

## A study of the efficacy of various home filtration substrates in the removal of microcystin-LR from drinking water

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### ABSTRACT

This study was conducted to determine whether common water filtration and purification systems bought by consumers and used in the home would remove cyanotoxins from water.

Commonly used universal filter housings and filter sizes were utilized to identify filter media that may be effective in the removal of microcystin-LR in deionized water.

Results suggest that the efficacy of home filtration devices in removing microcystin-LR varies considerably with the type of device being used. Carbon filters successfully removed microcystin-LR allowing only 0.05–0.3% of the toxin load to pass through the filter. On the other hand, pleated paper and string wound filters allowed >90% of microcystin-LR present in the sample to pass through the filters. Theoretically, the use of carbon home filtration devices tested in this study may provide protection against human exposure to cyanotoxin in addition to protection provided by water treatment methodologies utilized in water treatment facilities.

Further studies need to be done to assess the efficacy of home filtration devices for various cyanotoxins and for other filtering conditions such as increased toxin load, the presence of other contaminants in drinking water, and the repetitive use of the same filter over longer time intervals.

**Key words** | cyanotoxin, filter, microcystin, removal, toxin

### INTRODUCTION

The occurrence of cyanobacterial blooms in lakes and reservoirs has been reported worldwide and has the potential to affect the quality of public drinking water originating from these surface water bodies (Ressom *et al.* 1994; Chorus & Bartram 1999; Chorus 2001; Haider *et al.* 2003). Cyanobacterial blooms can occur sporadically and prolifically and result in an overgrowth of algae-like organisms in the water. When these blooms occur, depending on the efficacy of water treatment and removal processes in place, cyanobacteria and their toxins can potentially make their way into surface-derived drinking water (Chorus 2001). The presence of cyanobacteria can affect both the odour and taste quality of drinking water, but even more importantly has been linked with human

illness (Falconer *et al.* 1983a, b; Teixeira *et al.* 1993; Jochimsen *et al.* 1998; Chorus 2001; Griffiths & Saker 2003; Haider *et al.* 2003). While studies have suggested some ability of commercial water treatment plants to remove cyanobacterial toxins from surface water (Chorus & Bartram 1999), little information is available to help consumers protect themselves should the commercial process break down, or if they are outside a commercial treatment facility area. The current laboratory study was undertaken to investigate the efficacy of various home water filtration methods in the removal of low levels of microcystin-LR, a cyanotoxin, from drinking water.

Cyanobacteria are small, unicellular or filamentous bacteria with some characteristics of algae such as cell

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wall structure, pigments and the ability to perform oxygenic photosynthesis (Chorus 2001). Many cyanobacteria species are capable of forming toxins when growing in favourable conditions. Cyanobacterial growth and the occurrence of blooms are supported by ample sunlight, warm temperatures and waters that contain high levels of nutrients (Ressom *et al.* 1994; Falconer 1998; Haider *et al.* 2003).

Unusual blooms of toxic algae have been implicated as the cause of illness and death in both animals and humans. Accounts of human illness and death have been documented subsequent to populations drinking inadequately treated water that originated from surface water contaminated with cyanobacteria and their toxins (Griffiths & Saker 2003; Haider *et al.* 2003).

*Microcystis*, one of many species of cyanobacteria, is commonly found in freshwater and is capable of producing toxins called microcystins. About 60 congeners of microcystin have been characterized (Botes *et al.* 1982a, b, 1985; Rinehart *et al.* 1994; Sivonen 1996) and microcystin-LR is one of the most common structural variations (Carmichael *et al.* 1988). *Microcystis* and its associated toxins are repeatedly found in studies of algal blooms worldwide and it is one of the most studied cyanobacteria in laboratory animals. In the various reported incidents of poisonings in humans and livestock caused by cyanobacteria and their toxins, *Microcystis* is the most frequently cited organism (Chorus & Bartram 1999).

