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**PAPER**

# Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes

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The cyanobacterial toxins, nodularin and microcystin, are highly efficient inhibitors of cellular protein phosphatases. Toxicity primarily evolves following ingestion of cyanobacterial material or toxins and results in liver and renal pathology. Ingestion is the main route of exposure in the World Health Organizations current risk assessment of nodularin and microcystins. Nasally applied microcystin appears to have a 10-fold higher availability and toxicity than orally ingested toxins, suggesting that aerosolized toxins could represent a major risk for human populations close to lakes with cyanobacterial blooms. In this study, nodularin and microcystin levels in aerosols were assessed using high and low volume air samplers for 4, 12 and 24 h periods at lakes Forsyth and Rotorua (South Island, New Zealand). These lakes were experiencing blooms of *Nodularia spumigena* and *Microcystis* sp., respectively. Using the high volume samplers up to 16.2 pg $m^{-3}$ of nodularin and 1.8 pg $m^{-3}$ of microcystins were detected in the air. Aerosolized nodularin and microcystins do not appear to represent an acute or chronic hazard to humans. The latter was concluded based on calculations using average human air intakes, the highest nodularin or microcystin concentrations measured in the air in this study, and assuming inhalatory toxicities comparable to toxicological data obtained following intraperitoneal applications in mice. However, as the toxin concentrations in the air were calculated over extended sampling periods, peak values may be underestimated. Aerosolized toxins should be considered when developing risk assessments particularly for lakeside populations and recreational users where inhalation of cyanotoxins may be a secondary exposure source to a primary oral exposure.

## Introduction

Harmful cyanobacterial blooms occur in marine, estuarine and freshwater ecosystems worldwide. The cyanobacteria responsible for these blooms often produce highly potent toxins that cause problems when the water is used for recreation, hygienic purposes, commerce and drinking. Microcystins are the most common cyanotoxins linked to animal and human poisonings.[1]

Microcystins and the structurally similar nodularin are cyclic peptides that irreversibly inhibit eukaryotic serine/threonine protein phosphatases.[2,3] An irreversible covalent bond forms between the toxin and protein phosphatases, which leads to subsequent cell structure damage[4,5] and results in liver disease as well as nephro- and neurotoxicity.[6–8]

Most microcystins are highly toxic with intraperitoneal mouse toxicities ranging between 50 and 300 μg $kg^{-1}$ body weight (mouse).[9] Oral administration of microcystin-LR to mice has demonstrated a 24 h $LD_{50}$ ranging between 5 and 10.9 mg $kg^{-1}$.[10,11] The latter has led the World Health Organization (WHO) to develop a drinking water guideline for the common hydrophilic variant microcystin-LR resulting in

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### Environmental impact

Cyanobacteria (blue-green algae) produce toxins that are a health hazard to humans and animals when consumed or through contact with them. This study investigated whether aerosolized toxins are a health risk to humans. Using high volume sampler's aerosolized toxins were detected in the air at two lakes experiencing cyanobacterial blooms. Using average human air intakes, the highest toxin concentrations measured in this study, and assuming inhalatory toxicities comparable to toxicological data obtained following intraperitoneal applications in mice, aerosolized cyanotoxins do not appear to represent an acute or chronic hazard to humans.

a guidance value of 1 μg microcystin-LR $l^{-1}$.[12] Toxicity by oral uptake is generally at least one order of magnitude lower than toxicity by intraperitoneal injection. However, Fitzgeorge *et al.* (1994) demonstrated that toxicity relevant doses of intranasal application were comparable to intraperitoneal injection. Moreover, membrane damage by intranasal microcystin augmented the toxicity of the neurotoxic cyanotoxin, anatoxin-a.[13] Thus this uptake route could represent a major risk for humans inhabiting the shores of lakes with frequent cyanobacterial blooms or for those using the water for hygienic purposes (showering and sauna)[14] or participating in recreational activities such as waterskiing or sailing where inhalation of spray is likely to occur.[15]

Epidemiological studies have demonstrated a significant increase in reports of minor symptoms, particularly respiratory symptoms, associated with visits to water bodies with elevated cyanobacteria concentrations.[16,17] Additionally, there are anecdotal and documented reports of respiratory illness linked to cyanobacterial blooms (see Stewart *et al.*, 2006 for an in-depth review).[17] For example, Turner *et al.* (1990) reports on the hospitalization of soldiers with pneumonia after canoeing through *Microcystis* blooms and Philipp and Bates (1992) noted an increase in upper respiratory and gastrointestinal symptoms among people dinghy sailing in rivers with elevated cyanobacteria concentrations.[18,19] Despite these reports very few studies have attempted to quantitatively measure cyanotoxin concentrations in aerosols. Cheng *et al.* (2007) and Backer *et al.* (2008) used high volume samplers (HVS) and personal air samplers (PAS) to detect levels of aerosolized microcystins.[20,21] The PAS worn by study participants for 89–134 min measured microcystin levels ≤80 pg $m^{-3}$, while the HVS (1.5 $m^3$ $min^{-1}$) placed on boats close to the edge of several small lakes in Ohio, USA, measured microcystin concentrations ≤37 pg $m^{-3}$ within 8 h, which was also the limit of detection for the analytical method employed.[21] Only low concentrations (≤4 μg $l^{-1}$) of microcystins were measured in the water bodies during these experiments. In the marine environment there is a well established association between toxin-containing aerosols from *Karenia brevis* and respiratory irritations in humans and mammals.[22,23]

Current recreational guidelines[24–26] do not take inhalation of aerosolized cyanotoxins into consideration when calculating microcystin health alert thresholds. An improved understanding of relationships between nodularin and microcystin, and cell concentrations in surface waters, wind strength and levels of aerosolized cyanotoxin may help in refining future risk assessments. The aim of this study was to assess cyanotoxins levels in aerosols at two lakes with contrasting concentrations of cyanobacterial species and cyanotoxins; Lake Forsyth (South Island, New Zealand), containing low levels of nodularin and *Nodularia spumigena* and Lake Rotorua (South Island, New Zealand), which was experiencing a dense bloom of microcystin-producing *Microcystis* sp.

