Ca^{2+} on polymers [32, 33]. All of the water samples were labeled with CTC (100 μM). The fluorescence measurements were carried out with a Shimadzu RF-5000U spectrofluorophotometer ($\lambda_{\text{ex}} = 390 \text{ nm}$, $\lambda_{\text{em}} = 530 \text{ nm}$).

2.5 Three-dimensional excitation–emission matrix fluorescence spectroscopy

All the three-dimensional EEM spectra were obtained using a luminescence spectrometry (F-4500 FL Spectrophotometer, Hitachi, Japan), following protocols presented in a previous study [34]. EEM spectra are a collection of a series of emission spectra over a range of excitation wavelengths, which can be used to identify the fluorescent compounds present in complex mixtures. In this study, the scanning wavelength range was set: excitation wavelength (Ex) 220–500 nm (every 5 nm), emission wavelength (Em) 260–700 nm (every 5 nm), and the scanning speed was set at 2400 nm/min. The spectrum of Milli-Q water was recorded as the blank. The software Surfer 8 (Golden Software, Inc., Golden, CO) was employed to process the EEM data.

2.6 Dissolved metal measurement

After water samples were PVDF filtered and UV-irradiated (releasing heavy metals from DOC-metal complexes) [35], the samples were concentrated based on the NIEA W308.22B method. Dissolved concentrations of selected metals (Cu, Ni, Mn) were then determined by a Z-2000 series polarized Zeeman Atomic Absorption Spectrometer (Hitachi, Japan), equipped with an auto-sampler. Spike recoveries of the metals analyses were in the range of 94% to 104%.

2.7 Statistical analysis

The data in this study were presented as means $\pm$ standard deviation (SD). Statistically, significant differences were based on results of Student's t-test analysis with p values of 0.05. Statistical analysis was assessed using Microsoft Office Excel and GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA).

3. Result and discussion

3.1 Self-assembly of riverine and marine DOC into microgel

As shown in Fig. 1, the self-assembly of the riverine and marine DOC polymers was demonstrated as monitored by FC for over ten days. This indicates DOC polymers in 0.22 μm filtered freshwater and seawater samples can spontaneously re-aggregate to form microgels. The microgel size and the self-assembly trend do not exhibit remarkable differences between the three water types at 240 hrs ($p > 0.05$), and the final size of the microgels reached approximately 3–4 μm within 72 hrs. The equilibrium size of the microgels growing no bigger than 4–5 μm is most likely because the average DOC polymer chain length is rather limited [36–38]. The equilibrium size of the microgels was also confirmed by SEM images of microgel samples collected at 240 hrs. These micrographs provided visual evidence that riverine and marine microgels appeared to be tangled and forming the microscopic gels (Fig. 2a–c), suggesting that aquatic DOC polymers can spontaneously assemble into polymer networks. This is consistent with previous riverine and marine microgel observations [18, 31]. We also examined the microgel concentration and that normalized by DOC concentrations, in order to assess the microgel abundance and the assembly effectiveness ($[\text{microgel}]/\text{DOC}$) of the DOC (Fig. 3). The concentration of upstream microgels was $0.99 \pm 0.12 \text{ mg L}^{-1}$ and has the highest DOC concentration of $10.67 \pm 0.73 \text{ mg L}^{-1}$ (Table 1). While mean microgel concentrations of downstream and marine samples observed $1.38 \pm 0.11 \text{ mgL}^{-1}$ and $0.62 \pm 0.11 \text{ mgL}^{-1}$, the downstream DOC concentration was low at $5.93 \pm 0.86 \text{ mgL}^{-1}$. The lowest DOC concentration was found in the marine sample ($0.44 \pm 0.01 \text{ mgL}^{-1}$). Figure 3 compares the $[\text{microgel}]/\text{DOC}$ ratio, representing the microgel abundance per each organic carbon unit, of the three different samples. It is noteworthy that the marine $[\text{microgel}]/\text{DOC}$ ratio was significantly higher than the other two samples ($p < 0.05$). This illustrates that although the marine sample has the lowest DOC content, it has the higher capability in self-assembling to form microgels. By contrast, the highest DOC was found in the upstream sample, but the highest microgel abundance did not appear on the site. Further investigation of the effects of environmental parameters could help in understanding the possible mechanism behind the phenomenon observed.

