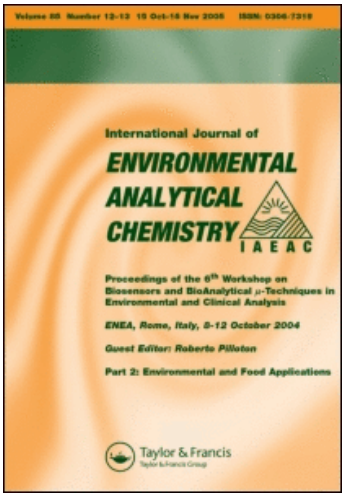

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Effect-directed analysis: a powerful tool for the surveillance of aquatic systems

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Effect-directed analysis: a powerful tool for the surveillance of aquatic systems

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The advantages of combined approaches for the evaluation of drinking-water quality, such as effect-directed analysis (EDA) consisting of biological and chemical analytical methods are illustrated in this work by comparing a classical and EDA approach applied to samples from China and Germany. The China samples contain polycyclic aromatic hydrocarbons, alkylanilines, chloroanilines, chloronitrobenzenes, chlorobenzothiazoles, chloroalkylphosphates, as well as pesticides ((4-chloro-2-methylphenoxy)ethanoic acid, metolachlor, atrazine, and the insect repellent *N*,*N*-diethyl-3-toluamide) in concentrations between 1 and 1000 $\mu g\,L^{-1}$. The toxicity profiles of two River Elbe samples and one sewage-treatment-plant sample, as determined by the luminescent bacteria test, selectively reflect the contamination background: the first profile only possesses two out of eight fractions well above the significance level of 10% inhibition, in the second profile, all but one fraction lie well above this level, exhibiting also a much higher total toxicity in each fraction as compared with the latter sample. In the third example, all fractions are far above the significance level and show a much higher total toxicity, but also the profile shifts towards less polar fractions.

Keywords: Effect-directed analysis (EDA); Bioassay-directed fractionation; GC–(HR)MS; Luminescent bacteria; Drinking water; Environmental early warning system (EEWS)

1. Introduction

Within the next decade, more than half of the world's population are expected to live in large metropolitan centres, due to increasing migration from rural areas. Limitation of pure drinking water and insufficient sanitation will be one of the upcoming problems in the growing process of these megacities. According to UN policy, measures have to be taken in order to overcome these problems. Existing resources have to be protected from contamination, and a controlled drinking-water cycle, including waste-water treatment, has to be established. The recently published World Water Development Report II (WWDR II) places emphasis on transdisciplinary approaches concerning the

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surveillance and evaluation of drinking water and its resources, including the assessment of verified contamination and the establishment of indicators that can be understood by political decision makers [1, 2].

In the focus of an upcoming transdisciplinary project lies Karachi, Pakistan, and its drinking-water supply as a classic example for a megacity. The objective of part of the project presented here is the analysis of the current state of drinking water, its resources, and waste-water effluents regarding contamination with organic substances. The method developed for this purpose consists of a combination of chemical and biological methods based on the toxicity identification evaluation (TIE) concept suggested by the US EPA [3–6]. This integrative method, also known as effect-directed analysis (EDA), is a holistic approach to characterize an environmental sample including a first assessment of the risk, which is able to meet the above-mentioned UN requirements [7, 8]. Contrary to solely analytical approaches, not only is the sample extract fractionated and characterized by chromatographic analytical methods (NP/RP-HPLC-DAD (Diode-Array-Detector), GC–MS), but from each step an aliquot is examined by accompanying bioassays. Thus, information about the status and possible risks of the aquatic ecosystem is gained, including mixture toxicity effects which can be overlooked by subsequent single-substance toxicity testing. However, it is worth noting that the above-mentioned US-EPA TIE concept at the beginning was aimed at establishing a causal link between *one substance* and *one specific effect*. This ambitious goal led to difficulties, especially in the case of surface waters, due to the complexity of the composition, the relatively small amounts of contaminants, as well as the mixture toxicity effects [7]. These obvious disadvantages gave rise to misunderstandings of the basic concept and hampered a broader application of this approach in the scientific community. However, though the establishment of a causal link between one substance and a specific effect may be too ambitious, an effect-directed concept is assumed to possess many advantages as compared with the classical approach [7–14]. It will be shown in the present article that this also holds for environmental surface-water samples. The method has been successfully developed and validated at the laboratory level and applied to many different kinds of aqueous samples from the local drinking-water cycle at Hamburg, Germany. In order to demonstrate the strength of this approach for reducing the complexity of an environmental sample, in this article the results for the fractionation of one River Elbe sample are presented. Furthermore, results of acute toxicity screening are exemplarily shown for the toxicity profiles of three aqueous Elbe river samples representing different origins of contamination, including surface water with diffuse sources, surface water with an additional point source (industrial effluent), and a sewage water sample representing very high contamination.

