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An alternative clean-up column for the determination of polychlorinated biphenyls in solid matrices†

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The need for continuous monitoring of polychlorinated biphenyls (PCBs) has necessitated the development of analytical techniques that are sensitive and selective with minimal reagent requirement. In light of this, we developed a column for clean-up of soil and sediment extracts, which is less demanding in terms of the amount of solvent and sorbent. The dual-layer column consists of acidified silica gel and molecularly imprinted polymers (MIPs). MIPs were synthesized *via* aqueous suspension polymerization using PCB 15 as the dummy template, 4-vinylpyridine as the functional monomer and ethylene glycol dimethacrylate as the cross-linker and the obtained particles characterized *via* SEM, BET, and batch rebinding assays. Pre-concentration of the spiked real-world water sample using MISPE gave recoveries between 85.2 and 104.4% (RSD < 8.69). On the other hand, the specific dual-layer column designed for clean-up of extracts from complex matrices provided recoveries of 91.6–102.5% (RSD < 4%) for spiked soil, which was comparable to clean-up using acidified silica (70.4–90.5%; RSD < 3.72%) and sulfoxide modified silica (89.7–103.0%; RSD < 13.0%). However, the polymers were reusable maintaining recoveries of 79.8–111.8% after 30 cycles of regeneration and re-use, thereby availing a cost-effective clean-up procedure for continuous monitoring of PCBs. Method detection limits were 0.01–0.08 ng g^{-1} and 0.002–0.01 ng mL^{-1} for solid matrices and water, respectively.

Environmental impact

Monitoring of polychlorinated biphenyls (PCBs) is of substantial interest due to the threat these chemicals pose to human and environmental health. Consequently, the development of advanced yet affordable analytical techniques will ensure that these compounds can be monitored at minimal cost. The present study demonstrates the advantages of utilizing molecularly imprinted polymers (MIPs) for the determination of polychlorinated biphenyls in complex matrices, thereby revealing the potential of tuneable sorbent materials for environmental analysis of such relevant pollutants.

1. Introduction

The chemical and physical stability exhibited by polychlorinated biphenyls (PCBs) made them major components in electrical equipment as coolants and lubricants, and additives in various open systems, thus rendering them ubiquitous environmental contaminants. It is the evidence that they cause adverse health effects in humans and animals that led to ban in production and use of these compounds in many countries in the 1970s. Though measures like adoption of the Stockholm convention on persistence organic pollutants (POPs) have been put in place to curb any further releases into the environment, monitoring of these compounds in the environment is of substantial interest, in order to protect humans and animals, and detect any illegal releases.

To this end, efforts have been geared towards developments of techniques that are rapid with minimal reagent requirement so as to facilitate monitoring of these compounds at an affordable cost. For analysis of PCBs in aqueous media, the conventional liquid–liquid extraction (LLE) has been largely replaced by solid phase extraction (SPE),[1] solid phase microextraction (SPME),[2] and star bar sorptive extraction (SBSE).[3] Although the last two techniques have almost eliminated the need for a solvent, SPE still remains a technique of choice attracting continuous development of new sorbent materials to replace the conventional C18 sorbent which is characterized by poor performance.[4] On the other hand, analysis of organic compounds in solid matrices has seen the introduction of ultrasonic assisted extraction (UAE),[5,6] microwave assisted extraction (MAE),[7,8] pressurized liquid extraction (PLE),[9–12] and super-critical fluid extraction (SFE),[13] as substitutes for the

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conventional Soxhlet extraction which is associated with long extraction hours and large amounts of solvent. Apart from PLE which has been designed to achieve both extraction and clean-up by incorporating clean-up sorbents in the extraction cell, all other techniques employ an additional purification step using silica gel, acidified silica gel, Florisil, alumina (or a combination of these materials), and Bio-beads S-X3.[14–16] In addition to these, alternative sorbents that impact selectivity and stability during sample purification have aroused scientific interest in the last 2 decades. The sorbents, *i.e.*, molecularly imprinted polymers (MIPs) are tailored to show selectivity to a specific compound or a group of similarly structured compounds, which then facilitates elimination of matrix interferences during sample purification.

Selectivity in these polymers is achieved by carrying out polymerization of functional monomers in the presence of a target analyte (a.k.a., template), followed by removal of the template leaving behind nanocavities that re-bind the target analyte with high specificity. In addition, MIPs have readily demonstrated their robustness as molecular recognition matrix in a variety of environments, and can be regenerated for multiple uses maintaining their performance even after 10 cycles of usage or more.[17]

MIPs in solid phase extraction, *i.e.*, MISPE, as either pre-concentration or clean-up sorbents is the most advanced application area, and some of the successful applications are in the study of citalopram in human serum and urine, PAHs in water, methamidophos in water and soil, and quercetin in red wine,[17–20] among many others. To the best of our knowledge, only three studies have reported imprinted polymers with recognition properties for PCBs using 1,2,3,4,5-pentachlorobenzene (1) and 1,2,3-trichlorobenzene (2),[21] 3,4-dichlorobenzene acetic acid,[22] and xylenes,[23] as dummy templates and porogenic templates, respectively. Although recognition of the PCBs was demonstrated, applications in environmental studies have only been reported for fish samples. Therefore, the focus of our study was to incorporate imprinted polymers in determination of 6 indicator PCBs in soil and sediments, which are important matrices for monitoring of these compounds. Our extraction technique was ultrasonic assisted extraction, followed by purification of the extracts on specific columns prepared using acidified silica gel and MIPs, and finally quantification using gas chromatography micro-cell electron capture detector (GC-μECD). The optimized method enabled determination of PCBs in real-world samples, provided well-defined chromatograms, and demonstrated minimal solvent requirement, thereby unveiling a reliable and affordable protocol.

2. Materials and methods

2.1 Chemicals and reagents

Chemicals and reagents were purchased from the following sources: PCB standards mixture (28, 52, 101, 138, 153, 180), PCB 14, 15 and 209, hexachlorobenzene (HCB), poly(vinyl alcohol) (PVA, M_w 13 000–23 000, 87–89% hydrolyzed), ethylene glycol dimethacrylate (EGDMA, >98%), 2,2′-azobis isobutyronitrile (AIBN), 4-vinylpyridine (4-VP, 95%), silver nitrate ($AgNO_3$), copper powder (<45 μm), Supelclean C18 SPE cartridges (6 mL, 500 mg, 51.7 μm, 490 $m^2\ g^{-1}$), and Supelclean sulfoxide SPE from Sigma-Aldrich (Steinheim, Germany); toluene, pesticide grade *n*-hexane (≥99%), methanol, dichloromethane (DCM), acetone, chloroform, empty SPE cartridges (6 mL) and frits (20 μm porosity) from Carl Roth Chemicals (Karlsruhe, Germany); silica gel 60 0.063–0.200 mm (70–230 mesh), sulphuric acid (95–97%) from Merck (Darmstadt, Germany); and nitrogen (99.999%) for gas chromatography from MTI IndustrieGase AG (Neu-Ulm, Germany). EGDMA and 4-VP were distilled under reduced pressure before use to remove the inhibitors, while water used was purified using a Milli-Q academic filter system (Millipore, Billerica, USA).

