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Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins

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Abstract:

Marine biotoxins are algal metabolites that can accumulate in fish or shellfish and render these foodstuffs unfit for human consumption. These toxins, released into seawater during algal occurrences, can be monitored through passive sampling.

Acetone, methanol and isopropanol were evaluated for their efficiency in extracting toxins from algal biomass. Isopropanol was chosen for further experiments thanks to a slightly higher recovery and no artifact formation. Comparison of Oasis HLB, Strata-X, BondElut C18 and HP-20 sorbent materials in SPE-mode led to the choice of Oasis HLB, HP-20 and Strata-X. These three sorbents were separately exposed as passive samplers for 24 h to seawater spiked with algal extracts containing known amounts of okadaic acid (OA), azaspiracids (AZAs), pinnatoxin-G (PnTX-G), 13-desmethyl spirolide-C (SPX1) and palytoxins (PlTXs). Low density polyethylene (LDPE) and silicone rubber (PDMS) strips were tested in parallel on similar mixtures of spiked natural seawater for 24 h. These strips gave significantly lower recoveries than the polymeric sorbents. Irrespective of the toxin group, the adsorption rate of toxins on HP-20 was slower than on Oasis HLB and Strata-X. However, HP-20 and Strata-X gave somewhat higher recoveries after 24 h exposure. Irrespective of the sorbent tested, recoveries were generally highest for cyclic imines and OA group toxins, slightly lower for AZAs, and the lowest for palytoxins.

Trials in re-circulated closed tanks with mussels exposed to *Vulcanodinium rugosum* or *Prorocentrum lima* allowed for further evaluation of passive samplers. In these experiments with different sorbent materials competing for toxins in the same container, Strata-X accumulated toxins faster than Oasis HLB, and HP-20, and to higher levels. The deployment of these three sorbents at Ingril French Mediterranean lagoon to detect PnTX-G in the water column showed accumulation of higher levels on HP-20 and Oasis HLB compared to Strata-X.

This study has significantly extended the range of sorbents for passive sampling of marine toxins. In particular, sorbents were included that had previously been evaluated for polyhalogenated

contaminants, pharmaceuticals, phytochemicals or veterinary residues. Moreover, this study has for the first time demonstrated the usefulness of the polymeric Oasis HLB and Strata-X sorbents in laboratory and field studies for various microalgal toxins.

Highlights

► First use of Strata-X, Oasis HLB, LDPE and PDMS as passive samplers for many toxins. ► Passive sampling of Ovatoxin-a was possible in laboratory trials. ►Presence of *Prorocentrum lima* indicated through passive sampling.► Toxins from benthic algae detected with passive samplers. ► HP-20 most appropriate for long exposure periods (>5 days).

Keywords : Passive sampling ; Marine toxins ; Oasis HLB ; Strata-X ; LDPE ; PDMS ; SPATT

1. Introduction

Marine toxins are an important international issue for public health and the fish and shellfish industry. Indeed, toxins produced by a number of naturally occurring planktonic and benthic/epiphytic microalgae can accumulate in seafood and render them improper for human consumption. To protect consumers from intoxication, many countries, essentially those with important shellfish industries, have set up monitoring programs. Traditional monitoring through phytoplankton monitoring and shellfish testing has proven to be an effective warning method. However, phytoplankton samples can only describe a 'snapshot' of the microalgal community at a single point in space and time (Lane et al., 2010), and some microalgal species are too small (<20 µm) or benthic/epiphytic. Such algae are hard to identify or detect as identification is difficult for organisms below 20 µm in size and benthic/epiphytic algae require specific, additional sampling protocols (MacKenzie, 2010). For these reasons, Solid Phase Adsorption Toxin Tracking (SPATT) has been introduced as a passive sampling technique to detect and accumulate toxins released into the water during algal blooms (MacKenzie et al., 2004). Another advantage of the SPATT technique is the fact that the targeted toxins do not undergo biotransformation unlike in shellfish (Jauffrais et al., 2013). Thus, the identification of microalgae through toxin profiles in passive samplers is simplified.

Many different sorbents have been used for passive sampling all over the world, from HP-20 to SEPABEADS type resins (Fux et al., 2008, Li et al., 2011, Pizarro et al., 2013 and Zhao et al., 2013), for the accumulation of microalgal or cyanobacterial toxins of different polarities (Kudela, 2011, Lacaze, 2012 and Zhao et al., 2013). In the environment, passive sampling has proven to be useful to detect toxins released into seawater by benthic or very small pelagic microalgae like Pinnatoxins (PnTXs) (MacKenzie et al., 2011) or Azaspiracids (AZAs) (Fux et al.,

2009), using HP-20 sorbent. This sorbent was also efficient in accumulating ciguatoxin and maitotoxin in *Gambierdiscus pacificus* cultures (Caillaud et al., 2011).

Besides SPATT designed for toxin monitoring, various passive samplers were developed for the monitoring of polar or hydrophobic organic contaminants (HOCs), such as Polar Organic Chemical Sampler (POCIS), or Low Density PolyEthylene (LDPE) and Polydimethylsiloxane (PDMS) strips. There are two different POCIS configurations: the pharmaceutical POCIS and the pesticide POCIS. In the first one the membrane encloses Oasis HLB sorbent, while in the second the sorbent is a triphasic mixture of Isolute ENV$^+$ polystyrene divinylbenzene and Ambersorb1500 carbon dispersed on S-X3 Biobeads (Alvarez et al., 2004; Harman et al., 2012). POCIS devices are often used in aquatic environments to monitor polar organic chemicals (Alvarez et al., 2004; Harman et al., 2012; Kaserzon et al., 2012). For hydrophobic compounds, the first passive sampling device developed was the Semipermeable membrane device (SPMD) which is made of a layflat low density polyethylene (LDPE) membrane tube that contains a liquid film of triolein (Huckins et al., 1990). However, the use of SMPD can lead to various difficulties related to possible tearing of the membrane (loss of triolein) or the separation of triolein from HOCs. Moreover, a disadvantage of triolein is that it is too specific to be generically used for the monitoring of compounds of different polarities (Lacaze, 2012). Next to SMPD, single-phase polymeric sheets and films like LDPE (Allan et al., 2013; Bao et al., 2012) or silicone rubber (PDMS) (Rusina et al., 2007; Shea et al., 2006) have also been used for the accumulation of lipophilic compounds.

This study aims to evaluate the ability of Oasis® HLB, Strata-X™, BondElut™ C18, LDPE and PDMS as passive samplers to accumulate marine toxins. Although passive sampling has been successfully used several times to monitor microalgal toxins using different bulk polymeric sorbents, Oasis HLB has, to our knowledge, never been evaluated as a passive sampling sorbent for phycotoxins, and Strata-X has been evaluated only once as a passive sampler for the accumulation of cyanotoxins (Wood et al., 2011). In this study we also evaluated the use of LDPE and silicone membrane strips for the accumulation of lipophilic toxins other than brevetoxins (BTXs) (Shea et al., 2006). This is also the first attempt to test samplers containing a low amount of sorbent (300 mg, ten times less than the amount typically used). Furthermore, in addition to lipophilic azaspiracids (AZAs), 13-desmethyl spirolide-C (SPX1), okadaic acid (OA), dinophysistoxin-1 (DTX1) and PnTX-G (Fig. 1), we have also tested our passive samplers for their ability to accumulate the amphiphilic palytoxins (PlTXs).

Different types of sorbents were investigated through various protocols: i) screening and optimisation of sorbents in SPE-mode, and choice of the sorbents appropriate for passive sampling; ii) passive samplers immersed in spiked seawater; iii) passive samplers in experimental tanks and iv) passive samplers in the field. Throughout the

different protocols, sorbents and samplers were compared according to the amount of toxins they had accumulated.

2. MATERIALS AND METHODS

2.1. Chemicals and sorbent material

Certified standard solutions of OA, 13-DesMe-C (SPX1), AZA-1, 2 and 3, and PnTX-G were obtained from the National Research Council in Halifax, Canada. Purified Palytoxin (PlTX) standard was from Wako (Japan). Methanol, acetonitrile, butanol, isopropanol, hexane, ethyl acetate and acetone were obtained as HPLC grade solvents from AtlanticLabo (Bordeaux, France) and Sigma Aldrich (Steinheim, Germany). Acetic acid eluent for LC-MS and ammonium hydroxide (28–30%) were also acquired from Sigma-Aldrich. Milli-Q water was produced in-house to 18MΩ/cm quality, using a Milli-Q integral 3 system (Millipore). For analyses with the high resolution mass spectrometry instrument, acetonitrile and water of very high purity grade were obtained from Fischer Scientific (Illkirch, France).

