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Monitoring of The Safety and Quality of Water of *La Ferme* Impounding Reservoir of Mauritius

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Abstract: Water supply from *La Ferme* impounding reservoir is primarily intended for agricultural use. Unfortunately, there is a concern that the reservoir water is potentially befouled by agrochemicals and pollutants from nearby farms, feeder canals and a solid waste transfer station. The nutrient influx into *La Ferme* could favour the growth of toxin-producing cyanobacteria which could eventually jeopardize human and animal health. This research is aimed at assessing the water quality of *La Ferme* reservoir. Water was sampled at the intake tower and two rivers that discharge into La Ferme during summer/rainy (November–April) and winter/dry (June–September) seasons. The mean cyanobacterial count and level of orthophosphate, nitrate and total organic carbon in the reservoir were 63,738 cells/mL, 0.065 mg/L, 0.125 mg/L and 4.8 ppm, respectively. Orthophosphate and total organic carbon levels were significantly positively correlated with cyanobacterial cell counts. The mean orthophosphate level in reservoir water was found to be significantly higher ($p \leq 0.05$) during rainy seasons (0.08 mg/L) compared to dry seasons (0.04 mg/L). Residues of herbicide tebuthiuron and cyanotoxin microcystin-LR were also detected with a mean concentration of 0.15 and 0.3 mg/L, respectively. This study highlights the potential eutrophic status of *La Ferme* reservoir and contamination of its watersheds by microalgal toxin and herbicide that could compromise the ecological integrity of the reservoir and negatively impact the sustainable use of this natural resource.

Key words: *La Ferme* reservoir, nutrient, eutrophication, cyanotoxin, herbicide.

Background

Pollution of freshwater bodies often originates from the discharge of pollutants that degrade the ecosystem. Prime contaminants are agrochemicals such as fertilisers, pesticides and herbicides which are commonly used in intensive agriculture. Sofarini et al. (2020) reported that phosphorous and nitrogen-based nutrients leaching from agricultural lands into watersheds may potentially accumulate into lakes and subsequently promote eutrophication. This induces an imbalance in aquatic biota and water quality (Chislock et al., 2013). Eutrophic microalgae bloom causes hypoxia of water systems and are harmful to aquatic life (Bellinger and Sigee, 2015).

Freshwater harmful algal blooms comprise the toxigenic species of cyanobacteria whose toxin impair vital organs in humans (WHO, 2020). Toxin-producing species include *Microcystis, Anabaena, Nostoc, Oscillatoria* and *Lyngbya* (Bellinger and Sigee, 2015). The most widespread toxin is microcystin and its occurrence at levels exceeding 1 μg/L in drinking water represents a serious threat to public health (WHO, 2020).

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Besides microcysins, herbicides represent another threat to freshwater ecosystems. Herbicides are used worldwide in agriculture to control weeds which tend to compete with crops for growth factors such as water and nutrients. Injudicious use of these agrochemicals can severely affect the environment (Meyer and Scribner, 2009) and also lead to multiple irreversible health problems in human (Mahmood et al., 2016). These organic pollutants may contaminate aquifers and surface water bodies causing harm to untargeted biota (WHO, 2020). In Mauritius, the prevalence of herbicides diuron, tebuthiuron (phenyl-urea family) and atrazine (triazine family) *residues* in watersheds are particular areas of concern. Diuron has been classified as a human carcinogen while tebuthiuron causes muscle spasms. Atrazine can cause breast cancer and tumours in the reproductive organs of human beings (Ory, 2015).

Water supply from *La Ferme* reservoir is intended for irrigation of crop plantations and livestock animal consumption. However, the scarcity of rainfall in the dry seasons may call local authorities responsible for potable water supply to resort to the purification of water from *La Ferme* for domestic use during water shortages. Unfortunately, the water of *La Ferme* reservoir is suspected to be contaminated by potentially toxic microalgae and herbicide contaminants. This is further compounded by nutrient loading from feeders Trianon Grosses Roches and La Fenêtre, which are susceptible to anthropogenic pollution as they flow in the vicinity of residential, industrial and agricultural zones. According to Nabybaccus et al. (2018), total organic carbon and nutrient levels at LF are superior to environmental standards due to contamination by surface runoff. This research study, therefore, is aimed at assessing the vulnerability of *La Ferme* reservoir to nutrient enrichment that may favour blooms of toxin-producing cyanobacteria species as well as contamination by specific herbicide residues commonly used in Mauritius.