The choice of using a combined approach is based on the results of a preceding project, concerning the drinking-water cycle of Shanghai, in which the classical approaches of chemical and biological investigations were applied consecutively. Aqueous samples from Shanghai tap water and drinking-water resources such as Tai Hu Lake were screened for mutagenicity by different bioassays (*Salmonella*/microsome assay, Ames test, Arabinose resistance test (Ara test) and SOS/umu test). Samples were taken from a tap in the south of the city and from its respective supply facility (which pumps directly out of the Yangtze River), and tap samples were also taken from the middle and south districts as well as their supply facility (which pumps out of the Huangpu River originating from nearby Tai Hu Lake). These tests showed remarkable results, particularly the mutagenic potential of the tap water samples, i.e. agents were

present in the drinking water of Shanghai capable of inducing transmitted genetic changes in humans [15, 16]. As a consequence of these results, additional water samples were taken to be analysed chemically via GC–MS, in order to determine any possible causes of the mutagenic effects mentioned above. The results of this second Shanghai campaign will be discussed herein.

This example clearly shows the advantages of a combinatorial approach, such as EDA, where aliquots of the same sample are being used both for biotests and chemical analyses, as compared with the classical approaches where these investigations are carried out in parallel using two different samples. The comparison of these approaches is the main objective of this work, illustrated by results for the two aforementioned examples.

2. Experimental

2.1 *Experimental strategy*

For the first example, the analyses of the Shanghai tap water (classical approach), the supply facilities, and its resources, were performed as follows. The samples were filtered and enriched via solid-phase extraction using a styrene-divinylbenzene-copolymer phase (SDB-1). The elution was performed sequentially using *n*-hexane, ethyl-ethanoate, and methanol. The first extract was directly used for GC–MS analysis, and the second extract underwent a solvent change to toluene before being injected. The third extract was derivatized with methyl-chloromethanoate (MCM) prior to analysis. In addition to GC–LRMS (low-resolution, ion-trap mass spectrometry) the analytes were separated and identified by GC–HRMS (high-resolution, magnetic sector field mass spectrometry), as well. The mutagenicity biotests were applied according to standard procedures.

The EDA method developed for the upcoming evaluation of the Karachi drinking-water system consists of the following steps (figure 1). The aqueous samples are filtered and enriched as described above. The sequential elution is performed with *n*-hexane and methanol, and both eluates are subsequently fractionated by HPLC. The *n*-hexane eluate is fractionated using NP–HPLC with a dichloromethane/*n*-hexane gradient, as the methanol eluate RP–HPLC is used with a water/methanol gradient. From each of the NP–HPLC fractions, an aliquot is taken for investigation by the luminescent bacteria test for acute aquatic toxicity, and the other aliquot is analysed with GC–MS. Each of the resulting RP–HPLC fractions is split into three aliquots, one of which is investigated using GC–MS after a solvent change to toluene, and the second aliquot is derivatized with MCM and then analysed using GC–MS. The third aliquot is investigated using the accompanying biotest.

2.2 *Sampling, filtration, extraction, and elution*

The water samples from China were taken by local cooperation partners as described previously [15, 16]. Water samples from the River Elbe were taken in Hamburg-Neumühlen (Hamburg harbour, right bank, depth 4 m), in the Bützfleth harbour (left bank, depth 4 m) and in the presedimentation pool at the sewage treatment plant,

