Original Article

Spatial Distribution and Temporal Change of PPCPs and Microbial Fecal Indicators as Sewage Markers after Rainfall Events in the Coastal Area of Tokyo

Chomphunut Poopipattana, Misaki Nakajima, Ikuro Kasuga, Futoshi Kurisu, Hiroyuki Katayama, Hiroaki Furumai

ABSTRACT
Water sampling was conducted in the coastal area of Tokyo following two rainfall events in October and November 2016. The coastal area receives, through urban rivers, a considerable amount of combined sewer overflow (CSO) pollutants from overflow chambers and pumping stations. Five pharmaceutical and personal care products (PPCPs) including acetaminophen, theophylline, crotamiton, carbamazepine, and caffeine were analyzed and used as chemical sewage markers. In addition, two types of bacteriophage were counted as markers for viral contamination as well as the fecal bacterial indicators Escherichia coli and total coliform. High contamination by PPCPs and microbial fecal indicators was observed after the rainfall events. Five key markers were selected among nine target markers using correlation analysis and were used to express the spatial distribution and temporal change in CSO pollutants. Escherichia coli showed relatively fast die-off behavior and decreased sharply from one day after the rainfall events, while bacteriophages persisted for several days after the events. Somatic coliphage showed more persistent behavior than F-specific bacteriophage. Labile markers such as caffeine also showed high rates of disappearance. In addition, monitoring results suggested that combined analyses of PPCPs and microbial fecal indicators can provide a more informed discussion on the distribution and diffusion of sewage contaminants in coastal waters following rainfall events.

Keywords: Combined sewer overflows (CSOs), sewage markers, pharmaceutical and personal care products (PPCPs), microbial fecal indicators, coastal water

INTRODUCTION
Combined sewer overflows (CSOs) have been known to be an important source of contamination to the receiving water. Sewage pollutants such as fecal-related microorganisms, micropollutants, organic matters, and nutrients are transported and discharged into water bodies through CSOs during heavy rainfall events [1–3]. These contaminants can potentially pose health risks to humans as well as marine organisms [4–6]. Consequently, more precise studies on the distribution of CSO pollutants in receiving waters are necessary.

The Tokyo coastal area receives a considerable amount of pollutants from urban rivers, from treated wastewater, and, occasionally, from CSOs. There are approximately 800 chambers releasing CSOs in the Tokyo 23 wards area, resulting in high contamination following rainfall events. The Odaiba seaside park, a famous waterfront for recreational activities, is situated within the coastal area affected by wet weather pollutants.

Representative pollutants have been introduced as sewage markers to evaluate the impact of CSO contamination. Microbial markers, including traditional fecal indicator bacteria such as Escherichia coli (E. coli), total coliforms (TCs), and Enterococcus, have been used and evaluated in most previous studies [7–9]. Nevertheless, these bacteria do not represent the contamination of persistent compounds derived from CSOs, because they show fast die-off rates. Additionally, viruses should be included in order to evaluate their potential to pose a health risk to humans as well as their...
different persistency compared to bacteria. Bacteriophages were purposed as potential indicators of viral contamination [10,11] and were included in this study.

Recently, micropollutants such as pharmaceuticals and personal care products (PPCPs) have gained more interest as potential sewage markers due to their specificity to sources of human pollution. Their suitability as chemical markers [12,13] and their fate [14,15] were investigated. In addition, the concentration of PPCPs in CSOs itself has been reported in several studies [16–18]. However, only a limited number of investigations have been undertaken on CSO pollution in receiving waters, especially in coastal environments.

As CSOs contain various kinds of pollutants having different behaviors, several kinds of sewage markers should be used to represent CSO pollutants and to evaluate their fate in receiving waters. A combination of fecal indicator bacteria, bacteriophages as viral contaminants, and PPCPs can certainly provide more in-depth information on the distribution of CSO pollutants and their temporal change.

In this study, a survey was conducted in the coastal area of Tokyo on consecutive days after two CSO events. Both chemical and microbial markers were measured and evaluated together to express the distribution of pollutants following the rainfall events. In addition, temporal change was also investigated among markers to describe their behavior in coastal waters.