2.2 Sample collection

Sampling was done in Kenya in January 2014 where soil was collected from Dandora and Mt. Kenya, and sediment at five sites along Nairobi River which included: Ondiri, Kijabe, Outering, Eastern bypass, and Ruai (Fig. 1). Tap water was collected from a laboratory at the Institute of Analytical and Bioanalytical Chemistry, University of Ulm, whereas river and lake water were collected from the Danube River (Ulm) and Ludwigfelder See (Neu-Ulm). Water samples were contained in glass bottles and transported to the laboratory where they were filtered through 0.45 μm pore filters and stored at 4 °C until analysis.

2.3 Preparation of molecularly imprinted polymers by suspension polymerization

PCB 15 imprinted microspheres were prepared following a previously reported protocol by Lai *et al.*,[24] with slight modifications. Thus, the continuous phase was prepared by dissolving 2.0 g of PVA in 50 mL Milli-Q water at 95 °C while stirring, which was then allowed to cool to room temperature. Then, the organic phase consisting of PCB 15 (dummy template, 0.3 mmol), 4-VP (functional monomer, 4.8 mmol), EGDMA (cross-linker, 24 mmol), and AIBN (radical initiator, 2% mol of the polymerizable double bond) as the radical initiator dissolved in a mixture of toluene (5 mL) and chloroform (2 mL) was added to the aqueous phase while stirring at 1000 revolutions per min (rpm). The mixture was stirred for 5 min, and the suspension left under UV irradiation (50 W, 365 nm) at room temperature for 4 hours to allow polymerization to take place. A control polymer (a.k.a., non-imprinted polymers; NIPs) and a blank were synthesized using the same procedure, however, in the absence of the template for the NIPs and without the functional monomer and the template for the blank. The resulting particles were then wet filtered under vacuum using borosilicate filters with a pore size of 10–16 μm.

Removal of the template and unreacted monomers was achieved on ULEX[25] (*i.e.*, an extraction device developed at the Institute of Analytical and Bioanalytical Chemistry, University of Ulm) under sonication using methanol : acetic acid (90 : 10, v/v) until no traces of PCB 15 were detected within the extraction solution *via* GC-μECD. To ensure complete removal and avoid template bleeding, the polymers were packed into SPE cartridges and further treated with 12 mL of methanol followed

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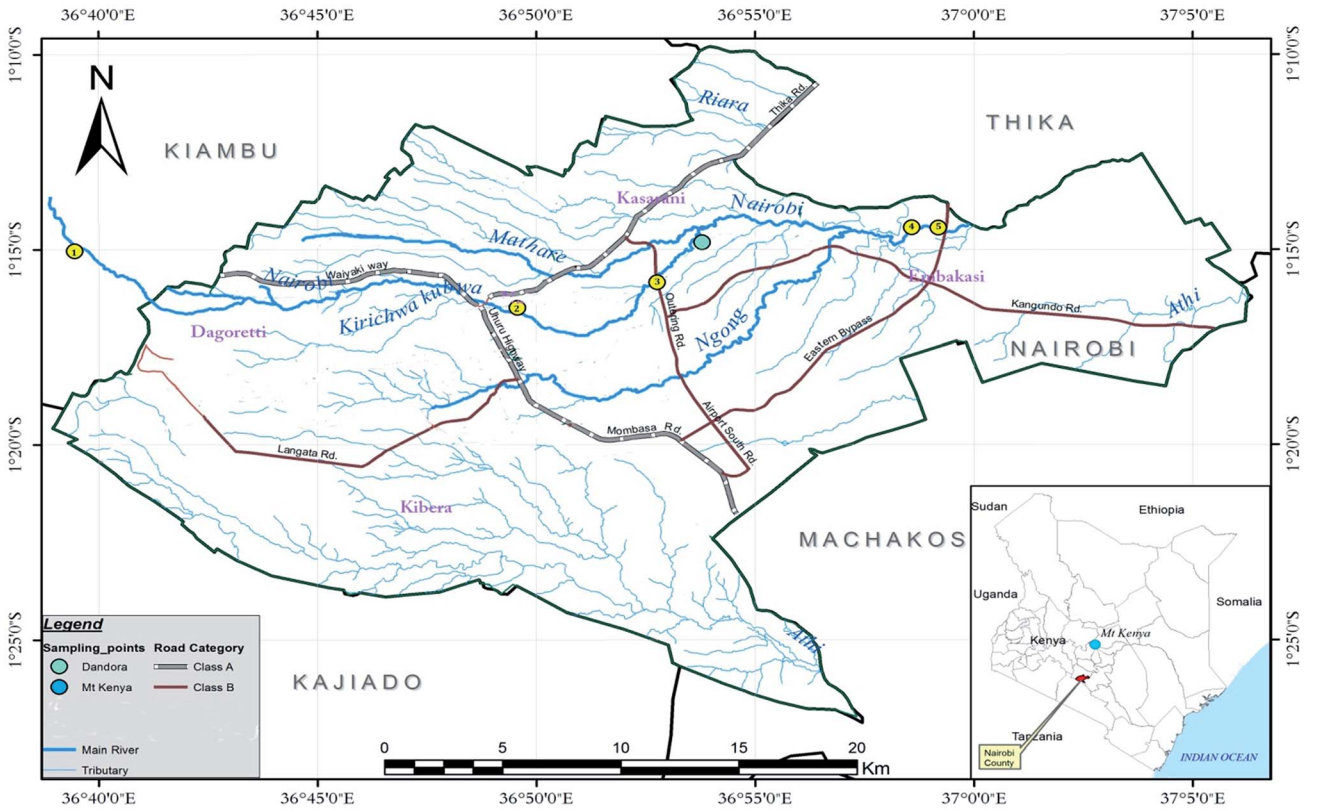

Fig. 1 Map of the study area showing 5 sampling sites along Nairobi River, *i.e.*, Ondiri (1), Kijabe (2), Outering (3), Eastern bypass (4), and Ruai (5); and two other sites, *i.e.*, Mt. Kenya and Dandora where soil was sampled.

by 12 mL of *n*-hexane : acetone (3 : 1, v/v). Twenty mg of the washed particles were then shaken with hexane for 4 h, and the supernatant was analyzed for the presence of the template, whereby absence of the peak of interest confirmed complete removal of the template. The MIP particles were then sieved under acetone to the desired size fraction using sieves of different mesh sizes, and dried in an oven under vacuum at 45 °C overnight.