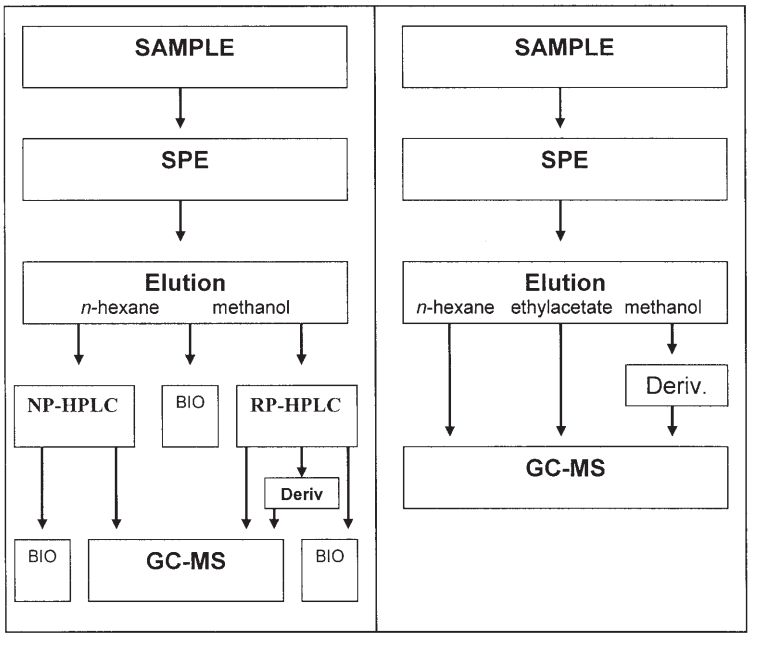

Figure 1. Flow diagram of the analytical procedure. Left-hand side: effect-directed method as applied to the River Elbe samples; right-hand side: classical approach as applied to the Tai Hu lake samples [17].

Dradenau (Hamburg, depth 0.5 m) with a sampler developed in the research group using 2.5-L amber glass bottles [18]. The China samples were filtered through a glass-fibre filter (Whatman, pore size 0.45 μm) coupled online to a preconditioned Bakerbond SDB-1 solid phase and an extraction unit (extraction unit constructed in the research group [18]; solid phase obtained from Baker Griesheim, Germany). Elution was performed after drying the solid phase with a gentle stream of nitrogen (grade 5.0, Linde, Germany) with *n*-hexane, ethyl-ethanoate and methanol (8 mL each) sequentially. The Hamburg samples were filtered and extracted as above but eluted with 7 mL of *n*-hexane and 70 mL of methanol. All eluates were reduced using a TurboVap 500 Evaporator (Zymark, Hopkinton, MA) to a final volume of approximately 1 mL, an aliquot of which was taken for the EDA method (Hamburg samples), i.e. the luminescent bacteria test, while the other aliquot was reduced to 200 μL under a gentle stream of nitrogen for the subsequent HPLC fractionation. All solvents used were SupraSolv grade purchased from Merck Darmstadt, Germany.

2.3 *NP-/RP-HPLC fractionation*

For the EDA method, the *n*-hexane eluate was injected onto a nitrophenyl-silica column (length 125 mm, i.d. 4 mm, particle size 5 μm, Macherey & Nagel, Düren, Germany) and separated with a dichloromethane/*n*-hexane gradient programme (6 min isocratic dichloromethane, in 30 min to 50/50 dichloromethane/*n*-hexane, holding for 6 min, in 6 min to 100% *n*-hexane). The methanol eluate was injected onto a Lichrosphere 100 RP-18 column (length 125 mm, i.d. 4 mm, particle size 5 μm, Merck, Darmstadt,

Germany) and separated with a water/methanol gradient programme (3 min 95/5 water/methanol, in 42 min to 100% methanol, holding for 3 min). The system consisted of an autosampler Gina 50, a Merck-Hitachi gradient pump (NP), an M 480 gradient pump (RP), a column oven STH 585 and a DAD-detector UV 340 S (all Gynkotek-Dionex, Germering, Germany, except the NP gradient pump Merck, Darmstadt, Germany). The column was kept at 298 K, the flow rate was 1 mL min^{-1}, fractions were taken every 6 min, and programmes lasted 48 min, resulting in eight fractions each. All solvents used were SupraSolv grade, except for water, which was LichroSolv grade, all purchased from Merck (Darmstadt, Germany).

2.4 *Sample preparation and GC–MS analysis*

The preparation and fractionation, including the elution order of the Shanghai samples, were already described in section 2.1. The HPLC fractions of the Hamburg samples, processed by the EDA method, were prepared according to the following procedures. The NP–HPLC fractions were split into two aliquots, one for the bioassay test and another reduced to a final volume of 150 μL under a stream of nitrogen, and then injected into the GC–MS for further analysis. The RP–HPLC fractions were split into three aliquots, the first aliquot diluted with water to keep the methanol content below 4% (v/v), reduced to a volume of 1 mL with the TurboVap Evaporator, extracted with toluene (3×300 μL) and dried over sodium sulfate, which was heated to 523 K overnight prior to use. The second aliquot was prepared for derivatization as detailed in section 2.5, and the third aliquot was prepared for the biotest, as detailed in section 2.6.

