Effects of anthropogenic inputs on the organic quality of urbanized streams

Kathryn N. Kalscheur, Rebecca R. Penskar, Allison D. Daley, Shannon M. Pechauer, John J. Kelly, Christopher G. Peterson, Kimberly A. Gray

Department of Civil & Environmental Engineering, Northwestern University, Evanston, IL 60208, USA
Department of Biology, Loyola University Chicago, Chicago, IL 60660, USA
Department of Environmental Science, Loyola University Chicago, Chicago, IL 60660, USA

Abstract

Due to arid conditions, population growth, and anthropogenic impacts from agricultural and urban development, wastewater effluent makes up an increasingly large percentage of surface water supplies, promoting concerns about the potential ecological and human health effects associated with the organic quality of surface waters receiving treated wastewater discharge. Anthropogenic inputs alter the quality and quantity of organic carbon and also affect the ability of aquatic ecosystems to retain or transform carbon and other nutrients. In this paper, we use pyrolysis-GC/MS (Py-GC/MS) as a tool to examine whether the dissolved organic carbon (DOC) in suburban streams influenced by anthropogenic inputs displays an organic signature that is structurally different from natural organic material (NOM). Py-GC/MS was not only able to differentiate among stream sites that received discharge from upstream wastewater treatment plants and those that did not, but also distinguished stream sites influenced significantly by storm water. Distinct organic signatures were evident in stream waters with upstream wastewater treatment plant discharges regardless of the distance from effluent discharge, indicative of the persistent nature of effluent-derived organic material (EfOM). The pyrolysis fragments of 3-methyl-pyridine, 2-methyl-pyridine, pyrrole, and acetamide were identified as indicators of EfOM, supporting previous research that has suggested that protein and aminosugar derivatives are possible wastewater markers. Furthermore, pyrolysis fragments associated with soil polycarboxylic acids correlated highly with stream sites having the least anthropogenic influences.

1. Introduction

Due to arid conditions, population growth, and anthropogenic impacts from agricultural and urban development, wastewater effluent makes up an increasingly large percentage of surface water supplies, causing concern about the potential ecological and human health effects associated with the organic quality of surface waters receiving treated wastewater discharge. Anthropogenic inputs (e.g., point and non-point sources of nutrients, inorganic and organic chemicals, microorganisms etc.) may alter the quality and quantity of organic carbon and affect the biogeochemical cycling of carbon and other nutrients by aquatic ecosystems. Over 80,000 chemicals are registered for commercial use in the...
United States, yet little to no data exist on their potential or actual health effects (Nel et al., 2006). It is clearly not feasible to assess the effects of all possible chemicals in wastewater or treated effluent on aquatic ecosystems, drinking water treatment processes and finished drinking water quality. Also lacking are techniques and standards to identify all these discrete compounds and their degradation products, as they form an extremely complex and highly variable mixture. As an alternative, methods have been developed in recent years to fingerprint the entire dissolved organic matrix in wastewaters and surface waters receiving wastewater discharge to identify and differentiate the possible sources of organic carbon (Bridgeman et al., 2011; Dignac et al., 2000; Litton et al., 2010; Peschel and Wildt, 1988; Poerschmann et al., 1997; Seredynska-Sobecka et al., 2011; Sirivedhin and Gray, 2005a). However, comprehensive comparisons of streams that vary in the types of anthropogenic influences are lacking. In this paper, we use pyrolysis-GC/MS (Py-GC/MS) as a tool to examine whether suburban streams sites influenced by anthropogenic inputs show organic signatures that are structurally different from those dominated by ‘natural’ organic material.

2. Background

2.1. Effluent-derived organic material

Municipal wastewater effluent contains numerous synthetic organic constituents derived from household chemicals (detergents, personal care products, paints, etc.) and pharmaceuticals (Heim et al., 2003). A nationwide reconnaissance of the presence of a broad suite of 95 organic wastewater contaminants (OWCs) in water resources detected OWCs in 80% of the streams sampled (Kolpin et al., 2002). Some insights into the general characteristics of effluent-derived organic material (EfOM) have been made, but many attributes are still poorly understood. EfOM has been found to have a chemical signature that is distinct from natural (non-anthropogenic) organic material (NOM) (Leinweber and Schulten, 1998; Poerschmann et al., 1997; Sirivedhin and Gray, 2005a) and can persist and control organic quality at downstream points in riparian systems (Sickman et al., 2007; Sirivedhin and Gray, 2005a).

