CAWTHRON

Development of Solid Phase Adsorption Toxin Tracking Technology (SPATT) for Monitoring Anatoxins

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Development of Solid Phase Adsorption Toxin Tracking Technology (SPATT) for Monitoring Anatoxins

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EXECUTIVE SUMMARY

Sampling and monitoring for cyanotoxins, especially in large waterbodies and rivers, can be problematic. Cyanotoxins levels can change very quickly with changing environmental and hydrological conditions. Current sampling practices (*e.g.* grab samples) provide only a snapshot of cyanotoxins present at one point in time and may miss areas or times of highest risk. This is particularly problematic when sampling rivers where continuous flows rapidly transport toxins. Recent investigations have revealed the widespread distribution of anatoxin-producing benthic cyanobacteria in New Zealand rivers, highlighting the need for development of new sampling technologies. To overcome the challenges associated with sampling and monitoring cyanotoxins, we have assessed the potential of solid phase adsorption toxin tracking technology (SPATT) for monitoring anatoxin-a (ATX). The technique involves the passive adsorption of cyanotoxins onto sorbents stored in porous membrane sachets and their subsequent extraction and analysis.

In this study, 15 different adsorption substrates were screened for their efficiency to uptake anatoxin-a. Four sorbents were selected and subjected to further laboratory studies which investigated their ability to adsorb anatoxin-a over an extended period in the SPATT bag format. A similar percentage (4-12%) of initial total water concentration of anatoxin-a was desorbed from all four substrates (powdered activated carbon (PAC) G-60, Strata-X (polymeric resin), AG 50W-X4 (strong cation exchange) and Amberlite IRP-64 (weak cation exchange)) after 24 hours. The greatest percentage reduction in initial total water concentrations was observed in the water containing the SPATT bag filled with PAC (56% reduction in 24 hours). However, the current elution method did not appear to effectively desorb all ATX from the PAC G-60 and further optimisation is required.

The results to date indicate that all four sorbents have promise to be utilised in a SPATT approach to monitor ATX. Activated carbon has additional advantages that it is a cheap and readily available substrate. We recommend further work to optimize SPATT for ATX using PAC, to undertake longer-term laboratory studies and to carry out field trials in selected rivers in the 2008-09 summer. SPATT has the potential to be integrated into current cyanobacterial monitoring programmes and would be a very useful and economical tool for early warning and monitoring of toxic cyanobacterial events.

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1. INTRODUCTION

Cyanobacteria (blue-green algae) produce natural toxins that can be harmful to animals and humans (Codd *et al.* 2005). Cyanotoxin production in New Zealand has commonly been attributed to planktonic cyanobacteria (Wood *et al.* 2005). Consequently there is a growing awareness amongst water-users of the health risks associated with toxic planktonic cyanobacteria, however, the dangers associated with benthic (attached to substrates) cyanobacteria are less widely acknowledged. Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and are found in a wide range of water quality conditions (Briggs & Kilroy 2000). A succession of dog poisonings in 2005 in the Wellington region led to the confirmation of anatoxin-a (ATX) and homoanatoxin-a (HTX; both neurotoxins) from benthic *Phormidium* sp. (Wood *et al.* 2007b). Microcystins (hepatatoxins) have also been detected in benthic material (Hamill 1999; Wood *et al.* 2005). Recent investigations have revealed the widespread distribution of these toxic benthic species (Wood unpubl. data) and this has created complex management issues.

Existing guidelines for planktonic cyanobacteria cannot be transferred directly to benthic species due to fundamental differences in biological requirements and monitoring methodologies. Consequently, public health/local authorities are issuing health warnings along large stretches of rivers on the basis of a very limited understanding of risks. The current New Zealand Drinking-Water Guidelines (Ministry of Health 2005) do not provide any practical advice on how to monitor or assess levels of benthic cyanobacteria/toxins in drinking water supplies. Likewise, international guidelines (currently used as surrogate New Zealand guidelines) for managing cyanobacterial risks in recreational use waters do not mention benthic cyanobacteria.

Analysis has identified anatoxins in cyanobacterial mat material and in stomach contents of dead dogs (Gugger 2005; Wood *et al.* 2007b), however, it is unclear whether extracellular toxins (*i.e.* toxin free in the water column) are being released in substantial quantities. Under certain environmental conditions mats may die or detach from the substrate (often accumulating along the edges of river) potentially causing lysing of cells and resulting in pulses of cyanotoxins being released into the water. Traditional sampling, *i.e.* taking a grab sample, only provides a snap-shot from the flow continuum and may underestimate the risk posed by benthic cyanobacteria.

