



## Research Article

# Cyanotoxins in drinking water supply reservoir (Legedadi, Central Ethiopia): implications for public health safety



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Received: 23 September 2020 / Accepted: 1 February 2021

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## Abstract

Cyanobacterial blooms in drinking water supply affect its quality, which ultimately impacts ecosystem and public health. Thus, this cross-sectional study was conducted to perform a preliminary study on cyanotoxins via analysis of samples collected only once from two sites during the month of peak algal bloom and to subsequently prompt a comprehensive risk assessment in a major drinking water source, Legedadi Reservoir, of Addis Ababa, the capital city of Ethiopia. Samples were collected during peak algal bloom month (January 2018) from two sampling sites, near the dam (S1) and at the center of the reservoir (S2). Identification and enumeration of phytoplankton taxa were done and the measurement of common hepatotoxin (MCs and NOD) concentrations was conducted using liquid chromatography-tandem mass spectrometry. In the reservoir, cyanobacteria made up to 98% of total phytoplankton abundance, with *Dolichospermum* and *Microcystis* spp, dominating the phytoplankton community. In these first cyanotoxin analyses conducted for a drinking water supply source in Ethiopia, six major MC variants, namely MC-dmRR, MC-RR, MC-YR, MC-dmLR, MC-LR, and MC-LA, were detected in both algal seston and water samples. MC-LR was the most dominant MCs variant, while nodularin was not detected for both sampling sites. Extracellular total MC concentrations ( $\mu\text{g L}^{-1}$ ) of 453.89 and 61.63 and intracellular total MC concentrations ( $\mu\text{g L}^{-1}$ ) of 189.29 and 112.34 were recorded for samples from S1 and S2, respectively. The high concentrations of extracellular MCs, with MC-LR constituting the greatest proportion, indicate the extremely high potential public health risk for end-users.

**Keywords** Cyanobacteria · Risk assessment · Microcystins · Drinking water supply · Legedadi Reservoir · Public health risk

## 1 Introduction

In developing countries like Ethiopia, surface water bodies are under severe threat due to uncontrolled disposal of industrial waste and application of chemical fertilizers on agricultural lands located near water bodies [1].

Industrial wastewaters and agricultural runoff ultimately elevate nutrient levels and promote cultural eutrophication of lakes/reservoirs [2]. Eutrophication of drinking water source reservoir degrades water quality through the induction of hazardous cyanobacterial blooms [3]. Climate change-induced high temperature further enhances the

**Supplementary Information** The online version contains supplementary material available at (<https://doi.org/10.1007/s42452-021-04313-0>).

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SN Applied Sciences

(2021) 3:328

| <https://doi.org/10.1007/s42452-021-04313-0>

Published online: 15 February 2021

SN Applied Sciences  
A **SPRINGER NATURE** journal

dominance of cyanobacterial species in reservoirs/lakes [4].

Cyanobacterial blooms in a drinking water supply source reduce water quality by increasing turbidity, affecting taste and odor, and producing toxins, which eventually influence ecosystems and affect public health [5]. Different environmental factors affect cyanotoxin production. For instance, temperature, light intensity, pH, nutrients, salinity, ultraviolet radiation, wind, trace metals, and environmental pollutants influence cyanotoxin production. [6, 7] pointed out that nutrient limitation (specifically N-limitation) and light regulate *mcy* gene and hepatotoxin production. [8] reported that when the concentration of nitrate increases, MC production was increased in non-nitrogen-fixing species.

According to [9, 10], a shortage of trace metals (iron, zinc, copper, and magnesium) in aquatic systems promotes the production of intracellular cyanotoxins as a means of acquisition or storage. When there is a high level of trace metal that leads to toxification in an aquatic system, cyanobacteria produce extracellular toxins to form metal complexes and detoxify metals [11].

Hepatotoxins (MCs and NODs), which are the most common toxic substances associated with blooms, are similar in structure, mode of action and are produced non-ribosomally from *mcy* and *nda* gene clusters, respectively [12].

MCs are cyclic heptapeptides, while NODs are cyclic pentapeptides; however, they both have the common amino acid, ADDA ( $\beta$ -amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), the most unusual substructure, which has not been reported except in MCs and NODs [13]. MC-LR and NOD-R are the most abundant congeners of MCs and NODs, respectively [13, 14]. MCs are stable and resistant to high temperature, extreme pH, sunlight and presence of common hydrolytic enzymes due to their cyclic structure [15]. These cyanotoxins are mostly associated with the acute intoxication of humans and livestock [3].