2.4 Characterization of the polymer particles

The particle shape, size, and surface morphology were determined using a DualBeam Helios Nanolab 600 (Hillsboro, OR, USA) scanning electron microscope (SEM), while specific surface area, pore size, and pore volume were determined *via* the nitrogen adsorption–desorption BET and BJH method on particles that had been degassed for 4 h at 100 °C under vacuum prior to analysis.

2.5 Kinetics and equilibrium rebinding experiments

Thirty mg of the polymer particles was weighed into 2 mL tubes and 1.0 mL of 0.44 μg mL^{-1} PCB 15 standard in *n*-hexane was added and then vortexed for 3 h. To determine the extent of adsorption with time, the supernatant was analyzed after every 30 min. The tubes were centrifuged at 4000 rpm for 10 minutes and the supernatant was filtered through a 0.45 μm PTFE filter (VWR International GmbH, Darmstadt, Germany) into 1.5 mL GC autosampler vials, and the concentration of PCB 15 determined using GC-μECD. The amount of the bound analyte was determined by subtracting the final concentration from the initial concentration and dividing by the mass of the polymer used. After establishing the time required to reach equilibrium, the procedure was repeated with PCB 15 over the concentration range 0.088–0.968 μg mL^{-1}. Kinetic data were applied on pseudo-first-order and pseudo-second order kinetics models, while the binding parameters were calculated using Langmuir and Freundlich adsorption isotherms (see ESI†).

2.6 Optimization of molecularly imprinted solid phase extraction (MISPE)

Five hundred mg of the polymer particles (size fraction: 32–60 μm) were suspended in methanol, and then slurry packed into a 6 mL empty polypropylene cartridge with a frit at the top and bottom (20 μm porosity). Prior to use, the column was conditioned with 12 mL of methanol followed by 12 mL of *n*-hexane : acetone (3 : 1, v/v), and finally equilibrated with 6 mL of *n*-hexane. During these steps, the column was not allowed to dry. One mL (20 ng mL^{-1}) of PCB standard mixture in *n*-hexane was then introduced to the column at a flow rate of 0.5 mL min^{-1}, and the column was dried for 10 min under full vacuum. Elution of the adsorbed analytes was performed using 5 mL *n*-hexane : DCM (9 : 1, v/v), and reduced to near dryness under a gentle flow of argon then reconstituted in *n*-hexane to

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a volume of 1 mL for GC-μECD analysis. For comparison, conventional C18 and sulfoxide modified silica columns were processed similar to the MISPE cartridges, as well as the control non-imprinted solid phase extraction (NISPE) columns.

Validation of the MISPE cartridges for analytes in aqueous media was done by first conditioning the columns with 6 mL of methanol, followed by equilibration with 6 mL of Milli-Q water. Five mL of Milli-Q water (containing 125 μL of methanol, organic modifier) spiked at a concentration of 0.2 ng mL^{-1} was loaded onto the column and washed with 2 mL of methanol then dried under full vacuum for 15 min. The elution step and eluate treatment before analysis were as described above. C18 and sulfoxide cartridges were treated *via* the same procedure, however, washed with 2 mL of 10% methanol in water. The optimized MISPE protocol was then applied for real-world water samples spiked at 0.2 ng mL^{-1}; these samples were analyzed before spiking using the validated protocol to determine the level of contamination.

2.7 Preparation of specific clean-up columns

After establishing the performance of the MISPE cartridges using PCB standards, the specific MISPE column (composite-MISPE) was prepared by adding 750 mg of sulphuric acid impregnated silica gel (SiO_2–H_2SO_4) on top of the MIP beads column and then subjected further to a validation process using PCB standards.

2.8 Study of matrix effect and sample analysis

In order to determine the performance of the specific column in the presence of interfering matrices, real-world soil and sediment samples from background sites, *i.e.*, Mt. Kenya & Ondiri were spiked with the constituents of interest, and taken through extraction and clean-up steps following the validated protocol. The samples had been analyzed prior to spiking following the validated protocol to ascertain the initial level of contamination. Therefore, 5 g of soil spiked at 4 μg kg^{-1} was sonicated for 5 min with 20 mL of *n*-hexane : acetone (8 : 2, v/v), and the extract separated from the soil by centrifugation. Then it was transferred into a round bottomed flask and extraction repeated twice using 10 mL of the solvent mixture. The final volume was reduced to 1 mL using a rotary vacuum evaporator with the water bath set at 30 °C. The extract was then subjected to a clean-up procedure using the validated specific columns with an additional sulphur removal step as described in the ESI.† The sediment sample was subjected to similar steps, yet, were dried using K_2SO_4 before extraction. Once the recoveries were determined, the collected field samples were analyzed accordingly for the levels of PCBs.

2.9 Quantification of PCBs by GC-μECD

Gas chromatography was performed using an Agilent 6890 GC (Agilent Technologies) coupled to a micro-cell ECD detector (GC-μECD). Separation of PCBs was achieved on a ZB5-MS capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness with a 1 m deactivated fused silica guard column, which was connected to the analytical column through a glass capillary connector. The temperature program applied was an initial temperature of 60 °C (hold time 2 min), ramped at 15 °C min^{-1} to 210 °C (hold time 2 min), and finally ramped at 10 °C min^{-1} to 275 °C (hold time 5 min) resulting in a total GC run time of 25.5 min. The detector temperature was set at 280 °C. One μL of standards and samples was manually injected using the on-column injection mode. Nitrogen (>99.999% purity) was used as both carrier gas at a flow rate of 2 mL min^{-1} and detector make-up at 30 mL min^{-1}. Data were processed *via* Chemstation software version A.01.08 supplied by Agilent Technologies.

