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Evaluation of cyanotoxin risk in shallow groundwaters using SPATT samplers

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Evaluation of cyanotoxin risk in shallow groundwaters using SPATT samplers

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Prepared for Tasman District Council

CAWTHRON

Glossary

Term	Definition
Benthic	Substrate-attached
dp	Decimal place
IANZ	International Accreditation New Zealand
MAV	Maximum acceptable value
MfE	Ministry for the Environment
MoH	Ministry of Health
ND	Not detected
Planktonic	Free-floating
SPATT	Solid-phase adsorption toxin tracking
Strata-X	The solid-phase resin used in these SPATT samplers (it is more effective at binding anatoxins than other resins)
TDC	Tasman District Council

Contents

Executive summary

Some cyanobacteria can produce harmful compounds (cyanotoxins) that pose a human health risk when present in drinking water supplies. While the risks from cyanobacteria and cyanotoxins are relatively well understood in surface waters, less is known about their impact on groundwaters used for drinking water. Groundwater supplies may be influenced by surface waters contaminated with cyanotoxins when they are not separated by an impermeable substrate; furthermore, it is not currently clear how much cyanotoxins are likely to be removed by aquifer sediments.

With the introduction of new drinking water regulations in Aotearoa New Zealand, drinking water suppliers, such as Tasman District Council, need to evaluate the level of risk associated with their drinking water supplies and produce contingency plans to minimise the risk. To better understand the cyanotoxin risk in groundwater supplies located close to cyanotoxin-contaminated surface waters, a literature evaluation was undertaken on cyanotoxin infiltration into groundwaters and the removal of cyanotoxins by aquifer sediments. This work also included a case study that assessed the cyanotoxin risk in the Wakefield water supply using solid-phase adsorption toxin tracking (SPATT) samplers.

The literature evaluation indicated that cyanobacteria cells are likely to be excluded by aquifer sediments, but less is known on how effective the sediments are at removing dissolved cyanotoxins. Overseas, microcystins have been detected in groundwater supplies in several studies, with concentrations sometimes breaching safe levels for drinking water. In river systems around Aotearoa New Zealand, anatoxins are the cyanotoxin class of most concern, However, there is very little information on the adsorption characteristics of anatoxins by aquifer sediments and no studies investigating the most prevalent anatoxin congeners in Aotearoa New Zealand. The literature evaluation also highlighted that different structural classes of cyanotoxins have different adsorption properties and that a 'one-size-fits-all' approach cannot be used.

The case study investigating whether anatoxins are likely to infiltrate the shallow groundwater system used for the Wakefield water supply was unsuccessful. This was due to a lack of anatoxin-producing cyanobacteria in the Wai-iti River during the sampling period. While the case study was unable to provide clarity on whether anatoxins can enter the groundwater supply, the sampling protocols and decision frameworks developed will be useful for future evaluations of the cyanotoxin risk in groundwater supplies close to cyanotoxin-contaminated surface waters.

1. Introduction

Cyanobacteria are an integral component of freshwater ecosystems, as they fix carbon dioxide and atmospheric nitrogen and form the basis of many food webs. However, when ideal conditions for growth occur, some cyanobacteria can form blooms where high levels of biomass can accrue very quickly (Figure 1). Some cyanobacteria are also capable of producing cyanotoxins – compounds that have negative health effects for humans and animals when they are exposed to sufficiently high amounts. Cyanotoxins are a serious human health consideration for drinking water supplies, with maximum acceptable values (MAVs) for four classes of cyanotoxins specified in the Drinking Water Standards for Aotearoa New Zealand (anatoxins, cylindrospermopsins, microcystins / nodularins and saxitoxins; Water Services [Drinking Water Standards for New Zealand] Regulations 2022).

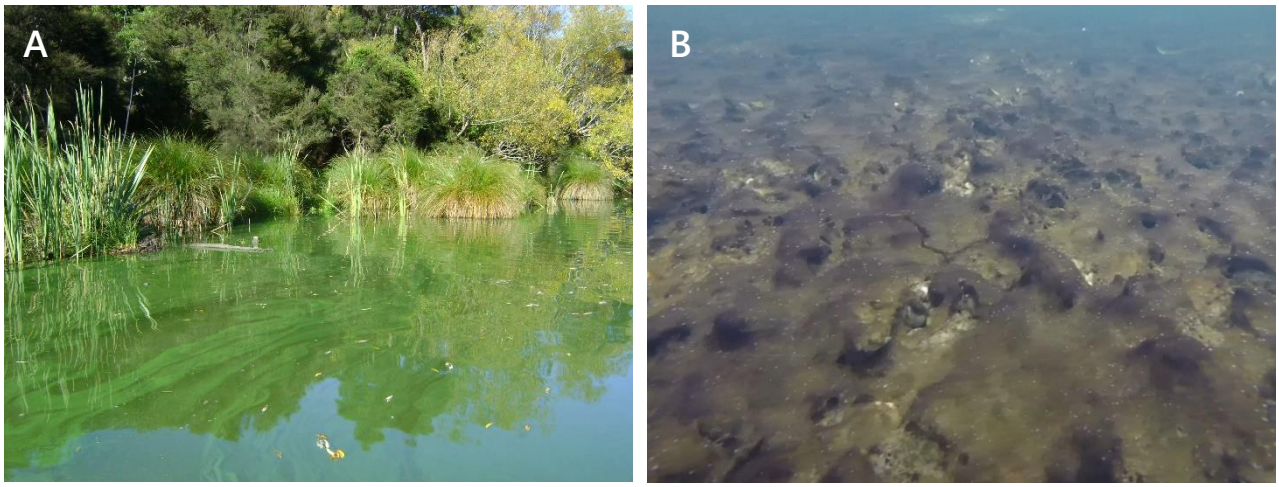

Figure 1. Images of freshwater cyanobacterial blooms. (A) Planktonic *Microcystis* bloom in Lake Rotorua (Kaikōura). (B) Benthic *Microcoleus* bloom on the base of the Mataura River (Southland).

While cyanobacteria and cyanotoxins are relatively well-understood human health risks in surface waters (MfE and MoH 2009; MoH 2020; WHO 2021), less is known about their impact on groundwaters used as drinking water supplies. Groundwater supplies may be influenced by surface waters contaminated with cyanotoxins when they are not separated by an impermeable substrate (e.g. solid rock). While multiple studies have demonstrated that aquifer sediments are able to filter out cyanobacteria cells from water (e.g. Ray et al. 2002; Pan et al. 2006; Romero et al. 2014; Harvey et al. 2015), there is limited understanding on the removal of dissolved cyanotoxins. To date, there has been little research undertaken in Aotearoa New Zealand on the risk posed by cyanotoxin-producing cyanobacteria to surface-water-influenced shallow groundwater supplies.

With the introduction of new drinking water regulations in Aotearoa New Zealand, Tasman District Council (TDC; and all drinking water suppliers) must prepare contingency plans if the level of risk in drinking water sources is 'medium' or 'high'. Currently, there is no guidance on how to categorise the

cyanotoxin risk to groundwater sources, and it falls to individual suppliers to conduct a robust risk assessment process. The Wai-iti River in the Tasman District regularly has blooms of potential cyanotoxin-producing benthic cyanobacteria (*Microcoleus*) and, therefore, is an ideal case study for researching the cyanotoxin risk in shallow groundwater systems.

This report provides:

- an evaluation of literature on cyanotoxin infiltration into groundwaters and the removal of cyanotoxins by aquifer sediments (Section 2)
- results from a case study using SPATT samplers to evaluate the risk of cyanotoxin infiltration in the groundwater supply at Wakefield during the summer of 2024 (Section 3)
- a protocol for undertaking similar SPATT sampler evaluations in other groundwater supplies potentially impacted by cyanotoxin-contaminated surface waters (Appendix 3).

2. Literature evaluation

Literature on cyanotoxins in groundwater supplies and the removal of cyanotoxins by aquifer sediments was identified through web searches using Google Scholar and supplied by TDC. The literature is evaluated and summarised in Sections 2.1–2.3.

2.1 Cyanotoxin infiltration of groundwater

No literature on the presence of anatoxins in groundwater systems was found, but seven international studies have noted microcystin infiltration into groundwater.

Ueno et al. (1996) measured microcystin concentrations in surface waters (ponds, ditches and rivers) and groundwaters (shallow and deep wells: approximately 3–5 m and 100–200 m in depth, respectively) in the city of Haimen and Fusui County (China). Microcystins were not detected in any of the deep well samples, but microcystins were detected in 4% of the shallow well samples.