For GC–MS analysis a Magnum ITD 40 (Finnigan MAT, Bremen, Germany) ion-trap mass spectrometer was used under the following conditions: electron impact (EI) mode at 70 eV, manifold temperature 473 K, transfer line at 523 K, emission current 10 μA, dwell time 100 μs, and scan range 90–500 amu (full scan mode). This was coupled to a Varian 3400 GC system (Sunnyvale, CA), and separation was performed on an HP-5MS column, analogue to DB-5 (J&W Scientific, Folsom, CA), length 15 m, i.d. 0.2 mm, film thickness 0.33 μm, carrier gas helium 5.0 run with an A 200 SE Autosampler (CTC Analytics, Zwingen, Switzerland), and injected volume 2 μL. The temperature programmes used included: 333 K (2 min) with 7 K min^{-1} to 533 K (kept for 7 min) for the Shanghai samples and 353 K (2 min) with 6 K min^{-1} to 523 K (kept for 15 min) for toluene and 333 K (2.5 min) with 6 K min^{-1} to 523 K (kept for 15 min) for *n*-hexane in the case of the Hamburg samples. For the identification of compounds in the Tai Hu lake samples, GC–HRMS measurements were performed on a VG 70250 SE mass spectrometer (Micromas, Manchester, UK), operated in EI mode, 70 eV at RP 5000 with PFK as a reference, coupled to an HP 5890 GC equipped with either a BPX-5 column (SGE, Austin, TX), i.d. 0.25 mm, film thickness 0.25 μm, length 30 m, or an Innowax column (Agilent, Palo Alto, CA), i.d. 0.25 mm, film thickness 0.25 μm. Temperature programmes: (BPX-5) 333 K (3 min) with 3 K min^{-1} to 573 K (kept for 20 min) and (Innowax) 333 K (3 min) with 5 K min^{-1} to 483 K (kept for 20 min); injection 1 s splitless. For the identification of the analytes, the MassLib software (MSP Kofel, MassLib V8.7E, Copyright 1996–2004, MPI für Kohleforschung) was used, and for comparison of MS spectra the following libraries were used: NIST 02, copyright NIST/EPA/NIH mass spectral database, US Dept. of Commerce; Wiley Registry of Mass spectral data, copyright John Wiley & Sons Inc.; Chemical Concepts Mass

spectral database 4th ed., copyright Chemical Concepts; Geochemicals 1st ed., copyright Chemical Concepts; MCR Collection 1st ed., copyright Chemical Concepts; Food Volatiles 1st ed., copyright TNO Zeist; K. Pfleger, H.H. Maurer, A. Weber, Mass spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their metabolites, 2nd ed., VCH, Weinheim, 1992, 2000.

2.5 *Derivatization*

For derivatization, all respective aliquots were evaporated to almost dryness and taken up again in 100 μL of a mixture of acetonitrile/methanol/water/pyridine (5/2/2/1; v/v/v/v). Then, 7 μL of methyl-chloromethanoate (MCM, Merck Darmstadt, Germany) was added, shaken, and set to rest for 10 min at room temperature. Afterwards, 100 μL of water and 500 μL of *n*-hexane were added, the phases were separated, and the organic phase was washed twice with 100 μL of water, then dried for 1 h over sodium sulfate, which had been heated overnight at 523 K prior to use and reduced to a final volume of 150 μL under a stream of nitrogen [18, 19]. Pyridine was used in p.a.-grade, water in LichroSolv grade, and all other solvents in SupraSolv grade, and all were purchased from Merck (Darmstadt, Germany).

2.6 *Bioassays*

The bioluminescence inhibition assay for acute toxicity with *Vibrio fischeri* is a standardized commercial bioassay based on the reduction in bioluminescence of the marine bacterium *V. fischeri* following exposure to toxicants [20]. The respective aliquots were reduced to almost dryness and diluted with 2% NaCl aq. solution to keep the methanol content below 2% (v/v); in the case of more volatile organic solvents DMSO (dimethyl sulfoxide, supraSolv grade, Merck Darmstadt, Germany) was used as a keeper, keeping its content below 1% (v/v) to exclude any false-positive results for toxicity exhibited by the organic solvents. Procedural blanks were performed in parallel and tests in duplicate; for additional quality assurance, positive controls (7.5% NaCl aq.) and negative controls (2% NaCl aq.) were always used prior to testing. The hardware used was the LUMISmini luminometer with the LUMIStherm thermostat and the bacteria strain NRRL-B-111777 (all Dr Lange GmbH, Düsseldorf, Germany) [12, 13, 21].

3. Results and discussion

As outlined in the introduction, in an earlier investigation [15, 16] aqueous samples from Shanghai tap water and drinking-water resources such as Tai Hu Lake were screened for mutagenicity using different bioassays (*Salmonella*/microsome assay, Ames test, Arabinose resistance test (Ara test) and SOS/umu test). These tests revealed that agents were present in the drinking water of Shanghai, capable of inducing transmitted genetic changes in humans. Therefore, additional water samples were taken, in order to determine the possible causes of the mutagenic effects mentioned above. The GC/MS analyses of the water sample extracts of this second Shanghai campaign were the focus

Table 1. Selection of organic contaminants identified in two surface-water sample extracts taken from Tai Hu Lake, China (compounds identified by spectra libraries).

