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Diagnosis of complex mixture toxicity in sediments: Application of toxicity identification evaluation (TIE) and effect-directed analysis (EDA)☆

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ABSTRACT

Determining causality of sediment toxicity is of great importance in aquatic risk assessment, but there are tremendous challenges due to joint toxicity of trace pollutants in complex sediment matrices. Two approaches, namely toxicity identification evaluation (TIE) and effect-directed analysis (EDA) have been developed. Conventional sediment TIEs take the advantage of environmental relevance by using whole organism bioassays; however, they suffer from lack of effective methods for specifically identifying major contributors as it typically only evaluates contaminant class rather than specific contaminants. Alternatively, EDA is a powerful tool in identifying causes of sediment toxicity with sophisticated fractionation and chemical analysis of targeted and non-targeted non-polar organic toxicants, but it is not always environmentally relevant due to the use of in-vitro bioassays and exhaustive solvent extraction. An integrated TIE and EDA method would provide an environmentally relevant and toxicant specific approach to effectively determine causality of sediment toxicity by combining the merits of the two methods. Bioavailability-based extraction and dosing techniques are recommended to be incorporated into the integrated method to improve the accuracy of toxicity diagnosis. Besides considering bioavailability in the integrated TIE and EDA approach, the premise of adverse outcome pathways should also be considered. Generally speaking, both TIE and EDA have focused on adverse effects at cellular and organism levels. The addition of trait-based approaches in screening multiple toxicological endpoints helps to extend effects on cellular and organism levels to population level, and provides a better understanding of potential impacts to the community and ecosystem. The outcome pathway underlies the critical role of determining causality in interpreting impacts of complex mixtures to benthic community and aquatic ecosystem.

1. Introduction

Sediments play a crucial role in ecosystem functioning, providing habitat for millions of species that are important in aquatic food webs (Burton and Johnston, 2010). At the same time, sediments act as a major sink for a variety of contaminants ranging from those that are well-known and regularly monitored to those of unknown identity (e.g. unidentified by-products and transformation products). Exposure to complex mixtures of contaminants in sediments may cause adverse effects to benthic and epibenthic organisms. Meanwhile, potential release of sediment-bound contaminants to overlying water puts aquatic ecosystems at risk (Josefsson et al., 2010; Pang et al., 2012). Knowing the magnitude of toxicity is of great importance in sediment risk assessment, and identifying the key toxicants responsible for the adverse effects from complex mixtures of contaminants is also critical for developing effective measures for release control and remediation and management of contaminated sediments. Due to the complexity of the sediment matrix, diagnosing key toxicants causing sediment toxicity and assessing their impacts on aquatic ecosystem have become one of the greatest challenges in the current ecological risk assessment (van den Brink et al., 2016).

Given a set of contaminants in the target lists which may be

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related to sediment toxicity, dose-response based approaches (top-down) have been developed to identify the major toxicants in sediment (Burgess et al., 2013a). Nevertheless, numerous studies combining targeted analysis and bioassays in ecological risk assessment of complex mixtures of contaminants indicate that the analytes in the target lists could only partially, or poorly, explain the observed effects, calling for advancing cause-effect based approaches and performing non-targeted analysis of complex mixtures (Brack, 2003; Brack et al., 2016). The recent boom of new biological and analytical technologies has made this approach viable. On one hand, a number of methods have been developed to assess sediment toxicity, ranging from cellular assay to adverse outcomes on individual, population and community levels (Ankley et al., 2010; Köhler and Triebskorn, 2013). Yet, the linkage and extension between each level of the adverse outcome pathway, e.g. extrapolation from high throughput cellular assays to more environmentally relevant whole-organism bioassays, need further research. On the other hand, sophisticated analytical tools have been developed to perform targeted and non-targeted analysis with high sensitivity and selectivity in combination with various fractionation procedures (Brack et al., 2016; Chibwe et al., 2017; Krauss et al., 2010; Weller, 2012), which have significantly improved understanding the causes of sediment toxicity.

To combine biological and chemical analyses in understanding cause-effect relationships of complex mixtures in sediment, toxicity identification evaluation (TIE) and effect directed-analysis (EDA) are two principal bioassay-directed diagnosing approaches (Burgess et al., 2013a). The two approaches share the same goal, i.e. to identify the key toxicants from tremendous numbers of contaminants in mixtures, but they have distinct assumptions, strategies, methodologies and endpoints as well as respective features (Table 1). The methodologies and applications of the two approaches have been individually reviewed (Brack et al., 2016; Ho and Burgess, 2013; Hong et al., 2016a). Burgess et al. (2013b) compared the performance, similarities, differences and applications of TIE and EDA, and proposed an example to complement the two approaches in diagnosing causality. Due to their different assumptions and methodologies, however, combinations of TIE and EDA in sediment toxicity identification are still limited to date.

In some cases, sediment toxicity diagnosis failed to identify the major toxicants, and one possible explanation is lack of investigation into bioavailability (Brack, 2003; Burgess et al., 2013a). Sediment characteristics, e.g. contents and types of organic carbon and grain size, strongly influenced the bioavailability of sediment-bound contaminants, and further altered their toxicity (Cornelissen et al., 2005; Mehler et al., 2011a). Therefore, it is imperative to take bioavailability of sediment-bound contaminants into account when diagnosing toxicants.

In the present review, we summarize the current status and applications of TIE and EDA in identifying key toxicants in sediment, with a focus on refining their complementary use and the benefits of incorporating bioavailability into the methods based on recent research findings. Finally, the role of diagnosing causality in sediment impact assessment is demonstrated.

2. TIE for classifying and diagnosing toxicants in sediment

Toxicity identification evaluation was firstly developed by the U.S. Environmental Protection Agency (USEPA) in the 1980s to identify the key toxicants in municipal and industrial effluents (USEPA, 1991). The TIE methods for effluent were then modified for use in sediment interstitial water in the 1990s (USEPA, 1992) and whole sediment in the mid-2000s (USEPA, 2007). In general, a TIE procedure includes three phases: characterization, identification and confirmation. Before phase I, screening toxicity testing using whole organisms is performed to select toxic sediments for TIEs. In phase I, possible toxicant classes are characterized by whole-organism toxicity testing after sediment manipulation with appropriate amendments. Toxicants are classified as nonionic organic contaminants, cationic metals, ammonia, sulfide, ionic imbalance and pH, and the first three classes of toxicants are the most frequently characterized in sediment TIEs (Ho and Burgess, 2013). After characterization, a phase II TIE is conducted to determine the concentrations and toxicity contribution of possible toxicants to the observed toxicity with a focus on the class(es) of toxicants characterized by phase I. Based on the findings in phases I and II, TIE phase III is devoted to confirming the identified toxicants using independent lines of evidence.