## Materials and methods

### Extraction efficiency

An extraction efficiency experiment was undertaken to determine if microcystins and nodularins (either "free" or within cells) could be reliably extracted from the EPM GF/C filters used in the HVS by standard cyanotoxin extraction methods routinely used in our laboratory. The extraction efficiency was evaluated in the presence of cyanobacterial cells using cultures of *Microcystis* sp. (CYN11) and *N. spumigena* (CYN43). Cell enumeration was carried out at 200× magnification (BX51, Olympus, Wellington, New Zealand) in a Sedgwick-Rafter chamber.[27] The following serial dilutions were prepared: *Microcystis* sp. 1000, 300, 50 and 0 cells $ml^{-1}$ and *N. spumigena* 500, 150, 50, 10 and 0 cells $ml^{-1}$. Each dilution series was prepared in duplicate and the second replicates were spiked with 25 pg of microcystin-YR and nodularin respectively (DHI, Denmark). Three replicates of 1 ml of each dilution were pipetted onto the EPM GF/C filters and placed in 15 ml Falcon tubes with 5 ml of 100% methanol. The samples were sonicated (30 min), the extraction was repeated and supernatants were combined and dried under nitrogen with heating at 35 °C. The dried extract was solubilized in 1 ml of 20% methanol and analyzed by ELISA as described below.

### Study sites and sample collection

Lake Forsyth is a small (5.62 $km^2$), shallow (max. depth 4.1 m), hypertrophic and slightly brackish coastal lake in the South Island (43°494′22 S, 172°42′30 E) of New Zealand.[28,29] The lake has a long history of *N. spumigena* blooms and associated stock deaths.[30] Lake Rotorua is a very small (0.55 $km^2$), shallow (max. depth 3 m), eutrophic lake in the upper South Island (42°24′05 S, 173°34′57 E) of New Zealand.[31] Blooms of *Microcystis* sp. and low levels of microcystins have been recorded.[32]

Surface waters samples (700 ml) were collected 1 m from the shore approximately every 6 h over a 37.5 h period (4 to 6 May 2009) at Lake Forsyth and approximately every 4 h over a 49 h period (6 to 8 May 2009) at Lake Rotorua. Sub-samples (200 ml) were preserved using Lugol's iodine for species identification and enumeration. Sub-samples (50 ml, unfiltered) were frozen (−20 °C) for analysis of total microcystins and nodularins. Fifteen millilitre sub-samples were filtered (0.45 μm, Acrodisk Minisart, Sartoriusstedium Biotech, Germany) and filtrates frozen (−20 °C) for extracellular microcystin and nodularin analysis.

Aerosol samples were collected with high volume air samplers (HVSs; flow rate 60 $m^3$ $h^{-1}$, 70 standard cubic feet per minute, Lear Siegler, Australia) fitted with 20.3 × 24.5 cm glass fibre filters (Whatman EPM 2000) and low volume air samplers (LVSs; flow rate 0.072 $m^3$ $h^{-1}$, Portable Aerosol Spectrometer #1.109, GRIMM, Germany) fitted with 47 mm PTFE-filters. The GRIMM LVS was used to count and measure size distribution of particulate matter (PM). At Lake Forsyth three HVSs and one LVS were deployed at 2 m spacings on the lake shore. Filters were collected after 12 h (0400, 5 May 2009) and the samplers were redeployed and filters collected 24 h later (0400, 6 May 2009). At Lake Rotorua one HVS and one LVS were deployed at the lake edge and the filter retrieved at the end of two consecutive 24 h periods (1200, 7 May 2009 and 1300, 8 May 2009). A second HVS was deployed on a peninsular, approximately 20 m from and 30 m above the lake, for a 4 h period (1300 to 1700, 7 May 2009). At the end of each sampling period the HVS filters were divided into quarters and LVS filters in half.

 

These were frozen (−20 °C) for later nodularin and microcystin analysis.

A portable weather station was deployed within 5 m of the HVS and recorded wind speed and direction, and air temperature (WS-2355, La Crosse Technology, WI). Data were collected at 5 min intervals. Hourly rainfall data from the weather station closest to each sampling location were obtained from the Cliflow database of the National Institute of Water and Atmospheric Research, New Zealand.

### Sample analysis

Cyanobacteria identification and enumeration was carried out using an inverted Olympus microscope (CKX41, Olympus, Wellington, New Zealand) and Utermöhl settling chambers.[33]

Water sub-samples collected for toxin analysis were freeze thawed, sonicated (20 min) and centrifuged (5000*g*, 10 min). The supernatant was diluted in MilliQ water and used directly for enzyme-linked immunosorbent assay (ELISA) or liquid chromatography–mass spectrometry (LC–MS) analysis.

One quarter of each EPM GF/C filter from the HVS was placed in 50 ml Falcon tubes containing 40 ml of 100% methanol. Samples were sonicated (30 min), the extraction was repeated and supernatants were combined and dried under rotary evaporation. Samples were resuspended in 10 ml of 100% methanol and dried under nitrogen with heating at 35 °C. One half of each PTFE-filter from the LVS was placed in 15 ml Falcon tubes with 5 ml of 100% methanol and the filters were treated and cyanotoxins extracted as described in the extraction efficiency section. The dried extracts were solubilized in 1 ml of 100% methanol, spin-filtered (Micro-Spin® Centrifuge Filter Tubes, Grace Davison Discovery Sciences, USA) and an aliquot was used for LC–MS. A 200 µl aliquot was dried under nitrogen with heating at 35 °C, resuspended in 20% methanol and used for ELISA.

The total ADDA containing microcystins and nodularins in the samples was quantified with a commercially available ELISA kit (Abraxis LLC, USA) based on the methods of Fischer *et al.* (2001).[34] Each sample was analyzed in duplicate and average values are reported.