2.10 Quality control

Quality control measures involved matrix spike, cleaning of silica gel before activation, analysis of blank samples, replicate analysis, rinsing of glassware with acetone before use, and use of high purity standards and solvents. The method detection limit (MDL) was determined following the EPA guidelines[26,27] with K_2SO_4 and Milli-Q water as blank matrices for solids and water, respectively. The method is based on collecting and analysing a series of blanks spiked at a concentration corresponding up to 5 times the expected MDL *via* the same protocol. The MDL is then calculated as

$$\mathrm{MDL} = T_{(n-1,1-\alpha=0.99)} \times \mathrm{SD} \quad (1)$$

where $T_{(n-1,1-\alpha=0.99)}$ is the student's *t* value at $n-1$ degrees of freedom and at a 99% confidence level, n is the number of replicates, and SD is the standard deviation of replicate analyses.

Quantification was based on an internal standard calibration method using PCB 209 as the injection standard. Seven point calibration curves covering low, middle, and high concentrations of 0.25, 2, 5, 50, 100, and 200 ng mL^{-1} for all the PCB congeners were prepared. A chromatogram of 100 ng mL^{-1} PCB mixture standard is shown in the ESI, Fig. S1.†

3. Results and discussion

3.1 Microsphere synthesis

Suspension polymerization was the synthetic protocol of choice, as in contrast to conventional bulk polymerization, which requires extended procedures of polymer grinding and sieving leading to losses in the process.[28,29] The choice of the polymerization constituents, *i.e.*, the solvent or porogen, the functional monomer, and the cross-linker was also taken into consideration since they play a role in determining the performance of the resulting polymer. The porogen is particularly important because it governs the polymer morphology, the strength of non-covalent interactions, in addition to solubilizing the functional monomers.[30–33] In this study, we optimized a mixture of toluene (less polar) and chloroform (more polar) as porogen. Since imprinting of constituents such as PCBs which are poorly functionalized is a challenge, we chose the electron-rich 4-VP as our functional monomer in order to facilitate the only possible interactions, which are π-stacking of the aromatic rings.

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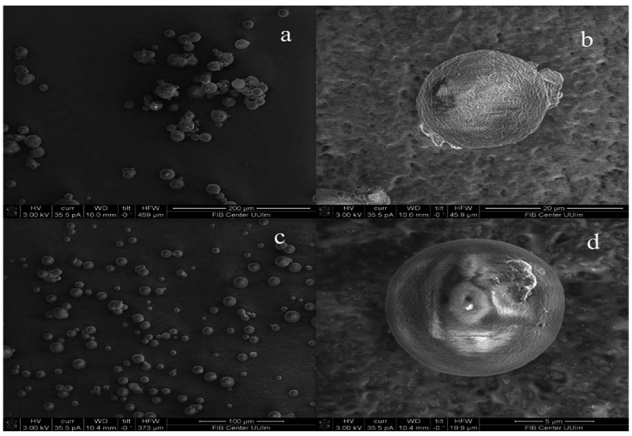

Fig. 2 Scanning electron micrographs of (a and b) MIP, and (c and d) NIP.

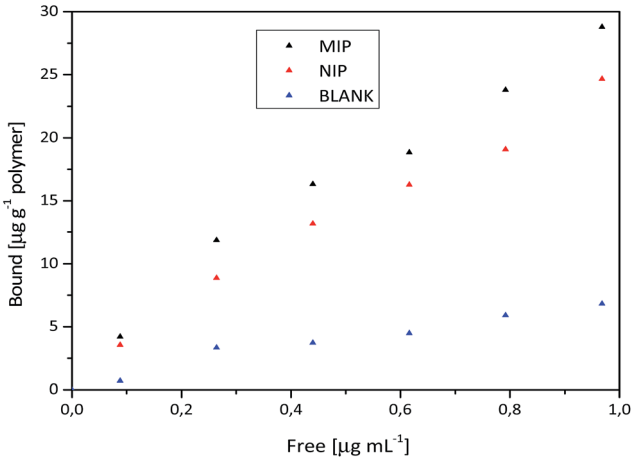

Fig. 3 Binding isotherms of MIP (black), NIP (red), and blank (blue).

The obtained particles were microspheres (Fig. 2) with dimensions ranging from 6 to 60 µm, and a repeat synthesis gave particles within this size range (RSD < 10%). The SEM images of the MIP revealed a comparatively rough surface as compared to the NIP, which was confirmed by the obtained BET surface area of 285.56 ± 20.36 and 134.45 ± 7.55 $m^2\ g^{-1}$ for the MIP and NIP respectively, and MIP pore volume of 0.69 ± 0.12 $cm^3\ g^{-1}$ *vs.* 0.33 ± 0.027 $cm^3\ g^{-1}$ for the NIP.

3.2 Template extraction

Template removal is a general challenge in molecular imprinting, and frequently complete removal is not achieved leading to template bleeding. A previous study established that up to 1.38% of the template remains incorporated within the polymer matrix even after extensive extraction,[34] which is detrimental for trace-level analytical applications of these materials. Incomplete removal of the template also limits the fraction of available binding sites for rebinding of the template. In the present study, template removal by the ULEX and monitoring by GC-µECD achieved almost complete removal (see ESI Fig. S2†). Owing to the low detection limit exhibited by the used GC-µECD (0.01–0.08 ng g^{-1}), the extraction process was considered sufficient for trace analytical applications of the generated MIPs. In addition, as a precautionary measure, we used PCB 15 as a dummy template to represent the 6 indicator PCBs targeted in our present study, meaning that any PCB 15 which bleeds out during the polymer applications does not affect the final quantification due to the different retention times exhibited by the molecules.

3.3 Binding characteristics of the polymer sorbents

To study the binding characteristics, batch rebinding assays were carried out at a concentration range of 0.088–0.976 µg mL^{-1}, where the adsorption capacities of all the polymers increased with increasing initial concentration; though slightly for the blank (Fig. 3). The MIP curve progressing slightly above the NIP, where the rather low imprinting effect realized was attributed to weak π–π interactions explored in the synthesis as PCBs are poorly functionalized molecules. On the other hand, the blank polymer prepared in the absence of both the template and the functional monomer revealed significantly lower binding capacities compared to both the MIP and NIP ($p < 0.05$). It is therefore concluded that 4-VP significantly increases the binding capacity by providing an electron-rich polymer matrix, which extensively interacts with PCB molecules. Introduction of the template during the imprinting process further enhances the binding capacity *via* the creation of binding sites specific to PCBs. The binding isotherms were well fitted on the Freundlich isotherm as was given by high correlation coefficients (Table 1). The MIP was slightly more heterogeneous than the NIP which is characteristic of imprinted polymers due to the formed binding cavities. In contrast, the blank gave a heterogeneity index almost equal to 1, indicating the homogeneous nature of the matrix due to lack of imprinting effect.