Materials and Methods

Site Description and Sampling

La Ferme (LF) reservoir was built for the purpose of irrigation in 1914. The catchment area of LF (19.61 km^2) receives mean annual precipitation of 1380 mm and the reservoir is supplied with water from the high-altitude feeder canals Trianon Grosses Roches and La Fenêtre. A hot and dry climate prevails in the catchment area of LF with a mean annual temperature of 28.8°C, 7.5 hours of sunshine per day and 72% humidity. Large acres of forested land around the reservoirs have been cleared to make way for agriculture, settlements, a solid waste transfer station and photovoltaic panels for electricity generation.

There were three investigated sampling stations in all (Table 1 and Figure 1). From the sampling station S1 located at the intake tower (deepest point and spillway of the reservoir), water was sampled from the euphotic zone at a depth of 2 m with a Van Dorn Volume Sampler. Grab samples were collected at sampling stations S2 and S3 from Feeder canals Trianon Grosse Roche and La Fenetre, respectively, to understand the extent of nutrient loading from the inflowing rivers into LF. Samples were collected in separate 1-L plastic containers for physical, chemical and biological analyses at 30-day intervals between 09.30 a.m to 13.00 p.m over a period of 12 months. The samples were transported in a light-proof isotherm box containing icepacks.

Table 1: Location of each sampling station

Sampling station	*Location*
S1	Spillway tower and euphotic zone of LF
S2	Feeder canal Trianon Grosse Roche at 10 m upstream of discharge point into LF
S3	Feeder canal La Fenêtre at 10 m upstream of discharge point into LF

Physico-chemical Analyses

Temperature measurements were made with a calibrated mercury-filled thermometer (Thermco ACC003C, USA), which was graduated for every 0.1°C. Dissolved oxygen and pH of the water were determined using a calibrated DO and pH meter (Mettler Toledo, USA), respectively. Levels of orthophosphate and nitrate were determined by UV VIS spectrophotometry (Hach, USA). Levels of total organic carbon (TOC) were determined in parts per million using the oxidative combustion method with a combustion analyser (Teledyne Tekmar, USA) (Eaton et al., 1995).

Microscopic Observation

Microscopic examination was done to enumerate and identify living individual algal cells. About 100 mL of algal suspension was treated with Lugol's solution and allowed to sediment in a stoppered 100 mL measuring