In pre-2013 TIE literature reviewed by Ho and Burgess (2013), 90% of the whole-sediment TIEs conducted by that time found organic pollutants, either singly (70%) or in combination with metals (10%) or ammonia (10%) were responsible for the observed sediment toxicity. Before the development of whole-sediment TIE, interstitial water TIE has been often applied to identify suspected toxicants in sediment. Compared with whole sediment TIE, interstitial water TIE takes more classes of toxicants into consideration thanks to more diverse methods available for toxicant characterization in phase I TIE, however, it is less environmentally relevant and disparate results have been found for the two methods in some cases. Ammonia and organics (i.e. polycyclic aromatic hydrocarbons, PAHs) were regarded as the major source of sediment toxicity in Illinois River in the U.S. when interstitial water and whole sediment TIE were applied, respectively (Mehler et al., 2010b). The process to obtain interstitial water from sediment may change the composition of contaminants and correspondingly cause biased toxicity contributions between whole sediment and interstitial water, resulting in erroneous identification when interstitial water TIE is used. Therefore, the more environmentally relevant whole-sediment TIEs are recommended to diagnose the causality of sediment toxicity in the presence of complex mixtures of contaminants. In addition to conventional TIEs, specific TIEs have been successfully developed to assess toxicity contribution of certain chemicals in sediment, e.g. pyrethroid, organophosphate and carbamate pesticides, using esterase and piperonyl butoxide addition as well as temperature manipulation (Amweg and Weston, 2007; Mehler et al., 2011b; Weston and Amweg, 2007; Weston and Jackson, 2009; Weston et al., 2009; Wheelock et al., 2004). Specific TIEs take the advantages of identifying and confirming specific chemicals, but they may fail to identify broader classes of toxicants, which limits their usage in identifying toxicants in complex mixtures.

Overall, nonionic organic contaminants are the most common in the list of major toxicants in sediments based on the last 20 years of TIE practices (Ho and Burgess, 2013). Of the 26 peer-reviewed TIE studies with nonionic organics contributing to sediment toxicity, only 11 studies identified the candidate toxicants by TIE methods (Ho and Burgess, 2013). The identified organic toxicants in the 11 studies included pyrethroids, organophosphate pesticides, PAHs and polychlorinated biphenyls (PCBs) (Anderson et al., 2006; Ho et al., 1997; Mehler et al., 2010a, 2010b), which are in the list of routinely analyzed contaminants. As reported in the American Chemistry Society's database, more than 10^8 chemicals have been indexed to date (https://www.cas.org/content/counter), and majority of these chemicals are synthetic organics with an ever-expanding structural possibilities (Daughton, 2005). However, only a small fraction of this vast pool of chemicals, i.e. several thousands of compounds, have ever been analyzed in environmental samples and even less (tens to hundreds) have been regularly monitored (Brack et al., 2016). Phase II TIE methods need to be further developed to identify contaminants which are not regularly

Table 1
The pros and cons of toxicity identification evaluation (TIE) and effect-directed analysis (EDA) in diagnosing complex mixtures of toxicants in sediment.

Parameter	TIE		EDA	
	Advantage	Disadvantage	Advantage	Disadvantage
Sample form	Whole sediment sample which accounts for bioavailability and all potential toxicity	Difficulty to identify toxicants in whole sediment sample due to the complexity	Organic solvent extract of sediment; complexity of the sample can be significantly reduced by repeated fractionations	Potential loss of some toxicants during extraction and fractionation; inconsistent composition of toxicants in original sediment and the extract Disregards contaminants that are not organic
Toxicity testing type and representative toxicological endpoint	Environmentally relevant whole organism bioassay: lethal and sublethal endpoints (e.g. growth, reproduction, behavior)	Labor and time consuming; relatively low throughput	High throughput in vitro bioassay with specific toxicological endpoints: e.g. endocrine disruption, mutagenicity and genotoxicity; sensitive. Can be linked to certain types of chemical structures	Less environmentally relevant; few studies using in vivo bioassay in EDA, which increased the environmental relevance but decreased the throughput
Toxicant of concern	All classes of potential toxicants are considered	Analysis of toxicants in target lists; common toxicants more easily identified. Possible loss of toxicants with unknown identities	Targeted & non-targeted toxicants	Emphasis on organic contaminants
Incorporation of bioavailability	Bioavailability is considered by toxicity testing using whole sediment and measuring bioavailable/bioaccessible concentrations of contaminants	Analyze the bioavailable concentrations of commonly analyzed contaminants	Bioavailability is not considered in conventional EDA; Recent EDAs have started to incorporate bioavailability in the steps of sediment extraction and extract dosing	Current bioavailability-based extraction and dosing methods are only suitable for contaminants with certain range of hydrophobicity, resulting in loss of potential toxicants; incorporation of bioavailability may decrease the throughput of bioassay
Toxicant identification	Identifying various classes of contaminants	Low specificity for organic contaminants due to sample complexity	High specificity by focusing on toxic fractions after repeated fractionation; sensitive; non-targeted analysis	Only focusing on organic contaminants
Cost	Relatively low cost for chemical analysis due to targeted analysis and small sample size (the same as the number of samples for TIE)	Relatively high cost for whole-organism bioassays using whole sediment; high cost for collecting large volume of samples	Relatively low cost for in vitro bioassays	Relatively high cost for chemical analysis due to non-targeted analysis and large sample size after fractionation; some reagents and cells for in vitro bioassays are expensive

monitored or with completely unknown identity, which would decrease the percentage of unexplained toxicity (Burgess et al., 2013a; Escher et al., 2013). It is practically impossible to directly assess the universe of chemicals in complicated sediment matrices to identify the active toxicants. Thus, it is imperative to develop alternative approaches that reduce the complexity of sediment extracts and target contaminants causing risk. These approaches are complementary to the phase II identification in TIE, especially for sediment samples in which organics have been characterized as the main class of toxicants (Burgess et al., 2013a). Like TIEs use bioassay directed fractionation to elucidate known and unknown toxicants in complicated matrices with a focus on organics, EDA's endpoints are often more rapid and can give information on the types of organic toxicants present. Combining EDA with TIE meets the challenge mentioned above (Brack, 2003; Brack et al., 2016; Hong et al., 2016a).