Eynard et al. (2000) evaluated cyanobacterial blooms in five lakes in the Baltezers area of Latvia, including microcystin measurements of the water from a nearby artificially recharged groundwater aquifer used for drinking water. Low dissolved microcystin concentrations (< 0.01 µg/L) were regularly detected in the groundwater, although higher concentrations were also measured (≥ 0.6 µg/L). The study noted a 1-month delay between the presence of microcystin-producing blooms in the nearby lake and increased microcystin concentrations in the groundwater system, which corresponded with the expected retention time between the infiltration basin and the pumping station.

Messineo et al. (2006) undertook a 5-year monitoring study of microcystin-producing cyanobacteria in Lake Albano (Italy), including dissolved microcystin measurements of 13 nearby wells. Microcystins were detected in two of the wells, although concentrations were very low (0.004–0.067 µg/L).

Mohamed and Al Shehri (2009) assessed microcystin concentrations in 10 groundwater wells located in the Asir region of Saudi Arabia. The wells were in the proximity of rainwater puddles and ponds that contained microcystin-producing cyanobacteria. Microcystins were detected in each of the wells at varying concentrations (0.3–1.8 µg/L). While the well waters were generally screened from the surface waters by aquifer sediments, large storm events had caused surface waters to spill directly into the wells.

Tian et al. (2013) detected microcystins in groundwater sources in the Huai River Basin (China), and the researchers linked the groundwater microcystin contamination with cyanobacteria in a nearby river. Microcystin contamination of groundwater supplies near Lake Chaohu (China) was also noted by Yang et al. (2016), with one microcystin-contaminated well located hundreds of metres from the lake.

Mohamed et al. (2022) evaluated microcystin infiltration of groundwater wells close to the Nile River (Egypt) and detected dissolved microcystins in the wells up to 35 m away. Microcystin concentrations in

the wells were linked to the distance from the river (i.e. lower toxin concentrations were detected in supplies that were further from the river) and the severity of the cyanobacterial bloom (i.e. higher toxin concentrations in the river water were linked to higher concentrations in the groundwater). While microcystins were detected in the groundwater wells, removal of some of the dissolved microcystins by the aquifer sediments was observed (i.e. lower dissolved toxin concentrations were observed in the wells compared to the river water). The authors of this study noted that the aquifer sediments were graded sand and gravel with thin interbeds of clay.

The maximum dissolved microcystin concentrations detected in groundwater supplies from the studies described above were as follows (ordered by decreasing microcystin concentrations):

- 1.8 µg/L (Mohamed and Al Shehri 2009)
- 1.5 µg/L (Eynard et al. 2000)
- 1.1 µg/L (Yang et al. 2016)
- 0.45 µg/L (Tian et al. 2013)
- 0.84 µg/L (Mohamed et al. 2022)
- 0.10 µg/L (Ueno et al. 1996)
- 0.07 µg/L (Messineo et al. 2006)

Three of these maximum measurements exceeded the 1 µg/L World Health Organization (WHO) guideline value for microcystins in drinking water (WHO 2020) and Aotearoa New Zealand's 1 µg/L MAV for microcystins / nodularins in drinking water (Water Services [Drinking Water Standards for New Zealand] Regulations 2022). In addition to the three measurements greater than 1 µg/L, two of the maximum measurements were close to or above the 50% MAV value (0.5 µg/L), which indicates when heightened risk management actions are implemented in Aotearoa New Zealand.

2.2 Cyanotoxin removal by aquifer sediments

Previous research has demonstrated that aquifer sediments are able to remove cyanobacterial cells from water (e.g. Ray et al. 2002; Pan et al. 2006; Romero et al. 2014; Harvey et al. 2015); however, there is less certainty on the removal of dissolved cyanotoxins by different types of aquifer sediments. While multiple studies have examined the adsorption of microcystins by aquifer sediments and several studies have investigated the adsorption characteristics of other cyanotoxins (cylindrospermopsins, nodularins and saxitoxins; Table 1), only one study has evaluated the adsorption of anatoxins (Klitzke et al. 2011). This is significant for the local context, as anatoxins are the most prevalent cyanotoxin observed in our river systems (McAllister et al. 2016). Furthermore, only one anatoxin congener was evaluated (anatoxin-a), but other structural congeners (e.g. homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a) are commonly observed in higher concentrations in the cyanobacterial mats that grow in rivers around Aotearoa New Zealand.

Table 1. Summarised findings from studies investigating cyanotoxin adsorption to aquifer sediments (including closely related studies).

Cyanotoxin class	Experimental design	Findings	Reference
Anatoxins	Batch	• Higher sediment clay content led to higher adsorption of anatoxin-a.	Klitzke et al. (2011)
Cylindrospermopsins	Column	• At pH~8, cylindrospermopsin did not adsorb to sand or sediment containing up to 4% 'fines'.	Klitzke et al. (2010)
	Batch	• Higher sediment organic carbon content led to higher adsorption of cylindrospermopsin.	Klitzke et al. (2011)
Microcystins	Batch	• Higher sediment clay content led to higher adsorption of microcystin-LR. • Highest adsorption of microcystin-LR occurred around pH 4.8.	Miller et al. (2001)
	Batch	• Sediment organic carbon content did not have a large influence on the adsorption of microcystin-LR.	Dillon et al. (2002)
	Batch	• Sediment clay content had the largest positive effect on adsorption of microcystin-LR. • At lower microcystin-LR concentrations, the organic carbon content of sediments was also correlated with higher adsorption. • Sediment sand content was not associated with adsorption of microcystin-LR.	Miller et al. (2005)
	Batch	• At pH 8, higher sediment clay content led to higher adsorption of microcystin-LR.	Mohamed et al. (2007)
	Batch	• Microcystin-RR adsorbed to lake sediments more strongly than microcystin-LR. • Sediment clay content had the largest positive effect on adsorption of microcystin-LR and microcystin-RR. • Sediment organic matter contents above 8% had a positive effect on adsorption of microcystin-LR and microcystin-RR. • Highest adsorption of microcystin-LR to lake sediments and kaolinite (a clay mineral) occurred around pH 2.5 and decreased at higher pH (7.2 and 9.7).	Wu et al. (2011) *

Cyanotoxin class	Experimental design	Findings	Reference
Microcystins	Batch	• Microcystin-RR adsorbed to filter sand more strongly than microcystin-LR and microcystin-YR. • Higher adsorption of microcystin-LR was observed in aquifer sediment (containing 'fines' and organic material) than in filter sand and quartz sand.	Grützmacher et al. (2020)
Nodularins	Batch	• Higher sediment clay content led to higher adsorption of nodularin-R. • Highest adsorption of nodularin-R occurred around pH 4.8. • Higher salinity generally increased adsorption of nodularin-R.	Miller et al. (2001)
	Batch	• Sediment clay content had the largest positive effect on adsorption of nodularin-R. • At lower nodularin-R concentrations, the organic carbon content of sediments was also correlated with higher adsorption. • Sediment sand content was not associated with adsorption of nodularin-R.	Miller et al. (2005)
Saxitoxins	Batch	• Saxitoxin adsorbed to different types of clay and to marine sediments. • High ionic strength reduced adsorption of saxitoxin. • Some saxitoxin was able to be desorbed from the sediment.	Burns et al. (2009) *
	Batch	• At pH~7, saxitoxin and neosaxitoxin adsorbed to sediments containing 4% clay / silt.	Romero et al. (2014)

* These studies focused on the adsorption of cyanotoxins to lake and marine sediments rather than aquifer sediments.

From the studies that investigated cyanotoxin adsorption (Table 1), sediment clay content was generally the dominant factor driving increased cyanotoxin adsorption. This was noted for anatoxin-a, microcystins, nodularin-R and saxitoxins. Cylindrospermopsin was the exception, as Klitzke et al. (2011) did not observe increasing adsorption effects with increasing sediment clay content. With cylindrospermopsin, increasing sediment organic matter had the largest positive effect on adsorption. Microcystins and nodularin-R demonstrated higher adsorption at lower pH (< 4.8), but the effect of solution pH was not evaluated for other toxin classes. While increased ionic strength promoted nodularin-R adsorption, it reduced adsorption of saxitoxin. This is likely due to saxitoxin adsorption to sediment being mediated through ion exchange, which is reduced with higher concentrations of ions in the solution. This process reinforces the need to individually evaluate the different toxin classes, as their structural differences will influence how effective aquifer sediments are at removing them.

Although it was not a focus of our literature evaluation, we also identified several studies that investigated the biodegradation of cyanotoxins by microbes associated with aquifer sediments. Several studies found that microbial degradation was effective at reducing the concentrations of anatoxin-a (Rapala et al. 1994), cylindrospermopsins (Klitzke et al. 2010; Ho et al. 2012; Klitzke and Fastner 2012) and microcystins / nodularin-R (Miller and Fallowfield 2001; Ho et al. 2012). Klitzke and Fastner (2012) noted that anoxic conditions inhibited the biodegradation of cylindrospermopsin. Ho et al. (2012) did not observe biodegradation of saxitoxins by microbial communities from two water sources in Australia but noted that the microbes capable of degrading saxitoxin may not have been present.

