

## CHAPTER VI

### SPECTROFLUOROMETRY ANALYSIS FOR CHARACTERIZING DOM AND DOM FRACTIONS IN INDUSTRIAL ESTATE WASTEWATER TREATED BY STABILIZATION PONDS

#### 6.1 Introduction and Literature Reviews

In Chapter 5, the chemical classes of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds and the chemical classes of the six dissolved organic matter (DOM) fractions from the respective waters were determined. The relationships between the chemical classes and specific THMFP values were discussed. The obtained results showed that different chemical classes in each DOM fraction led to significantly different specific THMFP. The complex compositions of DOM, therefore, are the most important factor contributing to the formation of potentially carcinogenic substances. Although pyrolysis GC/MS is one of the most notable methods used to determine the organic composition of DOM, interpreting its results is complicated. Presently, spectrofluorometry analysis (fluorescent excitation-emission matrix, FEEM) has been proposed by many researchers as another promising technique for characterizing the organic compositions of DOM. The FEEM has the advantage of simplicity due to its minimal sample amount, pretreatment and analysis time requirements. A FEEM is obtained by fluorescent spectrometry, recording the matrix of fluorescent intensity in coordinates of excitation and emission wavelengths. The FEEM provides information on the putative origin of fluorescent organic matter in water; it may identify the matter as a tyrosine-like substance, tryptophan-like substance, humic and fulvic acid-like substance, and so on (Coble 1996; Nakajima *et al.* 2002; Chen *et al.* 2003; and Sierra *et al.* 2005). However, the application of FEEM on wastewater and treated wastewater and their DOM fractions has not been widely studied. On this basis, it would be useful and meaningful to utilize FEEMs for characterizing the organic compositions of the DOM and DOM fractions in all water samples in this study. This new information could help and support the use of FEEMs for characterizing DOM in water.

## 6.2 Objective of This Chapter

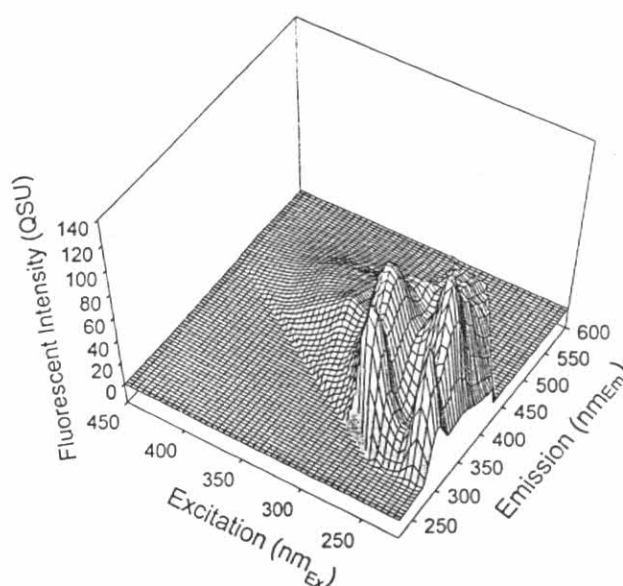
The main objective of this chapter was to use spectrofluorometry analysis to determine the characteristics of the DOM and DOM fractions in the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds.

## 6.3 Material and Methods

### 6.3.1 Sample Collections and Experimental Procedure

Water samples were collected in February 2005 from the sampling points as presented in Chapter 3 (Section 3.3).

Influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds, collected in February 2005, were filtered through a pre-combusted (550 °C for 2 h) Whatman GF/F (nominal pore size 0.7  $\mu\text{m}$ ) filter. All filtered waters were measured using FEEMs. The details of the FEEM analysis were presented in Chapter 3 (Section 3.7). A resin adsorption procedure was used to fractionate all filtered waters into six DOM fractions: hydrophobic neutral (HPON), hydrophobic base (HPOB), hydrophobic acid (HPOA), hydrophilic base (HPIB), hydrophilic acid (HPIA) and hydrophilic neutral (HPIN). The six DOM fractions of all filtered waters were analyzed for their FEEM. A sample of the three-dimensional view of a FEEM of influent wastewater is depicted in Figure 6.1.



**Figure 6.1:** Example of a three-dimensional view of a FEEM of influent wastewater

### 6.3.2 Interpretation of FEEM Data

The fluorescent peaks of the fluorescent excitation-emission wavelengths (Ex/Em), which exhibit the outstanding fluorescent emission intensities, were classified as shown in Figure 6.1. In this study of the influent and effluent of stabilization ponds and the six DOM fractions, eight major fluorescent peaks were detected: 230nm<sub>Ex</sub>/295nm<sub>Em</sub> (peak A), 275nm<sub>Ex</sub>/300nm<sub>Em</sub> (peak B), 240nm<sub>Ex</sub>/355nm<sub>Em</sub> (peak C), 280nm<sub>Ex</sub>/350nm<sub>Em</sub> (peak D), 275nm<sub>Ex</sub>/380 nm<sub>Em</sub> (peak E), 255nm<sub>Ex</sub>/410nm<sub>Em</sub> (peak F), 275nm<sub>Ex</sub>/410nm<sub>Em</sub> (peak G), and 330nm<sub>Ex</sub>/410nm<sub>Em</sub> (peak H). The putative fluorescent organic matters of the respective peaks were classified according to the summary of data from literature as shown in Table 6.1. The Ex/Em of fluorescent peaks A, B, C, D, E, F, G and H were compared with the Ex/Em of the fluorescent organic matter in Table 6.1. It was determined that peaks A and B were tyrosine-like; peaks C and D were tryptophan-like; and peaks E, F, G, H and I were humic and fulvic acid-like substances. The locations of the fluorescent peak positions from all water samples are presented in Figure 6.2.

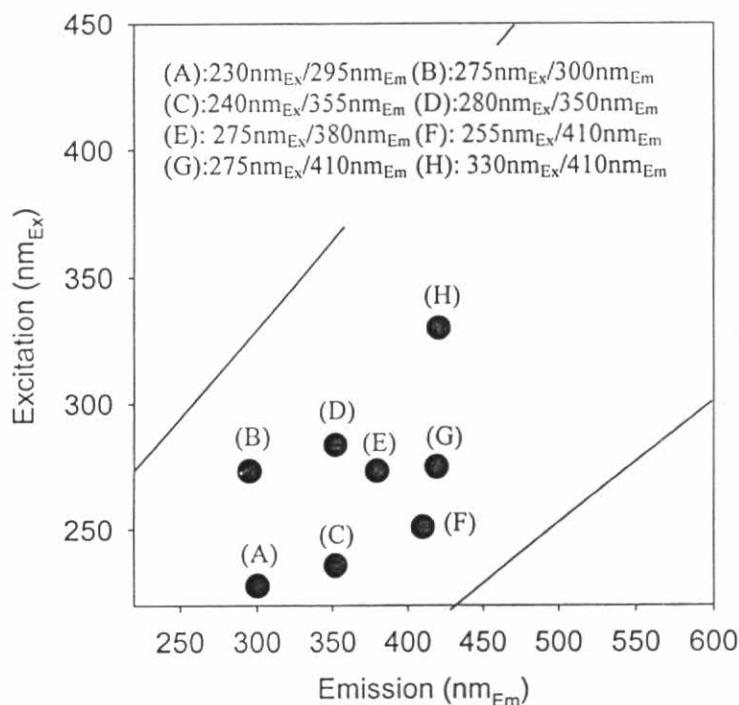


Figure 6.2: Location of fluorescent peak positions from all water samples in this study

**Table 6.1:** Fluorescent peak positions and major pyrolysis fragments of major fluorescent components from literature data