A passive *in situ* methodology known as solid phase adsorption toxin tracking technology (SPATT) has been shown to be as a simple and sensitive means of warning of toxic micro-algal bloom development and associated shellfish contamination in the marine environment (MacKenzie *et al.* 2004). SPATT involves suspending in the waterbody small bags containing adsorption substrates which accumulate toxins. These can then be extracted and measured, providing information on toxins over an extended period. Applicability has been demonstrated for a range of lipophillic (fat soluble) toxins but a range of technical problems remain to be solved for use of SPATT with highly water soluble toxins such as anatoxins and cylindrospermopsin.

The aims of this project were to assess the ability of a range of adsorption substrates to uptake anatoxin-a and determine the potential applicability of using SPATT in rivers for tracking of the highly water soluble cyanobacterial toxins.

2. METHODS

2.1. Cyanobacterial cultures

Anatoxin-a producing *Aphanizomenon issatschenkoi* (CAWBG02; Wood *et al.* 2007a; Selwood *et al.* 2007) cultures were grown in 2 L Erlenmeyer flasks containing 1600 mL of MLA media (Bolch & Blackburn 1996). Flasks were incubated under a light regime of 100 µmol m^{-2} s^{-1} with a 12:12 h light:dark cycle, at a temperature of 18 ± 1°C. After 21 days the cyanobacterial cultures were gently centrifuged (1800 g, 10 minutes) to sediment cells without rupturing. The supernatant was decanted and the pellets and overlying liquid combined and freeze-thawed. Samples were then centrifuged (3000 g, 10 minutes) to sediment cell material. The supernatant was decanted and stored at -20°C. The ATX concentration was determined using liquid chromatography mass spectrometry (LC-MS) as described in Section 2.2. This material was used for all laboratory experiments.

2.2. Anatoxin-a determination by LC-MS

Anatoxins were separated by LC (Acquity uPLC, Waters Corp., MA) using a 50 × 1 mm Acquity BEH-C18 (1.7 µm) column (Waters Corp., MA). Mobile phase A (water) and mobile phase B (95% acetontrile) both containing 0.1% ammonium formate pH 4 was used at a flow of 0.3 mL/min^{-1} and a rapid gradient from 100% A to 100% B over 2.5 minutes. Injection volume was 5 µL. The Quattro Premier XE mass spectrometer (Waters-Micromass, Manchester) was operated in ESI+ mode with capillary voltage 0.5 kV, desolvation gas 900 L/hr^{-1}, 400 °C, cone gas 200 L/hr^{-1} and cone voltage 25 V. Quantitative analysis was by multiple reaction monitoring (MRM) using MS-MS channels set up for anatoxin-a (166.1 > 131.0; 166.1 > 149.1; Rt 1.3 minutes). The instrument was calibrated with dilutions in 0.1% formic acid of authentic standards of anatoxin-a (A.G. Scientific, CA).

2.3. Adsorption substrate screening

Fifteen adsorption substrates were assessed for their ability to bind ATX (Table 1) in a solid phase extraction (SPE) format using 3 mL cartridges each containing 50-200 mg of material (Table 1). The materials utilise several different sorption mechanisms. Seven were commercially available pre-packed SPE cartridges. Four activated carbons were evaluated (see Section 2.3.1). Additionally AG 50W-X4 (BioRad, New Zealand), AG 50W–X8 (BioRad, New Zealand), Diaion® HP-20 (Supelco) and Amberlite IRP-64 (Aldrich) were

available as bulk beads. Each was packed (200 mg) into 3 mL cartridges with frits bottom and top (Phenomenex, New Zealand).

2.3.1. Preparation of granulated and powdered activated carbon cartridges

Granulated activated carbon (GAC; GAC -830, -1020, -1240, NORIT, Swift NZ Ltd, New Zealand) and powdered activated carbon (PAC; Darco® G-60, Aldrich, USA) were pre-conditioned by boiling in HCl (4M, 30 minutes). After cooling, the acid was decanted and samples were washed repeatedly (more than 40 times) to remove fine particulates and acid (increase pH of wash to greater than five). Samples were oven dried at 65 °C. The PAC G-60 was mixed 2:1 (v/v) with untreated ∝-cellulose powder (Sigma, USA). The adsorbents were packed (200 mg) into 3 mL cartridges with frits bottom and top (Phenomenex, New Zealand).