The main objectives of this study are: i) to investigate the increase in concentrations of PPCPs and microbial fecal indicators following rainfall events in comparison to dry weather concentrations; ii) to investigate the relationship among microbial and chemical markers; and iii) to investigate the spatial distribution and temporal change in microbial fecal indicators and PPCP concentrations following rainfall events.

**MATERIALS AND METHODS**

**Research area and potential sources of CSO pollutants**

The coastal area of Tokyo, a part of Tokyo Bay that is located close to Tokyo City, is the target area of this study, as shown in Fig. 1. The area includes the Odaiba seaside park, which serves as a place for recreational activities. Therefore, water quality in this area is of high concern. Sampling locations and potential sources of CSO pollutants are shown together in Fig. 2.

The three main urban rivers flowing into the coastal area are the Sumida River, the Furu River, and the Meguro River.

The Sumida River has several tributaries, including the Kanda River. The total base flow rate from the three main rivers is estimated at $9.3 \times 10^4$ m$^3$/d. In addition, some portion of Ara River flow is diverted at the upstream end of the Sumida River, as can be seen in Fig. 1. The diversion flow rate under dry weather conditions was estimated at $17 \times 10^4$ m$^3$/d. These urban rivers usually receive a considerable amount of contamination following rainfall events through pumping stations, overflow chambers, and secondary effluent discharge from sewage treatment plants located along the rivers. The Kanda River is strongly influenced by the many overflow chambers located along the river.

Primary-effluent CSOs stemming from sewage treatment plants also contribute to CSO volume. The location of several STPs is shown in Fig. 1. Located on the upstream of the Kanda River is the Nakano STP, with a capacity of $4.6 \times 10^4$ m$^3$/d. The Ochiai STP ($45 \times 10^4$ m$^3$/d), discharges into the Kanda River and the Myoshoji River, one of major tributaries of the Kanda River. The Shingashi STP ($70.5 \times 10^4$ m$^3$/d), Ukima STP ($16.5 \times 10^4$ m$^3$/d), Miyagi STP ($35 \times 10^4$ m$^3$/d), and the Mikawashima STP ($70 \times 10^4$ m$^3$/d) also discharge into upstream segment of the Sumida River and
its tributaries. The Higashiogu STP treats secondary effluent from the Mikawashima STP (20 × 10^4 m^3/d), which is used for water reclamation.

There are two sewage treatment plants in the coastal area, namely, the Shibaura and Ariake. The Shibaura STP has a capacity of 70 × 10^4 m^3/d, while the Ariake has a capacity of 4.7 × 10^4 m^3/d but only receives sewage from a separate sewer system.

Coastal surface water sampling following rainfall events

Surface water samples were collected (at a depth of about 0.5 m) within 2 h from seven locations around the coastal area of Tokyo (Fig. 2). Sampling work was performed on several consecutive days following two rainfall events. The first sampling was done on the 18th, 19th, 20th, 22nd, and 25th October 2016 (hereafter referred to as Day1 to Day8) following the rainfall event that occurred on the 17th and 18th October 2016 (30 mm of total precipitation, maximum hourly rainfall intensity of 7.5 mm/h). The second sampling was done on the 12th, 14th, and 16th November 2016 (hereafter referred to as Day1 to Day5) following the rainfall event that occurred on the 11th November 2016 (49 mm of total precipitation, maximum hourly rainfall intensity of 12.0 mm/h). During the second sampling, 6 mm of rainfall, with intensity of 2.0 mm/h, occurred. Information on the sampling event, precipitation, and tide level can be found in Table S1 and Figs. S1 and S2 in the supplementary materials. As shown in Figs. S1 and S2, the tidal water levels in the first and second sampling periods were in the range of −0.5 m to +0.7 m and −0.5 m to 0 m, respectively.

To compare the increase in contaminant concentrations following the rainfall events, samples for microbial fecal indicators were also collected under dry weather conditions on the 12th July 2015. The dry weather condition is defined as at least several days following rainfall events, when the effect of CSOs on water quality is considered negligible. A light rainfall of 22.5 mm was recorded at the Tokyo meteorolog-
cal observatory station (Otemachi) from July 8th to 9th with a weak maximum rainfall intensity of 2.5 mm/h. Therefore, CSO discharge was considered very small.