Selectivity studies by competitive batch rebinding assays showed that the MIP was indeed selective for PCB 15 among other related compounds as was given by the high binding capacities (see ESI Fig. S3†). PCB 14 – which has the same number of chlorine atoms as PCB 15 though a different substitution pattern – could also be recognized by the MIP in contrast to HCB, which has only one benzene ring. The different behaviour for the PCBs and HCB can be attributed to the dimensions of the binding sites entailed *via* the imprinting process binding well-fitting PCBs more tightly compared to HCB, thus yielding reduced binding capacities for the latter.

3.4 Optimization of the MISPE

This step focused on six "indicator PCBs" which have been globally proposed for monitoring in the environment.

Table 1 Langmuir and Freundlich adsorption isotherm constants

		MIP	NIP	Blank
Langmuir	Q_m (µg g^{-1})	60.2	54.9	23.5
	K_L (mL $µg^{-1}$)	0.8539	0.7344	0.4233
	R^2	0.9037	0.8924	0.3695
Freundlich	K_F (µg g^{-1})	29.5	24.3	7.60
	n	0.7742	0.7845	0.9035
	R^2	0.9882	0.9966	0.9412

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Optimization was done in both organic and aqueous media using *n*-hexane and Milli-Q water, respectively. In the organic media, *n*-hexane was used as the loading solvent due to its non-polar nature, and the fact that it is commonly applied as the extraction solvent for the analysis of chlorinated compounds in solid matrices. The elution solvents investigated herein were 3 mL of *n*-hexane : acetone (3 : 1, v/v) followed by 2 mL of DCM, and 5 mL of *n*-hexane : DCM (9 : 1, v/v), which yielded almost similar recoveries, *i.e.*, 89.4–99.15% and 90.4–99.0% respectively; the latter solvent was selected because of its reduced polarity, which should limit the elution of polar interferences. MISPE and NISPE cartridges provided recoveries of 94.9–99.0% and 90.4–98.9%, respectively, which were higher than 69.2–78.9% for C18 (see ESI Table S1†). The inability of C18 to retain non-polar compounds is also confirmed by recoveries of 25.5%, 58.8%, 1–97% for caffeine in green tea, benzo[*a*]pyrene (BAP) in instant coffee, and 16 PAHs in acetonitrile.[24,35,36] C18 adsorbent retains molecules through hydrophobic forces while the MIPs employed additional π–π interaction forces, and molecular recognition thus increasing chances of high recoveries. On the other hand, commercially available sulfoxide-modified silica gave recoveries between 83.8 and 90.2% in organic media. These high recoveries were attributed to interactions between the π-electron cloud of the PCBs aromatic moieties and the rather electrophilic sulfur.

For potential applications of the polymers in enrichment of PCB in contaminated aqueous samples, optimization was done using Milli-Q water at a pH of 7 giving recoveries >70%. C18 and sulfoxide modified silica gave recoveries of 68.1–73.0% and 35.6–83.1% respectively, which decreased to 33.9–67.7% and 17.9–56.5% with increase in flow rate from 1 mL min^{-1} to 5 mL min^{-1}. The performance of the two sorbents in aqueous phase was contrary to what was realized in organic phase, thus ruling out their application in aqueous media. The MISPE cartridge was further applied for pre-concentration of PCBs in real-world samples which consisted of tap (pH = 7.69), river (pH = 8.42), and lake water (pH = 8.12). The samples had been analyzed before spiking providing no measurable signals. Recoveries >80% were recorded for all the water samples (Table 2). The reproducibility of the method expressed as relative standard deviation (RSD) was in the range of 0.3–8.7%. The water samples had been spiked at a concentration of 0.2 ng mL^{-1} which is less than 0.5 ng mL^{-1} maximum allowable contaminant levels for PCBs in drinking water (as given by US environmental protection agency, EPA),[27] meaning that the polymers can be applicable in monitoring of PCBs. Molecular recognition by the polymers is further demonstrated by the high recoveries given by PCB 138, 153, and 180 which have two chlorine atoms at the *para* positions just like the template used, *i.e.*, PCB 15. In addition, the high chlorinated compounds are much electron poor and therefore have got enhanced π–π interaction with the electron-rich polymer matrix.

Table 2 Recovery (% ± SD) of 6 PCBs in real-world water samples spiked at 0.2 ng mL^{-1}[a]

	Tap water	River water	Lake water
PCB 28	83.6 ± 5.20	96.0 ± 8.34	83.7 ± 5.87
PCB 52	85.2 ± 2.68	88.2 ± 1.06	83.5 ± 4.03
PCB 101	95.6 ± 0.28	94.1 ± 1.70	92.4 ± 5.80
PCB 153	98.9 ± 1.59	103.1 ± 3.11	95.9 ± 2.67
PCB 138	100.9 ± 2.04	97.1 ± 2.97	95.8 ± 6.01
PCB 180	97.8 ± 5.48	104.4 ± 4.31	98.5 ± 1.06

[a] SD is the standard deviation of replicate analysis.

3.5 Optimization of the specific clean-up columns and study of the matrix effect

Due to the complexity of certain matrices, a simple MISPE may not be sufficient for removal of all interfering components, and some studies have proposed a two-step process whereby the sample extract is first passed through a pre-column packed with either the non-imprinted polymer, C18, or even restricted access materials (RAMs), and then further cleaned-up using MISPE.[37–39] In our case, we modified the MISPE cartridge for clean-up of soil and sediment extracts by incorporating acidified silica gel (SiO_2–H_2SO_4) which helped in the removal of lipids that may be present in soil and sediment extracts.[40,41] Validation of the composite-MISPE column using PCB standards gave recoveries ranging from 89.6–96.0%, and an optimized elution volume of 5 mL *n*-hexane : DCM (9 : 1, v/v). Acidified silica, which is among the conventionally applied sorbents in clean-up was also optimized resulting in recoveries of 92.1–94.9%, and elution volumes of 10 mL *n*-hexane : DCM (9 : 1, v/v). To optimize the whole procedure from extraction to clean-up, we used spiked real-world samples. We tested two solvent systems in the extraction step, *i.e.*, 9 : 1 and 8 : 2 of *n*-hexane : acetone which were less polar compared to the commonly reported *n*-hexane : acetone (3 : 1 or 1 : 1)[1,42,43] or *n*-hexane : DCM (1 : 1).[44] PCB recoveries were determined by subtracting the peak values of non-spiked soil and sediments from the spiked samples which resulted in recoveries in the range of 62.9–79.5% and 91.6–102.5% for the two solvent systems studied herein. The second solvent system (*n*-hexane : acetone; 8 : 2) gave the best recoveries and was adopted for sediment resulting in recoveries of 70.2–94.6%. In addition to increasing the polarity of the extraction solvent, the extraction time was reduced to 15 min with three extraction cycles of 5 min each, compared to one continuous extraction cycle of 30 min using the first solvent system (*n*-hexane : acetone; 9 : 1). Acidified silica resulted in recoveries between 70.4 and 90.5% for spiked soil samples. In addition, commercially available sulfoxide-modified silica was tested for clean-up, which resulted in recoveries between 89.7 and 103.0% (Table 3). This sorbent was initially developed for the extraction of PCBs from oil transformers, waste, and mineral oils. The results obtained in the present study confirm that it is also applicable as a clean-up sorbent for the determination of PCBs in solid matrices.