2.3.2. SPE screening procedure

Cartridges were conditioned with methanol (6 mL, HPLC grade, Biolab, New Zealand) and MilliQ water (6 mL). Water samples containing 1 µg of ATX were loaded onto cartridges in two volumes: 10 mL (100 ng/mL), samples A and B, and 100 mL (10 ng/mL), samples C and D. The ability of ATX to bind to the adsorbent substrate was assessed using the entire 10 mL break-through from samples A and B and the second 10 mL and final 10 mL of break-through for the 100 mL samples C and D.

Bound ATX was eluted from the cartridges with a variety of solvents (Table 1; 6 mL). Cartridges 1-5 were eluted twice, the second time with a strong acid to ensure maximum ATX desorption. The eluants from Cartridges 10-13 were directed into 50% formic acid in MilliQ (1 mL) to immediately dilute the strong base. Eluants for 1-5 and 9-14 were diluted 1:10 in MilliQ. Eluants for 6-8 and 15 were diluted 1:10 in ammonia (0.67M). Each cartridge type and sample volume was evaluated in duplicate. ATX concentrations were determined as described in Section 2.2.

2.4. Evaluation of field trial sites

Moderate abundance (>30% coverage) of *Phormidium* spp. were observed in September 2008 at two sites; the outlet of Lake Henley (Masterton; 40° 56'S, 175° 41 E) and Wainuiomata River at Richard Prouse Park (Wellington; 41° 16'S, 174° 57 E). Sub-samples (~20 g) of *Phormidium* spp. mats were collected and frozen (-20 °C). Anatoxin-a and homoanatoxin-a concentration in the mats were determined to assess the suitability of these sites for field trials with SPATT bags.

Table 1. Adsorbent media, water volume applied (containing 1 µg ATX) and elution solvents. * Commercially available pre-packed cartridge, # PAC and cellulose, WCX weak cation exchange, SCX, strong cation exchange.

Sample No.	Product	Manufacturer	Type/Mode	Adsorb. (mg)	Sample Vol. (mL)	Elute 1	Elute 2
1A/B	GAC 830	Norit	carbon, granular	200	10	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
1C/D	GAC 830	Norit	carbon, granular	200	100	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
2A/B	GAC 1020	Norit	carbon, granular	200	10	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
2C/D	GAC 1020	Norit	carbon, granular	200	100	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
3A/B	GAC 1240	Norit	carbon, granular	200	10	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
3C/D	GAC 1240	Norit	carbon, granular	200	100	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
4A/B	G-60	Darco	carbon, powder	200#	10	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
4C/D	G-60	Darco	carbon, powder	200#	100	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
5A/B	Carbograph *	Alltech	carbon, graphite	150	10	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
5C/D	Carbograph *	Alltech	carbon, graphite	150	100	5% Formic acid in 70% MeOH	5% conc. HCl in MeOH (6ml)
6A/B	WCX *	Supelco	Silica-COOH, WCX	500	10	5% Formic acid in 70% MeOH	
6C/D	WCX *	Supelco	Silica-COOH, WCX	500	100	5% Formic acid in 70% MeOH	
7A/B	WCX *	Bakerbond	Silica-COOH, WCX	500	10	5% Formic acid in 70% MeOH	
7C/D	WCX *	Bakerbond	Silica-COOH, WCX	500	100	5% Formic acid in 70% MeOH	
8A/B	Strata-X-CW *	Phenomenex	Polymer-COOH, WCX	200	10	5% Formic acid in 70% MeOH	
8C/D	Strata-X-CW *	Phenomenex	Polymer-COOH, WCX	200	100	5% Formic acid in 70% MeOH	
9A/B	Strata-X *	Phenomenex	Polymeric	200	10	100% MeOH	
9C/D	Strata-X *	Phenomenex	Polymeric	200	100	100% MeOH	
10A/B	MCX *	Oasis	Polymeric	50	10	5% NH_3 in MeOH (5 mL)	
10C/D	MCX *	Oasis	Polymeric	50	100	5% NH_3 in MeOH (5 mL)	
11A/B	Strata-X-C *	Phenomenex	Polymer-SO_3H, SCX	200	10	5% NH_3 in MeOH (5 mL)	
11C/D	Strata-X-C *	Phenomenex	Polymer-SO_3H, SCX	200	100	5% NH_3 in MeOH (5 mL)	
12A/B	AG 50W-X4	BioRad	Polymer-SO_3H, SCX	200	10	5% NH_3 in MeOH (5 mL)	
12C/D	AG 50W-X4	BioRad	Polymer-SO_3H, SCX	200	100	5% NH_3 in MeOH (5 mL)	
13A/B	AG 501-X8	BioRad	Polymer-SO_3H, SCX	200	10	5% NH_3 in MeOH (5 mL)	
13C/D	AG 501-X8	BioRad	Polymer-SO_3H, SCX	200	100	5% NH_3 in MeOH (5 mL)	
14A/B	HP-20	Supelco	Polymeric	200	100	100% MeOH	
14C/D	HP-20	Supelco	Polymeric	200	10	100% MeOH	
15A/B	Amberlite IRP-64	Rohm & Haas	Polymer-COOH, WCX	200	100	5% Formic acid in 70% MeOH	
15C/D	Amberlite IRP-64	Rohm & Haas	Polymer-COOH, WCX	200	10	5% Formic acid in 70% MeOH	