Fluorescent organic matter	Excitation (nm <sub>Ex</sub> )/Emission (nm <sub>Em</sub> )	Majors pyrolysis fragments
Tyrosine-like and protein-like substances	220-275/300-305 (Wolfbeis, 1985), [A] <sup>1</sup> , [B] 275/310 (Coble, 1996), [B] 270-280/300-320 (Leenheer <i>et al.</i> , 2003), [B] 270-275/300-302 (Yamashita and Tanoue, 2003), [B]	Phenol and <i>p</i> -cresol (phenol, 4 methyl), (Bruchet <i>et al.</i> 1990) Pyridines, pyrroles, indoles, nitriles, phenol and <i>p</i> -cresol (equal quantities from tyrosine), (AWWA, 2000)
Tryptophan-like and protein-like substances	220-275/340-350 (Wolfbeis, 1985), [C], [D] 275/340 (Coble, 1996), [D] 270-280/320-350 (Leenheer <i>et al.</i> , 2003), [D] 280/342-346 (Yamashita and Tanoue, 2003) [D]	Indole (AWWA, 2000), acetronitrile, benzonitrile, phenylacetoneitrile, pyridine, methylpyridine, pyrrole, indole and methylindole (Leenheer and Croues, 2003)
Fulvic acids and fulvic-like substances	330-350/420-480 (Coble, 1996), [H] 290-340/395-430 (Baker and Genty 1999), [E], [G] 320-340/410-430 (Baker, 2001), [H] 315/437-441 (Yamashita and Tanoue, 2003) 260/460, 310/440 (Sierra 2005) [F], [H]	<b>Humic substances consist of humic and fulvic acids</b> <b>Humic acids;</b> Bruchet (1986) and Gadel and Bruchet (1987): highly aliphatic. Faure <i>et al.</i> 2006: (1) n-alk-1-ene/n-alkane with 8-29 carbon presenting the limited odd over predominance in the C <sub>24</sub> -C <sub>29</sub> range (2) alkyl-benzenes, naphthalene, alkyl-naphthalene (3) phenol.
Humic acids and humic-like substances	250-260/380-460, 330-350/420-480 (Coble, 1996) [E], [F], [G], [H], 235-255/435-465 (Nakajima <i>et al.</i> , 2002) [E] 250-260/380-480, 330-350/420-480 (Leenheer <i>et al.</i> , 2003) [E], [F] [G], [H] 350-365/446-465 (Yamashita and Tanoue, 2003) 265/525, 360/520 (Sierra, 2005)	<b>Fulvic acids;</b> Bruchet (1986) and Gadel and Bruchet (1987): highly peaks of butenal, acetic acid, 2-fufural, methyl furfural, levoglucosenone, 5-(Hydroxymethyl) 2 fufaral. Croue <i>et al.</i> (1993): large peaks of phenol and cresol <b>Humic substances:</b> Lu <i>et al.</i> 2006: (1) Nitrogen containing compounds (such as pyrrole and pyridine), aliphatic products (such as n-alk-1-ene/n-alkane with 5-29 carbon) (2) aromatic products (such as alkyl-benzenes, naphthalene and alkyl-naphthalene, (4) lignin products (such as o-cresol, m cresol, catechol, dimethylphenol) (5) carbohydrate product (such as furan, 2-methyl proppenal, dimethylfuran, vinyfuran, 2-fufaraldehyde.

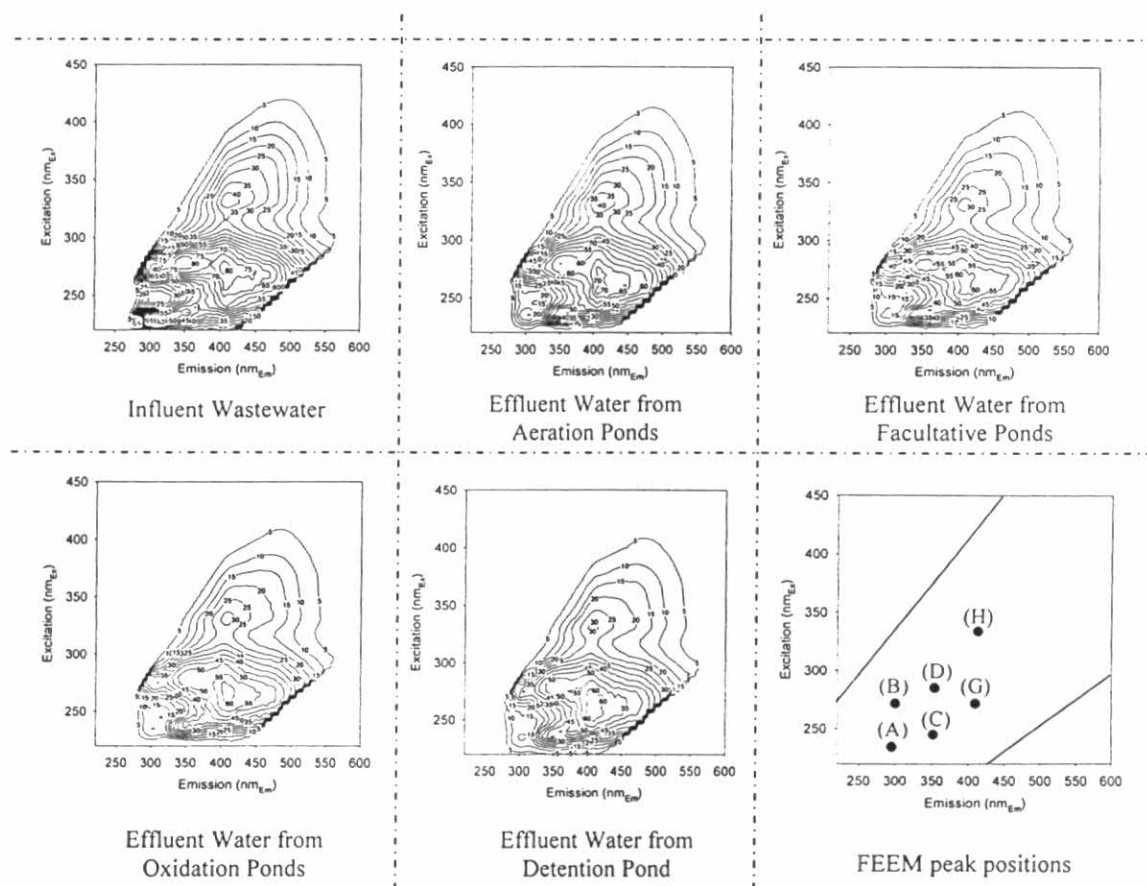
Remark: 1[ ] = fluorescent peaks positions, Bruchet *et al.* 1990 proposed that tyrosine-like substances lead to similar quantities of phenol and *p*-cresol.

As can be seen from Table 6.1, the major pyrolysis fragments of fluorescent organic matter, including tyrosine-like, tryptophan-like, fulvic acid-like, and humic acid-like substances, have been proposed by numerous researchers. From Chapter 4, pyrolysis GC/MS analysis was utilized to measure the chemical classes of all water samples in this study. The obtained pyrolysis fragments from Chapter 4, therefore, could be compared with the pyrolysis fragments of the fluorescent organic matter from the literature data in Table 6.1 in order to preliminarily confirm the appearance of tyrosine-like, tryptophan-like, fulvic acid-like, and humic acid-like substances in the water samples.

## 6.4 Results and Discussions

### 6.4.1 FEEM of Influent Wastewater and its Treated Wastewater

The FEEMs of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds are illustrated in Figure 6.3.



**Figure 6.3:** FEEMs (contour interval of 5 QSU) of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds.

**Table 6.2:** Fluorescent peak positions and their putative fluorescent organic matter with major pyrolysis fragments of unfractionated waters

Water sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater	•	•	•	•			•	•	<p><i>Tyrosine-like substance at peaks A and B:</i> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><i>Tryptophan-like substances at peaks C and D:</i> Indole</p> <p><i>Humic and fulvic acids-like substances at peaks G and H:</i> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{20}</math>, alkyl-benzenes, naphthalene, and nitrogen containing compounds</p>
Effluent water from aeration ponds	•	•	•	•			•	•	<p><i>Tyrosine-like substances at peaks A and B:</i> Nitrile</p> <p><i>Humic and fulvic acids-like substances at peaks G and H:</i> <math>C_4 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{19}</math> and naphthalene</p>
Effluent water from facultative ponds	•	•	•	•			•	•	<p><i>Tyrosine-like substances at peaks A and B:</i> Pyrrole and nitrile</p> <p><i>Tryptophan-like substances at peaks C and D:</i> Acetonitrile, benzonitrile, pyrrole</p> <p><i>Humic and fulvic acids-like substances at peaks G and H:</i> <math>C_6 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{23}</math>, phenol, nitrogen containing compound and furfural</p>
Effluent water from oxidation ponds		•	•	•			•	•	<p><i>Tyrosine-like substances at peak B:</i> Phenol and <i>p</i>-cresol, pyrrole and nitrile</p> <p><i>Tryptophan-like substances at peaks C and D:</i> Pyrrole</p> <p><i>Humic and fulvic acids-like substance at peaks G and H:</i> <math>C_6 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{21}</math> and nitrogen containing compound.</p>
Effluent water from detention pond	•	•	•	•			•	•	<p><i>Tyrosine-like substances at peaks A and B:</i> Pyrrole and nitrile</p> <p><i>Tryptophan-like substances at peaks C and D:</i> Benzonitrile and pyrrole</p> <p><i>Humic and fulvic acids-like substances at peaks G and H:</i> <math>C_6 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{19}</math>, alkyl-benzenes, naphthalene, phenol, nitrogen containing compound, phenol, and aldehydes</p>

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks E, F, G and H = Humic and fulvic acid-like substances

