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Assessment of a novel device for onsite integrative large-volume solid phase extraction of water samples to enable a comprehensive chemical and effect-based analysis

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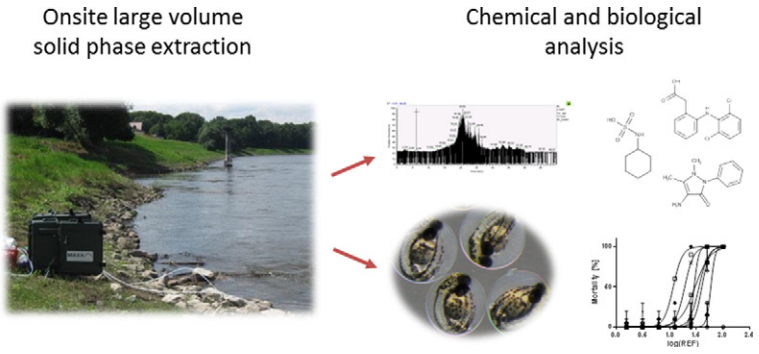
HIGHLIGHTS

- A novel solid phase extraction device for chemical and effect-based analysis was developed
- Good recoveries for organic contaminants in a large log D range were obtained for 159 out of 251 compounds
- Samples were successfully evaluated using a set of seven different bioassays for ten endpoints
- The device is applicable of sampling of up to 50 L of water

GRAPHICAL ABSTRACT



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ABSTRACT

The implementation of targeted and nontargeted chemical screening analysis in combination with in vitro and organism-level bioassays is a prerequisite for a more holistic monitoring of water quality in the future. For chemical analysis, little or no sample enrichment is often sufficient, while bioanalysis often requires larger sample volumes at a certain enrichment factor for conducting comprehensive bioassays on different endpoints or further

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effect-directed analysis (EDA). To avoid logistic and technical issues related to the storage and transport of large volumes of water, sampling would benefit greatly from onsite extraction. This study presents a novel onsite large volume solid phase extraction (LVSPE) device tailored to fulfill the requirements for the successful effect-based and chemical screening of water resources and complies with available international standards for automated sampling devices. Laboratory recovery experiments using 251 organic compounds in the log D range from −3.6 to 9.4 (at pH 7.0) spiked into pristine water resulted in acceptable recoveries and from 60 to 123% for 159 out of 251 substances. Within a European-wide demonstration program, the LVSPE was able to enrich compounds in concentration ranges over three orders of magnitude (1 ng L^{-1} to 2400 ng L^{-1}). It was possible to discriminate responsive samples from samples with no or only low effects in a set of six different bioassays (i.e. acetylcholinesterase and algal growth inhibition, androgenicity, estrogenicity, fish embryo toxicity, glucocorticoid activity). The LVSPE thus proved applicable for onsite extraction of sufficient amounts of water to investigate water quality thoroughly by means of chemical analysis and effect-based tools without the common limitations due to small sample volumes.

1. Introduction

In Europe, the protection of natural water resources is regulated by the Water Framework Directive (WFD; European Union, 2000) and the Groundwater Daughter Directive to WFD (GWD; European Union, 2006) that are implemented in European member states' legislations and international river basin management. The monitoring and regulation of the chemical status of surface and ground waters refer to the priority substances listed in WFD and amended by the GWD and the Environmental Quality Standards (EQS) Directive (European Union, 2008, 2013). However, it has been demonstrated that monitoring of priority pollutants is not sufficient, because mixtures of many more known and unknown chemicals contribute to adverse environmental effects (Malaj et al., 2014; Moschet et al., 2014; Neale et al., 2015; von der Ohe et al., 2009).

The combination of targeted and nontargeted chemical screening analysis with in vitro and organism-level bioassays has been recommended for the identification of (eco-)toxicologically active compounds and mixtures by a number of more recent studies to supplement the existing concepts towards a holistic effect-based and chemical analyses approach (Altenburger et al., 2012; Brack et al., 2015; Creusot et al., 2013; Di Paolo et al., 2016; Krauss et al., 2010; Silva et al., 2002; Wernersson et al., 2015). Generally, the amount of sample enrichment required for chemical analyses and bioassay depends on the sensitivity of individual methods as well as the physicochemical properties, bioavailability, exposure concentrations, toxic potentials and mixture toxicity effects of the compounds contained in the sample. Modern chemical analytical instrumentation allows for the analysis of small water volumes with no or only low sample enrichment for most of the typical water pollutants (Bahlmann et al., 2015; Berset et al., 2010; Brack et al., 2015, 2016; Dyer et al., 2004; Fernández-Ramos et al., 2014; Seitz et al., 2006), while the analysis of some priority substances with very low EQS values as well as in vivo and in vitro tests may require greater enrichment and larger water volumes (Neale et al., 2015; OECD, 2004; OECD, 2012).

The implementation of integrated chemical and effect-based monitoring strategies (Brack et al., 2017) would greatly benefit from automated onsite sampling techniques for efficient and successful real-time collection and extraction of large water volumes. Such techniques can prevent logistic, technical, economic and scientific issues related to the storage and transport of large volumes of water to the laboratory. Furthermore, this approach allows time-integrated sampling of a water body over days or weeks to yield representative samples (Roll and Halden, 2016).