The altered quality of the organic matter resulting from the presence of EfOM can affect surface water quality (Sirivedhin and Gray, 2005b). Research comparing disinfection by-product formation potentials (DBPFP) between wastewater effluent and surface waters surprisingly found negative correlations between phenolic signatures of the samples and DBPFP, but strong positive relationships between DBPFP and organic nitrogen and halogenated signatures (Sirivedhin and Gray, 2005b). In addition, this research showed that EfOM produced distinct proportions of DBP and products were shifted to brominated DBF, which may be associated with greater health risks.

EfOM may also affect a variety of biological processes within aquatic ecosystems. For example, in wetland sediments Sirivedhin and Gray (2006) found a positive relationship between denitrification potential (DNF) and biodegradable organic carbon concentration. While EfOM may increase levels of DOC within a wetland, which could stimulate denitrification, EfOM quality may be more refractory to further microbial degradation since it has been through biological treatment (Dignac et al., 2000). Denitrification rates in wetland sediments have also been observed to be negatively affected by phenolic compounds (Dodla et al., 2008; Sirivedhin and Gray, 2006) and positively influenced by polysaccharides (Dodla et al., 2008). In an experimental study, bacteria preferentially assimilated fresh exudates from planktonic diatom blooms, but they did not rely exclusively on the exudates as a source of organic carbon as predicted (Norman et al., 1995). Chemical components of algal exudates vary considerably in their availability to bacteria (Norman et al., 1995), with a substantial fraction found to be resistant to bacterial mineralization over a 2.5-year study (Fry et al., 1996). While periphytic biofilms are heterotrophic, they are, on annual average, a net consumer of organic carbon (Romani et al., 2004) and the fraction of bacterial carbon requirements that algal extracellular release can meet varies (Raines and Pace, 1991). EfOM may serve as an alternative carbon source for bacteria within periphyton. Based on preliminary data, it is hypothesized that stream waters enriched with EfOM displays a decoupling of the relationship between bacterial denitrifier and algal species within periphyton, which may alter the ability of these communities to perform critical ecosystem services (Peterson et al., 2011).

2.2. Organic characterization by pyrolysis-GC/MS

Pyrolysis coupled with GC/MS allows characterization of the chemical features of complex mixtures of non-volatile, macromolecular organic material that cannot be analyzed by traditional methods. It also facilitates the determination of organic sources and tracking of molecular transformation within organic matrices in biofilms. In the pyrolysis step the organic material is thermally cleaved into volatile fragments, which are then swept into the GC for separation and the MS for identification. When pyrolysis is carried out under a set of controlled conditions, the parent structures produce predictable and reproducible sets of fragments creating a chemical fingerprint reflecting the combined organic characteristics of the mixture. The fragments in the pyrochromatogram can provide structural information about the chemical building blocks of the organic material. This structural information allows samples to be differentiated based on material origin, system inputs and/or biogeochemical processing (Irwin, 1982; Moldoveanu, 1998). Data interpretation relies on multivariate analyses, which are used to reduce the large amount of highly correlated data to a small number of independent variables that can then be used to extract chemical markers.

3. Materials and methods

3.1. Sampling sites

The seven sampling sites used in this study are located within DuPage County (Illinois, USA) (Fig. 1). DuPage County is a part
of the Chicago metropolitan area, and is the second most populous county in Illinois.

3.1.1. Restored Prairie
Located within the city of Naperville, the Restored Prairie site is within the 1849-acre Spring brook Prairie Forest Preserve, where Spring Brook (#1) originates. Two non-contiguous streams used in this study are named ‘Spring Brook’, hereafter denoted as Spring Brook (#1) and Spring Brook (#2). This sampling site is located in a prairie at the downstream end of a two-mile stream-restoration project that created a meandering stream from a section that had been channelized for agricultural use.