While the various toxins produced by cyanobacteria have been described functionally as hepatotoxins, neurotoxins or cytotoxins, the microcystins have been shown to be primarily hepatotoxins. In animal studies, acute intravenous or intraperitoneal exposure to microcystin has resulted in severe liver damage while the kidneys and lungs were less affected (Chorus & Bartram 1999). Microcystins also show tumour promoting activity through inhibition of protein phosphatases 1 and 2A (PP1 and PP2A) (Falconer *et al.* 1988; Falconer 1991a, b; Fujiki *et al.* 1996).

This study was conducted to determine whether common, commercially available water filtration and purification systems bought by consumers and used in the home would remove cyanotoxins from water. Microcystin-LR, one of the most common and ubiquitous cyanotoxins, was chosen to be studied. While home filtration would not be recommended as the sole protection against cyanotoxins,

such filters, if effective, could add an additional level of protection and assurance to persons simply concerned about, or legitimately susceptible to, adverse health effects resulting from cyanotoxin ingestion. This is of interest, since prior experiences indicate that standard water treatment methods and procedures used by water treatment facilities may be overburdened during unusually heavy blooms (Chorus & Bartram 1999). Additionally, the concern has been raised that, since water treatment methods vary in their ability to remove cyanotoxins (Chorus & Bartram 1999), various water treatment facilities may not routinely remove 100% of cyanotoxin contamination. This theoretically could result in acute or chronic low-level exposure to various cyanotoxins of unknown health significance. However, it should be noted that environmental data indicate that cyanobacterial blooms are episodic in nature, which precludes the likelihood of persistent chronic exposure.

## METHODS

### Experimental design

A study was designed in a phased approach to isolate and control experimental variables in a step-wise manner. In the first phase described in this report, standard, universal filter housings and filter sizes were utilized to identify filter media that may be effective in the removal of microcystin in deionized water with subsequent evaluation of filtration efficiency. To eliminate variables in design and size, standard 9.75 inch (24.77 cm) filter cartridges and universal filter housings, which are readily available to consumers throughout the US, were used.

Microcystin-LR was selected as the toxin for use in the initial set of experiments. This specific aquatic toxin was chosen as it has been extensively studied in experimental animals for its role in potential health effects in humans. In addition, the World Health Organization (WHO 1998) has issued a provisional exposure guideline of  $1.0 \mu\text{g l}^{-1}$  microcystin-LR in drinking water. Finally, this toxin is readily available at a reasonable cost.

In addition to blank controls, the protocol utilized concentrations of 1 mg microcystin-LR solution on carbon block (CB) and carbon wrap (CW) filters and 0.5 mg microcystin-LR solution on pleated paper (PP) and fibre

wound (FW) filters. These concentrations were chosen to meet method analytical detection limits and to serve as a reasonable challenge to the filter media. While the amounts within the concentrations observed in surface water, relevance to concentrations that might be found in distributed treated water is unknown. At the initial phase of the study, smaller concentrations of microcystin-LR were planned; however after an initial set of experiments with lower toxin concentrations (200 µg), the concentration was adjusted to account for filter efficiency in toxin removal as well as to accommodate analytical detection limits.

Each filter type used in this study was tested in triplicate using a new filter for each test. In addition, each filter test consisted of ten (500 ml) filtrate samplings. Prior to each filtration event, the apparatus was rinsed with 4 litres of deionized water to saturate and flush the filter. This volume was voided prior to sample collection to account for dead volume within the apparatus. The first two samples were taken immediately prior to the introduction of the toxin and were followed by eight additional samples taken immediately after the toxin was introduced to the filter. The system flow rate averaged  $2.5 \text{ l min}^{-1}$ . All ten filtrate samples were collected, processed and analysed to determine whether the filter permanently absorbed the toxin or if it was only temporarily retaining the toxin with a subsequent slow release. To ensure that filter apparatus used in this study did not contain background contamination a filtrate sample was collected and analysed prior to each series of test samplings.