2.3 Summary

While aquifer sediments appear to be effective at removing cyanobacteria cells from surface waters, their effectiveness in removing dissolved cyanotoxins is not fully understood. Multiple studies have reported microcystins (a cyanotoxin commonly observed in lakes) in groundwater systems located close to surface waters affected by cyanobacterial blooms, with concentrations exceeding safe levels for drinking water on several occasions. The amount of sediment between the surface water supply and the groundwater supply (i.e. the distance and level of connectivity) as well as the type of sediment appear to play a role in the effectiveness of aquifer sediments in removing dissolved cyanotoxins.

The studies reviewed on cyanotoxin adsorption by sediments mainly focused on the removal of microcystins. While these data will prove useful for groundwater supplies closely related to lakes that suffer from microcystin-producing cyanobacterial blooms (e.g. *Microcystis*), microcystins are not a prevalent toxin class in rivers around Aotearoa New Zealand (unless the river is the outflow of a lake experiencing a microcystin-producing cyanobacterial bloom). Anatoxins are more likely to be present in our rivers, and only one study investigated the adsorption of anatoxin-a. This work indicated that aquifer sediments containing clay were more effective at anatoxin-a removal. Other insights from the literature review included the finding that different structural classes of cyanotoxins demonstrate different adsorption properties and that a 'one-size-fits-all' approach cannot be safely adopted.

For groundwater supplies closely related to river systems in Aotearoa New Zealand, more studies investigating the adsorption of anatoxin congeners frequently observed here (homoanatoxin-a,

dihydroanatoxin-a and dihydrohomoanatoxin-a) are required to better understand the effectiveness of aquifer sediments and associated microbial communities for removing anatoxins. This future work will contribute to an improved evaluation of how cyanotoxin risk is minimised through aquifer sediment filtration and biodegradation.

3. Case study on the Wakefield water supply

The Wakefield water supply provides reticulated water to the urban Wakefield area, Pigeon Valley and Main Road Spring Grove (as far as the Spring Grove church; TDC 2024a; Figure 2). Water is taken from a well, bore and infiltration gallery close to the Wai-iti River in Wakefield. Following abstraction, the water undergoes cartridge filtration, aeration, chlorination and ultra-violet (UV) disinfection. The Wai-iti River regularly contains benthic *Microcoleus* blooms during the summer months (TDC 2024b), which can sometimes produce anatoxins (McAllister et al. 2016).

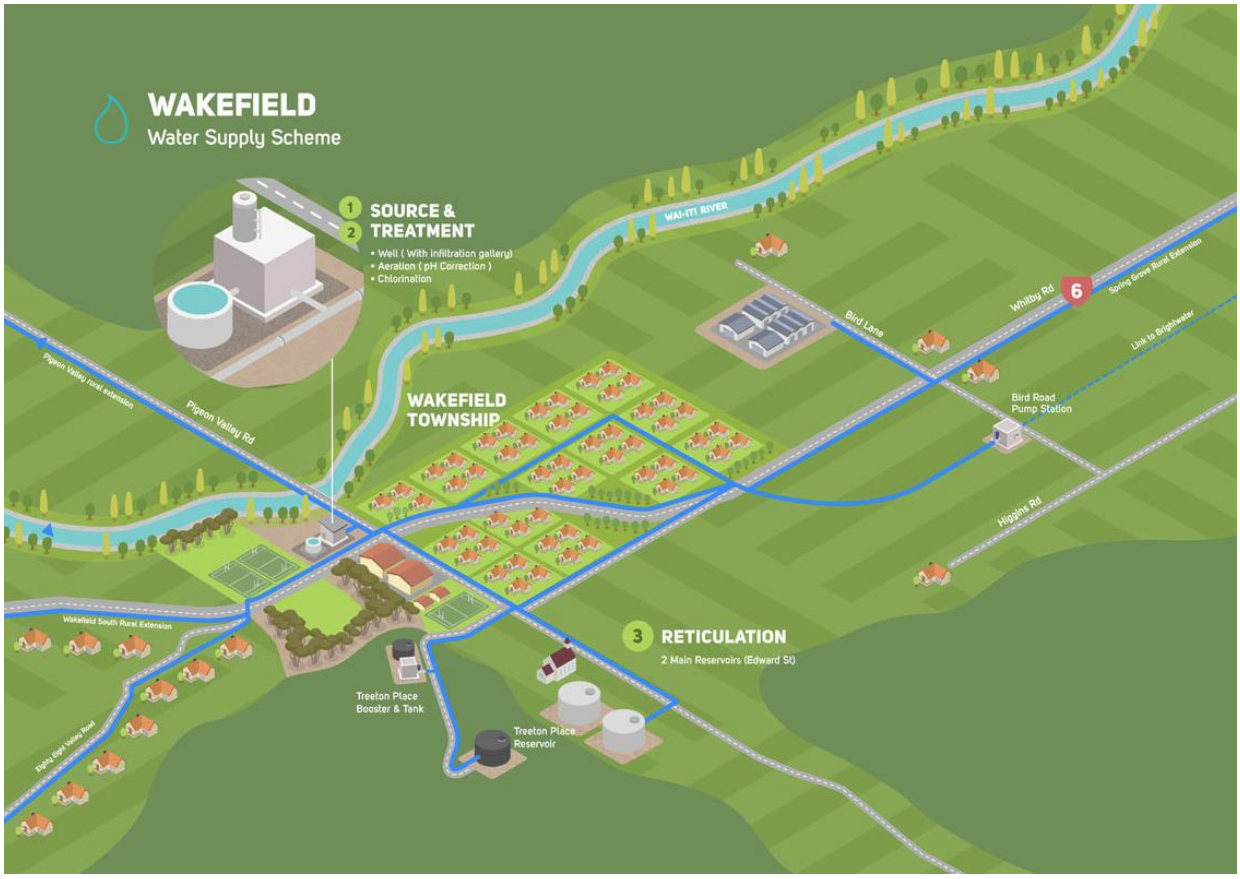

Figure 2. Schematic of the Wakefield water supply, which draws source water from the Wai-iti River through an infiltration gallery and into a shallow well (TDC 2024a; note that this schematic may no longer be current).

3.1 Sampling plan

All sample collection and observations were undertaken by TDC staff after training from the report author.

Sampling sites

All sampling sites were near the Wakefield Recreation Reserve where Pigeon Valley Road crosses the Wai-iti River (Figure 3). Weekly source water samples and SPATT samples were collected from the Wakefield well (at the southwestern corner of the parking lot and near the eastern corner of the field; Figure 4). When possible, water samples were collected from the Wakefield bore (at the northeastern corner of the field and closer to the river than the Wakefield well site; Figure 5), but this required the presence of an engineering team member to activate a pump system. Weekly river water and cyanobacterial mat samples were collected from a river site (Figure 6), where the benthic cyanobacterial mat coverage was also evaluated. The river site was a 30-m stretch of the Wai-iti River located at the fork in the river found near the access way at the northwestern corner of the field (Figure 3). The location of the river sampling site was just upstream from the infiltration gallery feeding the Wakefield Well.

Figure 3. Location of sampling sites used during the Wakefield water supply case study.

Figure 4. Images of the Wakefield well sampling site.

Figure 5. Images of the Wakefield bore sampling site.

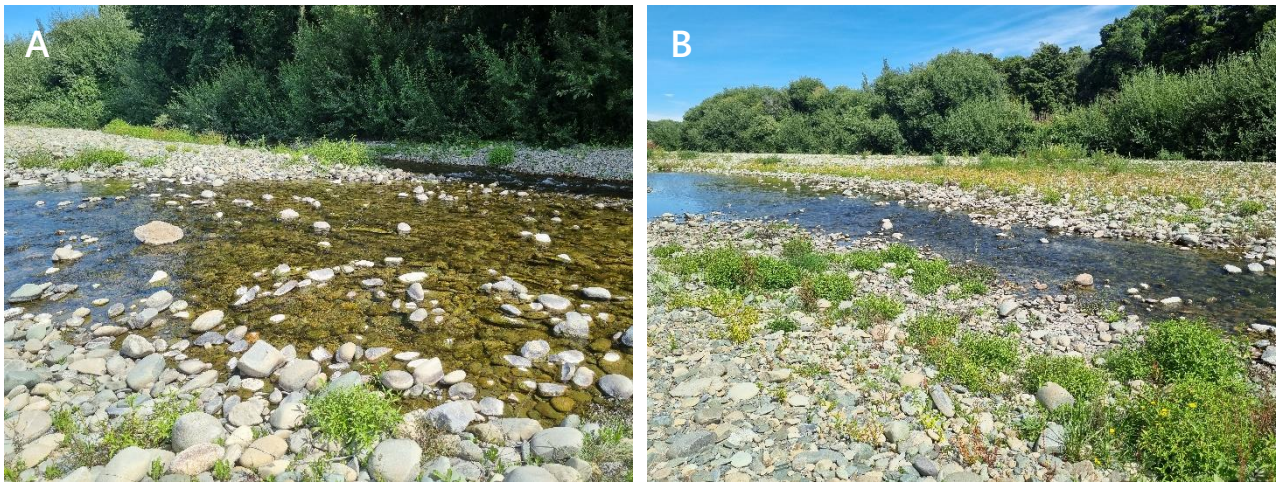

Figure 6. Images of the downstream (A) and upstream (B) ends of the river site used for cyanobacterial mat sampling and measuring of the benthic cyanobacterial mat coverage in the Wai-iti River.

