Suspect screening analysis of the occurrence and removal of micropollutants by GC-QTOF MS during wastewater treatment processes

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ARTICLE INFO

Keywords:
Suspect screening
Wastewater treatment
Micropollutants
Transformation products
Occurrence and removal

ABSTRACT

The presence of micropollutants in wastewater treatment plant (WWTP) poses potential risks to the aquatic system and human health. This study comprehensively characterized the presence, elimination, and transformation of micropollutants in the WWTP using the suspect screening approaches by employing the gas chromatography coupled with quadrupole time-of-flight mass spectrometry. Overall, 5724 features were identified in influent, while only 3418 features existed in effluents. Hierarchical cluster analysis (HCA) was used to group features based on intensity profiles to investigate the behavior and fate of detected features. Four representative trends were defined with the corresponding features. Among 117 compounds identified during the treatment process, there were 99 compounds in influent, 10 biological transformation products (TPs), 3 ozone TPs, and 5 ultraviolet TPs. Furthermore, the removal efficiencies for the detected compounds were determined. The study findings highlight the importance of the removal of emerging pollutants and TPs such as fipronil, fipronil sulfide, and fipronil sulfone.

https://doi.org/10.1016/j.jhazmat.2019.05.031

Received 27 February 2019; Received in revised form 8 May 2019; Accepted 15 May 2019

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1. Introduction

Thousands of micropollutants from industrial and domestic sources are discharged to municipal wastewater treatment plants (WWTPs). These compounds can be either removed by adsorption or degradation during the wastewater treatment process or remain untreated [1]. The discharge of WWTP effluent introduces untreated micropolllutants into receiving waters, which pose a risk to water quality and human health [2,3]. Therefore, investigating the presence and removal efficiency of micropolllutants in WWTPs is important to evaluate the potential risk of effluents to the aquatic environment. Several studies have reported the occurrence of various chemicals, e.g. pharmaceuticals and personal care products (PPCPs) [4,5], plasticizers [6,7], pesticides [8–10], and steroid hormone [11–13] during the wastewater treatment. Since these studies only focused on certain target compounds, the occurrence and removal of micropolllutants during the wastewater treatment processes cannot be assessed comprehensively [14,15].

High-resolution mass spectrometry (HRMS) can be used for suspect screening of expected compounds using databases and non-target screening of unknown compounds, providing efficient and practical approaches for fast identification of compounds [16,17]. Databases containing chemical formulas and mass spectrum information can result in an efficient suspect screening to tentatively identify compounds without reference standard in the aquatic environment [14,18,19]. Schymanski et al. [20] proposed a communicating confidence system depending on the amount of available compound information for the identification of unknown compounds. They pointed out that all the detected compounds through the suspect screening method can be marked as level 2 (probable structure). However, the confidence level of the compound can be up to level 1 (confirmed structure) if the reference standard is available.

Previous studies detected various chemicals with diverse physico-chemical properties in wastewater. The polarity of detected chemicals ranged from very polar to nonpolar. Several studies used the liquid chromatography-high-resolution mass spectrometry (LC-HRMS) for screening polar organic compounds in wastewater [18,19,21–23], whereas the nonpolar and semi-volatile micropolllutants, which are preferred to be analyzed by gas chromatography HRMS (GC-HRMS), were underestimated. The advantages of GC-MS coupled with electron ionization (EI) mode are the highly reproducible fragmentation pattern, the availability of some databases and reproducible retention time (RT). Then, the normalized retention index (RI) can be calculated and used to tentatively identify the unknown compound with high confidence [17]. However, only limited studies have investigated micropolllutants in aquatic systems using gas chromatography coupled with quadrupole time-of-flight mass spectrometry (GC-QTOF MS). A previous study showed that many micropolllutants such as 2,4-dimethylphenol identified through GC-HRMS were not detected by LC-HRMS, demonstrating that GC-HRMS was crucial in screening micropolllutants [14]. However, their GC-QTOF MS suspect screening procedure was based only on a relatively small database (Agilent GC/Q-TOF-Pesticide PCDL) of 750 pesticides. Consequently, only limited compounds can be identified by a library search.

This study used the screening workflow combined with GC-QTOF MS to investigate the occurrence, behavior, removal and possible transformation products (TPs) of micropolllutants during the WWTP treatment procedure based on a large database. Furthermore, this work presented an approach for the identification of noticeable compounds that may pose a risk to the water environment and humans via the effluent disposal from WWTP.