## MATERIALS

### Chemicals

Microcystin-LR standard was purchased from Sigma-Aldrich (Sigma M 2912); other reagents were HPLC or AR grade. Formic acid was purchased from Sigma-Aldrich (Sigma # F-0507). Other reagents were obtained from Fisher Scientific: HPLC grade water (Fisher # W5-4), acetonitrile (Fisher A996-4) and methanol (Fisher A454-4).

### Equipment

A Waters HPLC system consisted of an Alliance 2695 Separation Module with a Waters 2996 Photodiode Array Detector (set for wavelength 210–350 nm). A Symmetry C<sub>18</sub>,

octadecylsilyl reverse phase cartridge (2.1 mm × 150 mm, 5 µm) column (Waters, WAT056975) at 35°C was used. Mass spectroscopic analysis was performed using Waters Micro-mass ZQ with ESI Probe (Waters # WAT700000289) running in positive ionization mode (ES<sup>+</sup>) with cone voltage set at 50 V and operating temperature at 250°C.

### Standard preparation

The standard microcystin-LR was diluted to a concentration of  $1 \text{ mg l}^{-1}$ . A standard calibration curve from  $1 \text{ µg ml}^{-1}$  microcystin-LR stock solution was created by injecting 10, 20, 30, 40, 50, 75 and 100 µl per run. The instrument was operated using MassLynx V3.5 software.

### Additional materials and equipment

24-Port Vacuum Manifold (Fisher, 11-131-32) with Aspirator (Sibata Scientific Technology, Japan), FloJet Pump (Model 430-042) with Gauge and Evaporator (Pierce ReactiVap III, Rockford, Illinois). Graduated glassware 500 ml, 1 l and 5 l (Wheaton). All tubing used was FDA/USDA/USP Certified (1/4" Nalgene and 1/2" VWR, Cat # 60985-556). Solid phase extraction columns were purchased from Fisher Scientific (PrepSep Extraction Columns, P-453).

### Filter cartridges used

All water filters and the filter housing used in the test apparatus (see Table 1) were marketed under the brand name OmniFilter<sup>®</sup> and were purchased from a local home improvement centre.

### Sample preparation

All 500 ml samples (including blank samples) were concentrated to the final volume of 1 ml using a solid phase concentration procedure. When concentrations exceeded assay limits, subsequent dilutions, particularly in pleated paper and fibre wound whole house water filters, were used. To concentrate microcystin-LR dissolved in 500 ml water the entire water sample (500 ml) was passed through C<sub>18</sub> solid phase extraction column (Fisher, PrepSepExtraction Columns, P-453), which separated the analyte

**Table 1** | Filter cartridges used in test apparatus. Note: Model number, descriptions and micron ratings were obtained from manufacturer's literature (OmniFilter®)

Filter type	Micron rating	Abbreviation
# CB3, carbon block 'undersink' cartridge	0.5 µm	CB-0.5
# CB1, carbon block 'undersink' cartridge	1.0 µm	CB-1.0
# TO1, carbon wrapped 'whole house' cartridge	2.0 µm	CW
# RS2, fibre wound 'whole house' cartridge	5.0 µm	FW
# RS1, pleated paper 'whole house' cartridge	20.0 µm	PP

(microcystin-LR) from its matrix (H<sub>2</sub>O) via a selective adsorption process. Through a series of tests, a high recovery rate for the analyte (microcystin-LR) using solid phase extraction process and 500 ml spiked samples was achieved. During this process, an average 96.4% of tested analyte was recovered. This recovery rate is similar to the recovery rate previously reported (Tsuji *et al.* 1994). After microcystin-LR was retained on the bonded phase sorbent bed, it was eluted with 5 ml of 100% methanol (repeated three times). The collected methanol wash fractions were dried at room temperature in an evaporator (Pierce, ReactiVap III, Rockford, Illinois) in a stream of nitrogen. Dried samples reconstituted with 100% methanol to a final volume of 1 ml, were capped and analysed by liquid chromatography coupled with mass spectroscopy (HPLC/MS) (Figure 1).