Diaon® HP-20 polymeric resin was purchased as bulk resin from Sigma-Aldrich. Strata-X™ (200 mg, 500 mg cartridges and bulk) and Oasis® HLB (30 mg, 200 mg and bulk) were supplied by Phenomenex (Le Pecq, France) and Waters (Saint Quentin Yvelines, France), respectively. Reverse phase BondElut™ C18 cartridges (500 mg) and 3 mL capacity 2 frits-Reservoirs were from Agilent Technologies. AlteSil™ translucent silicone rubber sheets (PDMS) of 500 µm thickness were purchased from Altec Products Limited (Cornwall, UK). LDPE strips (70 µm) were prepared from additive-free LDPE lay-flat tubing purchased from Brentwood Plastics (MO, USA).

2.2. Microalgae cultures and algal extracts

2.2.1. Microalgae cultures

Algae paste from *Azadinium spinosum* and *Alexandrium ostenfeldii* were available from previous studies (Jauffrais et al., 2012b; Medhioub et al., 2011). *Prorocentrum lima*, *Vulcanodinium rugosum* and *Ostreopsis ovata* were cultured at the laboratory. *V. rugosum* (IFR-VRU-01I strain) was cultured using L1 medium without silicate, prepared with filtered (0.2 µm) Mediterranean sea water. The culture was in batch mode (200 mL), with a photon flux density of 292 µmol/m^2/s. Temperature was set at 22°C. *P. lima (PL4V* and *PL2V strains)* was

cultured using F/2 medium, prepared with filtered (0.2 µm) Atlantic sea water. The culture was in batch mode (300 mL), with a photon flux density of 181 µmol/m^2/s. Temperature was set at 16°C. *O. ovata* strains, isolated in the bay of Villefranche-sur-Mer (France), were cultured using L1 medium without silicate, prepared with filtered (0.2 mm) Mediterranean seawater. Soil extract (2 mL/L of medium) (Andersen, 2005) was added to the medium. The culture was in batch mode (200 mL), with a photon flux density of 370 µmol/m^2/s. Temperature was set at 22°C. All microalgae were cultured with a photoperiod of 16 h of light and 8 h of dark, and cells were collected during stationary phase by 20 min centrifugation at 3500 *g* and 4°C.

2.2.2. Algal extracts

For all spiking experiments, algal extracts were used rather than certified toxin standards. *Ostreopsis ovata* extracts were obtained according to a previously published procedure (Ciminiello et al., 2010), with the following modifications: sonication times were increased to 1 h with pulse mode (on ice) to ensure all cells were broken and three consecutive extractions were carried out, with 5 mL MeOH/H_2O (1:1, *v/v*) for each extraction step. The extracts were combined and evaporated to dryness under nitrogen stream before reconstitution in 5 mL of methanol/water (1:1, *v/v*). A protocol adapted from Jauffrais *et al.* (2012a and b) was used to obtain algal extracts for all other microalgae. Up to 1 g of algal paste was bath-sonicated and serially extracted using 3 x 5 mL of extraction solvent (organic solvent:water, 9:1, *v/v*). The combined supernatants were then gently evaporated to dryness under nitrogen at 45°C, and the residues reconstituted with 5 mL of methanol. Triplicate extractions with acetone, isopropanol or methanol as organic solvents, were investigated on *Vulcanodinium rugosum*, *Alexandrium ostenfeldii* and *Azadinium spinosum* to choose the most suitable solvent for maximum toxin recovery. Algal pellets (around 100mg) were accurately weighed before extraction and toxin concentrations were expressed as a function of algal mass (wet weight).

2.3. Instrumentation and analytical methods

Analyses were performed using a UHPLC system (1290 Infinity, Agilent Technologies) coupled to a 6540 UHD Accurate-Mass Q-ToF (Agilent Technologies) equipped with a dual ESI source. Chromatographic separation was achieved on a Phenomenex Kinetex C18 (150 x 2.1 mm, 1.8 µm) column, maintained at 40°C and with a flow rate of 500 µL/min. The binary mobile phase consisted of water (A) and acetonitrile (B), both containing 0.1% acetic acid. The total run time of one chromatographic run was 21 min. The elution gradient started with 5% of B held constant for 2.4 min, then 100% B was reached within 11.6 min. After a hold time of 2.5 min at 100% B, 5% B was reached within 50 s, followed by 4 min re-equilibration of the column at 5% B. To avoid

cross contamination of samples, the needle was washed for 10 s in the flush port with MeOH 90% before each injection.

The instrument was operated in positive mode and full-scan analysis was performed over *m/z* 65 to 1700 range and with an acquisition range of 2 spectra/s. Capillary voltage was 2000 V and fragmentor voltage 200 V. The temperature of the Jet Stream Technologies™ source was set at 200°C with drying gas at 5 L/min and sheath gas at 12 L/min at 355°C. The resolution achieved was generally between 25,000 and 40,000 depending on mass. In full-scan mode, the instrument had a mass error of 1 ppm, which can increase to less than 5 ppm for very small molecules or low abundance.

The exact mass-to-charge ratios (*m/z*) used for toxin analyses on the Q-ToF instrument were as follows: SPX1 692.4521; PnTX-G 694.4678; AZA1 842.5049; AZA2 856.5206; OA 805.4733; DTX1 819.4889; PlTX 906.4858; OvTX-a (896.1573); PTX2 (881.4658).

Instrument limit of detection was 4.5 ng/mL for OA, 0.35 ng/mL for AZA1, 0.27 ng/mL for SPX1, 0.34 ng/mL for PnTX-G and 100 ng/mL for PlTX.

Statistical evaluations were carried out using SigmaPlot 12.5. The significance test used to discriminate between the accumulation capabilities of the sorbents was the t-test.

2.4. Screening of sorbent material and optimization of toxin recovery

A wide range of cartridges and sorbents from different manufacturers are available to extract compounds with various chemical properties. To choose sorbents suitable for passive sampling, Oasis HLB, Strata-X, BondElut C18 and HP-20 were evaluated. The properties of these sorbents are listed in Table 1. Algal extracts (2.2.2) were quantified to determine the exact amount of toxin and used to prepare spiking solutions. Comparison was based on toxin recoveries obtained for each sorbent.

Screening of the sorbent materials.

Commercially available Oasis HLB (30 mg), Strata-X (200 mg), BondElut C18 (200 mg) and HP-20 (200 mg) SPE cartridges were investigated. Spiked seawater (1 mL) was loaded onto cartridges previously preconditioned with MeOH followed by Milli-Q water. The spiked seawater contained microalgal extracts with quantified toxin amounts as follows: 2.2 ng of SPX1, 11.5 ng of AZA1, 15.5 ng of PnTX-G, 104 ng of OA and 3639 ng of OvTX- a. Each cartridge was then washed with adapted volumes of 5% MeOH (500 µL to 2 mL depending on the amount of sorbent), and eluted with 100% MeOH (2 to 3 mL). The MeOH was evaporated to dryness under a

nitrogen stream at 45°C. Finally, the dry residue was then reconstituted in 500 µL of MeOH, or in 500 µL of a mixture of MeOH/H_2O (1:1, *v/v*) for OvTX-a, before analysis.

Choice of eluent to improve OvTX-a recoveries

Two different solvents were tested in triplicate as elution solvents on Oasis HLB (30 mg) and Strata-X (200 mg) cartridges: MeOH and MeOH/H_2O (1:1, *v/v*). Both cartridges were first conditioned with methanol then Milli-Q water. Spiked seawater (1 mL) was loaded onto each cartridge. The seawater had been spiked with microalgal extracts to yield the following final amounts in the spiked seawater: 2.2 ng of SPX-1, 12 ng of AZA-1, 15 ng of PnTX-G, 105 ng of OA and 161 ng of OvTX-a. After a washing step with 5% methanol (500 µL for HLB and 2 mL for Strata-X), cartridges were eluted with 6 mL of each solvent to be investigated. Eluents were evaporated to dryness under nitrogen stream at 45°C. The dry residue was reconstituted in 250 µL of MeOH/H_2O (1:1, *v/v*) and transferred into HPLC vials before analysis.

Evaluation of toxin adsorption on SPE cartridges

Cartridges (Oasis HLB, Strata-X, BondElut C18) were carefully emptied and only the bottom frit was left. The cartridges were thoroughly cleaned with MeOH and Milli-Q water to ensure all residual adsorbent was removed. Reservoirs of 3 mL capacity and with 2 frits (Agilent) were also tested (Supplementary material S1). Samples (1 mL seawater) containing 2.2 up to 161 ng of toxins (OA, AZA1, PnTX-G, SPX1, OvTX-a) were loaded onto the emptied and pre-cleaned cartridges. Washing was done with 1 mL MeOH (5%) and elution with 2 x 1 mL MeOH. Load, wash and elution were all recovered and directly analysed.

