Investigation and assessment of micropollutants and associated biological effects in wastewater treatment processes

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A B S T R A C T

Currently, the wastewater treatment plants (WWTPs) attempt to achieve the shifting from general pollution parameters control to reduction of organic micropollutants discharge. However, they have not been able to satisfy the increasing ecological safety needs. In this study, the removal of micropollutants was investigated, and the ecological safety was assessed for a local WWTP. Although the total concentration of 31 micropollutants detected was reduced by 83% using the traditional biological treatment processes, the results did not reflect chemicals that had poor removal efficiencies and low concentrations. Of the five categories of micropollutants, herbicides, insecticides, and bactericides were difficult to remove, pharmaceuticals and UV filters were effectively eliminated. The specific photosynthesis inhibition effect and non-specific bioluminescence inhibition effect from wastewater were detected and evaluated using hazardous concentration where 5% of aquatic organisms are affected. The photosynthesis inhibition effect from wastewater in the WWTP was negligible, even the untreated raw wastewater. However, the bioluminescence inhibition effect from wastewater which was defined as the priority biological effect, posed potential ecological risk. To decrease non-specific biological effects, especially of macromolecular dissolved organic matter, overall pollutant reduction strategy is necessary. Meanwhile, the ozonation process was used to further decrease the bioluminescence inhibition effects from the secondary effluent; $\geq$ 0.34 g O_3/g DOC of ozone dose was recommended for micropollutants elimination control and ecological safety.

Introduction

The Global Chemicals Outlook II (GCO-II) states that global chemical sales (excluding pharmaceuticals) are projected to grow from € 3.47 trillion in 2017 to € 6.6 trillion by 2030 (United Nations Environment Programme, 2019). Global supply chains, as well as the trade of chemicals and products are becoming increasingly complex in emerging economies. Unfortunately, hazardous chemicals continue to be released into the environment in large quantities. The wastewater discharged from domestic wastewater treatment plants (WWTPs) is one of the most important sources of environmental pollution (Gavrilescu et al., 2015). Following the advancement of instrumental analysis and bioassays, an increasing number of micropollutants and the adverse effects from secondary effluent, namely health hazards and ecosystem hazards, are revealed (Escher et al., 2014; Luo et al., 2014; Link et al., 2017). Reducing the discharge of micropollutants from WWTP effluents is an effective solution in controlling water pollution. Hence, a new water protection act entered firstly into force in Switzerland in 2016, which aimed to update WWTPs in order to achieve an average 80% removal (from influent to effluent) of organic micropollutants (Bourgin et al., 2018).

Organic micropollutants from domestic wastewater are of low concentrations (usually at the level of ng/L) and high complexities (Pereira et al., 2017). More than 100 organic micropollutants have been detected in domestic wastewaters and surface waters

– Table 1 – Traditional water quality parameters of wastewater from WWTP.

Parameter	COD (mg/L)	BOD_5 (mg/L)	TN (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	SS (mg/L)
Influent	476 ± 182	228 ± 91	44.80 ± 14.2	35.85 ± 7.5	6.96 ± 3.8	426 ± 217
Effluent	19.41 ± 2.7	6.63 ± 1.2	9.65 ± 1.5	0.93 ± 0.57	0.27 ±0.04	5.20 ± 1.6
Removal	95.92%	97.09%	78.46%	97.41%	96.12%	98.78%

COD: chemical oxygen demand; BOD_5: five-day biochemical oxygen demand; TN: total nitrogen; TP: total phosphorus; SS: suspended solids.

(Bu et al., 2015; Wang et al., 2018). It is difficult to control all the detected micropollutants in the WWTP effluent; moreover, the removal of micropollutants from wastewater in WWTPs is not completely clear. Thus, a short list of 12 indicator chemicals was proposed by the Swiss Federal Office for the Environment (FOEN). Despite a shift in the function of WWTPs, that is from controlling general pollution parameters, such as COD, TN, TP, to decreasing several organic micropollutants discharge, they are unable to satisfy the increasing safety needs related to the aquatic ecosystems (Välitalo et al., 2017; Pedrazzani et al., 2019; Zhang et al., 2019). It is difficult to control the biotoxicity from wastewater only by decreasing the concentration of micropollutants. The biological effect of mixture, which consist of individual chemicals with the concentrations within the acute and chronic quality standards, need to be considered for the safety of aquatic ecosystems (Silva et al., 2002). Thus, bioassays, which reflect the joint biological response of all active chemicals in the sample, could provide vital supplementary for ecological safety (Zhang et al., 2011; Abbas et al., 2018; Gehrmann et al., 2018).

It is impossible to control all the detected biological effects from the domestic wastewaters (Escher et al., 2014; Xiao et al., 2015). A sample with detectable biological response does not mean that this is necessarily unacceptable. There is a need to identify the priority biological effect from discharged wastewater for WWTP management. Comparing the specific and non-specific biological effects, a decrease in responsible chemicals in the sample could effectively reduce the specific biological effects but could not control the non-specific biological effects (Escher et al., 2013; Tang and Escher, 2014; Hashmi et al., 2018). For example, decreasing the herbicide content or hormonal steroids in wastewater could reduce the PSII photosynthesis inhibition effect or endocrine disruption effects. Thus, this study investigated both non-specific and specific biological effects from wastewater in the WWTP.

Furthermore, the threshold values for relevant biological responses need to be identified to assess the ecological safety of wastewater. Presently, there are two main methods to derive threshold values. The first method involves deriving threshold values from chemicals in the existing list of environmental quality standard (EQS). However, the established EQS of many countries and regions do not fully consider potential chemicals that have a clear biological effect on aquatic organisms. Another method is to derive the threshold value from HC5 (hazardous concentration where 5% of aquatic organisms are affected) which was obtained from the species sensitivity distribution (SSD) model. This method makes up for the shortcoming of the threshold values derivation from EQS. Meanwhile, it was revealed that the assessment results obtained using threshold values from EQS were similarly consistent with those from HC5 (Ma et al., 2019). The ecological safety assessment based on the threshold values of the priority biological responses, will aid in policy-making during the operation and management of WWTP.

