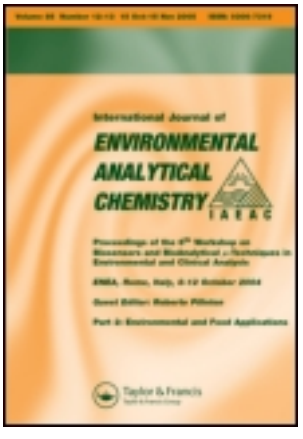

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An automatic field sampler utilising supported liquid membrane (SLM) for on-site extraction of s-triazine herbicides and degradation products: applied to an agricultural region of Ethiopia

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An automatic field sampler utilising supported liquid membrane (SLM) for on-site extraction of *s*-triazine herbicides and degradation products: applied to an agricultural region of Ethiopia

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A portable and time-integrating field sampler based on the supported liquid membrane (SLM) extraction technique was constructed. Using two programmable syringe pumps and one programmable valve, the sampler could carry out automatic unattended extraction for up to seven extracts, combining the steps of sampling, trace enrichment and clean-up. The sampler was applied to the extraction of four *s*-triazine herbicides (atrazine, cyanazine, prometryn and terbutryn) and six major degradation products of *s*-triazines, including three dealkylated products (deethyl deisopropyl atrazine (DDA), deisopropyl atrazine DIA and deethyl atrazine (DEA)) and three hydroxylated products (hydroxy atrazine (ATOH), hydroxy propazine (PROH) and hydroxy terbutylazine (TZOH)). The donor solution was obtained by mixing sample and buffer, consisting of 1 M phosphate buffer at pH 7.0 and 1.7 M NaCl, in the ratio of 19 : 1 (v/v). Extraction was performed by continuously pumping 10 mL portions of donor along the SLM until 3 L of sample had been extracted. The SLM consisted of di-*n*-hexylether and the acceptor was 1 M HCl. After extract collection, extracts were neutralised with NaOH and buffered with phosphate. Extracts were analysed with HPLC, using a gradient elution consisting of 3.5 mM phosphate and acetonitrile and UV-detection at 220 nm. Enrichment factors in reagent water ranged from 1.3 (for DIA) to 2739 (for terbutryn). The developed field sampler was tested by carrying out 24-h time-weighted on-site extraction of the ten *s*-triazine target compounds in Hawassa Lake and its tributary river, located in the agricultural region of the Southern Rift Valley of Ethiopia. Atrazine, cyanazine and terbutryn were generally below the method detection limit, while prometryn was frequently found. Overall, *s*-triazines were not persistent in the studied environment and degradation products of *s*-triazines were found in higher concentrations than the parent herbicides in both the river and the lake.

Keywords: pesticides; *s*-triazines; degradation products; field sampler; automated sampling; on-site extraction; supported liquid membrane; liquid chromatography

1. Introduction

Pesticide residues from agriculture are commonly found in recipient water bodies. In order to protect human health, the European Union (EU) dictates that the concentration of pesticides or relevant metabolites, degradation and reaction products in drinking water

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should not exceed a maximum admissible concentration of $0.1\,\mu g\,L^{-1}$ for a single pesticide, and $0.5\,\mu g\,L^{-1}$ for total pesticide concentration (European Council Directive, 98/83/EC). For *s*-triazines, a group of herbicides acting as inhibitors of photosynthetic electron transport [1,2], the United States Environmental Protection Agency (EPA) dictates maximum contaminant levels (MCL) in drinking water of $3\,\mu g\,L^{-1}$ for atrazine and $4\,\mu g\,L^{-1}$ for simazine.

In surface waters, *s*-triazine herbicides are commonly detected in concentrations below $2\,\mu g\,L^{-1}$ (e.g. [3–8]). Among *s*-triazines, atrazine is usually the compound with the highest detection frequency and also the compound found in the highest concentrations [1]. Some reported EC_{50} toxicity data (in $\mu g\,L^{-1}$) of selected *s*-triazines towards different algal species range from 28 to 135 for atrazine [1,2,9], 27 to 60 for cyanazine [1,9], 12 to 13 for prometryn [1,9] and 7.8 for terbutryn [1]. For these compounds, no observed effect concentrations (NOEC) (in $\mu g\,L^{-1}$) have also been reported, being 7.9 for atrazine, 5.0 for cyanazine, 0.82 for prometryn and 1.6 for terbutryn [1]. Typically, *s*-triazine concentrations found in environmental waters are below NOEC values from toxicity tests. However, even if toxicity to non-target aquatic plants and algae is low, combined effects cannot be ruled out [10]. It has been found that the toxicity of *s*-triazine mixtures follows the concept of toxicity concentration addition well, even if concentrations of individual herbicides are below NOEC levels [1]. Environmental conditions will determine the fate of the applied herbicides. For example, following UV irradiation, *s*-triazine degradation products in the form of dealkylated and hydroxylated degradation products have been found [11,12]. Thus, both parent *s*-triazine herbicides and degradation products are frequently found in the environment [3–7,13,14].

In water analysis, sampling is commonly done by collecting discrete spot samples. The disadvantage of this method is that it only gives the concentrations relevant for the particular time when the samples were taken. An important alternative to spot sampling is therefore the gathering of composite samples, which are made up by pooling a number of spot samples proportional either to time or flow of the sampled water body [15]. The main advantage of composite sampling is that it allows more representative water samples to be characterised with far fewer analyses than would be required using spot sampling [15].