Optimization of load volumes on Oasis HLB (30 mg) SPE cartridges.

Load volumes of 5 mL, 15 mL and 25 mL were tested on HLB 30 mg cartridges with samples containing from 2.2 ng up to 160.7 ng of various toxins (OA, AZA1, PnTX-G, SPX1, OvTX-a). Before loading, the cartridges were first conditioned with 1 mL MeOH followed by 1 mL Milli-Q water. Then they were washed with 500 µL MeOH (5%) and eluted with 6 mL MeOH. Elution fractions were evaporated to dryness under nitrogen stream at 45°C and reconstituted in 250 µL of MeOH/H_2O (1:1, *v/v*) before analysis.

Evaluation of Oasis HLB, Strata-X, BondElut C18 and HP-20 (200 mg) inside Agilent Reservoirs

Each adsorbent (200 mg) was packed inside Agilent reservoirs with 2 frits. Spiked seawater (2 mL) was loaded onto cartridges previously preconditioned with MeOH followed by Milli-Q water. The spiked seawater

contained extracts with quantified toxin amounts as follows: 14 ng of SPX1, 19 ng of AZA1, 18 ng of PnTX-G, 238 ng of OA and 359 ng of OvTX- a. Each cartridge was then washed with adapted volumes of 5% MeOH (2 mL to 3.5 mL depending on sorbent type), and eluted with 100% MeOH (10 mL). The MeOH was evaporated to dryness under a nitrogen stream at 45°C. Finally, the dry residue was then reconstituted in 250 µL of a mixture of MeOH/H_2O (1:1, *v/v*) before analysis.

2.5. Laboratory experiments: design, handling and deployment

HP-20, Strata-X and Oasis HLB SPATT bags and discs were essentially identical to those from Mackenzie *et al.* (2004) and Rundberget *et al.* (2009), respectively. Lower amounts of resin were used (100 mg to 300 mg weight as delivered), and a 30 µm nylon mesh (Mougel, France) was used with regards to the small particle size of Strata-X and Oasis HLB sorbents (Table 1). For HP-20 resin, we noted a significant water content in the product as delivered, the dry weights for this resin are only ca. 40% of the weights as delivered. Before exposure, SPATT bags and discs were soaked overnight in methanol. They were then gently washed with Milli-Q water to remove methanol residues, and soaked again during 2 hours in Milli-Q water. After conditioning, care was taken to keep the SPATTs in Milli-Q water at 4-6 °C, for not more than 2 days before use.

LDPE and PDMS membranes were cut into 2.8 x 5 cm strips (14 cm^2 surface area). Conditioning method of LDPE strip was adapted from Shea *et al.* (2006), with an extra pre-extraction step with methanol during 4 hours. PDMS strips were thoroughly pre-extracted with ethyl acetate during 48 hours in a Soxhlet apparatus (Rusina et al., 2007). Both membrane strips were then allowed to dry, weighed and transferred separately into clean glassware and kept at -20 °C before use.

Extraction protocol for Oasis HLB, Strata-X and HP-20 passive samplers

After 24 h exposure, polymeric resins were extracted using a modified version of the extraction protocol developed by Fux *et al.* (2008) . Briefly, the SPATTs bags and discs were rinsed twice in 500 mL Milli-Q water and the remaining surface water removed by blotting with paper. The resin was transferred into polypropylene reservoirs of 3 mL capacity with 2 frits (Agilent) placed on a manifold. Vacuum was applied to get rid of remaining water and the resin was eluted with 15 mL methanol drop wise. The extracts were evaporated to dryness under a nitrogen stream at 45°C. The dry residue was finally reconstituted with 500 µL of MeOH/H_2O (1:1, *v/v*), filtered on Nanosep MF centrifugal filters 0.2µm (Pall) and transferred into LC vials for analysis.

***Extraction protocol for LDPE and PDMS* sheets**

At time 0 and after 1h, 7h, 24h and 72h of exposure, LDPE and PDMS strips were removed from the seawater, left to dry and then sequentially extracted twice with methanol. The fractions were gathered and evaporated to dryness under a stream of nitrogen at 45°C and the dry residue was reconstituted in 500 µL of MeOH/H_2O (1:1, *v/v*).

SPATTs, LDPE and PDMS sheets deployed in spiked seawater

Algal extracts from *A. spinosum*, *A. ostenfeldii*, *V. rugosum*, *P.lima* and *O. ovata* were used to spike seawater. The total content of methanol in seawater was approximately 0.2 % to ensure no modification of partition mechanisms. Spiking concentrations for each toxin were as follows: 22.4 ng/mL for OA, 1.36 ng/mL for SPX1, 1.76 ng/mL for AZA1, 1.70 ng/mL for PnTX-G and 33.7 ng/mL for OvTX-a.

Conditioned SPATT bags containing 100 mg, 200 mg or 300 mg of adsorbent (Oasis HLB, HP-20 or Strata-X) were individually placed in spiked seawater inside a closed Erlenmeyer flask. During the experiment beakers and Erlenmeyer flasks were agitated at ca. 90 rpm.

Each membrane strip was installed on a holding frame and placed inside a closed beaker containing spiked seawater solution. In order to avoid any cross contamination of membrane strips with unwanted molecules, glassware had carefully been cleaned and heated for 6 h at 400°C before use.

To monitor toxin accumulation using passive samplers, 1 mL (for SPATT experiment) and 3 mL (for LDPE/PDMS sheet experiment) of seawater were extracted on Oasis HLB SPE cartridges with the previously optimised protocol (2.5). Samples (1 mL to 3 mL) of seawater were withdrawn at times 0 h, 1 h, 7 h, and 24 h for SPATTs and up to 72 h for membrane strips.

SPATT exposure in cylindro-conical tanks: seawater containing mussels and microalgae

Mussels (*Mytilus galloprovincialis*, obtained from Galicia, Spain, and *Mytilus edulis* from Pen Bé, France) were allowed a 3-day period of adaptation in separate 128 L seawater tanks, maintained in the laboratory at 18°C. *Mytilus galloprovincialis* mussels (9 kg) were fed with a culture *of V. rugosum*, while *Mytilus edulis* mussels (8 kg) were fed with a culture of *P. lima*. Both cultures had previously been concentrated by filtering them over a 100 µm nylon mesh to remove culture media and mucus. In each cylindro-conical tank, conditioned SPATT discs (300 mg) of Oasis HLB, Strata-X and HP-20 were immersed simultaneously in triplicate and extracted after 3-day exposure.

2.6. Field trials at Ingril Lagoon

Ingril is a French Mediterranean lagoon, where the presence of the dinoflagellate *Vulcanodinium rugosum* has already been reported (Hess et al., 2013; Nezan and Chomerat, 2011). Conditioned SPATT discs containing 300 mg Oasis HLB, Strata-X or HP-20 were deployed on three consecutive weeks at South Ingril Lagoon during July 2013. Three SPATT discs of each type of sorbent were deployed at approximately 50 cm depth. The samplers were deployed inside cages typically used for POCIS™ deployment. After deployment, the samplers were kept at -20°C until extraction.

3. RESULTS

3.1. Microalgae extraction: solvent efficiencies

A. ostenfeldii, *A. spinosum* and *V. rugosum* algal pellets were extracted in triplicate and the amount of toxin extracted was calculated based on SPX1, AZA1 and PnTX-G calibration curves. Toxin concentration was reported to the mass (g) of algal pellet used for each extraction. Isopropanol was more efficient than methanol or acetone for the extraction of SPX1, PnTX-G and AZA1 (Table 3). Overall, for each toxin, isopropanol extracted 4% more toxin than methanol and 7% more toxin than acetone.

3.2. Comparison of sorbents in SPE-mode

For the first experiment with commercially available Oasis HLB (30 mg), Strata-X (200 mg), BondElut C18 (500 mg) and home packed HP-20 (200 mg) SPE cartridges, recoveries of toxins were generally above 50% except for OvTX-a. Indeed, no OvTX-a was recovered from BondElut C18 and HP-20, and only 13% was recovered from Oasis HLB and 25% from Strata-X (Table 4).

Choice of eluent to improve OvTX-a recoveries

Average recoveries of toxins (three replicates) from Oasis HLB and Strata-X cartridges using MeOH and MeOH/H_2O (1:1, *v/v*) indicated that no toxin was recovered from Strata-X using the latter solvent (Table 5). Only OA (15%) could be recovered from Oasis HLB when MeOH/H_2O (1:1, *v/v*) was used and best recoveries were obtained with MeOH. For pure MeOH, recoveries from Oasis HLB ranged from 20% (OvTX-a) to 97%

(for OA) and those from Strata-X ranged from 9% (OvTX-a) to 85% (OA). AZA-1 and 2 had low recoveries compared to the other toxins, except for OvTX-a, the recovery of which was even lower.