In the present study, the removal of organic micropollutants was surveyed at the WWTP where traditional biological treatments were applied. To identify the priority biological effect and to achieve the ecological safety, non-specific bioluminescence inhibition effect and specific photosynthesis inhibition effect from wastewater were investigated and evaluated using HC5, which was obtained using SSD model. Finally, ozonation process was conducted to control the priority biological effect. By combining the responsible chemical investigation and ecological safety assessment, this study provides a feasible measure to manage and update WWTPs for ecological safety.

1. Materials and methods

1.1. Sampling and pretreatment

The WWTP locates in Xi'an, China, with a treatment capacity of 2×10^5 m^3/day. The municipal domestic wastewater that is collected from the service area is treated using anaerobic-anoxic-oxic biological treatment processes and UV disinfection, and is later discharged into the nearby Bahe river. The treatment efficiency of the WWTP is stable. The traditional water quality parameters of the influent and effluent are shown in Table 1. The sampling site included the influent (IN), aerated grit chamber effluent (AGE), primary settling tank effluent (PSE), secondary settler effluent (SSE), and UV chlorination (UV) (Fig. 1). During the monitoring period, water samples were collected once per month from each site during the winter (n=15) and transported to the laboratory immediately. Each sample was filtered through 0.7 μm glass microfiber (Φ 150 mm, Whatman™, UK), and its pH was adjusted to 2.3 before the solid-phase extraction (SPE). Briefly, the prepared water samples were pumped through Waters Oasis HLB (500 mg, 6 mL) cartridges, which were preconditioned with 10 mL of 1:1 (*V/V*) *n*-hexane-dichloromethane mixture, 10 mL of methanol, and 10 mL of milli-Q water, respectively. After extraction, the cartridges were eluted with 10 mL of methanol and 10 mL of the 1:1 (*V/V*) *n*-hexane-dichloromethane mixture, successively. The eluates were evaporated at 40 °C until dry using a nitrogen evaporator (Sample concentrator MD200, ANPEL, China) and were re-dissolved in 1.0 mL of methanol. The residues were divided into two equal parts for chemical and biological analyses. For biological analysis, the solvent of the residues was exchanged with 1% dimethyl sulfoxide (DMSO). For every sampling and pretreatment, ultrapure water was set and analyzed following the same procedures for quality assurance and quality control.

1.2. Chemical and biological analysis

Thirty-one chemicals were selected and analyzed based on ACQUITY ultra-performance liquid chromatography – Xevo TQ mass spectrometrometry (UPLC/MS, Waters, USA) screening results. The detected chemicals were categorized into pesticides (herbicides, insecticides, bactericides), pharmaceuticals, and UV filters. The analytical procedures were conducted based on our previous studies (Ma et al., 2019). The recovery of 31 chemicals with individual concentrations of 5 μg/L and 50 μg/L were 62%-130% and 58%-127%, respectively. The recovery and method limits of quantification (LOQs) are shown in Appendix A Table S1.

The combined algae test (2 hr-PSII) and luminescent bacteria toxicity test were conducted to evaluate the biological responses of wastewater. The combined algae test (2 hr-PSII) was conducted according to previous studies (Ma et al., 2019). *Chlorella vulgaris* was used as the testing organism, which was purchased from

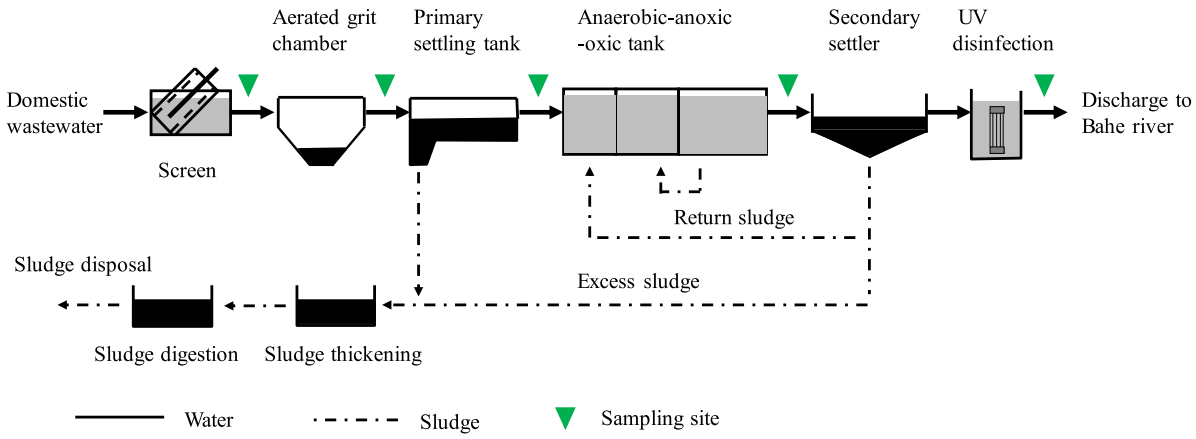

Fig. 1 – Outline of the wastewater treatment processes –

Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB, China) and grown in a BG11 medium in an illumination incubator (Blue pard, China) at 24±2 °C under a 12 h light/12 h dark cycle at 4000 lux illumination intensity. It was shocked 3–5 times a day. Before the test, *C. vulgaris* grown in the logarithmic growth phase was inoculated into fresh medium and cultured about 3 days to attain a 3×10^6–4×10^6 cells/mL density (Cellometer Auto T4, Nexcelom, USA). During the test, a 50 μL SPE extract was exposed to 300 μL algal suspension in a black 96-well plate (Corning, USA). After 2 h of exposure, PS II quantum yield, which is expressed as Y(II), was detected using Maxi-Imaging-PAM (WALZ, Germany) fluorometry. Ten concentrations of the sample or positive reference compound (diuron) with a 1:7 (*V/V*) dilution ration was tested in duplicate. 1% DMSO was set as the negative control. The tests for each water sample were conducted in triplicate in different microplates.