Class	Compounds	Sample 1 ($\mu g\,L^{-1}$)[a]	Sample 2 ($\mu g\,L^{-1}$)[a]
Hydrocarbons	Various PAHs	1–100	1–100
	Various alkanes	0.1–100	0.1–100
Anilines	Mono- to tetrachloroaniline	0.1–10	0.1–10
	Alkylanilines	0.1–100	0.1–100
	Alkylnitroanilines	0.1–10	0.1–10
Benzenes	Chlorobenzenes	0.1–100	n.d.[b]
	Alkylbenzenes	0.1–100	0.1–100
	Chloronitrobenzenes	1–100	1–100
	Phenylethanoic acids	1–500	1–500
	Di- and trichlorobenzoic acids	1–1000	1–1000
Benzothiazoles	Benzothiazol	100–1000	100–1000
	Alkylbenzothiazoles	10–100	10–100
	Methoxybenzothiazoles	0.1–10	0.1–10
	Mono- to trichloro-benzothiazoles	1–1000	1–1000
Pesticides/	Metolachlor	1–10	1–10
Insect repellent	DEET	1–10	1–10
	Triazines	10–100	10–100
	Permethrinic acid	100	100
	MCPA	10	10
Organophosphates	Tributylphosphate	1–10	n.d.
	Tris(2-chloroethyl)phosphate	10–100	10–100
	Tris(2-chloropropyl)phosphate	10–100	n.d.
	Triphenylphosphinsulfid	0.1–1	n.d.
Various unknown mono- to pentachloro compounds		1–1000	1–1000

[a]Semi-quantitative estimation, range of congeners.
[b]n.d. not detected.

of the present investigation, in the course of which two 0.7-L samples were taken from Tai Hu Lake, one at a heavily industrialized location, the other in a presumably pristine area.

In general, a vast array of hazardous substances were encountered, many reaching the upper $\mu g\,L^{-1}$ range. In particular, chlorinated substances dominate the picture, particularly regarding quantity. A rough summary of the contaminants found in the Tai Hu lake samples, analysed by the classical approach, is presented in table 1. Concentration ranges are given for different congeners by a rough semi-quantitative estimation using an external standard.

The samples contain polycyclic aromatic hydrocarbons (PAHs), known industrial pollutants such as alkylanilines, chloroanilines, chloronitrobenzenes, chlorobenzothiazoles, chloroalkylphosphates as well as pesticides, e.g. (4-chloro-2-methylphenoxy)ethanoic acid (MCPA), metolachlor, atrazine, and the insect repellent *N*,*N*-diethyl-3-toluamide (DEET). Most interesting from the analytical point of view is the high content of organochlorine compounds, ranging in concentrations between 1 and 1000 $\mu g\,L^{-1}$. These were mainly found in the methanol eluate after derivatization, this means that they represent highly polar substances, often hydroxy or carboxy derivatives. The reproducibility of these results was very good, as inferred from a comparison between the GC–LRMS and the GC–HRMS measurements. The major part of these compounds is not included in existing spectra libraries (see section 2.4). However, it is obvious that the unknowns belong to a series of structurally similar compounds with a considerable number of isomers and homologues. One series of