Assessment of optimum load volume

Increasing sample volumes were loaded onto Oasis HLB (30 mg) cartridges to determine maximum load capacity. For all toxins, recoveries slightly increased from 5 mL to 15 mL load but declined with 25 mL load (Table 6). The load itself was also analysed for 5 and 15 mL volumes after passage through the cartridge and no toxins had been detected for these load volumes. Yields ranged from 45% (AZA2) to 102% (DTX1). For OvTX-a, recoveries were significantly lower than for other toxins (around 10%) and quite similar regardless of the volume of sample loaded.

Evaluation of Oasis HLB, Strata-X, BondElut C18 and HP-20 inside 2-frits-reservoir (Agilent)

To make sure toxin adsorption on cartridge reservoirs would not jeopardize the comparison between Oasis HLB, Strata-X, BondElut C18 and HP-20 sorbents, the same cartridge reservoir (Agilent) and the same amount of sorbent (200 mg) were used for all sorbents. Recoveries equal to or higher than 90% were obtained for OA, DTX1 and PnTX-G on BondElut C18, Oasis HLB and Strata-X (Fig. 2). For AZA1 and SPX1, recoveries ranged from 55 to 80%. Compared to the other toxins, OvTX-a (35%) and AZA-2 (55%) recoveries were somewhat lower on all cartridges, especially for OvTX-a. BondElut C18 results were comparable to those of Oasis HLB or Strata-X, except for OvTX-a for which Oasis HLB recoveries were significantly lower ($P = 0.006$). It is noteworthy that HP-20 gave the lowest recoveries for PnTX-G, OA and DTX-1, with statistically significant differences from the other sorbents ($P < 0.001$). The recoveries of SPX1 and AZA-1 and 2 from HP-20 were also lower compared to the other resins, but the difference observed was not statistically significant ($P > 0.06$).

3.3. Comparison of passive samplers at laboratory scale

3.3.1. Spiked seawater

Monitoring of toxin disappearance from spiked seawater exposed to Oasis HLB, Strata-X, HP-20, LDPE and PDMS strips

Oasis HLB, Strata-X, HP-20, LDPE and PDMS passive samplers have been exposed to spiked seawater during 24h. It is important to note that spiking concentrations were voluntarily very high (five times each toxin

LOD/mL) to ensure decrease of toxin in the water could be followed. Toxin content in seawater was monitored over the 24 h of exposure (Fig. 3A-E). For the polymeric sorbents, first there was a rapid decrease until time 7h, and then the concentration continued to slowly decrease from 7h to 24h. For each toxin, similar decrease was observed for Oasis HLB, Strata-X and PDMS, with the exception of OvTX-a. For HP-20, the decrease was more linear than for the other samplers. Comparing these four passive samplers, it was obvious that during the first 7 hours of exposure, and from top to down, Strata-X seemed to accumulate faster than Oasis HLB, HP-20 and PDMS. Despite the somewhat slower decrease with HP-20, the amount of toxin remaining in the seawater after 24 h was similar for Strata-X, HP-20 and Oasis HLB (i.e 0% for OvTX-a and between 10 and 30% for the other toxins). This amount was also comparable in the case of PDMS for PnTX-G, but significantly higher for the other toxins. Of all the samplers, LDPE seemed to be the least effective. Indeed, only AZA1 and OvTX-a appeared to be removed from the seawater by this sampler.

Toxin recovery on each sampler after 24h exposure

After 24h exposure to spiked seawater, passive samplers were extracted to determine the amount of toxin accumulated by each sampler. Recoveries from LDPE were negligible for all toxins (Fig. 4). For AZA1, PnTX-G and SPX1, HP-20 was the most suitable sampler. OA and OvTX-a recoveries were slightly better on Strata-X resin. Of the three resin-type passive samplers, Oasis HLB was the one that gave the lowest but acceptable recoveries. Although recovery using PDMS was generally significantly lower compared to the resin phases, PnTX-G was significantly better recovered by PDMS membrane strips than by Strata-X and Oasis HLB. In summary, Oasis HLB, Strata-X and HP-20 were all able to accumulate OA, AZA1, PnTX-G, SPX1 and OvTX-a, with different efficiencies. PDMS membrane strips showed a good accumulation of PnTX-G, and were also able to accumulate AZA1 and SPX1 but with unsatisfactory yields. LDPE was the poorest passive sampler of all those investigated. All toxins and samplers considered, HP-20 consistently produced recoveries above 50%, apart from OvTX-a, which had poor recoveries on all phases except on Strata-X.

3.3.2. Tank trials

Oasis HLB, Strata-X and HP-20 SPATTs (300 mg) were simultaneously exposed inside tanks containing mussels and *P. lima* or *V. rugosum* microalgal cultures. As a consequence, those SPATTs were in competition. Strata-X and Oasis HLB exceptionally accumulated more toxins than the HP-20 sampler (Fig. 5). The amount of

PnTX-G, OA and DTX-1 was slightly higher on Strata-X than on Oasis HLB, however, this difference was not statistically significant ($P = 0.462$).

3.4. Comparison of Oasis HLB, Strata-X and HP-20 samplers at Ingril lagoon

For assessment of the passive samplers in the field, Oasis HLB, Strata-X and HP-20 SPATTs were deployed in July at Ingril Lagoon on a weekly basis. All three samplers were able to accumulate five toxins in this lagoon: SPX1, PnTX-G, OA, DTX1 and PTX2 (Fig. 6A). The three samplers also had similar accumulation patterns for these five toxins, with SPX1 present at trace levels while PnTX-G, OA and DTX1 were present at higher concentrations than PTX2. Furthermore, there was no significant difference between Oasis HLB, HP-20 and Strata-X for accumulated SPX1 levels. HP-20 appeared to be more efficient for OA and DTX1, followed by Oasis HLB. Oasis HLB accumulated PnTX-G at levels equal to those on HP-20. Unexpectedly, Strata-X was the sampler that gave the lowest PnTX-G and OA amounts. The time of exposure had a slight effect on the amounts of OA accumulated. Also, adsorption of OA on Oasis HLB and Strata-X decreased with increasing time of exposure (Fig. 6B). As a result, the gap between OA levels on HP-20 compared to Strata-X and Oasis HLB, statistically significantly increased ($P < 0.05$) with longer exposure times.

4. DISCUSSION and CONCLUSION

Microalgal extracts were used for all our spiking experiments at the laboratory. To investigate the solvent that yielded better toxin recoveries, methanol, isopropanol and acetone were tested. Methanol, acetone and acetonitrile had already been evaluated for *A. spinosum* extraction (Jauffrais et al., 2012a), and *V. rugosum* and *A. ostenfeldii* are commonly extracted using methanol (Geiger et al., 2013; Munday et al., 2012; Otero et al., 2010). Only isopropanol had not yet been tested as an extraction solvent for these microalgae. After estimation of toxin recoveries, isopropanol was chosen because it enabled extraction of higher amounts of compounds of interest and did not yield methylester artifacts as previously reported (Jauffrais et al., 2012a).

4.1. Choice of extraction solvent and appropriate SPE type resin

HP-20 is a commonly used sorbent for passive sampling of toxins. The other three resins investigated (Oasis HLB, Strata-X and BondElut C18) are common SPE sorbents used to remove matrix effects (Gerssen et al.,

2009; Kilcoyne and Fux, 2010) or to purify toxins before structural elucidation (Uchida et al., 2013). On BondElut C18 cartridges, retention of the toxins may be based on hydrophobic interactions. On the other hand, with the polymeric sorbents, the retention of toxins depend on either hydrophobic or dipole-dipole, hydrogen bonding and π-π interactions.

For the first SPE screening experiment with different resins, recoveries for OvTX-a were the lowest and no toxin was found in the loading and washing solvents, suggesting the elution solvent was either inappropriate or the eluent volume used was not sufficient. Therefore, optimisation was carried out to improve these recoveries. Two different extraction solvents were investigated on Oasis HLB and Strata-X SPE cartridges which were more efficient after the first trial. Best OvTX-a recoveries were obtained with methanol, while MeOH/H_2O (1:1, *v/v*) was not suitable to recover OvTX-a. Indeed, in studies by Uchida *et al.* (2013) and Selwood *et al.* (2012) using Oasis HLB and Strata-X, PlTXs were eluted with acidified organic solvents. This could explain why we were not able to recover OvTX-a under basic conditions. However, we did not tested acidic conditions since another toxin group i.e. AZAs had been shown to be easily degraded during evaporation of acidic eluents (Alfonso et al., 2008). Maximum capacity on 30 mg Oasis HLB cartridges was around 15 mL loading volume, and recoveries decreased with higher sample load volume.