LC–MS analysis of selected samples was undertaken using a Waters Acquity ultra-performance liquid chromatography system (Waters, Milford, MA) coupled with a Waters-Micromass Quattro Premier triple quadrupole mass spectrometer (Manchester, UK). Separation was achieved with a Phenomenex Luna C18 2.5 µm 50 × 1 mm column at 40 °C, eluted at 0.25 ml $min^{-1}$ with linear gradients of 10% acetonitrile (A) and 90% acetonitrile (B), each containing 50 mM of formic acid and 2 mM of ammonia. The gradients consisted of 10 to 20% B over 0.5 min, increasing to 40% B at 3 min, 100% B at 3.5 min and held for 0.5 min, before returning to initial conditions. The electrospray ionization source was operated in positive-ion mode (100 °C, capillary 1 kV, nitrogen desolvation gas 900 l $h^{-1}$ (350 °C), cone gas 50 l $h^{-1}$) with multiple reaction monitoring (MRM) using MS–MS channels set up for microcystin-RR, didesmethyl-RR, desmethyl-RR, LR, YR, didesmethyl-LR, desmethyl-LR, FR, WR, AR, LA, LY, LW, LF, and nodularin. The *m*/*z* 135 fragment ion from the protonated molecular cation was selected for each toxin ($[MH_2]^{2+}$ for microcystin-RR and variants; $MH^+$ for the others). Cone voltage and collision energy were optimized for each individual variant. Primary standards of -RR, -YR, -LR, -LW, -LY, -LF and nodularin were purchased from DHI (Denmark).

## Results

### Extraction efficiency

The extraction efficiency experiments with pure microcystin-YR and nodularin showed that concentrations of ≥25 pg $filter^{-1}$ could be accurately extracted from the filters and measured using the ADDA-ELISA (Table 1). The dilution experiments demonstrated that microcystin and nodularin could be extracted from *Microcystis* sp. and *N. spumigena* when present on glass fibre filters at low levels (50 cells for *Microcystis* sp. and 10 cells for *N. spumigena*, Table 1). Spiking samples with known concentrations of microcystins-YR or nodularin showed that extraction efficiencies ranged between 71 and 116% for *Microcystis* sp. and 67.3 to 135.5% for *N. spumigena* (Table 1).

### Lake Forsyth

Four cyanobacterial species were identified in the Lake Forsyth water samples: *Aphanocapsa* sp., *Merismopedia* sp. (2 species) and *N. spumigena*. *Aphanocapsa* sp. and *Merismopedia* spp. comprised the majority of the phytoplankton with cell concentrations exceeding 3 500 000 and 5500 cells $ml^{-1}$ respectively in all samples (data not shown). These species are not known to produce nodularin and therefore were not considered in relation to nodularin aerosol concentrations. *Nodularia spumigena* cell concentrations varied between 190 and 5150 cells $ml^{-1}$ (Fig. 1). Nodularin was detected in all water samples and total (intra and extracellular) nodularin concentrations (measured using ELISA) varied as cell concentrations of *N. spumigena* changed in the water column (Fig. 1). The highest concentration of nodularin (9.9 µg $l^{-1}$) was measured in the sample collected at 1430, 4 May 2009 (Fig. 1). The percentage of extracellular nodularin was low (<15%) in all samples (Fig. 1) except for the sample collected at 2200 (5 May 2009). In this sample extracellular nodularin accounted for 28% of the total nodularin, however, cell concentrations were also very low when this sample was collected (Fig. 1).

Nodularin was detected using ELISA on all EPM GF/C filters from the HVS. At the end of the 12 h sampling period there were marked variations in concentrations of nodularin on each filter (1.04 to 11.6 ng $filter^{-1}$, Fig. 1). Variation in nodularin on the EPM GF/C filters was minimal at the end of the 24 h period, however, concentrations were also lower (0.34 to 0.42 ng $filter^{-1}$, Fig. 1). Using LC–MS, nodularin was detected on the glass fibre filters from the 12 h experiment (albeit slightly lower than the values measured *via* ELISA: 0.7, 1.9, 10.9 ng $filter^{-1}$). The ADDA-ELISA used during the present study measures the total amount of ADDA-containing compounds in the sample. Free ADDA fragments can persist in a water body after cyanotoxin biodegradation and this may account for some of the variability between results. No nodularin was detected *via* LC–MS on the filters from the 24 h experiments. Using an average nodularin quota of 2.67 pg $cell^{-1}$ (based on the intracellular levels in the water and cell concentrations as determined by ELISA) the maximum number of cells was estimated to be 4630 on the filter

**Table 1** Mass of microcystin or nodularin per EPM GF/C filter with added *Microcystis* sp. (CYN11) or *Nodularin spumigena* (CYN43) cells. In the spiked experiments microcystin-YR (25 pg filter$^{-1}$) and nodularin (25 pg filter$^{-1}$) were added to each filter. Results are an average of three replicates ± one standard deviation

| No. of cells added to filter | Mass of microcystin/nodularin in non-spiked experiment/pg | Mass of microcystin/nodularin extracted in spiked experiments/pg | Extraction efficiency (%) |
|---|---|---|---|
| Microcystin | | | |
| 0 | 0.0 ± 0.0 | 19.6 ± 0.4 | 78.4 |
| 50 | 31.4 ± 1.5 | 49.1 ± 17.7 | 70.8 |
| 300 | 96.9 ± 15.0 | 125.9 ± 52.6 | 116.1 |
| 1000 | 426.6 ± 29.2 | 454.9 ± 11.7 | 113.1 |
| Nodularin | | | |
| 0 | 0.00 ± 0.0 | 33.8 ± 23.4 | 135.5 |
| 10 | 12.8 ± 12.8 | 88.3 ± 9.6 | 82.1 |
| 50 | 44.2 ± 11.0 | 61.0 ± 5.8 | 67.3 |
| 150 | 247.6 ± 62.4 | 274.18 ± 6.2 | 106.1 |