Luminescent bacteria toxicity test was conducted according to a modified ISO 11348 (2008) procedure using *Aliivibrio fischeri*, which was purchased from the China Center of Industrial Culture Collection (Ma et al., 2016). In the test, 100 μL of bacterial suspension which was prepared from logarithmically growing bacteria, was exposed to 100 μL of the testing sample in a cell of a white 96-well plate for 15 min. The relative light units were measured on a Centro LIApc LB962 Microplate Luminometer (Berthold Technologies Company, Germany). The tests for each sample were conducted in triplicate in different microplates. Eight concentrations of the sample and a positive reference compound (phenol) with a 2:3 (*V/V*) dilution ratio was tested in triplicate. The solution containing 1% DMSO and 2% NaCl was set as the negative control.

For the two bioassays, effective concentration (EC_{50}) was calculated from the concentration-response curve, which was quantified as the relative enrichment factor (REF) of the extracted sample, as shown in Eq. (1). To make the biological responses comparable, the biotoxicity of the water sample was standardized to the bioanalytical equivalent concentration (BEQ) using positive reference compound, as demonstrated in Eq. (2). It was expressed as BEQ_{diuron} and BEQ_{phenol} for photosynthesis inhibition effect and bioluminescence inhibition effect of the water samples, respectively.

$$REF = ER_{SPE} \times DR_{bioassay} \tag{1}$$

$$BEQ = \frac{EC_{50} \text{ of positive reference compound}}{EC_{50} \text{ of water sample}} \tag{2}$$

where, ER_{SPE} is the enrichment ratio of the water sample during the SPE process; $DR_{bioassay}$ is the dilution ration of the extracted water sample during the bioassay.

1.3. Derivation of HC5

SSD modeling was used to derive the HC5 of the positive reference compound as the threshold value of the biological response. To construct SSD curves, single-species acute toxicity data (EC_{50}) were collected from the US EPA ECOTOX database (http://www.epa.gov/ecotox). Only acute toxicity data of primary producers (e.g., diatoms, green algae, and cyanobacteria) were collected for estimating the HC5 of diuron which was the reference compound used in the combined algae test. When multiple toxicity values were collected from one species, the arithmetic mean of these values was used as the toxicity for that species to remove the unequal weighting of different species (Arias-Andrés et al., 2018). The HC5 values and their 95% confidence intervals were estimated using the SSD Generator software provided by the US EPA CADDIS (https://www.epa.gov/caddis).

1.4. Biological effect prediction based on known chemicals

The non-specific effect of mixtures with respect to *A. fischeri* was predicted using concentration addition (CA) model, which was expressed as $EC_{50,CA}$ (mg/L) as shown in Eq. (3) (Tang et al., 2013).

$$EC_{50,CA} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{50,i}} \right)^{-1} \tag{3}$$

where, n is the number of mixture components, and p_i and $EC_{50,i}$ are the fraction and EC_{50} value of component i in the mixture, respectively. The EC_{50} values of individual chemicals toward *A. fischeri* were obtained from the quantitative structure-activity relationships which was estimated using the liposome-water distribution coefficient K_{lipw} at a defined pH of 7 (expressed as D_{lipw} (pH 7)). The details are provided in our previous studies (Ma et al., 2017). The predicted BEQ_{phenol} (expressed as BEQ_{phenol}-pre) of the water sample was calculated from Eq. (4).

$$BEQ_{phenol} - pre = \frac{EC_{50,phenol}}{EC_{50,CA}/C_{Total}} \tag{4}$$

where, C_{Total} (mg/L) is the sum concentration of the known chemicals in the mixture.

With respect to the specific photosynthesis inhibition effect, the effect of individual herbicides i was standardized against diuron, which is defined as the relative potency (RP) of the individual herbicides i (Eq. (5)). The predicted BEQ_{diuron} (expressed as BEQ_{diuron}-pre) of the water sample was calculated from Eq. (6) (Magnusson et al., 2013; Vermeirssen et al., 2009).

The EC_{50} values of individual herbicides used for RP_i derivation were obtained from their dose-response curves which were determined using the combined algae test (2 hr-PSII) as described above. The EC_{50} values of detected herbicides obtained from their dose-response curves are shown in Appendix A Table S2.

$$RP_i = \frac{EC_{50,diuron}}{EC_{50,i}} \quad (5)$$

$$BEQ_{diuron-pre} = \sum_{i=1}^{n} RP_i \times C_i \quad (6)$$

1.5. Ozonation treatment

The ozonation process was carried out at a bench-scale in the laboratory at room temperature (25 ± 1 °C). Ozone was produced from an ozone generator (SanKang, Jinan, China) which was fed with pure oxygen continuously pumped into the glass reactor (with an effective volume of 5 L) and reacted with 3.5 L of secondary effluent. At the end of the reaction, high-purity nitrogen was fed into the reactor for 5 min to strip the remaining ozone in the reactor. All the unreacted ozone in the off-gas was destroyed in two sequential 350 mL 2% KI trap bottles in the terminal ozonation reactor. The concentrations of ozone in the in-gas and off-gas of the reactor were measured based on the method specified by Kasprzyk-Hordern et al. (2006). Under a reaction time of 0–30 min, the reacted ozone dosage was in the range of 0–1.31 g O_3/g DOC.