As evaluation of toxin adsorption on cartridges had shown that toxins adsorbed less on the Agilent reservoirs with 2-frits (Supplementary material, S1), all the sorbents were tested inside this reservoir in a second experiment. HP-20 gave the lowest recoveries for most of the toxins compared to Oasis HLB, Strata-X and BondElut C18 (Fig. 2). This was in agreement with other studies that had demonstrated a low accumulation rate for HP-20, compared to polymeric resins other than Oasis HLB and Strata-X (Fux et al., 2009; Li et al., 2011). Thus, HP-20 was not appropriate for use in SPE-mode. BondElut gave higher recoveries than Oasis HLB and Strata-X but this sorbent was not retained because it was impractical for field use. Indeed, unlike polymeric sorbents which are readily wettable, BondElut C18 can never be allowed to dry once conditioned. The different resins we have tested are commercialised for their differences in retention mechanisms and thus we could have expected significant differences in the recoveries of the adsorbed toxins. However, apart from HP-20, all polymeric resins and even the lipophilic BondElut C18 performed quite similarly (Fig. 2). Indeed, all toxins tested are compounds with both polar and apolar features. Moreover, as the targeted toxins have various physico-chemical properties, simultaneous optimisation of recoveries for all the toxin groups was hard to achieve as previously noted by Gerssen *et al.*(2010).

4.2. Comparison of passive samplers at laboratory and field scale

As already mentioned, Oasis HLB, and Strata-X had already been tested in SPE mode to remove mussels matrix effects, but never as passive samplers for phycotoxins. These sorbents, as well as HP-20, were investigated as passive samplers in spiked seawater, experimental tanks and in the environment. LDPE and PDMS membrane strips were additionally tested as passive samplers in spiked seawater. Monitoring of toxin decrease in spiked seawater during 24h of samplers exposure confirmed the slow accumulation rate of HP-20 compared to Oasis HLB and Strata-X polymeric resins (Fig. 3; Supplementary material S2). Similar decrease in toxin levels in the seawater was observed with Oasis HLB, Strata-X and HP-20, except for OvTX-a. This difference could be explained by the amphiphilic property of OvTX-a as well as its large molecular size. Also, apparently almost all of OvTX-a had been adsorbed after only 7h exposure. OvTX-a recoveries from passive samplers were poor. These low recoveries were, however, consistent with the low recoveries in SPE mode described above. As of yet, there is no indication whether generally low recoveries for this compound are due to instability in solution or due to irreversible adsorption. Accumulation of toxins on LDPE and PDMS was lower than on Oasis HLB, Strata-X and HP-20. This may be due to the physical difference between the sorbents, thus, difference in sorption mechanisms. Furthermore, PDMS appears to be slightly more efficient for toxin sampling than LDPE. The diffusivity of large molecular size compounds like phycotoxins could be lower in LDPE than in PDMS (Rusina et al., 2010). As reported by Allan *et al.* (2013) silicone (PDMS) also appears to be less effective than LDPE in sorption and is suitable for the sampling of a large range of compounds of interest. Surprisingly, adsorption of PnTX-G seemed to be easier on PDMS than the adsorption of SPX1, despite their structural similarities. An explanation for this difference could not be found. Levels of toxins accumulated on LDPE and PDMS were significantly lower than on Oasis HLB, Strata-X or HP-20, except for PnTX-G. HP-20 showed higher recoveries than every other sampler in the field, confirming observations about its higher sorption capacity than other polymeric resins (Lane et al., 2010). The simultaneous exposure of Oasis HLB Strata-X and HP-20 inside the experimental tanks (Fig. 5) led to results different to those from the field trials and from the SPATTs exposed separately to spiked seawater. Indeed, levels of OA, DTX1 and PnTX-G accumulated were higher on Strata-X and Oasis HLB than on HP-20. This was probably a result of the competition between the samplers, which was detrimental to HP-20 because of its low accumulation speed.

The amount of resin used for the passive sampling experiment was approximately ten times lower than the amount commonly reported in the literature (3 g). We also noted that, contrarily to Strata-X and HLB resins, HP-20 resin is delivered with a significant water content (i.e. 60% H_2O); dry weight equivalents should be used to report results to facilitate standardisation and comparison across studies. Nevertheless, the SPATT deployed

showed good accumulation efficiency. Indeed levels of OA per gram of sorbent were comparable to, or even higher, than those from SPATT deployed in similar semi-closed environments on the South Coast of Norway (Rundberget et al., 2009) or in New Zealand (MacKenzie et al., 2004; MacKenzie et al., 2005).

While this paper focussed on the technical aspects of passive sampling sorbents, it should be noted that other factors may also influence the choice of sorbent. For use in routine monitoring, the cost of sorbent material is also significant. Despite the high water content of HP-20 resin as product, its cost is significantly less than that of sorbent materials with similar efficacy (ca. 20-fold less). Also, the average particle size of HP-20 is relatively large, which facilitates collection using coarse phytoplankton mesh; possibly this reduces also the amount of bio-fouling observed on some of the finer mesh sizes.

As found by Mackenzie *et al.* (2011), our field experiments have also demonstrated the ability of passive sampling to accumulate and thus enable detection of toxins from benthic or epiphytic microalgae in environmental conditions. Accumulation of SPX1 and PnTX-G in our study indicated the presence of *A. ostenfeldii* and *V. rugosum*, respectively. On the other hand, the presence of OA, DTX1 and PTX2 (Fig. 6A) in the SPATTs deployed suggested that both *Dinophysis species* and *P. lima* were present in the water during the field trial at Ingril Lagoon.

4.3. Conclusion

As recommended by Lacaze (2012), we have attempted to extend the range of sorbents suitable for passive sampling. This is the first report of Strata-X, Oasis HLB, LDPE and silicone (PDMS) investigation as passive samplers for lipophilic phycotoxins other than Brevetoxins (BTXs). In addition, investigation of polymeric sorbents had shown that passive sampling of OvTX-a was possible. However, more development is needed to improve toxin recoveries of this toxin from polymeric resin after exposure. This study has also confirmed the possibility of detecting toxins from benthic microalgae in the environment. Moreover, this is the first time passive sampling has efficiently been used in France and has pointed towards the presence of different microalgae species including *P. lima*. As the sorbents we have investigated have shown different efficiencies and accumulation speed, we can conclude that Strata-X and Oasis HLB, which are fast accumulators, are probably better for daily or on-board evaluation of toxin presence. HP-20 however should be more appropriate for long exposure periods (> 5 days). Furthermore, the feasibility of down-scaling the amount of sorbent used in the passive sampling and thus decreasing the amounts of extraction solvents for a more economical and environmental friendly technique has been demonstrated for semi-enclosed coastal areas.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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573

574 **FIGURES LEGEND**

575 Fig 1: Structures of the toxins of interest: Azaspiracids 1 and 2 (AZA-1, AZA- 2), Okadaic acid (OA),
576 Dinophysistoxin-1 (DTX1), 13-desmethyl spirolide-C (SPX1), Pinnatoxin-G (PnTX-G), Palytoxin
577 (PlTX) and Ovatoxin-a (OvTX-a)

578 Fig 2: Average toxin recoveries (%) from Oasis HLB, Strata-X, BondElut C18 and HP-20 packed in the same
579 SPE reservoir with 2-frits (Agilent). Error bars are standard deviations (n=3).

580 Fig 3: Toxin disappearance from seawater of OA, AZA1, PnTX-G, SPX1 and OvTX-a for Oasis HLB (300 mg),
581 Strata-X (300 mg), HP-20 (300 mg), PDMS and LDPE passive samplers.

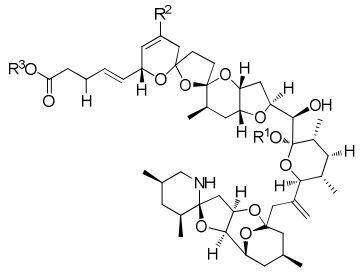
582 Fig 4: OA, AZA1, PnTX-G, SPX1 and OvTX-a recoveries from Oasis HLB, Strata-X, HP-20, PDMS and LDPE
583 after 24h immersion in spiked seawater. Values for Oasis HLB, Strata-X and HP-20 are mean values
584 from three SPATT bags containing 100 mg, 200 mg or 300 mg of resin. Error bars represent standard
585 deviations (n=3).* Strata-X (n = 2) significantly higher than Oasis HLB ($P < 0.001$, n = 3) and HP-20 ($P = 0.043$, n = 3).

587 Fig 5: Amount of toxin (average in [ng/g dry resin] ± SD) accumulated on HP-20, Oasis HLB and Strata-X
588 exposed for 3 days in two cylindro-conical tanks (tank 1: *M. edulis* fed with *V. rugosum*; tank 2: *M.*
589 *galloprovinciallis* fed with *P. lima*).

