Effect of the Recycling Process on Drinking Water Treatment: Evaluation Based on Fluorescence EEM Analysis Using the Peak-Picking Technique and Self-Organizing Map

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Abstract: The recycling process is applied in many water treatment plants (WTPs), although this process can lead to adverse effects. The effect of the recycling process on the characteristics of dissolved organic matter was evaluated based on a fluorescence excitation-emission matrix using the peak-picking technique and self-organizing map (SOM). In this study, an evaluation of two WTPs, one with and one without a recycling system, was carried out. Both WTPs show moderate efficiency during the coagulation–flocculation process in removing DOC, fulvic acid-like, humic acid-like, and tryptophan-like substances. The recycling process causes increased values of fulvic acid-like, humic acid-like, and tryptophan-like substances and specific ultraviolet absorbance (SUVA) after the filtration process of about 31.0%, 35.7%, 22.2%, and 6%, respectively. Meanwhile, the WTP without recycling showed a reduction in the level of fulvic acid-like, humic acid-like, and tryptophan-like substances and SUVA by 23.3%, 52.9%, 27.8%, and 21.1%, respectively. Moreover, SOM analysis based on the peak-picking technique can determine differences in sample clusters due to the recycling process.

Keywords: fluorescence EEM; self-organizing map; recycled water; water treatment plants

1. Introduction

The presence of natural organic matter (NOM) in source water leads to many problems in the water treatment process. Among these problems are an increase in coagulant doses [1], membrane fouling [2,3], changes in taste and odor stimulation [4], and the production of precursors of disinfection by-products (DBPs) [5,6]. DBPs negatively impact human health, resulting in miscarriages, children born small for gestational age, and bladder cancer [7]. The sources of NOM in water treatment plants (WTPs) are derived from soil particles carried by water flows, residual decomposition of plants and animals, and other organic sources around water bodies [8]. Biological activities in water bodies contribute to the release of NOM into the source water. The recycled water from drinking water treatment sludge (DWTS) is another NOM source if the WTPs apply a recycling process.

The recycling process in drinking water treatment plants is applied to minimize waste generated during the production process and, at the same time, to increase the total operational efficiency. Backwashing water from sand filters and the sludge from the sedimentation units are the primary targets of the recycling process. Sludge from the sedimentation units is generally collected in a storage tank for gravity thickening, mechanical dewatering, and the generated supernatant. Mechanical dewatering using a centrifuge filter or a pressure filter produced dewatered sludge with a solids content of 19.0% and 23.2%, respectively, as documented in a report by USEPA in 2011 [9]. Meanwhile, the supernatant from the DWTS treatment unit can be discharged into the environment or reused as raw water in WTPs employing the recycling process.
The supernatant from DWTS contains pollutants from the source water (including soils, organic matter, and microorganisms) and chemicals added during water treatment (including coagulant, acid, or alkali for pH adjustment). Zhou [10] found that organic matter in DWTS has the potential to degrade the quality of settling water. Other studies have suggested that the concentration of DBPs (trihalomethanes and haloacetic acids) and the levels of protozoa, such as Giardia and Cryptosporidium, in filter backwashing water are considerably higher than those in raw water [11,12]. In contrast, other researchers have found positive impacts, such as increased coagulation efficiency, reduced coagulant dosage, and enhanced turbidity removal [13,14]. However, there is still a need for discussion on the issue of the effect of recycling using recycled water from DWTS treatment units, particularly concerning the characteristics of DOM, such as humic-like and protein-like substances.

An appropriate technique to obtain a considerable amount of information on the composition and characteristics of DOM is the use of fluorescence excitation-emission matrix (EEM) data. Even though this method only identifies organic components containing fluorescence chromophores, it works effectively and is extensively used [15]. Fluorescence spectroscopy has been used in many studies, such as to evaluate water treatment processes [4,16], to track DOM during wastewater treatment [17], to monitor recycled water systems [18], to monitor groundwater quality [19], to investigate the impact of sewage effluent discharge [20], to track DOM in aquatic environments [21], to estimate biological activity in coastal zones [22], to track organic matter during nitrification [23], and to characterize soil DOM [24]. Fluorescence spectroscopy is widely used in water quality analysis because of its benefits, including high sensitivity, small sample sizes, non-invasiveness, relatively low cost, quick measurement times, and potential for online monitoring [4,25,26].