3. EDA focusing on diagnosing organic toxicants in sediment

Effect-directed analysis combines biological assays, physico-chemical fractionation and chemical analysis to identify bioactive organic toxicants in complex mixtures (Brack, 2003). Under the guidance of biological effects, EDA reduces the sample complexity and narrows down the number of possible toxicants by repeated (if needed) fractionation and bioassay. The processes eliminate fractions with no and low effects and analyzes fractions with high biological activity. Finally, the candidate toxicants identified by EDA are confirmed by re-establishing dose-response relationship. Unlike TIE performing toxicity testing using whole sediment, organic solvent extracts of sediment are generally used for biotesting, fractionation and chemical analysis in EDA. The performance, requirements and research of EDA processes, i.e. bioassay, fractionation, identification and confirmation, are detailed in Table 2. The EDA method was firstly proposed in the 1980s (Samoiloff et al., 1983; Schuetzle and Lewtas, 1986). Since then, the methodology has continually progressed and been applied in diagnosing toxicants in environmental samples (Brack, 2003; Brack et al., 2007, 2008). Although non-targeted analysis of toxicants with unknown identity has always been highlighted in EDA, it has become being more routinely implemented in EDA for environmental samples since the 2010s when various cutting-edge instruments, e.g. high resolution gas/liquid chromatography-mass spectrometer (GC/LC-MS), have been rapidly developed and widely used (Brack et al., 2016; Hong et al., 2016a).

Recently, several studies have reviewed the status and challenges of applying EDA in identifying key toxicants in complex mixtures of environmental samples (e.g. Brack et al., 2016; Hong et al., 2016a). Among their environmental applications, over 60% of EDA studies were conducted for sediment matrices (Hong et al., 2016a), implying wide applications of this powerful technique in

Table 2
A summary of the performance, requirements and status of effect-directed analysis (EDA) processes.

EDA process	Performance, requirements and status
Bioassay	● High throughput in-vitro bioassays are applied to meet the demand for a great deal of bioassays, because EDAs are designed to isolate and identify individual toxicants from a universe of contaminants (Brack et al., 2016; Burgess et al., 2013a) ● In-vitro bioassays, e.g. cellular toxicity testing, with endpoints like mutagenicity, genotoxicity and endocrine disruption (Creusot et al., 2013; Lubcke-von Varel et al., 2011; Reifferscheid et al., 2011; Weiss et al., 2009) help elucidate modes of action of contaminants ● The link is relatively weak between in-vitro toxicity and adverse outcomes on individual and population levels. To solve the problem, whole organism toxicity testing are started to be used in EDAs, however, increasing throughput of in-vivo testing is necessary for its use in EDA (Qi et al., 2017; Schmitt et al., 2011a)
Fractionation	● To reduce the complexity of toxic mixtures, sediment extracts are fractionated according to their physicochemical properties, e.g. polarity and molecular size of the component ● Typically, sediment extracts are fractionated using preparative chromatography, and the separation is based on adsorption, partition, ion exchange, size exclusion and affinity (Bandow et al., 2009a; Creusot et al., 2013; Lubcke-von Varel et al., 2008) ● Selection of an appropriate chromatographic system to fractionate sediment extracts is crucial to the accuracy of EDA outcomes, and the main elements considered are representativeness of original sediment sample, sufficient amount of sample for bioassay, reproducibility and precision
Identification	● After certain rounds of fractionation and bioassay, the components in the biologically active fraction(s) are qualified and quantified using chemical analysis by combining targeted analysis of contaminants with known identity (including target contaminants and those not routinely analyzed but known chemicals) and non-targeted screening of chemicals with unknown identity by structure elucidation ● Chemical identification is one of the biggest challenges in EDAs, which mostly relies on GC-MS and LC-MS for environmental samples (e.g. sediment) due to the small sample size and high detection sensitivity needed. ● Extensive spectral libraries of MS support the identification of unknown toxicants. The mass spectra given by GC/LC-MS, especially the newly developed (ultra) high resolution analyzers (e.g. time-of-flight (TOF) MS and Orbitrap MS), can be combined with additional structure generation tools and computer models to elucidate chemical structures of the suspect toxicants with unknown identity based on the fragmentation and retention (Brack et al., 2016; Burgess et al., 2013a)
Confirmation	● After the candidate toxicants have been identified by EDA, it is crucial to confirm their contributions to the noted toxicity by establishing quantitative cause-effect relationships between the toxicant(s) and observed toxicity ● It is required that the identified toxicant(s) explain at least part of the observed toxicity and are detected in the study samples with toxic units correlating to the observed toxicity ● A tiered approach has been recommended in EDA confirmation, including analytical confirmation of toxic candidates, effect confirmation using effect data from the literature and/or in-vivo and/or in-vitro bioassays and hazard confirmation of in situ effects under realistic exposure conditions (Brack et al., 2008)

determining causality of toxicity in complex sediment matrices.