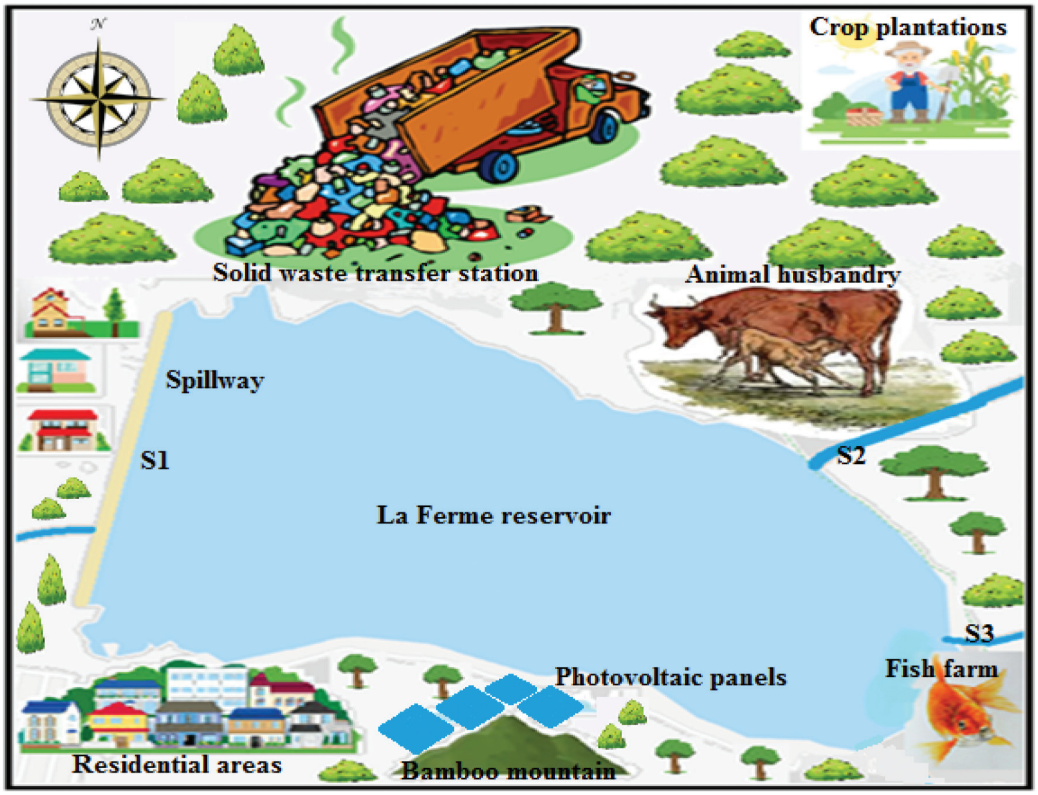

Figure 1: Simplified representation of land use and anthropogenic activities in the catchment area of *La Ferme* impounding reservoir (S1, S2 and S3 represent stations where sampling was conducted).

cylinder for 48 h in darkness. After sedimentation, the top 90% of the volume was siphoned off without disturbing the settled algae. The remainder was then shaken gently and a 1 mL sub-sample was transferred to the Sedgwick-Rafter counting chamber. The sample was left to settle for 30 min before counting with the 20x objective of a phase-contrast microscope (Euromex, Holland). The 40x objective was used for identification at the genus level on the basis of noticeable morphological features such as shape, motility, cell wall structure and colonial form. These features were compared with illustrations from standard identification keys to enable the accurate identification of algal cells (Prescott, 1954).

Toxicological Analysis

500 mL water samples were subjected to Reversed-Phase Solid-Phase Extraction (SPE) and Ultra-Performance Liquid Chromatography (UPLC) for detection of microcystin-LR (ISO 20179:2005), atrazine (Trajkovska et al., 2001) and diuron and tebuthiuron (EPA Method 532). A reversed-phase ACQUITY BEH-C18 UPLC column (2.1 × 100 mm, particle size 1.7 µm) (Waters Acquity, USA) was mounted and UPLC analyses were performed using a Waters Acquity system (Acquity™, Milford, MA, USA) coupled to a photodiode array (PDA) λ detector. Chromatographic gradient elution was conducted for microcystin-LR, atrazine and polyureas (diuron and tebuthiuron) using the mobile phases acetonitrile/aqueous trifluoroacetic acid, acetonitrile/water and 25 mM phosphate/acetonitrile, respectively. After the chromatographic separation, the detection was performed by UV absorbance at a wavelength of 220 nm (atrazine), 245 nm (diuron, tebuthiuron) and 238 nm (microcystin-LR), respectively. LC acquired data on retention time (s) and UV absorbance (nm) from target compounds was processed and quantified using the Waters MassLynx™ v4.1 software. Levels were recorded in milligrams per litre.

Statistical Analysis

All analyses were conducted in two replicates. Where appropriate, a Pearson's correlation was applied using Graph-Pad Prism 8 software (GraphPad, 2018) as a measure of the linear correlation between the concentration of algae and other water quality parameters. Statistical significance was attributed to $p < 0.05$.