Raw EEM data contain a considerable amount of fluorescence information, which comprises thousands of excitation/emission (Ex/Em) pairs and intensity responses. Various methods have been used to analyze EEM data, such as peak picking [27], fluorescence regional integration [28], principal component analysis [29], parallel factor analysis (PARAFAC) [30], and self-organizing map (SOM) analysis [31]. To date, no studies have attempted to combine peak picking with self-organizing map analysis for the assessment of fluorescence data. SOM is used to explore the data relationships by translating highly dimensional data into a simple, less dimensional map with topological and metric relationship data [32]. The application of SOM to assess DOM characteristics in WTPs based on fluorescence EEM data was first carried out by Bieroza in 2009 [31]. The fluorescence data for SOM analysis are mainly pre-processed using parallel factor analysis (PARAFAC) [33–38]. PARAFAC modeling is vulnerable to fitting models with few or too many components because it relies on selecting a sufficient number of components [39]. Previous studies only showed two components of humic-like substances (C1 and C2) generated by PARAFAC [40]. Meanwhile, when using a peak-picking technique, all components of organic matter, such as humic-like substances (peaks A and C) and protein-like substances (peaks B and T), can be analyzed. The peak-picking technique can capture the components of DOM and assist in analyzing EEM data [15]. Moreover, this technique shows a significant correlation between peaks and water quality parameters and has the potential to be used for real-time monitoring [41].

The objectives of this study were (i) to evaluate the effect of the recycling process on the characteristics and composition of DOM in the water treatment process and (ii) to explore the relationship between EEM data and clustering samples based on fluorescence intensity using the peak-picking technique and SOM analysis.

2. Materials and Methods
2.1. Drinking Water Treatment Plants and Water Samples for Study

Water samples were collected from two WTPs (hereafter referred as Plant A and Plant B) in Japan that use a rapid sand filtration system to treat surface water. The quality of the surface water used as the raw water of the two plants is similar in terms of turbidity, color, and DOC. Plant A uses a recycling system, i.e., supernatant obtained from the sludge
treatment unit is pumped back into the receiving well and mixed with raw water, while Plant B does not (Figure 1).

**Plant A**

![Water Treatment Flowchart]

**Plant B**

![Water Treatment Flowchart]

Figure 1. Flowchart of the water treatment processes of Plant A and Plant B.

Water samples included raw water in the receiving well, sedimentation effluent, and purified water after the disinfection process. Each sample was collected in a 1 L glass bottle pre-washed and cleaned with chlorine and pure water. Samples were stored in a cooling box, and analysis was conducted soon after the samples were transported to the laboratory. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was added to the samples containing residual chlorine (i.e., the samples from the sedimentation effluent and the purified water) immediately after sampling to suspend the process of the oxidation of DOM by chlorine.

### 2.2. Water Quality Analysis

Direct in situ measurement was conducted for pH, temperature, dissolved oxygen (DO), electrical conductivity (EC), oxidation–reduction potential (ORP), and residual chlorine (free and total chlorine). After the samples arrived at the laboratory, turbidity and color were immediately measured. Water samples were then filtered using 0.2 μm cellulose acetate membrane filters (Toyo Roshi, Tokyo, Japan) and stored at 4 °C for DOM analysis.

DOC was measured using a TOC analyzer (TOC-Vwet, Shimadzu, Kyoto, Japan). Samples were acidified using phosphoric acid (85%) with acid addition rate of 3% to make the pH lower than 3. Samples were injected with 1.5 mL of oxidizing agent (mixture of 60 g of sodium peroxodisulfate and 15 mL of phosphoric acid 15% in 500 mL of Milli-Q water) and then sparged with nitrogen for 3 min to remove inorganic carbon. Ultraviolet absorbance at a wavelength of 260 nm ($\text{UV}_{260}$) was measured using an UV-vis spectrophotometer (UV-1600, Shimadzu, Kyoto, Japan). $\text{UV}_{260}$ has been widely used in Japan since it was introduced by Tambo [42]. This parameter is capable of detecting humic substances...
in water, and the values are less than 5% higher than UV$_{254}$ [43]. SUVA was obtained as the ratio of UV$_{260}$ to DOC. This ratio is useful in categorizing the hydrophobicity and hydrophilicity of NOM in water [44].