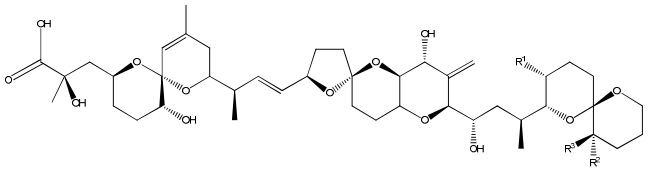
590 Fig 6: (A) Accumulation of SPX1, PnTX-G, OA, DTX1 and PTX2 on Oasis HLB, Strata-X and HP-20 SPATTs
591 exposed in July at Ingril Lagoon on a weekly basis (expressed as [ng/g dry resin]). (B) OA adsorption
592 on Oasis HLB, Strata-X and HP-20 for 5d, 6d and 7d of exposure during three consecutive weeks. Error
593 bars represent standard deviations (n=3).* Statistically significantly different ($P < 0.05$).

594

Fig 1

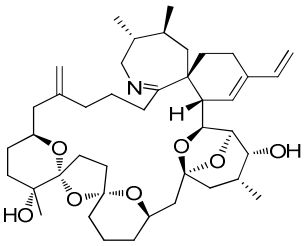


	R^1	R^2	R^3
AZA1	H	H	H
AZA2	H	CH_3	H

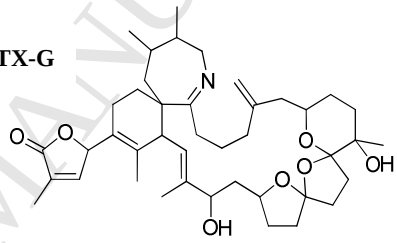


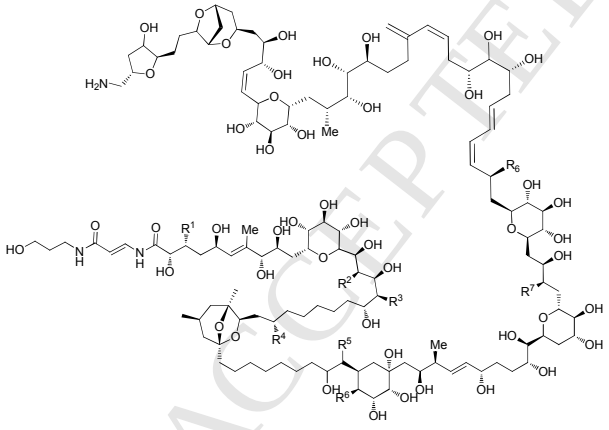
	R^1	R^2	R^3
OA	CH_3	H	H
DTX1	CH_3	H	CH_3