Another commonly used water sampling technique is passive sampling, in which the analytes of interest diffuse through a membrane from the sample medium to a collecting medium or to another material, like a solid-phase microextraction (SPME) fibre [16,17]. In this sampling method the flow continues until equilibrium is achieved or until the investigator terminates the sampling session. One of the advantages with passive sampling is that it gives a time-weighted average (TWA) of the concentration during a known time period [18–20]. The state-of-the-art of passive sampling and/or extraction methods for long-term monitoring of pollutants in different environmental compartments has been reviewed extensively [17].

Usually, solid phase extraction is used for sample pre-treatment when studying *s*-triazines [3–5,7,13,14,21–23]. However, considerably cleaner extracts have been obtained with supported liquid-membrane (SLM) extraction than with SPE for *s*-triazines [24] and acidic drugs [25]. SLM is based on the same chemical principles as liquid–liquid extraction (LLE), but uses only minimal amount of organic solvents, making it a greener technique than LLE. An SLM can be formed by impregnating the pores of a supporting porous membrane material with water immiscible solvent, which is held in place by capillary forces, and thus prevents physical mixing of aqueous sample and acceptor (extract) solutions, when housed in a membrane holder. Such a 3-phase system is useful for

extraction of ionisable compounds in flow systems [26,27]. Donor pH is set so that analytes are predominantly in neutral form and can enter the organic membrane where they diffuse into the acceptor. Acceptor pH is set so that analytes become charged and thereby are trapped in the acceptor, which over time leads to enrichment. Preferably, acceptor pH is set 3.3 units below analyte pK_a for basic analytes, rendering the fraction of neutral analyte <0.0005, which is considered sufficient for complete trapping [26,28]. In general, the highest degree of extraction is obtained when analyte log K_{ow} is between $\sim$2.4 and $\sim$3.3 [29]. At these intermediate log K_{ow} values, diffusion in and flow rate of the donor will most greatly influence the extraction. At lower or higher log K_{ow}, extraction is limited either by too low dissolution into the membrane from the sample or by low stripping of the analytes from the membrane into the acceptor.

SLM has been used for extraction of various compounds, including haloacetic acids [30], biogenic amines [31], phenolic compounds [32,33], surfactants [34], bipyridilium herbicides [35] and *s*-triazines [24,28,29], and is described in reviews by Jönsson and Mathiasson [26,27]. With membrane extraction, trace level enrichment and clean-up can be performed in a single step. Moreover, membrane extraction is also well suited for automation [36]. Thus, SLM also has a potential for TWA sampling.

In our earlier work [8] involving the agricultural region of the Ethiopian Rift Valley lakes, 2-h composite sampling was utilised followed by SLM extraction of *s*-triazine herbicides to study lake pollution by these pesticides. The samples collected from the two lakes were transported 300–500 km to the laboratory, where they were stored in a cold room until analysis. After some pre-treatments like sample pH adjustment and filtration, extraction was carried out and concentrations of atrazine and terbutryn ranging from 0.02 to 0.05 $\mu g\,L^{-1}$ were determined. However, it is known that decomposition of these compounds may occur during transport and storage, and analytes can also be lost in the filter papers used during filtration [37]. In order to decrease such sources of error, on-site extraction and clean-up is therefore desired.

TWA field sampling utilising SLM extraction in an agricultural region has been performed by Mathiasson *et al.* [38] for determination of MCPA and by Knutsson *et al.* [39] for determination of six phenoxy acid herbicides. However, these works [38,39] were not based on fully automated extraction as they needed daily manual harvest of the enriched extracts. More recently, the use of an entirely automated in-lab SLM extraction system for carrying out unattended trace enrichment of *s*-triazine herbicides and their breakdown products from various aqueous matrices was demonstrated by Megersa *et al.* [40].

The purpose of this work was to develop a portable SLM-based field sampler, suited for environmental studies. A sampler was specially constructed for automated on-site sampling and SLM extraction. The construction of the sampler and its operation will be briefly presented. The sampler was applied to extraction of *s*-triazine herbicides and degradation products thereof from Hawassa Lake and its tributary river, which is located in the agricultural region of the Southern Ethiopian Great Rift Valley.

2. Experimental

2.1 *Reagents and chemicals*

Standards of *s*-triazine herbicides and their metabolites used in this study were purchased from Dr Ehrenstorfer GmbH (Wesel, Germany). Abbreviations used, log K_{ow}- and

pK_a-constants are given in Table 1. The skeletal structure of *s*-triazine herbicides is given in Figure 1 with additional structural information appearing in Table 1.

Analytical grade di-*n*-hexylether (Sigma Chemicals, St. Louis, MO, USA) was used as the SLM solvent. Other chemicals, including sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, acetonitrile, hydrochloric acid and sodium hydroxide, were purchased from Merck (Darmstadt, Germany). For SLM, donor buffer concentrate consisted of 1 M phosphate buffer at pH 7.0 to which sodium chloride (BDH Laboratory Supplies, Poole, England) had been added to obtain a final concentration of 1.7 M NaCl. The acceptor solution was 1 M HCl. All solutions were prepared from analytical-grade reagents in either (in Sweden) high

Table 1. Summary of structure and physical properties for the investigated herbicides and degradation products.