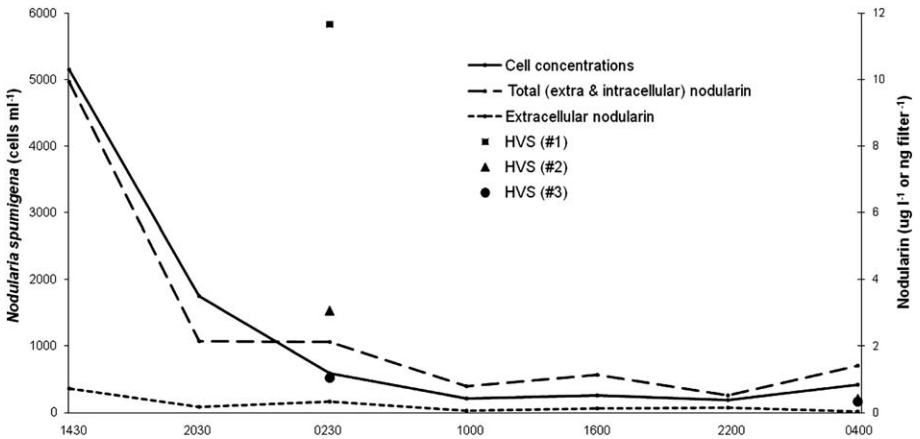


**Fig. 1** Nodularin and *Nodularia spumigena* concentrations in water and on EPM GF/C filters from high volume samplers (HVSs) deployed at the edge of Lake Forsyth (South Island, New Zealand) over a 37.5 h period (4 to 6 May 2009).

**Table 2** Mass of nodularin (Lake Forsyth) or microcystin (Lake Rotorua) in air, and estimated number of cells of *Nodularia spumigena* or *Microcystis* sp. on each filter, during experiments at lakes Forsyth and Rotorua (New Zealand) in May 2009. Nodularin and microcystin concentrations were measured using ELISA. —, sampler not deployed during sampling period

| | | Air concentration of nodularin or microcystin/ng m$^{-3}$ | | | Estimated number of cells of *N. spumigena* or *Microcystis* sp. on filter | | |
|---|---|---|---|---|---|---|---|
| Location | Sampling period/h | HVS#1 | HVS#2 | HVS#3 | HVS#1 | HVS#2 | HVS#3 |
| Lake Forsyth | 12 | 0.0162 | 0.0043 | 0.0014 | 4360 | 1150 | 390 |
| Lake Forsyth | 24 | 0.0002 | 0.0003 | 0.0002 | 130 | 160 | 130 |
| Lake Rotorua | 24 | 0.0003 | — | — | 3200 | — | — |
| Lake Rotorua | 4 | — | — | 0.0018 | – | — | 4920 |
| Lake Rotorua | 24 | 0.0009 | — | — | 9520 | — | — |

from HVS#1 at the end of the 12 h experiment (Table 2). No nodularins were detected on the PTFE-filters from the LVS, however, wind speed analyses and associated PM measurements (Fig. 2) demonstrated a higher concentration of aerosolized PM ≤10 μm at wind speeds <10 km h$^{-1}$, suggesting the LVS nozzles used may have prevented detection of PM at higher wind speeds.

Wind speed increased gradually during the 12 h experiment from approximately 10 km h$^{-1}$ to 38 km h$^{-1}$ (Fig. 2). The wind speed varied between 15 km h$^{-1}$ and 43 km h$^{-1}$ for the first 12 h of the 24 h experiment. The wind then subsided, before gradually increasing to 30 km h$^{-1}$ (Fig. 2). The wind direction throughout the experiments was generally onshore (northerly), however, gusts up to 90° from the east and west were recorded. Precipitation only occurred during the last 6 h (2.7 mm), which unfortunately in conjunction with severe gusts of wind led to the inundation of the LVS and thus prevented logging of PM data over the 24 h period.

## Lake Rotorua

Three cyanobacterial species were identified in the Lake Rotorua samples: *Aphanothece* sp., *Anabaena* sp. *Nova* and *Microcystis*

 

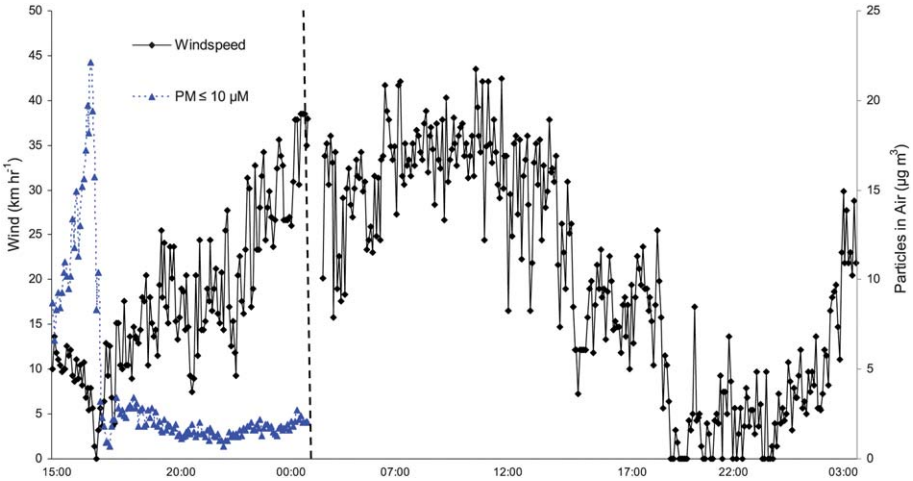


**Fig. 2** Wind speed and particulate matter (measured by the GRIMM low volume sampler) recorded at the shore of Lake Forsyth (South Island, New Zealand) over a 37.5 h period (4 to 6 May 2009). Dashed line indicates end of the 12 h experiment. Precipitation in conjunction with severe gusts of wind led to the inundation of the GRIMM low volume sampler and thus prevented logging of particulate matter over the second (24 h) sampling period.



sp. *Aphanothece* sp. was present in very low (<1 cell ml$^{-1}$) concentrations. *Anabaena* sp. *Nova* concentration varied between 80 000 and 550 000 cells ml$^{-1}$ throughout the study period (data not shown). In a parallel study[35] these samples were analysed for *mcyE* genes using an *Anabaena* specific *mcyE* QPCR assay. No *Anabaena* specific *mcyE* genes were detected confirming that this species does not produce microcystins in this lake. Therefore, *Anabaena* sp. *Nova* was not considered in relation to concentrations of microcystins in the aerosols. *Microcystis* sp. concentrations were high (>120 000 cells ml$^{-1}$) during the entire 49 h. There was a significant increase in *Microcystis* sp. concentrations between 1100 and 1700, 7 May 2009 (Fig. 3). This occurred after a period of no wind followed by very light onshore winds. Using ELISA microcystins were detected in all water samples and changes in concentrations followed a similar pattern to the shifts in *Microcystis* sp. with the maximal microcystin concentration (2140 µg l$^{-1}$) recorded at 1300, 7 May 2009 (Fig. 3). LC–MS analysis of this sample revealed the presence of microcystin variants: microcystin-YR (1.2 µg l$^{-1}$), microcystin-LR (3.3 µg l$^{-1}$), desmethyl-microcystin-LR (719 µg l$^{-1}$), didesmethyl-microcystin-LR (60 µg l$^{-1}$), and microcystin-WR (0.2 µg l$^{-1}$). The percentage of the total microcystin in the water column that was extracellular varied markedly from 0.7% to 45%. The lowest percentages were measured when *Microcystis* sp. cell concentrations were high (Fig. 3).