Results

Variation in the physicochemical parameters of LF water is shown in Figure 2. Surface water temperature oscillated between 28.9 and 30.0°C throughout the study period with a mean of 29.4°C. Mean pH and dissolved oxygens were 8.67 and 9.97 mg/L, respectively. Over the study period, the level of total organic carbon (TOC) ranged from 2.2 to 9.2 ppm with a mean of 4.8 ppm (Figure 3). Water sampling for the enumeration of total cyanobacteria revealed a mean algal cell density as high as 63738 cells/mL at S1. The mean cell count for specific toxin-producing cyanobacterial species were 8100, 40959 and 4925 cells/mL for *Lyngbya, Oscillatoria* and *Microcystis*, respectively (Figure 4). A statistically significant and moderate positive correlation ($r = 0.5804$, $p < 0.05$) between TOC levels and cyanobacteria cell counts in LF was noted. During the 12-month period, the input of orthophosphate and nitrate from Trianon Grosses Roches (S2) and La Fenêtre (S3) into the LF reservoir fell in the range of 0.06–0.24 mg/L and 1.9 – 4.1 mg/L, respectively. Mean orthophosphate input from feeder Trianon Grosses Roches (S2) and La Fenêtre (S3) were 0.16 and 0.12, respectively, while their nitrate influx was 2.4 mg/L and 3.0 mg/L, respectively (Table 2). The orthophosphate concentration at the deepest point of the reservoir (S1) was found to exceed the safe limit of 0.05 mg/L on several occasions and even the mean orthophosphate concentration (0.07 mg/L) for the period of interest was higher than the safe limit. The average concentration of nitrate recorded at S1 was 0.1 mg/L, which also largely exceeded the minimum level (0.01 mg/L) necessary to support the growth of most algal species. Peaks in total algal cell count recorded in October (100500 cells/mL) and February (120000 cells/mL) were found to coincide with the occurrence of high orthophosphate levels of 0.09 and 0.21 mg/L, respectively (Figure 5). Pearson's correlation revealed a statistically significant and strong positive correlation ($r = 0.7862$, $p < 0.05$) between algal cell density and orthophosphate levels in LF during the 12-month study period. LF can thus be perceived as constantly under the threat of eutrophication due to its chronically high mean orthophosphate level compounded by regular influx from the feeding rivers (mean of 0.1 mg/L). Throughout the study period, the variation in the concentration of microcystin-LR closely followed the trend of blue-green algal cell density at the LF reservoir (Figure 6). The mean level of blue-green algae and microcystin-LR was found to be 63738 cells/mL and 0.3 µg/ML, respectively, thus posing a risk to human, animal and environmental health. With regard to herbicide residues, diuron, tebuthiuron and atrazine were detected in the waters at a mean concentration

Table 2: Mean annual levels of nutrient loading from feeder canals Trianon Grosses Roches and La Fenêtre

	Trianon Grosses Roches	*La Fenêtre*	*Guidelines level*
Mean annual level of orthophosphate (Reactive) loading (mg/L)	0.16	0.12	0.05 mg/L TP for streams discharging into a lake (MGG, 1998)
Mean annual level of nitrate loading (mg/L)	2.4	2.97	0.03 mg/L for streams discharging into a lake (Bellinger and Sigee, 2010)

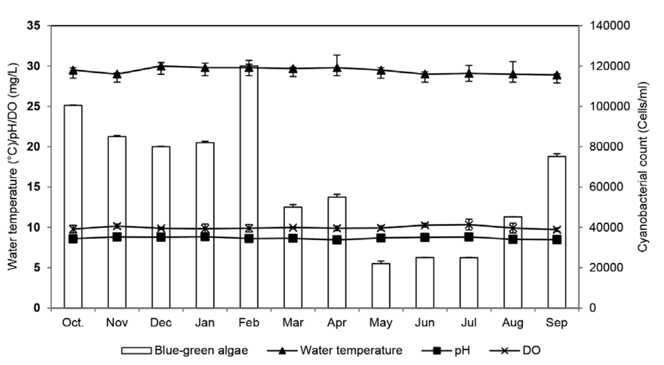

Figure 2: The effects of temperature, pH and DO level on blue-green algal cell density at LF reservoir.