2. Results and discussion

2.1. Occurrence of micropollutants in the WWTP

A total of 31 target chemicals including 6 herbicides, 5 pesticides, 3 bactericides, 12 pharmaceuticals, and 5 UV filters, were detected in the water samples (Appendix A Table S2). The number of detected chemicals in all the samples rarely changed, which suggested that almost all the chemicals detected in the WWTP influent were also detected in the effluent. The total concentration of 31 target chemicals decreased from (2517.28±223.92) ng/L to (435.45±104.80) ng/L during the wastewater treatment processes (Fig. 2f). The micropollutants in the wastewater from WWTP were dominated by pharmaceuticals, which accounted for >70% of the total concentration of micropollutants, while insecticides only accounted for the smallest fraction among the five categories (0.42%–2.72%).

The detected chemicals in the influent of the WWTP had average concentrations ranging from 0 to 619.33 ng/L. Of the 31 chemicals, 5 pharmaceuticals, namely ibuprofen (619.33±268.98 ng/L), diphenhydramine hydrochloride (376.92±183.56 ng/L), sulfamethoxazole (374.92±348.73 ng/L), clarithromycin (329.00±126.43 ng/L), and roxithromycin (222.25±83.17 ng/L) were the dominant micropollutants in the influent, with their average concentration exceeding 200 ng/L (Appendix A Table S2). Most of them were observed in the WWTP effluent at high concentrations. Thus, pharmaceuticals were the dominant micropollutants in domestic wastewater. Many statistical studies have highlighted the high frequency and high load of pharmaceuticals in wastewater obtained from WWTP (Miège et al., 2009; Zhang et al., 2018). The concentrations of all the detected individual insecticides were < 10 ng/L during the whole treatment process. This resulted in insecticides having the least concentration among the five categories of micropollutants. The concentration of the detected micropollutants was within the reported concentration range of micropollutants in domestic wastewater (Loos et al., 2013; Luo et al., 2014; Petrie et al., 2015).

2.2. The removal of micropollutants in the WWTP

Fig. 2a–e summarizes the overall removal of the five categories of micropollutants during the wastewater treatment processes. The concentrations of herbicides and bactericides in the influent were (62.19 ± 11.89) and (82.17 ± 45.13) ng/L, respectively (Fig. 2a and c). The aerated grit chamber treatment process decreased 24% of herbicides and 22% of bactericides; the primary settling tank treatment process had almost no effect on their removal. The A^2O biological treatment process further decreased their concentrations to 32.00 ± 11.07 (49% removal) and 37.86 ± 23.24 ng/L (54% removal), respectively, and they remained stable despite UV disinfection. Surprisingly, the total concentration of five insecticides saw a 20% reduction after the aerated grit chamber treatment process, but increased in the subsequent treatment processes, even exceeding that in the influent (−13% removal), as seen in Fig. 2b. The insecticides were the smallest components among the five categories of detected micropollutants and the concentration of all the individual insecticides were below 10 ng/L. Moreover, concentrations of chlorpyrifos, dipterex, and acetamiprid increased after the aerated grit chamber process, and the other insecticides decreased slightly or remained stable (Appendix A Table S2). Many studies also demonstrated that the removal of pesticides in WWTPs was variable and often poor, with concentrations in effluents sometimes higher than those in influents (Stamatis et al., 2010; Kock-Schulmeyer et al., 2013; de Oliveira et al., 2020). This may be attributed to the deconjugation of metabolites and/or transformation products of the pesticides, hydrolysis, and desorption from particulate matter during wastewater treatment, etc. (Kock-Schulmeyer et al., 2013). The toxicity of herbicides, bactericides, and insecticides to microorganisms, which were largely present in activated sludge, may be the primary reason for their low removal efficiency in wastewater treatment processes (Ma et al., 2018; Zeyad et al., 2019).

As seen in Fig. 2d and e, pharmaceuticals and UV filters decreased from (2170.60 ± 131.14) to (1622.09 ± 420.53) ng/L (25% removal) and from (191.83 ± 70.36) to (122.78 ± 26.11) ng/L (36% removal), respectively, after the aerated grit chamber treatment. Furthermore, their removal increased to 72% and 77% after A^2O biological treatment, respectively. UV disinfection, which as the last unit, had a certain positive effect on the removal of pharmaceuticals and UV filters. The detected pharmaceuticals included non-steroidal anti-inflammatory drugs, macrolide antibiotics, sulfonamide antibiotics, antiepileptic drugs, and antihistamine drugs, etc., thereby suggesting their complex characteristics. This resulted in different removal mechanisms of detected pharmaceuticals in the wastewater treatment processes. It has been revealed that the removal efficiency of pharmaceuticals in WWTP had a close relationship with their molecular properties (Joss et al., 2006; Tadkaew et al., 2011; Arola et al., 2017). Based on their $\log K_{ow}$, $\log K_{oc}$, and K_{biol}, the high removal efficiency of pharmaceuticals in the aerated grit chamber and A^2O biological treatment processes was attributed to oxidation, adsorption and biodegradation (Ma et al., 2018). However, the high removal of UV filters (with high $\log K_{ow}$ value) may be attributed to the adsorption action and biodegradation in the wastewater treatment processes (Appendix A Table S2). It was revealed that the biological removal efficiencies of BP-3, EHMC, and OC were >60%, and BP-4 with low $\log K_{ow}$ value achieved low removal efficiency in the traditional secondary effluent (Tsui et al., 2014). This was in accordance with the present study.