2.5. Evaluation of selected substrates in SPATT bag format

Four substrates were selected for further evaluation in the SPATT bag format.

- PAC G-60 (powdered activated carbon, Darco, acid washed) - exhibited no breakthrough, moderate recovery of ATX and is a cheap and readily available substrate. The powder was mixed 1:2 with cellulose powder to improve its flow characteristics.
- Strata-X-CX (polymeric resin with strong cation exchange, Phenomenex) - exhibited no breakthrough, high recovery of ATX, however, bulk material is not available and pre-packed cartridges are expensive. Pre-packed cartridges were disassembled and the substrate used for SPATT experiments.
- AG 50W-X4 (polymeric resin with strong cation exchange, BioRad) - exhibited little breakthrough, high recovery of ATX, bulk material is available; however, it is moderately expensive.
- Amberlite IRP-64 (polymeric resin with weak cation exchange, Rohm & Haas) - exhibited little breakthrough, high recovery of ATX, bulk material is available; however, it is moderately expensive.

SPATT bags with dimensions of approximately 40 x 60 mm were constructed from 5 µm polyester mesh, which was heat sealed along seams using an impulse heat sealer. Each bag contained 1 g of substrate (For PAC = PAC and cellulose). A length of polyester string was fixed to each bag so that it could be secured easily to laboratory or field equipment. Each substrate was evaluated in duplicate and control bags containing no substrate were included.

SPATT bags were conditioned by suspension in 100% MeOH (overnight) followed by reverse osmosis (RO) water (10 minutes). Each bag was then suspended in a beaker (1 litre) containing 1000 mL RO water spiked with 10 µg of ATX (10 ng/mL). Samples were kept in the dark at 18 °C and aerated continuously with sterile filtered air to circulate water. Water samples (1 mL) were collected prior to addition of SPATT bags and at 10 minutes, 1 hour, 3 hour, 8 hour and 24 hour intervals. Formic acid (5 µL) was added to each sample and these were kept in the dark at 4 °C until analysis for ATX by LC-MS.

After 24 hours SPATT bags were removed. The top of each SPATT bag was cut open and the contents and SPATT bag placed into scintillation vials containing the elution solvent for desorption of ATX (Table 2). Vials were vortexed briefly then placed on a shaker with occasional vortexing. After 15 minutes an aliquot (1.5 mL) was centrifuged (14000 rpm, 2 minutes). An aliquot (100 µl) of the supernatant was collected and diluted as described in Table 2. Formic acid (5 µL) was added to each sample. Scintillation vials were kept in the dark at 4 °C and a second aliquot was taken after 96 hours and processed as described above. Anatoxin-a concentrations in all samples were determined as described in Section 2.2.

Table 2. Adsorbent substrates for SPATT bag trials, desorption solvents and dilution for analysis.

Substrate	Desorption	Dilution
DARCO,PAC G-60	5% formic acid in 70% MeOH (12 mL)	1:10 0.67M NH_3
Strata-X	100% MeOH (12 mL)	1:10 MilliQ
AG 50W-X4	5% NH_3 in MeOH (10 mL) then 50% formic acid (2 mL)	1:10 MilliQ
Amberlite IRP-64	5% Formic acid in 70% MeOH	1:10 0.67M NH_3
Control	100% MeOH (12 mL)	1:10 MilliQ

3. RESULTS

3.1. Cyanobacterial cultures

The concentration of anatoxin-a in the harvested *Aph. issatschenkoi* (CAWBG02) supernatant was 15 µg/mL by LC-MS.

3.2. Evaluation of adsorption substrates by SPE

Six substrates (GAC 830, 1020, 1240; Carbograph, AG 501-X8, HP-20) exhibited average to low retention of the applied ATX (Table 1). All other substrates retained high proportions (>70%) of ATX. Four substrates were selected, based on ease of availability and to cover a range of substrate types, for trials in the SPATT bag format (see Section 2.4).