3.2 Possible microgel formation mechanism

Based on previous research, various possible mechanisms have been suggested for the aquatic DOC aggregation, including that the self-assembly of marine DOC polymers is driven mainly by Ca^{2+} bonds, which served as cross-linkers in stabilizing aggregates [13, 39, 40]. Therefore, the contribution of Ca^{2+} binding was the first

examined [1, 14] and CTC staining was used. CTC staining has been found useful in probing the relative contribution of Ca^{2+} cross-linking in marine microgels [32, 33]. In this measurement, three samples of aquatic microgels with different origins were stained with CTC to determine the gel fluorescence intensity. Samples collected at the beginning (0th day, original DOC polymer) and at the 10th day (the microgels formed in equilibrium) were stained and measured. The CTC fluorescence intensity was also normalized by DOC concentration to show the fluorescence intensity per each organic carbon unit. The fluorescence intensity/DOC ratio at the 10th day was remarkably higher than at the 0th day ($p < 0.05$) in all sites (Fig. 4), which is consistent with previously published observations that DOC polymers can form microgels via Ca^{2+} cross-linking [13, 33]. While the marine gel fluorescence intensity/DOC ratio was also much higher than the other two samples ($p < 0.05$), the data also followed the trend similar to Ca^{2+} concentration, salinity and microgel concentration. As the lowest CTC fluorescence intensity and Ca^{2+} concentration was found in the upstream site, it was speculated that upstream DOC polymers at a lower electrolyte concentration could result in fewer Ca^{2+} ions available in binding polymer surfaces and less microgel formed. Therefore, extra NaCl and CaCl_2 were added in the native upstream sample to examine the effect of salinity levels and Ca^{2+} concentrations on the upstream microgel formation. As displayed in Fig. 5a, the addition of extra NaCl can decrease microgel concentration ($p < 0.05$). This observation may be consistent with our previous suggestion that the presence of extra NaCl can hinder marine microgel formation, because a large number of Na^+ ions out-compete Ca^{2+} for the binding sites on polymers [31]. It was found that increasing CaCl_2 concentration did not exhibit differences in the native upstream microgel concentration ($p > 0.05$). Comparing [microgel]/DOC ratio, the ratio clearly showed that upstream samples with extra electrolytes (close to seawater levels) were still significantly smaller than the marine sample ($p < 0.05$). The relative CTC fluorescence intensity (native upstream sample as 1) may provide one of the possible mechanisms that the addition of extra NaCl or CaCl_2 to the upstream sample cannot effectively facilitate Ca ions as cross-linking on the polymer surface (CTC intensity much lower than the marine's sample, $p < 0.05$) (Fig. 5b). These results suggest that although the upstream sample has the highest DOC concentration, it does not seem to self-aggregate effectively, possibly indicating that non-assembled organic substances are the main portion of this site. However, most of the DOC polymers (like exopolymeric substances (EPS) or other biological secretion) are released by aquatic phytoplankton, microalgae, and bacteria. Their main chemical components containing acidic functional groups (carboxylate, sulfate, and phosphate) can readily chelate ions such as Ca ions forming microgels, playing a key role in DOC assembly. Therefore, the production of gel particles is not only