Analyte Type	Compound	R_1	R_2	R_3	Abbreviation	log K_{ow}[a]	pk_a[a]
Dealkylated degradation products	Deethyl deisopropyl atrazine	Cl	NH_2	NH_2	DDA	0	1.5
	Deisopropyl atrazine	Cl	NH_2	CH_2CH_3	DIA	1.2	1.3
	Deethyl atrazine	Cl	$CH(CH_3)_2$	NH_2	DEA	1.6	1.3
Hydroxylated degradation products	Hydroxy atrazine	OH	$CH(CH_3)_2$	CH_2CH_3	ATOH	1.4	5.15
	Hydroxy propazine	OH	$CH(CH_3)_2$	$CH(CH_3)_2$	PROH	–	5.2
	Hydroxy terbutylazine	OH	CH_2CH_3	$C(CH_3)_3$	TZOH	–	–
Parent herbicides	Cyanazine	Cl	CH_2CH_3	$CCN(CH_3)_2$	CYZN	1.8	1.0
	Atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$	ATZN	2.7	1.68
	Prometryn	CH_3S	$H(CH_3)_2$	$CH(CH_3)_2$	PRYN	3.34	4.05
	Terbutryn	CH_3S	CH_2CH_3	$C(CH_3)_3$	TRYN	3.74	4.4

Note: [a]Log K_{ow} and pK_a obtained from [40].

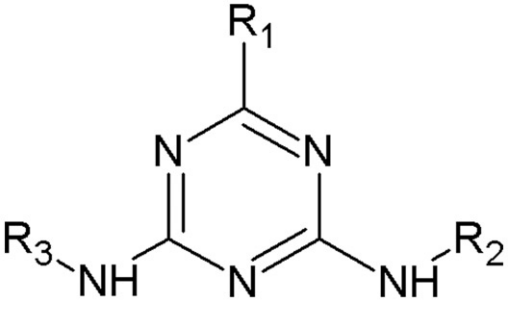

Figure 1. Skeletal structure of *s*-triazine herbicides. For R_1, R_2 and R_3, see Table 1.

purity water obtained from a Milli Q-RO4 unit (Millipore, Bedford, MA, USA) or (in Ethiopia) doubly-distilled water.

100 mg L^{-1} stock solutions of the *s*-triazine compounds and their degradation products were prepared in polypropylene bottles. Solid DDA was dissolved in 5 mL acetonitrile and 5 mL water. Hydroxylated products were dissolved in 1 mL of 1 M HCl, while the other analytes were dissolved in 1–2 mL acetonitrile. All stock solutions were diluted to the final volume with acetonitrile. Sample solutions for extraction and standards for HPLC were diluted from the stock solutions with reagent water. Stock and standard solutions were stored at 4°C in darkness when not in use.

2.2 *Chromatographic system*

HPLC with gradient elution using a mobile phase consisting of 3.5 mM phosphate buffer (pH 7.0) and acetonitrile was employed for analysis. The gradient elution was the same as the conditions previously developed for the mixture of the *s*-triazines herbicides and their degradation products [40]. Separation was carried out on a C18 analytical column (Ace 5 C18 Silica; 250 mm × 5 μm id; Advanced Chromatography Technologies, Aberdeen, Scotland). The mobile phase flow rate was adjusted to 1 mL min^{-1} and detection was performed with a UV detector at 220 nm.

Two different HPLC systems were employed. For the determination of enrichment factors and analysis of extracts from sampling campaign 1, the chromatographic system included an on-line degasser, a Varian pump Model 9012 (Varian Analytical Instruments, Sunnyvale, USA) and a Spectroflow detector Model 783 (ABI Analytical Kratos Division, Ramsey, USA). Injection was manual. The data were collected with JCL 6000 chromatographic data system for Windows (revision 27, Jones Chromatography Ltd., Hengoed, Mid-Glamorgan, UK). For the determination of extracts from sampling campaigns 2–4, the employed HPLC was an Agilent Model 1100 (Agilent Technologies Waldbronn, Germany), comprising on-line degasser, autosampler with the vial compartment theromstatted to 4°C, column compartment thermostatted at 20°C and diode array detector. Data from the 1100 system were collected and managed with the ChemStation software (Agilent).

2.3 *Membrane apparatus and flow system*

A small sized membrane holder (smaller than the one used earlier [40]) was designed to fit in the portable sampler box. It was made up of two polytetrafluoroethylene (PTFE) blocks with Archimedes spiral grooves with the dimensions of 0.25 mm depth, 1.5 mm width and 438 mm length, yielding a channel volume of about 164 μL. Two aluminium blocks with six screws and an O-ring made the assembly stable and leak tight.

A portion of the liquid membrane support, Fluoropore™ FG (Millipore, Bedford, MA, USA) was cut and made to fit into the membrane holder described above. Characteristics for the membrane support include an average pore size of 0.2 μm, a total thickness of 175 μm of which about 115 μm is polyethylene backing and a porosity of 70%. It was then immersed into the di-*n*-hexylether for about 30 min, and then the soaked membrane was placed between the two PTFE blocks. When the whole construction is clamped, it forms two separate channels, i.e. the donor and the acceptor compartments.

For sample flow, fluorinated ethylene propylene (FEP) tubes and flangeless fittings from Genetec (Gothenburg, Sweden) were used. In order to ensure mixing of sample and donor buffer before extraction, the tube from the donor pump to the membrane was coiled. FEP tubes with an inner diameter of 0.75 mm were used, except for the acceptor channel between the membrane and the vials where FEP tubes with an inner diameter of 0.25 mm were used. During on-site extraction a larger Teflon® tube was used for aspiration of surface water sample.