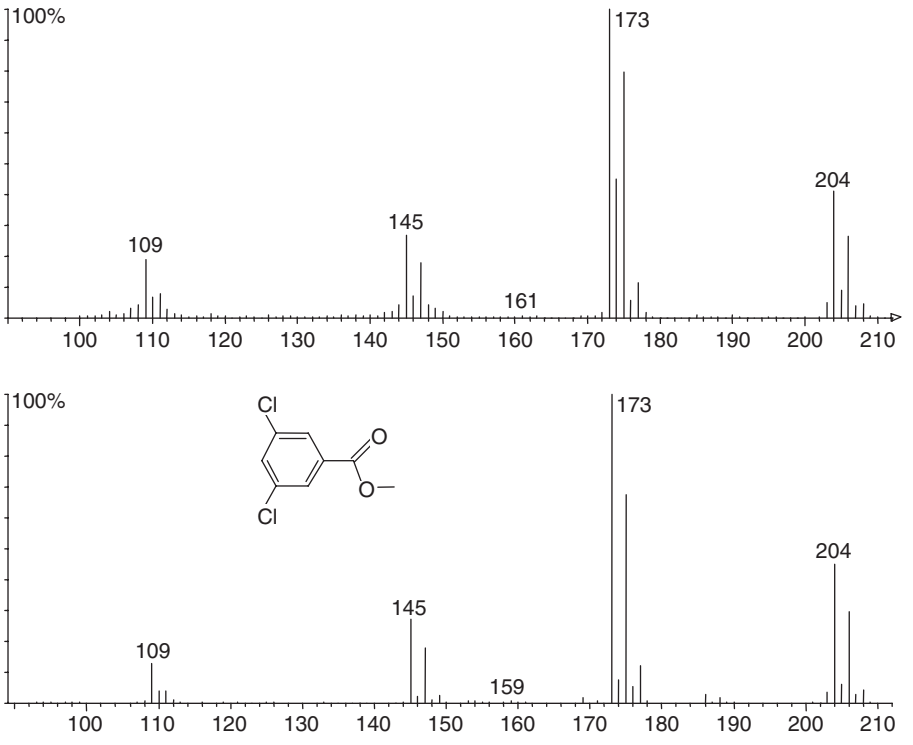

Figure 2. Mass spectra of a dichlorobenzoic acid methyl ester from the Tai Hu lake (above) and the corresponding library spectra (beneath).

related known compounds are di- and trichlorobenzoic acids, as shown in figure 2. Further identification of the novel compounds and verification by measurements of reference compounds are needed. Yet this will be a task not relevant to the main object of this work—the comparison of the classical and combined approaches (EDA) in terms of evaluating the potential danger of these contents in drinking water—and will be published elsewhere.

The classical approach would include the evaluation of toxicity data, if available, and inference of the risk of the contamination situation by modelling. The drawbacks of this classical approach are obvious: many single substance toxicity tests have to be performed in the case of novel or toxically evaluated compounds that are yet to be evaluated. Even more important is the fact that no matrix influences or mixture toxicity effects would be included, which are often the cause of the observed effects [7]. At this point, the superiority of the combined approach becomes even clearer, especially with regard to comparability with the real-world situation, because chemical analyses and toxicity tests are performed using aliquots of the same sample extract including the above-mentioned matrix related and mixture toxicity effects.

In order to make these advantages clearer and to show which additional information can be gained in one single experimental approach, results for the development and validation process of the combined (EDA) method developed for the evaluation of drinking-water cycles will be presented. These experiments have been performed locally

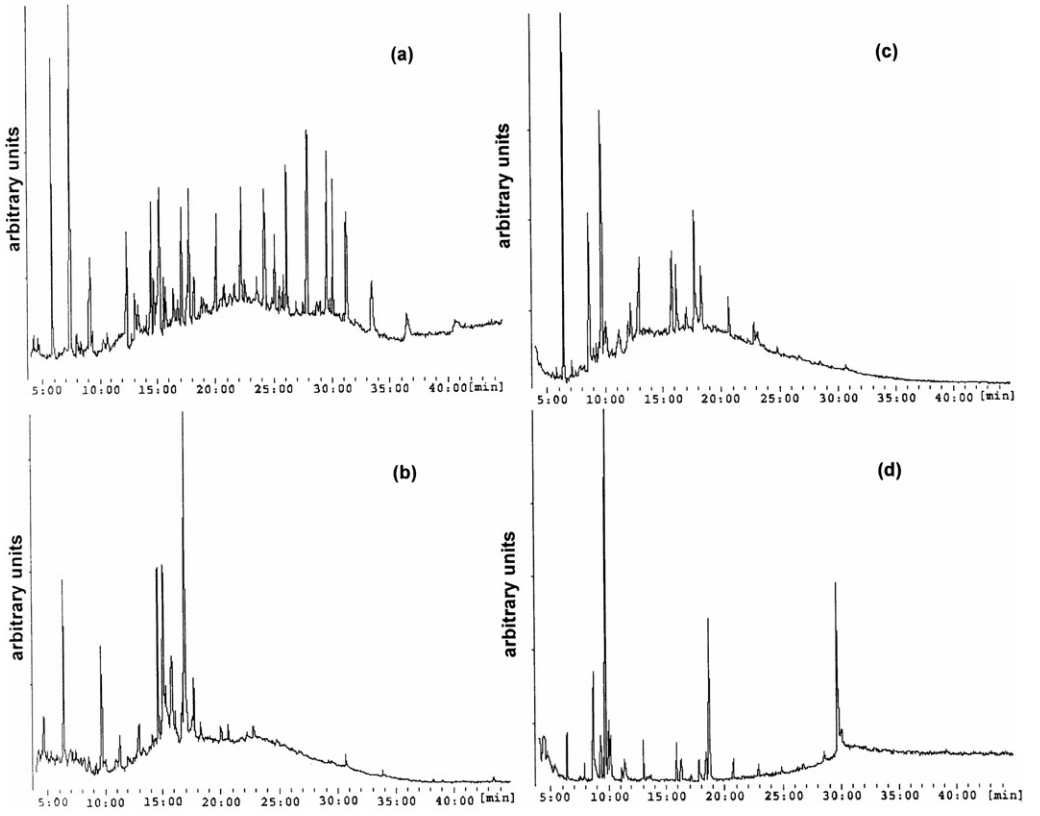

Figure 3. Gas chromatogram of the methanol eluate of a River Elbe sample (a) and three of its respective fractions after RP–HPLC separation (b–d), taken from Hamburg harbour (Oevelgoenne). GC–LRMS, EI at 70 eV, full scan mode.