A fluorescence spectrophotometer (RF-5300, Shimadzu, Kyoto, Japan) was used to record the fluorescence EEM of DOM. The excitation (Ex) and emission (Em) wavelengths were set from 220 to 550 nm with a scanning interval of 5 nm. According to previous research, this wavelength range covers all positions of fluorescence intensity in samples from water treatment processes [40]. The fluorescence intensity was normalized to the quinine sulfate unit (QSU) by dividing all EEM data with 10 ppb quinine sulfate in 0.05 M H$_2$SO$_4$ at the specified wavelength of Ex/Em = 350/450. Peak-picking analysis was used to identify the humic-like, fulvic-like, and protein-like substances based on the literature [27]. The position of the fluorescence peak was obtained for humic acid-like substances (peak C) at Ex 350 nm and Em 420–480 nm; that for fulvic acid-like substances (peak A) was obtained at Ex 260 nm and 380–460 nm; that for tyrosine-like substances (peak B) was obtained at Ex 275 nm and Em 310 nm; and that for tryptophan-like substances (peak T) was obtained at Ex 275 nm and Em 340 nm.

Self-organizing map analysis, or Kohonen map analysis, is a machine learning tool using a two-layered artificial neural network (ANN) based on the biological nervous system [45]. SOM is an iterative process in which map vectors are adjusted to be more like sample vectors. The Euclidean distance metric is used to determine the similarity of map vectors and sample vectors. After identifying the most comparable map vector, the weights of the vector and the weights of its surrounding neurons are changed to move closer to the sample vectors [37]. The EEM data are converted to the best-matching unit (BMU) at the end of the learning phase, which is the unit that is most comparable with the EEM prototype. Sample patterns can be investigated by projecting native EEM on their BMUs on the SOM grid [45]. Fluor_SOmap toolbox in Matlab R2018b was used for classification and pattern recognition of the fluorescence peaks of the samples following the tutorial of SOM analysis by Cuss et al. [46]. SOM was run for two different datasets from Plant A (n = 48) and Plant B (n = 75). The lowest levels of mean quantization error (mqe) and topographical error (tge) were used to generate the BMU and interneuron distance matrix (U-matrix) maps. One of the linearly initialized maps and ten randomly initialized maps were used to produce the U-matrix and BMU maps in this study.

Other parameters, such as the fluorescence index (FI), biological index (BIX), and freshness index ($\beta / \alpha$), were determined for the purpose of comparison. FI was used to identify the relative contribution of terrestrial and microbial sources to the DOM [47,48]. BIX, as an indicator of autotrophic productivity with high values (>1), was correlated to the recently produced DOM of autochthonous origin [49]. The freshness index ($\beta / \alpha$) is an indicator of the freshness of the produced DOM, with higher values representing a higher proportion of fresh DOM [22,50], where more recently derived DOM is represented by $\beta$ and highly decomposed DOM is represented by $\alpha$. The following equation was used to calculate these parameters:

\[
FI = \frac{\text{Em} (450 \text{ nm})}{\text{Em} (500 \text{ nm})} \text{ at Ex } 370 \text{ nm} \tag{1}
\]

\[
\text{BIX} = \frac{\text{Em} (380 \text{ nm})}{\text{Em} (430 \text{ nm})} \text{ at Ex } 310 \text{ nm} \tag{2}
\]

\[
\frac{\beta}{\alpha} = \frac{\text{Em} (380 \text{ nm})}{\text{Em} (420 - 435 \text{ nm})} \text{ at Ex } 310 \text{ nm} \tag{3}
\]

3. Results and Discussion
3.1. Characteristics of Water Samples

Table 1 shows general water quality parameters measured during the sampling period at Plant A and Plant B. The raw water of the WTPs indicates a low concentration of DOM.
and is dominated by transphilic fractions (SUVA values between 2 and 4). Therefore, the performance of Plants A and B shows moderate efficiency in reducing the DOC concentration by 36.9% ± 7.2% and 48.8% ± 5.0%, respectively. The DOC which is dominated by the transphilic fraction can be removed by coagulation process at a rate of about 25–50%, which is in accordance with the findings of another researcher [51–53]. The UV$_{260}$ value of Plant A and B decreased after water treatment by 74.4% and 66.1%, respectively. This proved that the efficiency of reducing UV$_{260}$ levels is higher than that of DOC [52]. The coagulation–flocculation process in both WTPs mostly removed humic acid-like and hydrophobic substances. Humic acid-like substances are more easily coagulated by aluminum and iron (III) salts compared to fulvic acid-like substances [54]. Humic acid-like substances have a higher UV absorbance and aromaticity than other substances, such as the hydrophilic fraction [55].