The most powerful sampling and enrichment approach for complex mixtures of known and unknown contaminants is solid phase extraction (SPE). Several well-tested and widely used solid phases that trap organic compounds with a broad range of properties (nonpolar to polar, neutral to charged) based on C_{18} or polystyrene-divinylbenzene (co-)polymers are commercially available (Fontanals et al., 2007; Fontanals et al., 2011; Hennion, 1999). A combination of complementary sorbents to cover a broad range of compounds with different properties has been successfully applied to surface water samples (Kern et al., 2009). It is an advantage of SPE to capture and stabilize the compounds on the sorbents when sampled (Hillebrand et al., 2013). Different approaches and devices for the sampling of large volumes of water have been developed since the 1970s (CIAgent, 2012; Coes et al., 2014; Dawson et al., 1976; de Lappe et al., 1983; Dean et al., 2009; Ehrhardt and Bums, 1990; Ellis et al., 2008; Gomez-Belinchon et al., 1988; Green et al., 1986; Hanke et al., 2012; Lakshmanan et al., 2010; McKenzie-Smith et al., 1994; Petrick et al., 1996; Reineke et al., 2002; Roll et al., 2016; Sarkar and Sen, 1989; SEASTAR INSTRUMENTS, 1984; Sturm et al., 1998; Suarez et al., 2006; Supowit et al., 2016; Thomas et al., 2004; Thomas et al., 2001; Weigel et al., 2001; Yunker et al., 1989). Briefly, many of the devices were best suited for low water volumes (for analytical purposes), are not (anymore) commercially available or do not operate in a fully automated mode (see Supporting material for detailed information).

Since none of the existing devices and approaches satisfies all of the above-mentioned requirements, a novel device for the onsite large-volume SPE (LVSPE) was developed. It fulfills the following technical characteristics:

- Automated device for the unattended and representative sampling according to international standards (e.g., ISO 5667-1, 2006);
- Combination of SPE with a pre-filtration cartridge to separate suspended particulate matter (SPM) from the water phase;
- Tailor-made columns that allow customizable selection and combination of sorbents to focus on chemical properties and quantities as determined by the goals of the research question;
- Implementation of a pressurized system to force the water through the extraction columns;
- Usage of 12 V electronic components (controller, pumps, valves) and low energy consumption, in such a way that the device can run with a car battery or a battery-buffered fuel cell, solar panel or wind turbine.

The successful implementation and application of sampling approaches in the chemical and biological assessment of complex environmental mixtures requires the assurance of the representativeness and integrity of the samples with minimized alteration and bias (Brack et al., 2016; Schulze et al., 2011). The aim was to assess whether the LVSPE device:

1. Is able to capture a wide-ranging set of known organic water contaminants (among them pesticides, biocides, pharmaceuticals, and artificial sweeteners) with good recoveries and high repeatability?
2. Can enrich a sufficient volume of water to perform a set of different bioassays even for minimally contaminated waters?

3. Does provide blank samples containing no or very low contamination and deriving no or minimal toxicological effects to be able to unequivocal distinguish the chemical and effect signals from background levels?

2. Material and methods

2.1. Technical description of the LVSPE device

The design of the LVSPE device allows for the collection of up to 50 L water (Fig. 1, LVSPE50). The main parts of the devices are the pre-filter, the sampling and dosing chamber, the ball valve, the pressure chamber and the controller. The devices are built into a Storm Case iM 2750 (L × W × D: 62.5 × 50 × 36.6 cm) purchased from Peli Products (Barcelona, Spain). An apparatus following the same principle but designed for the extraction of up to 1000 L is presented in SM.

Briefly, water is sucked by vacuum into the borosilicate glass dosing system (1). The water enters the Sartopure GF+ MidiCap pre-filter (Sartorius) (2) in the inflow pipe to remove suspended particulate matter. A conductivity sensor controls the maximal water level in the glass tube (volume: 600 mL) and a dip tube allows exact dosing of the sample volume (500 mL). The ball valve (3) keeps the water in the dosing system and releases it into the pressure chamber (4) when opened. After release, the ball valve closes and the water is pumped with a positive pressure of approximately 100 kPa through one cartridge (5) or a sequence of cartridges with different sorbents (Fig. 1a). The cartridges are filled from the bottom to avoid preferential flow paths through the solid phase bed.

The controller allows a customized programming of the sampling frequency and the total number of sub-samples of 500 mL each until the desired total volume is reached (e.g., 50 L). The extraction cartridge of the LVSPE50 device is built of polyvinylidene fluoride (PVDF) (Fig. 1b). Cartridges made of stainless steel can also be used, but fine threads in such parts are prone to malfunctions due to the brittleness of this material. The cartridges are available in different sizes (4 to 10 g of sorbent). The solid phases are packed between the glass filter plates, and the cartridges are closed with two screw caps with O-ring type silicone tights.