Microcystins were detected using ELISA on all EPM GF/C filters from the HVS. The lowest concentration was measured at the end of the first 24 h period (0.42 ng filter$^{-1}$) and the highest at the end of the second 24 h period (1.24 ng filter$^{-1}$; Fig. 3). Despite only being deployed for 4 h and situated 20 m away from and 30 m above the lake, microcystins were detected on the EPM GF/C from HVS#3 (Fig. 3). Using an average microcystin quota

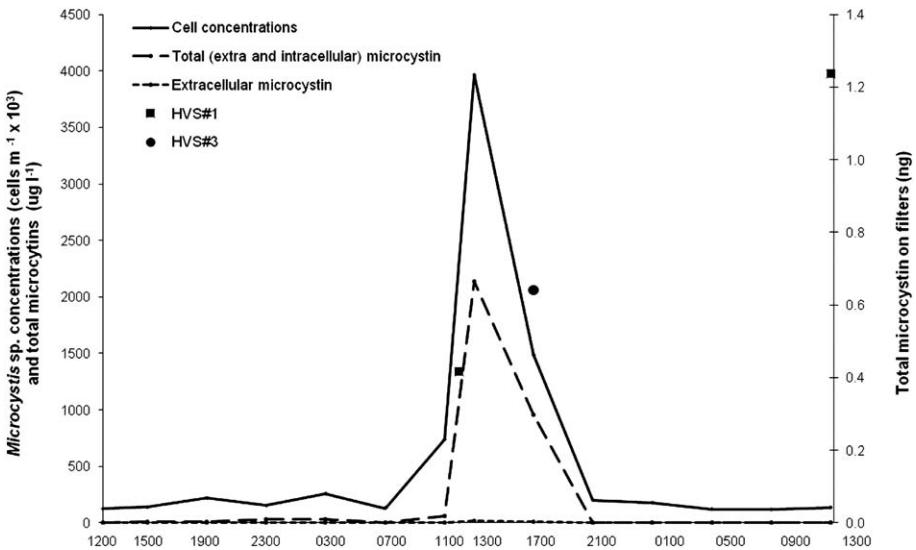


**Fig. 3** Total microcystins and *Microcystis* sp. concentrations in water and on EPM GF/C filters from high volume samplers (HVSs) deployed at Lake Rotorua (South Island, New Zealand) over a 49 h period (6 to 8 May 2009).

of 0.13 pg (based on the intracellular levels in the water and cell concentrations) the maximal number of cells on a filter was estimated to be 9520 on the filter from HVS#1 at the end of the second 24 h experiment (Table 2). The LC–MS detected only trace levels (0.02 ng filter$^{-1}$) of microcystin-LR on the filter from HVS#3. This discrepancy probably occurred because the *Microcystis* sp. in Lake Rotorua produces multiple microcystin variants. These individual variants would have been below the reliable limits of detection of the LC–MS method (0.2 μg l$^{-1}$). Thus in this study the ADDA-ELISA, which measures total ADDA-containing compounds, proved to be a more suitable method for measuring low levels of total microcystins on the filters. No microcystins were detected on the PTFE-filters.

During the first 24 h wind was variable ranging between 0 and 28 km h$^{-1}$ (Fig. 4). In general, the wind remained onshore (easterly) during this period. This sampling period coincided with a period of heavy rain (26.4 mm in 10 h). Wind speed in the second 24 h period was low (<5 km h$^{-1}$), until the final four hours when the wind gradually increased from the south (Fig. 4). There was periodic light rainfall during the second 24 h (1.4 mm).

## Discussion

Adverse effects in humans following exposure to cyanotoxins *via* contaminated food or water are well documented.[36] Health risks associated with aerosolized cyanotoxins have been presumed to occur following anecdotal reports from persons swimming or canoeing on surface waters with intense cyanobacterial blooms.[18] However, few studies have specifically attempted to quantify actual levels of aerosolized toxins.[21,22] In contrast to the earlier studies, this study monitored cell numbers and toxin content in the water, wind, precipitation and size differentiated particulate matter at regular time intervals and compared these data with toxins (nodularin and microcystins) in the aerosols.

At both lakes, Forsyth and Rotorua, higher levels of toxins were detected on HVS filters following periods of elevated toxins in surface waters. Further experiments with shorter sampling periods may allow this association to be refined such that the toxin concentrations in the water could be used as a proxy for levels that are likely to be present in aerosols. Physical variables *i.e.*, wind speed and direction, may also affect this correlation. In our study increasing wind strength in the direction of the samplers did not result in increased levels of aerosolized toxins with the maximum concentrations on the filters following extended periods with relatively little wind (<10 km h$^{-1}$). Periods of no-or-light winds often promote cyanobacterial surface scums, with even light to moderate winds preventing the upward migration of buoyant cyanobacteria such as *Microcystis*.[37,38] These surface scums would allow greater entrainment of cyanobacteria and their toxins in aerosols, whereas in moderate-to-strong winds the blooms are mixed throughout the top part of the water column reducing entrainment as concentrations of cyanobacteria close to the water surface are reduced. Additionally heavy rainfall, at both lakes Rotorua and Forsyth, may have reduced the toxin levels in the aerosols during the second 24 h sampling periods.