Table 3. Recovery of anatoxin-a from adsorbent substrates used in a SPE format. All results are the average of duplicate samples.

Sample no.	Product	Manufacturer	Sample vol. (mL)	ATX in break through	ATX in elute 1	ATX in elute 2
1A/B	GAC 830	NORIT	10	60%	13%	1%
1C/D	GAC 830	NORIT	100	74%	13%	1%
2A/B	GAC 1020	NORIT	10	63%	19%	2%
2C/D	GAC 1020	NORIT	100	73%	16%	2%
3A/B	GAC 1240	NORIT	10	43%	21%	2%
3C/D	GAC 1240	NORIT	100	66%	18%	1%
4A/B	PAC G-60	Darco	10	0%	77%	5%
4C/D	PAC G-60	Darco	100	0%	67%	4%
5A/B	Carbograph	Alltech	10	19%	50%	1%
5C/D	Carbograph	Alltech	100	68%	11%	1%
6A/B	WCX	Supelco	10	0%	99%	
6C/D	WCX	Supelco	100	1%	111%	
7A/B	WCX	Bakerbond	10	0%	98%	
7C/D	WCX	Bakerbond	100	0%	106%	
8A/B	Strata-X-CW	Phenomenex	10	0%	95%	
8C/D	Strata-X-CW	Phenomenex	100	0%	96%	
9A/B	Strata-X	Phenomenex	10	1%	89%	
9C/D	Strata-X	Phenomenex	100	2%	80%	
10A/B	MCX	Oasis	10	0%	99%	
10C/D	MCX	Oasis	100	0%	102%	
11A/B	Strata-X-C	Phenomenex	10	0%	98%	
11C/D	Strata-X-C	Phenomenex	100	0%	101%	
12A/B	AG 50W-X4	BioRad	10	0%	94%	
12C/D	AG 50W-X4	BioRad	100	2%	110%	
13A/B	AG 501-X8	BioRad	10	72%	9%	
13C/D	AG 501-X9	BioRad	100	73%	4%	
14A/B	HP-20	Supelco	10	42%	48%	
14C/D	HP-20	Supelco	100	38%	26%	
15A/B	Amberlite IRP-64	Rohm & Haas	10	0%	91%	
15C/D	Amberlite IRP-64	Rohm & Haas	100	1%	111%	

3.3. Evaluation of adsorption substrates in SPATT format

3.3.1. Water samples

No decrease in ATX was observed in the control water over time (Figure 1, Table 4). The greatest decrease in ATX (percentage of initial total water concentration) in the water samples was observed in the beaker containing the PAC G-60 SPATT bag. After 24 hours the ATX in the water was 46% of the original concentration (Figure 1, Table 4). This decrease was approximately linear over the 24 hour period. Similar percentages (85-87%) of ATX remained in the water samples containing the Strata-X, AG 50W-X4 and Amberlite IRP-64 SPATT bags and adsorption appeared to occur gradually over the study period (Figure 1, Table 4).

Figure 1. Comparison of anatoxin-a (percentage of initial total water concentration) in water samples at each time interval in SPATT bag experiment. + Control, ▲ AG 50W-X4, ♦ powdered activated carbon, ■ Strata-X, × Amberlite IRP-64.

