Science of the Total Environment 650 (2019) 10–17

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Extended suspect screening strategy to identify characteristic toxicants in the discharge of a chemical industrial park based on toxicity to *Daphnia magna*

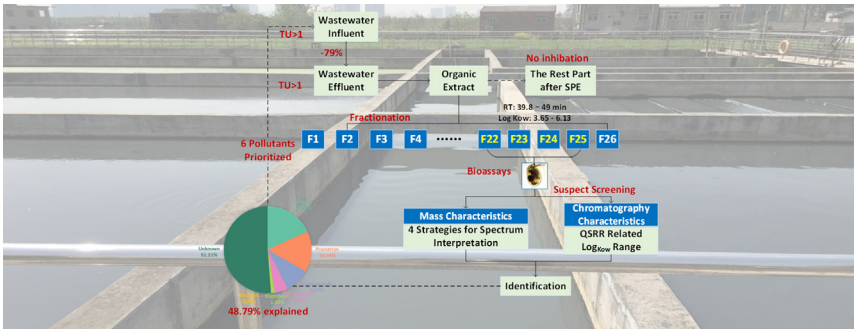
Jing Guo [a], Dongyang Deng [a], Yuting Wang [a], Hongxia Yu [a], Wei Shi [a,b,c,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiangsu 210023, China
[b] Jiangsu Environmental Monitoring Center, Nanjing University, Nanjing, Jiangsu 210023, China
[c] Jiangsu Key Laboratory of Chemical Pollution Control and Resources Reuse, Nanjing University, Nanjing 210023, China

HIGHLIGHTS

- An extended suspect screening strategy was developed by *in vivo* bioassay-directed fractionation and suspect screening.
- 53.6% and 53.8% of the false positive structures were removed by mass and chromatography characteristics, respectively.
- Imazalil, prometryn, propiconazole and tebuconazole were identified as key toxicants with toxicity contributions near 50%.
- Such suspect screening explained 2.48 more times of the total toxicity and saved 90% of the labor.
- Such suspect screening approach provides more efficient identification of key toxicants in complex environmental matrices.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 May 2018
Received in revised form 15 August 2018
Accepted 17 August 2018
Available online 18 August 2018

Editor: Jay Gan

Keywords:
Wastewater
Daphnia magna
Fractionation
Effect-directed analysis, EDA
Potency balance

ABSTRACT

With an increasing amount of industrial wastewater being discharged and the numerous chemicals existed in, methods to identify toxicants in such complex matrices are urgently needed for source control and quality management. *In vivo* toxicity to *Daphnia magna* was evaluated in the effluent of a wastewater treatment plant (WWTP). An extended suspect screening strategy was performed by bioassay-directed fractionation, accompanied with suspect screening of 228 suspect chemicals in toxic fractions based on their mass characteristics and chromatography characteristics. A toxicity evaluation of the original samples, organic components extracted by solid-phase extraction (SPE) and the filtered samples showed that organic compounds extracted by SPE were the main toxic components. Four of the 26 fractions of the organic extracts exhibited a toxic unit (TU) > 1.0, with hydrophobic organic compounds contributing most to the toxicity. Twenty-eight of the 228 suspects were identified in four toxic fractions, with 53.6% of the suspects elucidated by spectrum interpretation based on mass characteristics and 53.8% more false positive suspects removed based on chromatography characteristics. Finally, 6 pollutants, including imazalil, prometryn, propiconazole, tebuconazole, buprofezin and diazinon, were further confirmed and explained 48.79% of the observed toxicity. With 2.48 times more of the toxicity explained and 90% of the labor saved, the extended suspect screening strategy enabled more efficient and reliable

* Corresponding author at: State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiangsu 210023, China.
E-mail address: njushiwei@nju.edu.cn (W. Shi).

https://doi.org/10.1016/j.scitotenv.2018.08.215
0048-9697/

identification compared to traditional quantitative analysis and non-target screening, especially for identification of characteristic toxicants in complex environmental matrices.

1. Introduction

Recently, with the vigorous development of China's chemical industry, over 60 national and provincial chemical industrial parks have been agglomerated (Tian et al., 2012), especially along rivers and in eastern coastal areas. Currently, over 50,000 chemicals have been used or produced in chemical industrial parks in China (Greenpeace, 2017), including raw materials, products and by-products, many of which are organic pollutants, including pesticides (Zapata et al., 2010), dyes (Yu and Wen, 2005), phenols (Livingston, 1993), halogenated organic pollutants and polycyclic aromatic hydrocarbons (PAHs) (Wang et al., 2007). These chemicals tend to be released into the environment through wastewater effluents, which may pose potential hazards to aquatic organisms. However, the techniques most frequently used for wastewater treatment, such as anaerobic digestion and aerobic composting, are ineffective for removing these pollutants (Prasse et al., 2015; Rahmanian et al., 2014). In addition, current standards on industrial wastewater discharge generally include common limits on several volatile organic compounds (VOCs), heavy metals, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and ammonia nitrogen (GB8978, 2017) that are far from enough in terms of numerous known or unknown chemicals that exist in industrial wastewater effluents. Therefore, methods to identify key toxicants are urgently needed.