2.4 *Portable field sampler*

Figure 2 shows a schematic diagram of the portable SLM sampler. It consists of the membrane (in the membrane holder), two pumps, one valve for handling different extracts, one vial rack, one power transformer, one cooling fan, a sampler probe with filter and necessary tubes and fittings, as well as a support construction in stainless steel. The SLM sampler, both for field and laboratory usage, is built into a plastic box ($42 \times 26 \times 37$ cm) with a handle on top. Batteries for field power supply and controlling computer are not fitted in the sampler box. The weight of the sampler box, including some minor spare parts but without buffer and acid solutions, is 12.8 kg. IP class of the closed box is estimated to 32. However, during the sampling campaigns reported here, no specific exit hole for the sample aspiring tube was made, which meant the box was slightly open during on-site sampling. To be on the safe side during on-site sampling, a plastic tarpaulin was used to protect the sampler from rain and dust.

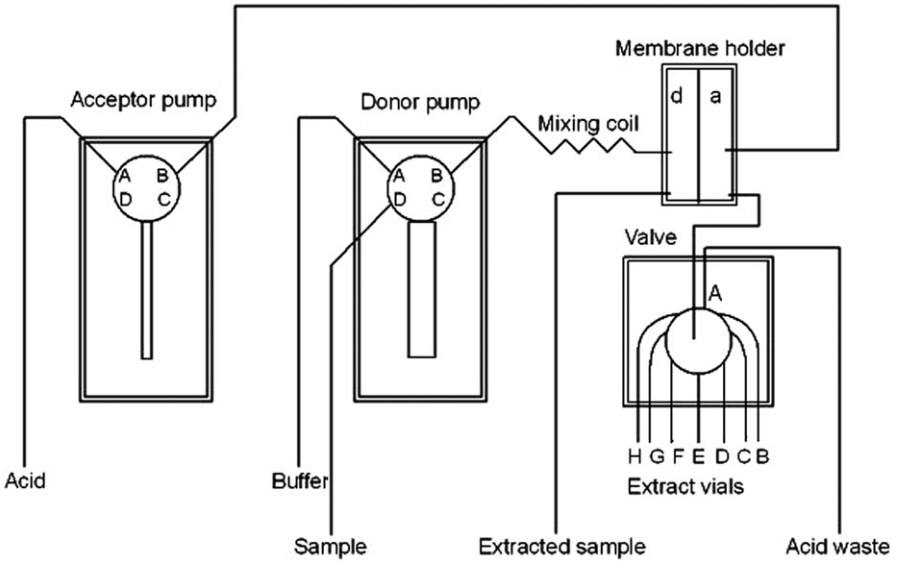

Figure 2. Schematic diagram of the portable field sampler. A–D on the pumps and A–H on the extract valve refers to channels on their 4- or 8-port valves, respectively. Lower-case a and d refers to the physically separated spiral-shaped acceptor and donor channel in the membrane holder, respectively. Communication lines from the donor pump to the acceptor pump and the extract valve are not shown.

The two syringe pumps, Model 50300 from Kloehn (Las Vegas, NV, USA), were used to independently control the flow rates of the donor and acceptor phases. The donor pump was supplied with a 10 mL syringe and the acceptor pump with a 1 mL syringe. The valve, Model 50120 (Kloehn), helped to distribute extracted samples from acceptor phase to the extraction vials through eight different ports, with one port reserved for acceptor waste, meaning that the sampler could perform up to seven extractions during unattended use. Two programs, WinPump® v. 3.3 and WinValve® v. 3.1 (Kloehn), were used to control the two pumps and the valve, respectively. The three devices enabled the SLM extraction either under direct control by a laptop computer or by programs saved in the memories of the devices. The latter strategy is obviously most suitable for unattended on-site extraction while the former is suitable for method development in the laboratory or for troubleshooting purposes in the field. Each device (the two pumps and the valve) was programmed separately. Details about the programming and technical set-up for SLM extraction can be obtained from the authors.

During on-site extraction, the sampler was powered from two serially connected 12 V batteries. In order to make the method more environmentally adapted and more convenient for long-time on-site extraction in the remote area of Hawassa Lake, two solar cell panels were used to charge the batteries. The solar cell panels, Model NR100G, and battery regulator, Model Mmini Pro, were purchased from Naps (Skärholmen, Sweden). However, the solar cell panels were rather bulky (1.3×0.65 m; 9.1 kg) and significantly larger than the more conveniently sized portable sampler itself. In remote areas that cannot be reached by terrain cars, more flexible equipment is obviously needed. Alternatively, extra batteries can be used, but these are also heavy. Thus, in order to make the use of solar cells more practical for portable samplers, smaller and more efficient panels would be convenient.

2.5 *Operation of the field sampler and extraction procedure*

Figure 3 shows the automated extraction procedure. For on-site extraction, the previously optimised automated method [40] was generally followed. During extraction the donor pump (Figure 2), equipped with a 10 mL syringe, was used to aspirate sample from the water body through an immersed filter (100 μm mesh size), and combine it with donor buffer concentrate from the bottle container. In this process the water from the lake was first aspirated into the 10 mL syringe through the attached 4-port valve and the buffer was aspirated into the same syringe via the same valve according to the set program. The donor pump aspirated 100 μL of buffer and 1900 μL of sample water five times, giving a mixture of in total 500 μL donor buffer concentrate and 9500 μl sample in the 10 mL syringe, i.e. a buffer : sample ratio of 1 : 19 (v/v). For practical reasons, it was not possible to use a 1 : 1 v : v ratio of buffer and sample as previously [8,40], which is why the buffer was added as a concentrate. Through port B of the donor pump valve, this mixture was then dispensed with final mixing in the mixing coil into the donor channel of the membrane holder (marked with 'd' in Figure 2), using a flow rate of 5 mL min^{-1}. After passing through the donor channel of the membrane unit, where extraction takes place, the donor was led to waste. This process was repeated continuously in a programmed extraction loop until 3 L of sample has been processed (i.e. 316 times taking into account the mixing of sample and buffer), which made up the total volume for one sample.