so far, on the basis of samples taken in the drinking-water cycle of the city of Hamburg (Germany). Basically, the method has been developed for the surveillance of complete drinking-water cycles, in order to act as an Environmental Early Warning System (EEWS). Therefore, different kinds of matrices contained in aqueous samples have to be processed. Thus far, the method has been successfully applied to samples from the tap, possible resources (River Elbe and Alster Lake surface water from different locations), and the waste-water-treatment process. With the chosen copolymer solid phase, the broadest range of compounds possible can be covered in a single extraction step at ambient pH, from non-polar substances to highly polar compounds [22, 23]. Subsequent fractionation by HPLC reduces the complexity of the resulting chromatograms often encountered in the case of environmental sample extracts. Also, no additional clean-up steps are necessary, since matrix compounds which elute in the methanol fraction are separated off by RP–HPLC fractionation. Examples of the reduction in complexity by means of a River Elbe methanol extract (figure 3a) and three of its respective RP–HPLC fractions (figure 3b–d) are displayed in figure 3.

The unequivocal advantage of combined (EDA) approaches as compared with solely chemical analytical approaches is exemplarily shown in figure 4(a–c). The toxicity profiles of the respective eight RP–HPLC fractions of the methanol eluates of two River

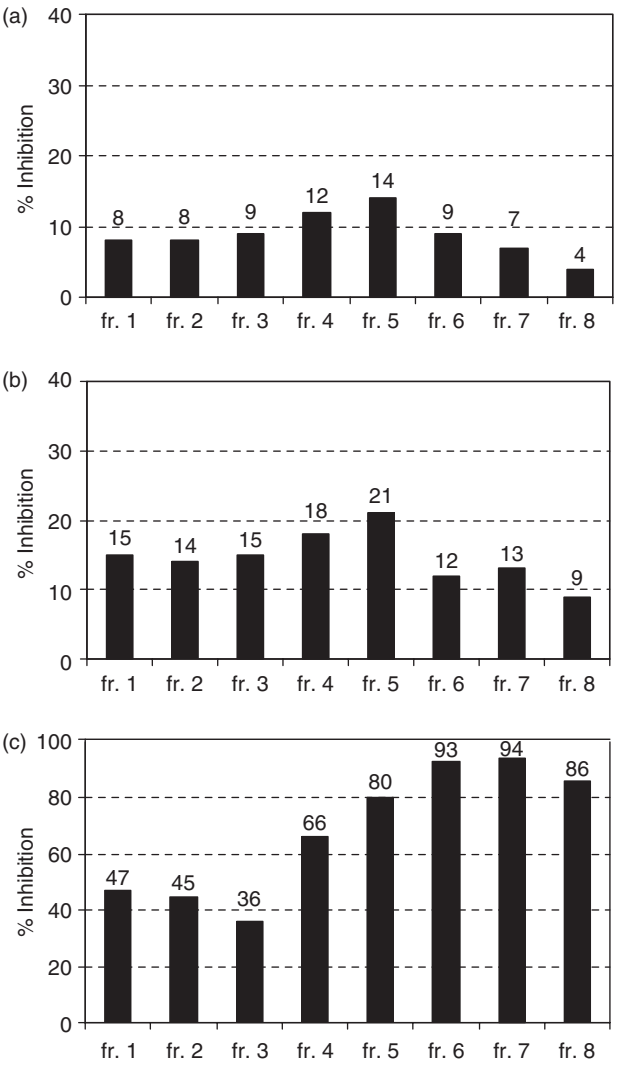

Figure 4. (a) Toxicological profile of eight RP-HPLC fractions (fr. 1 to fr. 8) resulting from the separation of the methanol eluate of a River Elbe sample, taken in the Hamburg harbour (Oevelgoenne) where diffuse sources prevail. LUMISTOX® bioluminescence inhibition assay, 30-min acute aquatic toxicity test with *Vibrio fischeri*. (b) Same as (a), but for a River Elbe sample, taken from the plume of an industrial point source (Buetzfleth). (c) Same as (a), but for a sample taken from the presedimentation pool at the wastewater-treatment plant Koehlbrand (a) Hamburg.