2.2. Preparation, conditioning and extraction of sampler cartridges and processing of samples

The quantity of sorbents used was up-scaled from an amount of 0.2 g of sorbent, which is commonly used to extract 1–2 L of water in case of Chromabond® HR-X (Macherey Nagel). Since the cartridges with ion exchange sorbents Chromabond® HR-XAW and Chromabond® HR-XCW were grouped in flow direction behind the column with HR-X, the half quantity those were considered. The cartridges were assembled, filled with the solid phase sorbents and conditioned separately according to Table 1. To account for a swelling of the sorbents, the amounts were slightly reduced to fit into the columns.

After conditioning and sampling, the openings were covered with aluminum foil to avoid contamination and drying of the wet sorbent. The columns were stored and transported at 4 °C before and after sampling. Later, the cartridges were connected separately to a nitrogen gas stream for 1 h to purge residual water and subsequently subjected to freeze drying for around 8 h. The extraction was carried out according to Table 1. The extracts of the different cartridges were kept separate for further analysis with HR-XAW and HR-XCW extracts being neutralized by adding formic acid or 7 N ammonia in methanol (MeOH) before storage. All extracts were reduced in volume using rotary evaporation and adjusted to a final enrichment factor of 1:250 (HR-X) and 1:500 (HR-XAW, HR-XCW) using a mixture of MeOH:ethyl acetate (EtAc;1:1,v:v) before preparation of aliquots for chemical and biological analyses.

2.3. Laboratory and field performance of the LVSPE50 device

Recoveries were tested under laboratory conditions. A 60 L grab water sample of a pristine creek (Wormsgraben, Harz Mountains, Germany; N 51.770167, E 10.696444) was collected on 14 January 2014 and stored in a clean stainless steel drum at 4 °C. The sample was divided into 6 × 10 L sub-samples in 10-L borosilicate glass beakers. Three out of six samples were spiked using a mixture of 251 organic compounds (500 ng each; Table S1) in the log D range of −3.6 to 9.7 (pH 7). The substances in the spike mix cover different compounds classes such as pharmaceuticals, pesticides, industrial chemicals and other chemicals of emerging concern which are typically analyzed in surface waters and wastewater treatment plant effluents (e.g., Hug et al., 2014; Loos et al., 2013a, 2013b; Richardson and Ternes, 2014; Ruff et al., 2015). The recoveries were calculated as the ratio between the amount of substance found in the extracts and the amount of substance spiked to the water samples. Beakers were coated and wrapped with aluminum foil to protect from light and contamination. The remaining three samples were used as unspiked ambient field controls in order to check for background concentrations of the targeted analytes. The samples were extracted using the LVSPE50 with the HR-X, HR-XAW and HR-XCW sorbents in sequence (Table 1). The beakers were rinsed with 1 L of original Wormsgraben water, which was extracted using the same cartridges to remove residual compounds from the glass walls.

Subsequently, the LVSPE50 device was applied on 35–50 L surface water samples collected at 18 sampling sites in six European countries (Croatia, Czech Republic, Germany, Hungary, Slovakia, Switzerland;

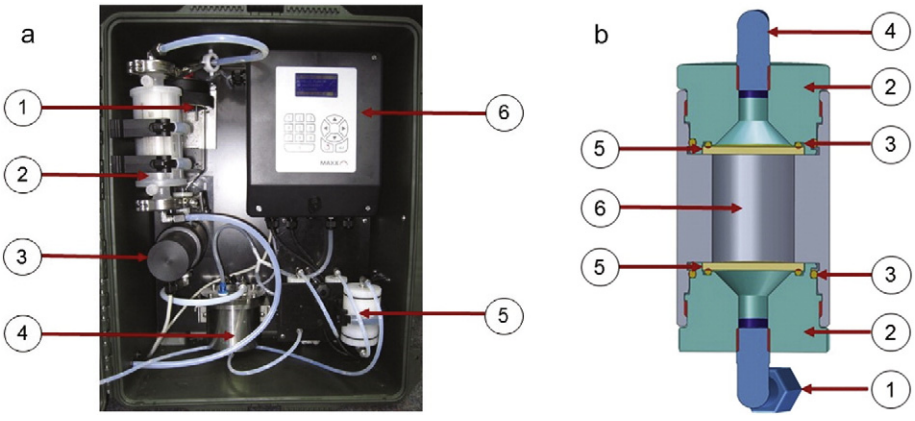

Fig. 1. (a) Picture of the LVSPE50 device; (1): Dosing system (500 mL), (2): pre-filter (3): ball valve, (4): pressure chamber (550 mL), (5): extraction cartridge, (6): controller (Photo by MAXX GmbH); (b) Scheme of the LVSPE50 cartridge, body and screw caps, made of polyvinylidene fluoride; (1): inlet fitting, (2) lower and upper screw caps with mortises to take in the (3) silicone tights (O-rings), (4) outlet fitting, (5) glass filter disc, (6) body containing the sorbent.

Table 1
Settings for sampler preparation, conditioning and extraction; MeOH: methanol, EtAc: ethyl acetate, LC-MS: liquid chromatography–mass spectrometry; HR-X: hydrophobic polystyrene-divinylbenzene copolymer; HR-XAW: weakly basic secondary and tertiary ammonium polymeric anion exchanger based on HR-X; HR-XCW: weak carboxylic acid modified polymeric cation exchanger for SPE; during sampling the sorbents are assembled in the order HR-X, HR-XAW and HR-XCW.