Overall, pesticides including herbicides, insecticides, and bactericides were difficult to remove, whereas pharmaceuticals and UV filters were effectively removed in the WWTP where the A^2O biological treatment was employed. Comparing the individual treatment processes, the aerated grit chamber achieved

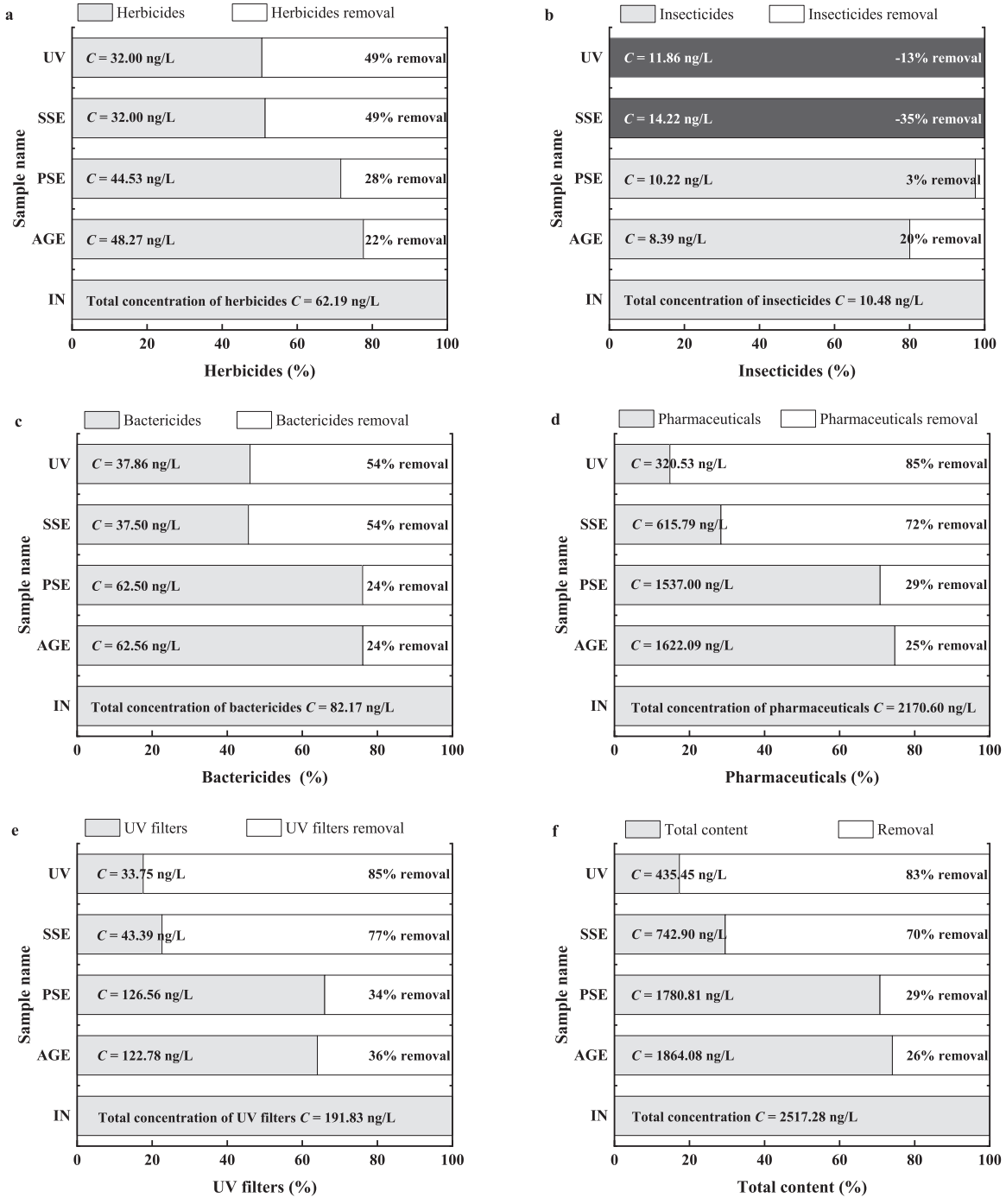

Fig. 2 – Removal of micropollutants in wastewater treatment units, including (a) herbicides, (b) insecticides, (c) bactericides, (d) pharmaceuticals, (e) UV filters, and (f) all detected micropollutants. The dark gray bar in b indicates an increase in the concentration of insecticides. IN: influent; AGE: aerated grit chamber effluent; PSE: primary settling tank effluent; SSE: secondary settler effluent; UV: UV chlorination –

approximately 20% removal of all the five categories of micropollutants; the A^2O biological treatment further decreased approximately 50%–80% of micropollutants (except insecticides). Primary settling tank treatment and UV chlorination process resulted in the total concentration of five categories of micropollutants remaining constant or decreasing slightly. In the present study, the total concentration of the detected micropollutants decreased by 83% during the wastewater treatment process without subsequent tertiary treatment. However, this concealed those micropollutants that had low removal efficiencies and those that accounted for a small part of all the detected micropollutants. These micropollutants may have a significant impact on the aquatic environment. For example, the remaining insecticides in the discharged wastewater may have adverse biological effect on invertebrates. Zhang et al. (2018) indicated that only the A^2O biological treatment process was ineffective in micropollutants removal, but advanced technologies employed in the treatment steps, such as membrane bioreactor and ozonation, were demonstrated to be potent micropollutants elimination techniques.