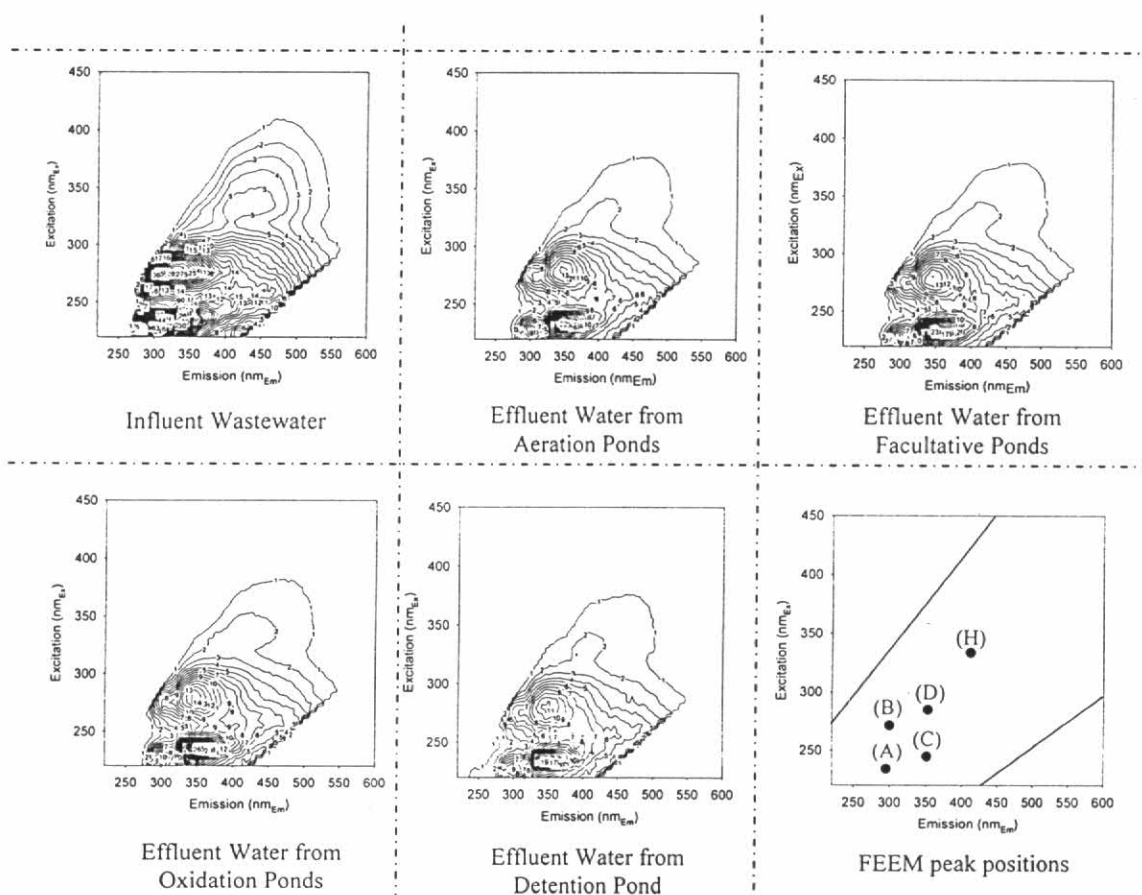
When the influent wastewater was analyzed phenol and *p*-cresol, pyrrole, nitrile and indole were detected as the major pyrolysis fragments of the tyrosine-like substances at peaks A and B; whereas indole was detected as the major pyrolysis fragment of the tryptophan-like substances at peak C and D; and  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{20}$ , alkyl-benzenes, naphthalene, and nitrogen containing compounds were found as the major pyrolysis fragments of the humic and fulvic acid-like substances at peaks G and H. On this basis, it was deduced that the tyrosine-like substances at peaks A and B, tryptophan-like substances at peaks C and D and humic and fulvic acid-like substances at peaks G and H were the major fluorescent organic matter in the influent wastewater.

After the influent wastewater was treated by the aeration ponds, the detected fluorescent peaks of the effluent were almost the same as those of the influent wastewater. However, a pyrolysis fragment of the tryptophan-like substances was not detected, whereas the pyrolysis fragment of tyrosine-like substances at peaks A and B was nitrile. For the humic and fulvic acid-like substances at peaks G and H, it was  $C_4 \leq$  aliphatic hydrocarbon fragments  $\leq C_{19}$  and naphthalene. The FEEMs of the facultative pond effluent were analyzed. The major pyrolysis fragments of the tyrosine-like, tryptophan-like and humic and fulvic acids-like substances are shown in Table 6.2. Interestingly, acetonitrile and benzonitrile were detected as the major pyrolysis fragments of the tryptophan-like substances at peaks C and D. This observation was considerably similar to the results of the study by Leenheer and Croues (2003) which presented that acetonitrile, benzonitrile, phenylacetonitrile, pyridine, methylpyridine, pyrrole, indole and methylindole were the specific fragments of tryptophan in water. After the effluent water from the facultative ponds was treated by the oxidation ponds, the fluorescent peak A disappeared, while other detected fluorescent peaks remained the same. The major pyrolysis fragments of the tyrosine-like, tryptophan-like and humic and fulvic acids-like substances are tabulated in Table 6.2. The tyrosine-like substances at peak A was completely removed after the oxidation ponds. Nevertheless, when the FEEM of the effluent water from the detention pond was analyzed, the fluorescent peak A was detected again. The tyrosine-like substances at peak A in the treated wastewater may have directly leached from organic sources in the detention pond or it may have come from the discharged storm water into this pond. Other detected fluorescent peaks were almost the same as those of the effluent water from the oxidation ponds. From the obtained results, it

can be concluded that the compositions of fluorescent organic matter in the influent wastewater and its treated wastewater were significantly similar when tyrosine-like, tryptophan-like and humic and fulvic acid-like substances appeared to be predominant in the fluorescent organic matter.

#### 6.4.2 FEEM of HPON

The FEEMs of HPON of the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds are illustrated in Figure 6.4, while the fluorescent peak positions of HPON and their putative fluorescent organic matter with pyrolysis fragments are tabulated in Table 6.3.



**Figure 6.4:** FEEMs (contour interval of 1 QSU) of HPON of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds



**Table 6.3:** Fluorescent peak positions of HPON and their putative fluorescent organic matter with major pyrolysis fragments

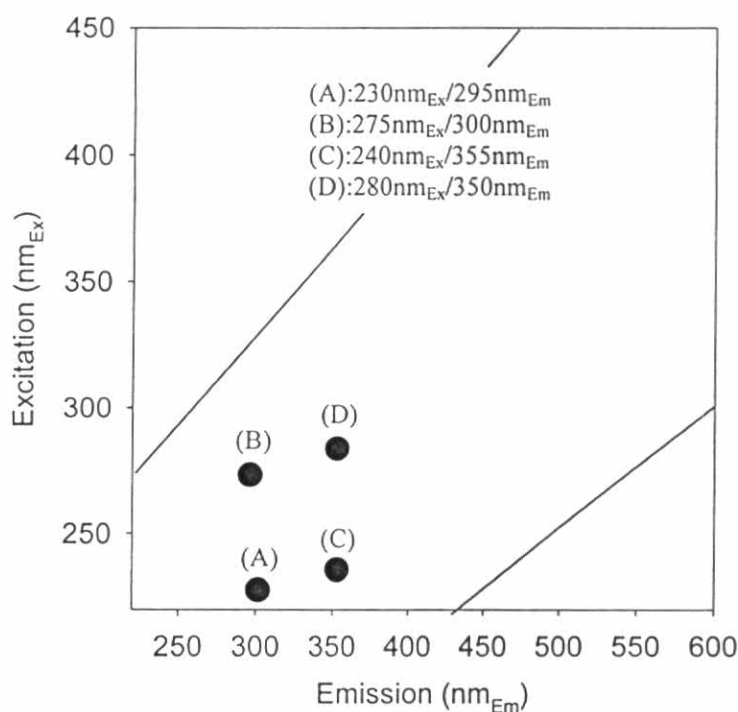
Water Sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater	•	•	•	•				•	<p><b>Tyrosine-like substance at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Tryptophan-like substances at peaks C and D:</b> Indole, 1H-pyrrole-2 methyl, 1H-pyrrole-3 methyl, pyrrole, pyridine-3methyl, acetronitrile and benzonitrile</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> C<sub>3</sub> ≤ aliphatic hydrocarbon fragments ≤ C<sub>15</sub>, alkyl-benzenes, naphthalene, alkyl-naphthalene, and nitrogen containing compounds and phenol.</p>
Effluent water from aeration Ponds	•	•	•	•					<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Tryptophan-like substances at peaks C and D:</b> Indole, pyrrole, pyridine-3methyl, and benzonitrile</p>
Effluent water from facultative Ponds	•	•	•	•					<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole and nitrile</p> <p><b>Tryptophan-like substances at peaks C and D:</b> Pyrrole and benzonitrile</p>
Effluent water from oxidation ponds	•	•	•	•					<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol and pyrrole</p> <p><b>Tryptophan-like substances at peaks C and D:</b> Pyrrole</p>
Effluent water from detention pond	•	•	•	•					<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, nitrile and pyrrole</p> <p><b>Tryptophan-like substances at peaks C and D:</b> Acetronitrile and pyrrole</p>

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks E, F, G and H = Humic and fulvic acid-like substances

In Figure 6.4 and Table 6.3, fluorescent peaks A, B, C, D, and H appear to dominate in the FEEMs of the HPON in the influent wastewater. A more detailed analysis of the pyrolysis fragments of the putative fluorescent organic matter of HPON in the influent wastewater was undertaken. Phenol and *p*-cresol, pyrrole, nitrile, and indole were identified as the pyrolysis fragments of tyrosine-like substances at peak A and B. 1H-pyrrole-2 methyl, 1H-pyrrole-3 methyl, pyrrole, pyridine-3 methyl, indole, acetronitrile and benzonitrile were the pyrolysis fragments of the tryptophan-like substances at peaks