**PPCPs analysis by high resolution Fourier transform mass spectrometer**

Five PPCP compounds were targeted in this study, including acetaminophen (ACE), theophylline (THEO), and caffeine (CAF) as labile compounds, plus carbamazepine (CMZ) and crotamiton (CTMT) as conservative compounds [13,19–21].

One-liter samples were collected in pre-combusted glass bottles containing 1 g/L of ascorbic acid for sample preservation. Samples were then transported to the laboratory under cool conditions and were filtered through glass fiber filters (GF/F, 0.7 μm) after arrival. The samples were stored in a refrigerator at 4°C.

The solid phase extraction procedure was undertaken on the samples within 48 hours after collection. Before extraction, a mixture of internal standards was spiked into the samples at concentrations of 100 ng/L for CAF and 40 ng/L for other PPCPs. The extraction procedure was based on a previous study [22], with some modifications. The Oasis HLB cartridges (6 cc) were preconditioned with 5-mL Methanol and 5-mL MilliQ water (pH = 4). Samples were then passed through the cartridges at a flow rate of 10 mL/min followed by flowing air for 30 s. The cartridges were washed with 2 × 5-mL of MilliQ water (pH = 4), and analytes were eluted with 2 × 5-mL of methanol at a flow rate of 1 mL/min. The eluents were dried under a gentle stream of nitrogen gas at 40°C until dry and were then reconstituted in a 1-mL mixtures of methanol:water, 50:50 (v/v) and stored at −20°C until analysis. Recovery during extraction was different for each compound, ranging from 55.8% to 101.9%.

A LC-MS system using Orbitrap Fourier transform mass spectrometer (Exactive, Waltham, Massachusetts, USA) was used for the PPCP detection and analysis. Analytes were separated in the HPLC system (Accela, Thermo-Fisher Scientific, Waltham, USA) equipped with a Thermo hypersil gold column 150 × 2.1 mm with a 5 μm particle size. Every compound was detected in positive ion mode. The mobile phase used included solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid) at a flow rate of 0.2 mL/min according to the following program: Initial conditions 100% A, decreased to 90% in 4.5 min, decreased to 60% in 22.5 min, decreased to 0% in 15 min, and finally returned to the initial conditions after 4.5 min with the re-equilibration of the column set at 7 min. Total acquisition time was 53.5 min. The MS instrumental setting and gradient elution program was set following the methodology presented in a previous study [22]. Data were acquired in full-scan mode with two scan events simultaneously over a mass range of 150.0 − 300.0 for molecular ions and 50.0 − 200.0 for fragment ions. In-source collision-induced dissociation at 20 eV was performed to produce fragment ions from molecular ions.

The detection and confirmation of target compounds was based on a mass-to-charge ratio (m/z) and retention time with criteria of 5 ppm mass tolerance and a 0.3 min retention time window. For each target compound, one molecular ion [M+H]+ and at least one fragment ion were acquired. Information on the retention time and mass-to-charge of each compound is shown in Tables S2 and S3 for the internal standards.

The limit of detection (LOD) and limit of quantification (LOQ) were determined for every set of samples (normally eight samples per set). The LOD is defined as the lowest detectable concentration with a signal-to-noise ratio of at least 3:1, whereas LOQ is defined as the lowest detectable concentration with a signal-to-noise ratio of at least 10:1. Information on analytical performance, including LOD, LOQ, recovery, and linearity can be found in Table S4 in the supplementary materials.

**Analysis of fecal bacteria and bacteriophages**

All samples for microbial analysis were collected with a stainless bucket, transported under cool conditions in pre-sterilized polyethylene bags or sterilized Corning centrifuge tubes (50 mL) to laboratory, and analyzed within 24 hours after sampling. All analysis was done in duplicate. One milliliter of sample was used when high concentrations were expected, while 5-mL of sample was used for low-concentration samples.

TC and E. coli were determined by the single agar layer method using a Chromocult® Coliform Agar (Merck, Darmstadt, Germany). After overnight incubation at 37°C, dark-blue to violet colonies were counted as E. coli, while all colonies (salmon to red colonies and dark-blue to violet colonies) were counted as TC.