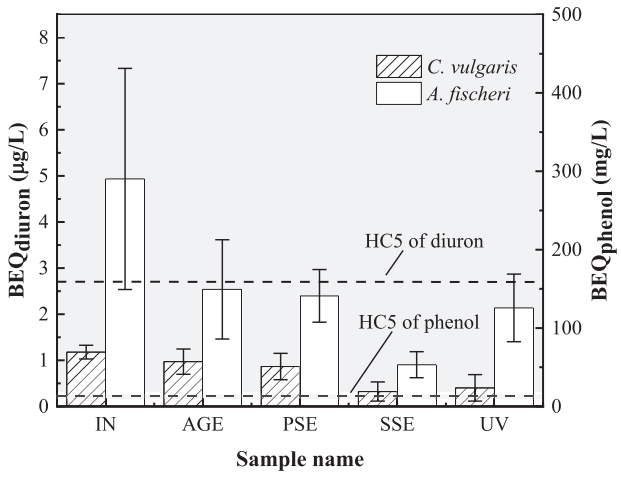

Fig. 3 – Variation in photosynthesis inhibition effect and bioluminescence inhibition effect during the wastewater treatment process. The gray zones represent 95% confidence interval of HC5 of diuron and phenol, respectively. The dotted lines represent their median HC5 –

2.3. Variation in biological effects and assessment

2.3.1. Variation in specific and non-specific biological effects

To evaluate the biological effects of pollutants from domestic wastewater, specific photosynthesis inhibition effect and non-specific bioluminescence inhibition effect were analyzed during the wastewater treatment processes. In the combined algae test (2 hr-PSII), BEQ_{diuron} decreased from 1.18 to 0.87 μg/L (27% removal) after the primary settling tank treatment, and then further dramatically decreased to 0.32 μg/L (73% removal) after the A^2O biological treatment (Fig. 3). Compared to A^2O biological treatment, UV chlorination process resulted in a marginal increase in the photosynthesis inhibition effect. Previous studies revealed that the photosynthesis inhibition effect from domestic wastewater could be reduced by approximately 39%–54% (Escher et al., 2008; Macova et al., 2011; Tang et al., 2014). In the case of the luminescent bacteria toxicity test, BEQ_{phenol} dramatically decreased from 290.26 to 149.30 mg/L (49% removal) after the first aerated grit chamber and then slightly decreased to 141.04 mg/L after the primary settling tank treatment. A^2O biological treatment further effectively reduced BEQ_{phenol} to 53.23 mg/L (82% removal). However, UV disinfection resulted in the BEQ_{phenol} increasing significantly to 125.68 mg/L. Variations in bioluminescence inhibition effect from wastewater during treatment processes in the present study were in accordance with previous studies (Wang et al., 2007; Ma et al., 2011; Tang et al., 2014). Välitalo et al. (2017) stated that there was no considerable difference in the toxicity removal efficiency of the seven treatment plants, and no correlation was observed with the operational parameters of the WWTP, such as process configuration, temperature, or sludge age.

2.3.2. Ecological safety assessment based on HC5

In order to evaluate the ecological safety of wastewater, HC5 values of the positive control regarding the biological toxicity test were deduced as the threshold values based on SSD models. In the case of diuron as the positive control of the combined algae test, 78 toxicity data regarding algae were collected and fitted with the SSD model. The fitted SSD curves of diuron are shown in Fig. 4a. The median HC5 of diuron obtained was 2.7 μg/L (0.32–8.23 μg/L at 95% confidence interval). In total, 491 toxicity data for phenols were collected, which were tested with 85 aquatic species belonging to five taxonomic groups, including fish, mollusca, vermes, insects/spiders, and crustaceans. The number of toxicity data for specific groups are shown in Appendix A Table S3. The fitted SSD curves of phenol are shown in Fig. 4a. The extrapolated median HC5 of phenol from the SSD curve was 6.04 μg/L (4.10–9.00 μg/L at 95% confidence interval).

The median HC5 and the 95% confidence interval of diuron and phenol are also shown in Fig. 3. The photosynthesis inhibition effect from all the detected water samples were below the median HC5 of diuron and exceeded the lower limit of its 95% confidence interval (except the SSE). This suggested that wastewater had a negligible photosynthesis inhibition effect, even the untreated raw wastewater. On the contrary, the bioluminescence inhibition effect of all the detected wastewater was over the higher limit of its 95% confidence interval of HC5 of phenol, indicating that this wastewater may pose a high risk for the aquatic organisms, despite undergoing secondary treatment. Hence, the bioluminescence inhibition effect from the domestic wastewater should be identified as the priority biological effect. Although, the secondary effluent achieved domestic wastewater discharge standards, the bioluminescence inhibition effect from the domestic wastewater should pay much attention. Xu et al. (2014) proposed an integrated toxicity assessment method and indicated that the bioluminescence inhibition effect from reclaimed water was also the dominant biological effect that needed to be controlled. Ma et al. (2019) indicated that compared to photosynthesis inhibition effect and genotoxicity, the non-specific bioluminescence inhibition effect from secondary effluent was the most difficult biological effect to be removed by advanced treatments, such as coagulation, ultraviolet photolysis and photocatalysis.

To guarantee the safety of aquatic organisms, the Ecotox centre in Switzerland proposed an acute and chronic quality standard. Appendix A Table S4 lists the numerical requirements of detected chemicals in the present study. Chemical risk assessments can also be carried out by comparing the detected concentrations with associated quality standards. It can be seen that the concentration of chlorpyrifos in the influent exceeds the chronic quality standard value (4.6×10^{-4} μg/L), even after undergoing a secondary biological treatment. This suggested that chlorpyrifos in the discharged wastewater posed a potential risk to aquatic organisms that faced long-term pollution. The concentration of clarithromycin and ibuprofen in the influent exceeded their chronic quality standards values (0.12 and 0.011 μg/L, respectively), and then they were reduced to within the standard values after biological treatment. However, it needs to be noticed that there may be a secondary intoxication risk due to their special toxicological characteristics. Hence, the ecological risk caused by micropollutants in the discharged wastewater need to be further reduced. This was in accordance with the biological effect assessment using HC5.