MCs are mainly liver toxicants causing inhibition of eukaryotic serine/threonine protein phosphatases 1 and 2A, which trigger extreme phosphorylation of cytoskeletal filaments leading to serious liver injury. Long-term exposure causes the promotion of liver tumors. Nodularins cause gastroenteritis, allergic irritation reactions, and liver diseases. Nodularin-R is the most notorious hepatotoxin that causes severe liver damage in humans and other animals [16, 17]. The WHO's tolerable limits for Nodularins and Microcystins-LR concentrations ( $\mu\text{g/L}$ ) in drinking water are 1.5 and 1.0, respectively [18]. The lethal dose 50 (LD50) of MC-LR is 25 to 150  $\mu\text{g/kg}$ , while the LD50 for NOD-R is 50 to 150  $\mu\text{g/kg}$  [19].

Under bloom condition, the problem becomes more pronounced in drinking water supply sources as

conventional drinking water treatments are ineffective in removing these toxins [20].

Legedadi Reservoir, the subject of the study reported here, is the major drinking water supply source for Addis Ababa, the capital city of Ethiopia. Year-round occurrence and dominance of potentially toxic cyanobacterial species in this reservoir have been reported by [21]. However, efforts made to restore the reservoir using environmentally sound and safe restoration techniques are virtually absent, while the application of algacides like copper sulfate has been a common practice in Legedadi Reservoir. The main drawback of using copper sulfate as an algacide is its induction of cyanobacterial cell lysis with consequent release of intracellular toxins [22].

Thus, the application of copper sulfate in a reservoir supporting toxic cyanobacterial blooms can lead to enormous public health risk. Furthermore, the Legedadi Reservoir's water supply system has conventional methods of water treatment including pre-chlorination (3.06 mg/l), coagulation (main coagulant polydimethyl-diallyl-ammonium-chloride), sedimentation, filtration, and post-chlorination (2.8 mg/l). This conventional treatment system is not effective in removing extracellular cyanotoxins [23], and the coagulants used in the treatment further induce cell lysis and consequent release of intracellular toxins [24]. All these factors incite high public health concern for using Legedadi Reservoir as drinking water supply source.

So far, few studies [25–28] have quantified MCs in a freshwater hydropower reservoir in Ethiopia. However, information on the concentrations of cyanobacterial toxins and associated public health risk in drinking water supply source reservoirs such as Legedadi Reservoir is nonexistent. Cyanobacterial blooms and cyanotoxins in drinking water sources are serious problems, particularly in countries like Ethiopia, where more than 62 million people lack access to safe water and alternative sources of drinking water supply are inadequate [29]. Thus, there was the need to undertake public health risk assessment of using water from Legedadi Reservoir. Therefore, this preliminary cross-sectional study on cyanotoxins was conducted via the analysis of samples collected once from two sites during the time of peak algal bloom, and to subsequently prompt a comprehensive risk assessment in a major drinking water source, Legedadi Reservoir.

## 2 Materials and methods

### 2.1 Study area

Legedadi Reservoir, one of the major drinking water supply sources for the capital city of Ethiopia, Addis Ababa, is located approximately 25 km east of Addis Ababa. It is

found at an altitude of 2450 m, and geographical position of 9° 01' N–9° 13' N latitude and 138° 60' E–39° 07' E longitude (Fig. 1).

The reservoir was constructed in 1967 and is administered by Addis Ababa Water and Sewerage Authority (AAWSA). It had an initial storage capacity of 45.9 million cubic meters and surface area of 5.33 km<sup>2</sup> [30]. The maximum and mean depths of the reservoir at 2466 meter above sea level are 30 and 4 m, respectively.