Sampling frequency

Sampling and monitoring were undertaken approximately weekly, but the timing was reduced or extended by several days when required. This frequency was based on the ideal duration that the SPATT samplers can be deployed before the build-up of biofilms, and to capture changes in cyanobacterial mat abundance and cyanotoxin concentrations.

The sampling frequency was extended up to 10 days when required (e.g. due to staff availability or poor weather conditions that limited sampling of the river).

Measuring benthic cyanobacteria percentage coverage

Refer to Appendix 1 for full instructions on evaluating percentage coverage of benthic cyanobacteria in wadeable rivers (adapted from MfE and MoH 2009).

Starting downstream, the percentage coverage was evaluated through an underwater viewer (to the nearest 5%) at five locations across four transects evenly spaced along the river monitoring site. Rocks containing cyanobacterial mats were collected from two locations across each transect after measuring the percentage coverage (for cyanobacterial mat samples). The average benthic mat coverage for each transect was calculated (the average of the five measurements) before calculating the average coverage of the four transects (the percentage coverage for the monitoring site).

Cyanobacterial mat samples

A composite cyanobacterial mat sample was collected from at least eight rocks using a 50 mL plastic tube and a metal spatula. The rocks were collected while measuring cyanobacterial mat percentage coverage (from two locations across each of the four transects). For transects that did not contain benthic cyanobacterial mats, we used rocks from other locations at the monitoring. If no benthic cyanobacterial mats were observed, then no cyanobacterial mat samples were collected. A portion of

the cyanobacteria mat (approximately 2 cm in diameter) was scraped into the sample tube using the spatula. This was repeated for all eight rocks, and all samples were placed into the same container.

Samples were frozen until extraction and anatoxin analysis.

River water samples

At the start of the visit, a river water sample was collected from the middle of the river at the downstream end of the monitoring site (to avoid disturbing the streambed during sampling). The sample was collected in a 50 mL plastic tube, which was rinsed with river water three times before the tube was filled to the 40 mL mark (to avoid tube breakage upon freezing).

Samples were frozen until extraction and anatoxin analysis.

Source water samples

Source water samples were retrieved from the Wakefield well by lowering a bailer (Solinst Model 428 BioBailer) into the water using the sampling line. A 50 mL plastic tube was rinsed three times using the bulk water sample before the tube was filled to the 40 mL mark (to avoid tube breakage upon freezing).

On occasion, source water samples were collected from the Wakefield bore. The water sample for the Wakefield bore was taken at the water treatment plant source water sample tap. As the bore operates intermittently to supplement the Wakefield well, water supply from the well was disengaged and supply from the bore was run for approximately 30 min (to flush the pipe) before a sample was taken. This was used to rinse and fill a 50 mL plastic tube as above.

Samples were frozen until extraction and anatoxin analysis.

SPATT samples

Pre-prepared SPATT samplers were provided to TDC staff, who primed them before deployment (see Appendix 2).

SPATT samplers were only deployed at the Wakefield well, where the SPATT sampler was attached to a sampling line and lowered into the water reservoir. The gap under the tightening clasp was used to attach the sampler to the sampling line using a carabiner. The sampling line reached far enough into the well that it remained underwater during its deployment, but not so far that it touched the base of the reservoir.

After approximately a week of deployment, the SPATT sampler was retrieved and replaced with a new sampler (except for the final sample). The retrieved SPATT sampler was placed in an individual resealable bag and frozen until extraction and anatoxin analysis.

The date that the sampler was deployed and retrieved was documented so that any anatoxin measurements could be normalised to the amount of time the SPATT sampler was deployed.

3.2 Anatoxin analysis methodology

River, well and bore water extraction

Water samples were defrosted, and a 10 mL aliquot was transferred to a 15 mL plastic tube containing 10 µL of formic acid to yield a final concentration of 0.1%. The anatoxins were extracted using three freeze-thaw cycles interspersed with sonication for 30 min. After the final freeze-thaw cycle, the extracts were clarified by centrifugation (3,000×*g* for 10 min) and transferred to glass autosampler vials and stored at −20 °C until analysis. This process was undertaken in case cyanobacteria cells were present in the samples. Analysis for dissolved anatoxins only would not require an extraction procedure.

SPATT sample extraction

The nylon mesh and Strata-X of the SPATT sampler were submerged in 20 mL of methanol + 0.05% formic acid and extracted at 4 °C in the dark for 2 hours with periodic mixing (every 30 min). The nylon mesh was removed using tweezers and the extract was clarified by centrifugation (3,000×*g* for 5 min). A 5 mL aliquot of the extract was transferred to a glass vial and dried at 40 °C under a stream of nitrogen gas. The dried extract was resuspended in 0.1% formic acid in milli-Q water (1 mL), transferred to a micro-centrifuge tube and clarified by centrifugation (12,000×*g* for 5 min). The supernatant was transferred to a glass autosampler vial and stored at −20 °C until analysis.

Cyanobacterial mat sample extraction

Wet mat samples were dried in a freeze-drier. The water content was then determined by weighing the wet sample and the dry sample (each to 4 dp) and calculating the amount of water that had been removed. The dried mat samples were milled with a metal spatula, and approximately 0.5 g of milled sample was weighed into a 15 mL plastic tube (with the sample weight recorded to 2 dp). 10 mL of 0.1% formic acid in milli-Q water was added to each sample, and the anatoxins were extracted using three freeze-thaw cycles interspersed with sonication for 30 min. After the final freeze-thaw cycle, the extracts were clarified by centrifugation (3,000×*g* for 10 min) and transferred to glass autosampler vials, which were stored at −20 °C until analysis.

Anatoxin analysis

Anatoxin concentrations in extracts were determined by liquid chromatography-tandem mass spectrometry as described in Kelly et al. (2019). The limit of detection was 0.05 ng/mL in water samples (the equivalent of µg/L) and 0.1 ng/mL in cyanobacterial mat and SPATT samples (due to the higher background in these extracts).

3.3 Case study results

From 12 January 2024 to 28 March 2024, approximately weekly measurements of benthic cyanobacterial mat coverage were made at the river site on the Wai-iti River close to the infiltration gallery for the Wakefield water supply (Table 2). At the beginning of the monitoring period, cyanobacterial mat coverage was above 30%, but this progressively decreased until it was less than 5% by March 2024.

Table 2. Benthic cyanobacterial mat coverage monitoring data from the Wai-iti River near to the infiltration gallery used for the Wakefield water supply.

Monitoring date	Average cyanobacterial mat coverage
12/01/2024	33%
18/01/2024	33%
26/01/2024	27%
01/02/2024	21%
09/02/2024	18%
15/02/2024	15%
23/02/2024	7%
01/03/2024	4%
08/03/2024	2%
15/03/2024	1%
22/03/2024	< 1%
28/03/2024	0%

From 1 February 2024 to 28 March 2024, SPATT samplers were retrieved from the Wakefield well after being deployed in the source water for approximately 1 week (6–8 days; Table 3). Anatoxins were not detected in any of the SPATT samples (Table 3).

Table 3. Anatoxin results for SPATT samplers deployed in the Wakefield well.

Sampling date	Client ID	Days deployed	ng of anatoxins / per g of Strata-X / day *				
			ATX	HTX	dhATX	dhHTX	Total
01/02/2024	TDC-RJN014	6	ND	ND	ND	ND	–
09/02/2024	TDC-RJN019	8	ND	ND	ND	ND	–
15/02/2024	TDC-RAW004	6	ND	ND	ND	ND	–
23/02/2024	TDC-RJN024	8	ND	ND	ND	ND	–
01/03/2024	TDC-RJN028	7	ND	ND	ND	ND	–

Sampling date	Client ID	Days deployed	ng of anatoxins / per g of Strata-X / day *				
			ATX	HTX	dhATX	dhHTX	Total
08/03/2024	TDC-RN032	7	ND	ND	ND	ND	–
15/03/2024	TDC-RJN037	7	ND	ND	ND	ND	–
22/03/2024	TDC-RN041	7	ND	ND	ND	ND	–
28/03/2024	TDC-RJN046	6	ND	ND	ND	ND	–

* ND = Not detected (< 0.1 ng/mL in the SPATT sampler extract).