2. Experimental

2.1. Chemicals and solvents

High purity organic solvents such as n-hexane, cyclohexane, methanol, dichloromethane and acetone were purchased from J.T. Baker (Center Valley PA, USA). Ultrapure water was supplied by a Milli-Q Millipore system. For the identification of pesticides, 9 reference standards were selected and listed in Table S1.

2.2. Information about the sampling WWTP and sample details

Selected WWTP serves about 850,000 people in Beijing, China, and has a maximum treatment capacity of 500,000 m³/day. The schematic flow chart of the WWTP is shown in Fig. 1. Wastewater mainly from residential sources first passes through the primary clarifier and then the effluent from the primary treatment unit flows into the activated sludge treatment unit that has a secondary clarifier. The activated sludge treatment adopts the phoredox (modified bardenpho) system, consisting of anaerobic, first-anoxic, first-aerobic, second-anoxic, and second-aerobic systems. The hydraulic retention times (HRT) in the primary clarifier, five steps of activated sludge treatment, and secondary clarifier are 15, 2, 3, 11, 2, 3 and 8 h, respectively. About 60% of the effluent from a secondary treatment unit is directly discharged to a receiving river, while the other 40% is treated in a tertiary treatment system, consisting of hyper-filtration, ozonation, and ultraviolet processes. The effluent of the advanced treatment system is used for irrigation water.

Samples were collected in locations along the wastewater treatment process under normal dry weather conditions in November 2016 (Fig. 1). At each location, 72-h flow-proportional composite samples were collected in triplicates, resulting in a total of 30 samples (three samples per sampling point). The wastewater influent to the WWTP was around 220,000 m³/day during the sampling period and the water temperature during the biological treatment process was 21–23 °C.
Samples were collected in 4 L amber glass bottles, filtered through a glass microfiber filter GF/C 0.7 μm (Whatman, Maidstone, U.K.) and stored at 4 °C until analysis. The samples were analyzed within a week of collection.

2.3. Sample pretreatment and analytical method

Samples were pretreated using solid phase extraction (SPE). C18 silica material is widely used as SPE sorbent because of its universality and low cost, though highly polar compounds (log Kow < 1) are prone to a breakthrough on the C18 column [24]. Hence, SPE sorbent was used to screen the nonpolar and semi-volatile micropollutants in this study. Water samples (1 L) were passed over an Elut-Bond C18 cartridge (Agilent) and the cartridge was dried for 1 h and eluted by 5 mL of hexane, followed by 5 mL hexane/dichloromethane (1:1 v/v), and by 5 mL dichloromethane. Extracts were mixed and transferred to autosampler vials and then concentrated to 200 μL. Laboratory procedure blanks (ultrapure water) were used and analyzed in the same way for each of the six samples.

The instrumental analysis was carried out on Agilent 7890B GC coupled to an Agilent 7200B QTOF-MS (HP-5MS 30 m × 0.25 mm, 0.25 μm column, Agilent Technologies, Inc.) running in the EI mode. Further information about the analysis is given in Table S2.

2.4. Suspect screening by GC-QTOF MS

30 samples and 5 procedure blanks were rerun in one randomized sequence using the analytical method as described above. 5 μL extracted solution was withdrawn from each sample and mixed to produce a quality control (QC) sample, which was rerun after every 5 sample injections. Spectral deconvolution with Agilent Unknown Analysis software (B.08.00) was used to extract the data acquired by GC-QTOF MS.

Database is a powerful tool to identify micropollutants. The advantages of GC-EI-MS are the highly reproducible fragmentation pattern and the availability of a comprehensive and reliable library containing over 220,000 compounds (NIST 14) [25]. Furthermore, an exact mass personal compound database and library (PCDL) (Agilent GC/Q-TOF-Pesticide) can reduce the false positive number in the library search. Thus, these two libraries were used to compare each feature of the spectra using the Unknown Analysis software. The matched compounds with a peak shape quality > 50, match factor > 60, and chromatographic peak width between 3 and 15 s were selected (Table S3). RT locking and RI were used to presumptively identify the unknown compounds with high confidence.

The Agilent GC/Q-TOF-Pesticide library (750 pesticides) contains RT for each compound and the GC-EI-MS analytical method described in Section 2.3 was adapted from the Agilent method. Thus, RT locking was used in the suspect screening procedure of this library. Firstly, the accepted deviations between measured RT and the library RT was set at 0.5 min. A mixed reference standard with 70 pesticides available in this library was analyzed with duplicate injections before and after the sample sequence to reduce the deviation between the measured RT and the library RT. The accepted RT deviations were calculated as 0.3 min.

For the NIST 14 library, RT value with a deviation of ± 2% between the calculated RI and experimental RI. Further, a deviation of ± 10% is acceptable if the library RI is only an estimate [17].