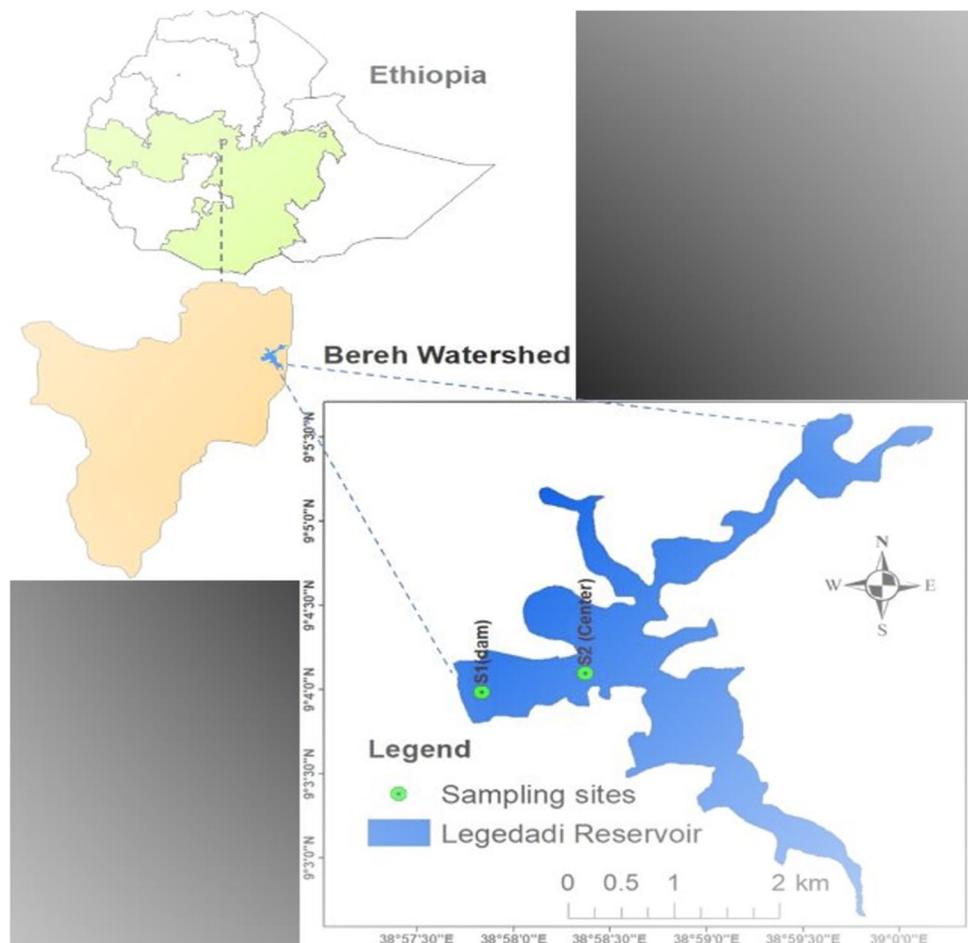
The reservoir has an outflow rate (to water treatment plant) of 126,666 (m<sup>3</sup>/day) and water retention time of 325 days [30]. The catchment of the reservoir has a total area of 205.7 km<sup>2</sup>. It is a sub-catchment of the Akaki River basin, which flows in a northeast–southwest direction and forms the northwest corner of the Awash River basin. The reservoir's catchment has an annual sediment yield of 845 t/km<sup>2</sup>/year. Expectedly, the huge sediment transport is accompanied by associated high nutrient load on the reservoir.

## 2.2 Sampling protocol

Two sampling sites were carefully selected considering the areas of frequent surface scum appearance and application of CuSO<sub>4</sub> by Addis Ababa water and sanitation authority (AAWSA).

The first site (S1) was located near the dam (deepest part of the reservoir), while the second one (S2) was located at the center of the reservoir. Due to budget limitations, sample collection was conducted only once during the peak bloom month (January 2018) using a Van Dorn bottle sampler Horizontal Model (Eijkelkamp Soil & Water supplier) to document baseline information on the contamination of the reservoir water with cyanotoxins. Samples were taken from the surface and 0.25 m depth and mixed in equal proportions to produce a composite sample. For the identification of major cyanobacterial genera, the composite samples were properly mixed, and 1000 ml aliquots were dispensed into bottles and fixed with Lugol's iodine (0.01% v/v).

**Fig. 1** Location map of the study area with sampling sites



### 2.3 In situ measurements

The transparency of the water (Secchi depth,  $Z_{SD}$ ) was estimated using a 20-cm-diameter black and white Secchi disk (United Scientific Supplies SCDSK1). Turbidity was measured using portable turbidity meter (model HI 93 703–11). Temperature and dissolved oxygen (DO) were measured using a portable digital oxygen meter (EUTECH instruments, model DO300), pH was measured using digital pH meter (HANNA instruments, model HI 9024), while conductivity was determined using multi-parameter meter (HQ40d). The specific conductivity ( $K_{25}$ ) was calculated according to [31].

### 2.4 Analysis of physicochemical parameters

Total suspended solids (TSS) were determined gravimetrically using a known volume of properly mixed sample, which was filtered on pre-weighed glass fiber filter paper (GF/F) and pre-dried at 105 °C to constant weight to measure total suspended solids [32]. The titration method was used to estimate total alkalinity (TA) and phenolphthalein alkalinity (PA). Water samples were titrated with 0.2 N  $H_2SO_4$  using phenolphthalein and bromocresol green-methyl red indicators within a few hours after sample collection according to [33] and expressed in  $meq L^{-1}$ . Except for total phosphorus (TP) and ammonia ( $NH_3 + NH_4^+ - N$ ), water samples were filtered with GF/F and used for spectrophotometric determination of inorganic nutrients. The ascorbic acid method was used to determine the soluble reactive phosphate-phosphorus (SRP) and total phosphorus (TP) after persulfate digestion [32]. The sodium salicylate method was used to determine nitrate ( $NO_3 - N$ ), while the molybdosilicate method was used to estimate silica ( $SiO_2$ ) [32]. Finally, the phenate method was used to estimate ammonia [33].

### 2.5 Identification and enumeration of phytoplankton taxa

Samples were well mixed and 100 ml aliquots were transferred to 1000 ml measuring cylinders and kept in the dark for 24 h. Then, 90 ml of the supernatant was carefully drawn off. The remaining 10 ml was mixed properly and 1 ml was placed in a Sedgewick-Rafter cell and allowed to settle. Identification to genus/species level was done using various identification guides, e.g., [34, 35]. According to [36], cell abundance was estimated by counting cells of algal units encountered in 40–50 grids.

$$\text{Cells ml}^{-1} = \frac{N * 1000 \text{ mm}^3}{A * D * F}$$

where  $N$  is the number of cells or units counted,  $A$  is area of field (area of each grid,  $1 \text{ mm}^2$ ),  $D$  is depth of Sedgewick-Rafter chamber (1 mm), and  $F$  is number of fields/grids counted.