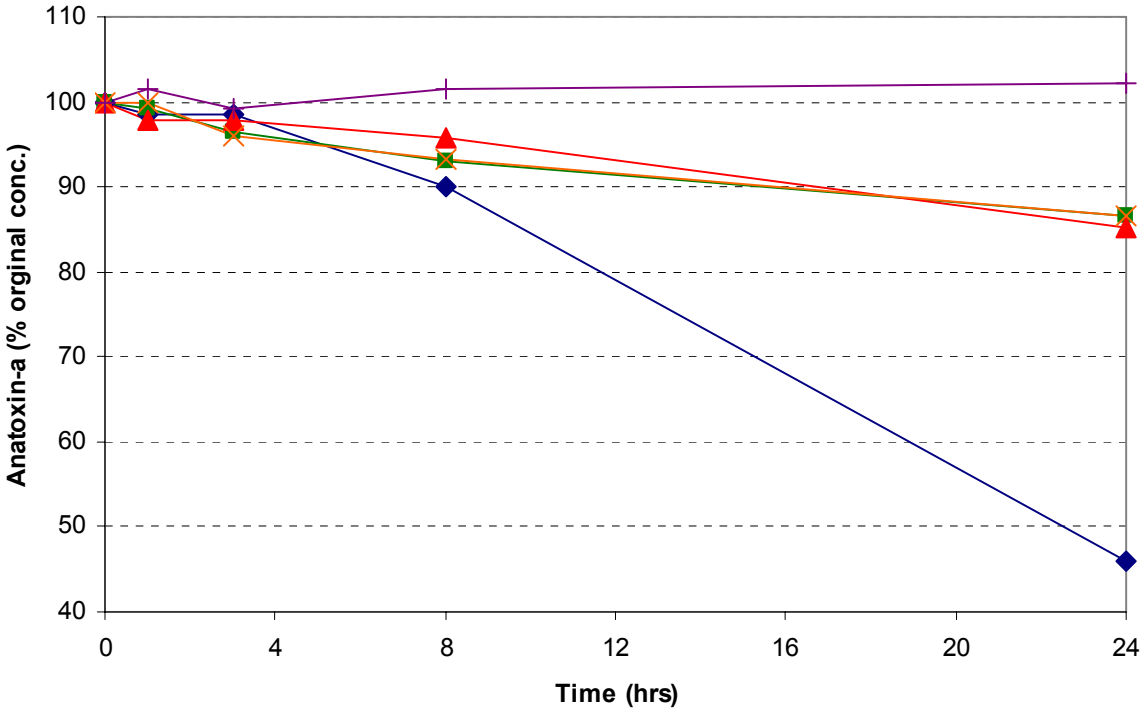


Table 4. SPATT bag experiment. Comparison of anatoxin-a in water at each time interval, amount released from the SPATT sorbent (% of initial amount) and % ATX desorbed from substrate.

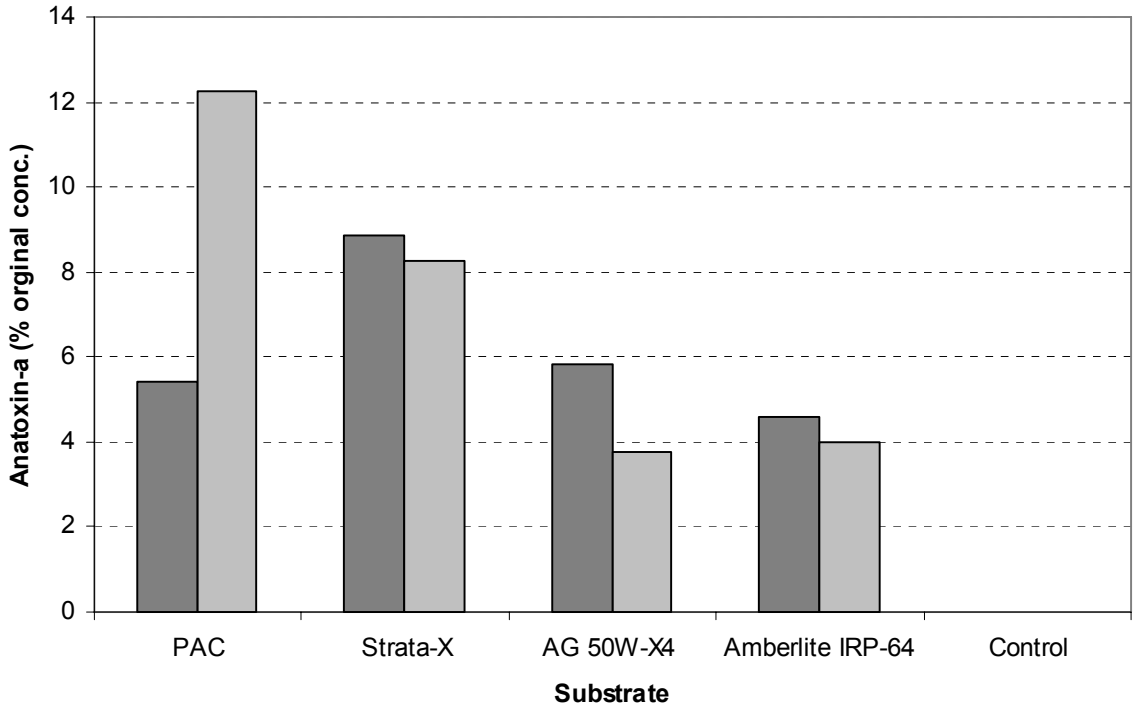
	Time (hrs)								
	Water						SPATT Bag		
Substrate	**0**	**0.6**	**1**	**3**	**8**	**24**	**24**	**96**	**% ATX desorbed from substrate**
PAC	100	99	99	99	90	46	5	12	23%
Strata-X	100	100	99	96	93	87	9	8	66%
AG 50W-X4	100	99	98	98	96	85	6	4	40%
Amberlite IRP-64	100	97	100	96	93	86	5	4	34%
Control	100	101	101	99	101	102	0	0	0%