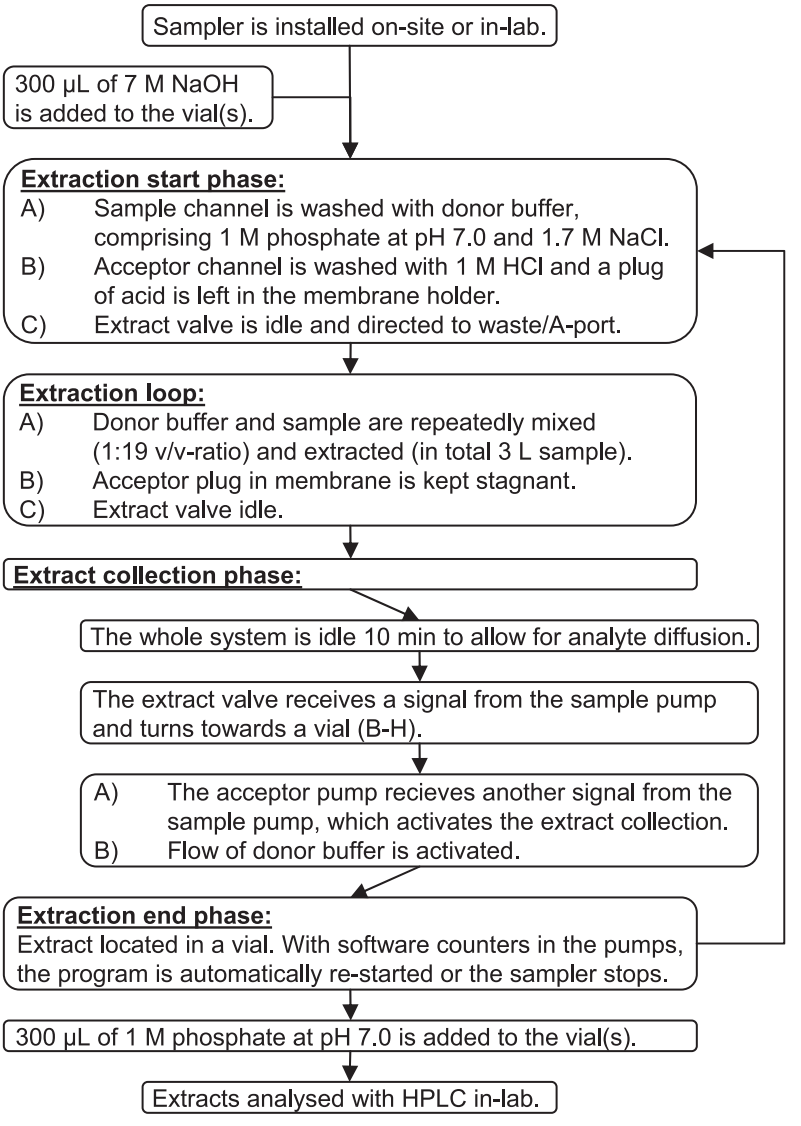

Figure 3. Simplified flow scheme of the automated extraction procedure.

The second pump was supplied with a 1 mL syringe, and controlled the flow of the acidic acceptor solution. Prior to extraction, the acid was first aspirated into the syringe through port A of the attached 4-port valve and then, through port B, dispensed into the acceptor channel of the membrane (marked with 'a' in Figure 2) with a flow rate of $0.5\,\mathrm{mL\,min^{-1}}$ [8,40]. The surplus acid was dispensed through port A of the 8-port extract valve. The acceptor solution was then kept stagnant in the membrane holder and the extract valve was directed against the A-port, until 3 L of sample had been extracted. Here, a decrease in method precision could arise, owing to pressure of the flowing donor on the membrane so that small volumes of the otherwise stagnant acceptor solution were pressed out. Therefore, we recommend that for future work with similar equipment the extract

valve has a port which is blocked. The acceptor channel shall be directed towards this blocked port during extraction.

When 3 L of sample had been extracted, the sample side of the membrane was rinsed with pure buffer for 10 min (effective time) and then left for diffusion of analyte into the acceptor for 10 min as previously optimised [40]. Thereafter, a signal was sent to the extract valve from the donor pump, causing the extract valve to direct towards one of the extract vials (B–H). Five seconds later, another signal was sent to the acceptor pump from the donor pump. Then, a fixed volume (1 mL × 2) of acid was aspirated by the acceptor pump from the bottle and dispensed into the acceptor channel of the membrane unit. The acceptor extract was thereby pumped through the valve and directed to one of the seven extract vials, where the extract was collected. During collection of the extract, donor buffer was kept flowing, which has been observed to increase overall enrichment [40]. 300 µL of 7 M NaOH had been manually added to the extract vials, prior to extraction start-up, in order to neutralise the obtained extracts directly as they were collected. After an extract had been collected into a vial, it was ensured that the extract would remain neutral until analysis by manually adding 300 µL of 1 M phosphate buffer at pH 7.0. Between extractions, both the donor and the acceptor channels were washed with fresh buffer or acidic acceptor solution, respectively. In total, the whole extraction procedure lasted about 24 h for each extract. Subsequent extraction(s) started automatically and the sampler terminated completely once all desired extracts had been obtained, by using software counters in the pumps.