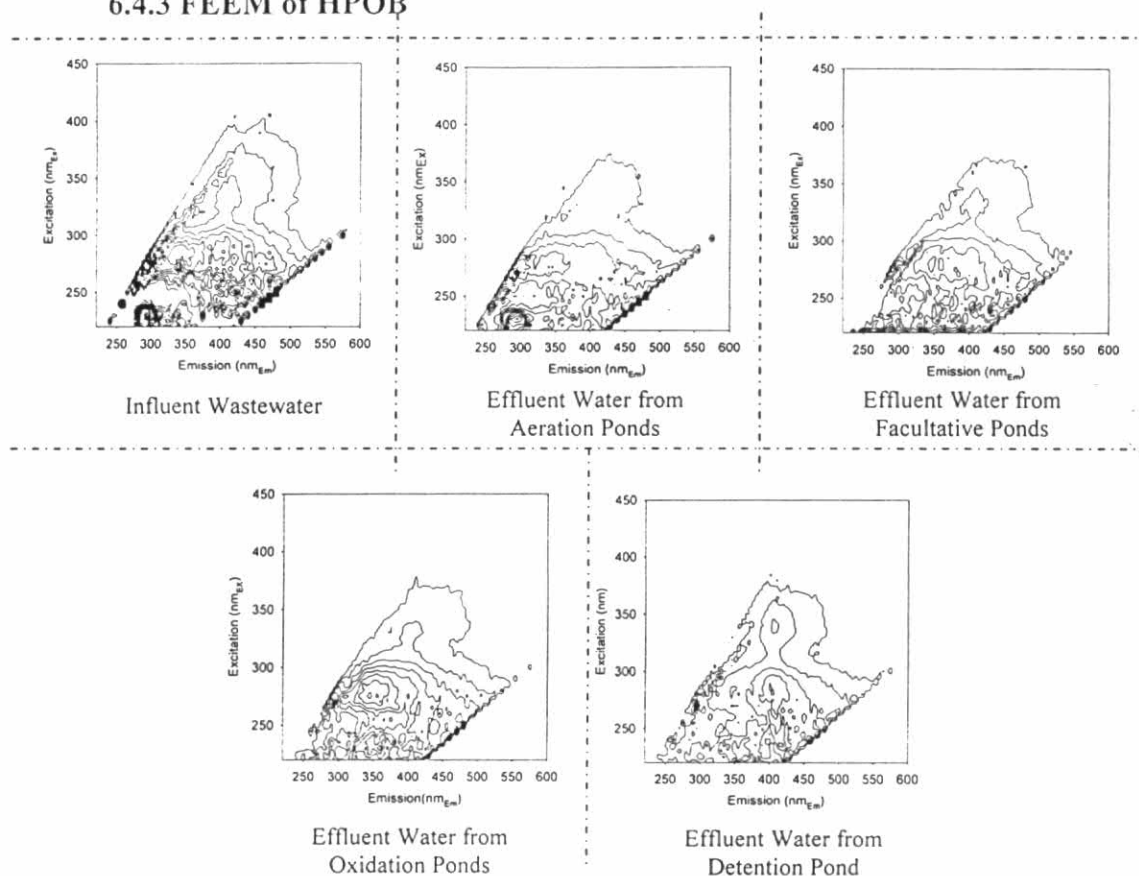
C and D; whereas  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{15}$ , alkyl-benzenes, naphthalene, alkyl-naphthalene, nitrogen containing compounds, and phenol were the pyrolysis fragments of the humic and fulvic acid-like substances at peak H.

When FEEMs of the HPON in the effluent from the aeration, facultative, oxidation, and detention ponds were performed, it was found that the fluorescent peaks A, B, C, and D were detected in all the mentioned water samples. Phenol and *p*-cresol, pyrrole, and nitrite were the major pyrolysis fragments of the tyrosine-like substances at peaks A and B, whereas pyrrole and benzonitrile were identified as the major pyrolysis fragments of the tryptophan-like substances at peaks C and D. It was concluded that tyrosine-like and tryptophan-like substances were the predominant fluorescent organic matter of HPON in the effluent water from the aeration, facultative, oxidation and detention ponds. Using the fluorescent peak positions as an index to distinguish HPON from other DOM fractions, a proposed FEEM signature of HPON was created as depicted in Figure 6.5.



**Figure 6.5:** The proposed FEEM signature of HPON

### 6.4.3 FEEM of HPOB



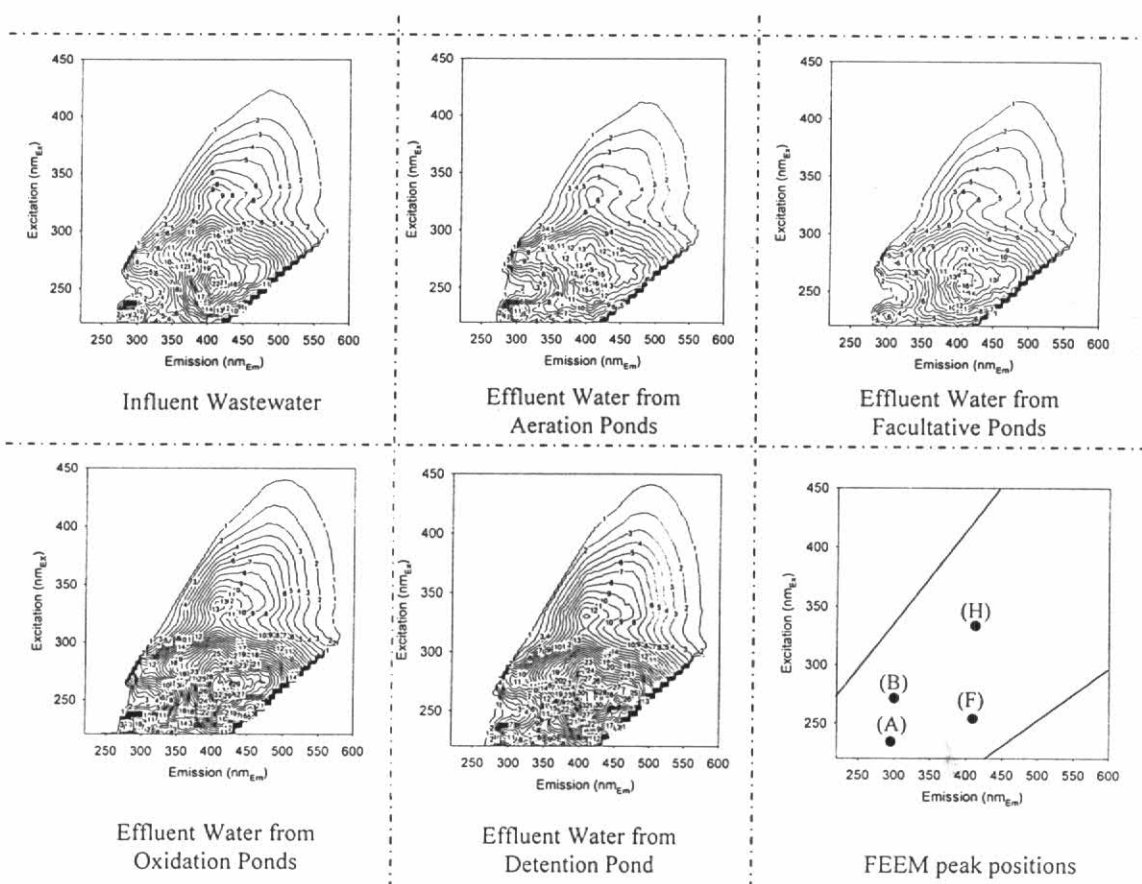
**Figure 6.6:** FEEMs (contour interval of 0.2 QSU) of HPOB of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds

The FEEMs of HPOB of the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds are shown in Figure 6.6. A fluorescent peak for HPOB was very difficult to identify because an outstanding fluorescent intensity could not be determined at any of the excitations/emissions in the FEEMs. This may have been due to the very low DOC concentrations of HPOB in all the water samples. From this result, it can be preliminary stated that spectrofluorometry analysis is unsuitable for determining the characteristics of DOM in water that has very low DOC concentrations.

### 6.4.4 FEEM of HPOA

The FEEMs of HPOA of the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds are illustrated in Figure 6.7, while

fluorescent peak positions of HPOA and their putative fluorescent organic matter with pyrolysis fragments are presented in Table 6.4.