3.1.2. Forest Preserve
Also located within the Spring brook Prairie Forest Preserve in Naperville, IL is the Forest Preserve site. This site is in a forested portion of the preserve downstream of the Restored Prairie site on Spring Brook (#1) and also receives flow from a small tributary originating on a neighboring golf course (F.P.D.D.C, 2009).

3.1.3. Upstream of Wheaton WWTP
The sampling site upstream of the Wheaton WWTP is at the headwaters of Spring Brook (#2) in Kelly Park, a 16-acre playground/open grass field surrounded by suburban development in Wheaton, IL.

3.1.4. Downstream of Wheaton WWTP
Farther downstream on Spring Brook (#2), is a sampling site within the St. James Farm Forest Preserve, 3.2 km downstream of the Wheaton Sanitary District Plant’s effluent discharge. The Wheaton Sanitary District Plant has a design dry weather treatment capacity (NPDES permitted discharge flow) of 8.9 mgd (0.39 m³/s), a peak daily design flow of 13.4 mgd (0.59 m³/s) and a peak wet weather flow treatment capacity of 45 mgd (1.97 m³/s). About 5 km downstream of the plant, Spring Brook (#2) joins with the West Branch of the DuPage River. During dry weather periods, an estimated 85 percent of the flow in the West Branch of the DuPage River is from wastewater treatment plants (W.S.D, 2009).

3.1.5. Salt Creek (upstream and downstream of a storm water discharge)
Salt Creek, a tributary of the Des Plaines River, originates in Northwest Cook County at Wilke Marsh in the city of Palatine and meanders southward through DuPage County to our sampling sites in the 90-acre Salt Creek Park Forest Preserve in Wood Dale, IL. Two samples were taken at this site, one upstream and one downstream of a storm water culvert. Most of the creek’s watershed is urbanized and densely populated. Since DuPage County’s massive growth in the 1960s, the creek has been flood-prone (F.P.D.D.C, 2009), as was observed during our sampling. Five municipal wastewater treatment plants are upstream of our sampling sites on Salt Creek with a total National Pollutant Discharge Elimination System (NPDES) permitted discharge flow of 37.1 mgd (1.63 m³/s) (Table 1).

3.1.6. East Branch DuPage River
The East Branch of the DuPage River, which joins with the West Branch of the DuPage River in Bolingbrook, begins in Bloomingdale, IL and flows southward through several urbanized and densely populated communities before reaching our sampling location in Woodridge, IL. Four municipal wastewater treatment plants are upstream of our sampling site on East Branch DuPage River with a total NPDES permitted discharge flow of 35.7 mgd (1.57 m³/s).

3.2. Water quality measurements
Water samples were collected from the seven study sites for nutrient analyses and organic material characterization on June 2, June 16, July 7, and August 4, 2009. On each sampling date, field measurements of velocity (Marsh-McBirney Flomate (TM) Model 2000 portable flow meter) and cross-sectional area were used to calculate stream discharge using the midsection method (Gore, 2006). U.S. Geological Survey (USGS) precipitation and discharge data in the sampling area showed that measurable storm events occurred throughout the study and within three days prior to each sampling date. However, due to stagnant flow conditions at the US of Wheaton WWTP site on 7-July, no samples were collected for organics analyses. Samples for nutrient analyses were frozen until filtered (pre-rinsed 0.45 µm Tuffryn™ membrane filters made of hydrophilic polysulfone) and analyzed for dissolved nitrate (NO₃⁻-N) via the ultraviolet second-derivative spectroscopy method (detection limit of 0.005 mg N/L with range of
0–15 mg N/L (Crumpton et al., 1992), dissolved ammonia (NH₃–N) via the phenate method (detection limit of 0.01 mg N/L with range of 0.2–10 mg N/L) (Solorzano, 1969), and dissolved phosphate (PO₄–P) via the ascorbic acid method (detection limit of 0.005 mg P/L with range of 0.01–0.25 mg P/L) (Murphy and Riley, 1962). Stream water was also collected in acid-washed amber glass jugs and filtered (pre-reins 0.45 μm Tuffryn membrane filters) for determination of dissolved organic carbon (DOC) by high temperature catalytic oxidation (APHA Standard Method 5310 B; Dohrmann Apollo 9000) and organic material characterization via Py-GC/MS (A.P.H.A., 1998).