The developed clean-up method produced highly refined extracts with substantially reduced matrix interferences, as demonstrated by the baseline of the associated chromatograms (see ESI, Fig. S4–S6†) and recoveries within the recommended range for analysis of PCBs (70–120%).[45] The protocol was

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Table 3 Recoveries (% ± SD) of PCBs in background soil and sediments spiked at 4 ng g^{-1}[a]

	Soil			Sediment
	MISPE	Acidified silica	Sulfoxide-modified silica	MISPE
PCB 28	92.6 ± 1.13	83.0 ± 0.28	97.0 ± 2.19	84.6 ± 0.86
PCB 52	102.5 ± 2.12	90.5 ± 0.49	103.0 ± 13.4	73.0 ± 1.47
PCB 101	91.6 ± 0.47	70.4 ± 2.62	89.7 ± 1.06	70.2 ± 9.49
PCB 153	97.0 ± 4.27	73.6 ± 0.57	91.9 ± 1.34	72.5 ± 6.98
PCB 138	94.5 ± 1.05	75.3 ± 1.34	94.7 ± 1.56	73.3 ± 8.27
PCB 180	92.0 ± 1.36	73.7 ± 2.33	91.5 ± 0.71	94.6 ± 3.19

[a] SD is the standard deviation of replicate analysis.

comparable to the acidified silica sorbent and sulfoxide-modified silica in terms of recoveries and removal of matrix interferences (see ESI, Fig. S7 & S8†), suggesting that it could be used as an alternative for the determination of PCBs. The polymers, however, offer an additional advantage of re-usability (see ESI, Fig. S10†), which therefore makes monitoring of PCBs affordable. In summary, to process one sample, a total of 40 mL solvent was required for extraction, and 9 mL for clean-up.

3.6 Levels of PCBs in real-world soil and sediments

Mt. Kenya is considered an ideal background site for the analysis of POPs in Kenya due to its remote location. Background PCB levels were below the detection limits except for PCB 153 and 138, which were determined at 0.07 and 1.43 ng g^{-1}, respectively (Table 4). The presence of these higher-chlorinated PCBs can be related to the fact that they are less volatile, and therefore bind more strongly to solid matrices. On the other hand, PCB 153, 138, and 180 have been reported to be more dominant in the environment.[46,47] Levels of PCBs in Dandora ranged from 0.22–6.74 ng g^{-1}, with PCB 28 giving the lowest concentration and PCB 153 the highest. Compared to the earlier survey in 2008 by UNEP on POP levels in Africa, which reported PCB levels below the limit of quantification (LOQ) for Mt. Kenya soils, and levels ranging from 0.7–1.9 ng g^{-1} in Dandora,[48] the levels of PCBs in the present study were high in Dandora which could be attributed to its proximity to a municipal waste disposal, where continuous human activities like burning of waste may be contributing to PCB burden in the environment close by.

The other matrix studied was sediment, which is important for monitoring of organics in aquatic systems as it is considered a sink for these compounds which bind to sediments owing to their high octanol–water partition coefficient (K_{ow}).[49,50] Sediment samples had contamination levels ranging from 0.04–1.94 ng g^{-1} (Table 4). The level of PCBs increased along the river profile except at the Eastern bypass, which recorded lower levels probably due to its location away from human influence. The sampling site downstream of the river, *i.e.*, Ruai had the highest ∑PCBs of all the samples collected along the Nairobi River profile, where the rather high levels were attributed to additional contributions of the PCBs by the industrial and municipal effluents discharged into the river. Outering recorded the second highest levels of PCB contamination. The site is subjected to numerous human activities in addition to burning of plastic waste – especially tyres – in the area, and also due to its close proximity to light industries. As was expected, Ondiri which was our background site and the source of Nairobi River was less contaminated mainly due to its location in the upstream where there is limited human influence.

Compared to past studies in East Africa, levels of PCBs in sediments in the present study were slightly higher than what has been reported along the Kenyan coast (0.15–1.16 ng g^{-1} dry weight),[51] Winam Gulf, Lake Victoria, Kenya (6.9×10^{-5} to 4×10^{-4} ng g^{-1}),[52] Napoleon Gulf, Lake Victoria, Uganda (3.6×10^{-4} to 8.4×10^{-4} ng g^{-1}),[16] and less than levels reported at the Kavirondo Gulf, Lake Victoria, Kenya (bdl-60 ng g^{-1}).[53] In relation to other parts of the world, PCB levels in the present study were comparable except in extremely polluted areas. The reported PCB levels are 0.33–8.08 ng g^{-1} in Lake Bosumtwi, Ghana,[42] 0.019–1.206 ng g^{-1} dry weight in Ghal El Melh lagoon in Tunisia,[54] and 0.03–1.00 ng g^{-1} dry weight in Xiamen offshore area of China.[10] Exceptionally, high levels of PCB contamination have been reported in Kentucky Lake, USA (11–660 ng g^{-1} for PCB 180), North west Persian Gulf, Iran (100–18 400 ng g^{-1}), Scheldt River, Belgium (14.8–46.4 ng g^{-1} for PCB 153), where the high levels were linked to a transformer manufacturing company and industrial waste water discharge.[43,46,55]

3.7 Method performance

K_2SO_4 and Milli-Q water were used as the blank matrices and subjected to the validated protocol for soil and aqueous samples respectively, revealing no peaks for the six PCBs, thus

Table 4 Levels of PCBs (ng g^{-1} dry weight) (mean ± SD) in surface soil and sediments (0–5 cm top layer); $n = 3$