3.3.2. SPATT bag desorbate

No ATX was bound to the SPATT mesh without adsorbent. The highest percentage of ATX desorbed from SPATT materials after the 15 minutes incubation in solvent was observed for Strata-X (9%, Figure 2, Table 4). This was closely followed by all other substrates (5-6%, Figure 2). Longer (96 hours) incubation in solvents did not markedly alter the percentage ATX desorbed from the Strata-X, AG 50W-X4 and Amberlite IRP-64 SPATT bags. However, there was a notable increase in the percentage ATX from the PAC G-60 (from 5 to 12%, Figure 2, Table 4). The failure of the sum of the ATX in the water (24 hour) plus SPATT bag

desorbate to reach 100% for PAC G-60 indicates that a proportion of ATX was very strongly bound to the carbon. Further optimisation of the PAC G-60 SPATT format and desorption could increase this recovery. The sum of ATX in water (24 hour) and bag desorbate was close to 100% for the other SPATT substrates, indicating that the majority of the bound ATX had been released from these substrates. ATX was desorbed from Strata-X with the highest efficiency (Table 4).

Figure 2. Comparison of anatoxin-a desorbed from SPATT substrate (percentage of initial total water concentration) into the SPATT bag after 24 hours. ■ 15 minutes in elution solvent ■ 96 hours in elution solvent.



3.4. Evaluation of field trial sites

No ATX or homoanatoxin-a were detected in the samples from Lake Henley and the Wainuiomata River. Therefore no field trials with SPATT bags were undertaken.

4. DISCUSSION

The results of the experiments described in this report demonstrate the potential that SPATT has for accumulation of anatoxins. We screened 15 different adsorption substrates for their efficiency to uptake anatoxin-a. Four adsorption substrates were selected and subjected to further laboratory studies which investigated their ability to adsorb anatoxin-a over an extended period in the SPATT bag format. The greatest reduction in initial total ATX concentration in the water was observed in the samples with SPATT bags filled with a powdered activated carbon (PAC G-60, 56% reduction in 24 hours). Additional advantages of PAC are that it is a cheap and readily available substrate. However, the current method for desorption did not appear to effectively unbind all ATX from the PAC.

A similar percentage (4-12%) of initial total water concentration of anatoxin-a was desorbed from all four substrates (powdered activated carbon (PAC) G-60, Strata-X (polymeric resin), AG 50W-X4 (strong cation exchange) and Amberlite IRP-64 (weak cation exchange)) after 24 hours. This is comparable to other studies that have assessed the ability of adsorbent materials to bind cyanotoxins (microcystins). Kohoutek *et al.* (2008) found that after 24 hours the percentage uptake of microcystins onto their optimised passive sampling devices was approximately 3-4%. After 14 days, their maximum percentage uptake was approximately 35%. It is plausible that a similar percentage uptake would be observed for the Strata-X, AG 50W-X4 and Amberlite IRP-64 substrates, if our study period was extended. ATX was desorbed from Strata-X with the highest efficiency and provided ATX uptake increases overtime this may prove an efficient substrate for the SPATT bags.

The lack of a potential field site during the project period (no anatoxins detected and only low benthic cyanobacterial abundance due to high winter flows) prevented the extension of the laboratory studies into field trials. We strongly recommend that further funding is given to this work so that field studies can be undertaken this summer. Significant increases in benthic cyanobacterial mats and toxins are anticipated in multiple rivers around New Zealand during the summer period.

Further research is required to determine the most suitable form of activated carbon to provide high uptake and the most efficient desorption technique. Further customisation of the SPATT design (*i.e.* the "tea bag" style) may be required to ensure efficiency and robustness in rivers. Anatoxin-a also occurs in lake environments in New Zealand (Wood *et al.* 2005; Wood *et al.* 2007a) and the methodologies developed in this study would be equally applicable to lentic systems.