| Parameter             | Plant A | Plant B |
|-----------------------|---------|---------|
|                       | Raw Water | Sedimentation Effluent | Purified Water | Raw Water | Sedimentation Effluent | Purified Water |
| pH                    | 7.10 ± 0.28 | 6.85 ± 0.13 | 6.88 ± 0.11 | 7.07 ± 0.28 | 6.83 ± 0.29 | 7.02 ± 0.26 |
| Temperature (°C)      | 18.4 ± 6.1 | 17.9 ± 6.5 | 18.5 ± 6.2 | 20.4 ± 4.9 | 20.5 ± 5.0 | 20.5 ± 5.0 |
| DO (mg·L$^{-1}$)      | 9.45 ± 1.63 | 9.56 ± 1.50 | 9.75 ± 1.55 | 7.94 ± 1.90 | 8.75 ± 1.38 | 8.83 ± 1.32 |
| Conductivity (mS·m$^{-1}$) | 6.28 ± 0.98 | 7.21 ± 1.04 | 7.33 ± 0.91 | 6.00 ± 0.50 | 6.49 ± 0.57 | 7.27 ± 0.49 |
| Turbidity (NTU)       | 2.95 ± 1.52 | 0.25 ± 0.07 | 0.05 ± 0.02 | 1.49 ± 0.68 | 0.16 ± 0.06 | 0.08 ± 0.03 |
| DOC (mg·L$^{-1}$)     | 0.75 ± 0.21 | 0.49 ± 0.08 | 0.48 ± 0.07 | 1.21 ± 0.13 | 0.60 ± 0.07 | 0.61 ± 0.06 |
| UV$_{260}$ (m$^{-1}$) | 2.51 ± 0.98 | 0.64 ± 0.18 | 0.67 ± 0.18 | 3.56 ± 0.44 | 1.07 ± 0.23 | 0.92 ± 0.28 |
| SUVA (L·mg$^{-1}$·m$^{-1}$) | 3.31 ± 0.65 | 1.33 ± 0.45 | 1.41 ± 0.39 | 2.96 ± 0.21 | 1.78 ± 0.27 | 1.47 ± 0.34 |

Figure 2 shows that the DOC of purified water decreased in Plant A, whereas the purified water of Plant B shows the opposite trend. Meanwhile, the UV$_{260}$ and SUVA of Plant A increased after the filtration process. Typically, the UV$_{260}$ decreases during the water treatment process because aromatic molecules, such as humic acid-like substances, can be removed by the coagulation–flocculation process. Based on the calculation of the Pearson correlation coefficient (Pearson’s r) at the significance level of 0.05, the increase in SUVA showed a relatively strong positive correlation with the increase in the fluorescence intensity of peaks A, C, and T, but only a moderately positive correlation for peak B (Table S2). The release of DOM from filtration media may be responsible for increasing the SUVA and fluorescence peaks in purified water. The recycling process may contribute to the accumulation of some fractions of NOM, such as fulvic acid-like substances in filter media. Fulvic acid-like substances are more difficult to remove during the coagulation–flocculation process because these substances have more fraction groups and categories as low-molecular weight substances (usually <1500 Dalton) [56]. The purified water at Plant B revealed the low fluorescence intensity of peaks A, C, B, and T. The SUVA values also decreased after the treatment process, although the correlation with the peak intensity did not show a tendency.