Samples without features unavailable in at least two of the triplicates or intensity 5 times less than that of the blank sample were discarded to reduce the possible false positive of identification. Since the library match name of the same feature in two samples can be different, the library matching process was manually inspected. Based on the detected compounds, reference standards were purchased for complete identification.

Then, a feature list was exported and imported into Mass Profiler Professional (MPP, version B.14.0, Agilent Technologies, Inc.) statistical analysis software. Further statistical analysis like data filtration, principal component analysis (PCA), and Hierarchical Cluster Analysis (HCA) was conducted by MPP. The trends of features across the WWTP were investigated and similar feature profiles were clustered together using HCA. The flowchart of extraction and data analysis methods is given in Fig. 2.

3. Results and discussion

3.1. Results of suspect screening

About 4000–6000 features per sample were extracted after the deconvolution procedure and the total number of detected features and matched number in each sample is given in Table S5. The detected features significantly decreased with the progress of treatment in WWTP (Fig. 3). A slight increase in the matched number (NIST) before and after the secondary clarifier (sample S6 and S7) was observed and it could be because some flocculant agents such as FeCl₃ added in the secondary clarifier could have acted as a catalyst to some compounds, leading to degradation of compounds and increase in the features. The decrease in PCDL matched number illustrates that fewer pesticides were generated during the catalytic process.
After discarding features that were found in only one of the triplicates and had 5 times less than that of the blank sample, 6956 individual features were reserved. The 3-dimensional principal component analysis (3D—PCA) (Figure S1A) indicated that the sample pretreatment, analysis, and data pre-processing are accurate and reproducible. The 2-dimensional principal component analysis (2D—PCA) (Figure S1b) indicates a significant differentiation between sample groups demonstrating an effective WWTP treatment process. The principal component 1 only explained approximately 20% of the variance due to the comparison of a relatively high number of features. The inter-group differences between samples from secondary treatment (S2, S3, S4, S5, and S6) and those from subsequent treatment processes (S7, S8, S9, and S10) are significant because the secondary treatment samples contained a large amount of activated sludge, resulting in a complex matrix.

The influent water extracts were screened using the PCDL library (750 pesticides) with RT locking and 41 software generated hits (criteria: more than two detections and intensities 5 times higher than the blank) were found. Further, conservative criteria: more than two detections and intensities 5 times higher than the blank sample (750 pesticides) with RT locking and 41 software generated hits (criteria: more than two detections and intensities 5 times higher than the blank) were used to tentatively identify the compound with RT deviation of 0.138 min (< 0.3 min). Finally, the compound was confirmed by a reference standard.

Table S6.2 presents information of all reserved suspect compounds and the elimination ratio (ER) calculated based on the peak area ratio in the secondary treatment process with conventional activated sludge and in the tertiary treatment with hyper-filtration, ozonation and ultraviolet. The ER of each compound class is given in Table 1 and Fig. 5.

3.2. Fate of micropollutants in influent during the whole wastewater treatment

During the treatment process, more than half of the identified compounds (n = 65) in influent were completely eliminated, whereas 57 of them were eliminated during the secondary treatment, indicating that both whole treatment and biological treatment were highly effective for the removal of micropollutants. Except for the eliminated 57 compounds, the ER for the remaining compounds (n = 8) were in the range from -102% to 99.1% (negative ER implies that the production of compounds during the treatment). Increase of 6 compounds after secondary treatment (negative ER) suggests that these compounds could act as biological TPs such as fipronil sulfoxide and fipronil sulfone [26]. A lower value for the ER after ozonation process compared to that after biological treatment indicates that these compounds could act as ozone TPs such as di-tert-butyl-p-benzoquinone and n-heptafluorobutyryl-de-sipramine. Further, a higher ER after ozonation process in comparison to the effluent ER suggests that these compounds could be ultraviolet TPs such as o-Xylene.

13 of 16 PPCPs detected were removed after biological treatment such as ibuprofen, the ER of which was 100%, close to 98% reported in previous studies for the same biological treatment process [27]. About 42% of D5 (decamethyl-cyclopentasiloxane) was removed during secondary treatment, while other studies found that the ER of D5 was 59% in a sequencing batch reactor with the activated sludge process [28]. Furthermore, this study found that ozonation and ultraviolet processes had no effect on the removal of D5.