### 2.6 Estimation of phytoplankton biomass

Phytoplankton biomass was estimated as chlorophyll-a (Chl-a) concentration ( $\mu\text{g L}^{-1}$ ) determined using a phytoplankton analyzer (PHYTO-PAM, Heinz Walz GmbH, Effeltrich, Germany).

### 2.7 Identification and quantification of cyanotoxins

Water samples were filtered using 47 mm GF/F filters. The seston retained by the filters was covered with aluminum foil and placed in Petri dishes and then kept in a freezer until analysis was conducted. The filtered water samples were kept in a freezer over night before analyses were conducted.

#### 2.7.1 Extraction of MCs and NOD from algal seston

Extraction of MCs and NOD was done according to [37]. First, known volume of sample was filtered through a glass microfiber filter papers (47 mm GF/F filters). Then, the filter papers that retained algal seston were folded in half and put in a 10-ml glass tube and freeze-dried on a freeze-drier (Alpha 1–2 LD, Martin Christ Gefriertrocknung sanlagen GmbH, Osterode am Harz, Germany) for two hrs. After that, 2.5 ml of 75% methanol-25% Millipore water (v/v) was used to extract sample three times in a water bath at 60 °C using the glass pipette squeeze method. Then, 7.5 ml samples on the speedvac tube were dried up using a Speedvac (Savant SPD121P, Thermo Scientific, Waltham, MA, USA) centrifuge pre-heat at 50 °C. Then, 300  $\mu\text{l}$  of 100% MeOH was used to reconstitute the sample three times after well vortexed and mixed. Afterward, the reconstituted 900  $\mu\text{l}$  samples were transferred to 2 ml Eppendorf vials with a cellulose-acetate filter (0.2  $\mu\text{m}$ , Grace Davison Discovery Sciences, Deerfield, IL, USA) and centrifuged for 5 min at  $16,000 \times g$  (Galaxy 16DH, VWR, International). Finally, filtrates were transferred to amber glass vials and caps were tightly closed and stored in a freezer till LC–MS/MS analysis. Prior to LC–MS/MS analysis, calibration standards were prepared [38].

#### 2.7.2 Extraction of extracellular microcystins and nodularin

Filtrates of water samples filtered using 47 mm GF/F filters and stored in a freezer were dried in a Speedvac (Savant SPD121P, pre-heat centrifuge at 50 °C). Then, 300  $\mu\text{l}$  of 100% MeOH was used to reconstitute the sample three

times after well vortexed and mixed. Afterward, the reconstituted 900  $\mu\text{L}$  samples were transferred to 2-ml Eppendorf vials with a cellulose-acetate filter (0.2  $\mu\text{m}$ , Grace Davison Discovery Sciences, Deerfield, IL, USA) and centrifuged for 5 min at 16,000  $\times g$  (Galaxy 16DH, VWR, International). Finally, filtrates were transferred to amber glass vials and caps were tightly closed and stored in a freezer till LC–MS/MS analysis. Prior to LC–MS/MS analysis, calibration standards were prepared [38].

### 2.7.3 LC–MS/MS analyses

The concentrations of nine MC variants (dm-7-MC-RR, MC-RR, MC-YR, dm-7-MC-LR, MC-LR, MC-LA, MC-LY, MC-LW, and MC-LF) and nodularin (NOD) were examined by LC–MS/MS according to the procedure of [39]. LC–MS/MS analyses were done using an Agilent 1200 LC and an Agilent 6410A QQQ (Agilent Technologies, Santa Clara, CA, USA). MCs variants were separated on an Agilent Eclipse XDB-C18 4.6\_150 mm, 5 m columns. Calibration standards were obtained from DHI LAB Products (Hørsholm, Denmark) [37] (supplementary material). The sample was injected with a flow rate of 0.5 ml/min; the column temperature was 40  $^{\circ}\text{C}$ . Eluents were Millipore water with 0.1% formic acid (v/v, Eluent A) and acetonitrile with 0.1% formic acid (v/v, Eluent B) that were run using an elution program of 0–2 min 30% B, 6–12 min 90% B, with a linear increase of B between 2 and 6 min and a 5-min post run at 30% B. Detailed information on MS/MS settings for each MC variant and NOD are shown in (supplementary material). Recovery of sample and analysis was estimated by spiking a cyanobacterial matrix in triplicates: The recovery rate was 100% for MC-LA, 99.5% for DM-7-MC-RR, 96.1% for MC-RR, 75% for MC-YR, 77.8% for DM-7-MC-RR, 79.1% for MC-LR, 72.8% for MC-LY, 53.5% for MC-LW, and 63.9% for MC-LF. Furthermore, repeatability, the limit of detection and limit of quantification of LC–MS/MS analyses are described in [38].