3.2. Fluorescence EEM and Index

The fluorescence EEM data were analyzed using the peak-picking technique to gain more information and to observe the intensity shifts of every peak. In both WTPs’ raw water, fulvic acid-like substances (peak A) were dominant, followed by humic acid-like substances (peak C) and tyrosine-like substances (peak B). The low intensity of fulvic acid-like and humic acid-like substances in sedimentation effluent indicates that coagulation–flocculation can more easily remove these substances when compared to the removal of protein-like substances (Table 2 and Figure 3). Plant A reduced the levels of fulvic acid-like, humic acid-like, and tryptophan-like substances by about 52.8%, 54.8%, and 50.0%, respectively. Meanwhile, Plant B reduced the levels of fulvic acid-like, humic acid-like,
and tryptophan-like substances by about 62.4%, 71.5%, and 41.2%, respectively. Both WTPs found it difficult to remove tyrosine-like substances, with low removal rates for Plants A and B of about 15.0% and 15.6%, respectively. Wang (2021) and Jones (2019) also found that the coagulation process effectively eliminated humic-like and tryptophan-like substances, but the tyrosine-like substances remained more resistant to coagulation [57,58]. Hydrophobic high-molecular weight humic substances predominate in raw water and are easily removed by the coagulation process [4]. Tryptophan-like substances are more readily removed than tyrosine-like substances because tryptophan-like substances have a greater adsorption capacity than tyrosine-like substances and have strong interactions with humic-like substances [59].

Figure 2. SUVA, UV260, and DOC changes (purified water and sedimentation effluent) in Plants A and B.
Table 2. Fluorescence EEM peaks and fluorescence index in WTPs.

| Parameter                              | Plant A Raw Water | Plant A Sedimentation Effluent | Plant A Purified Water | Plant B Raw Water | Plant B Sedimentation Effluent | Plant B Purified Water |
|----------------------------------------|-------------------|-------------------------------|------------------------|-------------------|-------------------------------|------------------------|
| Fulvic acid-like substances/Peak A (QSU) | 0.78 ± 0.26       | 0.29 ± 0.09                   | 0.38 ± 0.10            | 1.16 ± 0.23       | 0.53 ± 0.13                   | 0.43 ± 0.16            |
| Humic acid-like substances/Peak C (QSU) | 0.41 ± 0.16       | 0.14 ± 0.05                   | 0.19 ± 0.05            | 0.61 ± 0.14       | 0.26 ± 0.08                   | 0.17 ± 0.07            |
| Tyrosine-like substances/Peak B (QSU)  | 0.52 ± 0.15       | 0.46 ± 0.04                   | 0.45 ± 0.06            | 0.45 ± 0.08       | 0.41 ± 0.10                   | 0.38 ± 0.13            |
| Tryptophan-like substances/Peak T (QSU)| 0.22 ± 0.13       | 0.09 ± 0.02                   | 0.11 ± 0.03            | 0.31 ± 0.10       | 0.23 ± 0.20                   | 0.18 ± 0.30            |
| Fluorescence index (FI)                | 1.49 ± 0.28       | 1.93 ± 0.30                   | 1.92 ± 0.13            | 1.57 ± 0.07       | 1.92 ± 0.13                   | 2.04 ± 0.55            |
| Biological index (BIX)                 | 0.57 ± 0.05       | 0.66 ± 0.05                   | 0.68 ± 0.03            | 0.59 ± 0.07       | 0.81 ± 0.10                   | 0.76 ± 0.09            |
| Fulvic acid-like Peak A (QSU)          | 0.78 ± 0.26       | 0.29 ± 0.09                   | 0.38 ± 0.10            | 1.16 ± 0.23       | 0.53 ± 0.13                   | 0.43 ± 0.16            |
| Humic acid-like Peak C (QSU)           | 0.41 ± 0.16       | 0.14 ± 0.05                   | 0.19 ± 0.05            | 0.61 ± 0.14       | 0.26 ± 0.08                   | 0.17 ± 0.07            |
| Tyrosine-like Peak B (QSU)             | 0.52 ± 0.15       | 0.46 ± 0.04                   | 0.45 ± 0.06            | 0.45 ± 0.08       | 0.41 ± 0.10                   | 0.38 ± 0.13            |
| Tryptophan-like Peak T (QSU)           | 0.22 ± 0.13       | 0.09 ± 0.02                   | 0.11 ± 0.03            | 0.31 ± 0.10       | 0.23 ± 0.20                   | 0.18 ± 0.30            |
| Freshness index (β/α)                  | 0.56 ± 0.05       | 0.64 ± 0.04                   | 0.67 ± 0.03            | 0.59 ± 0.07       | 0.79 ± 0.09                   | 0.73 ± 0.08            |
| Biological index (BIX)                 | 0.57 ± 0.05       | 0.66 ± 0.05                   | 0.68 ± 0.03            | 0.59 ± 0.07       | 0.81 ± 0.10                   | 0.76 ± 0.09            |
| Fluorescence index (FI)                | 1.49 ± 0.28       | 1.93 ± 0.30                   | 1.92 ± 0.13            | 1.57 ± 0.07       | 1.92 ± 0.13                   | 2.04 ± 0.55            |