F-specific bacteriophage or F-phage (FPH) was determined by the single agar layer plate counting method using Salmonella typhimurium WG 49 as the host strain, modified from the standard method [23]. Somatic coliphage (SOM-CPH) was determined by the double agar layer plate counting method using E. coli WG5 as the host strain, following the Standard Methods [24].
In cases where 1-mL and 5-mL of sample were used for the analyses, the detection and enumeration limits of bacteria and bacteriophage were 100 CFU/100 mL and 20 CFU/100 mL or PFU/100 mL, respectively.

**RESULTS AND DISCUSSION**

**Contamination by PPCPs and microbial fecal indicators following rainfall events**

Table 1 shows the concentration range of PPCPs in surface coastal waters on Day1 and Day5 following rainfall events, while Table 2 shows the range of microbial fecal indicators on Day1 following the events and on a dry day. All detailed concentration data from the seven locations for both October and November events can be found in Tables S5 to S8. Due to the unavailability of PPCP data under dry weather conditions, PPCP concentration levels on Day1 were compared to Day5 following the rainfall events instead. The Day1 concentrations were significantly higher than those of Day5, which clearly indicated that there was contamination by untreated wastewater discharge through CSOs. ACE and THEO showed relatively similar concentration ranges with 4 to 7 times elevated concentrations on Day1. CAF showed the highest concentrations among the five PPCPs with 7 to 9 times higher concentrations. While CMZ, showed the lowest concentrations among the five PPCPs with a lower concentration change of around two times. Likewise, CTMT showed a less than 2 to 4 times concentration change. Reported concentrations of PPCPs in sewage treatment plant influents ranged from 16.5 to 270 ng/L, from 382 to 3,030 ng/L, from 50 to 10,900 ng/L and from 5,200 to 65,600 ng/L) for CMZ, CTMT, ACE, and CAF, respectively, in other studies [18,25].

In the cases of ACE and CAF, 2 log lower concentrations than for other PPCPs were observed. The higher decreases were possibly caused by degradation and sorption.

Comparing the Day1 data from the October and November events, concentrations in November showed a narrower range than those in October, except for CTMT. More uniform distribution patterns appeared in November due to higher total precipitation denoted by a heavier intensity and longer duration. More volume of CSOs seemed to be discharged with longer rainfall periods, which affected a broader range

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Table 1 Concentration of PPCPs in surface coastal waters following rainfall events.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Day</th>
<th>Concentration range (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACE</td>
</tr>
<tr>
<td>Oct. 2016</td>
<td>Day1</td>
<td>48 – 531</td>
</tr>
</tbody>
</table>

Table 2 Concentration of microbial fecal indicators in the coastal area of Tokyo following rainfall events and under dry weather conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sampling period</th>
<th>E. coli (CFU/100 mL)</th>
<th>TC (CFU/100 mL)</th>
<th>FPH (PFU/100 mL)</th>
<th>SOMCPH (PFU/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After rainfall event, Day1</td>
<td>Oct. 2016</td>
<td>3.59 – 4.54</td>
<td>4.07 – 5.09</td>
<td>Below DL</td>
<td>2.11 – 2.95</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After rainfall event, Day1</td>
<td>Nov. 2016</td>
<td>3.00 – 4.43</td>
<td>4.04 – 5.09</td>
<td>Below DL</td>
<td>2.63 – 3.45</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weather</td>
<td>July 2015</td>
<td>1.86 – 2.66 (MPN/100 mL)</td>
<td>-</td>
<td>Below DL</td>
<td>Below DL – 1.95</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weather (n = 9)*</td>
<td>Sept. 2014</td>
<td>1.00 – 2.30 (MPN/100 mL)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Data from Tokyo Metropolitan Government (DL was not mentioned) [33]
DL=20 CFU or PFU/100 mL
of coastal area.

As shown in Table 2, concentrations of fecal bacteria indicators and bacteriophages on Day1 following the rainfall events were compared with those under dry weather conditions. Regarding E. coli, dry weather concentrations, which were comparable to other reported values by the Tokyo Metropolitan Government, were 1 to 2 log lower than those following rainfall events. The results corresponded to a previous study reporting 1.4 log higher concentrations following a rainfall event [26]. Regarding bacteriophages, limited data are available, but dry weather concentrations were also lower than those measured following rainfall events. While FPH was not detected under dry weather conditions, its concentrations were 1 to 2 log higher following rainfall events in most of the stations. SOMCPH was also not detected in some locations under dry weather conditions, but its log concentrations were 2 to 3 times higher following rainfall events at all locations. A relatively narrower concentration range and higher concentrations were observed in bacteriophages in November as compared to October.