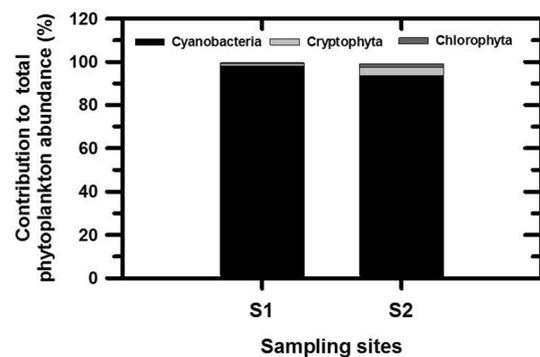
## 3 Results

### 3.1 Physicochemical parameters

The clarity of the reservoir, expressed in terms of mean Secchi depth (cm), was 11 and 10 at S1 and S2, respectively. The mean levels of turbidity of the reservoir (NTU) were 311 and 315 at S1 and S2, respectively, while those of TSS ( $\text{mg L}^{-1}$ ) were 0.24 at both S1 and S2. The mean water temperature ( $^{\circ}\text{C}$ ) of S1 and S2 were 17.86 and 17.5 respectively. The reservoir was well oxygenated, with mean DO concentrations ( $\text{mg L}^{-1}$ ) of 7.22 and 6.85 at S1 and S2, respectively.

**Table 1** Levels of physicochemical parameters measured at S1 and S2 of Legedadi Reservoir in January 2018

Parameters	Concentration	
	S1	S2
$Z_{SD}$ (cm)	11	10
DO ( $\text{mg L}^{-1}$ )	7.22	6.85
Temperature ( $^{\circ}\text{C}$ )	17.9	17.5
TSS ( $\text{mg L}^{-1}$ )	0.24	0.24
Alkalinity ( $\text{meq L}^{-1}$ )	1.05	0.95
pH	7.91	7.94
Conductivity ( $K_{25}$ , $\mu\text{S cm}^{-1}$ )	29.89	31.33
Turbidity (NTU)	311	315
TP ( $\text{mg L}^{-1}$ )	597	605
SRP ( $\mu\text{g L}^{-1}$ )	116.6	130
Nitrate ( $\mu\text{g L}^{-1}$ )	276	314
Ammonia ( $\mu\text{g L}^{-1}$ )	3.06	3.09
Silica ( $\text{mg L}^{-1}$ )	9.33	9.86
Chl-a ( $\mu\text{g L}^{-1}$ )	6510	128.07



**Fig. 2** Relative abundance of major algal groups

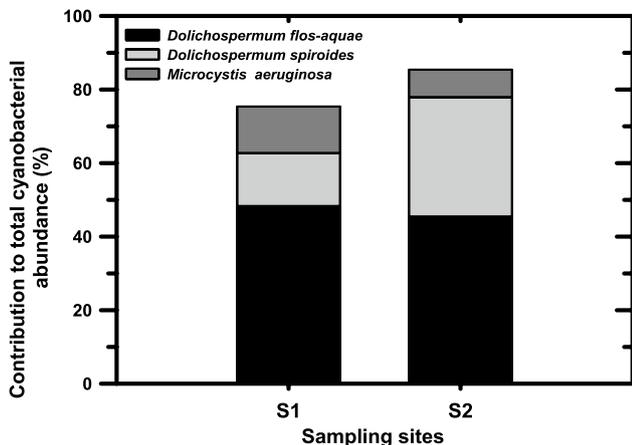
The mean concentrations ( $\mu\text{g L}^{-1}$ ) of SRP at S1 and S2 were 166.6 and 130, respectively (Table 1) while the mean total phosphorous (TP) concentrations ( $\mu\text{g L}^{-1}$ ) were 597 and 605 at S1 and S2, respectively (Table 1). The mean concentrations ( $\mu\text{g L}^{-1}$ ) of nitrate at S1 and S2 were 276 and 314, respectively, while mean concentration ( $\mu\text{g L}^{-1}$ ) of ammonia was 3.06 at S1 and 3.09 at S2.

### 3.2 Major phytoplankton taxa

Cyanobacteria constituted 98% and 93.5% of total phytoplankton species at S1 and S2, respectively. Cryptophyta and Chlorophyta were the second and third most abundant groups at both sampling sites, with percentage contributions of 0.73% and 0.64% at S1 and 3.9% and 1.66% at S2, respectively (Fig. 2).