After preparation, the sample or standard was placed inside the platinum filament coil of the pyrolysis probe (Chemical Data Systems Pyroprobe, 2000) which was then inserted into the interface (Chemical Data Systems 1500 valved GC interface). Conditions of the pyrolysis unit used to ensure reproducibility of the analysis include: (1) pyrolysis interface temperature of 250 °C, (2) final pyrolysis temperature of 625 ± 5 °C, (3) total pyrolysis time of 1 min, and (4) ramping rate of 20 °C/minute. After flash pyrolysis, the volatile pyrolyzates were directly swept onto a gas chromatography column to be separated (60-m, 0.25-mm internal diameter, crossbond, carbowax column; Restek: Stabilwax®). The gas chromatograph (Fisons 8030) was operated in a splitless injection mode with a column head pressure of 25 psi. Oven temperature was held at 45 °C for 15 min, then ramped up to 240 °C at 2 °C/min, and finally, held at 240 °C for 10 min. The separated fragments were identified by mass spectrometry (Fisons MD 800) that operated at 70 eV and scanned from 20 to 400 amu at 1 scan per second. Positive electron ionization (EI+) mode was used as an ion source. The source temperature was set at 200 °C and the GC/MS interface temperature was 250 °C. A GC/MS data acquisition software, Xcalibur version 1.2, was used to collect the mass to charge (m/z) scan and produce a pyrochromatogram. The National Institute of Standard Technology (NIST) Library (match at >800/1000) was then used to identify the fragments in the pyrochromatogram.

### 3.3 Interpretation of pyrolysis-GC/MS data using multivariate analyses

While a common approach to analyzing the data generated by Py-GC/MS is to focus on individual fragments or groups of fragments (Biber et al., 1996), the number of chemical fragments produced by pyrolysis of periphyton can range from 50 to >300. These fragments are highly correlated with one another because multiple parent structures can produce overlapping sets of fragments (Irwin, 1982). To reduce the large amount of highly correlated data to a small number of independent variables while preserving most of the variance in the data, multivariate analyses, such as principal component analysis (PCA) and factor analysis, are used. These methods are an improvement over previous semi-

### Table 1 – Physical and chemical stream characteristics.

<table>
<thead>
<tr>
<th>Site</th>
<th>Discharge (m³/s)</th>
<th>Upstream WWTP permitted Flowsa (m³/s)</th>
<th>Potential percent WWTP Effluentb</th>
<th>DOC (mg/L)</th>
<th>NO₃–N (mg/L)</th>
<th>PO₄–P (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restored Prairie</td>
<td>0.02–0.18</td>
<td>0.09</td>
<td>–</td>
<td>5.3–6.1</td>
<td>0.003–0.25</td>
<td>0.003</td>
</tr>
<tr>
<td>Forest Preserve</td>
<td>0.03–0.17</td>
<td>0.14</td>
<td>–</td>
<td>5.1–6.5</td>
<td>0.003–2.31</td>
<td>0.003</td>
</tr>
<tr>
<td>US of Wheaton WWTP</td>
<td>0.00–0.42</td>
<td>0.11</td>
<td>~100%</td>
<td>4.1–5.1</td>
<td>0.52–2.68</td>
<td>0.003</td>
</tr>
<tr>
<td>DS of Wheaton WWTP</td>
<td>0.11–0.48</td>
<td>0.34</td>
<td>0.39</td>
<td>~90%</td>
<td>5.9–7.6</td>
<td>9.19–14.35</td>
</tr>
<tr>
<td>Salt Creek</td>
<td>0.74–3.05</td>
<td>1.82</td>
<td>1.63</td>
<td>~90%</td>
<td>6.7–7.3</td>
<td>3.59–11.22</td>
</tr>
<tr>
<td>Salt Creek + Stormwater</td>
<td>0.74–3.05</td>
<td>1.82</td>
<td>1.63</td>
<td>7.3–7.5</td>
<td>5.39–11.22</td>
<td>7.17</td>
</tr>
<tr>
<td>E Branch DuPage River</td>
<td>0.95–3.89</td>
<td>2.12</td>
<td>1.57</td>
<td>~74%</td>
<td>6.1–7.5</td>
<td>4.87–12.45</td>
</tr>
</tbody>
</table>

---

a Sum of the National Pollutant Discharge Elimination System (NPDES) permitted flows of the upstream wastewater treatment plants (WWTP). b Based on upstream WWTP permitted flows: average stream discharge.
quantitative methods because they reveal patterns in data of high dimensionality and are the first steps in identifying and extracting as chemical markers key pyrolysis fragments from the entire suite of pyrolysis fragments generated by a complex organic matrix (Sirivedhin and Gray, 2005a).