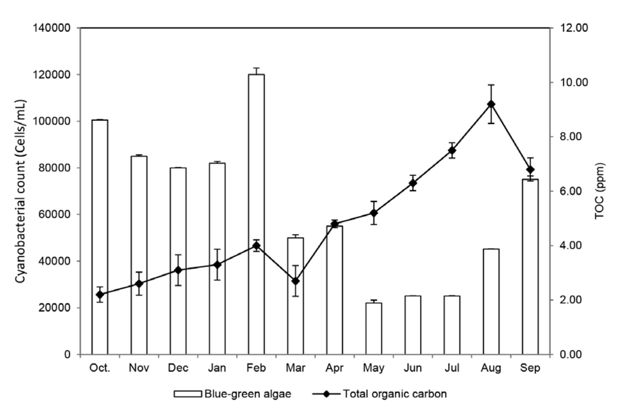

Figure 3: The relationship between TOC levels and cell counts of blue-green algae identified at LF reservoir.

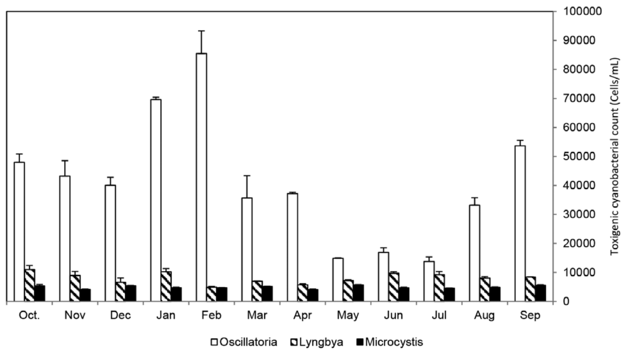

Figure 4: Trend in the level of three potentially toxigenic cyanobacterial species enumerated at LF reservoir.

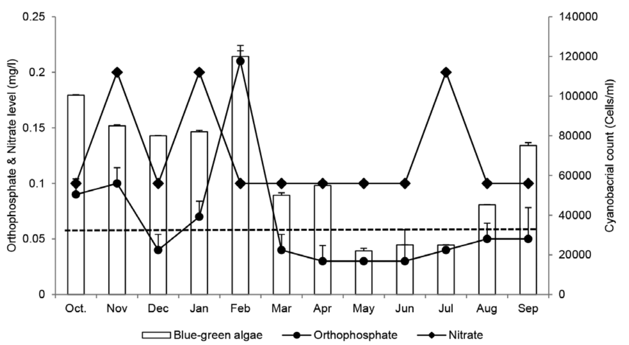

Figure 5: The relationship between nutrient levels and blue-green algae cell count at LF reservoir.

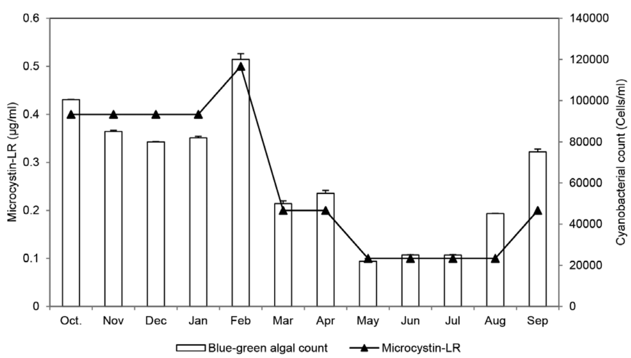

Figure 6: The relationship between blue-green algae cell density and Microcystin –LR levels at LF reservoir.

of 0.05, 0.15 and 0.05 mg/L, respectively, during the overall study period (Figure 7).