7 plasticizer or flame-retardant compounds were identified, including four organophosphorus esters (OPEs) such as tris(2-butoxyethyl) phosphate, triethyl phosphate, tri(2-chloroethyl) phosphate (TCEP), and tris(1,3-dichloroisopropyl) phosphate (TDCPP). ER for chlorinated OPEs (TCEP and TDCPP) increased gradually during secondary treatment, hyper-filtration, and ozonation, while they decreased after ultraviolet treatment, indicating that the two OPEs could be ultraviolet TPs and the whole WWTP treatment process played a limited role on the elimination of chlorinated OPEs [29,30]. In addition, the other two OPEs were removed after secondary treatment, suggesting that it is easier to remove chlorine-free OPEs than chlorinated OPEs. Furthermore, the presence of Cl atoms in TCP and TDCPP decreased...
their reactivity toward biological, ozone and ultraviolet treatments. Other classes of compounds were not shown because of almost complete removal after secondary treatment.

The remaining 3 plasticizers or flame-retardant compounds were phthalate esters (PAEs), including bis(2-ethylhexyl) phthalate (DEHP), di-n-butylphthalate (DBP), 1,4-benzenedicarboxylic acid, and bis(2-ethylhexyl) ester (DOTP). About 82% of DHEP were removed after the whole process, while the biological treatment was the most effective part with 77% efficiency. The ER for DBP and DOTP after biological treatment were -43% and -82%, respectively, indicating that biological treatment played a limited role in the removal of DBP and DOTP. Similarly, ozonation process had -31% for DBP and -61% for DOTP. 63% of the DOTP were removed after ultraviolet treatment and the ER for DBP was still -21%. An increasing trend of these plasticizers or flame-retardant compounds after the water treatment may be the result of the contamination of water pipe or filter materials used in the WWTP process.

The biological treatment removed 7 pesticides, insecticide TPs, di-chlorvos and climbazole. Biological treatment and ozonation played an important role in the removal of diethyltoluamide, 2,4-isopropoxyphenol and fipronil. As TPs of fipronil, the level of fipronil sulfone during the whole treatment almost had no significant change, while fipronil could degrade into fipronil sulphide during the biological treatment (ER = -102%). Almost half of the fipronil sulphide in S7 was removed during ozonation. Compared to fipronil, fipronil sulphide and fipronil sulfone exhibited greater environmental stability during the biological treatment process, indicating a significant environmental risk because of their persistence [26]. In addition, ultraviolet treatment almost had no effect on the removal of all 7 compounds mentioned above. Biological treatment was effective for the removal of PAHs, except for naphthalene, because if its stable molecular structure with low molecular weight. The ultraviolet process removed naphthalene with high efficiency.

Biological treatment removed 10 steroids and hormones, indicating that biodegradation was the primary processes for the removal of steroids and hormones in WWTPs in agreement with the findings of the previous study [32]. The ER of estrone and androsterone was almost 100%, which is relatively higher than that in seven WWTPs located in Beijing [32]. Similarly, the phenols, food additives and most of the medical intermediates were eliminated after the biological treatment.

### 3.3. Behavior and fate of micropollutants during the treatment process

Features were grouped based on intensity profiles using HCA to investigate the behavior and fate of detected features during wastewater treatment (Fig. 6; the normalization algorithm in Text S1). The intensities of each feature could either decrease, remain the same, or even increase between sampling sites, resulting in numerous possible trends. Therefore, the description of various trends during the secondary treatment (S2—S6) was omitted because of the complicated activated sludge treatment mechanism. Finally, four trends corresponding to four types of representative compounds were defined (Fig. 6) and features belonging to different trends were identified. Alkane and their esters, acids, and similar compounds were neglected since they were irrelevant to this study.

Trend 1 in Fig. 6 refers to features that were present in all sampling sites. The dotted line represents the intensity of features of trend 1, which could either decrease, remain the same, or even increase during the whole wastewater treatment process. 34 of 42 features in Trends 1 (Table S7.1) were identified as confidence level 2 and compounds such as Tri(2-chloroethyl) phosphate (TCEP) were not removed completely during the complicated treatment steps suggesting that they are more persistent than other compounds in the aquatic ecosystem. A limited number (n = 6) of compounds such as fipronil sulfide, fipronil sulfone, TDCPP, and di-n-butylphthalate were not significantly eliminated (ER < 20%), while 18 of them were eliminated significantly (ER > 70%).

Features with trend 2 represent compounds formed during the biological treatment process and were not removed in the sampling site. Subsequently, 10 of 48 features assigned to trend 2 were identified (Table S7.2). The 10 compounds had low ER at 5% on average after hyperfiltration because hyperfiltration had little effect on the elimination of compounds that are persistent in ozonation and ultraviolet treatment. Some compounds such as atrazine could be ultraviolet transformation products, resulting in lower ER after ultraviolet treatment compared to that after ozonation treatment.