In this research, PCA was employed to assess between-system differences in organic signatures, which were comprised of the 50 major (by area) pyrolysis fragments in each sample, creating a list of 137 unique pyrolysis fragments identified in each stream water pyrochromatogram. PCA of the organic profiles was performed using prcomp function in R (R Development Core Team, 2008). The peak areas were normalized by the maximum peak area of the identified fragments in each sample. Pyrolysis fragments having the highest correlations with the PCA eigen values were then analyzed by factor analysis of their normalized peak areas to characterize organic signatures. Factor analysis examined the proportion of the variance shared by a given fragment with the other fragments and grouped the fragments into factors. A maximum-likelihood factor analysis was executed with the factanal function in R using a varimax rotation (R Development Core Team, 2008).

4. Results and discussion

4.1. Stream water characteristics

The results of conventional stream water measurements are presented in Fig. 2. Discharges rates were higher during the wet month of June than during July and August when precipitation decreased. For all streams, the discharge flow rate on the final sampling date (08/04/09) was less than a quarter of that measured on the first sampling date (06/02/09). By comparing the average measured flow rates to the sum of the upstream NPDES permitted discharge flows (i.e. sum of upstream wastewater treatment plants’ designed average flows), it is evident that a considerable portion of the stream water was potentially comprised of wastewater effluent downstream of Wheaton WWTP and at the Salt Creek, Salt Creek + Storm water and E Branch DuPage River sites (Table 1). With average DOC concentrations in all the stream waters ranging from 4.7 mg/L C to 7.6 mg/L C, these values are on the higher end of the typical range measured in freshwaters (Buffle et al., 1988; Volk et al., 2002). However, the mean

Fig. 2 – Physical and chemical stream characteristics: discharge, dissolved organic carbon, nitrate nitrogen, and phosphate phosphorus. Salt Creek and Salt Creek + Stormwater are represented by a single point for discharge. Symbols for stream sites with upstream wastewater effluent discharge(s) have solid fill.
DOC and nutrient levels of all stream waters downstream of WWTP discharges was significantly higher than the mean of all the stream waters not receiving WWTP discharge (7.0 ppm C, 8.26 mg/L NO₃⁻N and 0.84 mg/L PO₄³⁻P compared to 5.4 ppm C, 0.80 mg/L NO₃⁻N and 0.01 mg/L PO₄³⁻P) (Wilcoxon rank sum test, $p = 8.4 \times 10^{-5}$, $p < 2.0 \times 10^{-6}$ and $p < 8.3 \times 10^{-7}$).

4.2. Pyrolysis-GC/MS

The first step interpreting Py-GC/MS results is to identify and compare major and unique pyrolysis peaks in each sample’s organic fingerprint. An example of this is illustrated in Fig. 3, where the primary peaks from the DS of Wheaton WWTP and Restored Prairie stream sites from the 7-July sampling event are labeled. This particular pyrochromatogram of DS of Wheaton WWTP site is representative of the results from all sites downstream of WWTP discharges, while the pyrochromatogram of the Restored Prairie site is representative of the Restored Prairie and Forest Preserve sites, which were the only sites on a stream that originated and was contained within a restored/preserved landscape. Even though the DS of Wheaton WWTP and Restored Prairie stream sites have differing anthropogenic influences, there are some dominant pyrolysis fragments, such as benzene, 1,2-dimethyl-, phenol, and phenol, 4-methyl-, that occur in both samples. In all samples, phenol was among the most dominant pyrolysis fragments, indicating a strong aromatic chemical nature. In addition, other typical aliphatic and polysaccharide products, such as acetic acid, furfural and propanoic acid, were fairly uniform among the samples and, as such, not useful for discrimination of organic sources. However, the organic signature of the Restored Prairie and Forest Preserve samples exhibited stronger peaks of 2-cyclopenten-1-one and 2-cyclopenten-1-one, 2-methyl-, which have been shown to be derived from soil polycarboxylic acids (Bracewell et al., 1980; Wilson et al., 1983), while the organic signature of the sites with upstream WWTP discharges had signatures more characteristic of wastewater effluent with dominant pyrolysis fragments such pyridine, pyrrole, and acetamide, products of protein and aminosugar parent structures (Biber et al., 1996; Moldoveanu, 1998; Nollet, 2006; Wilson et al., 1983).