Different from TIEs, EDAs not only can identify contaminants that are relatively common, e.g. PAHs, PCBs, polybrominated diphenyl ethers (PBDEs), regulated pesticides and pharmaceuticals and personal care products (Brack et al., 2002, 2005; Regueiro et al., 2013) and those that are known yet not routinely monitored, e.g. sterols and estrone (Fetter et al., 2014; Higley et al., 2012; Schmitt et al., 2012), but also contaminants with unknown identities, even though some suspects remain unqualified at the end in some cases (Lei and Aoyama, 2010; Vrabie et al., 2012; Weiss et al., 2009). As discussed before, the bioassays in EDAs to date were mostly in-vitro testing, i.e. cellular toxicity testing, targeting the toxicants acting in certain mode of actions (MoAs) (Brack et al., 2016). On one hand, this specificity in in-vitro assays would guide identifying unknown toxicants in EDA to some extent. For example, the unidentified contaminants/peaks were characterized by the authors as new endocrine disruptors based on their androgen receptor agonistic disrupting potency in bioassays (Lei and Aoyama, 2010; Weiss et al., 2009). On the other hand, this characteristic limited EDA to solely analyze the bioactive fraction(s) containing the toxicants with certain MoAs which were predefined by the researchers but might not be responsible for adverse effects at individual and population levels. Consequently, the choice of assay endpoints may have biased toxicant identification. Compared with in-vitro bioassay, whole-organism toxicity testing reflects entire rather than specific effects of the exposed organisms and makes it possible to screen toxicants associated to multiple types of MoAs, and thus is more relevant to the toxicity at individual and population levels. However, toxicant identification may also be biased in whole-organism toxicity testing due to varying species susceptibility to certain toxicants (Li et al., 2013a). For example, the 50% lethal concentrations (LC50) of cypermethrin to insects ranged three orders of magnitude (Li and You, 2015), suggesting that the choice of testing organism could significantly influences toxicity evaluation and subsequently toxicant identification. Therefore, it is recommended to apply multiple species (whole-organism bioassays) and multiple endpoints (whole-organism and in-vitro bioassays) in TIE and EDA.

Overall, in-vivo toxicity testing is mainly conducted in TIEs, and it would supplement the development of appropriate toxic endpoints in EDAs. Although EDA is a powerful tool in identifying main toxicants in complex mixtures, it focuses on organics and overlooks other potential toxicants like metals and ammonia. Therefore, it is preferable to develop a comprehensive method for determining causality of sediment toxicity by complementing the features of TIE and EDA.

4. Integrating TIE and EDA to diagnose toxicants in sediment

Both TIE and EDA are designed to identify main contributors to observed toxicity from complex mixtures of contaminants with known and unknown identities, but they have distinct assumptions, strategies and methodologies. A detailed comparison of pros and cons of using TIE and EDA in diagnosing toxicants in sediment is presented in Table 1. In summary, TIE has the advantages of applying more environmentally relevant whole-organism testing to identify different classes of toxicants, yet it suffers from lack of effective phase II methods for identifying key toxicants especially in the case of organic contaminants, particularly those are not in the target lists of analytes. Alternatively, EDA has the potential to identify targeted and non-targeted organic toxicants through cycled fractionation and biotesting. However, the use of in-vitro bioassays with exhaustive organic solvent extracts instead of toxicity testing using whole organism and whole sediment may remove the association of EDA results with real environment risk assessment. In terms of cost, TIE is cost-effective in analyzing contaminants in the target lists but the cost of running whole-organism bioassays is high. On the contrary, EDA is relatively high cost in performing fractionation and non-targeted analysis for a great deal of fractionated samples, while it is cost-effective in

running in-vitro bioassays (Table 1).

As such, the two methods complement each other. The TIE considers environmental relevance by performing whole-sediment toxicity testing, which is not able to be fulfilled by the in-vitro bioassays using fractionated sediment extracts as in EDA. Cycled fractionation in EDA significantly reduces sample quantities so that it is challenge to collect sufficient amount of samples to perform whole-organism bioassays. Therefore, it is profitable to complement the merits of TIE and EDA and establish an integrated approach to more comprehensively diagnose the toxicants in complex mixtures.

As shown in Fig. 1, an integrated method of TIE and EDA starts from whole sediment toxicity screening with whole-organism testing as in regular TIE. For sediments showing adverse effects, phase I TIE is performed to evaluate toxicant contributions from individual classes of contaminants, i.e. ammonia, metals and organics, by exposing test organisms to the corresponding manipulated sediments. If ammonia and/or metals are characterized as the key toxicants, phase II TIE is performed to determine their concentrations in sediment and the respective toxicity contributions (usually expressed as toxic units). If organic contaminants are characterized as the main contributing toxicants, the regular phase II TIE is replaced by a toxicant identification method integrating EDA to better reveal the major toxicity contributor(s). When toxic candidates are identified by either phase II TIE or EDA, toxicant confirmation (phase III TIE and EDA confirmation) is performed according to a tiered procedure including analytical confirmation, effect confirmation and hazard confirmation, and finally obtains the confirmed toxicant(s) (Brack et al., 2008; Burgess et al., 2013a).

Compared with individual TIE or EDA, the integrated method complements each other and is more effective and environmentally realistic. Whole-organism toxicity testing in phase I TIE provides useful information on adverse effects at different levels, e.g. toxic endpoints like behavior and enzymatic activity, for selecting appropriate fractionation and bioassay methods in the following EDA procedures. Upon realizing the lack of environmental relevance by using in-vitro bioassays only, EDA researchers have applied in-vivo bioassays in water medium dosed with sediment extracts and their fractions and test species included algae (Bandow et al., 2009b), daphnia (Brack et al., 1999), fish embryo (Fetter et al., 2014) and midges (Qi et al., 2017). Sediment contact test with snails was also performed using sediments spiked with organic solvent extracts (Schmitt et al., 2011a, 2011b). In addition, in-vivo and in-vitro bioassays have been performed simultaneously as a weight of evidence to identify toxicants. For example, bisphenol-A was regarded as the main toxicant responsible for the observed malfunctions on snail reproduction by both sediment contact tests and cell-based estrogenic potency in the ER-LUC assays (Schmitt et al.,