2.6 *Description of sampling sites*

In Ethiopia, a variety of pesticides are seasonally applied in the country as a whole, but mostly in the lake regions [41]. Triazines have been used for the control of various weed species in sugarcane, cotton, maize and sorghum farming under different brand names [42] and are applied as pre- and post-emergence control between April and July, depending on the duration and intensity of the rainy season [8]. Atrazine (in combination with ametryne) is registered for control of grass weeds in sugarcane [42]. Prometryn (in combination with metolachlor) was registered for use against broadleaf weeds and grass weeds in cotton, but its registration has now been cancelled in 2009. Terbuthylazine (in combination with glyphosate) was registered for control of broadleaf weeds in coffee production, but its registration was also cancelled in 2009.

Hawassa Lake is located 270 km south of the capital of Ethiopia, Addis Ababa, in the Ethiopian Great Rift Valley, at an altitude of 1600–1700 m above sea level. It has a surface area of about 129 km^2, it holds 1.34 km^3 water and has a maximal depth about 21.5 m [43]. In the Hawassa area, sugarcane is produced in large amounts and coffee is also grown. Farms which annually consume plenty of pesticides are situated in close proximity to Hawassa Lake. The lake receives water mainly from a small river, Tikur Wuha, and from a number of small streams [43]. Low optical transparency in Great Rift Valley lakes is probably due to a high concentration of inorganic material [43] (Tikur Wuha means black water in the local language [8]). The rainy season in the Rift Valley is between June and September, with precipitation peaks in July and August. Carbonate dominates in terms of anions and the pH of the lake is about 8.8 [43]. Sum of common cations such as Na^+, K^+, $Mg^{2+}$and Ca^{2+} is rather low and the lake is rather dilute, which may be attributed to low concentrations in the inflow to the lake, possibly combined with underground seepage.

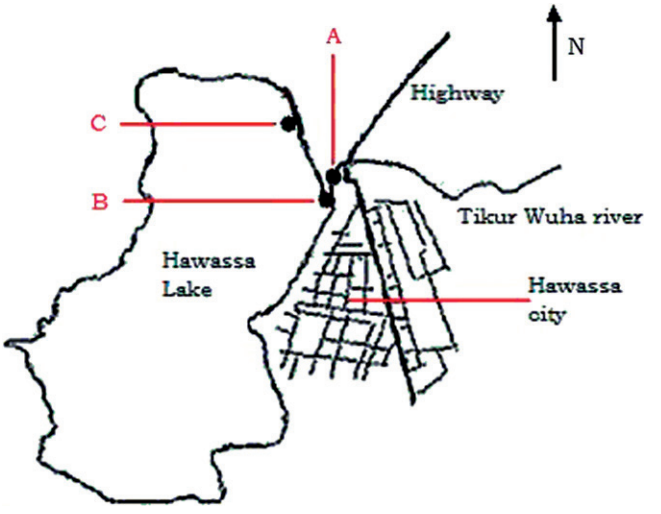

Figure 4. Hawassa Lake and the sampling sites, represented by •. A = Tikur Wuha river, B = outlet of the river into Hawassa Lake (junction) and C = lake body 1 km away from the river outlet.

Hawassa Lake otherwise appears to be a closed water basin with no surface water outflow [43]. The lake is used for fishery, but not as a resource for drinking water (private communication). The lack of surface water outflow could imply contamination build-up, which may harm the fishery. Underground seepage implies a risk for groundwater contamination.

On-site SLM extraction of the ten target *s*-triazines and their degradation products was carried out at Hawassa Lake and the river Tikur Wuha. Three sampling sites were selected in the northern part of the lake system (Figure 4). The first sampling site (A) was in the tributary river, ca. 200 m from its outlet in the lake. The second sampling site (B) was at the junction of the river and the lake. The depth of the water at this point was approximately 2 m and the sampler probe was stabilised at a depth of around 1 m. The third sampling site (C) was in the lake, about one kilometre away from sampling site B. It was considered to represent the free water mass of the lake (even though exact hydrological conditions were not studied). The water was clearer and shallower than at site 1, roughly 1 m deep, and the sampler probe was stabilised at a depth of about 0.5 m. Four sampling campaigns were carried out during the period from March 2003 to July 2004. The times and sites of each sampling campaign are summarised in Table 2.

2.7 *Extraction and enrichment factor*

The donor solution (subscript D) is the sample solution (S) to which buffer has been added in order to control sample pH. The enrichment factor, E_e, is the ratio of analyte concentration in the (stagnant) acceptor phase (C_A), to the concentration in the donor phase (C_D), as follows:

$$E_e = \frac{C_A}{C_D}. \quad (1)$$

Table 2. Times of sampling campaigns and sample sites where on-site extraction was conducted, marked by the number of replicates for that campaign and site. For location of sampling sites, see Figure 4.

Sampling campaign	Time of sampling	River (A)	Junction (B)	Lake (C)
1	March 2003		1	2
2	December 2003	3	2	3
3	February 2004	3		3
4	July 2004	3		1

The original sample concentration prior to buffer addition, C_D, will therefore be lower than C_S due to the slight dilution when buffer is added. Due to the dilution of the acceptor extract in connection with the harvesting of an extract into a vial and the addition of phosphate and sodium hydroxide, the extract concentration which is finally analysed, C_E, will be about 15.8 times lower than C_A.

3. Results and discussion

3.1 *Method optimisation for on-site extraction*

In order to adjust the sampler for on-site extractions, some modifications to the automated laboratory method optimised in our group [40] were made. Previously, the volume of buffer used to adjust the pH was as large as the sample itself. For practical reasons, the relative amount of buffer was significantly reduced, to 5% of the total volume. Consequently, the pH adjusting buffer had to be added in a more concentrated form.