To further compare the Py-GC/MS results, a semi-quantitative technique (Gray et al., 1995) was used to assess differences in organic carbon signatures among stream samples analyzed. After the chemical peaks were identified in each sample, they were classified among four categories (aromatic, aliphatic, nitrogen-containing aromatic, and nitrogen-containing aliphatic) with the percentage in each category based on the percentage of the total identified peak area. A comparison of the average percentage in each category for those stream waters downstream of WWTP discharges and those stream waters without upstream WWTP discharges shows that in both cases the waters had a stronger aromatic (50%–56%) than aliphatic (32%–33%) signature (Fig. 4). That the stream waters downstream of WWTP discharges had a stronger aromatic than aliphatic signature agrees with results reported by Dignac et al. (2000), who found municipal wastewater to be dominated by aromatic compounds, however other researchers have found EfOM to be aliphatic-dominated (Peschel and Wildt, 1988; Sirivedhin and Gray, 2005a). The mean organic nitrogen content (NAL + NAR) of all stream waters downstream of WWTP discharges was

![Fig. 3 – Pyrochromatograms of DS of Wheaton WWTP and Restored Prairie stream sites from the 7-July sampling.](image-url)

![Fig. 4 – Semi-quantitative analysis of organic signatures (ALI = aliphatic, NAL = nitrogen-containing aliphatic, ARO = aromatic and NAR = nitrogen-containing aromatic). (For the color version of this figure see the online version.)](image-url)
significantly higher than the mean of all the stream waters not receiving WWTP discharge (19% compared to 11%) (Wilcoxon rank sum test, \( p = 0.0027 \)). Similar to previous research on EfOM, stream waters influenced by wastewater treatment plants (DS of Wheaton WWTP, E Branch DuPage River, Salt Creek and Salt Creek + Storm water) had marked nitrogen-containing signatures associated with parent structures such as proteins and aminosugars (Peschel and Wildt, 1988; Sirivedhin and Gray, 2005a).

PCA was performed to reduce the large number of highly correlated pyrolysis fragments describing the organic quality of these waters to a small number of independent variables, or principal components (PCs), that would then reveal the internal structure of the data while preserving as much as possible the variation in the data set (Jolliffe, 2002). By graphing the PCs to create score plots (Fig. 5), it is possible to visualize how associated groups of pyrolysis fragments vary relative to one another (e.g., PC 1 vs. PC 2). It is also possible to identify patterns, separation and/or groupings in the data since the distances between the data points are approximately proportional to the dissimilarities between the organic fingerprints (Fig. 5) (McCune et al., 2002). PC1 accounts for 30.7% of the total variance of the data, while PC2 accounts for 11.3% of the total variance. The chemical fragments that had the strongest correlations with the PC1 axis were cyclopentenones, while acetamide, pyridines and pyrrole had strong negative influences on PC2.