**Figure 6.7:** FEEMs (contour interval of 1 QSU) of HPOA in the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds

Fluorescent peaks A, B, F and G were detected in the HPOA of influent wastewater and effluent water from the aeration, facultative oxidation and detention ponds. The fluorescent peaks A and B were representative of tyrosine-like substances, in which phenol and *p*-cresol, pyrrole, and nitrile were detected as the major pyrolysis fragments. The fluorescent peaks F and G were representative of humic and fulvic acid-like substances, in which  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{21}$ , alkyl-benzenes, naphthalene, alkyl-naphthalene, nitrogen containing compounds, and phenol were classified as the major pyrolysis fragments. Based on the obtained results, it can be stated that the tyrosine-like substances at peaks A and B and humic and fulvic acid-like substances at peaks F and G were the major fluorescent organic matter of HPOA in both the influent wastewater and treated effluent.

**Table 6.4:** Fluorescent peak positions of HPOA and their putative fluorescent organic matter with major pyrolysis fragments

Water Sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater	•	•				•		•	<p><b>Tyrosine-like substance at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{19}</math>, alkyl-benzenes, naphthalene, alkyl-naphthalene, nitrogen containing compounds and phenol.</p>
Effluent water from aeration ponds	•	•				•		•	<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{21}</math>, alkyl-benzenes, naphthalene, alkyl-naphthalene, and nitrogen containing compounds and phenol.</p>
Effluent water from facultative ponds	•	•				•		•	<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{21}</math>, alkyl-benzenes, naphthalene, alkyl-naphthalene, nitrogen containing compounds and phenol.</p>
Effluent water from oxidation ponds	•	•				•		•	<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{21}</math>, alkyl-benzenes, naphthalene, alkyl-naphthalene, and nitrogen containing compounds.</p>
Effluent water from detention pond	•	•				•		•	<p><b>Tyrosine-like substances at peaks A and B:</b> Nitrile, pyrrole and indole</p> <p><b>Humic and fulvic acids-like substances at peaks F and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{21}</math>, alkyl-benzenes, naphthalene, alkyl-naphthalene, and nitrogen containing compounds.</p>

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks, E, F, G and H = Humic and fulvic acid-like substances

As mention earlier, HPOA was the major DOM fractions in all water samples. Therefore a FEEM signature of HPOA was created in order to distinguish HPOA, the major DOM fraction, from the other DOM fractions. The proposed FEEM signature for HPOA is illustrated in Figure 6.8.

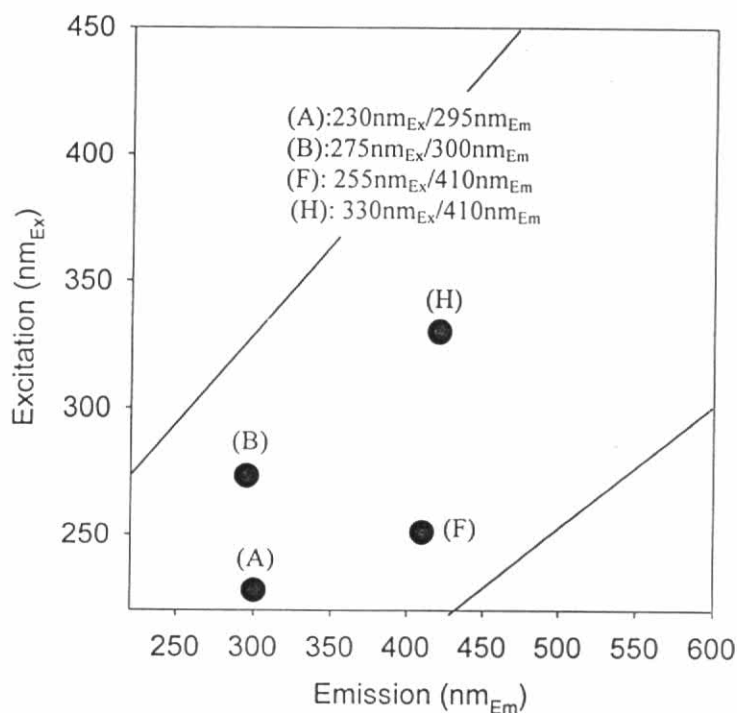
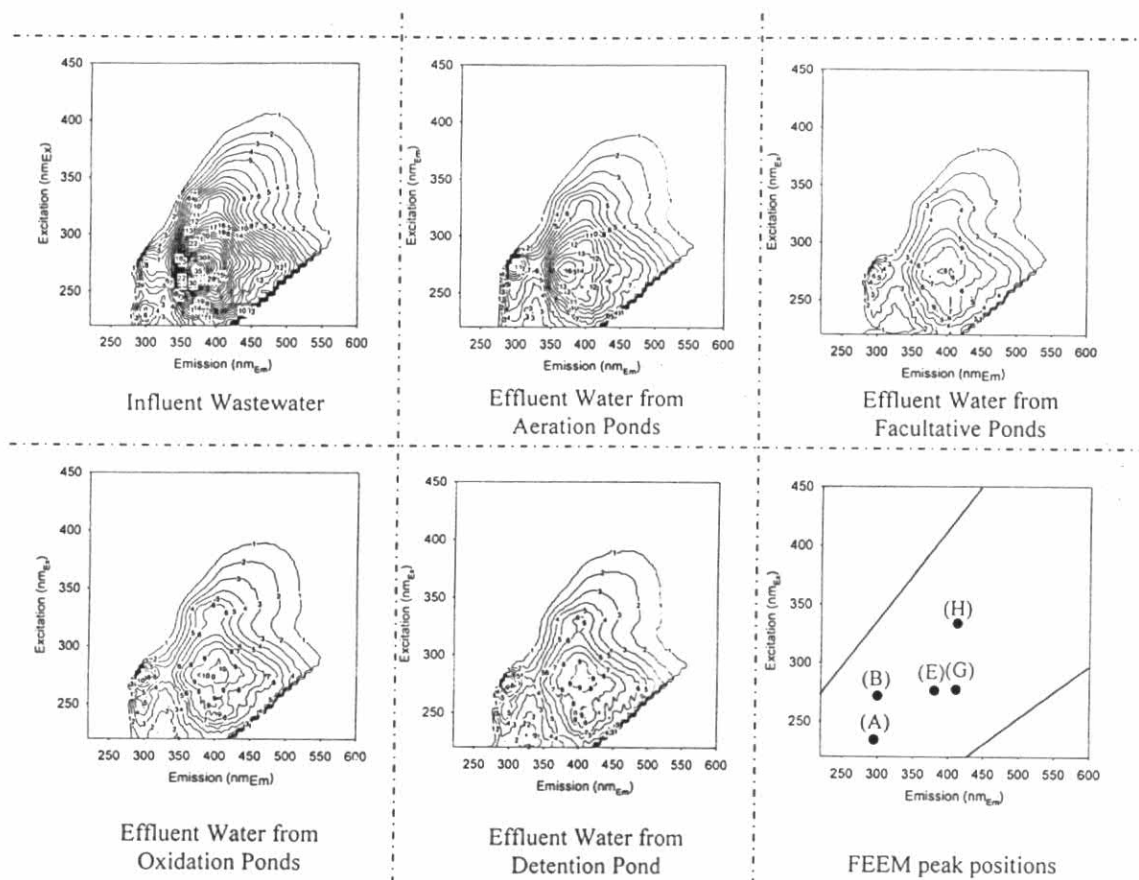


Figure 6.8: The proposed FEEM signature of HPOA

#### 6.4.5 FEEM of HPIB

Figure 6.9 presents the FEEMs of HPIB in the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds. The fluorescent peak positions of HPIB and their putative fluorescent organic matter with major pyrolysis fragments are shown in Table 6.5. Fluorescent peaks A, B, E and H appear to dominate in the FEEMs of HPIB in the influent wastewater. When the pyrolysis fragments of the putative fluorescent organic matter of HPIB in the influent wastewater were studied in more detail, nitrile was classified as the pyrolysis fragment of tyrosine-like substances at peaks A and B, whereas  $C_5 \leq$  aliphatic hydrocarbon fragments  $\leq C_{15}$ , alkyl-benzenes, nitrogen containing compounds, and phenol were the pyrolysis fragments of humic and fulvic acid-like substances at peaks E and H.



**Figure 6.9:** FEEMs (contour interval of 1 QSU) of HPIB of influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds

When the FEEM of HPIB in the effluent water from the aeration ponds was taken into consideration, fluorescent peak A disappeared, while fluorescent peaks B, E and H were detected. Nitrile was classified as a pyrolysis fragment of tyrosine-like substances at peak B; whereas  $C_4 \leq$  aliphatic hydrocarbon fragments  $\leq C_{19}$ , nitrogen containing compounds and phenol were classified as pyrolysis fragments of humic and fulvic acid-like substances at peaks E and H.

The detected fluorescent peaks of effluent water from the facultative, oxidation and detention ponds were practically the same. Fluorescent peak B, representative of tyrosine-like substances, was detected when nitrile was the major pyrolysis fragment. Fluorescent peaks G and H, the representatives of humic and fulvic acid-like substances, were determined where  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{23}$ , alkyl-benzenes, nitrogen containing compounds, and phenol were the major fragments.