Microcystins were detected on the filter from HVS#4 at Lake Rotorua despite it being deployed for a shorter exposure period (4 h), back from the lake shore (20 m) and at a higher elevation (30 m). Microcystins are extremely stable compounds[39,40] and once airborne could be transported many kilometres without degrading. Therefore health implications of aerosolized toxins need to be considered not only for lake-users but also for nearby populations. Dietrich and Hoeger (2005) have suggested that the WHO guidelines (0.04 μg microcystin kg$^{-1}$ body weight day$^{-1}$ for food, and 1 μg l$^{-1}$ for drinking water) need to be applied conservatively, as they do not consider the contribution of multiple sources of microcystin contamination.[41] Such considerations may also be required for lakeside residents where inhalation of cyanotoxins could be a secondary exposure source if the lake water was also used for drinking or hygienic purposes. Recent research has indicated a link between the cyanotoxin

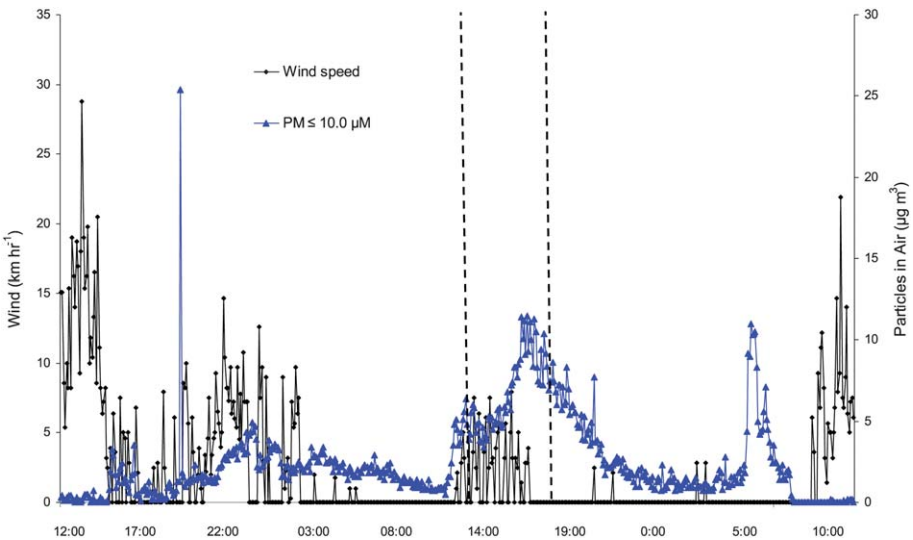


**Fig. 4** Wind speed and particulate matter (measured by the GRIMM low volume sampler) recorded at the shore of Lake Rotorua (South Island, New Zealand) over a 49 h period (6 to 8 May 2009). First dashed line indicates end of the first 24 h experiment, second dashed line indicates end of the 4 h experiment.

 

**Table 3** Potentially inhaled nodularin and microcystins during different types of activity at lakes Forsyth and Rotorua (New Zealand) in May 2009. Results use the maximum concentrations of nodularin (0.0162 ng $m^{-3}$) or microcystins (0.0018 ng $m^{-3}$) measured in the air during experiments. Minute ventilation values are those given in Anderson *et al.* (2006).[50] MDIC, maximum daily inhaled concentration

| Type of activity | Light work | Moderate work | Heavy work |
|---|---|---|---|
| Minute ventilations (l $min^{-1}$) | 30.3 | 43 | 72.3 |
| Total nodularin inhaled/ng | | | |
| 1 h | 0.0294 | 0.0418 | 0.0703 |
| 24 h | 0.7068 | 1.0031 | 1.6866 |
| Potentially inhaled toxin (24 h) to MDIC (200 ng $day^{-1}$) ratio | 283 | 199 | 119 |
| Total microcystin inhaled/ng | | | |
| 1 h | 0.0032 | 0.0046 | 0.0077 |
| 24 h | 0.0776 | 0.1101 | 0.1851 |
| Potentially inhaled toxin (24 h) to MDIC ratio | 5578 | 1817 | 1081 |



β-*N*-methylamino-L-alanine (BMMA) and amyotrophic lateral sclerosis (ASL).[42] In New Hampshire there is some evidence to indicate that exposure to BMMA may have resulted in higher than normal incidences of ASL in a town situated close by a lake prone to cyanobacterial blooms. Aerosolized BMMA is proposed as a possible exposure route.[43]

Toxin levels on filters from Lake Forsyth were markedly higher than those from Lake Rotorua (Fig. 1 and 3, and Table 2) despite the shorter sampling period (12 h *versus* 24 h) and generally lower levels of toxins in the surface water. This indicates possible variations in the ability of different species or toxins to become aerosolized. Waterborne toxins can be aerosolized *via* a bubble bursting process. Bubbles are formed by trapped air rising to the surface, and as the bubbles burst cyanobacteria (and other microorganisms) are ejected with and carried into the air by the droplets.[44,45] The ability to produce aerosols containing microcystin *via* this bubble-bursting process has been demonstrated in laboratory based experiments.[22] The different morphologies of *Microcystis* (large colonies) and *N. spumigena* (long filaments) may cause different dispersal efficiencies, affecting the quantities of toxins becoming aerosolized. It is plausible that other smaller or more fragile species *e.g.*, *Cylindrospermopsis raciborskii*, may be aerosolized to an even greater extent.

In this study, we did not determine if the toxins on the filters were from aerosolized nodularin or microcystin within the cyanobacterial cell or from free or particle adsorbed toxin. This has important implications for toxicity and in future studies this could be assessed using labelled antibodies. *Nodularia spumigena* cells with diameters 7–10 μm[46] and *Microcystis* sp. with diameters 3–9 μm[47] would not be expected to be delivered into the deep bronchiolar or alveolar cavities, but more likely deposited in the upper respiratory tract.[48] However, particle bound or free nodularin or microcystin would be expected to be deposited into the deepest bronchiolar or alveolar cavities. Thus toxicity from *N. spumigena* cells and *Microcystis* cells would most likely only involve the upper respiratory tract and result from nodularin, microcystin and/or lipopolysaccharides released during macrophage mediated cells lysis.