### Selection of representative markers based on correlation analyses

The relationship among markers was investigated for data of Day1 following rainfall events. There are four groups of markers: (1) fecal bacteria E. coli and TC; (2) bacteriophages of FPH and SOMCPH; (3) labile PPCPs of ACE, THEO and CAF; and (4) conservative PPCPs of CMZ and CTMT. Microbial and PPCPs data were log-transformed prior to analysis, because the concentrations showed log-normal distributions. Correlation coefficients among markers can be found in Tables 3 and 4. A coefficient greater than 0.9 was considered a high correlation as indicated by a green highlight.

It was confirmed that there was a high correlation within groups of labile PPCPs, conservative PPCPs, and fecal bacteria. However, FPH was less correlated with SOMCPH in November. Conservative PPCPs showed less correlation with other markers, and labile PPCPs also showed less correlation with both fecal bacteria and bacteriophages in most cases. Therefore, the five markers with the least correlation were selected, which were E. coli, FPH, SOMCPH, CAF, and CMZ. E. coli was selected due to their higher specificity to

### Tables

**Table 3** Correlation coefficients among markers (Day1, October).

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>TC</th>
<th>FPH</th>
<th>SOMCPH</th>
<th>ACE</th>
<th>THEO</th>
<th>CAF</th>
<th>CMZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPH</td>
<td>0.98</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOMCPH</td>
<td>0.85</td>
<td>0.86</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>0.93</td>
<td>0.86</td>
<td>0.89</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THEO</td>
<td>0.87</td>
<td>0.84</td>
<td>0.84</td>
<td>0.58</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAF</td>
<td>0.83</td>
<td>0.81</td>
<td>0.81</td>
<td>0.53</td>
<td>0.98</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMZ</td>
<td>0.20</td>
<td>0.25</td>
<td>0.10</td>
<td>0.22</td>
<td>0.02</td>
<td>-0.05</td>
<td>-0.11</td>
<td></td>
</tr>
<tr>
<td>CTMT</td>
<td>0.35</td>
<td>0.44</td>
<td>0.29</td>
<td>0.37</td>
<td>0.22</td>
<td>0.18</td>
<td>0.14</td>
<td>0.90</td>
</tr>
</tbody>
</table>

**Table 4** Correlation coefficients among markers (Day1, November).

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>TC</th>
<th>FPH</th>
<th>SOMCPH</th>
<th>ACE</th>
<th>THEO</th>
<th>CAF</th>
<th>CMZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPH</td>
<td>0.59</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOMCPH</td>
<td>0.87</td>
<td>0.89</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>0.73</td>
<td>0.81</td>
<td>0.96</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THEO</td>
<td>0.35</td>
<td>0.51</td>
<td>0.92</td>
<td>0.23</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAF</td>
<td>0.44</td>
<td>0.53</td>
<td>0.97</td>
<td>0.27</td>
<td>0.92</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMZ</td>
<td>0.86</td>
<td>0.96</td>
<td>0.61</td>
<td>0.93</td>
<td>0.79</td>
<td>0.53</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>CTMT</td>
<td>0.84</td>
<td>0.95</td>
<td>0.63</td>
<td>0.90</td>
<td>0.81</td>
<td>0.56</td>
<td>0.54</td>
<td>1.00</td>
</tr>
</tbody>
</table>
human sources pollution than TCs. CAF was more abundant and showed more confidence in the measurement. FPH and SOMCPH showed less correlation and both were included. Lastly, CMZ was selected, as it was a more common target PPCP and was measured globally [27–29].

**Spatial distribution of five selected markers**

The five selected markers were used to express the distribution of CSO pollutants on Day1 following the November rainfall event, as shown in **Fig. 3**. The top three markers shown on the profile are *E. coli* and the two bacteriophages, while the two bottom markers are labile and conservative PPCPs. In order to discuss the spatial distribution of these markers, the sampling time lag among different sampling points should be considered, because hydrodynamic conditions in the area depend on tidal changes. Nonetheless, the tide level changed only 0.5 m or less during the 2-h sampling period, and only 10% or less of the total water volume in the area moved according to the tidal change. Therefore, water mass movement by tidal effects was considered negligible during the sampling period.