From 1 February 2024 to 28 March 2024, source water samples were collected from the Wakefield well approximately weekly. Anatoxins were not detected in any of the Wakefield well samples (Table 4).

Table 4. Anatoxin results for source water from the Wakefield well.

Sampling date	Client ID	µg/L of anatoxins *				
		ATX	HTX	dhATX	dhHTX	Total
26/01/2024	TDC-RJN009	ND	ND	ND	ND	–
01/02/2024	TDC-RJN011	ND	ND	ND	ND	–
09/02/2024	TDC-RJN016	ND	ND	ND	ND	–
15/02/2024	TDC-RAW001	ND	ND	ND	ND	–
23/02/2024	TDC-RJN020	ND	ND	ND	ND	–
01/03/2024	TDC-RJN025	ND	ND	ND	ND	–
08/03/2024	TDC-RJN031	ND	ND	ND	ND	–
15/03/2024	TDC-RJN036	ND	ND	ND	ND	–
22/03/2024	TDC-RJN040	ND	ND	ND	ND	–
28/03/2024	TDC-RJN045	ND	ND	ND	ND	–

* ND = Not detected (< 0.05 µg/L).

From 1 February 2024 to 22 March 2024, water samples were collected from the Wakefield bore approximately fortnightly. Anatoxins were not detected in any of the Wakefield bore samples (Table 5).

Table 5. Anatoxin results for water from the Wakefield bore.

Sampling date	Client ID	µg/L of anatoxins *				
		ATX	HTX	dhATX	dhHTX	Total
01/02/2024	TDC-RJN010	ND	ND	ND	ND	–
09/02/2024	TDC-RJN015	ND	ND	ND	ND	–
23/02/2024	TDC-RJN023	ND	ND	ND	ND	–
08/03/2024	TDC-RJN033	ND	ND	ND	ND	–
22/03/2024	TDC-RJN042	ND	ND	ND	ND	–

* ND = Not detected (< 0.05 µg/L).

From 12 January 2024 to 28 March 2024, river water samples were collected from the Wai-iti River approximately weekly. Anatoxins were not detected in any of the river water samples (Table 6).

Table 6. Anatoxin results for river water from the Wai-iti River.

Sampling date	Client ID	µg/L of anatoxins *				
		ATX	HTX	dhATX	dhHTX	Total
12/01/2024	TDC-RJN004	ND	ND	ND	ND	–
18/01/2024	TDC-RJN006	ND	ND	ND	ND	–
26/01/2024	TDC-RJN008	ND	ND	ND	ND	–
01/02/2024	TDC-RJN013	ND	ND	ND	ND	–
09/02/2024	TDC-RJN018	ND	ND	ND	ND	–
15/02/2024	TDC-RAW002	ND	ND	ND	ND	–
23/02/2024	TDC-RJN021	ND	ND	ND	ND	–
01/03/2024	TDC-RJN026	ND	ND	ND	ND	–
08/03/2024	TDC-RJN029	ND	ND	ND	ND	–
15/03/2024	TDC-RJN034	ND	ND	ND	ND	–
22/03/2024	TDC-RJN038	ND	ND	ND	ND	–
28/03/2024	TDC-RJN043	ND	ND	ND	ND	–

* ND = Not detected (< 0.05 µg/L).

From 12 January 2024 to 28 March 2024, composite cyanobacterial mat samples were collected from the Wai-iti River approximately weekly. Anatoxins were not detected in any of the cyanobacterial mat samples (Table 7). The water content of the cyanobacterial mat samples ranged from 89% to 96% (Table 7), which is typical for *Microcoleus* dominated mat samples (Puddick et al. 2017).

Table 7. Anatoxin results for cyanobacterial mat samples from the Wai-iti River.

Sampling Date	Client ID	Water Content	µg/kg of anatoxins (dry weight) *				
			ATX	HTX	dhATX	dhHTX	Total
12/01/2024	TDC-RJN003	93%	ND	ND	ND	ND	–
18/01/2024	TDC-RJN005	94%	ND	ND	ND	ND	–
26/01/2024	TDC-RJN007	93%	ND	ND	ND	ND	–
01/02/2024	TDC-RJN012	93%	ND	ND	ND	ND	–
09/02/2024	TDC-RJN017	93%	ND	ND	ND	ND	–
15/02/2024	TDC-RAW003	96%	ND	ND	ND	ND	–
23/02/2024	TDC-RJN022	93%	ND	ND	ND	ND	–
01/03/2024	TDC-RJN027	96%	ND	ND	ND	ND	–
08/03/2024	TDC-RJN030	89%	ND	ND	ND	ND	–
15/03/2024	TDC-RJN035	96%	ND	ND	ND	ND	–
22/03/2024	TDC-RJN039	96%	ND	ND	ND	ND	–
28/03/2024	TDC-RJN044	94%	ND	ND	ND	ND	–

* ND = Not detected (< 2 µg/kg, when 0.5 g of sample is extracted in 10 mL).

3.4 Case study insights

While benthic cyanobacteria were present in the Wai-iti River for much of the sampling period, anatoxins were not detected in any of the samples. The absence of anatoxins in the SPATT samples and source water samples from the Wakefield well, and the water samples from the Wakefield bore, does not confirm that anatoxin infiltration into the groundwater is not occurring at this site. The absence of anatoxins in the cyanobacterial mat and river water samples collected from the Wai-iti River suggests that anatoxins were not present in the river during the sampling period, or that they were present at such low concentrations that they were not detectable using the techniques adopted in this study.

Anatoxin production in *Microcoleus* mats can be variable (Wood et al. 2010; Kelly et al. 2019); therefore it is possible that cyanobacteria proliferations further upstream were capable of producing anatoxins and potentially released the toxin into the overlying river water (Wood et al. 2018). If this occurred in sufficiently high concentrations, it would be captured in the river water samples, which were all negative.

Overall, the results from the study indicate that the cyanobacterial mats present in the Wai-iti River, both at the sampling site and upstream, during the sampling period were either not able to produce anatoxins or did not produce substantial levels of the toxin. However, this may not be the case in subsequent years. To limit testing costs, repeat assessments of this type should follow the guidance provided in Appendix 3 and confirm that toxin-producing cyanobacteria are present in the surface water system before evaluating for cyanotoxin infiltration in the groundwater system.

4. Conclusions

While cyanobacteria cells are likely to be excluded by aquifer sediments, it is not clear how effective they are at removing dissolved cyanotoxins and limiting their infiltration of groundwater supplies. Overseas, microcystins have been detected in groundwater supplies in several studies, with concentrations sometimes breaching safe levels for drinking water. The amount of sediment between the cyanotoxin-contaminated surface water and the groundwater appeared to increase the removal efficacy; therefore, shallow groundwater systems located close to rivers may be at-risk from cyanotoxin infiltration.

In rivers systems around Aotearoa New Zealand, anatoxins are the largest cyanotoxin of concern for groundwater used as drinking water supplies. There is very little information on the adsorption characteristics of anatoxins by aquifer sediments (only one study to date using anatoxin-a), making it difficult to predict the efficacy of removal. To the best of my knowledge, there is no information on this for the anatoxin congeners most prevalent in Aotearoa New Zealand (homoanatoxin-a, dihydroanatoxin-a and homoanatoxin-a).

A case study investigating whether anatoxins are likely to infiltrate the groundwater system used for the Wakefield water supply was unsuccessful due to a lack of anatoxin-producing cyanobacteria in the Wai-iti River during the sampling period. While the case study was unable to provide clarity on whether anatoxins can enter this groundwater supply, the sampling protocols developed will be useful for future evaluations in the Tasman District and other regions seeking to understand similar issues. While a systematic sampling regime was used for this case study, I suggest that future studies employ the staged approach described in Appendix 3. In the staged approach, SPATT samplers are not deployed in the water supply unless toxins are detected in the river system, reducing the likelihood of producing inconclusive results.

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6. Appendices

Appendix 1. Measuring benthic cyanobacteria mat percentage coverage in wadeable rivers

The following equipment is required to undertake a benthic cyanobacterial mat assessment:

- Underwater viewers or bathyscopes (see image below) are commercially available. These viewers allow a clear view of the stream bed with no interference from surface turbulence and reflection. They also enable a more or less standard area of the stream bed to be defined at each survey point (equivalent to a quadrat in terrestrial ecology). Photographs can be taken through these viewers for improved documentation of mat coverage.
- Clipboard, pencils and monitoring forms.
- Sampling containers and permanent marker pen or equivalent (for labelling).

Using an underwater viewer (or bathyscope) to visually assess periphyton growth. Photo credit: S.A. Wood, Cawthron Institute. Source: New Zealand guidelines for cyanobacteria in recreational fresh waters.