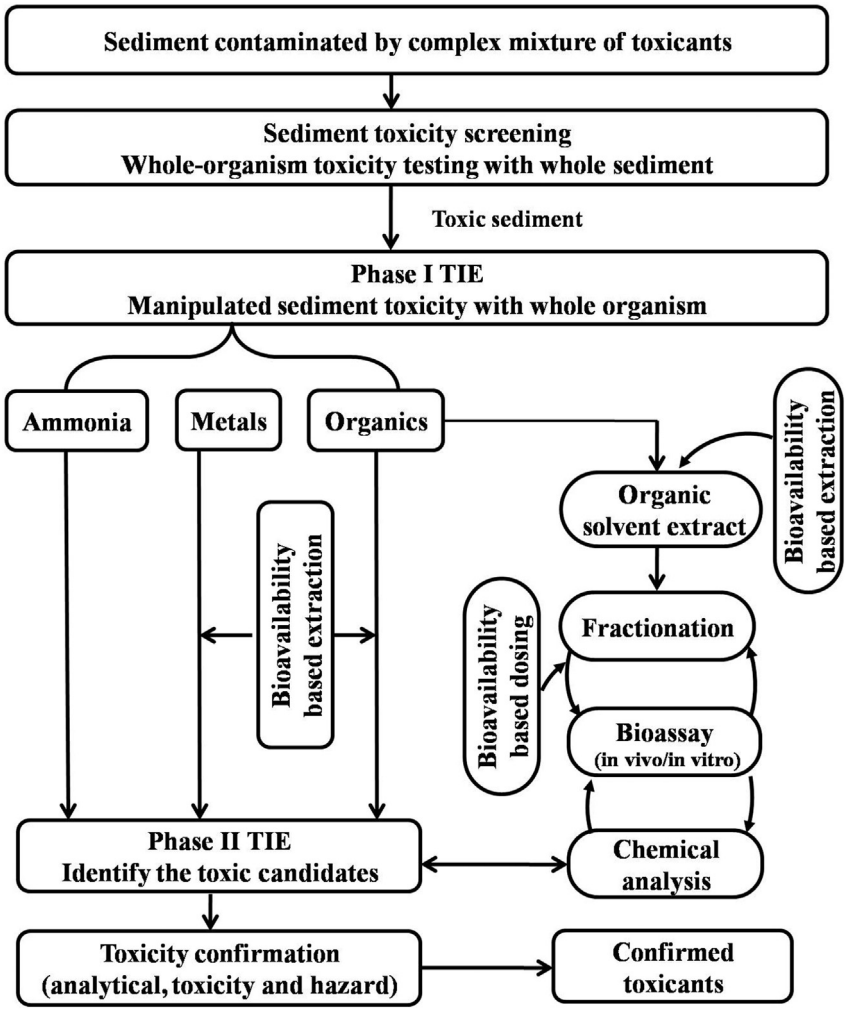

Fig. 1. A framework to illustrate the complementary nature of toxicity identification evaluation (TIE) and effect-directed analysis (EDA) in diagnosing causality of complex mixtures in sediment by incorporating bioavailability-based methods.

2011a). Furthermore, using the same test species in whole sediment screening toxicity testing and the following bioassays in EDA process makes toxicity interpretation clearer. Qi et al. (2017) applied altered enzymatic activity of surviving midges as endpoints in both screening sediment toxicity testing and EDA bioassays, which correlated well and made the canonical correlation analysis applicable in effect confirmation. Vice versa, adverse effects observed in whole-organism bioassays in EDA can be tested in whole organism and whole sediment toxicity testing in TIE. By doing so, toxic candidates identified by EDA can be quantified in sediment samples and their toxicity contributions to sediment toxicity estimated in TIE.

5. Incorporating bioavailability into identifying key toxicants in sediment

Bioavailability significantly influences sediment toxicity and it is necessary to consider bioavailability in diagnosing causality of toxic sediments (Brack et al., 2009; Burgess et al., 2013b). In the history of TIE and EDA, "disappointing" identification results have been reported in some cases, and overlooking the bioavailability of sediment-bound contaminants is one of the critical reasons for the failure of TIE and/or EDA applications (Brack et al., 2016; Burgess et al., 2013a; Ho and Burgess, 2013). As summarized in Table 1, conventional whole-sediment TIE takes bioavailability into consideration by exposing whole organisms to whole sediment. Sediment manipulation in phase I TIE changes the bioavailability of toxicants in sediment to differentiate toxicity contribution of a certain class of contaminants by treating sediment with appropriate amendments, e.g. coconut charcoal, serving as an additive to characterize toxicity of non-polar organics by sequestrating them in the charcoal (Yi et al., 2015). Conversely, conventional EDA often ignores bioavailability of sediment-associated contaminants by using exhaustive solvent extraction, which collects the total amount of organic contaminants from sediment instead of bioavailable fractions and accordingly overestimates their toxicity.

Although bioavailability is included in the effect/toxicity data, conventional sediment TIE ignores bioavailability in the exposure data due to the use of exhaustive extraction before instrumental analysis in phase II TIE (identification), possibly producing bias in assessing toxicity contribution from sediment-bound contaminants, especially highly toxic but poorly bioavailable contaminants (Ho and Burgess, 2013). To mimic more realistic exposure scenarios, bioavailability-based extraction methods are recommended to be applied in phase II TIE (Yi et al., 2015). The speciation of a chemical impacts its bioavailability and toxicity. In phase II of whole-sediment TIE, total ammonia rather than un-ionized ammonia in porewater was more frequently analyzed, possibly causing bias when evaluating ammonia toxicity (Mehler et al., 2010a; Perron et al., 2010; Yi et al., 2015). Since ammonia is readily dissolved in water, to identify sediment-bound toxicants using porewater TIE tends to overestimate ammonia's toxicity due to the direct exposure of organisms to the porewater in which concentrations of water soluble ammonia were elevated (Mehler et al., 2010b). For the other two main classes of possible toxicants (non-polar organics and metals), their bioavailability directly determines sediment toxicity, i.e. the sequestration of these compounds with sediment particles would dramatically reduce their freely dissolved concentrations in sediment porewater, and subsequently reduce their toxic potency (Brack and Burgess, 2011; Perron et al., 2010). Based on the equilibrium partitioning theory, the affinity of organic contaminants for organic carbon (black carbon included) controls their bioavailable concentrations in sediment (Accardi-Dey and Gschwend, 2003; Di Toro et al., 1991), while both organic carbon and acid volatile sulfide govern the partitioning of metals in sediment (USEPA, 2005).

Chemical speciation of metals greatly influences their bioavailability and toxicity, thus the total sediment concentrations poorly indicate metal toxicity (Ahlf et al., 2009; Bonnail et al., 2016). Bureau Commune de Reference (BCR) procedure has been widely adopted to speciate metals into several chemical mineralogical forms which represent various mobility and bioavailability, e.g. acid-soluble, reducible, oxidizable and residual fractions (Rauret et al., 2000; Sutherland, 2010). The acid-soluble fraction of metals was considered the most bioavailable. Studies have found the correlation between the speciation of sediment-bound metals and their bioaccumulation and toxicity in organisms (Bonnail et al., 2016; Rosado et al., 2016), but the implication of metal bioavailability in sediment TIEs is rare (Yi et al., 2015). Yi et al. (2015) incorporated bioavailable concentrations of metals estimated by BCR measurements in phase II of TIE for sediments from urban waterways, which narrowed the list of toxicity contributors from five metals (Cr, Cu, Ni, Pb and Zn) to three (Zn, Ni and Pb).