The highest contamination was found at the Sumida River mouth. The Sumida River receives a considerable amount of CSO pollutants from several STPs, pumping stations, and overflow chambers located upstream and in its tributaries. According to the literature, about 40% of the total overflow chambers are located in the Kanda River drainage area, contributing to a large volume of CSO discharge in Tokyo [30].

The contaminant concentrations gradually decreased in the direction toward Hinode and the Rainbow Bridge. Samples from Harumi as well as Odaiba and the Meguro River mouth showed less contamination. The different trends in contami-
nation levels seem to be related to the location of potential CSO sources. There are more pumping stations located along the Hinode and the Rainbow Bridge route, showing higher contamination in this coastal area. Although Odaiba is located near the Rainbow Bridge point, this recreational park was partially blocked and protected by surrounding embankments and was thus less polluted.

A relatively unique profile of markers was found at Shibaura, which is located near the Shibaura sewage treatment plant and pumping station. The concentration of CAF at this location was as high as that found at the Sumida River mouth. More than 98% of CAF, one of the labile markers, can be removed during the treatment process. Thus, the presence of CAF indicates recent contamination by untreated wastewater. It strongly implies that there was a significant effect of CSO discharge by the Shibaura sewage treatment plant and the accompanying pumping station prior to the sampling event. However, other markers did not show the same increasing trend as CAF. The reason should be further discussed in the context of information on PPCPs and microbial concentrations of influent sewage.

Investigation of similarity among locations by cluster analysis

Cluster analysis was conducted for Day1 of the November event in order to investigate the similarity in contamination of the five selected markers. Standardization of the data was necessary prior to analysis, because the five markers have different units. Log-based concentrations were standardized into a zero to one scale \((x – \text{min})/(\text{max} – \text{min})\). A cluster dendrogram is shown in Fig. 4.

The seven locations can be primarily divided into two groups. The Sumida River mouth, the Hinode and the Rainbow Bridge were clustered together, as they are located in the same stream line. The contamination was found to be from the same source and with higher concentrations as compared to other locations. Harumi, the Meguro River mouth and Odaiba were clustered together, as they had low contamination levels. A clustered subgroup was found for the Meguro River mouth and Odaiba. This implied that Odaiba was also affected by the contamination from the Meguro River. Shibaura, located in the canal system, was found to be in a single-member group. This supports the unique profile of markers that was found at this location denoted by the high concentration of CAF.

Cluster analysis, using the five selected markers with lower correlations, was found to be very effective for discussing the spatial distribution focusing on the similarity and dissimilarity among locations.

Fig. 4 Cluster dendrogram among locations (November).

Temporal change of microbial markers and labile markers

To investigate the changes in the spatial distribution over time, a radar chart of the selected markers, excluding CMZ, on Day1, Day3, and Day5 following the rainfall event was plotted for the November event (Fig. 5). E. coli and CAF are shown in the top and bottom of the plot, while bacteriophages are shown on the left and right sides of the chart. CMZ, a conservative PPCP marker, was excluded from the plot because only a slight change was observed.

In order to discuss temporal change, conditions of water mass movement and mixing with seawater should be considered as influencing factors. In addition, decay and microbial degradation of PPCPs and inactivation of microorganisms could affect their temporal changes. Total inflow volume from urban rivers and effluent discharge in the Sumida River system was estimated as \(1.5 \times 10^6\) m\(^3\)/d. Meanwhile, inflow volumes from the Furu River, the Meguro River, and effluent discharge from Shibaura STP located downstream of the Sumida River were estimated at \(5.9 \times 10^5\) m\(^3\)/d. Figure 6 shows the accumulated water volume at the seven sampling locations from the Sumida River mouth to the offshore in the North-South direction. We calculated the 5 m depth water volumes as well as total depth volume, because freshwater inflow might move within the surface layer in coastal waters that have vertical salinity gradients.