Figure 3. Fluorescence intensity of each peak during water treatment in Plants A and B.

Table 2 shows that the peaks of fulvic acid-like, humic acid-like, and tryptophan-like substances in purified water at Plant A were elevated by about 31.0%, 35.7%, and 22.2%, respectively, after the filtration process. In contrast, Plant B showed a decrease in the intensity of peaks A, C, and T by 23.3%, 52.9%, and 27.8%, respectively. The ratios of protein-like substances and humic-like substances in Plant A and Plant B are 0.4:0.6 and 0.3:0.7, respectively (Figure 3). Tyrosine-like aromatic proteins were found in large amounts in the raw water of Plant A. The high concentration of aromatic protein in raw water can be attributed to recycled water from DWTS, since aromatic protein is microbial-derived DOM. During sludge storage, anaerobic reactions may occur, resulting in the production of protein-like substances from the breakdown of extracellular lysis and lignin [60]. The low capability of the coagulation–flocculation processes to reduce protein-
like compounds causes these compounds to be carried into purified water. Collins [61] proposed an explanation for the increase in organic matter in filtration units. The low organic molecules increase in number because of the degradation of larger molecules of DOM into smaller molecules by microorganisms in sand media filters. Small molecules of DOM are collected and repolymerized into larger molecules, and this may contribute to the production of a high number of molecules of DOM in filtration units. These results also demonstrate that the increase in fluorescence intensity after filtration may suggest that filtration media could be a host for microbial communities and release extracellular polymeric substances that are attributed to the long-term operation of WTPs. A study by Bridgeman et al. [62] showed an indigenous source of organic contents derived from the abrasion of adsorption media, biofilm development, and filtration, capable of contributing to the concentration of DOM.

Table 2 and Figure 4 show the fluorescence index (FI) of the raw water for Plants A and B, approximately 1.4, indicating that the source of DOM was terrestrial in nature. Meanwhile, an index above 1.9 after the treatment process indicates DOM release from microbial activities [47]. The biological index (BIX) clearly shows a value between 0.6 and 0.7 for treated water, which expresses organic matter in water contributed to by the biological/microbial process [49,63]. The freshness index also demonstrated an increase in value as the water treatment process was carried out. While conventional water treatment is more efficient at removing organic matter from terrestrial origin (lower FI and β/α), the NOM of microbial-derived DOM often has a greater concentration in treated water [64,65].

![Figure 4](image-url)  
**Figure 4.** Fluorescence index for Plants A and B during the water treatment process.

### 3.3. Self-Organizing Map Analysis

Figure 5 shows the distribution of samples at Plant A (n = 48 sample) and Plant B (n = 75 sample) based on data from the peak-picking technique of fluorescence EEM. The U-matrix map has a color scale showing the distance between neighboring neurons of EEM data [45,46]. The low value of the U-matrix refers to high similarities between samples. The U-matrix and BMU maps were generated from the lowest number of mqe and tge to obtain the best results (Table S1). The value of mqe represents the average distance between the sample vectors. The lowest values of mqe for Plants A and B are 0.4215 and 0.3728, respectively. The value of tge represents the proportions of sample vectors to which the map vectors are the second most similar. The lowest values of tge for Plants A and B are 0.0208 and 0.000, respectively. The lower corner part of the U-matrix for Plants A
and B shows a yellow-brown color, indicating high dissimilarity. A blank hexagonal grid around samples ORW-5, TSW-6, and TPW-6 shows a notable difference in these samples compared to the others. The high fluorescence intensity of protein-like substances in the raw water of Plant A sample 5 (ORW-5) caused this sample to differ from the other samples (the most similar sample is ORW-15 as a reference). This sample was obtained in August, the summer season in Japan; therefore, it may contain algae that grow in the reservoir of the source water.