**Table 2** List of cyanobacteria species identified in the Legedadi Reservoir during the month of January

<i>Chroococcus dispesus</i> , Keissler	<i>Dolichospermum spiroides</i> Kleb
<i>Cylindrospermopsis curvispora</i> , Watanabe	<i>Gloeocapsa</i> sp.
<i>Cylindrospermum</i> sp.	<i>Microcystis aeruginosa</i> , Kutz
<i>Dolichospermum circinalis</i> Rabenhors	<i>Microcystis flos-aquae</i> , Wittrock
<i>Dolichospermum crassa</i> Lemmerman	<i>Microcystis novacekii</i> , Komárek
<i>Dolichospermum flos-aquae</i> , Brebisson ex Bornet & Flahault) P, Wacklin, L. Hoffmann and J. Komarej	



**Fig. 3** Relative abundance of the major cyanobacterial species expressed as percentage of the total cyanobacterial abundance

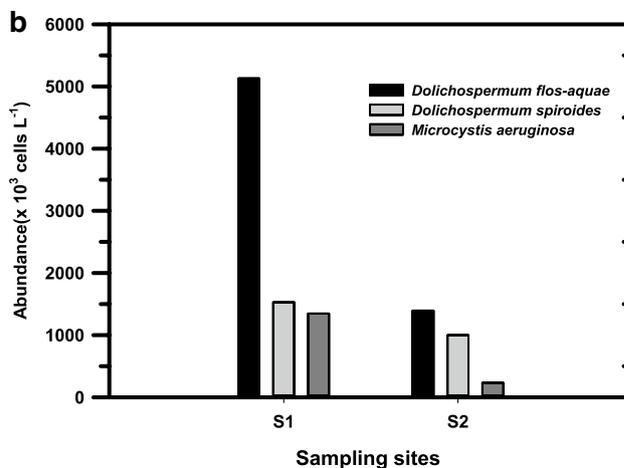
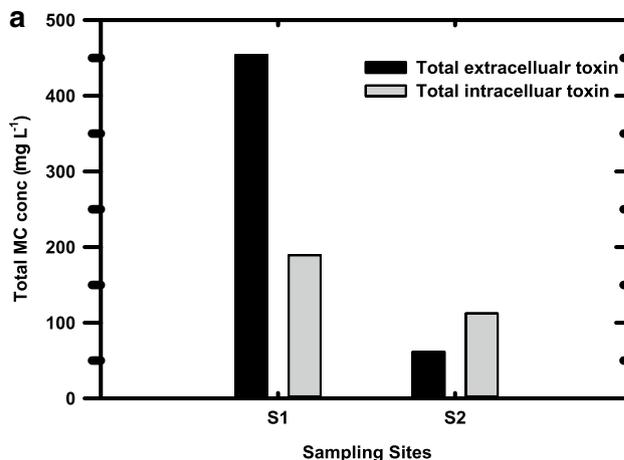
Eleven species of potentially toxic cyanobacteria were identified in samples at the two sampling sites of the reservoir (Table 2). The most dominant cyanobacterial genera in this study were *Dolichospermum* and *Microcystis* (Fig. 3), with cell densities of 6,951,000 cells L<sup>-1</sup> and 1,918,000 cells L<sup>-1</sup>, respectively. The percentage contributions of the dominant species, *Dolichospermum flos-aquae*, *Dolichospermum spiroides* and *Microcystis aeruginosa*, to the total cyanobacterial abundance were 48.3%, 14.3%, and 12.5%, respectively (Fig. 4a).

### 3.3 Cyanotoxins

LC-MS/MS analyses of cyanobacterial extracts revealed the occurrence of substantial levels of extracellular MCs (µg L<sup>-1</sup>) of 453.89 and 61.63 and of intracellular MCs (µg L<sup>-1</sup>) of 189.29 and 112.34 at S1 and S2, respectively (Table 3). The observed results indicate that total extracellular MCs concentration at S1 was higher than the total intracellular MC concentration at S2 (Fig. 4b).

Six variants of MC were identified in Legedadi Reservoir, the main MC variants being MC-dmRR, MC-RR, MC-YR, MC-dmLR, MC-LR, and MC-LA. The concentrations of all MC's variants except MC-LA were higher at S1 than at S2.

MC-LR was the most dominant MC variant, with percentage contributions to the total MC concentrations (µg



**Fig. 4** Total microcystins concentrations in relation to the total abundance of microcystin-producing species

L<sup>-1</sup>) of 72.6% and 74.9% in water and 75.2% and 59.4% in algal seston at S1 and S2, respectively. Among the different MC variants, MC-YR was the second most dominant MC's congener in water and algal seston samples at both sampling sites. The percentage contributions of MC-YR to total MCs were 12.7% and 15.3% in water samples and 9.3% and 13.2% in algal seston samples, at S1 and S2, respectively. Nodularin was not detected in any of the samples analyzed.