The distance of water mass movement in the surface layer can be approximately estimated by considering the inflow volumes for 2 days and 4 days. The inflow volumes were used differently for the calculation at each sampling location.
depending on their corresponding sources. As shown in Fig. 6 and considering the total inflow volume in the Sumida River system, surface water mass at the Sumida River mouth on Day 1 might move around the Hinode point up to 3 km in distance from the river mouth over 4 days. However, the distance of water mass movement for other sampling locations did not seem to be as long as the Sumida River mouth point. This estimation of water mass movement indicated that the polluted water mass likely remained within the targeted coastal area even 4 days following the rainfall event. Therefore, the temporal change might be mainly caused by dispersion, dilution by seawater, decay/inactivation, and microbial degradation.

At locations affected by higher contamination levels, which are the Sumida River mouth, Hinode, and the Rainbow Bridge, CAF and E. coli showed a greater change from Day 1 to Day 5. CAF decreased drastically from Day 1 to Day 3 and still showed a decreasing trend from Day 3 to Day 5. While E. coli showed a drastic decrease from Day 1 to Day 3, almost no decrease was observed from Day 3 to Day 5. A previous study showed that E. coli can be inactivated by high osmotic pressure in seawater [31]. In addition, sunlight inactivation can also be a major mechanism impacting their disappearance. On the contrary, less temporal change was observed at Harumi and Odaiba, whose contamination levels were lower. By comparison, both bacteriophages were found to be more persistent than E. coli and CAF in all locations.

Focusing on the Sumida river mouth, both types of bacteriophages showed higher persistency than the bacterial markers as mentioned above. In addition, most locations showed SOMCPH having more persistency than FPH. FPH showed a greater decrease at Hinode and the Rainbow Bridge, which
might imply their lower persistence under high salinity. There was a significant increase at the Meguro River mouth on Day5, which might have been caused by the river pollution from additional precipitation on December 14.

As was noted in a previous study, SOMCPH is susceptible to solar wavelengths below 342 nm (UV-B and shorter UV-A). A higher percent of inactivation was attributed to shorter solar wavelengths, while FPH is more susceptible to longer wavelengths than SOMCPH, but not particularly susceptible to UV-B [32]. In seawater, the attenuation of sunlight takes place at lower wavelengths. Therefore, longer solar wavelengths tend to predominate in seawater, resulting in a longer period of SOMCPH survival than that of FPH.

In addition, while FPH was not found, SOMCPH was present, up to 90 PFU/100 ml, under dry weather conditions, as shown in Table 2. Thus, the dry weather data also emphasize the longer survival of SOMCPH than that of FPH. The order of persistent behavior, from high to low, of microbial fecal indicators in seawater can be deduced to be as follows; SOMCPH > FPH > E. coli.

Lastly, Shibaura showed a unique pattern of temporal change in CAF and FPH. There was a much greater decrease in CAF from Day1 to Day3 than that observed at other locations. This can be explained by the special location of Shibaura, which is near a sewage treatment plant and continuously receives secondary effluent. The decrease in CAF and FPH might be attributed to flushing by the effluent as well as by the dilution by seawater. However, SOMCPH concentrations remained constant throughout 5 days, the source of which remains unclear.

CONCLUSIONS

High contamination by PPCPs and microbial fecal indicators was observed from one day following rainfall events. Three labile PPCPs concentrations were found to be 4 to 9 times higher than those on Day5, while two conservative PPCPs concentrations were found to be less than 4 times higher. For fecal bacteria and bacteriophages, 1 to 2 log higher concentrations were observed.

A high correlation was found within each group of markers on the first day following the rainfall events. However, SOMCPH was found to be less correlated to FPH in the November event. Five markers with lower correlations among nine target markers were selected to characterize the spatial distribution. The highest contamination of the five selected markers was found at the Sumida River mouth. The concentration gradually decreased toward offshore. The Hinode route was found to contribute most of the contamination to the coastal area of Tokyo. In addition, both the Harumi route as well as Odaiba, were less contaminated. A unique profile of key markers was found at a location near the Shibaura
sewage treatment plant. The monitoring data emphasize the usefulness of evaluating both chemical and microbial markers. Cluster analysis was found to be effective and can be used to quantify the similarity among locations.

The results of the temporal changes in the selected markers indicated that CAF and E. coli had the highest rates of disappearance, followed by bacteriophages. SOMCPH showed more persistent behavior than FPH in most locations.

REFERENCES


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