	LVSPE50
Solid phases	*HR-X* (10 g)
	HR-XAW (4 g)
	HR-XCW (4 g)
Conditioning	*HR-X*
	−200 mL EtAc
	−200 mL MeOH
	−100 mL water (LC-MS grade)
	HR-XAW
	−200 mL MeOH
	−100 mL water (LC-MS grade)
	HR-XCW
	−200 mL MeOH
	−100 mL water (LC-MS grade)
Extraction	*HR-X*
	−100 mL EtAc
	−100 mL MeOH
	HR-XAW
	−100 mL MeOH with 2% 7 N ammonia in MeOH
	HR-XCW
	−100 mL MeOH with 1% formic acid

Table S2) as part of the European Demonstration Program (EDP) of the EDA-EMERGE project (Brack et al., 2013).

2.4. Ambient unspiked field control and laboratory blank

Unspiked ambient field controls and a laboratory blank were processed in parallel to the laboratory recovery test and the field sampling campaign in the Saale river basin, respectively. The control samples were subjected to the whole preparation and elution procedure without any enrichment step. For the ambient unspiked control related to the recovery test, three sub-samples of each 10 L of a 60 L pristine water from Wormsgraben were assessed by chemical analysis to account for possible interference with spiked compounds. For the blank sample related to the field sampling during the EDP, the concept of a circulation blanks was used to evaluate leaching of compounds from the sampling device, filters, tubing and sorbents and to reduce the efforts of regular processing of control samples. Typically, the extraction of larger volumes of water to obtain blank samples is very expensive with regards to obtain large amounts of clean laboratory water or may be affected by background contamination and artifacts originating from the water sample itself, even of high quality such as LC-MS grade. To check for background contamination or leaching form the machine, one circulation blank (sample EDP4091) was processed using 5 L of liquid LC-MS grade water (Chromasolv, Sigma-Aldrich) mineralized with analytical grade sodium chloride (0.2 g L^{-1}, Merck) to avoid problems with the conductivity-based dosing system. The water was stored in a 5 L brown glass bottle as a reservoir. From this reservoir, LVSPE50 extracted a 500 mL sub-sample per cycle and discharged the extracted water back into the reservoir. Overall, 100 cycles resulted in a blank sample representing 50 L of water.

2.5. Chemical and biological analysis

Briefly, liquid chromatography high-resolution mass spectrometry (LC-HRMS) analysis was carried out using an Agilent 1200 LC coupled to a Thermo LTQ Orbitrap XL mass spectrometer with electrospray ionization (ESI) according to Hug et al. (2015). A Kinetex™ core-shell C18 column (100 mm × 3.0 mm; 2.6 μm; Phenomex) with a linear gradient with water and methanol (both containing 0.1% formic acid to account for anionic species) at a flow rate of 0.2 mL min^{-1} for chromatographic separation was used. To account for compound losses, we used matrix-matched calibration and processed calibration standards using a down-scaled SPE method corresponding to that for the LVSPE samples. To demonstrate the applicability of the LVSPE approach for the effect-based analysis of surface water samples, aliquots of the LVSPE samples were subjected to a set of in vitro and organism-level bioassays (Table 2). The results of the bioassays were reported as relative enrichment factors (REF) which express the enrichment of the mixture of organic pollutants in a sample to achieve a specific effects in a bioassay (Escher and Leusch, 2011; Escher et al., 2006; Escher et al., 2014). The methods for chemical and biological analysis are detailed in the Supplementary data (Section S2.4).

2.6. Data analysis

Log D values at pH 7.0 and other physicochemical descriptors were calculated using the PhysChem Profiler of ACD/Percepta (ACD, 2015). Open Babel v2.3.2 (O'Boyle et al., 2011) was used to generate InChIKey for the compound identification. Statistical analysis was performed with R 3.3.0 (R Core Team, 2016). The Venn diagram was drawn with the R package VennDiagram (Chen, 2016). The elbow method retrieved the optimal number of clusters used in *k*-means clustering (Ketchen and Shook, 1996). Descriptive curve functions were calculated using the R package e1071 (Meyer et al., 2015). Processing of the bioassay data and calculation of concentration-response curves was performed with GraphPad Prism v6.07 (2015). The estrogenic assay data was assessed using the REGTOX Excel™ Macro (http://www.normalesup.org/~vindimian/fr_index.html) as previously described (Kinani et al., 2010).

3. Results and discussion

3.1. Chemical and biological analysis of the circulation blank

The extraction procedure was tested for any undesired chemical contamination as well as toxicological effects to exclude false positives during monitoring. This step included the recovery and the circulation blanks. None of the targeted compounds ($N = 251$) were detected in either blank. For the HR-X extract of the circulation blank, the lowest observed effect concentrations (LOEC) elicited a REF of 100 for the ER- and AR-mediated activity (expressed as cytotoxicity at this LOEC) and a LOEC at REF 250 and REF 500, respectively, for the AChE inhibition and the (sub-)lethal endpoints in FET. For the algal growth inhibition assay, the no observed effect concentration (NOEC) was at REF 100 for all three sorbents used. The (sub-)lethal effects in the FET showed a LOEC and NOEC of REF 500, respectively, for the HR-XAW and HR-XCW. These minor effects of the circulation blank appeared only at high REFs and hence they are unlikely to interfere with the evaluation of effects of environmental water samples. However, a thorough cleaning and conditioning (Table 1) of the sorbents used is highly recommended to remove production residues and contamination due to absorption of background air contaminants.