Elbe samples and one sewage-treatment-plant sample exemplify the feasibility of EDA as an EEWS. The first sample was taken in the Hamburg harbour representing only diffuse sources of contamination (figure 4a). In this case, fractions 4 and 5 show significant (inhibition >10%) acute toxic effects. Obviously, toxic effects prevail in fractions 4 and 5, which is typical for samples taken at this site, as shown in previous investigations [13, 14]. A different toxicity profile of a River Elbe sample taken around 70 km downstream in the plume of an industrial point source (DOW Chemical plant, Bützfleth) is shown in figure 4(b). In the latter case, the profile is different to that where

diffuse sources prevail, and all fractions except number eight cause significant toxic effects in the biotest. Fractions 4 and 5 are the most toxic also, but higher acute toxicity effects than in the previous example can be inferred from the luminescent-bacteria test. However, fractions 1–3 especially show effects which usually are not observed at sampling sites where diffuse sources are the main reason for contamination. In order to underline the exceptionally broad range of characteristics that may be used to help interpret risk assessments for different contamination backgrounds, the profile of a sample taken from the presedimentation pool at the sewage treatment plant Dradenau, Hamburg, is shown (figure 4c). In this case, the picture is totally different; first of all, a much higher total acute toxicity is suggested, due to higher concentrations of contaminants as compared with surface water samples. More important, however, is the completely different profile shown here. Fractions 1–3 are much lower in toxicity than the others, fractions 6 and 7 show the highest toxicity, and even fraction 8 is more toxic according to the luminescent bacteria biotest than fractions 4 and 5, which dominate the picture in the surface water samples described above.

In detail, the toxicity profile characteristics of the three locations exhibit pronounced differences: the first profile (figure 4a) has only two fractions (4 and 5) well above the significance level of 10% inhibition; in the second profile (figure 4b), all but one fraction lie well above the 10% level, exhibiting also a much higher total toxicity in each fraction compared with the latter sample. In the third example (figure 4c), not only are all fractions far above the significance level, showing a much higher total toxicity, but also the profile shifts from fractions 4 and 5, being the most toxic in the case of the River Elbe samples, to fractions 5–8 exhibiting the greatest toxic effects.

These examples may underline that the combined (EDA) approach offers a simple and conceivable tool for decision makers to set premises for chemical analyses. Furthermore, fast information concerning a pre-risk assessment and the origin of contamination can be gained by only looking at the toxicity profiles of the fractions. These few examples illustrate the possibility of the present approach to discriminate between environmental samples with regard to their origin and their respective contamination. The distinction made on the basis of the results of a fast, high-throughput screening assay such as the luminescent-bacteria test applied here is far less complicated and time-consuming than the distinction by chromatograms. The biotests were performed in duplicate, taking aliquots of the same sample differing by about 1% inhibition. Replicates of samples taken at the same site at a different time differ by about 2% inhibition. However, it should be mentioned that, in this case, only one screening assay was used in order to ensure a fast and high throughput of samples, though limiting the number of different effects detectable by this method.

4. Conclusions

Effect-directed analysis (EDA) is a promising tool for assessing potential risks related to safe drinking-water supplies in peri-urban regions of fast-growing megacities. This method represents a transdisciplinary approach, as suggested by the UN in the WWDR II issued this year, the advantages of which are obvious. In the classical approaches, the chemical analytical part results in identified compounds with unclear effects, and the biological investigations result in quantified effects due to unknown sources. These two

results are combined afterwards only by theoretical modelling, not including effects due to the mixture of toxicants or possible enhancing effects of biogenic components which are often a relevant cause of total toxicity. These are included in EDA approaches by performing chemical and biological investigations of aliquots of the same sample. The classical approach as performed in the case of the present Shanghai samples supplied reliable results with regard to toxicological effects. But regarding the requirements of an EEWS, which is supposed to provide an alert when the drinking-water quality is reduced by contamination (and possibly hazardous to human health), e.g 'mutagenicity', EDA is by far the best choice. The premise here should be not to interrupt the water supply any longer than is absolutely necessary. EDA includes bioassays which support the responsible decision makers on the basis of unequivocal and conceivable effects, without being obliged to identify all compounds and evaluate their respective toxicity. Herein, examples were presented for three water samples, two of which were taken in the River Elbe samples exhibiting different contamination backgrounds and one sewage-treatment-plant sample. EDA surveillance systems and their application to an EEWS are far less time-consuming and far more cost-effective, and it should be kept in mind that the application of these systems is needed more often in developing nations where the problems addressed by the UN prevail.

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