**Table 6.5:** Fluorescent peak positions of HPIB and their putative fluorescent organic matter with pyrolysis fragments

Water sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater	•	•			•			•	<i>Tyrosine-like substances at peaks A and B:</i> Nitrile <i>Humic and fulvic acids-like substances at peaks E and H:</i> $C_5 \leq$ aliphatic hydrocarbon fragments $\leq C_{15}$ , alkyl-benzenes, nitrogen containing compounds and phenol.
Effluent water from aeration ponds		•			•			•	<i>Tyrosine-like substances at peak B:</i> Nitrile <i>Humic and fulvic acids-like substances at peaks E and H:</i> $C_4 \leq$ aliphatic hydrocarbon fragments $\leq C_{19}$ , nitrogen containing compounds and phenol.
Effluent water from facultative ponds		•					•	•	<i>Tyrosine-like substances at peak B:</i> Nitrile <i>Humic and fulvic acids-like substances at peaks G and H:</i> $C_5 \leq$ aliphatic hydrocarbon fragments $\leq C_{15}$ , and nitrogen containing compounds
Effluent water from oxidation ponds		•					•	•	<i>Tyrosine-like substances at peak B:</i> Nitrile <i>Humic and fulvic acids-like substances at peaks G and H:</i> $C_6 \leq$ aliphatic hydrocarbon fragments $\leq C_{14}$ , and nitrogen containing compounds.
Effluent water from detention pond		•					•	•	<i>Tyrosine-like substances at peak B:</i> Nitrile and indole <i>Humic and fulvic acids-like substances at peaks G and H:</i> $C_3 \leq$ aliphatic hydrocarbon fragments $\leq C_{15}$ , alkyl-benzenes, nitrogen containing compounds, and phenol.

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks, E, F, G and H = Humic and fulvic acid-like substances

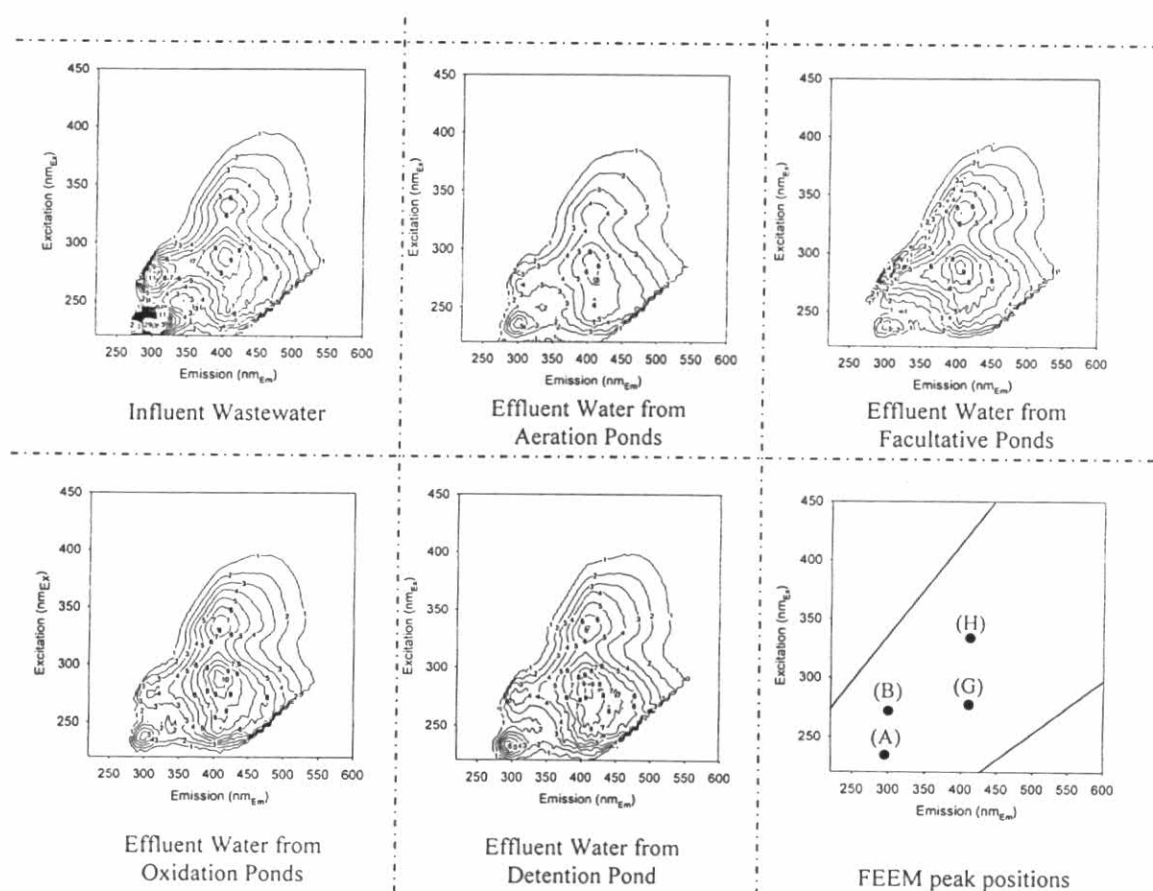
It must be noted that tyrosine-like substances were the major fluorescent organic matter in HPON, HPOA and HPIB. However, when the major pyrolysis fragments of tyrosine in HPIB were compared with those of HPON and HPOA, they were significantly different. In the case of HPON and HPOA, phenol and *p*-cresol, pyrrole, and nitrile were detected as the major pyrolysis fragments; while in the case of HPIB, only nitrile was found. It can be deduced that although the putative fluorescent organic matters of the DOM fractions were almost the same, they were dominated by different pyrolysis fragments.



Based on the obtained results, it can be summarized that tyrosine-like substances at peaks A and B and humic and fulvic acid-like substances at peaks E, G and H were the dominant fluorescent organic matter of HPIB in the influent wastewater and treated effluent. Since different FEEM peak positions in the FEEM of the influent wastewater and treated effluent were found, a FEEM signature for HPIB could not be established.

#### 6.4.6 FEEM of HPIA

The FEEMs of HPIA in the influent wastewater and effluent water from the aeration, facultative, oxidation and detention ponds are illustrated in Figure 6.10, while the fluorescent peak positions of HPIA and their putative fluorescent organic matter with pyrolysis fragments are presented in Table 6.6.



**Figure 6.10:** FEEMs (contour interval of 1 QSU) of HPIA in influent wastewater and effluent water of the aeration, facultative, oxidation and detention ponds

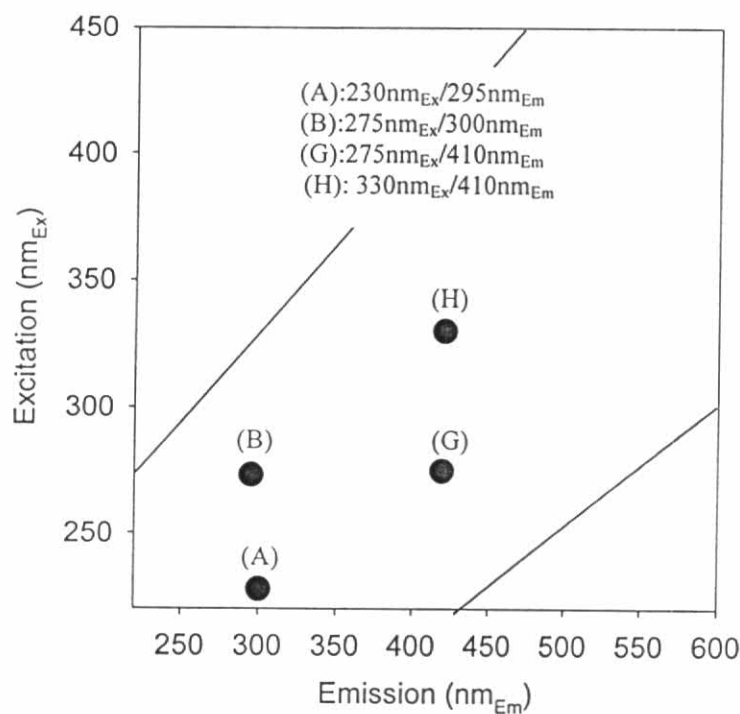
From Figure 6.10 and Table 6.6, it was found that fluorescent peaks A and B, and G and H appeared to be the respective dominant fluorescent peaks of HPIA of all water samples. After a more detailed analysis of the pyrolysis fragments of the putative fluorescent organic matter of HPIA, pyrrole, nitrile and indole were classified as the pyrolysis fragments of the tyrosine-like substances at peaks A and B, whereas  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{23}$ , alkyl-benzenes, nitrogen containing compounds and phenol were the major pyrolysis fragments of the humic and fulvic acid-like substances at peaks G and H.