The concept of the circulation blank was based on the assumption that contamination originates from the device, filters, sorbent or tubing and not from the "pure" high-grade water used for the processing of the blank. This approach allowed testing the potential mobilization of problematic contamination from filters, sorbents and tubing under realistic conditions. As a compromise, the circulation blank allowed simulating the extraction of for instance, 50 L of water by pumping 5 L of LC-MS grade water ten times through the instrument. Nevertheless, the circulation blank of 5 L is a simulation rather than an actual extraction of a 50 L "pure" water sample. If contamination results from the enriched water, it may mask the contaminants leached from the device and consumables.

Table 2
Bioassays used for assessment of LVSPE samples; AChE: inhibition of acetylcholinesterase, AR: androgen receptor-mediated activity, ER: estrogen receptor-mediated activity, GR: glucocorticoid receptor mediated activity.

Bioassay	Type	Target compound groups	Endpoint	Reference
AChE inhibition	Enzymatic reaction	Insecticides, miscellaneous	Inhibition of AChE enzyme activity	(Ellman et al., 1961; Froment et al., 2016; Galgani and Bocquene, 1991)
Algal growth inhibition with *Raphidocelis subcapitata*	Organism-level	Herbicides, disinfectants, miscellaneous	Inhibition of algal growth	(OECD, 2011; Rojíčková et al., 1998)
Ames fluctuation assay with TA98	In vitro	Natural and synthetic mutagenic compounds	Induction of reverse mutations	(Ames et al., 1975; Reifferscheid et al., 2011; Reifferscheid et al., 2012)
AR-mediated activity - MDA-kb2 cells	In vitro	Natural and synthetic (anti)androgens	(Anti-) androgenic response	(Creusot et al., 2015; Wilson et al., 2002)
ER-mediated activity - MELN cells	In vitro	Natural and synthetic (anti)estrogens	(Anti-) estrogenic response	(Balaguer et al., 1999; Creusot et al., 2015; Kinani et al., 2010)
GR-CALUX®	In vitro	Natural and synthetic (anti)glucocorticoids	(Anti-) glucocorticoid receptor mediated response	(Sonneveld et al., 2005)
Zebrafish embryo acute toxicity	Organism-level	Biocides, pharmaceuticals, miscellaneous	Survival, sublethal responses (e.g., heartbeat)	(ISO 15088, 2007; OECD, 2013)

3.2. Chemical assessment of spiked water samples

In the recovery test, three replicates of each 10 L of a pristine natural water sample spiked with 251 compounds were subjected to extraction with LVSPE50 and analysis with LC-HRMS, to assess the extraction efficiency and accuracy of LVSPE.

The Venn diagram in Fig. 2 shows the distribution of the compounds between the three different solid phases. The majority of compounds were recovered from the HR-X (98%; 246 out of 251), the first material in flow direction. For most chemicals in the intersection of the three solid phases, the main part of spiked substances was found in the HR-X ($N = 48$ out of 69) with <10% of recovery in HR-XAW and HR-XCW, respectively, the second and third material in flow direction. Only few substances recovered mainly in the HR-XAW (e.g., benzenesulfonic acid, chloridazon-desphenyl, perfluorobutanoic acid, salicylic acid) or in the HR-XCW (e.g., gabapentin, metformin). The average recoveries of the spiked compounds were 88 ± 43% (average and standard deviation; median: 96%; $N = 246$ out of 251) for the HR-X, 9 ± 21% ($N = 59$ out of 251) for the HR-XAW and 4 ± 6% ($N = 49$ out of 251) for the HR-XCW (Fig. 2, Table S4). The entire repeatability of the recoveries was 11%, 3% and 2%, respectively, for the HR-X, HR-XAW, and HR-XCW sorbents (with $N = 3$ replicates of spiked water samples). Two compounds, ethion and triclocarban were not found in any of the three fractions. The reason was maybe a strong irreversible adsorption to surfaces or the sorbents for which the solvation power of the solvents used was not sufficient.

Fig. 3 depicts the distribution of recoveries for the HR-X sorbent. The recoveries exceeded 50% for 204 out of 251 spiked chemicals. The density function retrieved a slightly super Gaussian (kurtosity = 0.3) and left-skewed (skewness = −0.3) distribution (see insert in Fig. 3). The calculation of the distribution and density functions for the HR-XAW and HR-XCW sorbents was impossible due to many observations with tiny recoveries and thus low variances of the values.