**Table 3** Intra- and extracellular MCs concentrations and ratio of total intracellular and extracellular MCs at the two sampling sites of the present study on Legedadi Reservoir

Sampling site	Toxins	Concentrations of MCs variants ( $\mu\text{g L}^{-1}$ )							Ratio (Total MCs (water): total MCs (in algal seston))
		DmRR	RR	YR	dmLR	LR	LA	Total MCs	
S1	Extracellular	2.60	7.75	57.68	22.42	329.07	34.38	453.89	2.39
S2	Extracellular	0	0	9.44	2.46	46.2	3.28	61.63	0.05
S1	Intracellular	3.42	2.20	17.67	10.06	142.38	13.56	189.29	
S2	Intracellular	0	0	14.86	3.54	67.12	26.82	112.34	

## 4 Discussion

The present results show that the reservoir had unusually high concentration of nutrients during the sampling time. The levels of nutrients recorded during the present study were high compared to those commonly recorded for tropical lakes including those reported previously for this study reservoir [21] and Koka Reservoir in Ethiopia [26, 28] during the sampling time. The present high levels of nutrients are associated with the high anthropogenic disturbance occurring in the catchment areas of the reservoir and the consequent nutrient-rich runoff to the reservoir. Moreover, the abundance and dominance of cyanobacterial species observed in this study have resulted primarily from the availability of high levels of nutrients. Total phosphorus and low water clarity were identified as the most important environmental factors associated with phytoplankton community structure [40].

In the case of high reservoir turbidity, the conflicting results were reported. Some studies have suggested that cyanobacteria can out-compete other phytoplankton species in turbid systems [41, 42], while others claim that the high level of non-algal turbidity reduces cyanobacterial growth [43, 44]. Yet, the gas vacuoles in cyanobacteria give them a competitive advantage to rendering them buoyant thereby enabling them to regain their vertical position where efficient photosynthesis is possible. Therefore, turbidity in water bodies cannot adversely affect cyanobacteria.

In the reservoir, there was a higher level of total extracellular MCs than total intracellular MCs. This extracellular MCs concentration could even be much higher when considering the argument by [28] that the procedure currently in use does not enable us to accurately and exclusively determine the two fractions, as MCs get bound to surfaces of suspended particulate matter and are subsequently retained by the filter paper. These particle-bound MCs, which are part of the extracellular fraction, are not available for inclusion in the measurement of MCs in the filtrate. This eventually results in an underestimation of

the extracellular MCs concentration. The total concentration of extracellular MCs at S1 was more than twice that of the intracellular MCs. The higher concentration of MCs at S1 may have resulted from the application of  $\text{CuSO}_4$  by AAWSA in the reservoir at S1.  $\text{CuSO}_4$  induces cell lysis and the consequent release of MCs. In a healthy cyanobacterial bloom, the highest proportion (98%) of microcystins is retained within the cells [45], while cell damage and death will lead to release of intracellular toxins to the environment [13].

The presence of such high concentrations of extracellular toxins in the reservoir and the ineffectiveness of the conventional water treatment process in removing the cyanotoxins from treated water causes a huge public health risk to the end-user. Furthermore, the primary oxidants such as potassium permanganate and chlorine could oxidize cyanotoxins in water bodies to form harmful by-products of trihalomethanes and mutagenic furanones [46].

The concentrations of MCs in this study are noticeably greater than those reported for most African lakes and reservoirs, including Lake Chivero, Zimbabwe [0.1–1.6  $\mu\text{g L}^{-1}$ ; [47], Ugandan freshwaters [0.02–10  $\mu\text{g L}^{-1}$ ; [48] and Koka Reservoir in Ethiopia [1.71 to 33  $\mu\text{g L}^{-1}$ , [28]. Freshwater cyanobacterial species including *Dolichospermum*, *Fischerella*, *Gloeotrichia*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria*, and *Planktothrix* produce MCs, which are common and tremendously toxic secondary metabolites in water bodies [49]. Therefore, any of cyanobacterial species identified in this study could be responsible for the observed MC concentration in the reservoir. To specifically identify which cyanobacterial genus was responsible for the toxin production, further molecular analysis of *mcy* encoding gene is obligatory. However, the few studies conducted on cyanotoxins in Koka Reservoir in Ethiopia have associated cyanotoxins production mainly with *Microcystis* and *Dolichospermum* spp. [25–28].

The largest portion of total MCs was constituted by MC-LR, which fundamentally causes a great concern for the end-user as MC-LR has been identified as the most

potent toxin and chemically stable in water [50]. Acute exposure to MC-LR leads to massive intra-hepatic hemorrhage, liver swelling, and death, whereas chronic exposure causes genotoxicity and carcinogenicity [51]. World health organization has also categorized MC-LR as “probably carcinogenic for humans” [52].

Conventional treatment systems cannot effectively remove the stable cyclic structure of MC-LR [53]. Therefore, the occurrence of high levels of extracellular MC-LR in the reservoir and the ineffectiveness of the treatment system in removing the toxin from the water indicate the high potential public health risk for the end-users.