For health and safety, and logistical reasons, the survey should be undertaken in teams of two: one observer and one scribe.

Monitoring should be undertaken under similar flow conditions (e.g. at no more than median flow). This ensures the surveys always cover the permanently wetted channel. Surveys in very low flows are acceptable, but higher flows should also be avoided due to the associated safety issues and reduced water clarity.

1) After arriving at a survey area, spend a few minutes looking along the riverbed for the presence of cyanobacterial mats. Mark out four transects in the selected area by placing marker rocks along the water's edge and evenly spaced along the monitoring site (e.g. approximately every 6 m along a 20 m monitoring site).

2) Complete the first section of the monitoring form with the site, date, time, etc., and note the general presence / absence of cyanobacterial mats and the presence of any detached mats along the shoreline.

3) Assemble the underwater viewer and, starting at the *downstream end*, wade into the stream at right angles to the water's edge. Go out to a depth of approximately 0.6 m (see graphics on the next two pages). In shallow rivers the transects may span the entire width. Wading into fast-flowing water can be dangerous and *extreme care* should be taken.

4) Record the maximum distance and depth in the boxes at the top of the column for Transect 1.

5) Hold the underwater viewer about 20 cm under the water, more or less on the transect line. The area of view should not be one that has just been walked over. Holding the viewer steady, and as vertical as possible, estimate to the nearest 5% the proportion of the area you see that is occupied by the cyanobacterial mat. Some examples are shown in the image on page 25. Cyanobacterial mats are usually dark black, dark brown or dark green in colour, leathery, and have an earthy, musty odour. Refer to appendix 7 in the 'New Zealand Guidelines for Cyanobacteria in Recreational Fresh waters – Interim Guidelines' for a photographic guide to benthic *Microcoleus* and for photos of other benthic algae commonly observed in rivers around Aotearoa New Zealand. Coverage should only be recorded if mats are greater than 1 mm thick, although it is useful to record the presence of thin mats.

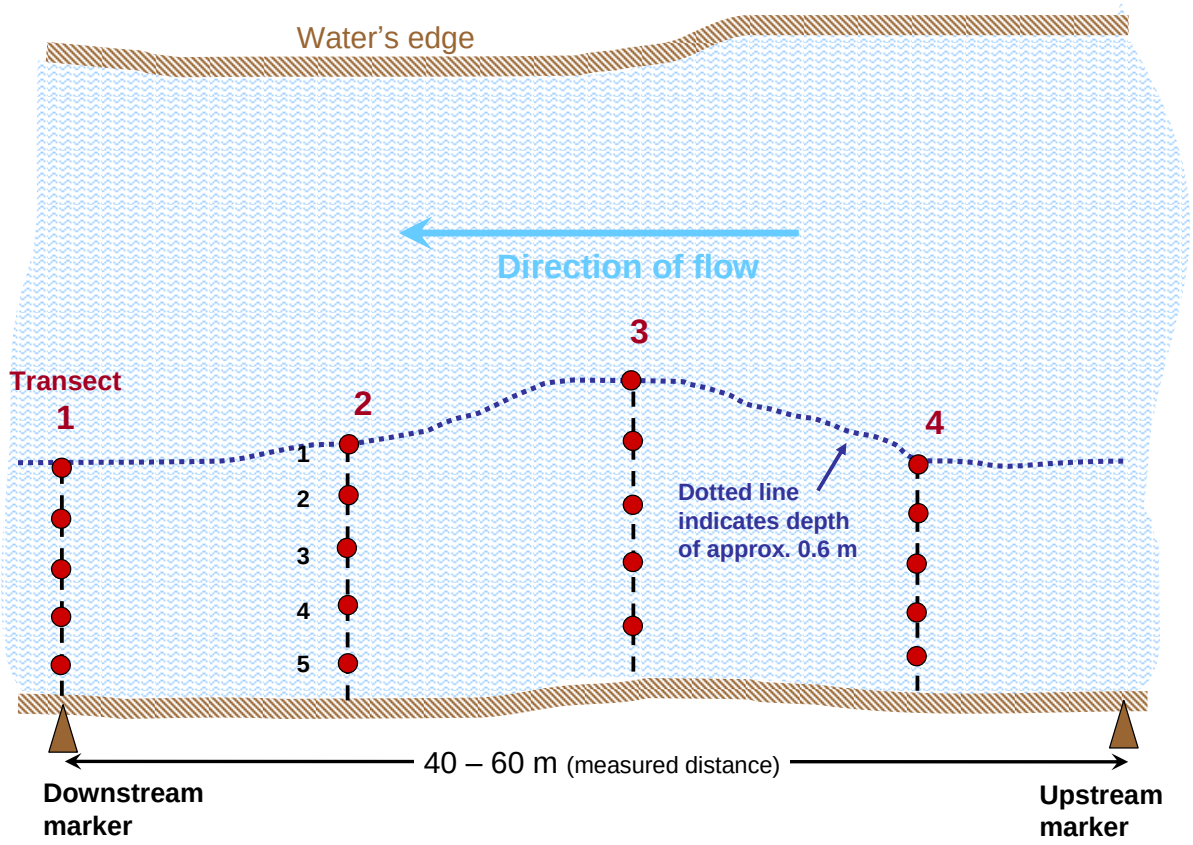
6) If there is any doubt about the identity of mat cover (i.e. whether it is *Microcoleus*) at any sampling point, take a sample for microscopic identification. Samples should be collected by scraping an egg-sized clump of mat into a sampling pottle. Samples for microscopy should be preserved with Lugol's iodine, and the cyanobacteria identification / enumeration testing provider will likely supply this – Cawthron offers this service at a fee, contact NaturalToxinsSection@cawthron.org.nz for more information.

7) Record the percentage cover in the appropriate boxes for each transect. Ideally, be consistent with the order of the survey points on each transect (e.g. Point 1 is always the deepest into the water and Point 5 is always closest to the waters' edge, see the graphics below). Record notes regarding other algal cover (e.g. green filaments overgrowing cyanobacterial mats).

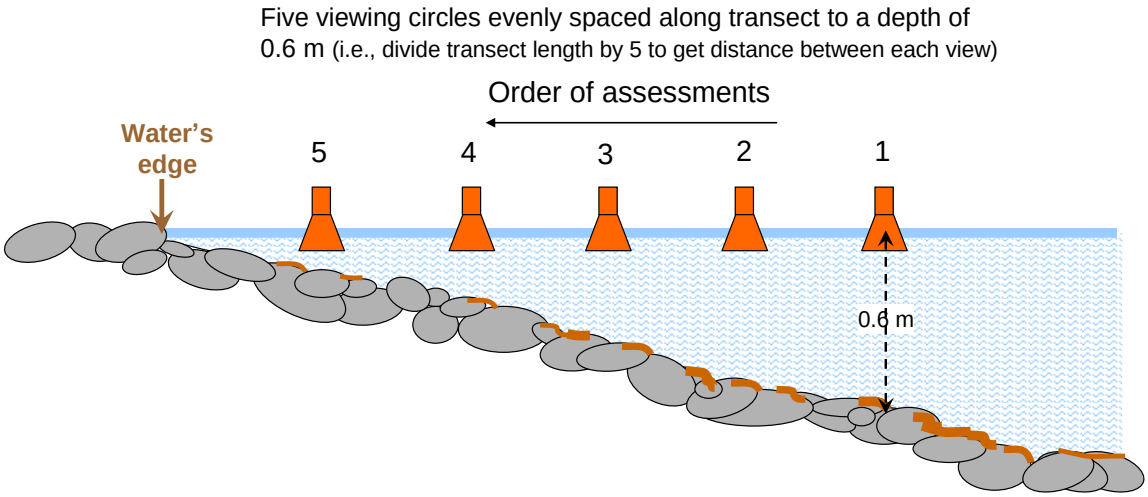
8) Space the points evenly along the transect to a depth of 0.1–0.15 m nearest to the water's edge, although this depth will vary according to the type of river. For example, if the riverbank is incised (channelised), the closest survey point will be deeper.

9) Move upstream to transects 2, 3 and 4, and repeat Steps 5 to 8 to complete the survey.