To evaluate the relationship between the recoveries and the physicochemical properties of the compounds, regression analysis and *k*-means clustering (with $k = 3$ centers) was performed (Fig. 4, Fig. S5, Table S4). Regression analysis did not resolve any systematic dependency between the recoveries and the log D and other descriptors (e.g., pKa, Kd, log P; data not shown). Since other analytical factors such as chromatography, ionization or irreversible adsorption to the sorbents or surfaces may affect the recoveries, this result might be different in another experimental setting.

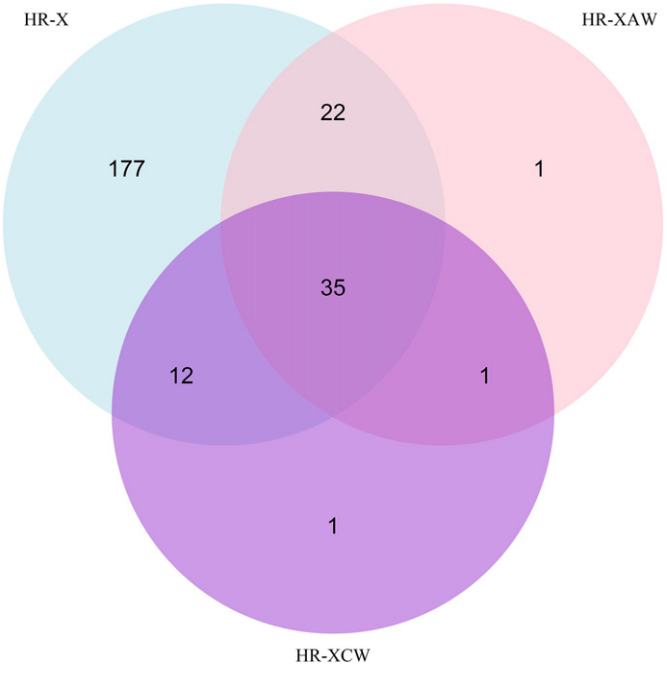

Fig. 2. Venn diagram of spiked compounds recovered in the three different sorbents in flow direction: HR-X: neutral solid phase material, HR-XAW: anionic exchanger solid phase material, HR-XCW: cationic exchanger solid phase material.

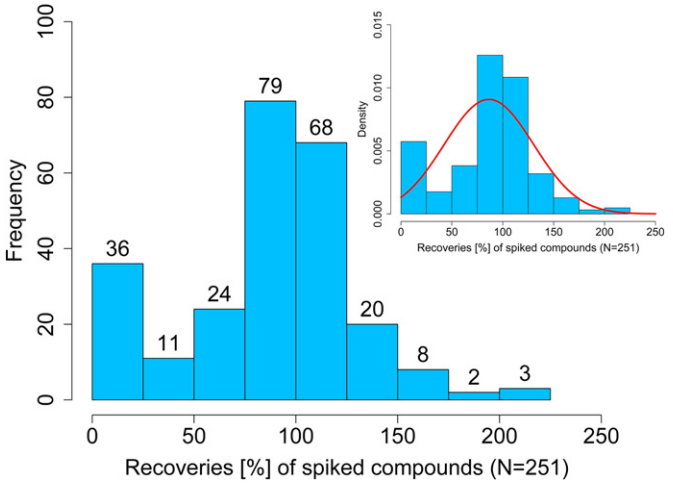

Fig. 3. Histogram of the recoveries (in %) of compounds ($N = 251$) spiked in a pristine water sample of Wormsgraben (Harz Mountains, Germany) and extracted with the LVSPE50 device using the neutral HR-X sorbent; the insert shows the density function of the distribution.

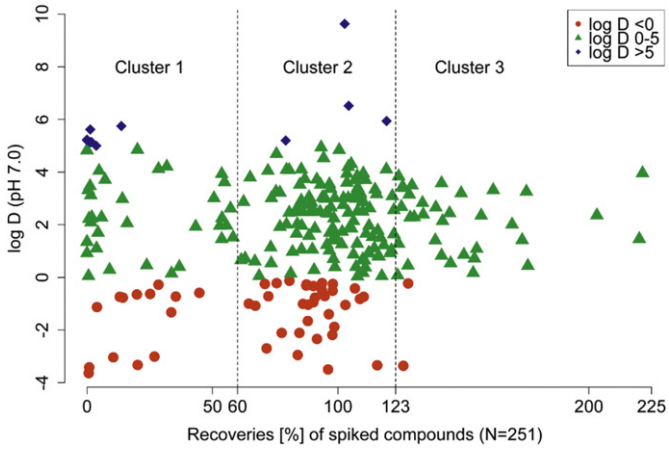

Fig. 4. Scatterplot of the total recoveries (in %) of compounds ($N = 251$) spiked in a pristine water sample of Wormsgraben (Harz Mountains, Germany) and extracted with the LVSPE50 device versus the water-octanol partition coefficient at pH 7.0 corrected for the speciation (log D); the dashed lines express the limits of the clusters derived from *k*-means cluster analysis ($k = 3$); the plot shows only the data for HR-X.