It is known that the use of carriers enhances the extraction of polar analytes [44]. For the laboratory optimised method, 10% (w/v) tri-*n*-octylphosphine oxide TOPO was therefore dissolved in the membrane solvent, which increased extraction of the more polar analytes, i.e. the metabolites, while decreasing uptake of the parent herbicides [40]. However, it was also observed that when the membrane solvent contained a carrier, the obtained results were less reproducible. This could be due to the gradual removal of carrier by the sample solution in the donor phase, i.e. the SLM becomes less stable when it contains a carrier. It was suspected that SLM stability would turn out to be a more crucial issue when lake water samples were to be extracted. Therefore, no carrier was used in the membrane solvent in the extractions reported here.

3.2 *Performance of the field sampler*

The elution order of the substances was the same as the order presented in the earlier work [40] and listed in Table 1. The HPLC system was calibrated by duplicate injections of standards of the ten analytes. Peak height yielded higher correlation coefficients for the first seven eluting compounds, i.e. DDA–CYZN, while for the three last eluting analytes, i.e. ATZN–TRYN, peak area gave better correlation coefficients. Therefore, peak height was used for any further calibration and data analysis for DDA–CYZN and peak area was used for ATZN–TRYN. Determination coefficients (r^2) typically ranged from 0.97 to

0.99 in the range of 1–50 μg L^{-1}. However, lower r^2 of 0.91 and 0.95 were obtained for DDA and CYZN, respectively.

In order to evaluate the performance of the field sampler, E_e was obtained in the laboratory by extracting 3 litres of spiked reagent water containing each of the ten target analytes at a concentration of 10 μg L^{-1}. Enrichment factors were calculated using Equation 1 and are presented in Table 3. The results for E_e obtained in this work differed from the previous ones [40], though this was expected due to the absence of a carrier in the membrane solvent.

For some compounds, primarily DDA, DEA, ATOH and CYZN, the precision was rather low (RSD for $E_e > 30\%$ for these compounds). A reasonable explanation for lower precision of these specific analytes may be that DDA elutes close to the solvent peak and that DEA and ATOH elute close to each other and thus are not completely resolved [40]. The lower precision of CYZN is probably due to the lower r^2 in the calibration. Low precision could also be partly due to that the extract valve was directed towards the open A-port when the donor was extracted and pumped through the membrane holder, even though such an error would affect all analytes rather equally. For future work with similar equipment, we anyway recommend that the extract valve has a port which is blocked to prevent even slight movement of the acceptor solution during extraction.

Method detection limit (MDL) was calculated as three times the standard deviation of the signal obtained for blank in the HPLC, corrected for dilutions and enrichment factors. For a sample volume of 3 L reagent water the calculated MDL-values are summarised in Table 3. It may be argued that E_e obtained from extraction of spiked reagent water cannot represent E_e for environmental samples, since there will probably be matrix effects in real samples. However, it was previously observed that humic matter and urine (which is a somewhat complex matrix) did not markedly decrease SLM extraction of *s*-triazines [40].

3.3 *Applications to the extraction of s-triazine herbicides and their metabolites at Hawassa Lake*

Extracts from Hawassa Lake were analysed three times. The HPLC signals of environmental extracts were interpolated towards the calibration curves, multiplied with appropriate dilution factors and divided by the enrichment factors in Table 3, to calculate

Table 3. Enrichment factor (with RSD (%) for three consecutive extractions in brackets) for extraction of the spiked water samples at the concentration of 10 μg L^{-1} and method detection limit in reagent water.

Analyte	Enrichment factor, E_e	MDL (μg L^{-1})
DDA	28 (54)	0.55
DIA	1.3 (11)	3.2
DEA	11.5 (30)	0.40
ATOH	7.4 (47)	0.37
PROH	218 (19)	0.01
TZOH	295 (18)	0.01
CYZN	4.0 (49)	0.67
ATZN	25 (4)	0.12
PRYN	2267 (3)	0.003
TRYN	2739 (5)	0.002

Table 4. Concentration levels of *s*-triazine herbicides and their metabolites in Hawassa Lake ($\mu g\,L^{-1}$). For uncertainty of the reported concentrations, see text and Table 3.

	March 2003		December 2003			February 2004		July 2004	
Compound	Junction	Lake	River	Junction	Lake	River	Lake	River	Lake
DDA	14	19	299	23	Nd x3	8	83	25	32
		15	31	11		Nd x2	19	Nd x2	
			Nd				Nd		
DIA	1.6	18	79	Nd x2	Nd x3	Nd x3	Nd x3	Nd x3	Nd
		29	Nd x2						
ATOH	Nd	8.1	408	23	92	106	144	100	568
		2.9	28	Nd	104	24	Nd x2	639	
			Nd		86	80		422	
DEA	5.2	2.0	47	27	Nd x3	42	55	112	108
		3.3	32	29		Nd x2	104	135	
			Nd				171	191	
PROH	Nd	Nd x2	22	8	9	6	Nd x3	Nd x3	Nd
			8	8	11	9			
			14		10	11			
TZOH	0.04	0.09	1	0.1	0.1	0.3	0.2	Nd x2	Nd
		0.21	0.8	0.2	0.6	1.4	0.2	0.18	
			Nd		0.5	0.9	0.1		
CYZN	7.6	7.2	Nd x3	Nd x2	7	Nd x3	Nd x3	Nd x3	42
		0.6			Nd x3				
ATZN	0.88	Nd x2	Nd x3	Nd x2	Nd x3	Nd x3	Nd x3	Nd x3	Nd
PRYN	Nd	Nd x2	0.05	0.03	0.03	0.03	0.03	Nd x3	Nd
			0.43	0.03	0.03	0.03	0.03		
			0.24		0.03	0.05	0.02		
TRYN	Nd	Nd x2	0.04	Nd x2	Nd x3	Nd x3	0.04	Nd x3	Nd
			0.07				0.04		
			Nd				Nd		