Based on the score plot illustrated in Fig. 5, the data were separated into three groups. The first group (with the most positive PC1 scores) included the Restored Prairie and Forest Preserve samples, which were the only stream sites that originated and were located within a restored/preserved landscape. PC1 divided the samples according to ‘natural’ (PC1 > 4) versus anthropogenic (PC1 < 4) influences. PC2 separated the sampling sites receiving high levels of wastewater effluent discharge from sites with more terrestrial/storm water runoff influences. The second group (with PC2 < 1 and –6 < PC1 < 3) captured the Salt Creek, DS of Wheaton WWTP and E Branch DuPage River samples. Unexpectedly, a third group displaying positive PC2 scores emerged and was made up of the stream samples that were most influenced by storm water flows, Salt Creek + Storm water and US of Wheaton WWTP. Based on traditional nutrient measurements, we would have expected the US of Wheaton WWTP site with low DOC and low nutrients (Fig. 2, Table 1) to be grouped with the Restored Prairie and Forest Preserve sites. Instead, the organic material in samples from this site was characterized by PC1 values much less (PC1 < –3) than the range of values observed for the Restored Prairie or Forest Preserve sites and on two sampling dates data from this site were grouped with the other site showing the greatest urban storm water influence. The pyrolysis fragment that had the strongest negative correlation with the PC1 axis, where this storm water grouping is located, is 6-tridecene. This long chain alkene (in association with a series of alkanes, alkenes and alkaadienes, some of which we also detected) has been identified by others as a pyrolysis product of polyethylene, which is the most widely used plastic (Moldoveanu, 2005). For instance, this fragment, which perhaps originated in our samples from synthetic construction or landscape materials, such as geomembranes, further defines the PC1 axis as an indicator of anthropogenic influence.

The two samples that fell outside of these groupings (but were closest to the effluent influenced group), 2-June US of Wheaton WWTP and 2-June Forest Preserve, were both collected under high flow/storm event conditions present on the first sampling date (Fig. 2, Table 1) and display features in their fingerprints that point to possible wastewater influences, which may, for instance, be due to influences from septic tanks or combined sewer overflows. Overall, samples collected on 2-June were shifted towards the storm water and wastewater groupings, which emphasizes how large storm events can have a major influence on the organic quality of surface waters.

In order to confirm statistically the data grouping identified by PCA, we applied a hierarchical clustering algorithm (hclust function in R using the average method), which shows that there are, indeed, three clusters (WWTP influenced, storm water influenced and “natural”, as illustrated in Fig. 5) and that the groupings or clusters are very similar between the two techniques (about 80–85% of the data are divided into the same groups). The only major differences are among a small group of samples that are at the edges of the PCA groups and that cluster analysis groups as wastewater affected (shown in a dotted outline in Fig. 5). Although distinct storm events are likely to have influenced the organic nature of these samples as discussed above (septic tank or combined sewer overflows), without detailed information about the quality and quantity of discharges to the streams we cannot resolve these small discrepancies between the two methods. The cluster analysis does, however, verify that PC1 is an indicator of anthropogenic influence and PC2 may serve an indicator of the nature of the anthropogenic influence with more negative values associated with wastewater and more positive values

![Fig. 5](image-url)
Table 2: Rotated factor pattern of stream water chemical fragments.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cyclopenten-1-one, 2,3-dimethyl-</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>2-Cyclopenten-1-one</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Benzene, 1-ethenyl-2-methyl-</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>2-Cyclopenten-1-one, 4,4-dimethyl-</td>
<td>0.95</td>
<td>0.17</td>
</tr>
<tr>
<td>2-Cyclopenten-1-one, 2-methyl-</td>
<td>0.95</td>
<td>0.17</td>
</tr>
<tr>
<td>2-Cyclopenten-1-one, 3,4-dimethyl-</td>
<td>0.94</td>
<td>0.19</td>
</tr>
<tr>
<td>1H-Indene, 2,3-dimethyl-</td>
<td>0.93</td>
<td>0.15</td>
</tr>
<tr>
<td>1H-Indene, 1,1-dimethyl-</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Ethanone, 1-(2-furfuryl)-</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Pyridine, 3-methyl-</td>
<td>−0.27</td>
<td>0.96</td>
</tr>
<tr>
<td>Pyridine, 2-methyl-</td>
<td>−0.19</td>
<td>0.86</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>0.33</td>
<td>0.82</td>
</tr>
<tr>
<td>Acetamide</td>
<td>0.21</td>
<td>0.55</td>
</tr>
</tbody>
</table>

associated with storm water runoff, although more data are needed to develop fully this relationship.