influenced by DOC concentrations [37, 41], but also affected by their physical-chemical characteristics and precursor types [8]. There have been many types of research using isolated EPS as an aquatic DOC polymers model, as self-assembled biopolymers are potentially produced by EPS and other biological secretions [42]. The other study also suggested that anthropogenic substances can inhibit the production of biopolymer, such as bismuth dimercaptopropanol, which could significantly reduce the production of EPS at a level just above the minimum inhibitory concentration [43]. In this case, our upstream and downstream samples were collected from Houjin River, the river passing through four major industrial parks. Lin et al. [28] pointed out that the high concentration of pollutants in water and sediment such as heavy metals, Di-(2-ethylhexyl) phthalate, and phthalate esters, indicate that the potential organic pollutants could have been discharged from these industrial parks. Additionally, rivers also deliver large quantities of terrestrial material such as humic or fulvic substances. Those relatively low molecular weight organic molecules (compared with DOC polymers) are only forming nano scale aggregates or micellar in suitable aqueous environments [44, 45]. Thus, we inferred that the upstream and downstream DOC substances might be mainly composed of non-assembled organic substances (pollutant or low molecular weight organic molecules) and might not be effectively self-assembled into microgels (low [microgel]/DOC ratio), causing the lower microgel concentration. Further evidence of chemical characteristics and possible sources of DOC substances can be illustrated by EEM contour graphs. EEM contour graphs are the result of merging a series of emission scans from excitations over a range of wavelengths and have been used as a tool for the characterization of chromophoric DOC [46, 47]. This technique provides information on the number and type of fluorophores present as well as their abundance. Fig. 6a–c demonstrate that the upstream and the downstream DOC samples have distinctive fluorescence properties and wavelengths of the humic-like fluorescence ($\lambda_{\text{ex/em}} = 280\text{--}285/380\text{--}400\text{ nm}$; $\lambda_{\text{ex/em}} = 325\text{--}330/410\text{--}425\text{ nm}$), and fulvic acid-like centers ($\lambda_{\text{ex/em}} = 250\text{--}255/445\text{--}450\text{ nm}$) [47]. The range of wavelengths observed here is in agreement with previously published results of wastewater, and polluted water [48]. Thus, the downstream sample has similar fluorescence fingerprints with upstream and the declined fluorescence intensities (compared with upstream). The reason for the decrease of downstream fluorescence intensity may be due to mixing with the sea water. Strait-water containing fulvic acid, marine humic acid is a specific fingerprint of marine samples, and fluorescence peaks were identified at excitation/emission wavelengths of 225–265/425–430 nm, 280–285/380–400 nm and 325–330/410–425 nm, respectively [47].

Our results demonstrated that the assembly effectiveness ([microgel]/DOC) of marine

samples was much higher than riverine. We infer that the upstream and downstream DOC polymers might be mainly composed of non-assembled organic materials. As results shown above, the contribution of Ca^{2+} binding on different DOC polymers (CTC fluorescence intensity), addition of extra Ca^{2+} to DOC polymers, and EEM contour graphs were used to support types and sources affecting microgel abundance in aquatic environments.

3.3 Sequestering of organic materials and heavy metals

The microgel formation may provide a tremendous reservoir for DOC. These microgels may interact with surrounding materials that can be removed from the water column by sedimentation. Therefore, in order to investigate the capability of riverine and marine microgels on sequestering organic materials and heavy metals, EEM fluorescence intensities and metals concentration were analyzed at the 0th day and at the 10th day. All samples were filtered through 0.22 μm filters to remove microgel from water samples. Comparing the 0th day and the 10th day EEM fluorescence intensity and metals concentration in the dissolved phase can assess the removal of CDOM and heavy metals via polymer aggregation. As illustrated in Fig 7, on the left are sample EEM contour graphs while bar charts on the right show changes in fluorescence intensities. EEM contour graphs showed that the main fluorescence peaks of humic acid-like, humic acid, and fulvic acid-like were typical in upstream and downstream samples; while fulvic acid, marine humic acid and humic acid polymer were characterized in seawater. These graphs provided evidence that the intensity of the main fluorescence peak decreased at the 10th day at all sites, and was effective in removing humic acid and fulvic acid-like from upstream and downstream samples. An average removal rate of 15–40 % was obtained, except humic acid-like (at less than 10 % at both sites). The averaged removal rates of chromophoric organic materials in upstream and downstream samples followed similar trends: fulvic acid-like $\approx$ humic acid > humic acid-like. In the marine sample, three main fluorescence peaks declined significantly, removing approximately 50 % of main chromophoric organic materials. Although removal rates of CDOM in upstream and downstream are less than the marine sample, but upstream and downstream microgels did capture a large quantity of CDOM, due to high CDOM concentration at river sites. The data were expected to show that the EPS biopolymer has many charged groups and polar groups that readily uptake organic materials, such as humic acids, dye, and organic pollutants [49-51]. These findings demonstrate the removal of chromophoric organic materials through the microgel phase resulting in a decrease of fluorescence intensity. Compared with assembled DOC polymers (more than 1000 kDa), low molecular weight chromophoric organic materials may be adsorbed or bound by microgels and could then be removed from the water column. However, biosorption