Note: Nd = not detected for one, two (x2) or three (x3) of the total replicates at the given sampling campaign and sampling site. The number of replicates are presented in Table 2.

analyte concentrations in the river and lake. Table 4 shows the concentration levels of *s*-triazine herbicides and degradation products in the river and Hawassa Lake.

For each sampling campaign and sampling site, replicate on-site extraction was performed (Table 2). In some cases the replicates agreed well, while in other cases they deviated from each other. We have chosen not to calculate averages in this situation, but present the data for each sampling separately.

There is a tendency that the concentrations are more disparate in the river compared to the lake, which can be seen most clearly in the data from December 2003. This could be due to the levelling effect of the lake body when different amounts of pollutants are released upstream. However, the precision in enrichment factor (Table 3) can also cause deviations between replicates. The precision was rather low for DDA, DEA, ATOH and CYZN, and consequently the reported values of these specific compounds must be regarded with caution.

The parent herbicide ATZN was observed in the river junction on only one occasion, while CYZN and TRYN were detected on a few occasions. In contrast, PRYN was

frequently detected at sub-$\mu g\,L^{-1}$-levels. Half-life in river water of only about 60 days for ATZN and PRYN has been reported [45,46]. With increasing temperature, degradation of ATZN and PRYN (and two other *s*-triazines, simazine and terbuthylazine) has been shown to increase in groundwater matrix, while increased temperature in sea and river water had little effect except for ATZN, for which degradation increased with temperature in all three kinds of aqueous matrices [45]. The same *s*-triazines (ATZN, PRYN, simazine and terbuthylazine) have been shown to photodegrade in sea and groundwater, while photodegradation played only a minor role in river water [46]. The combined effect of temperature and photodegradation could explain why ATZN was generally not detected at Hawassa Lake, in spite of it being one of the most commonly used *s*-triazines in Ethiopia. The values for PRYN were more or less constant between sampling sites, but it was only detected for two of the sampling campaigns.

The dealkylated and hydroxylated metabolites were more frequently found and overall also in markedly higher concentrations than the parent *s*-triazines. This is consistent with degradation of the parent pesticides in the lake environment. Comparing with other studies on the environmental fate of *s*-triazines, ATZN is usually detected in higher concentrations than dealkylated degradation products (in terms of DEA and DIA) in river samples, as has been observed in the USA [7], Germany [6] and China [5]. However, in a Swiss monitoring study, concentrations of DEA equalled or surpassed those of ATZN in surface water [3]. In an Italian coastal lagoon, concentrations of hydroxylated metabolites (e.g. ATOH and TZOH) often surpassed the concentrations of the parent compounds [13]. In groundwater, only 0.2% of the total measured concentration of cyanazine compounds (including parent compound and degradation products) comprised the parent compound itself [14].

The higher presence of the metabolites may also stem from other *s*-triazines than the four parent herbicides included in this study, as degradation routes of different *s*-triazines are interconnected [12]. For example, TZOH can be formed following UV-photodegradation of TRYN, terbuthylazine and terbumeton [11], and terbuthylazine is probably used in the region of Hawassa Lake (see Section 2.6).

Regarding the actual levels of observed concentrations of both parent herbicides and degradation products, it can be noted that the concentrations of degradation products are markedly high. For example, in December 2003, DIA and PROH were detected in the river at levels up to 79 and 22 $\mu g\,L^{-1}$, respectively. In surface water, triazine herbicides are commonly detected in concentrations below 2 $\mu g\,L^{-1}$ (e.g. [3–8]). However, concentrations of up to 108 $\mu g\,L^{-1}$ atrazine and 61 $\mu g\,L^{-1}$ cyanazine in river water in an agricultural region in the USA have been reported [7]. In Croatia, stream water concentration of up to 8.3 $\mu g\,L^{-1}$ atrazine in stream water was reported [4]. Thus, the rather high concentrations reported here fall in the upper range of previously reported concentrations of *s*-triazines.

4. Conclusions

We have successfully demonstrated the practical utilisation of unattended automated on-site SLM extraction for environmental analysis. Such a system may be of use for on-site extraction in remote areas or in situations in which spot sampling is not sufficient. Depending on the sampling time, different resolutions in time-weighted average sampling can be obtained, but there will also be a trade-off between the sampled volume and the degree of enrichment.

In the case study of the Hawassa Lake system, it is indicated that atrazine, cyanazine and terbutryn do not accumulate in the studied aqueous environment, while prometryn was frequently found. Overall, degradation products of *s*-triazines were found in higher concentrations than parent herbicides. Even though reported concentrations of four of the target analytes were quantitatively somewhat uncertain, it still seems evident that *s*-triazines degrade and are not accumulated in Hawassa Lake. The degradation products sometimes occur in rather high concentrations in the lake, which has no surface outlet. The question thus arises whether degradation products, mainly DIA, PROH and TZOH, can be more toxic to the ecosystem than the parent *s*-triazines and whether they are more persistent.

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