	Mt. Kenya[a]	Dandora[a]	Ondiri[b]	Kijabe[b]	Outering[b]	Eastern bypass[b]	Ruai[b]
PCB 28	Nd	0.22 ± 0.05	0.23 ± 0.07	0.68 ± 0.10	0.73 ± 0.43	1.03 ± 0.35	1.21 ± 0.43
PCB 52	Nd	0.88 ± 0.10	Nd	nd	0.49 ± 0.16	0.39 ± 0.10	1.94 ± 0.36
PCB 101	Nd	1.78 ± 0.005	Nd	nd	0.37 ± 0.0004	nd	0.66 ± 0.06
PCB 153	0.07 ± 0.03	3.51 ± 0.15	Nd	0.05 ± 0.01	0.13 ± 0.05	nd	0.54 ± 0.04
PCB 138	1.43 ± 0.20	6.74 ± 0.26	Nd	1.68 ± 0.14	1.54 ± 0.27	0.09 ± 0.02	1.15 ± 0.34
PCB 180	Nd	0.78 ± 0.02	Nd	0.44 ± 0.36	0.11 ± 0.0006	0.04 ± 0.007	0.20 ± 0.02
∑PCBs	1.50	13.91	0.23	2.85	3.37	1.41	5.70

[a] Soil. [b] Sediment, nd = not detected, SD = standard deviation.

Table 5 Analytical figures of merit for the proposed method

Congener	Linear range (ng mL^{-1})	Equation	Linearity (R^2)	Method detection limit (MDL)	
				Solids (ng g^{-1})	Water (ng mL^{-1})
PCB 28	0.465–186	$y = 0.015x + 0.0217$	0.9997	0.08	0.01
PCB 52	0.465–186	$y = 0.0073x + 0.014$	0.9998	0.05	0.007
PCB 101	0.26–104	$y = 0.0102x + 0.0159$	0.9996	0.02	0.003
PCB 153	0.21–85	$y = 0.0153x + 0.0176$	0.9997	0.02	0.003
PCB 138	0.23–92	$y = 0.0254x + 0.0242$	0.9987	0.02	0.003
PCB 180	0.22–89	$y = 0.0279x + 0.0174$	0.9998	0.01	0.002

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indicating no cross-contamination originating from the solvents and glassware. The calibration functions were established by plotting normalized peak heights (*i.e.*, ratio of the analyte peak and the PCB 209 response) *vs.* the concentration of the analyte. An exemplary sample calibration function for PCB 180 is shown in the ESI, Fig. S9.† Excellent linearity was obtained for all the compounds in the relevant concentration range of 0.21–186 ng mL^{-1} with correlation coefficients (R^2) ranging from 0.9987–0.9998. Method detection limits were 0.01–0.08 ng g^{-1} and 0.002–0.01 ng mL^{-1} for solid matrices and water respectively, while limit of quantification ranged between 0.05–0.28 ng g^{-1} and 0.008–0.04 ng mL^{-1} (Table 5). The detection limits realized were within those reported using other clean-up methods,[1,12,56] meaning that our developed protocol could as well be applied for the determination of PCBs in the environment. Since the cartridge was reusable, after the validation steps and sample analysis, it was subjected to recovery tests using the background samples which resulted in recoveries of 79.8–111.8% (see ESI, Fig. S10†), thus confirming the stability of the cartridge for environmental applications.

4. Conclusions

The present study reports a first time clean-up cartridge which incorporates imprinted polymers in the determination of PCBs in complex matrices. The developed protocol from extraction to instrumental quantification reported minimal solvent requirement, *i.e.*, approximately 50 mL for both the extraction and clean-up. In addition, the adopted clean-up method resulted in neat extracts with minimal interference levels, as indicated by well-defined chromatograms. The method was comparable to other conventional clean-up strategies, meaning that the cartridge can serve as alternative to conventional techniques, offering additional advantages of reusability. For the determination of PCBs in aqueous samples, the MISPE outperformed both the C18 and sulfoxide modified silica, thereby availing a reliable cartridge for monitoring of these compounds at their environmentally relevant low concentrations.

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References

1 I. Javedankherad, A. Esmaili-Sari and N. Bahramifar, *Bull. Environ. Contam. Toxicol.*, 2013, **90**, 285–290.
2 X. Song, J. Li, L. Chen, Z. Cai, C. Liao, H. Peng and H. Xiong, *J. Braz. Chem. Soc.*, 2012, **23**, 132–141.
3 E. Pérez-Carrera, V. M. L. León, A. G. Parra and E. González-Mazo, *J. Chromatogr. A*, 2007, **1170**, 82–90.
4 C. E. Mackintosh, J. A. Maldonado, M. G. Ikonomou and F. A. P. Gobas, *Environ. Sci. Technol.*, 2006, **40**, 3481–3488.
5 M. E. Aydin, S. Ozcan and A. Tor, *Clean*, 2007, **35**, 660–668.
6 M. Martínez-Parreño, J. Llorca-Pórcel and I. Valor, *J. Sep. Sci.*, 2008, **31**, 3620–3629.
7 P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding and G. Jiang, *Anal. Chim. Acta*, 2010, **663**, 43–48.
8 R. M. Criado, R. I. Pereiro and C. R. Torrijos, *J. Chromatogr. A*, 2003, **985**, 137–145.
9 R. Barra, P. Popp, R. Quiroz, C. Bauer, H. Cid and W. Tümpling, *Chemosphere*, 2005, **58**, 905–915.
10 Q. Li, Z. Luo, C. Yan and X. Zhang, *Bull. Environ. Contam. Toxicol.*, 2011, **87**, 372–376.
11 E. Cocco, C. Guignard, L. Hoffmann and T. Bohn, *Int. J. Environ. Anal. Chem.*, 2011, **91**, 333–347.
12 M. A.-E. Abdallah, D. Drage and S. Harrad, *Environ. Sci.: Processes Impacts*, 2013, **15**, 2279–2287.
13 S. Sporring, S. Bøwadt, B. Svensmark and E. Björklund, *J. Chromatogr. A*, 2005, **1090**, 1–9.
14 M. Nichkova, E.-K. Park, M. E. Koivunen, S. G. Kamita, S. J. Gee, J. Chuang, J. M. Van Emon and B. D. Hammock, *Talanta*, 2004, **63**, 1213–1223.
15 P. Suchan, J. Pulkrabová, J. Hajšlová and V. Kocourek, *Anal. Chim. Acta*, 2004, **520**, 193–200.