2.3.3. Contribution of known chemicals to the observed biological effects

In order to reveal the dominant chemicals that were responsible for the detected biological effects, a CA model was conducted to predict the specific photosynthesis inhibition effect and non-specific bioluminescence inhibition effect. As seen in Fig. 5a, the photosynthesis inhibition effect caused by the wastewater in the WWTP could be well predicted using the detected herbicides. For all the domestic wastewater collected during the entire treatment process, the contents of the detected herbicides contributed 2.47%–7.22% to the total concentration of all the detected chemicals; but the photosynthesis inhibition effects caused by the detected herbicides could explain 41%–101% of photosynthesis inhibition effects from the whole sample (Fig. 5a). The specific photosynthesis inhibition effects that was detected using bioassay could be well explained by the determined herbicides. Moreover, atrazine, which had high RP and accounted for a large part of detected herbicides (Appendix A Table S2), dominated the mixture effect

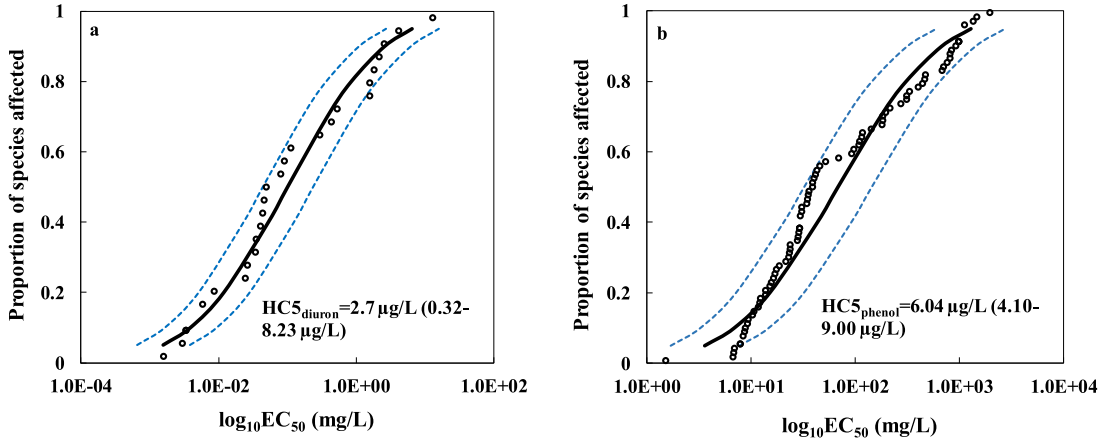

Fig. 4 – The SSD curves of (a) diuron and (b) phenol –

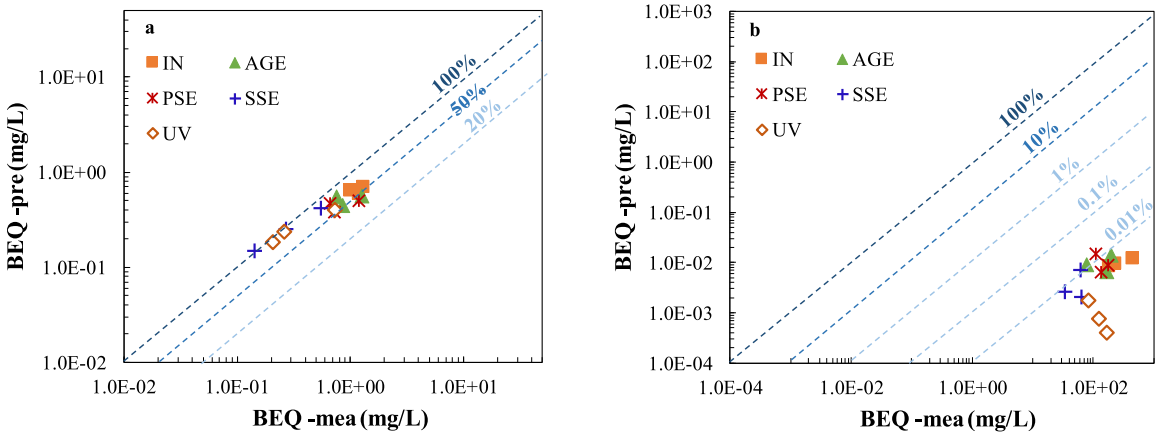

Fig. 5 – Contribution of detected chemicals to (a) specific photosynthesis inhibition effect and (b) non-specific bioluminescence inhibition effect. BEQ-pre and BEQ-mea are the predicted and measured bioanalytical equivalent concentration (BEQ) of the water sample in the two bioassays –

from domestic wastewater. Tang and Escher (2014) stated that herbicides dominated the algal toxicity in environmentally realistic mixtures from secondary effluent, reclaimed water, drinking water, or storm water, and the contribution of the non-herbicides was negligible (Tang and Escher, 2014). Escher et al. (2011) showed that herbicidal activity could be well explained by chemical analysis, with atrazine and simazine dominating the mixture effect.

On the contrary, all the detected chemicals only explained less than 0.1% of the measured bioluminescence inhibition effect (Fig. 5b). It has been confirmed that chemicals typically present in wastewater act as concentration-additives in the applied bioassays (Tang et al., 2014). The luminescent bacteria toxicity test reacted to most chemicals, but most chemicals acted as base-line toxicants in this assay (Escher et al., 2018). Hence, the deviation between the measured and predicated toxicity results may be because of "toxic unknowns", which were not detected by instrumental analyses, and were responsible for the bioluminescence inhibition effect, such as macromolecular dissolved organic matter (DOM). It has been seen that DOM in wastewater effluent, particularly humic substances with acidic nature, functioned as a toxicity inducer of residual chemicals in the secondary effluents, instead of a protector of toxicants (Hara-Yamamura et al., 2020). Additionally, the bioluminescence inhibition effect from industrial wastewater, which contained high concentration of particular chemicals, such as semi-coking wastewater, could be explained by the detected chemicals using the CA model (Ma et al., 2017). Overall, if it is to decrease the specific biological effect from the wastewater, the primary method should focus on controlling specific micropollutants; if non-specific biological effects from the wastewater attract much attention, all the contaminants should be decreased, especially the macromolecular DOM.