Comparatively, the use of bioavailable concentrations to predict their sediment toxicity is more common for organic contaminants and many reviews have summarized the current applications of biomimic techniques for estimating bioavailability of organics in sediment (Cui et al., 2013; Lydy et al., 2014; You et al., 2011). Two approaches, namely desorption-based (e.g. Tenax extraction) and equilibrium partitioning-based (e.g. passive sampling) methods have been extensively used to determine bioavailable concentrations of organics in sediment (Li et al., 2013a; You et al., 2008). A few studies have applied bioavailability/bioaccessibility-based extraction in phase II sediment TIEs (identification) and correlated bioavailable concentrations to sediment toxicity. Yi et al. (2015) eliminated one suspect from the list of candidate toxicants using Tenax extractable concentrations of organic contaminants instead of the total concentrations because this compound had much lower bioavailability compared with other toxicants. To take bioavailability into consideration, Phillips et al. (2006) analyzed the concentrations of organics in Ambersorb absorbent (carbonaceous resin) after isolating the absorbent from the manipulated sediment and used resin concentrations instead of total concentrations for evaluating sediment toxicity in a TIE. While not popular so far, bioavailability/bioaccessibility-based extraction has also been used to extract bioavailable fraction of organics for further bioassays and fractionations in sediment EDAs, including desorption-based Tenax extraction (Hong et al., 2016b; Schwab et al., 2009) and equilibrium-based passive sampling (Bergmann et al., 2017; Creusot et al., 2014; Seiler et al., 2006).

As shown in Fig. 1, bioavailability is not only dealt with during sediment extraction in TIEs and EDAs, but also in the step of dosing the extracts and fractions into test medium for EDA bioassays. In conventional EDA, extracts are dosed into the medium using organic solvents as carriers, e.g. dimethylsulfoxide or methanol. Upon the development of partitioning-based passive dosing techniques (Brown et al., 2001; Smith et al., 2010), passive dosing with polydimethylsiloxane (PDMS) has been applied in EDA bioassays (Bandow et al., 2009a; Jahnke et al., 2016). According to the hydrophobicity of potential toxicants and the purpose of the study, the fractionated sediment extracts are generally preloaded onto PDMS polymers using two methods (Table 3). To reduce compound loss in the bioassays, it is preferable to load sediment extracts and their fractions to PDMS directly. The preloaded PDMS then acts as a surrogate for organic carbon in sediment and serves as a partitioning delivery system transferring chemical mixtures into test medium through partitioning (Li et al., 2013b; Qi et al., 2017). The ideal scenario is that passive dosing provides constant exposure concentrations throughout the bioassays because preloaded PDMS polymer acts as an infinite source of chemical mixtures and

Table 3
Assumptions and challenges of using passive sampling and dosing approaches in sediment effect-directed analysis (EDA).

Challenges	Details	Study status and/or solutions
	● Lack of universal polymer	Various passive samplers have been developed for highly hydrophobic organic compounds (i.e. log K_{ow} > 5) (Di Filippo and Eganhouse, 2010; Lohmann, 2012), moderately hydrophobic organic compounds (i.e. 2 < log K_{ow} < 5) (Lao et al., 2016) and polar and ionizable compounds (Morin et al., 2012). Simultaneous use of two or more polymers in the extraction and dosing processes may help to include as more contaminants as possible in sediment EDAs. However, despite all attempts, passive sampling may miss some contaminants that are not compatible with passive sampler materials.
	● PDMS loading efficiency inadequacies (Endo et al., 2013; Pei et al., 2017; Qi et al., 2017)	Optimize the two loading methods: one is soaking PDMS in methanol/water solution containing sediment extracts and the chemicals in the extracts are loaded to PDMS based on equilibrium partitioning (Endo et al., 2013); the other is spiking sediment extracts directly into PDMS precursor (elastomer component and curing agent), mixing thoroughly and curing the PDMS with sediment extracts (Pei et al., 2017).
	● Bias of low hydrophobicity compound	To date, application of passive dosing in the bioassays has been validated for certain types of exposure scenarios and compounds, mainly for moderately hydrophobic compounds (e.g. PAHs) in small-volume in-vitro bioassays (Fischer et al., 2016; Seiler et al., 2014; Smith et al., 2010). Intensive efforts are required to validate the current passive dosing methods to determine if they are suitable for evaluating the toxicity of highly hydrophobic compounds using large-volume in-vivo bioassays, especially in the progress of developing environmentally realistic sediment EDAs accounting for known and unknown compounds with wide ranges of hydrophobicity.
Assumptions (Brack et al., 2016)	● Equilibrium is achieved between individual phases via partitioning ● The three partition coefficients, i.e. K_{oc} (organic carbon-water), $K_{pdms\text{-}w}$ (PDMS-water)and K_{lw} (lipid-water) are the same for the compound considered in the illustration ● No loss of chemicals or significant change in the composition of chemical mixtures in original sediment are occurred during extraction, loading and exposure processes	

compensates for any chemical losses caused by organismal uptake, evaporation, degradation and glassware adsorption (Brown et al., 2001). Overall, passive dosing mimics partitioning behaviors of the chemical mixtures in sediment-water-organism system, and is believed to simulate bioavailability-based exposure to benthic organisms, therefore it is superior to conventional solvent dosing enforcing all chemicals into exposure medium without considering their disparate partition behaviors in sediment.