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16 P. Ssebugere, M. Sillanpää, B. T. Kiremire, G. N. Kasozi, P. Wang, S. O. Sojinu, P. O. Otieno, N. Zhu, C. Zhu, H. Zhang, H. Shang, D. Ren, Y. Li, Q. Zhang and G. Jiang, *Sci. Total Environ.*, 2014, **481**, 55–60.
17 R. J. Krupadam, M. S. Khan and S. R. Wate, *Water Res.*, 2010, **44**, 681–688.
18 M. Abdouss, S. Azodi-Deilami, E. Asadi and Z. Shariatinia, *J. Mater. Sci.: Mater. Med.*, 2012, **23**, 1543–1552.
19 Z.-L. Shen, D. Yuan, Q.-D. Su, H. Zhang, J. Wang, J.-H. Zhu and Y.-M. Liu, *Biosci., Biotechnol., Biochem.*, 2011, **75**, 473–479.
20 A. Molinelli, R. Weiss and B. Mizaikoff, *J. Agric. Food Chem.*, 2002, **50**, 1804–1808.
21 D. Cleland and A. McCluskey, *Org. Biomol. Chem.*, 2013, **11**, 4646–4656.
22 X. Du, S. Lin, N. Gan, X. Chen, Y. Cao, T. Li and P. Zhan, *J. Sep. Sci.*, 2014, **37**, 1591–1600.
23 K. Hosoya, K. Yoshizako, H. Sasaki, K. Kimata and N. Tanaka, *J. Chromatogr. A*, 1998, **828**, 91–94.
24 J. Lai, R. Niessner and D. Knopp, *Anal. Chim. Acta*, 2004, **522**, 137–144.
25 S. Eppler, M. Stegmaier, F. Meier and B. Mizaikoff, *Anal. Methods*, 2012, **4**, 2296–2299.
26 K. Gomez-Taylor, M. Kahn, H. D. Telliard, W. A. Ditthavong, K. Kopylev, L. McCarty, H. Riddick, L. Miller, K. Cuddeback, J. Rushneck, D. Dedah and S. Stralka, *Technical Support Document for the Assessment of Detection and Quantitation Approaches*, US Environmental Protection Agency, Washington, DC, 2003.
27 D. Muir and E. Sverko, *Anal. Bioanal. Chem.*, 2006, **386**, 769–789.
28 L. Chen, S. Xu and J. Li, *Chem. Soc. Rev.*, 2011, **40**, 2922–2942.
29 B. Danielsson, *Adv. Biochem. Eng./Biotechnol.*, 2008, **109**, 97–122.
30 F. Horemans, A. Weustenraed, D. Spivak and T. J. Cleij, *J. Mol. Recognit.*, 2012, **25**, 344–351.
31 C.-Y. Liu and C.-C. Lin, *Electrophoresis*, 2004, **25**, 3997–4007.
32 P. Cormack and A. Elorza, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2004, **804**, 173–182.
33 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812–1832.
34 F. Meier, S. M. Elbert and B. Mizaikoff, *Anal. Methods*, 2012, **4**, 2755–2758.
35 Y. Jin and K. H. Row, *Bull. Korean Chem. Soc.*, 2007, **28**, 276–280.
36 W.-L. Ho, Y.-Y. Liu and T.-C. Lin, *Environ. Eng. Sci.*, 2011, **28**, 421–434.
37 F. G. Tamayo, J. L. Casillas and A. Martin-Esteban, *Anal. Bioanal. Chem.*, 2005, **381**, 1234–1240.
38 W. M. Mullett, M. Walles, K. Levsen, J. Borlak and J. Pawliszyn, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2004, **801**, 297–306.
39 C. Cacho, E. Turiel, A. Martín-Esteban, C. Pérez-Conde and C. Cámara, *Anal. Bioanal. Chem.*, 2003, **376**, 491–496.
40 F. Smedes and J. de Boer, *TrAC, Trends Anal. Chem.*, 1997, **16**, 503–517.
41 L. De Química and S. Agrícola, *J. Braz. Chem. Soc.*, 2013, **24**, 743–748.
42 S. Afful, J. Awudza, S. Twumasi and S. Osae, *Chemosphere*, 2013, **93**, 1556–1560.
43 A. Covaci, A. Gheorghe, S. Voorspoels, J. Maervoet, E. Steen Redeker, R. Blust and P. Schepens, *Environ. Int.*, 2005, **31**, 367–375.
44 J. Szlinder-Richert, Z. Usydus and A. Drgas, *J. Environ. Monit.*, 2012, **14**, 2100–2107.
45 L. Webster, P. Roose, B. Bersuder, M. Kotterman, M. Haarich and K. Vorkamp, *ICES Tech. Mar. Environ. Sci.*, 2013, **53**, 1–19.
46 M. Zahed, N. Bidhendi, A. Pardakhti, A. Esmaili-Sari and S. Mohajeri, *Bull. Environ. Contam. Toxicol.*, 2009, **83**, 899–902.
47 F. Wong, M. Robson, M. L. Diamond, S. Harrad and J. Truong, *Chemosphere*, 2009, **74**, 404–411.
48 J. D. Nzila, M. Ali, V. O. Madadi, H. K. Traore, E. Masanja and N. Belmikki, *Global monitoring plan for persistent organic pollutants: First regional draft monitoring report Africa region*, UNEP, 2009.
49 E. Hiller, L. Zemanová, M. Sirotiak and L. Jurkovič, *Environ. Monit. Assess.*, 2011, **173**, 883–897.
50 T. Floehr, H. Xiao, B. Scholz-Starke, L. Wu, J. Hou, D. Yin, X. Zhang, R. Ji, X. Yuan, R. Ottermanns, M. Roß-Nickoll, A. Schäffer and H. Hollert, *Environ. Sci. Pollut. Res. Int.*, 2013, **20**, 6934–6971.
51 J. Everaarts, V. Weerlee, C. Fischerm and T. J. Hillebrand, *Mar. Pollut. Bull.*, 1998, **36**, 492–500.
52 S. Omwoma, J. O. Lalah, M. Virani, K.-W. Schramm and B. Henkelmann, *Chemosphere*, 2015, **118**, 143–147.
53 S. O. Wandiga and V. O. Madadi, in *Handbook of Water Purity and Quality*, ed. A. Satinder, Academic Press, UK, 1st edn, 2009, pp. 39–65.
54 W. Ameur, S. Trabelsi, B. El Bedoui and M. Driss, *Bull. Environ. Contam. Toxicol.*, 2011, **86**, 539–544.
55 B. G. Loganathan, K. S. Kumar, S. Masunaga and K. S. Sajwan, *Arch. Environ. Contam. Toxicol.*, 2008, **54**, 20–30.
56 P. M. Hoai, N. T. Ngoc, N. H. Minh, P. H. Viet, M. Berg, A. C. Alder and W. Giger, *Environ. Pollut.*, 2010, **158**, 913–920.

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