**Table 6.6:** Fluorescent peak positions of HPIA and their putative fluorescent organic matter with pyrolysis fragments

Water Sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater	•	•					•	•	<p><b>Tyrosine-like substances at peaks A and B:</b> Phenol and <i>p</i>-cresol, pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks E and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{23}</math>, alkyl-benzenes, nitrogen containing compounds and phenol.</p>
Effluent water from aeration ponds	•	•					•	•	<p><b>Tyrosine-like substances at peaks A and B:</b> Pyrrole, nitrile and indole</p> <p><b>Humic and fulvic acids-like substances at peaks E and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{19}</math>, naphthalene, and nitrogen containing compounds.</p>
Effluent water from facultative ponds	•	•					•	•	<p><b>Humic and fulvic acids-like substances at peaks G and H:</b> <math>C_5 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{16}</math></p>
Effluent water from oxidation ponds	•	•					•	•	<p><b>Tyrosine-like substances at peak A and B:</b> Nitrile</p> <p><b>Humic and fulvic acids-like substances at peaks G and H:</b> <math>C_6 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{16}</math>, and nitrogen containing compounds and furfural.</p>
Effluent water from detention pond	•	•					•	•	<p><b>Tyrosine-like substances at peak A and B:</b> Pyrrole and nitrile</p> <p><b>Humic and fulvic acids-like substances at peaks G and H:</b> <math>C_3 \leq</math> aliphatic hydrocarbon fragments <math>\leq C_{15}</math>, nitrogen containing compounds and aldehydes.</p>

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks, E, F, G and H = Humic and fulvic acid-like substances

As stated previously, HPIA was the major DOM fraction in the effluent water of the facultative, oxidation and detention ponds, the proposed FEEM signature of HPIA could be used to distinguish HPIA from the other fractions. On this basis, it was concluded that the tyrosine-like substances at peaks A and B and humic and fulvic acid-like substances at peaks G and H were the dominant fluorescent organic matter of HPIA in influent wastewater and effluent water of the aeration, facultative, oxidation and detention ponds. The proposed FEEM signature for HPIA is depicted in Figure 6.11.

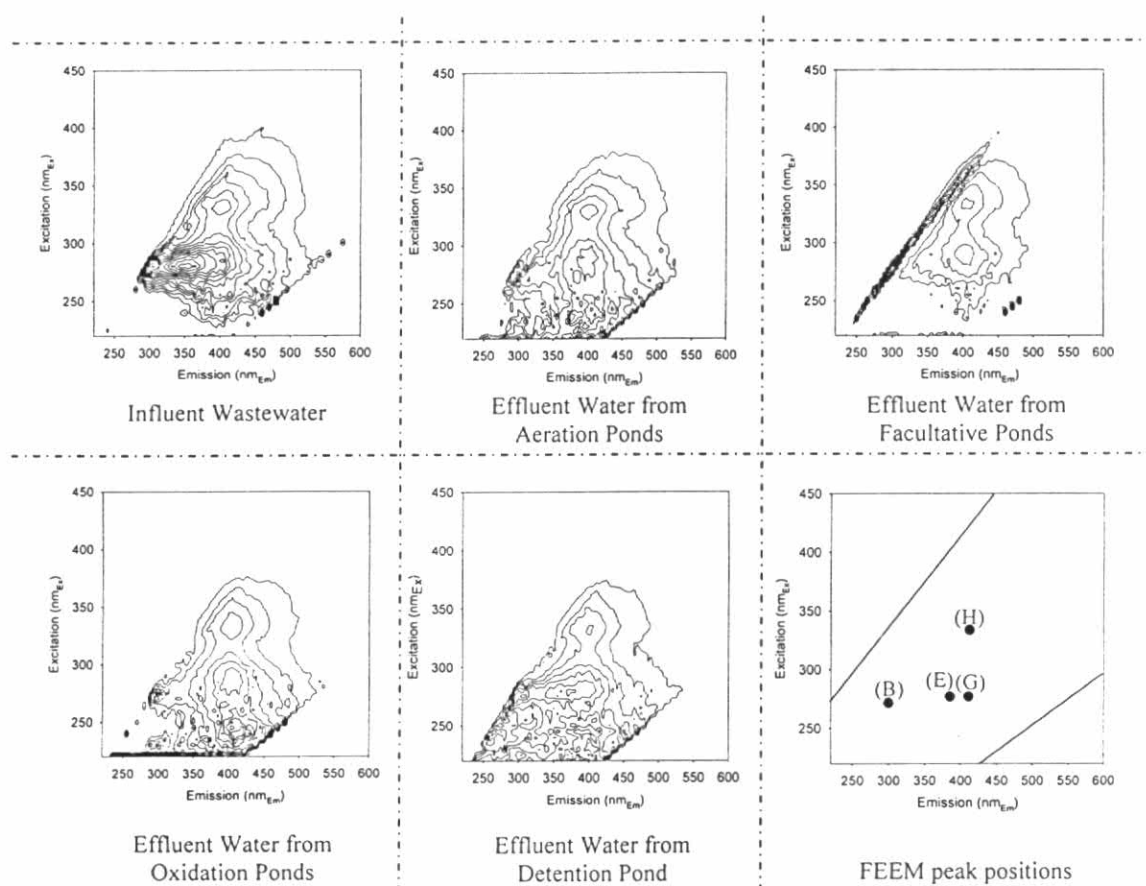


**Figure 6.11:** The proposed FEEM signature of HPIA

#### 6.4.7 FEEM of HPIN

Figure 6.12 presents the FEEMs of HPIN in the influent wastewater and effluent water of the aeration, facultative, oxidation and detention ponds. The fluorescent peak positions of HPIN and their putative of fluorescent organic matter with pyrolysis fragments are shown in Table 6.7. The fluorescent intensity of HPIN was considerably low when compared with the fluorescent intensities of the other fractions. The fluorescent peaks for HPIN, therefore, were slightly difficult to determine.

In FEEMs of HPIN in the influent wastewater, fluorescent peaks B, E and H were dominant. When the pyrolysis fragments of the putative fluorescent organic matter of HPIN in the influent wastewater was studied further, phenol and *p*-cresol, pyrrole, and nitrile were determined as the pyrolysis fragments of the tyrosine-like substances at peak B, whereas  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{23}$ , alkyl-benzenes, nitrogen containing compounds, phenol, and aldehyde were determined as the pyrolysis fragments of the humic and fulvic acid-like substances at peaks E and H.



**Figure 6.12:** FEEMs (contour interval of 0.3 QSU) of HPIN in the influent wastewater and the aeration, facultative, oxidation and detention pond effluents

Fluorescent peaks B, G and H were detected in the FEEMs of HPIN in the effluent water from the aeration ponds. Pyrrole and nitrile were classified as the major pyrolysis fragments of the tyrosine-like substances at peak B, whereas  $C_3 \leq$  aliphatic hydrocarbon fragments  $\leq C_{21}$ , naphthalene, nitrogen containing compounds and phenol were classified as the pyrolysis fragments of the humic and fulvic acid-like substances at peaks G and H.

Only peaks G and H were detected in the effluent water from the facultative ponds. Therefore,  $C_4 \leq$  aliphatic hydrocarbon fragments  $\leq C_{21}$ , alkyl-benzenes, nitrogen containing compounds, and phenol were the pyrolysis fragments of the humic and fulvic-acid like substances.

**Table 6.7:** Fluorescent peak positions of HPIN and their putative fluorescent organic matter with pyrolysis fragments

Water Sample	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F	Peak G	Peak H	Obtained pyrolysis fragments of putative fluorescent organic matter
Influent wastewater		•			•			•	<b>Tyrosine-like substances at peaks A and B:</b> Phenol and p-cresol, pyrrole and nitrile <b>Humic and fulvic acids-like substances at peaks E and H:</b> $C_3 \leq$ aliphatic hydrocarbon fragments $\leq C_{23}$ , alkyl-benzenes, nitrogen containing compounds, phenol and aldehydes.
Effluent water from aeration ponds		•					•	•	<b>Tyrosine-like substances at peaks A and B:</b> Pyrrole, and nitrile <b>Humic and fulvic acids-like substances at peaks G and H:</b> $C_3 \leq$ aliphatic hydrocarbon fragments $\leq C_{21}$ , naphthalene, and nitrogen containing compounds.
Effluent water from facultative ponds							•	•	<b>Humic and fulvic acids-like substances at peaks G and H:</b> $C_4 \leq$ aliphatic hydrocarbon fragments $\leq C_{21}$ , alkyl-benzenes, nitrogen containing compounds, and phenol
Effluent water from oxidation ponds		•					•	•	<b>Tyrosine-like substances at peak A and B:</b> Phenol and p-cresol, pyrrole and nitrile <b>Humic and fulvic acids-like substances at peaks G and H:</b> $C_5 \leq$ aliphatic hydrocarbon fragments $\leq C_{24}$ , nitrogen containing compounds and phenol.
Effluent water from detention pond		•					•	•	<b>Tyrosine-like substances at peak A and B:</b> Phenol and p-cresol, pyrrole and nitrile <b>Humic and fulvic acids-like substances at peaks G and H:</b> $C_4 \leq$ aliphatic hydrocarbon fragments $\leq C_{23}$ , naphthalene, nitrogen containing compounds, phenol and furfural.