The concentration of extracellular MC-LR was about 330 times higher than the permissible level established for drinking water [ $1 \mu\text{g L}^{-1}$ ], [18]. MC-LR is relatively persistent in the aquatic environment, after algaecide treatment [54]. Jones and Falconer [54] observed the presence of MC-LR up to 21 days after algaecide treatment of *Microcystis aeruginosa* bloom. This fact reflects the serious health risk associated with the use of Legedadi Reservoir as a source of drinking water supply as year-round occurrence and dominance of cyanobacteria has been observed in the reservoir. The use of  $\text{CuSO}_4$  by water quality managers to control the year-round occurrence of cyanobacterial bloom has exacerbated the problem.

Arginine-containing microcystins congeners (MC-dmRR and MC-RR) were detected at lower concentrations in algal seston and water samples from S1. Furthermore, they were not detected in samples from S2. The use of GF/C filter paper during sample extraction could be responsible for the observed low concentration of MC-dmRR and MC-RR. According to [55], compared to those samples whose filtration involved use of filter papers of other grades, in GF/C filtered samples, all microcystins that contained arginine were present at significantly lower concentrations because arginine-containing microcystins adhere to GF/C filters.

Nodularin was not detected in samples from both sites. This could be due to the fact that the genus *Nodularia*, the most common NOD producer, was not encountered in samples examined. Moreover, nodularin is also more commonly detected in brackish water than freshwater water [56]. Yet, [57] detected trace amounts of NOD in the absence of *Nodularia* sp. and nodularin was also detected in freshwater bodies dominated by *microcystis* spp. [58, 59].

Public health risk assessment of Legedadi Reservoir indicated the occurrence of extracellular MC-LR concentrations ( $\mu\text{g L}^{-1}$ ) of 329.07 and 46.2 at S1 and S2, respectively, and intracellular MC-LR concentrations ( $\mu\text{g L}^{-1}$ ) of 142.37 and 67.12 at S1 and S2, respectively. These concentrations are much higher than the WHO guideline value for drinking water. The occurrence of high concentration of MC-LR near the raw water intake site (S1) and the application

of chlorine in the water treatment process twice, at pre-chlorination (3.06 mg/L) and post-chlorination (2.8 mg/L), induces cyanobacterial cell lysis and the consequent release of MCs to the treated water could immensely augment the public health risk. This reservoir provides 52% of the domestic water supply for 2.75 million people. Therefore, the public health risk of using Legedadi Reservoir as a source of drinking water supply is great. Furthermore, the local communities in the catchment area of the reservoir directly use the reservoir water without further treatment for domestic purposes and this causes great concern for the health of local inhabitants.

## 5 Conclusion

This preliminary study revealed the occurrence of high concentrations of intracellular and extracellular MCs in the reservoir. The high concentrations of total extracellular MCs, especially at S1, could be associated with the application of  $\text{CuSO}_4$  and the associated cell lysis. Additionally, the high levels of intracellular and extracellular MC in the reservoir indicate the high potential public health risk associated with the use of the Legedadi Reservoir as a source of drinking water supply. The concentrations of intracellular and extracellular microcystins (total MCs and MC-LR) are much higher than the permissible level set by WHO for drinking water ( $1 \mu\text{g L}^{-1}$ ). Moreover, the occurrence of high concentrations of extracellular MCs constituted largely by MC-LR at both sites and the inefficiency of the conventional treatment system in removing microcystins from treated water are suggestive of the high potential public health risk.

Despite the limited data, our findings provide information on the possible public health risk of MC in the drinking water source. The results are expected to serve as an alarm for the water quality manager of the reservoir signaling the need to undertake year-round water quality monitoring of cyanotoxins and to look for effective and environmentally safe algal bloom control methods and water treatment system that can effectively remove cyanotoxins from the treated water.

The study is a cross-sectional survey and could not designate the observed toxin levels as maximum or minimum concentrations. Therefore, systematic monitoring and analysis of MC throughout the year for at least a couple of years along with quantitative assessment of dominant species connected to toxin production are required.

**Acknowledgements** We thank Addis Ababa water and sanitation authority for assistance during the sampling. We would also like to thank the Aquatic Ecology and Water Quality Management Group at Wageningen University for providing all the laboratory facilities for toxin analysis, SIDA Project, for covering travel and accommodation

costs to Wageningen University and Addis Ababa University thematic research Projects (Project No. TR/012/2016 and RD/PY-609/2016) for covering the cost of sampling in Legedadi Reservoir.

**Author contributions** HH, ML, conception and design, HH, WB, investigation, ML data curation, HH, DK, SL, ML data analysis, HH, draft the manuscript, DK, SL edit the manuscript, HH revised the manuscript.

**Code availability** Not applicable.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethics approval** Not applicable.

**Availability of data and material** All data and material used in this manuscript are available.

**Consent to participate** All authors have made substantial contributions to conception, design, investigation, data curation, data analysis, drafting, editing, and revising it critically, give final approval of the version to be published.

**Consent for publication** All authors have contributed from conception to approval of the final version and agreed to be accountable for all aspects of the work.

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