In contrast, free and particle bound nodularin and microcystin would be expected to mediate cytotoxicity in alveolar macrophages and epithelial cells. In addition, and provided the required organic anion transporting peptides (OATPs) were present,[49] nodularin and microcystin could enter the blood stream and be distributed systemically. The lack of respective *in vitro* studies with human cells and *in vivo* studies with improved personal samplers currently prevent proper assessment of potential risk by aerosolized nodularin and microcystin and/or toxin containing *N. spumigena* and *Microcystis* cells. Such *in vitro* studies are presently being carried out at our laboratory in Konstanz. Despite the latter impediment, earlier studies in mice suggest that nasally applied microcystins may be as toxic as intraperitoneal injections.[11] Using the current WHO guidance value for microcystin in drinking water (1 μg MC-$LR^{-1}$),[10] which assumes that an adult consumes 2 l water per day, the daily maximal oral exposure would be 2 μg microcystin-LR. Assuming 10-fold higher availability of nodularin and microcystin *via* an inhalatory rather than the oral route, the maximum daily inhaled concentration (MDIC) should not exceed 200 ng for 60 kg adult or should not exceed an aerosol concentration of 4.58 ng microcystin-LR $m^{-3}$ (when assuming a minute ventilation of 30.3 l $min^{-1}$ over 24 h).[50] Using different types of activities and the corresponding inhalation rates, the potentially inhaled nodularin and microcystins at lakes Forsyth and Rotorua were calculated, compared with the MDIC and a MDIC to 24 h toxin inhaled ratio calculated (Table 3). This comparison demonstrates that the concentrations of aerosolized nodularin or microcystin measured in air in this study should not pose an acute nor a chronic risk to humans exposed even during heavy work. The MDIC to 24 h toxin inhaled ratios calculated demonstrated that the amount of toxin potentially inhaled is a minimum of a factor 119 away from the 200 ng toxin still considered safe when using the WHO toxicological risk assessment for microcystin-LR as a basis. However, as the toxin concentrations in the air were calculated over extended sampling periods, the latter calculations may serve as an initial evaluation only. As the latter evaluation assumes continuous toxin exposure, intermittent peak toxin exposure may provide for a completely different toxicological scenario. Indeed, peak toxin values could have occurred in this study and thus may have been underestimated in this evaluation.

## Conclusions

This study and other recent research[21,22] have demonstrated that nodularin and microcystins can become aerosolized. Based on the concentrations measured in studies to date acute or chronic risks to humans from aerosolized toxins appear low. However, the data suggest a need for more systematic studies investigating

shorter sampling intervals, correlations between concentrations in water and aerosols, and other cyanotoxins produced by smaller or more fragile species. This research carried out in concert with epidemiological studies to determine causative symptoms would provide valuable data to assist in refining risk assessments for recreational use of water bodies and for lakeside populations.