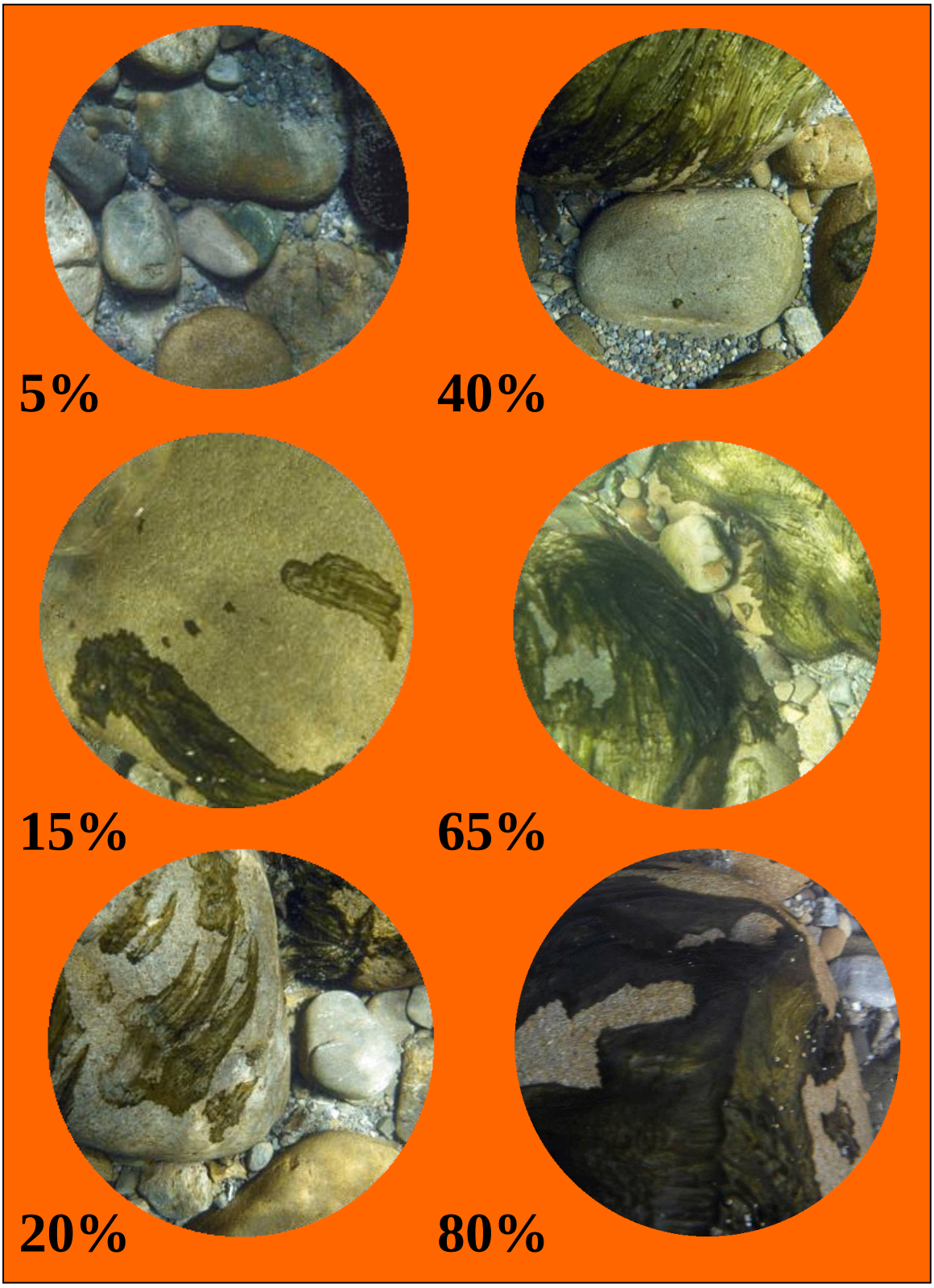
10) Calculate the average percentage cover per transect and then the average percentage cover per site (the average of the four transects).



Schematic of layout of transects (numbered in red) and survey areas (red circles, numbered in black) at a site (not to scale).Notes: The numbering indicates the order in which assessments are made and should correspond to the numbers on the monitoring form. The transects are spaced evenly along the survey reach. It may not always be possible to have five viewer results (e.g. in steep-sided rivers); in these circumstances, take as many views as practical per transect. If the river does not exceed 0.6 m in depth, the transect should span its entire width. Image credit: C. Kilroy, NIWA. Source: New Zealand guidelines for cyanobacteria in recreational fresh waters.



Schematic of transect cross-section showing arrangement of sampling points (not to scale). Notes: Assessment 1 will cover a greater area than Assessment 5 because of the greater water depth. However, this will be the case at all sites, and therefore assessments should be comparable. Image credit: C. Kilroy, NIWA. Source: New Zealand guidelines for cyanobacteria in recreational fresh waters.



Examples of different levels of cyanobacterial cover viewed through an underwater viewer. Photo credit: M. Heath, Victoria University. Source: New Zealand guidelines for cyanobacteria in recreational fresh waters.

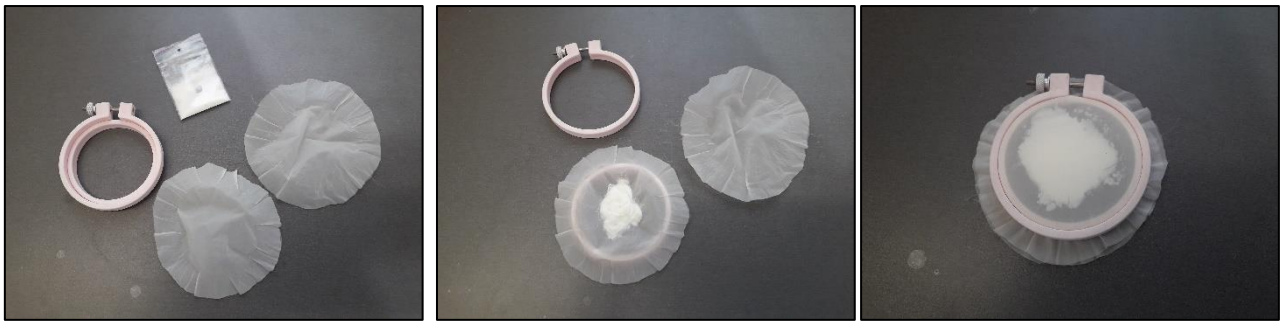
Appendix 2. Priming SPATT samplers for deployment

Solid-Phase Adsorption Toxin Tracking (SPATT) is a sampling system that uses a resin to passively sample for cyanotoxins dissolved in water over time. A 'solid-phase' resin is held between two pieces of nylon mesh and suspended in a waterbody. Toxins dissolved in the water will bind to the resin, providing a time-integrated sample. Compared to 'grab samples', SPATT sampling does not detect an accurate concentration of toxin in the water; instead, SPATT sampling provides early warning of toxins in a waterbody (as the toxin levels on the sampler build up over time) and allows for pulses of toxin released into flowing water to be detected more easily.

Because other organic compounds also bind to the solid-phase resin, the samplers will eventually become saturated and will no longer bind additional toxins in the waterbody. We recommend that the samplers not be left in a waterbody for longer than 10 days, but this will be dependent on the location and the concentration of dissolved organic compounds present.

The SPATT samplers are provided pre-assembled but will need to be 'primed' and 'equilibrated' prior to deployment. Following this process, do not allow the sampler to dry out, as the resin is hydrophobic. After it dries out, it will not necessarily become 'wet' again if placed directly in water and will need to be re-primed. During deployment, make sure that the sampler stays under the water surface so that the toxin results can be related to the amount of time the sampler spent in the water; this will also ensure that the sampler does not dry out.

When you remove the sampler from the water, it can be stored in a resealable bag in a freezer or fridge until shipping. Once the sampler is received back at Cawthron, we will remove the resin and extract the toxins bound to it. The resulting extract will be analysed for cyanotoxins by LC-MS/MS (liquid chromatography-tandem mass spectrometry).



Images illustrating SPATT sampler assembly. Note: SPATT samplers are provided pre-assembled.

Preparing the SPATT samplers for deployment

- Prime the required amount of SPATT samplers in methanol or ethanol.
    - Make sure that the samplers are covered with solvent.
    - Leave the samplers in the methanol / ethanol for at least 1 hour.
    - Samplers can be left for longer, so long as the solvent doesn't evaporate.
    - This process 'wets' the resin and washes off compounds left over from the manufacturing process.
- Transfer the primed samplers into a container of 'clean' water (Milli-Q water, RO-water or water treated with a carbon filter) to equilibrate.
    - Make sure that the samplers are covered with water.
    - Leave the samplers in the water for at least 30 min.
    - Samplers can be left for longer (e.g. several days) but will need to be monitored to avoid microbial growth.
    - This process washes the priming solvent from the resin and keeps the resin wet until deployment.