Discussion

Surface water temperature fell in the range of 28.9-30.0°C and it is thought to be highly conducive for the growth of certain mesophilic cyanobacterial species (Harada et al., 1999). Since a weakly alkaline pH prevailed in LF during the 12 months and since blue-

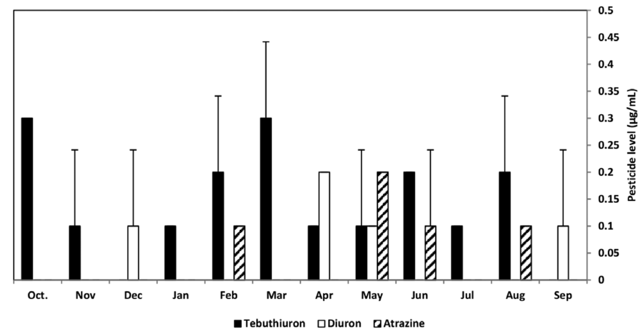

Figure 7: Trend in the level of pesticide contaminants detected in the water at LF reservoir.

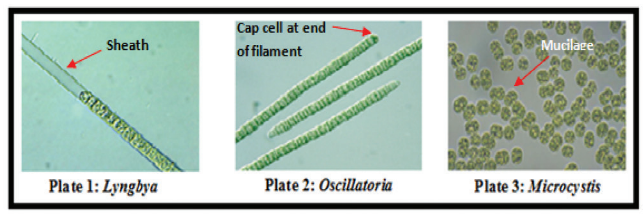

Figure 8: Colour plates of three blue-green algae species which are potentially toxigenic identified in LF impounding reservoir.

green algae are known to tolerate a broad pH range, the reservoir could support algal growth year-round (Bellinger and Sigee, 2015). The mean DO level of 9.97 mg/L recorded was indicative of oxygen saturation within the water column which could be attributed to prolific microalgal activity and photosynthesis. However, despite the prevalence of high DO levels, water bodies can occasionally become anoxic due to the decomposition of algal blooms by aerobic bacteria leading to the death of aquatic biota. Periodic fish mortality observed at LF could be attributed to a drop in DO levels due to aerobic decomposition of algal cells after a blooming event (Metcalf and Codd, 2014).

LF is thought to be under the constant threat of an algal bloom with a mean orthophosphate and nitrate influx of 0.14 and 2.7 mg/L, respectively, from its feeders. It is suspected that nutrient enrichment of these high-altitude feeders is related to run-off from agriculture, pasture lands and pollution. According to the Guidelines for Inland Surface Water Quality, the upper limit for phosphate levels of streams and rivers replenishing a lake is 0.05 mg/L (MGG, 1998). Moreover, the nitrate levels should not exceed 0.03 mg/L for streams, rivers and feeder canals discharging water into a lake (Bellinger and Sigee, 2015). Hence, we can infer that the mean nutrient influx of the feeders was above permissible levels. The annual average concentration of nitrate recorded at sampling station

S1 largely exceeded the minimum level (0.01 mg/L) necessary to support the growth of most algal species. With a mean nitrate level of 0.1 mg/L and an annual average influx of 2.7 mg/L from river waters, there is an impending risk of an algal bloom. The excessive level of nitrate could be attributed to over-fertilising which is known to reduce plants' ability to uptake nutrients and phosphates, leading to leaching of nutrient-rich run-offs into adjacent streams and rivers.

The mean orthophosphate level over the 12 months was found to be 0.07 mg/L. Natural levels of orthophosphate typically range from 0.005 to 0.05 mg/L and a higher level may trigger periodic excessive proliferation of algae in freshwater lakes (Kristiansen, 1996), leading to algal blooms. It is also possible that the high orthophosphate level observed in the months of October to February could have been attributed to periods of heavy rainfall. According to the MMS (2019), precipitation ranging as high as 153.2 -181.8 mm was registered during this period and could have resulted in nutrient-rich run-off from agricultural lands draining into the feeders.

Over the study period, the level of total organic carbon (TOC) ranged from 2.2 to 9.2 ppm. The periodic rise in TOC could be associated with pollution by soluble organic wastes leaching from nearby animal farms or other anthropogenic activities. According to Palmer (1969), the presence of soluble organic pollutants in freshwater bodies tends to influence cyanobacterial flora. Algae proliferation and TOC levels above 4 ppm could likely produce elevated amounts of disinfection by-products such as carcinogenic trihalomethanes (THMs) in the drinking water distribution system (Cox, 1965).