We used factor analysis to extract chemical markers that could be used to differentiate samples based on system inputs and to compare with other chemometric or biometric data (Sirivedhin and Gray, 2005a). The rotated factor pattern, which contains the loading or contribution of each common factor to each chemical fragment, is presented in Table 2. The first nine chemical fragments, which are largely cyclopentenones, contribute highly to Factor 1 and the next four chemical fragments contribute highly to Factor 2. Factor 1 may be associated with natural systems as the majority of these pyrolysis fragments have been shown to be derived from soil pyrolysis marker, such as furans, did not correlate with cyclopentenones in our samples (Moldoveanu, 1998). In addition, all these pyrolysis fragments were highly correlated with the positive PC1 axis, where we observe highly positive PC1 scores correlated with less anthropogenic influence and hence higher organic quality. Accordingly, as PC1 becomes more negative, there is an increasing anthropogenic signature. Factor 2 is likely associated with EfOM as these pyrolysis fragments are indicative of proteins (Biber et al., 1996; Moldoveanu, 1998; Nollet, 2006; Wilson et al., 1983), which have previously been found to dominate wastewater effluent samples (Peschel and Wildt, 1988; Sirivedhin and Gray, 2005a). In addition, all these pyrolysis fragments had a strong negative correlation with PC2, which corresponded to those sites having the greatest influence from wastewater treatment plant discharges, Salt Creek, DS of Wheaton WWTP and E Branch DuPage River. Acetamide may have had a weaker association with PC2 due to its more ubiquitous source in the N-acetylamino sugars of bacterial cell walls (Dignac et al., 2000).

5. Conclusions

The ability to identify inputs that degrade stream water organic quality is of utmost importance for a number of reasons. These include the ability to identify point and non-point sources in order to control inputs, monitor restoration efforts, restore or preserve ecological functions and protect human health. The organic quality of stream waters receiving discharge from wastewater treatment plants was found to be distinct from waters with little point-source anthropogenic influence based on PCA treatment of Py-GC/MS data. The distinct nature of the effects of effluent discharge on the organic fingerprint was evident regardless of the distance to the nearest upstream wastewater effluent discharge, indicating the persistent nature of EfOM. Stream waters with upstream wastewater treatment plant discharges also had the highest organic nitrogen content, which may contribute to elevated levels of disinfection by-product formation potentials and associated, potentially adverse, health effects were these waters to be used as a source for drinking water (Sirivedhin and Gray, 2006). In addition, the stream waters that had the greatest urban storm water runoff also exhibited an organic signature distinct from those dominated by wastewater or natural or restored ecological processes. The pyrolysis fragments of 3-methylpyridine, 2-methyl-pyridine, pyrrole, and acetamide were identified as indicators of EfOM, which supports previous research suggesting that these protein derivatives are possible wastewater markers (Peschel and Wildt, 1988; Sirivedhin and Gray, 2005a). Furthermore, pyrolysis fragments associated with soil polycarboxylic acids highly correlated with our stream sites having the least anthropogenic influences.

The integrated use of PCA and factor analysis, then, provides an insightful way in which to probe the organic quality of a variety of surface waters and potentially monitor inputs to aquatic systems. In this way we determined indicators of anthropogenic influence (PC1) and extracted a set of chemical pyrolysis markers to separate waters with organic quality dominated by human influences from those under more natural control. In addition, we are also able to discriminate among the types of anthropogenic influences (wastewater effluent vs. storm water runoff).

With previous research showing a denitrification rates negatively affected by phenolics (Dodla et al., 2008; Sirivedhin and Gray, 2006) and an overall preference for aliphatic over aromatic carbon sources (Sirivedhin and Gray, 2006), our findings may indicate that these urbanized streams have an impaired ability to denitrify. Furthermore, streams enriched with EfOM may exhibit a decoupling of the algal/bacterial relationships within periphyton, which may alter their ability to perform critical ecosystem services (Peterson et al., 2011). The significance of the structural differences in EfOM on algal and bacterial interactions within periphyton and denitrification rates is currently being explored.

Acknowledgements

We thank John Oldenburg for allowing access to study sites within the DuPage County Forest Preserve system. NahNah Kim, Peter Daniels, Valerie Bosscher and Allegra Mount are students who assisted in the laboratory and field. This research was funded by NSF Ecosystem Grant DEB 0640717 to Peterson and Kelly, NSF Ecosystem Grant DEB 0640459 to Gray, and an REU Supplement to NSF Ecosystem Grant DEB 0640717.
REFERENCES