of chromophoric organic materials by microgels showed the same trends in upstream and downstream. The lower extent in biosorption of humic acid-like substances than fulvic acid-like onto microgels can be explained in terms of their molecular size differences. Among these, fulvic acid is comprised of lower molecular weight and less aromatic structure than humic substances [52]. Literature has reported that low molecular weight compounds could be removed more efficiently than high molecular weight materials onto the sorbent surface [53]. In fact, CDOM has a significant effect on biogeochemical activity in aquatic environments and also involves many processes affecting organic matter. Despite photochemical alteration, coagulation, flocculation, CDOM-mineral particle sedimentation, and biological modification are considered to be a loss of riverine CDOM in the oceans [54, 55]. The pathway and fate for the loss of some fraction of CDOM are still attractive [56, 57]. In this study, our results suggest that fractions of CDOM are able to be sequestered through re-aggregation of different aquatic DOC polymers, which may provide an alternative route for the loss of some fraction of CDOM. One possible mechanism is that DOC polymers are amphiphilic materials, which have hydrophobic and hydrophilic sectors in one polymer molecule [1, 42]; therefore, they readily interact with both polar and non-polar substances. In our inference, the pH range of our samples was 7.21-8.12, the surface charge of dissolved organic materials (microgels or CDOM) is negative [58]. The hydrophilic sectors of microgels (self-assembled DOC) may tangle between microgels and CDOM via cation bridge; while the hydrophobic parts may be adsorbed through hydrophobic interaction.

Moreover, with respect to selected heavy metals, the concentration of metals in upstream is generally higher than that downstream, which evidently suggests discharge from upstream to downstream (Fig. 8). Further investigation is much needed to explore the self-removal of heavy metals via polymer aggregation in aquatic systems. Results from our study demonstrate that aquatic polymers are able to scavenge selected heavy metals via polymer aggregation and two-stage removal (metals binding with CDOM and then CDOM sequestered by microgel) (Fig. 8). Comparing the removal rate of metals can reveal the difference in affinity for metal ions—the affinity in order of $\text{Cu} \approx \text{Ni} > \text{Mn}$, $\text{Cu} \approx \text{Ni} > \text{Mn}$, and $\text{Cu} > \text{Ni} > \text{Mn}$ for upstream, downstream and marine samples, respectively. The surface charge of DOC polymers is negative in the range of pH 5–8; while the pH values of our samples were found within 7.21-8.12 at river-sea sites. The presence of these anionic functional groups on the surface of polymers offers cation exchange capabilities for complexing heavy metals [59]. The metal affinity order is in agreement with results of other studies. For example, a higher colloidal Cu than colloidal Ni was found in the San Francisco Bay estuary, California [60], Narragansett Bay, Rhode Island [61] and in

the Danshuei River estuary, Taiwan [62]. These affinity characteristic of metals are also similar to Irving-Williams Series ($\text{Cu(II)} > \text{Ni} > \text{Mn}$). The slight differences may be due to different DOC composition, the background concentrations of the metals, competition and other environmental parameters [63]. In fact, the Irving-Williams Series refers to the relative stability of complexes formed by a metal ion, which also explains the wide variety of organic ligands. This may indicate that microgels-metals sequestering capacity is generally affected by ligand interaction [11].