Sedimentation effluent sample 6 and purified water sample 6 from Plant B (TSW-6 and TPW-6) show a peak for protein-like substances (Ex/Em = 230/340 nm and Ex/Em = 285/340 nm, respectively). This result is supported by the values of the biological and freshness indices of those samples above 1, corresponding to the recently produced DOM of autochthonous origin and higher proportion of fresh DOM [22,49,50]. Sample TSW-6 had biological and freshness indices of about 1.24 and 1.20, respectively. Meanwhile, sample TPW-6 had values of 1.11 and 1.06 for the biological and freshness indices.

DOM released from algae may occur during the sedimentation process. Problems such as short-circuiting currents can generate floc floating, and the floc carries over to the filter. The same process occurs due to oxygen production during photosynthesis by algae in sludge blanket stimulate floc buoyant. Moreover, a V-notch weir (a triangular weir with sharp-crested, thin plates, and a V-shaped opening) is installed in order to measure the real-time flow of water in the effluent system and is often the location of the algae growth in the sedimentation unit. Those problems were suggested to stimulate DOM release in the sedimentation unit.

The raw water of Plants A (ORW) and B (TRW) was predominantly at the bottom area of the BMU with high fluorescence intensity. Meanwhile, the sedimentation effluent (SW) and purified water (PW) samples were predominantly observed in the middle and upper areas, suggesting high similarities. Some of the samples had an overlapping position of sedimentation water, and the purified water exhibited the same fluorescence intensity among the samples. Raw water, sedimentation effluent, and purified water are expected to be present in the bottom, middle, and upper areas; for example, Plant B, but not Plant A, showed purified water (OPW) in the middle area. The vertical direction in the SOM map represents the reduction in fluorescence intensity [32]. Figure 6 supports this phenomenon, as it shows that the humic-like substance peak emerged in purified water in Plant A.

The component planes show the distribution of variables around the map for the samples depicted on the BMU map (Figure 7). The component planes associated with each map show that peaks A (fulvic acid-like substances) and C (humic acid-like substances) have similarities for Plants A and B. The protein-like substances, such as peaks B (tyrosine-like substances) and T (tryptophan-like substances), have different patterns for Plants A and B. Peak B of Plant A shows dissimilarity in the bottom area, while it occurs in Plant B until the middle area. Most of the similarities are found in Plant B for peak T, except in the bottom corner area.
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Figure 5. The U-matrix and BMU map for Plants A and B (RW = raw water; SW = sedimentation water; PW = purified water).
Figure 6. Fluorescence EEM spectra for water samples.

Plant A

Plant B

Figure 7. Cont.
Plant B

Figure 7. Component planes for the peak of fluorescence intensity.

4. Conclusions

Based on fluorescence EEM analysis using the peak-picking technique and self-organizing map analysis to evaluate the recycling process in drinking water treatment, the following conclusions can be drawn from this study:

- The recycling process changed the composition of DOM by increasing the percentage of protein-like compounds in raw water. Tyrosine-like molecules were protein-like substances with a high percentage in raw water of WTPs with a recycling system; however, these substances were still present in significant amounts in the treated water because the coagulation process struggled to reduce this substance. In addition, there was an increase in humic substances such as humic acid-like and fulvic acid-like substances in treated water, although these substances were reduced through the coagulation–flocculation process.

- The fluorescence intensity from the peak-picking technique can be used as an input for SOM analysis to determine differences in sample clusters due to the recycling process. Through the U-matrix and best-matching unit from SOM analysis, WTPs with a recycling process showed different clusters for sedimentation effluent and purified water samples. The component planes showed that the peak of humic-like substances had similarities in both WTPs; meanwhile, the peak of protein-like substances showed different patterns, which the recycling system may cause.

- This study shows that implementing a recycling system in the water treatment process may make removing DOM, particularly humic-like and protein-like substances, more challenging for WTP operators. More research will be conducted to determine the effectiveness of recycled water pretreatment in reducing the levels of humic-like and protein-like substances in the water treatment process.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13233456/s1, Table S1: Fitting parameters of linearly and randomly initialized maps, Table S2: Pearson correlation results for Δ SUVA with other parameters of DOM.

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