Brack et al. (2016) demonstrated the impact of dosing techniques on the outcome of sediment EDAs by describing the partitioning and dosing scenarios of chemical mixtures in a system with sediment, porewater, PDMS, exposure medium and biota. In theory, the release of chemical mixtures from preloaded PDMS mimics organismal uptake, so the equilibrium partitioning-based passive dosing methods can reduce the bias of changing chemical composition when using conventional solvent dosing. Conventional solvent dosing may cause loss of lipophilic chemicals in water-only bioassays due to their significant absorption to test vessels. On the contrary, the loss can be compensated by continuous release of chemicals from preloaded PDMS when passive dosing is applied. Rather than using sediment extracts by exhaustive extraction to initiate the dosing procedure, bioavailability/bioaccessibility-based extraction is recommended to be combined with the dosing procedure in EDA (Fig. 2). This scenario illustrates a bioavailability-based EDA, which in theory more realistically reflects the exposure of test organisms to sediment-associated organic contaminants (Brack et al., 2016). However, it is difficult to control experimental deviations in EDA practices with complex mixtures of known and unknown chemicals, which may impact the accuracy of identifying toxicants. As shown in Table 3, three practical challenges are to be addressed for using passive sampling and dosing methods in sediment EDAs, i.e. looking for appropriate polymer(s), increasing loading efficiencies of low and high hydrophobic contaminants and developing effective passive dosing approaches for large-volume in-vivo bioassays.

Incorporation of bioavailability in sediment TIEs and EDAs helps to avoid or reduce bias in sediment risk assessment, helping the remediation to focus on actively toxic contaminants and eliminate those with limited exposure. Sediment TIEs are designed to include bioavailability by working with whole organism and sediment manipulation in phase I, however, phase II TIEs rarely consider bioavailability (Brack and Burgess, 2011; Yi et al., 2015). Conventional EDAs do not consider bioavailability issues, but more recent studies have focused on developing EDA methods that include bioavailability-based extraction and dosing techniques (Brack et al., 2016; Brack and Burgess, 2011; Burgess et al., 2013a; You and Li, 2017).

In the integrated method for TIE and EDA, bioavailability is highly recommended to be incorporated throughout the entire process to establish ecologically relevant and toxicity-specific methods for diagnosing the causality of sediment toxicity (Fig. 1). To achieve this, two challenges need to be fulfilled. One is to extract sufficient sample mass for fractionation and ecologically relevant in-vivo bioassays, and the other is to establish bioavailable thresholds (e.g. 50% lethal and effective concentrations) for as many toxicants as possible to perform more accurate toxicity evaluation (Burgess et al., 2013b; Maruya et al., 2012; USEPA, 2005).

6. Role of sediment toxicity identification in mixture impact assessment

Diagnosing the causality of toxicity is an indispensable step in ecological risk assessment framework for complex mixtures of contaminants (van den Brink et al., 2013). A framework namely “solution-oriented monitoring” has been proposed recently to deal with joint toxicity issues in water quality monitoring and management by integrating the analysis of priority mixtures (exposure) and mixture effects (effect) to capture the main drivers of mixture toxicity (Altenburger et al., 2015). To mechanistically elucidate the role of cause diagnosis in mixture impact assessment, adverse outcome pathway (AOP) has been applied (Fig. 3). An AOP is a conceptual framework proposed to extrapolate a direct molecular initiating event to “an adverse outcome at a biological level of organization relevant to risk assessment” (Ankley et al., 2010). In contrast to the original AOP concept, researchers are making effort to deal with mixture impacts by measuring common adverse outcomes to capture potential impacts of complex mixtures (Altenburger et al., 2015; Ankley et al., 2010). In brief, bioanalytical

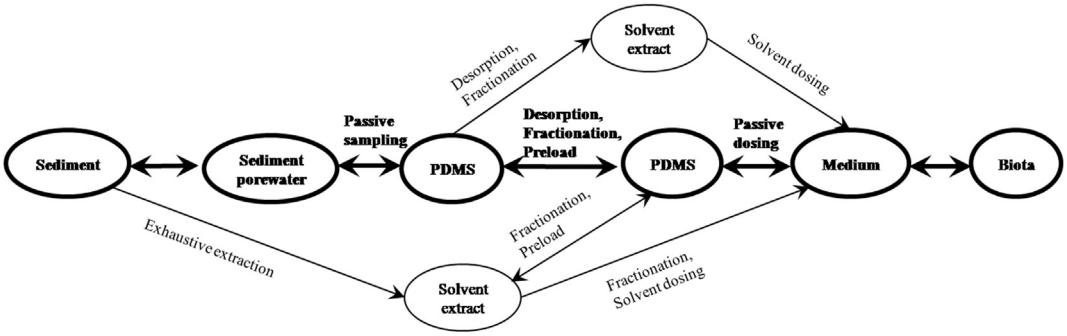

Fig. 2. Incorporation of bioavailability-based extraction and dosing techniques in sediment effect-directed analysis. Polydimethylsiloxane (PDMS) is used as a representative polymer for passive sampling and passive dosing techniques. Four options are included: (1) bioavailable extraction-bioavailable dosing; (2) bioavailable extraction-solvent dosing; (3) exhaustive extraction-bioavailable dosing; (4) exhaustive extraction-solvent dosing.