### Experimental filtration apparatus

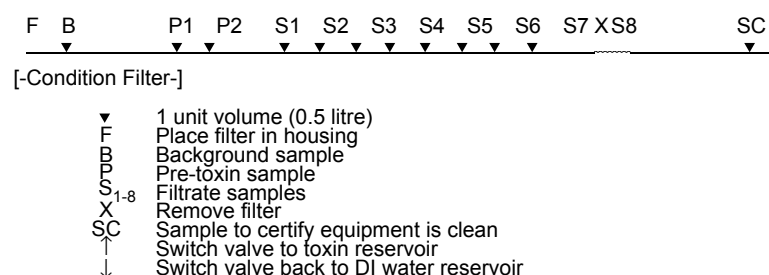
The experimental apparatus (Figure 2) consisted of a graduated glass (4l) water reservoir connected to a water pump, regulated to maintain a constant pressure of 205 kPa (30 psi) on a filter contained within a filter housing.

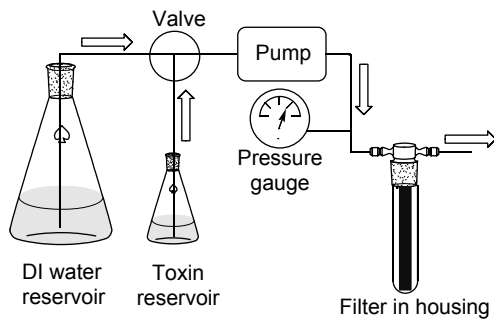
An additional, smaller, glass (1l) water reservoir for introduction of the toxin was connected to the system between the large water reservoir and the pump. All tubing used for connections was FDA/USDA/USP certified. All 10 filtrate samples were taken after the filter housing.

After initial assembly of the apparatus, blank deionized water was passed through the system without a filter in place, to assure that the apparatus was free from background toxins or other interferences. In addition, as previously mentioned, each filter tested had a pre-toxin sample taken to ensure that the system and filter were not contributing to subsequent measurements.

### Analysis summary

Immediately before injection, samples were reconstituted with 100% methanol. After injection of the sample, initial composition of the gradient (88% H<sub>2</sub>O, 2% acetonitrile (ACN) and 10% of 1% formic acid ran for 8 minutes with constant flow rate of 0.27 ml min<sup>-1</sup>. Over the next 27 minutes, it changed linearly to 65% H<sub>2</sub>O, 25% acetonitrile (ACN) and 10% of 1% formic acid. After 35 minutes, the

**Figure 1** | Diagrammatic representation of the sampling protocol.



**Figure 2** | Filtration apparatus.

composition of the gradient was changed for 1 minute to 35% H<sub>2</sub>O, 55% acetonitrile (ACN) and 10% of 1% formic acid, then immediately returned to the initial composition settings of 88% H<sub>2</sub>O, 2% acetonitrile (ACN) and 10% of 1% formic acid.