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## References

1 R. Ressom, F. S. Soong, D. J. Fitzgerald, L. Turczynowicz, O. E. Saadi, D. Roder, T. Maynard and I. R. Falconer, *Health Effects of Toxic Cyanobacteria (Blue-Green Algae)*, National Health and Medical Research Council, Canberra, Australia, 1994.
2 C. MacKintosh, K. A. Beattie, S. Klumpp, P. Cohen and G. A. Codd, *FEBS Lett.*, 1990, **264**, 187–192.
3 R. E. Honkanen, M. Dukelow, J. Zwiller, R. E. Moore, B. S. Khatra and A. L. Boynton, *Mol. Pharmacol.*, 1991, **40**, 577–583.
4 J. Goldberg, H. B. Huang, Y. G. Kwon, P. Greengard, A. C. Nairn and J. Kuriyan, *Nature*, 1995, **376**, 745–753.
5 J. T. Maynes, H. A. Luu, M. M. Cherney, R. J. Andersen, D. Williams, C. F. Holmes and M. N. James, *J. Mol. Biol.*, 2006, **356**, 111–120.
6 A. Milutinović, M. Živin, R. Zorc-Plesković, B. Sedmak and D. Šuput, *Toxicon*, 2003, **42**, 281–288.
7 D. Feurstein, K. Holst, A. Fischer and D. R. Dietrich, *Toxicol. Appl. Pharmacol.*, 2009, **234**, 247–255.
8 D. Feurstein, J. Kleinteich, A. H. Heussner, K. Stemmer and D. R. Dietrich, *Environ. Health Perspect.*, 2010, **118**, 1370–1375.
9 K. Sivonen and G. Jones, in: *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring, and Management*, ed. I. Chorus and J. Bartram, E&FN Spon, London, UK, 1999, ch. 2, pp. 41–111.
10 J. K. Fawell, R. E. Mitchell, D. J. Everett and R. E. Hill, *Hum. Exp. Toxicol.*, 1999, **18**, 162–167.
11 T. Yoshida, Y. Makita, S. Nagata, T. Tsutsumi, F. Yoshida, M. Sekijima, S. I. Tamura and Y. Ueno, *Nat. Toxins*, 1997, **5**, 91–95.
12 World Health Organisation, *Cyanobacterial Toxins: Microcystin-LR in Drinking-Water. Background Document for Preparation of WHO Guidelines for Drinking-Water Quality. (WHO/SDE/WSH/03.04/57)*, World Health Organization, Geneva, 2003.
13 R. B. Fitzgeorge, S. A. Clark and C. W. Keevil, in: *Detection Methods for Cyanobacterial Toxins*, ed. G. A. Codd, T. M. Jefferies, C. W. Keevil and E. Potter, Royal Society of Chemistry, Cambridge, UK, 1994. pp. 69–74.
14 K. Hoppu, J. Salmela and K. Lahti, *Clin. Toxicol.*, 2002, **40**, 309–310.
15 G. A. Codd, S. G. Bell, K. Kaya, C. J. Ward, K. A. Beattie and J. S. Metcalf, *Eur. J. Phycol.*, 1999, **34**, 405–416.
16 L. S. Pilotto, R. M. Douglas, M. D. Burch, S. Cameron, M. Beers, G. J. Rouch, P. Robinson, M. Kirk, C. T. Cowie, S. Hardiman, C. Moore and R. G. Attewell, *Aust. New Zeal. J. Publ. Health*, 1997, **21**, 562–566.
17 I. Stewart, P. M. Webb, P. J. Schluter and G. R. Shaw, *Environ. Health*, 2006, **5**, 6.
18 P. C. Turner, A. J. Gammie, K. Hollinrake and G. A. Codd, *Br. Med. J.*, 1990, **300**, 1440–1441.
19 R. Philipp and A. J. Bates, *Water Environ. J.*, 1992, **6**, 613–617.
20 Y. S. Cheng, Y. Zhou, C. M. Irvin, B. Kirkpatrick and L. C. Backer, *Mar. Drugs*, 2007, **5**, 136–150.
21 L. C. Backer, W. Carmichael, B. Kirkpatrick, C. Williams, M. Irvin, Y. Zhou, T. B. Johnson, K. Nierenberg, V. R. Hill, S. M. Kieszak and Y. S. Cheng, *Mar. Drugs*, 2008, **6**, 389–406.
22 Y. S. Cheng, T. A. Villareal, Y. Zhou, J. Gao, R. H. Pierce, D. Wetzel, J. Naar and D. Baden, *Harmful Algae*, 2005, **4**, 87–94.
23 R. H. Pierce, M. S. Henry, P. C. Blum, S. L. Hamel, B. Kirkpatrick, Y. S. Cheng, Y. Zhou, C. M. Irvin, J. Naar, A. Weider, L. E. Flemming, L. C. Backer and D. G. Baden, *Harmful Algae*, 2005, **4**, 965–972.
24 World Health Organisation, *Guidelines for Safe Recreational Water Environments, Volume 1: Coastal and Fresh Waters*, World Health Organization, Geneva, 2003, www.who.int/water_sanitation_health/bathing/srwe1/en/.
25 Ministry for the Environment and Ministry of Health, *New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters—Interim Guidelines*, Ministry for the Environment, Wellington, 2009.
26 National Health and Medical Research Council, *Guidelines for Managing Risks in Recreational Water*, Canberra, Australia, 2008.
27 B. McAlice, *Limnol. Oceanogr.*, 1971, **16**, 19–28.
28 J. Irwin. *Lake Forsyth Bathymetry 1 : 10 000*, New Zealand Oceanographic Institute Chart: lakes series. 1979.
29 C. A. Woodward and J. Shulmeister, *J. Paleolimnol.*, 2005, **34**, 481–501.
30 H. E. Connor, in: *The Poisonous Plants in New Zealand*, ed. H. E. Connor, Government Printer, Wellington, 2nd edn, 1977, pp. 209–212.
31 E. A. Flint, in: *New Zealand Lakes*, ed. V. H. Jolly and J. M. A. Brown, Auckland University Press, Auckland, 1975, pp. 163–192.
32 K. Christoffersen and C. W. Burns, *Verh. - Int. Ver. Theor. Angew. Limnol.*, 2000, **27**, 3222–3225.
33 H. Utermöhl, *Verh. - Int. Ver. Theor. Angew. Limnol.*, 1958, **9**, 1–38.
34 W. J. Fischer, I. Garthwaite, C. O. Miles, K. M. Ross, J. B. Aggen, A. R. Chamberlin, N. R. Towers and D. R. Dietrich, *Environ. Sci. Technol.*, 2001, **35**, 4849–4856.
35 S. A. Wood, A. Rueckert, S. C. Cary, D. P. Hamilton and D. R. Dietrich, *Environ. Microbiol. Rep.*, 2010, **3**, 118–124.
36 T. Kuiper-Goodman, I. Falconer and J. Fitzgerald, in: *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring, and Management*, ed. I. Chorus and J. Bartram, E&FN Spon. London, UK, 1999, ch. 4, pp. 113–153.
37 B. W. Ibelings, M. Vonk, H. F. J. Los, D. T. Molen and W. M. Mood, *Ecol. Appl.*, 2003, **13**, 1456–1472.
38 C. S Reynolds, R. L. Oliver and A. E. Walsby, *N. Z. J. Mar. Freshwater Res.*, 1987, **21**, 379–390.
39 K. Tsuji, T. Watanuki, F. Kondo, N. Ishikawa, M. F. Watanabe, S. Suzuki, H. Nakazawa, M. Suzuki, H. Uchida and K. I. Harada, *Toxicon*, 1995, **33**, 1619–1631.
40 K. I. Harada, K. Tsuji, M. F. Watanabe and F. Kondo, *Phycologia*, 1996, **35**, 83–88.
41 D. R. Dietrich and S. Hoeger, *Toxicol. Appl. Pharmacol.*, 2005, **203**, 273–289.
42 P. A. Cox, S. A. Banack and S. J. Murch, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 13380–13383.
43 T. A. Caller, J. W. Doolin, J. F. Haney, A. J. Murby, K. G. West, H. E. Farrar, A. Ball, B. T. Harris and E. W. Stommel, *Amyotrophic Lateral Scler.*, 2009, **10**, 101–108.
44 D. C. Blanchard and L. D. Syzdek, *J. Geophys.*, 1972, **77**, 5087–5099.
45 D. C. Blanchard and L. D. Syzdek, *Limnol. Oceanogr.*, 1975, **20**, 762–774.
46 R. Congestri, R. Federici and P. Albertano, *Aquat. Microb. Ecol.*, 2000, **22**, 283–290.
47 J. Komárek and K. Anagnostidis, in: *Susswasserflora von Mitteleuropa*, ed. H. Ettl, G. Gartner, H. Heynig and D. Molllenhauer, Gustav Fisher, Berlin, 2000, vol. 19, p. 548.
48 B. Holmberg, J. Högberg, G. Johanson, in: *Encyclopaedia of Occupational Health and Safety*, ed. J. M. Stellman, International Labour Office, Geneva, Switzerland, 4th edn, 1998, pp. 33.3–33.6.
49 A. Fischer, S. J. Hoeger, K. Stemmer, D. J. Feurstein, D. Knobeloch, A. Nussler and D. R. Dietrich, *Toxicol. Appl. Pharmacol.*, 2010, **245**, 9–20.
50 N. J. Anderson, P. E. Cassidy, L. L. Janssen and D. R. Dengel, *J. Int. Soc. Respir. Prot.*, 2006, **23**, 53–62.

 