2.3.4. Ozonation process to improve the ecological safety

To eliminate the ecological risk of the secondary effluent, especially the non-specific bioluminescence inhibition effect, ozonation process was conducted in the laboratory. Fig. 6 demonstrates that the BEQ_{diuron} was lower than the detection limit (<4.2 ng/L BEQ_{diuron} for the combined algae test) when the ozone dosage increased to 0.71 g O_3/g DOC, suggesting that the ozonation process performed well to reduce photosynthesis inhibition. Ozonation process could effectively remove herbicides, which were identified as the chemicals responsible for photosynthesis inhibition effect (Fig. 5a); the oxidation by-product of wastewater could not cause photosynthesis inhibition effect. Jia et al. (2015) indicated that the ozonation process (3.0 mg O_3/L) could reduce approximately 35% of the photosynthesis inhibition effect. Bhuvaneshwari et al. (2019) revealed that ozonation of wastewater effluent (3–4 mg O_3/L) could reduce the phytotoxicity regarding cyanobacteria *Spirulina*. Actually, in ozonation processes, the removal efficiency of photosynthesis inhibition effect was affected by ozone dosage, oxidation time, and wastewater effluents. Considering ecological safety, the ozonation process for biological effect reduction were also assessed using the threshold values of the two bioassays. The photosynthesis inhibition effect from the wastewater was still

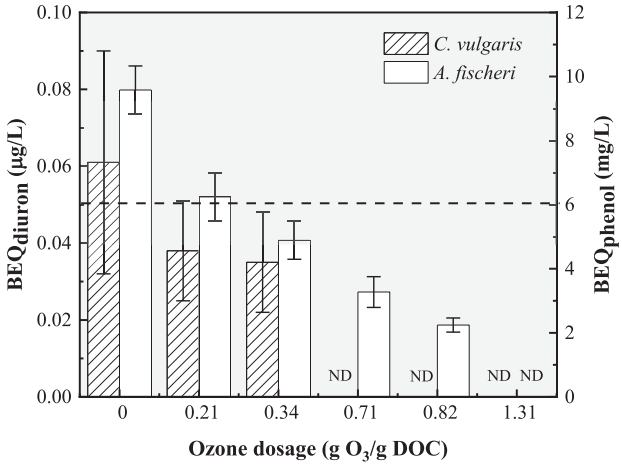

Fig. 6 – Variation in biotoxicity posed by secondary effluent during the ozonation process. The gray zone represents the scope of HC5 of phenol. ND: below the detection limit –

much lower than the HC5 of the diuron (2.7 μg/L; not shown in the Fig. 6) along the ozone dosage increase, indicating that the photosynthesis inhibition effect caused by the wastewater after ozonation processes was negligible.

The BEQ_{phenol} was lower than the detection limit (<1.3 mg/L BEQ_{phenol} for the luminescent bacteria toxicity test) when the ozone dosage increased to 1.31 g O_3/g DOC. This suggested that the ozonation process had a robust capacity to reduce non-specific bioluminescence inhibition effect, which was identified as the priority biological effect from the secondary effluent. According to the ecological safety assessment, $\geq$ 0.34 g O_3/g DOC of ozonation dosage could reduce the bioluminescence inhibition effect to an ecological safe level. Among the advanced treatment processes, ozonation is currently one of the recommended technologies to economically and effectively remove micropollutants and decrease toxicity from wastewater (Margot et al., 2013; Schindler Wildhaber et al., 2015; Bourgin et al., 2018). Ozonation process includes a direct reaction with ozone and an indirect reaction with the formation of hydroxyl radicals. Ozonation process was highlighted because ozone itself has a high oxidation potential (though lower than the hydroxyl radicals) and a high capacity to react with other oxidants or radiations to generate hydroxyl radicals. A specific ozone dose of 0.55 g O_3/g DOC is recommended at the Switzerland WWTP to ensure an average abatement of the 12 selected micropollutants by $\geq$ 80% after the entire treatment, and then an additional post-treatment (sand filtration, moving bed, fixed bed or granular activated carbon filtration) is required to eliminate the possible negative effects generated during ozonation (Bourgin et al., 2018). Hence, the recommended ozone dose for micropollutants reduction control was basically in line with that for the biological effect reduction control, although the detected micropollutants were not responsible for the measured bioluminescence inhibition effect (Fig. 5b).

3. Conclusions

The emerging micropollutants in wastewater treatment processes and their associated biological effects were investigated and assessed. The total concentration of the 31 detected micropollutants decreased by 83% after the entire treatment process. Of the five categories of micropollutants, herbicides, insecticides, and bactericides were not easily removed (especially insecticides); however, pharmaceuticals and UV filters were effectively removed. Of the individual treatment unit, the A^2O biological treatment could achieve approximately 50%-80% removal (except insecticides); aerated grit chamber removed approximately 20% for all the five categories of micropollutants. Based on the ecological safety assessment using HC5, the photosynthesis inhibition effect from wastewater was negligible, even in the case of raw wastewater without any treatment. However, the bioluminescence inhibition effect from the WWTP effluent, which was defined as the priority biological effect, posed potential ecological risk. Chemical risk assessments also proved that the WWTP effluent had an adverse effect on aquatic organisms, with chlorpyrifos being the dominant micropollutants. To further treat the WWTP effluent, $\geq$ 0.34 g O_3/g DOC of ozone dose was recommended for micropollutants reduction and ecological safety control. Hence, in order to improve the quality of discharged wastewater, advanced treatment processes should be used appropriately.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.03.054.

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