SPX1



PnTX-G





	R^1	R^2	R^3	R^4	R^5	R^6	R^7
PlTX	CH_3	OH	OH	CH_3	H	OH	OH
OvTX-a	CH_3	H	OH	CH_3	OH	H	H

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Fig 2

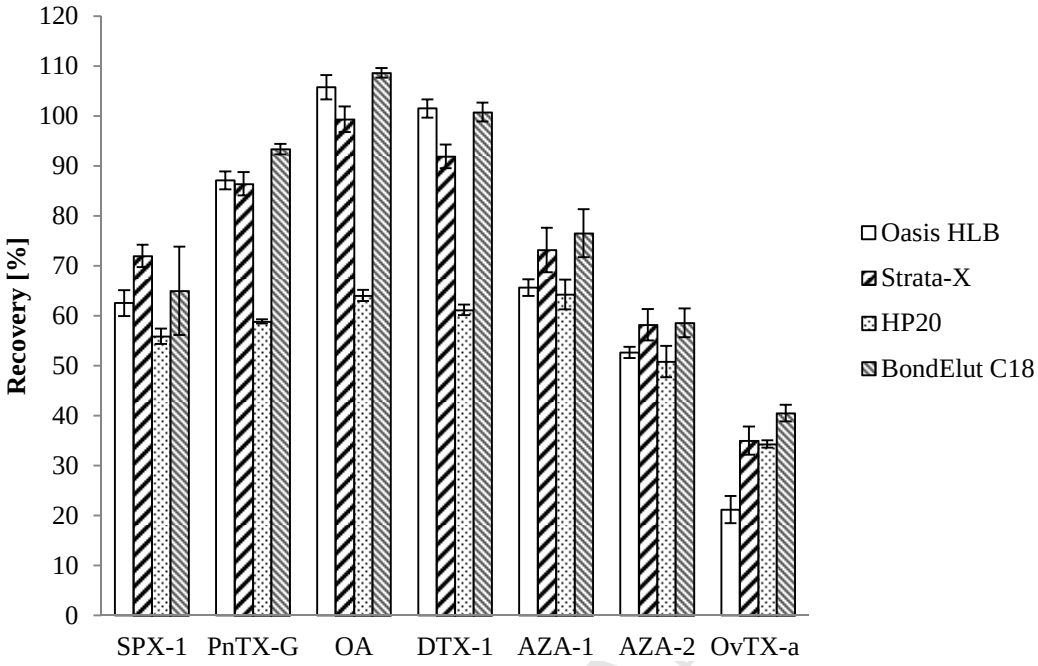

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Fig 3

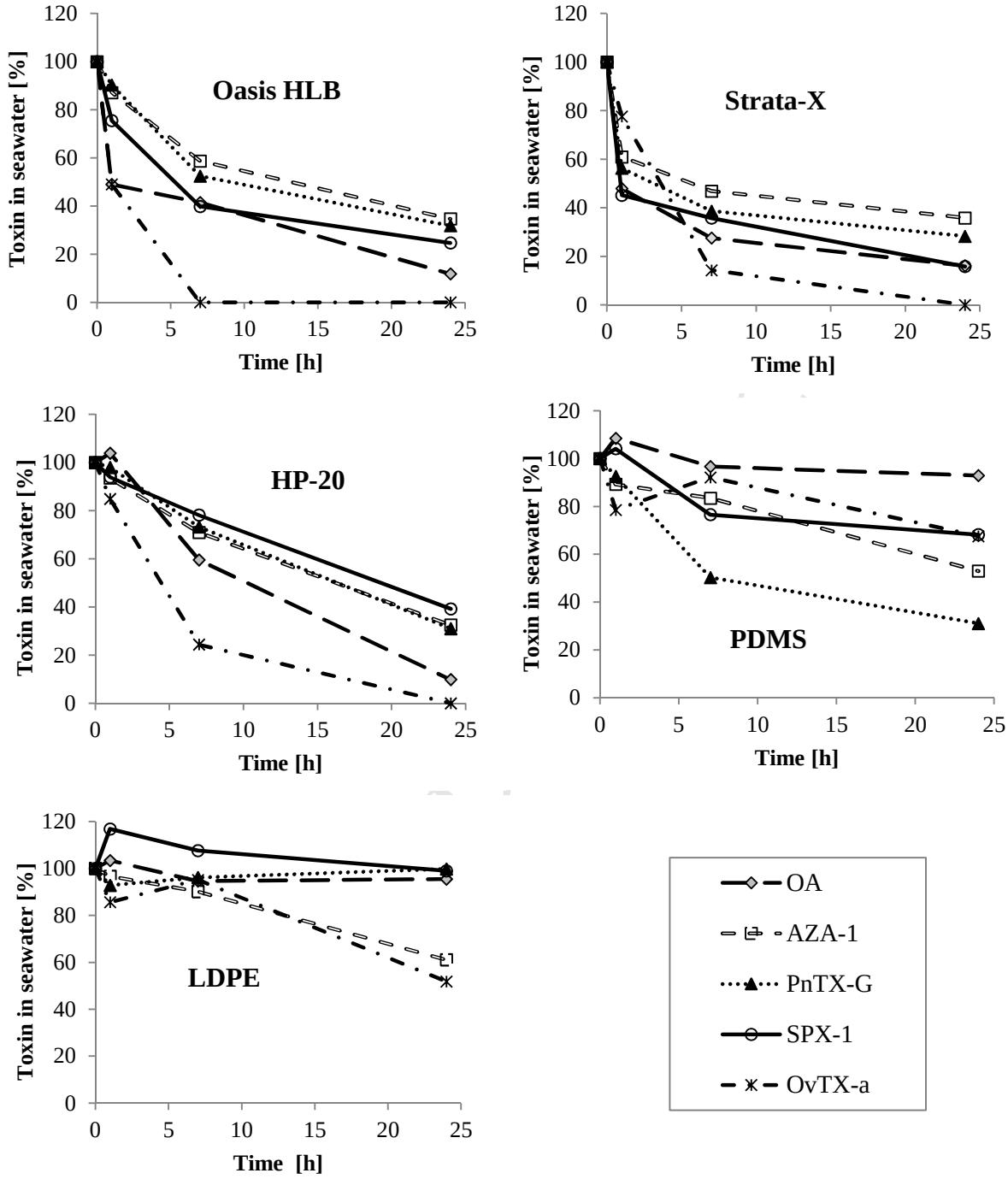

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Fig 4

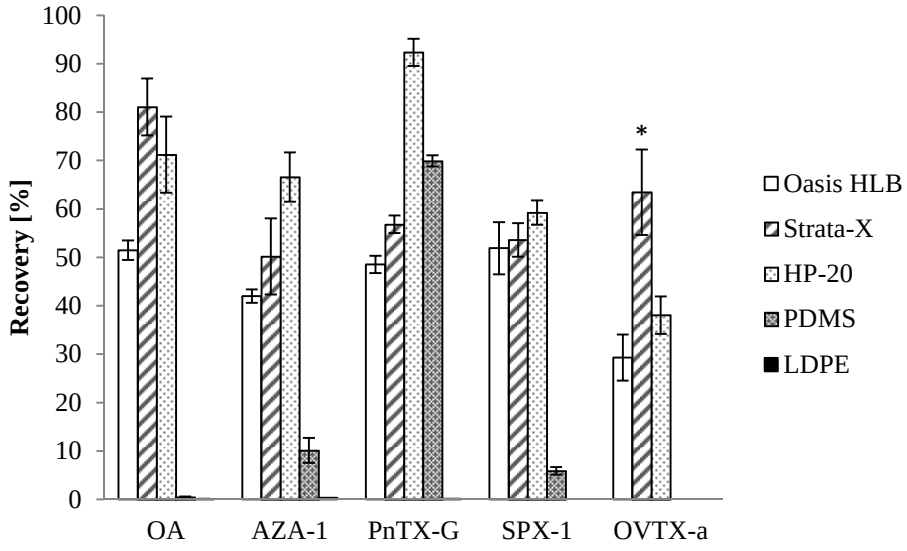

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Fig 5

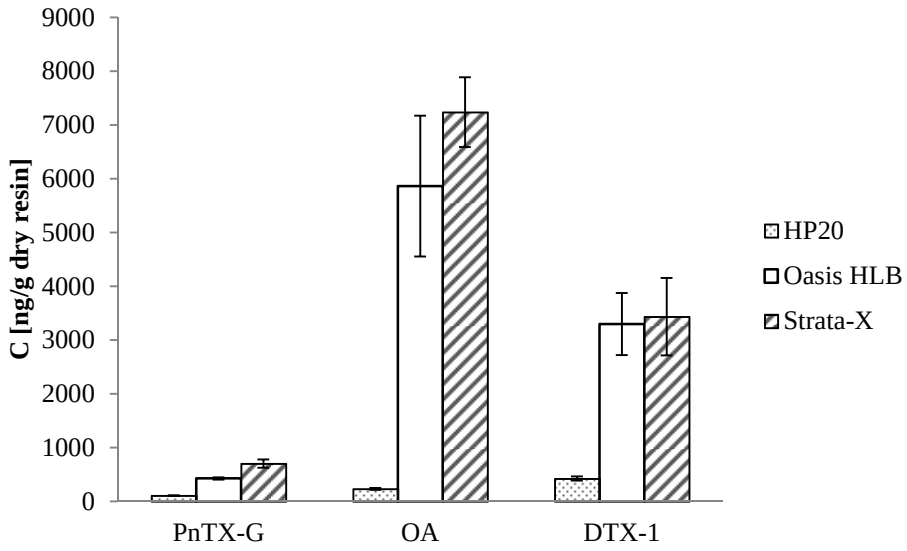

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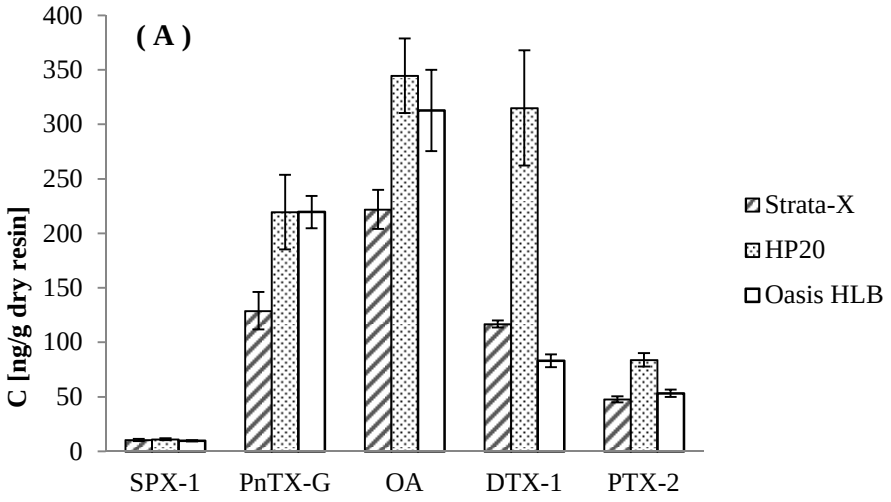
Fig 6



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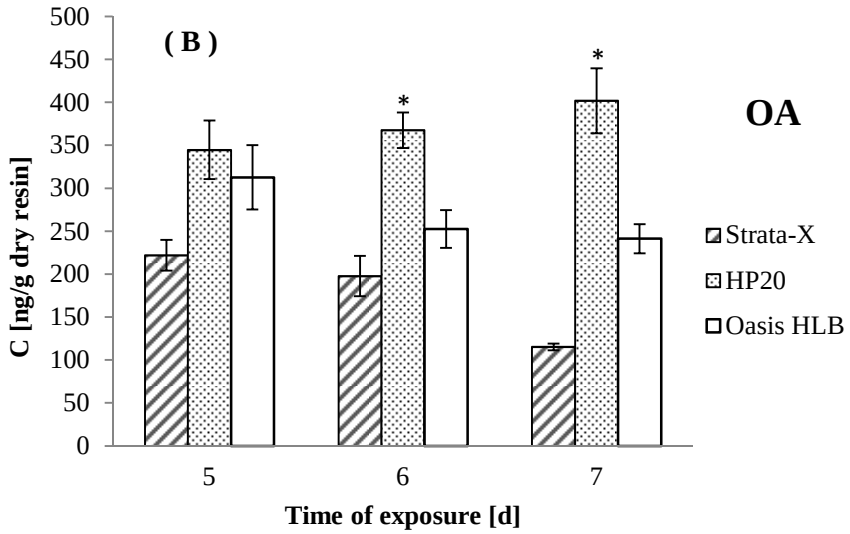

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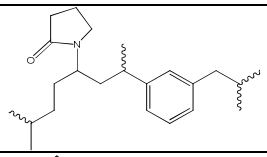
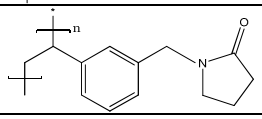
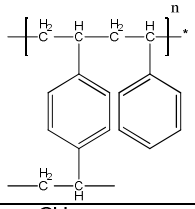
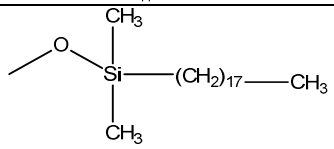
620 **Table 1:** Oasis HLB, Strata-X, HP-20 and BondElut C18 properties.

Sorbent	Structure	Surface area (m^2/g)	Pore volume (cm^3/g)	Average pore radius (Å)	Particle size (µm)
Oasis HLB		767	1.21	81	50 - 65
Strata-X		799	1.24	90	28 - 34
HP-20		500	1.3	200	397 - 840
BondElut C18		500	-	60	40 and 120 and irregular

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623 **Table 2:** LDPE and PDMS properties

Sorbent	*LDPE*	*PDMS*
Supplier	Brentwood Plastics	Altec Products
Thickness (µm)	70	500

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626 **Table 3:** Average amounts (mean ± standard deviation (=SD), n=3) of toxin extracted from *A. ostenfeldii*, *A.*

627 *spinosum* and *V. rugosum* using acetone, methanol and isopropanol

		Acetone	Methanol	Isopropanol
Toxin concentration (ng/g algae pellet)	***SPX-1***	5207 ± 136	5422 ± 183	5788 ± 137
	AZA-1	23269 ± 245	24220 ± 1234	24651 ± 1090
	PnTX-G	26008 ± 1306	26680 ± 243	27732 ± 1143

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Table 4: Toxin recoveries (%) from commercially available sorbents for the first screening experiment

	OASIS HLB (30 mg)	STRATA-X (200 mg)	BONDELUT C18 (500 mg)	HP-20 (200 mg)
SPX-1	88	85	83	59
PnTX-G	100	92	48	51
OA	111	100	95	83
DTX-1	119	103	95	86
AZA-1	67	66	46	48
AZA-2	57	60	34	44
OvTX-a	13	25	n.d	n.d

n.d: not detected

Table 5: Recoveries (average ± SD, n=3) obtained from Oasis HLB (30 mg) and Strata-X (200 mg) cartridges eluted with MeOH and MeOH/H_2O (1:1, *v/v*).