3.4 Environmental implication

The aggregation process is an interrelated pathway that determines the fate and sedimentation of particle-surrounding material within aquatic systems. Knowledge of their environmental impact would be highly important and useful. Therefore, assessing the self-assembly of DOC polymers in aquatic ecosystems is of paramount importance to the removal and transport of organic matters and pollutants. Previous studies described the essential role of biopolymers including EPS or colloid in providing association and adsorption sites for organic/inorganic pollutants binding, yet the role of these polymers in mediating the sequestering of surrounding inorganic/organic substances via the “re-aggregation” of DOC in the dissolved phase is largely unexplored. Our data clearly demonstrated that chromophoric organic materials and heavy metals were sequestered through a microgel phase in different water types. Thus, aquatic microgels are highly important in regulating the source strengths of pollutants as they could be removed through the sedimentation of gels. It might be attributed that the number of free or potentially free negatively charged functional groups and hydrophobic sites on the aquatic DOC polymer and CDOM surface is likely responsible for the strong binding, adsorption, and cross-linking of positively charged metal ions [64, 65]. Moreover, similar types of chromophoric materials in upstream and downstream possibly resulted in a similar trend of heavy metals removal, suggesting type and concentration of chromophoric materials may affect the sequestering capacity of metals. The results successfully confirm the idea that microgel phase plays a major role in its capturing, self-cleaning, and affecting the ultimate fate of organic and metal pollutants in aquatic systems.

4. Conclusion

In this study, we clearly found that riverine and marine DOC polymers have the capacity for abiotic aggregation in fresh and seawater, which are able to spontaneously form microscopic gels. Sources and physical-chemical characteristics of DOC polymers in the different aquatic environments, probably influence the microgel abundance and behavior. The microgel formation may be directly exported

as sinking particles, which also have potential applications in CDOM and heavy metal sequestering through microgel phase and removal from the water column. By determining the sequestering of organic materials and heavy metals through DOC polymer aggregation, we have been able to show that microgels play a critical role in determining the sequestering of organic/inorganic substances in aquatic systems. It can be seen that a good understanding of the ecological risk and fate of pollutants should consider the microgel phase besides traditional phase partitioning (truly dissolved, particulate, colloidal). These self-assembled microgels facilitate the accumulation of organic and inorganic materials, and this the accumulation may affect the exposure of higher-trophic-level predators such as filter-feeding fish, invertebrates, and even humans. Additionally, mechanisms of aquatic gel formation may provide potential implications in membrane biofouling and blocking (seawater desalination and wastewater reclamation). Consequently, much detailed information on the interaction between pollutants and microgels (speciation of metal ions, selective adsorption, affinity, and competition) in the aquatic environment will be sought in future studies.

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Figure captions

Figure 1. The self-assembly of DOC polymer as a function of time. DOC samples were collected from upstream and downstream of the Houjin River and Taiwan Strait. Each point is the average $\pm$ SD in more than five samples ($n \geq 5$).

Figure 2. SEM images of microgel at different aquatic systems: (a) upstream, (b) downstream, (c) Taiwan Strait (scale bar = 4 μ m).

Figure 3. The concentration of microgels and [microgel]/DOC ratio. Microgel samples were collected at the 10th day ($n=3$).

Figure 4. Fluorescence intensity measurements of CTC for fresh and marine microgels ($n \geq 3$).

Figure 5. Effects of extra NaCl and CaCl₂ on the microgel concentration (a) and CTC fluorescence intensity (b) of upstream water samples ($n=3$).

Figure 6. EEM contour graphs of (a) upstream (b) downstream and (c) Taiwan Strait DOC samples (X axis: excitation wavelength; Y axis: emission wavelength).

Figure 7. Characterization of the different DOC in the (a) upstream (b) downstream and (c) Taiwan Strait. Bar charts show the mean of each component fluorescence maximum and their removal rate via polymer aggregation ($n=3$).

Figure 8. Sequestering of heavy metals via polymer aggregation (a) upstream (b) downstream and (c) Taiwan Strait ($n=3$).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8