During the overall study period, the total cyanobacterial cell count ranged from 22, 000 to 120, 000 cells/mL. According to the WHO (2020), a population density of cyanobacteria exceeding 100,000 cells/mL of water can have a long-term deleterious effect on human health (Harada et al., 1999). The plates of the three specific genera of potentially toxigenic cyanobacteria are shown in Figure 8. Trichomes of *Lyngbya* were observed to be enclosed in a sheath and occurred as solitary cells or filamentous colonies. Trichomes of *Oscillatoria* lack a sheath and have gas vacuoles thus rendering them buoyant and enabling them to colonize the surface of water bodies. *Microcystis* is sub-spherical cells that also contain gas vacuoles and form dense colonies that are embedded in mucilage. *Microcystis* blooms have been reported to cause filter clogging at water-works and produce off-taste and off-odour in drinking water (Bellinger and Sigee, 2015).

The maximal cell count observed in February was also found to coincide with the peak in the level of microcystin-LR (0.5 µg/mL) registered in the same month. Microcystin-LR is a hepatotoxin produced by 40 species of cyanobacteria including *Microcystis*, *Oscillatoria* and *Lyngbya* which affect vital organs in humans upon exposure (Fawell et al., 1999). Microcystin-LR bears its name from the fact that leucine (L) and arginine (R) represent the two variable amino acids and is considered to be the most commonly encountered variant of the toxin (Fawell et al., 1999). WHO's current guidance on cyanotoxins in drinking water recommends a value not exceeding 1 µg/L (0.001 µg/mL) for microcystin-LR (free plus cell-bound) (Harada et al., 1999). The mean level of 0.3 µg/mL detected in the reservoir water indicates that the level of cyanotoxin was 300-fold higher than the guideline value. This points to its possible persistence in the aquatic ecosystem, bio-accumulation in the benthic fauna and eventual entry into the aquatic food chain. Actually, the toxicants are absorbed in zooplankton that forms the diet of several fish species in LF and subsequent uptake may lead to accumulation in muscle and fat tissues (Hardy et al., 2015). Thus, fishing at inland freshwater bodies could result in cases of human intoxication due to accidental ingestion of neurotoxins through consumption of fish contaminated with toxic microalgal species (Hardy et al., 2015).

In addition to cyanotoxin, traces of herbicides were also detected in the reservoir water. The herbicides were suspected to be percolating from agricultural lands, solid wastes transfer stations and areas of anthropogenic activities along the banks of rivers Trianon Grosses Roches and La Fenêtre. According to the US Environmental Protection Agency (US EPA, 2018), the Drinking Water Equivalent Level (DWEL) for diuron, tebuthiuron and atrazine are 0.1, 2.0 and 0.7 mg/L, respectively. Therefore, the levels of diuron (0.05 mg/L), tebuthiuron (0.15 mg/L) and atrazine (0.05 mg/L) in LF were within DWEL acceptable limits although there is a potential threat of their bioaccumulation in the aquatic food chain (Mahmood et al., 2016).

Conclusion

This study indicates that LF is under the constant threat of an algal bloom which may engender a degradation of its water safety and quality. The anthropogenic activities and land use developments within the catchment area of LF reservoir appear to be impacting adversely on the aquatic ecosystem of this natural resource. However, in order to ascertain the risks of bioaccumulation of cyanotoxins and herbicide residues, levels should be monitored in water and resident aquatic biota concurrently. In an attempt to sustainably conserve this resource and safeguard the water quality at LF, it is highly recommended to re-locate the solid waste transfer station and agricultural activities downstream surface watercourses. Moreover, water catchment areas of lakes should be reforested since trees are known to act as natural bio-filters against pollutant discharges into watercourses. An innovative water resource conservation strategy for LF would be the creation of artificial wetlands on feeders Trianon Grosses Roches and La Fenêtre absorb nutrients entering the reservoir and fully mature plants could be transformed into bio-fertiliser once composted.

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