Features with trend 3 represent ozone TPs. The red and black lines between S9 and S10 refer to ozone TPs that were not removed during the UV process, while the green line represents TPs that were removed. 1705 features out of 1751 features assigned to trend 3 were removed by

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**Table 1**

Elimination ratio for each compound class in influent.

<table>
<thead>
<tr>
<th>Compound Classa</th>
<th>Compounds Number</th>
<th>ER-1 (%) (after secondary treatment)</th>
<th>ER-2 (%) (after ozonation)</th>
<th>ER-3 (%) (effluent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPCPs</td>
<td>16</td>
<td>93%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Plasticizers, Flame retardants (FRs)</td>
<td>7</td>
<td>33%</td>
<td>44%</td>
<td>52%</td>
</tr>
<tr>
<td>Insecticide TPs</td>
<td>2</td>
<td>-54%</td>
<td>4%</td>
<td>9%</td>
</tr>
<tr>
<td>Pesticides</td>
<td>6</td>
<td>45%</td>
<td>82%</td>
<td>77%</td>
</tr>
<tr>
<td>PAHs</td>
<td>4</td>
<td>53%</td>
<td>60%</td>
<td>87%</td>
</tr>
<tr>
<td>Steroids &amp; Hormones</td>
<td>10</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Phenols</td>
<td>5</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Food Additives</td>
<td>4</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Medical intermediate</td>
<td>7</td>
<td>96%</td>
<td>100%</td>
<td>99%</td>
</tr>
</tbody>
</table>

* Many compounds (n = 38) present in influent cannot be classified appropriate. Hence, their ER are not shown here.

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**Fig. 5.** Elimination ratio for different classes of compounds (PPCPs, plasticizers, flame retardants, insecticide TPs, pesticides, and PAHs) after secondary treatment (ER-1), after ozonation (ER-2) and after ultraviolet (effluent, ER-3) treatments. Other classes of compounds were not shown because of almost complete removal after secondary treatment.
the ultraviolet treatment process and only 3 features were identified (Table S7.3). Similarly, only 5 out of 2031 features were identified in trend 4 (Table S7.4). The reason probably is that ozone TPs were too polar to remain in the SPE sorbents because very polar compounds (log Kow < 1) are prone to break on the C18 column. Additionally, it could be because ozone TPs were too polar to detect with GC–MS. Further, two libraries contained limited TPs and were difficult to detect using the suspect screening method. Additionally, several compounds such as alkanes, unsaturated hydrocarbons and their acids, and esters were produced after ozonation and ultraviolet processes and the identification of their precursor ions was difficult when using EI as the ion source. Further, these compounds significantly interfered with the mass spectrometry of the small molecule transformation products (molecular weight < 150), making them difficult to identify.

3.4. Limitations and future directions

The accuracy, precision, sensitivity, selectivity, and reproducibility are important for a screening method [33]. Additionally, the extraction, instrument analysis, and data processing method have a significant influence on the reproducibility of the suspect screening results. In this work, more than half of the peaks were in bad shape (< 50) after spectral deconvolution. Saturated peaks with a wide retention time significantly affected the detection of the compounds with low concentration, leading to the omission of a significant amount of mass spectrum information. Thus, compounds separation in the chromatography is important. Furthermore, the lack of reliable accurate mass spectroscopy library could result in the inaccurate matching of features like isomers in the NIST library. In summary, a good spectral deconvolution algorithm and the deconvolution parameter setting are critical for accurate mass spectrum identification. Moreover, reliable and accurate mass databases and libraries are required to accurately identify micropollutants.

4. Conclusions

This study is a pioneering study to comprehensively analyze the occurrence and removal of micropollutants during wastewater treatment processes using GC-HRMS. Several thousands of features were detected in each sample site and 117 compounds were identified during the treatment process. 34 of 99 compounds detected in the influent were not completely removed (trend 1), while 10 biological TPs (trend 2), 3 ozone TPs (trend 3), and 5 ultraviolet TPs (trend 4) were detected. 52 compounds including flame retardants, pesticides, antioxidant, and PAHs were present in the effluent and could pose the aquatic system and human health risks. Furthermore, ozonation and ultraviolet processes had no effect on the removal of D5, providing a reference for future research. Finally, this work presents an approach for the identification of noticeable compounds like persistent micropollutants and TPs.

Acknowledgements

We thank the National Natural Science Foundation of China (21625702 and 21621064), the National Basic Research Program of China (2015CB453102) and the Strategic Priority Research Program of
the Chinese Academy of Science (XDB14010400) for financial support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.05.031.

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