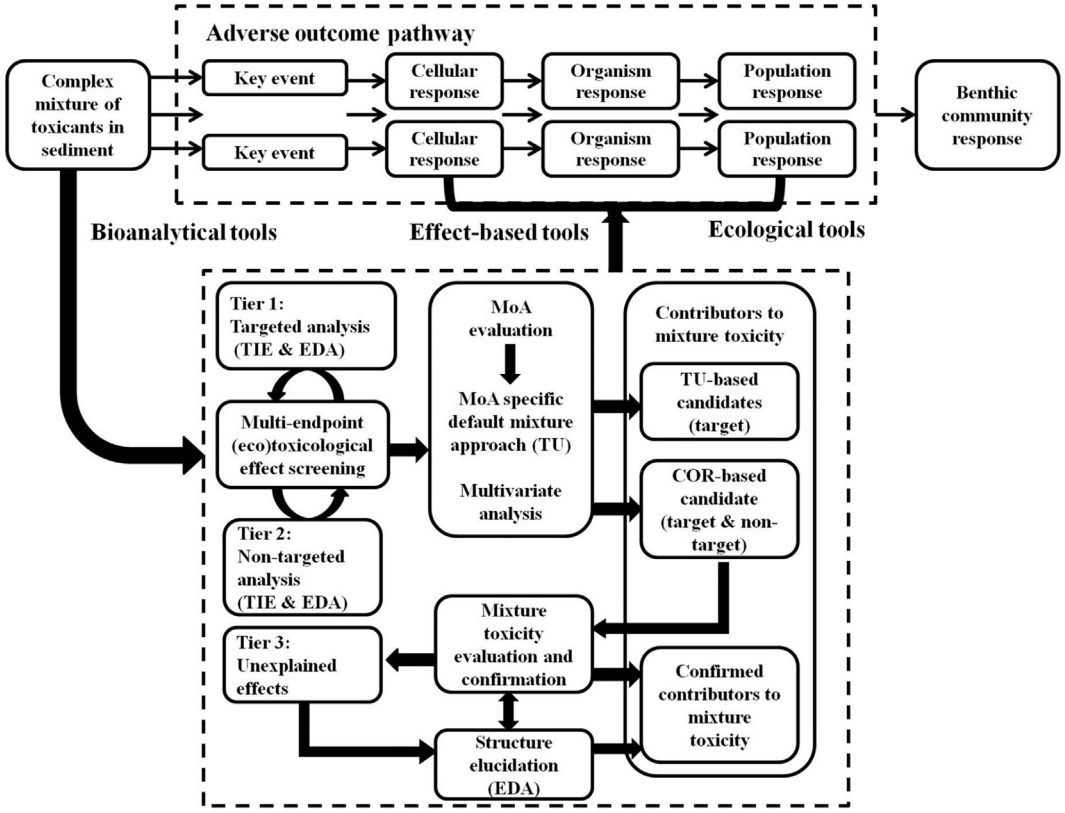

Fig. 3. Conceptual framework showing potential role of effect-based tools (toxicity identification evaluation (TIE) and effect-directed analysis (EDA)) in impact assessment of complex mixture in sediment (Altenburger et al., 2015; Brack et al., 2016). MoA: mode of action; TU: toxic unit; COR: correlation.

tools are applied to capture key events of biological reactions and molecular initiating events in organisms, e.g. Escher et al. (2014) applied 103 unique in-vitro bioassays to capture biological responses to complex mixtures in wastewater, recycled water and drinking water. Effect-based methods, including toxic endpoints at the cell and organism levels were performed to diagnose the joint effects of environmental samples, e.g. Qi et al. (2017) applied effect-based bioassays at the organism level to identify major toxicity contributors to the benthic invertebrate, *C. dilutus* in sediments from a large urban river in South China. Finally, ecological tools are applied to link toxicological effects and chemical mixtures to field surveys of ecological functions (Altenburger et al., 2015), e.g. Archaimbault et al. (2010) applied ecological traits-based approaches to examine the mixture effects of sediment-bound toxicants to benthic microinvertebrate communities. Using this scheme (Fig. 3), identification of key drivers of mixture toxicity is the critical basis which directs the further use of trait-based and eco(toxico)logical approaches in ecological risk assessment, and in reverse, guides the development of bioanalytical tools in recognizing the MoA of the main toxicants. Traits-based bioassessment uses traits (phenotypic or ecological characters of an organism which are measured at the individual level but usually applied as the average state of a species) to explain or predict the physical characteristics, ecological niche, and functional role of a species within the ecosystem (van den Brink et al., 2011; van den Brink et al., 2013).

Effect-based approaches, e.g. TIE and EDA, are developed and applied to determine causality of complex mixtures by combining

the exposures (concentrations of concerned chemical mixtures) and their effects (joint toxicity of the chemical mixtures) (Altenburger et al., 2015; Ankley et al., 2010; Brack et al., 2016; Burgess et al., 2013a; Qi et al., 2017). A tiered effect-based tool including the integrated approach of TIE and EDA is proposed to interpret the responses on cellular, organismal and population levels in the mixture impact assessment (Fig. 3). According to the identities of identified toxicants, targeted, non-targeted and unknown analyses are named as tiered 1, 2 and 3, respectively in the tiered method. Tier 1 deals with targeted analysis which is directed by the MoA of known chemicals using a specific default method based on summation of toxic units (Backhaus and Faust, 2012). Tier 2 screens the unexplained biological effects by using multi-targeted and non-targeted analyses based on multivariate analysis. Tier 3 addresses the remaining unexplained toxicological effects by elucidating chemical structure of unknown chemicals using mass fragmentation and chromatographic retention information (Brack et al., 2016).

Sediment toxicity identification using effect-based approaches provides information on the active toxicants and their associated toxicity on cellular and organismal levels in mixture impact assessment. Incorporation of traits-based approaches into targeted and non-targeted analyses (tiers 1 and 2 in Fig. 3) provides a way to expand effects on cellular and organism levels to population level (Altenburger et al., 2015; van den Brink et al., 2013). Further ecological-based evaluation extrapolates the mixture impact assessment to the benthic community and ecosystem level, which helps to assess the effects of multiple toxicants in ecosystems (van den Brink et al., 2016). In summary, the tiered effect-based toxicity identification approaches (i.e. an integrated method of TIE and EDA) help to identify the toxicants posing the highest risk to benthic community and even aquatic ecosystems, which may benefit regulators to take measures for evaluation, remediation, management and protection of the environment through mixture impact assessment.

7. Conclusions and perspectives

As the need to assess mixture impacts of multiple chemical stressors in aquatic ecosystems is apparent, diagnostic tools have been developed to identify key toxicity drivers and further support prioritizing chemicals concerning ecological risk assessment and management strategies. An integrated method complementing the environmentally relevant TIE and toxicant specific EDA is recommended to diagnose complex mixture toxicants in sediment. Regarding the current understandings and limitations, bioavailability-based extraction and dosing techniques are preferably to be incorporated in diagnostic tools to more accurately identify major toxicants. Extension of sample matrices from whole sediment and organic solvent extracts to organism tissues and biological fluids is another effective approach to consider bioavailability of sediment-associated contaminants in toxicity identification. Moreover, extension of chemical analysis from targeted to non-targeted analysis is a significant progress in sediment toxicity identification which helps to more comprehensively prioritize toxicants with known and unknown identities. To improve the efficiency of non-targeted analysis, three aspects should always be considered, including sufficient contaminants for extraction and fractionation procedures, appropriate in-vivo and in-vitro bioassays, and the use of improved analytical instruments and qualitative-quantitative methods. Future research is needed to standardize the procedures and criteria, which would help to obtain consistent and comparable results. Finally, effect-based approaches could be potentially combined with traits-based approaches to expand biological effects from cellular and organismal levels to population level, and provided the basis to develop new approaches to prioritize and regulate multiple toxicants in aquatic ecosystems.

Conflicts of interest

The authors declare no conflicts of interest.

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