**Remark:** Peaks A and B = Tyrosine-like substances, Peaks C and D = Tryptophan-like substances, Peaks, E, F, G and H = Humic and Acids-like substances

The detected FEEM peaks in the effluent water from the oxidation and detention ponds were practically the same. Fluorescent peak B, which represented tyrosine-like substances, was detected. Phenol and *p*-cresol, pyrrole, and nitrile were the

major pyrolysis fragments related to the tyrosine-like substances. Fluorescent peaks G and H, representing humic and fulvic acid-like substances, were determined.  $C_4 \leq$  aliphatic hydrocarbon fragments  $\leq C_{23}$ , naphthalene, nitrogen containing compounds, phenol and furfural were the major fragments related to the humic and fulvic acid-like substances. It must be noted that the major pyrolysis fragment of the tyrosine-like substances of HPIN was compared with the major pyrolysis fragments of the tyrosine-like substances of HPIB and HPIA and was found to be significantly different. For HPIB and HPIA, pyrrole and nitrile were detected as the major pyrolysis fragments, while in the case of HPIN, phenol and *p*-cresol, pyrrole and nitrile were the major pyrolysis fragments. It can be stated once again that although the putative fluorescent organic matter of the DOM fractions were almost the same, it could have been dominated by different pyrolysis fragments. From the obtained results, it can be proposed that tyrosine-like substances at peak B and humic and fulvic acids-like substances at peaks E, G and H were the dominant fluorescent organic matter of HPIN in the influent wastewater and treated effluent. Since different FEEM peak positions were found for the influent and effluent, a proposed FEEM signature for HPIB could not be established.

#### 6.4.8 Summary of the Locations of the FEEM Peak Positions of the DOM Fractions

Table 6.8 shows the fluorescent peak positions of the six DOM fractions obtained in this study and compares them with the literature data. In the case of HPON, the fluorescent peak positions at peak B ( $275\text{nm}_{\text{Ex}}/300\text{nm}_{\text{Em}}$ ) and peak C ( $240\text{nm}_{\text{Ex}}/355\text{nm}_{\text{Em}}$ , tryptophan-like substances) were considerably similar to the fluorescent peak positions of HPON in other studies. Peaks at  $280\text{nm}_{\text{Ex}}/310\text{nm}_{\text{Em}}$ , were recorded by Kimura *et al.* (2004),  $240\text{nm}_{\text{Ex}}/355\text{nm}_{\text{Em}}$  were reported by Chen *et al.* (2003), and  $230\text{-}300\text{nm}_{\text{Ex}}/340\text{-}520\text{nm}_{\text{Em}}$  were reported by Janhom (2004). Fluorescent peak position at peak D ( $275\text{nm}_{\text{Ex}}/300\text{nm}_{\text{Em}}$ ) was the same as the fluorescent peak positions of HPON at  $280\text{nm}_{\text{Ex}}/310\text{nm}_{\text{Em}}$  and  $230\text{-}300\text{nm}_{\text{Ex}}/340\text{-}520\text{nm}_{\text{Em}}$  that were report by Janhom (2004). In the case of HPOA, only the fluorescent peak position at peak F was similar to the fluorescent peak position of HPOA detected by Janhom (2004) at  $255\text{nm}_{\text{Ex}}/420\text{nm}_{\text{Em}}$ .

**Table 6.8:** Comparison of the fluorescent peak positions of each DOM fraction obtained in this study with those found in literature data

Organic fractions	FEEM peak positions (nm <sub>Ex</sub> /nm <sub>Em</sub> )	
	This study	Literature value data
HPON	Peak A: 230/300 Peak B: 275/300 ▲ Peak C: 240/355 ■ Peak D: 280/350 ■	225/609-621 (Marhaba <i>et al.</i> 2000), 225/350 (Chen <i>et al.</i> 2003) ■ [peak C] 280/310 (Kimura <i>et al.</i> 2004) ▲ [peak B] 230-300/340-520 (Janhom, 2004) ■ [peak C and D]
HPOA	Peak A: 230/300 Peak B: 275/300 Peak F: 255/410 ■ Peak H: 330/410	225-237/345-357 (Marhaba <i>et al.</i> 2000) 215/415 (Chen <i>et al.</i> 2003) 220/355 (Chen <i>et al.</i> 2003) 290/455 (Kimura <i>et al.</i> 2004) 255/420 (Janhom, 2004) ■ [peak F]
HPIB	Peak A: 230/300 Peak B: 275/300 Peak E: 275/380 ▲ Peak G: 275/410 ▲ Peak H: 330/410	225-237/369-381 (Marhaba <i>et al.</i> 2000) 290/380 (Kimura <i>et al.</i> 2004) ▲ [peak E and F]
HPIA	Peak A: 230/300 Peak B: 275/300 ▲ Peak G: 275/410 ▲ Peak H: 330/410 ▲	237-249/417-429 (Marhaba <i>et al.</i> 2000) 205/405 (Chen <i>et al.</i> 2003) 220/335 (Chen <i>et al.</i> 2003) 275/325 (Chen <i>et al.</i> 2003) ▲ [peak B] 300/400 (Chen <i>et al.</i> 2003) ▲ [peak H] 310/430 (Kimura <i>et al.</i> 2004) ▲ [peak H] 318/424 (Goslan <i>et al.</i> 2004) ▲ [peak H] 270/410 (Janhom, 2004) ■ [peak G] 330/410 (Janhom, 2004) ■ [peak H]
HPIN	Peak B: 275/300 Peak E: 275/380 ▲ Peak G: 275/410 ■ Peak H: 330/410 ■	225/309-321 (Marhaba <i>et al.</i> 2000) 295/355 (Kimura <i>et al.</i> 2004) ▲ [peak E] 230-300/340-520 (Janhom, 2004) ■ [peak G and H]

Remark: ■ = not more than 10 nm difference between the excitation and emission wavelengths of the FEEM peak in this study and those of the literature data; and ▲ = not more than 30 nm difference between the excitation and emission wavelengths of the FEEM peak in this study and those of the literature data.

With regard to the fluorescent peak positions of HPIB, Kimura *et al.* (2004) reported that HPIB exhibited a major fluorescent peak at 290nm<sub>Ex</sub>/380nm<sub>Em</sub>. This observation was relatively similar to the fluorescent peak positions of HPIB obtained in this study at peak E (275nm<sub>Ex</sub>/380nm<sub>Em</sub>) and peak G (275nm<sub>Ex</sub>/410nm<sub>Em</sub>). In the case of HPIA, the fluorescent peak position at peak B of 275nm<sub>Ex</sub>/300nm<sub>Em</sub> was relatively close to the one proposed by Chen *et al.* (2003) at 275nm<sub>Ex</sub>/335nm<sub>Em</sub>; whereas the fluorescent peak position at peak G of 275nm<sub>Ex</sub>/410nm<sub>Em</sub> was comparable to that the reported peaks of both Chen *et al.* (2003) at 300nm<sub>Ex</sub>/400nm<sub>Em</sub> and Janhom (2004) at 275nm<sub>Ex</sub>/410nm<sub>Em</sub>. The fluorescent peak position at peak H of 300nm<sub>Ex</sub>/400nm<sub>Em</sub> was considerably similar to those of Chen *et al.* (2003) at 300nm<sub>Ex</sub>/400nm<sub>Em</sub>, Kimura *et al.* (2004) at

310nm<sub>Ex</sub>/430nm<sub>Em</sub>, Goslan *et al.* (2004) at 318nm<sub>Ex</sub>/424nm<sub>Em</sub>, and Janhom (2004) at 330nm<sub>Ex</sub>/410nm<sub>Em</sub>. For HPIN, Kimura *et al.* (2004) reported that HPIN exhibited a major fluorescent peak at 295nm<sub>Ex</sub>/355nm<sub>Em</sub>. This observation corresponded well with the fluorescent peak position of HPIB obtained in this study at peak E: 275nm<sub>Ex</sub>/380nm<sub>Em</sub>. Janhom (2004) reported that the fluorescent peak of HPIN was detected at 230-300nm<sub>Ex</sub>/340-520nm<sub>Em</sub>. This fluorescent peak location was comparable to fluorescent peaks G and H of HPIN that were obtained in this study.

### 6.5 Concluding Remarks

The aim of this chapter was to investigate the characteristics of the DOM and DOM fractions in the influent wastewater and effluent waters from the aerations, facultative, oxidation and detention ponds by using spectrofluorometry analysis. Tyrosine-like, tryptophan-like, and humic and fulvic acid-like substances were the major fluorescent organic matter in the influent wastewater and treated effluents. The resin fractionation process was employed to all water samples. In the case of the hydrophobic organic fraction, tyrosine-like and tryptophan-like substances were classified as the major fluorescent organic matter in HPON. For HPOA, which was the major DOM fraction in all the water samples, tyrosine-like and humic and fulvic acid-like substances were found to be its major fluorescent organic matters. With regard to the hydrophilic organic fractions, tyrosine-like and humic and fulvic acid-like substances were the dominant fluorescent organic matter of HPIB, HPIA, and HPIN. It must be noted that even though the major fluorescent organic matter of HPOA, HPIB, HPIA, and HPIN were similar, their dominant pyrolysis fragments were considerably different. Each DOM fraction, therefore, was found to have its own FEEM signature that could be utilized to distinguish one from another. The next chapter will discuss the use of FEEM for evaluating the DOM reductions from each process of the stabilization pond system.