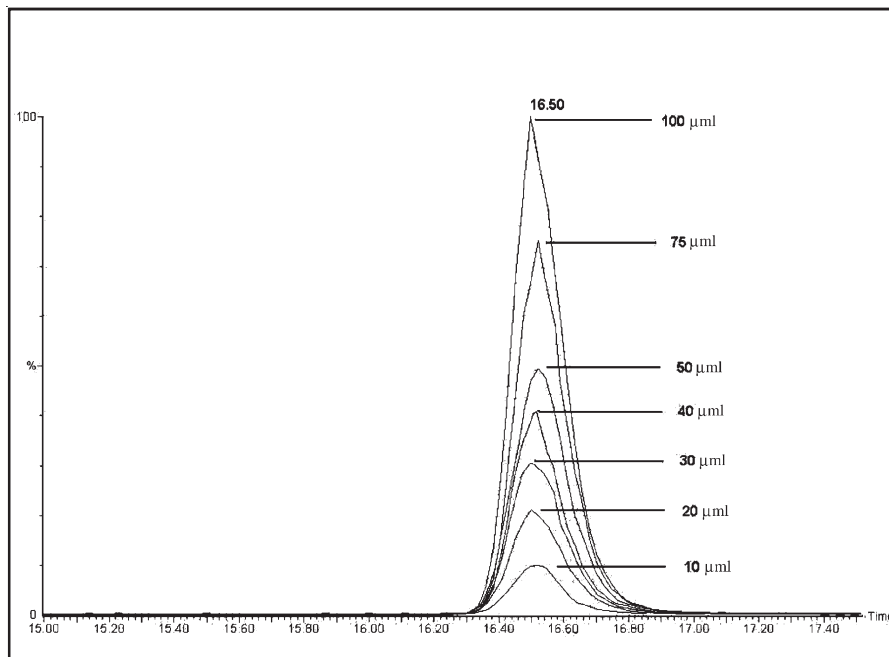
## RESULTS

In all sample extracts analysed by HPLC/MS only one single chromatographic peak displayed at 16.50 minutes. Identity of the peak was confirmed by mass spectra monitored at *m/z* 995.2. Adequate sensitivity was obtained

throughout the concentration range of the calibration curve. **Figure 3** represents an overlay of chromatographs obtained for a representative set of calibration injections ranging from 10 to 100 ng.

Results are outlined in **Table 2** and graphically represented in **Figure 4**. Results obtained were distinguished by a distinct and dramatic difference in observations from non-carbon and carbon containing filters. While both carbon-based filter media were effective in absorbing microcystin-LR, both the pleated paper and fibre wound filters retained almost none of the toxin. It should be noted that measurable levels of toxin were unexpectedly observed in the first two samples (P1 and P2; see **Table 2** and **Figure 4**) taken in the sampling protocol. This is most likely explained by a diffusion of the toxin into adjacent sample partitions due to dead volume within the test apparatus.

Data expressed as percentage recovered is represented in **Table 3**. When viewed in this manner, the overall effectiveness of the removal of microcystin-LR toxin from the water by filter cartridges containing carbon block (CB-1.0, CB-0.5) and carbon wrapped (CW) was the highest, removing over 99% of toxin. In contrast, the effectiveness of fibre wound (FW) and pleated paper (PP) filters was significantly less, removing only 5.84% and 4.65% of the toxin, respectively.



**Figure 3** | Overlay of chromatographs obtained for a representative set of calibration injections ranging from 10 to 100 ng.

**Table 2** | Micrograms of microcystin-LR recovered after filtration. Each row reports the results obtained for the sample point indicated in the sampling protocol. Columns report the micrograms of microcystin (average and standard deviation) for the various filter types studied. (PP = pleated paper, FW = fibre wound, CW = carbon wrapped, CB-1.0 = carbon block 1  $\mu\text{m}$ , CB-0.5 = carbon block 0.5  $\mu\text{m}$ , P<sub>1-2</sub> = pre-toxin samples, S<sub>1-8</sub> = filtrate samples)

Sample	PP		FW		CW		CB-1.0		CB-0.5	
	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
P1	4.83	2.89	4.14	4.01	0.82	0.070	0.027	0.030	0.020	0.009
P2	22.12	5.81	177.13	18.78	0.91	0.18	0.14	0.057	0.045	0.018
S1	154.69	43.11	162.07	15.59	1.21	0.14	0.23	0.21	0.039	0.015
S2	121.14	21.97	61.28	8.96	0.31	0.066	0.56	0.74	0.042	0.016
S3	96.35	13.37	40.45	8.59	0.24	0.037	1.03	1.66	0.041	0.013
S4	58.10	38.43	17.01	5.31	0.12	0.040	0.61	0.91	0.043	0.016
S5	15.79	23.53	5.09	1.18	0.078	0.036	0.48	0.74	0.081	0.074
S6	1.79	0.51	2.76	3.09	0.068	0.037	0.087	0.076	0.042	0.017
S7	1.32	1.00	0.45	0.19	0.063	0.035	0.041	0.0064	0.12	0.13
S8	0.64	0.50	0.40	0.41	0.056	0.033	0.035	0.026	0.049	0.021