Additionally we advocate that further laboratory and field trials are undertaken to assess the applicability of SPATT for monitoring other cyanotoxins. In particular lipophilic toxins such as microcystins are more amenable to passive sampling than highly water soluble toxins such as anatoxin-a. Kohoutek *et al.* (2008) showed that passive samplers provide a viable tool for long-term monitoring of microcystins. Likewise, previous work undertaken by this research group has demonstrated the advantages of using SPATT for monitoring microcystins in Lake

Rotoiti (Rotorua). In this work, the synthetic polymeric adsorbent, DIAION® HP20 (Mitsubishi Chemical Corporation) was added to mesh bags as described by MacKenzie *et al.* (2004). SPATT bags were deployed in Te Weta Bay, Lake Rotoiti. Surface water samples were collected at 9 am and 5 pm and SPATT bags were collected in triplicate once daily (9 am) for seven days. Samples were frozen until further analysis. SPATT bags were extracted as described in MacKenzie *et al.* (2004) and the total ADDA containing microcystin content in the extracts from the SPATT bags and water samples were quantified with a competitive indirect ELISA using the methods of Fischer *et al.* (2001).

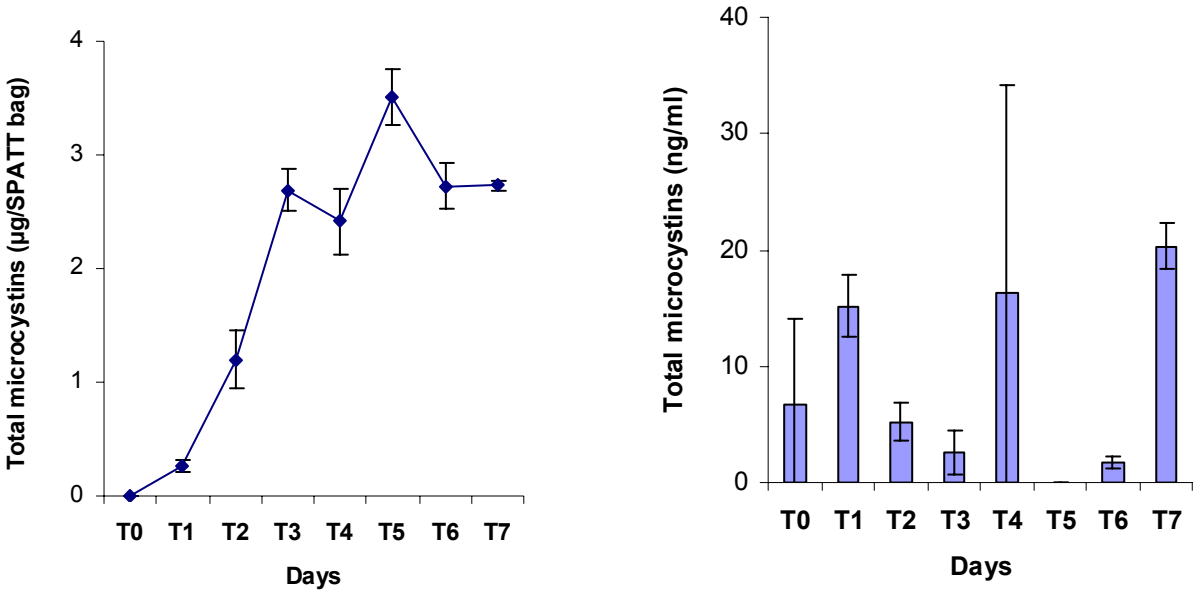


Figure 3. Total microcystin concentrations in (a) SPATT bags, and (b) lake water (average of 9 am and 5 pm samples, error bars show range). Adapted from Wood *et al.* 2008.

Microcystins were detectable in SPATT bags after one day and levels steadily increased over a five day period (Figure 3). Microcystins were detected in 12 of the 16 water samples. However, there was often a marked difference in concentrations between the morning and afternoon sampling period, highlighting the temporal variation of these toxins. The absence of microcystins in the water samples on Day 5 and at one time point on Day 0 and 4 demonstrates how misleading one time point samples can be for assessing potential health risks. SPATT provides a more integrated assessment of toxin concentrations over extended time periods.

The results of our laboratory studies on ATX and previous field work undertaken on SPATT and microcystins demonstrates that this approach represents a promising tool which can account for temporal and spatial variations in anatoxins and microcystin and avoid the requirement for extensive and expensive large-scale sampling regimes. In contrast to conventional monitoring (*i.e.* grab sampling) SPATT simulates handling of large volumes of water during a period of several days or weeks. The materials from which the SPATT bags are constructed are cheap and, analytical costs aside, significant cost savings within cyanotoxin monitoring programmes could be achieved. We recommend further research is undertaken to

optimize SPATT for ATX using activated carbons, to undertake longer-term laboratory studies and to carry out field trials in selected rivers (and lakes) in the 2008-09 summer.

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