Recoveries (%)		SPX-1	PnTX-G	OA	DTX-1	AZA-1	AZA-2	OvTX-a
MeOH	***HLB***	80 ± 4	76 ± 1	97 ± 3	90 ± 2	57 ± 3	50 ± 3	20 ± 1
	Strata-X	72 ± 3	70 ± 2	85 ± 2	79 ± 2	66 ± 4	59 ± 4	9 ± 4
MeOH/H_2O (1:1, v/v)	***HLB***	n.d	n.d	15 ± 2	n.d	n.d	n.d	n.d
	Strata-X	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d: not detected

Table 6: Recoveries (average in % ± SD, n=4) with an elution step of 6 mL MeOH for increasing sample volume (5 – 25 mL) onto Oasis HLB (30 mg) cartridges.

Load volumes	SPX1	PnTX-G	OA	DTX1	AZA1	AZA2	OvTX-a
5 mL	72 ± 2	79 ± 3	82 ± 2	96 ± 3	54 ± 2	45 ± 2	14 ± 2
15 mL	74 ± 2	80 ± 1	86 ± 4	102 ± 3	60 ± 2	48 ± 2	13 ± 5
25 mL	61 ± 3	68 ± 3	71 ± 2	81 ± 5	48 ± 1	37 ± 1	9 ± 2

SUPPLEMENTARY MATERIAL:

Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins

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S1: Evaluation of toxin adsorption on SPE cartridges and glassware

To evaluate toxin adsorption on empty cartridges (procedural losses), known amounts of toxin were loaded onto the cartridges and directly recovered just as wash and elution solvents. After loading, 100% recovery was obtained for none of the cartridges. However, the reservoir with 2 frits (Agilent) and the one of Oasis HLB yielded better recoveries than BondElut C18 and Strata-X reservoirs, indicating that there was less toxin adsorption on those cartridges. The washing step enabled recoveries to increase to up to 100% for all toxins except for AZA1. Indeed, for AZA1, the first elution step was required to reach 100% recovery. It is noteworthy that most of OvTX-a was retrieved after the loading step. Very high recoveries were obtained with the reservoirs containing 2 frits and Strata-X reservoirs gave the lowest OvTX-a recovery. Overall, all cartridges enable satisfactory recovery of nearly all toxins throughout the procedure. This is not the case for OvTX-a for which top down yields are obtained from 2-frits-reservoir, then BondElut C18, Oasis HLB and Strata-X, respectively.

Toxin adsorption evaluated from recovery (average ± SD) after load (1 mL spiked seawater) wash (1 ml of 5% MeOH) and elution (2*1 mL MeOH) through empty SPE cartridges. Standard deviation calculated from 3 replicates.

	Steps	**SPX-1**	**PnTX-G**	**OA**	**DTX-1**	**AZA-1**	**AZA-2**	**OvTX-a**
BondElut C18 reservoir	*Load*	89 ± 3	85 ± 6	89 ± 6	83 ± 8	61 ± 8	88 ± 6	73 ± 9
	Wash	8.8 ± 0.3	7.1 ± 0.4	10 ± 1	7.8 ± 0.3	1.6 ± 0.1	9 ± 1	n.d
	Elution 1	10 ± 1	17 ± 3	10 ± 2	15 ± 1	**37 ± 6**	10 ± 2	8 ± 7
	Elution 2	0.8 ± 0.5	2 ± 0.4	n.d	2 ± 1	5 ± 1	0.6 ± 0.3	n.d
	Total recovery	**109 ± 3**	**110 ± 2**	**109 ± 5**	**109 ± 8**	**104 ± 1**	**107 ± 5**	**81 ± 7**
Strata-X reservoir	*Load*	87 ± 2	85 ± 4	83 ± 1	86 ± 2	80 ± 5	83 ± 3	85 ± 7
	Wash	10.2 ± 0.4	9 ± 1	11 ± 1	9.8 ± 0.2	3.3 ± 0.4	11 ± 1	5 ± 5
	Elution 1	5.7 ± 0.2	9 ± 1	5.0 ± 0.6	7 ± 1	**15 ± 2**	4.8 ± 0.5	n.d
	Elution 2	0.1 ± 0.1	0.7 ± 0.1	n.d	n.d	1.9 ± 0.1	n.d	n.d
	Total recovery	**103 ± 2**	**104 ± 3**	**99 ± 2**	**103 ± 2**	**100 ± 4**	**99 ± 2**	**90 ± 9**
Oasis HLB reservoir	*Load*	99 ± 2	99 ± 6	92 ± 3	96 ± 2	86 ± 5	96 ± 4	82 ± 9
	Wash	15 ± 7	15 ± 7	17 ± 8	17 ± 7	7 ± 4	16 ± 7	n.d
	elution 1	6 ± 2	6 ± 2	4 ± 2	7 ± 2	**13 ± 5**	4 ± 1	n.d
	Elution 2	0.2 ± 0.1	0.7 ± 0.1	n.d	n.d	1.7 ± 0.1	n.d	n.d
	Total recovery	**109 ± 2**	**111 ± 7**	**103 ± 3**	**109 ± 2**	**99 ± 5**	**105 ± 4**	**82 ± 9**
Agilent 2-frits-reservoir	*Load*	96 ± 1	97 ± 5.4	97 ± 3	93 ± 2	89 ± 2	98 ± 4	95 ± 9
	Wash	12.2 ± 0.2	11.2 ± 0.5	14.0 ± 0.2	12.3 ± 0.6	4.4 ± 1.3	13.5 ± 0.2	n.d
	Elution 1	2.2 ± 0.3	2.8 ± 0.3	1.5 ± 0.2	0.4 ± 0.4	**7 ± 1**	0.4 ± 0.4	n.d
	Elution 2	n.d	0.7 ± 0.0	n.d	n.d	1.9 ± 0.3	n.d	n.d
	Total recovery	**111 ± 1**	**111 ± 6**	**113 ± 3**	**106 ± 2**	**102 ± 3**	**112 ± 4**	**95 ± 9**

n.d: not detected

685 **S2: Monitoring of toxin decrease (%) in spiked seawater for Oasis HLB, Strata-X, HP-20, LDPE and**

686 **PDMS**

	Time (hrs)	**SPX-1**	**PnTX-G**	**OA**	**AZA-1**	**OvTX-a**
HLB 100 mg	***0***	100	100	100	100	100
	1	49	44	32	48	7
	7	21	16	0	23	0
	24	33	28	12	28	0
HLB 200 mg	***0***	100	100	100	100	100
	1	105	105	103	99	124
	7	58	62	35	62	14
	24	36	36	17	33	0
HLB 300 mg	***0***	100	100	100	100	100
	1	75	90	63	87	49
	7	40	52	41	59	0
	24	25	32	12	35	0
Strata-X 100 mg	*0*	100	100	100	100	100
	1	87	88	83	95	-
	7	46	53	41	64	0
	24	28	32	0	36	0
Strata-X 200 mg	***0***	100	100	100	100	100
	1	118	81	62	75	-
	7	53	39	13	48	0
	24	37	30	0	33	0
Strata-X 300 mg	***0***	100	100	100	100	100
	1	45	56	48	61	78
	7	36	39	28	47	14
	24	16	28	16	36	0
HP-20 100 mg	***0***	100	100	100	100	100
	1	-	81	60	80	22
	7	112	33	6	41	0
	24	79	24	0	26	0
HP-20 200 mg	***0***	100	100	100	100	100
	1	81	95	100	90	56
	7	83	84	76	88	0
	24	44	44	23	42	0
HP-20 300 mg	***0***	100	100	100	100	100
	1	94	98	104	93	85
	7	78	73	59	71	24
	24	39	31	10	32	0
PDMS	***0***	100	100	100	100	100
	1	104	89	105	93	94
	7	82	44	95	78	72
	24	82	27	100	54	65

LDPE	***0***	100	100	100	100	100
	1	111	99	102	94	101
	7	122	112	110	102	109
	24	103	99	99	64	68

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First use of Strata-X, Oasis HLB, LDPE and PDMS as passive samplers for many toxins

Passive sampling of Ovatoxin-a was possible in laboratory trials

Presence of *Prorocentrum lima* indicated through passive sampling

Toxins from benthic algae detected with passive samplers

HP-20 most appropriate for long exposure periods (> 5 days)