## DISCUSSION

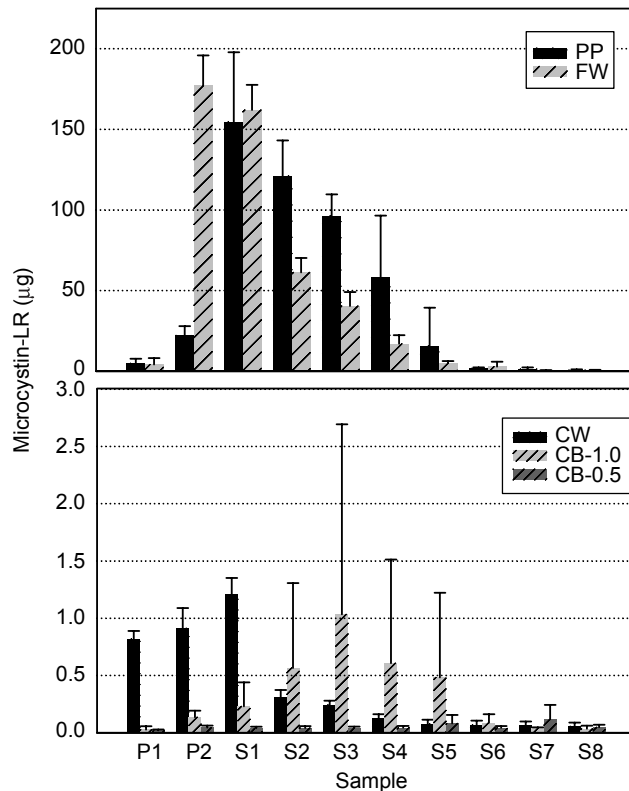
The purpose of this study was to investigate, in the laboratory, the efficacy of several home filtration substrates in the removal of microcystin-LR. This was done to better understand whether these filtration devices could provide additional protection for those concerned about cyanobacterial contamination in their drinking water.

Increases in human populations lead to increased demands on groundwater supplies that in turn may create the need to further utilize surface waters as a source of drinking water. This increased use of surface waters, such as rivers, lakes and dams, coupled with the eutrophication of these water bodies, increases the possibility that cyanobacteria and cyanotoxins could find their way into finished drinking water. As noted previously, the occurrence of cyanobacterial blooms in rivers, lakes and reservoirs has already been reported worldwide and has the potential to affect the quality of public drinking water originating from these surface water bodies.

Methodologies used by public water treatment facilities for purification of surface water may vary from site to site and may have different levels of success in removing cyanobacteria and their toxins (Chorus & Bartram 1999). Although unlikely because of the episodic nature of cyanobacterial blooms, it is possible that long-term low levels of cyanotoxin contamination could exist in drinking water and create a situation of chronic exposure for those drinking the water.

There is a paucity of data available to characterize the health risk of long-term exposure to cyanotoxins. Yu (1989) reported an association between drinking surface water (particularly ditch water) contaminated with cyanotoxin and an increase of liver cancer in rural China. However, interpretation of this data was limited by other confounding exposures including aflatoxin, hepatitis B infection and poor nutrition in the population studied.

Fleming *et al.* (2000) explored the possibility of long-term low-level exposure to cyanotoxins and its association with the occurrence of primary liver cancer by investigating a population living near a drinking water facility that utilized surface water as a source. Rates of primary liver cancer in the



**Figure 4** | Micrograms of microcystin recovered for the various filters studied vs. filter sequence number. (PP = pleated paper, FW = fibre wound, CW = carbon wrapped, CB-1.0 = carbon block 1  $\mu\text{m}$ , CB-0.5 = carbon block 0.5  $\mu\text{m}$ ).

area were slightly greater than surrounding populations but less than the statewide rate. Again, interpretation of the results was limited by the ecologic nature of the study and the potential for confounding exposures.