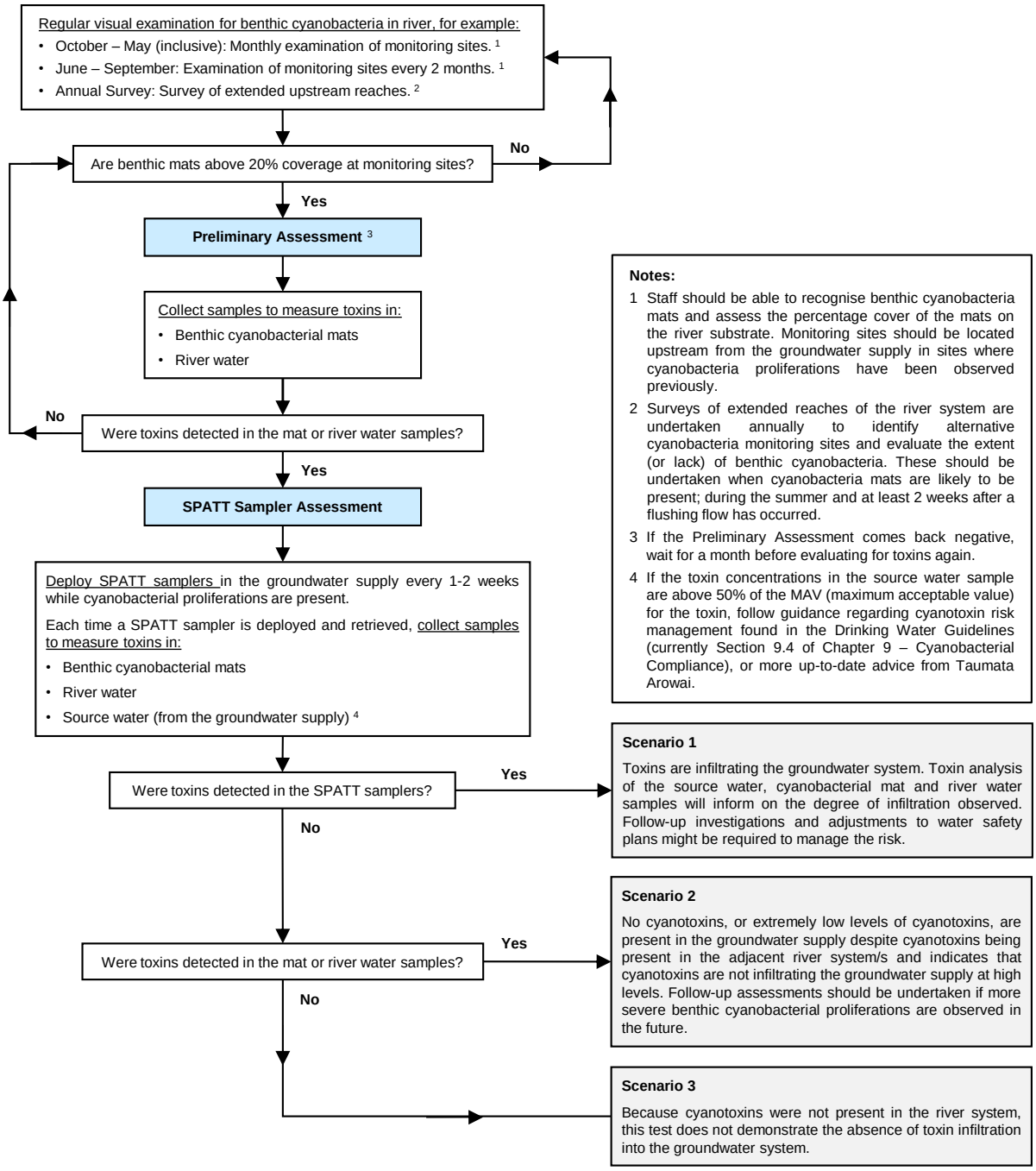
Appendix 3. Sampling and decision-making protocol for evaluating cyanotoxin risk in groundwater supplies with likely connectivity to surface waters containing potentially toxic benthic cyanobacteria

Solid-phase adsorption toxin tracking (SPATT) samplers (Wood et al. 2011) can be used to help establish whether cyanotoxins are infiltrating groundwater supplies potentially connected to surface waters. SPATT samplers use a resin to passively sample for cyanotoxins dissolved in water. A 'solid-phase' resin is held between two pieces of nylon mesh and is suspended in a waterbody. Cyanotoxins dissolved in the water will bind to the resin, providing a time-integrated sample. Compared to 'grab samples' of river water, SPATT sampling does not detect an accurate concentration of toxin in the water; instead, SPATT sampling provides increased sensitivity for detecting cyanotoxins in a waterbody (as the cyanotoxin levels on the sampler build up over time) and allows for pulses of cyanotoxin released into flowing water to be detected more easily.

Prior to deployment, SPATT samplers need to be primed with an organic solvent (e.g. ethanol, methanol) and then equilibrated in 'clean' water (e.g. reverse-osmosis water, distilled water). The deployment of the SPATT samplers will depend on the configuration of the groundwater system:

- For systems with an above-ground storage system (e.g. a holding tank), the SPATT sampler would usually be deployed here.
- For systems with a wide bore access, the SPATT sampler might be deployed using a piece of line to suspend it below the water level.
- For systems where source water access is largely restricted (e.g. can only be accessed through a tap), a sampling reservoir would need to be introduced; for example, a slow and steady flow of water is dispensed from the tap into a container where the SPATT sampler is deployed.

Because toxic cyanobacteria are not always present in rivers, the sampling and analysis framework described below should be used in response to benthic cyanobacteria being detected in the river, rather than for regular monitoring of groundwater supplies.



Decision-making framework for evaluating cyanotoxin risk in groundwater supplies with likely connectivity to surface waters containing potentially toxic benthic cyanobacteria.

1) If > 20% benthic cyanobacterial mat coverage is observed in rivers, undertake a **Preliminary Assessment** evaluating mat samples and river water samples:

   a) Collect 10 mat samples from different rocks and combine into a composite sample for cyanotoxin testing. If possible, analyse samples from several sites upstream from the groundwater supply.

b) Collect a river water sample near the groundwater site for toxin testing.

c) If possible, test for anatoxins, cylindrospermopsins, microcystins, nodularin and saxitoxins. Alternatively, the toxin classes that are most likely to be present can be assessed through analysing the mat samples for cyanotoxin production genes or by evaluating the cyanobacterial taxa present using microscopy.

d) These samples should be tested by an International Accreditation New Zealand (IANZ)-accredited laboratory.

2) Make decisions around further testing based on toxin results:

a) If cyanotoxins are detected in the mat or the water samples, then undertake a SPATT sampler assessment to determine if low levels of cyanotoxins are infiltrating the groundwater.

b) If cyanotoxins are not present in the mat or the water samples, then do not undertake a SPATT sampler assessment, as cyanotoxins are not evident in the river system.

3) To undertake a **SPATT Sampler Assessment**:

a) Deploy SPATT samplers in the groundwater supply every 1–2 weeks while cyanobacterial proliferations are present in the river.

b) Each time a SPATT sampler is deployed and retrieved, collect mat and river water samples as described in Step 1 above.

c) Each time a SPATT sampler is deployed and retrieved, also collect a source water sample from the groundwater supply.

d) Cyanotoxin testing will be guided by the types of toxins observed during the initial sampling.

e) SPATT samplers can generally be pooled for extraction and testing at the conclusion of the sampling period. This may also be possible for other samples collected, but this should be decided in consultation with Taumata Arowai or local Public Health Staff.

The combined results from the different samples collected for the SPATT sampler assessment provide evidence on whether cyanotoxin infiltration is occurring in the groundwater supply:

Scenario 1 – Cyanotoxins **are detected** in the SPATT samples. This suggests that toxins are infiltrating the groundwater system. Toxin analysis of the source water, cyanobacterial mat and river water samples will inform on the degree of infiltration observed. Follow-up investigations and adjustments to water safety plans might be required to manage the risk.

Scenario 2 – Cyanotoxins are **not detected** in the SPATT samples but **are detected** in the cyanobacterial mat / river water samples. This would demonstrate that no cyanotoxins (or extremely low levels of cyanotoxins) are present in the groundwater supply despite cyanotoxins being present in the adjacent river system/s and indicate that cyanotoxins are not infiltrating the groundwater supply at high levels. The level of infiltration would be affected by the concentration of cyanotoxins in the river system (more toxins in the surface water could mean more toxins in the groundwater), so follow-up assessments could be undertaken if more severe benthic cyanobacterial proliferations are observed in the future.

Scenario 3 – Cyanotoxins are **not detected** in the SPATT samples and are also **not detected** in the cyanobacterial mat / river water samples. Because cyanotoxins were not present in the river system, this test does not demonstrate the absence of toxin infiltration into the groundwater system. This is why an initial assessment for cyanotoxins in the river system should be undertaken prior to undertaking a SPATT Sampler Assessment.

The SPATT sampler testing for cyanotoxins undertaken at Cawthron is not accredited by IANZ and should only be used for research purposes (e.g. to understand connectivity between surface waters and groundwater supplies). It should not be used to understand the immediate risk to human health in a drinking water supply – in these instances, direct testing of source water samples should be undertaken by an IANZ-accredited testing provider.

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