The resulting three groups of *k*-means clustering include (1) one group of compounds with low recoveries in HR-X (<60%) and a larger overlap with HR-XAW and HR-XCW (56 out of 251 compounds), (2) one group with recoveries in HR-X in the range of 60% to 123% with only small overlap with both other sorbents (159 out of 251 compounds), and (3) one group with recoveries in HR-X >123% with only very small overlap with the ion exchanging phases (36 out 251 compounds, Fig. 4, Fig. S5, Table S4).

Among the causes for recoveries assigned to the first or third group are chromatographic reasons such as elution during dead time and matrix effects in ESI-MS analysis. The matrix effect is caused by co-extracted dissolved organic matter (DOM). The DOM is a heterogeneous mixture of compounds with a wide range of different structures and hence a higher load of DOM related compounds with affinity to polystyrene-divinylbenzene co-polymers can be expected (Raeke et al., 2016; Swenson et al., 2014) that co-elute with similar compounds in LC. However, correction with spiked internal standards and matrix-matched calibration often cannot compensate matrix effects. In the case of very nonpolar or hydrophilic compounds, an irreversible adsorption to surfaces and the sorbents or breakthrough is reasonable, respectively. The latter was observed for 4-aminobenzamide, acetaminophen, chloridazon-desphenyl, chlormequat, mepiquat, and *N*,*N*-dimethylsulfamide, which were qualitatively detected in the effluent water after extraction.

The chemical assessment of spiked water samples revealed that the LVSPE approach using the hydrophobic sorbent HR-X was suitable to capture a larger number of the spiked compounds with good recoveries between 60% and 123% without apparent dependency on their physicochemical properties. The usage of any other general purpose solid phase (e.g., Oasis® HLB or Amberlite® XAD) or resins with specific functional groups such as ionic exchangers might be an opportunity for tailored applications. However, in this study, the latter considerably enhanced the recoveries of only a few compounds (e.g., benzenesulfonic acid, benzothiazole, gabapentin, metformin, *N*-nitrosomorpholine, perfluorobutanoic acid, salicylic acid). Certainly, in the setting of the recovery experiment using a relative low volume of spiked water (10 L) and a low expected content of dissolved organic carbon (DOC), the amount of 10 g of HR-X (or similar sorbents) as the first sorbent in flow direction could be enough to trap large amounts of spiked compounds. In another setting with larger volumes of spiked water with higher content of DOC, a larger breakthrough and distribution over the three phases is possible.

In marine applications, the salt content of the water can be an issue to be considered. Higher salinity caused by co-extracted inorganic salts can effect (1) the extraction of charged organic compounds due to competitive ionic interactions of the ionic exchangers with inorganic cations ($Li^+ < Na^+ < NH_4^+ < K^+ < Mg^{2+} < Ca^{2+}$) and anions ($Cl^- < Br^- < NO_3^- < SO_4^{2-} < ClO_4^-$) (Bäuerlein et al., 2012), (2) the chemical analysis due to matrix effects (Mallet et al., 2004; Wu et al., 2010), and (3) the results of bioassays due to salinity intolerances of the test species (Gonçalves et al., 2007; Dinnel et al., 1987; Haque et al., 2014; Sawant et al., 2001). Therefore, proper washing of the cartridges with ultra-clean water after extraction is recommended to avoid the carryover of a higher load of inorganic salts to the organic extract (Loos et al., 2013a, 2013b; Wu et al., 2010).

3.3. Biological assessment of field samples

A major reason for developing the LVSPE approach was the lack of appropriate sampling equipment for the effect-based screening analysis and monitoring of water resources. Enrichment of a larger volume of water is required to deliver enough extract for the subsequent testing in a set of different bioassays or even to perform effect-directed analysis. To investigate whether the LVSPE approach is applicable for effect-based analysis, extracts of samples collected during the EDP were assessed using seven in vitro and organism-level bioassays representing diverse modes of action (MOA) and adverse effects of pollutants (Table 2). Since HR-XAW and HR-XCW extracts of those samples were only effective in a few assays and endpoints, only the results for the HR-X extracts are represented in this study (Tousova et al., unpublished data). Using the observation of a biological response at a REF of 100 as a criterion of decision, 8 out of 10 toxic endpoints (Table 2) allowed a discrimination of active from non-active surface water samples with 5% (endpoint mutagenicity) to 77% (endpoint estrogen receptor mediated activity) of the samples exhibiting significant responses. A REF of 100 is an enrichment level that can be easily achieved in effect-based and chemical monitoring using LVSPE in a reasonable period and without any problems of blank toxicity (Fig. 5). Anti-AR activity and AChE inhibition did not respond to any of the samples, the latter up to a REF of 500. For range finding and avoidance of masking effects of the targeted specific endpoints, the occurrence of cytotoxicity was tested in all cell-based tests beforehand.