**Table 3** | Percentage of microcystin recovered from the filtrate using the various filter types studied. (PP = pleated paper, FW = fibre wound, CW = carbon wrapped, CB-1.0 = carbon block 1  $\mu\text{m}$ , CB-0.5 = carbon block 0.5  $\mu\text{m}$ ). Note, differences in amount injected were dictated by observed amount recovered to accommodate detection limits

Filter type	Amount injected ( $\mu\text{g}$ )	Amount recovered ( $\mu\text{g}$ )	Percentage recovered (%)
PP	500	476.77	95.35
FW	500	470.78	94.16
CW	1000	3.88	0.39
CB-1.0	1000	3.25	0.32
CB-0.5	1000	0.52	0.05

There are multiple retrospective accounts of acute human illness occurring in populations after drinking water originating from surface waters experiencing heavy cyanobacterial blooms (Falconer *et al.* 1983a, b; Teixeira *et al.* 1993; Jochimsen *et al.* 1998; Chorus 2001; Griffiths & Saker 2003; Haider *et al.* 2003). Reported signs and symptoms have included those of gastroenteritis, liver damage, pneumonia, diarrhoea and death. These situations apparently involved the use of inadequately treated water for drinking and in one tragic scenario inadequately treated water was used for haemodialysis (Jochimsen *et al.* 1998). It should be noted that the use of copper sulfate to lyse cyanobacteria and control blooms actually increased the release of toxin and is thought to have increased the risk for illness in populations drinking water from such sources (Haider *et al.* 2003).

These reports of adverse human health effects associated with drinking water contaminated with cyanotoxin have raised concerns for researchers and public health professionals. Based on these concerns, we set out to study the efficacy of home filtration devices in removing cyanotoxin as a possible additional safeguard for consumers.

In this laboratory study, microcystin-LR standard was added to water at known concentrations with subsequent investigation of the effectiveness of several types of filter in removing the microcystin-LR. Laboratory results show that the common home filters tested in this study show significant variation in their ability to remove microcystin-LR. The carbon filters were quite efficient, with the filtrate containing only 0.05–0.3% of the microcystin-LR that was dissolved in the original test solution. On the other hand, the pleated paper and wound fibre filters allowed large percentages of the microcystin-LR to pass through the filter and into the filtrate (>90%).

While these findings are promising for the efficacy of carbon filters, further work needs to be done to fully assess the ability of these filters to add additional protection against cyanotoxin contamination in drinking water. Similar investigations will need to be done on cyanotoxins other than microcystin-LR in order to appreciate the generality of these findings. Additional investigations need to be done to assess other filtration processes, to assess the effect of filter load when large amounts of toxins are present, and to assess the continued effectiveness of filtration over longer periods of use. Also of interest would be the effect of

other contaminants in the water and how the water quality affects the short and long-term efficiency of filters.

## CONCLUSION

The results of this laboratory-based study provide initial findings to suggest that the efficacy of home filtration devices in removing microcystin-LR, a cyanotoxin, from drinking water, varies considerably with the type of device being used. Carbon filters successfully removed microcystin-LR allowing only 0.05–0.3% of the toxin load to pass through the filter. On the other hand, pleated paper and string wound filters allowed >90% of microcystin-LR present in the sample to pass through the filters.

Theoretically, the use of carbon home filtration devices tested in this study may provide protection against human exposure to cyanotoxin in addition to protection provided by water treatment methodologies utilized in water treatment facilities.

Further studies need to be done to assess the efficacy of home filtration devices for various cyanotoxins and for other filtering conditions such as increased toxin load, the presence of other contaminants in drinking water, and the repetitive use of the same filter over longer time intervals.

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