Current strategies to identify key pollutants rely heavily on the monitoring and hazard evaluation of conventional pollutants. However, the number of monitored chemicals is far less than the number of chemicals that actually exist. For example, a Greenpeace East Asia investigation at a chemical industrial park in Jiangsu, China showed that among all the 226 chemicals detected, only 26% are monitored as "hazardous chemicals" under current regulations for safety management (Greenpeace, 2017). Moreover, the evaluation of very limited conventional chemicals could only explain a limited part of the potential hazards to aquatic organisms (Brack et al., 2017). In addition to the regularly monitored chemicals, there exist thousands of known or unknown chemicals (Sobus et al., 2017). Thus, it is difficult to evaluate potential hazards by this type of top-down approach based on individual chemicals. Even if all the chemicals are possibly identified, the toxicological information and environmental behavior for such a huge number of pollutants are still hard to evaluate (Gavrilescu et al., 2015). As an alternative, a bottom-up method that measures adverse effects can be used in conjunction with chemical analysis. However, although the evaluation of toxicity indicates potential adverse effects on aquatic organisms, information on contributions of individual chemicals to the observed toxicity remains unknown (Brack et al., 2015). Therefore, bioassay-directed fractionation and identification, which combines toxicological and chemical analyses, is an effective way to identify key toxicants in wastewater, so that enables more appropriate treatments and management.

Effect-directed analysis (EDA), which combines chemical analysis and toxicity evaluation (Brack, 2003), is such a bioassay-directed fractionation and identification process mentioned above. EDA has become more and more useful in hazard evaluation and identification of toxicants and has successfully been used in the identification of key toxicants in various matrices (Brack et al., 2016), such as surface waters (Thomas et al., 2009), soils (Legler et al., 2011), sediments (Brack et al., 2005) and biota (Simon et al., 2011). However, several issues have emerged in key steps, including toxicity evaluation and toxicant identification, which have limited its further applications. Currently, toxicity evaluations in EDA analyses are mainly performed by *in vitro* bioassays, which are convenient and sensitive. However, such evaluations fail to reflect actual adverse effects on aquatic organisms, especially without consideration of the bioavailability of these pollutants in aquatic organisms, which indicates the importance of *in vivo* bioassays for more accurate evaluations. Moreover, current identification of toxicants in EDA generally consists of the quantitation of target pollutants and qualitative analyses of non-target pollutants in fractions. However, quantitation of target pollutants in industrial wastewater, which is a highly complex environmental matrix, is too costly and time-consuming to complete, with too many targets to be quantitated. Also, traditional quantitative analysis of monitored chemicals generally shows a low toxicity contribution, even <0.1% (Escher et al., 2013; Tang et al., 2013), which is inefficient on consideration of the cost and time spent. For the characterization of non-target pollutants, which has been proved to be a powerful tool in screening of suspect chemicals (Gago-Ferrero et al., 2015; Schymanski et al., 2015), although key toxic fractions are easy to identify, the key toxicants that contribute most to the toxicity of toxic fractions are hard to identify due to the difficulties in peak prioritization and structure elucidation (Hug et al., 2014). Thus, EDA has rarely been used in the identification of key toxicants in complicated matrices such as industrial wastewater.

The objectives of the present study were to evaluate the potential hazards of discharge from industrial wastewater to aquatic organisms using *Daphnia magna*, to develop an extended suspect screening strategy based on *in vivo* bioassay-directed fractionation and high-throughput suspect screening which will efficiently simplify the target analysis and provide more reliable information for non-target screening to identify characteristic pollutants in industrial wastewater.

2. Materials and methods

2.1. Materials and chemicals

All the synthetic chemicals, including PAHs, phenols and pesticides, were purchased from AccuStandard (New Haven, CT, USA), Sigma-Aldrich (St. Louis, MO, USA), Supelco (Bellefonte, PA, USA) and Fluorochem (Derbyshire, UK). All solvents used in the study, including *n*-hexane (Hex), dichloromethane (DCM) and methanol (MeOH), are liquid chromatography (LC) grade and were purchased from Merck (Darmstadt, Germany). Detailed information of the standards for instrumental analysis is summarized in Table S1 in the Supporting information (SI).

The workflow of the sample preparation and the extended suspect screening strategy of key toxicants in wastewater are shown in Fig. 1. Briefly speaking, original wastewater was evaluated acute toxicity to *Daphnia magna*. Key toxic components were identified by comparison of the original sample, the organic component extracted by solid-phase extraction (SPE) and the filtered sample after extraction, respectively. If organic extracts were the key toxic components, bioassay-directed fractionation was performed, and key toxicants were identified by suspect screening of toxic fractions based on mass characteristics and chromatography characteristics.

2.2. Sample collection and preparation

Influent and effluent wastewater samples (24 h flow-proportional samples) were collected from a wastewater treatment plant (WWTP) in NJ, Jiangsu, which is the second-most important chemical industrial aggregation after Shanghai. Moreover, there are key drinking water source areas and nature reserves located in the lower reaches. Four liters of wastewater samples were sampled in cleaned-up brown glass