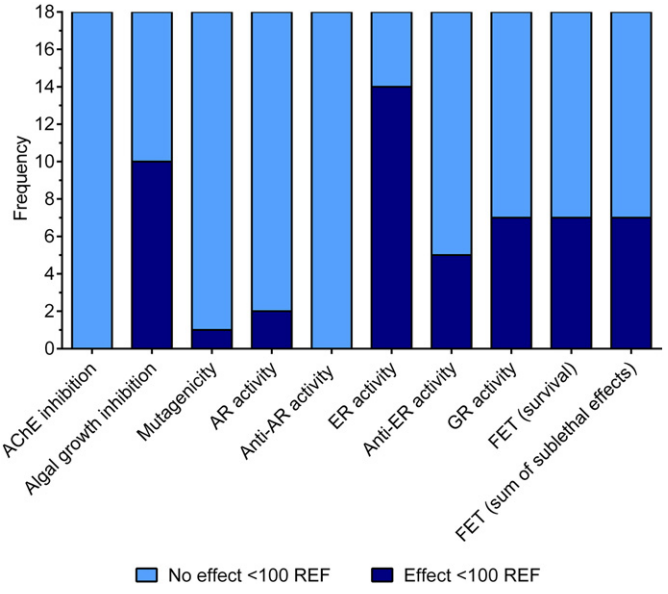

Fig. 5. Occurrence of responses in bioassays to 18 LVSPE samples collected during the European demonstration program; most samples were tested in most bioassays up to a REF of 100, except AChE (up to REF 500); REF: relative enrichment factor; AChE: acetylcholinesterase enzymatic inhibition, AR: androgenic mediated activity, ER: estrogenic mediated activity, GR: glucocorticoid receptor mediated signalling, FET: zebrafish embryo test (Tousova et al., unpublished data).

3.4. Chemical assessment of field samples

Fig. 6 shows a selection of concentrations of chemicals analyzed in the EDP water samples extracted using the HR-X sorbent. The analytes cover a wide range of substance classes such as pesticides, pharmaceuticals or industrial chemicals and their transformation products. The concentration levels were in the range from 0.2 ng L^{-1} to 2360 ng L^{-1}. The minimal and maximal concentration levels span over one to two orders of magnitude for most compounds. Once widely used legacy pesticides such as atrazine or simazine were among the identified substances. The overall concentration levels were comparable to those found frequently in European surface waters (Loos et al., 2013a, 2013b; Ruff et al., 2015; ter Laak et al., 2010). The chemical assessment of real water samples showed that the LVSPE approach was applicable to water samples containing compounds in a wide span of concentrations.

4. Conclusions

This study demonstrated LVSPE as a promising tool for the high quality sampling and extraction of pollutants for chemical and effect-based screening of water resources in field applications. LVSPE allows for onsite extraction of large volumes of water up to 50 L from natural or artificial water sources and thus provides sufficient sample volumes at the required enrichment factors for biological screening in a set of different bioassays and for chemical screening. Unequivocal distinction between likely effects of a blank sample and the effects of even only marginally polluted surface water samples was possible in this investigation. Furthermore, LVSPE appears to be suitable for the enrichment of complex mixtures of known water contaminants with no or only low systematic dependence from physicochemical properties with “good” recoveries. The flexible concept of the device allows for tailoring the configuration to the user's needs to reach the goals of a particular study. The device will facilitate the development of holistic effect-based and chemical assessment strategies to supplement the existing concepts of water quality assessment manifested in, e.g., the European Union Water Framework Directive. For example, the samples can be subjected to a first screening in a broad set of bioassays and afterwards used for effect-directed analysis in specific assays to unravel cause-effect relationships for the prioritization of effects and pollutants.

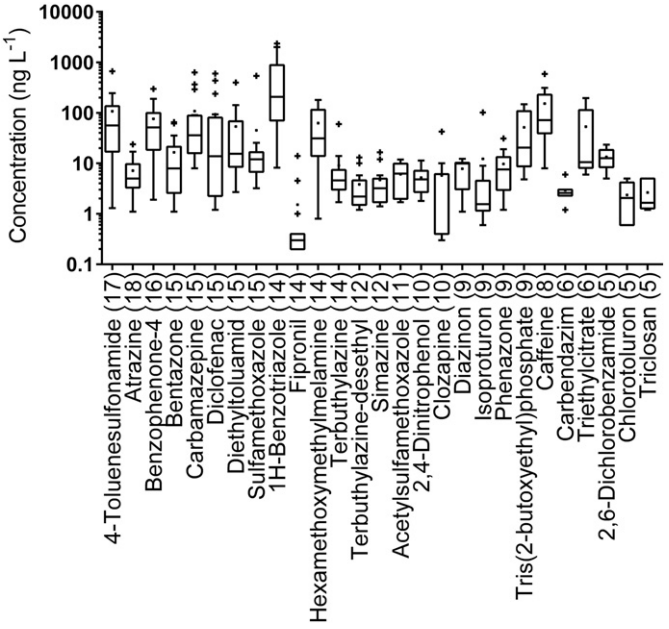

Fig. 6. Concentrations of frequently found organic compounds in 18 samples collected during the European demonstration program with the LVSPE50 device (in ng L^{-1}) grouped by occurrence of findings given in brackets; HR-X: neutral solid phase; data shown as box and whisker plot (box: median, 5^{th}/95^{th}-percentiles; whiskers: minimum and maximum; dot in box: mean), pluses represent outliers.

Conflicts of interest

The authors declare no conflicts of interests. However, we emphasized that the described device is considered for market release and commercial application.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2016.12.140. These data include the Google map of the most important areas described in this article.

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