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Full Length Research Paper

Monitoring of microcystin-LR in Luvuvhu River catchment: Implications for human health

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Cyanotoxins in surface drinking water sources are known to pose a threat to human health, of which microcystin-LR is the most investigated. The main aim of this study is to assess the levels of microcystin-LR in Luvuvhu River catchment and to assess the physicochemical parameters that may promote the growth of cyanobacteria. The level of microcystin-LR in some of the sampling sites was <0.18 µg/l except for one site (Luvuvhu River just before the confluence of Dzindi and Mvudi Rivers) which had a reading of 2 µg/l during August, 2009. Though the results indicated that some of the sites, especially on the Mvudi River system, had high nutrient levels, alkaline pH and water temperature <24°C, the levels of microcystin-LR were <0.18 µg/l. The production and release of microcystin-LR into water bodies by different strains of cyanobacteria involves a complex relationship between environmental variables. The water quality of the shallow hand dug wells and reservoir water were almost similar. The outflows had slightly high levels of nitrates and no soluble reactive phosphates in comparison with inflows, suggesting that the phosphates were being incorporated into the sediments. This could be a potential danger if the climatic conditions were to change and as this will promote the proliferation of nitrogen fixing cyanobacteria. Maybe, the non availability of phosphorous which is known to be a limiting nutrient in freshwater systems, could have contributed to no cyanobacteria blooms. Thus, the domestic consumption of these surface water sources may be a potential health hazard to the rural communities as it exposes the users to low levels of cyanotoxins over a long term.

Key words: Nutrient enrichment, microcystin, sediments, cyanobacteria.

INTRODUCTION

The enrichment of dams and lakes with nutrients is the major cause of eutrophication of freshwater sources. Nutrient enrichment is usually by nitrogen and phosphorus compounds, either from point sources such as the inflows of storm water drainage, industrial effluents, municipal wastewater and sewage effluents or non-points sources such as inorganic fertilizer runoffs and agricultural animal waste (WHO, 1999). In many countries throughout the world, the propagation of algae and cyanobacteria in surface water(s) sources such as reservoirs and rivers have an impact on the quality of drinking water from such sources, and South Africa is no exception. When there are massive growths of cyanobacteria in the river, cyanotoxins are usually produced. An occurrence of cyanobacterial blooms mostly appears in eutrophic freshwater systems. The most frequently occurring and wide spread cyanotoxins are microcystin-LR. They are mostly associated with *Microcystis* spp. which form scums in the water body (Mogakabe and Van Ginkel, 2008; Gumbo et al., 2008; 2010). Microcystin-LR are cyclic heptapeptides containing a specific amino acid. If a freshwater system contains microcystin-LR, it possesses a serious water quality problem which is a threat to human and animal health (Carmichael, 1994;

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Figure 1. (A) Water collection at Nandoni Dam (dug out well); (B) cooking a meal for the fishermen; (C) taking water for human consumption at home and (D) washing clothes.

Codd et al., 1997; Codd, 2000; Oberholster et al., 2009).

Nandoni Dam is a freshwater reservoir that was constructed to provide raw water for domestic use for urban areas of Makhado and Thohoyandou and the rural communities in the northern part of the Limpopo, from Malamulele and Lambani in the east to Sinthumule/Kutama in the west and alleviation of water shortages in Giyani (DWAF, 2004). The Nandoni Dam receives inflows from Luvuvhu River (water releases from Albasini dam) and its tributaries of Mambedi, Latonyanda and Mvudi. The water quality of the Mvudi River has over the years deteriorated due to nutrients enrichment from Thohoyandou sewage treatment plant (Mungufhala, 2004). The local communities surrounding the Luvuvhu River catchment have utilized the surface waters for bathing, swimming, cooking and washing clothes (Figure 1) and this may expose these communities to microbial poor waters (DWAF, 1996c; Mailula, 2010). This is due to erratic supplies of municipal water for human consumption.

The major objective of the study is to monitor the levels of microcystin-LR in surface water sources of the Luvuvhu River catchment. The specific objectives were to assess the levels of the microcystin-LR in Luvuvhu River catchment; to determine efficiency of riverbank filtration in microcystin-LR removal in the shallow hand dug wells and to assess the levels of physico-chemical parameters that may promote the growth of cyanobacteria.

MATERIALS AND METHODS

Sampling points

Samples were collected from eight sites along Luvuvhu River and its tributaries. Table 1 shows the sampling points, a description and Global Positioning System (GPS) coordinates.

Sampling methods and analysis

Water samples were collected monthly from July to December, 2009. Water samples were collected in two different sampling bottles. For microcystin-LR analysis, water samples were collected in dark brown 100 ml glass bottles and were transported in a cooler box to Rand Water laboratories (SANAS accredited) for analyses of microcystin-LR.

Onsite measurements were pH and temperature and these are analysed using a pH meter (Multi 340! /set Instruments, Germany) supplied by Wissenschaftlich-Technische werkstatten GMBH. It was first calibrated according to the manufacturer's guidelines using pH buffers of four and seven. For phosphates and nitrates, the water samples were collected in 250 ml colorless plastic bottles. All chemical analyses were conducted in triplicate. The Ion Chromatography Metrohm 850 Professional (Metrohmn) equipped with air-actuated 8 port injection valve with one sample loop (20 ul) was used to determine the following anions: Cl^-; Br^-; NO_3^-; NO_2^-; PO_4^{3-} and SO_4^{2-}. The eluent mixture was then prepared and used according to the manufacturer Metrohmn protocol. The eluent solution was then degassed. The anion standard solutions were prepared according to procedures outlined in Standard Methods for the Examination of Water and Wastewater (APHA, 2008). Dilute working solutions were prepared daily. For quality control purposes,

Table 1. Description of the sampling points.

Site no.	Site name	Description	GPS position
1	Mvudi River	Upstream of Thohoyandou sewage plant	S22°59' 37.5'' E030°27' 59.3"
2	Mvudi River	Downstream of Thohoyandou sewage plant	S23°00' 10.4'' E030°28' 46.0''
3	Dzindi River	River that join Mvudi River	S23°00' 37.9'' E030°28' 37.2"
4	Hand dug well 1	A shallow well which is approximately 30 cm deep and 1.5 m to the edge of Nandoni lake water	S23°00' 11.3'' E030°31' 5.8''
5	Nandoni lake water	Nandoni lake water is close to the hand dug well	S23°00' 1.6'' E030°30' 59.5''
6	Hand dug well 2	A shallow well which is approximately 30 cm deep and 1.5 m to the edge of Nandoni lake water	S23°00' 12.5'' E030°30' 58.9''
7	Luvuvhu River	Just before the confluence of Dzindi and Mvudi rivers	S23°00' 37.9'' E030°31' 0.5''
8	Luvuvhu River	Downstream of Nandoni Dam well	S23°58' 19.4'' E030°36' 10.9''

a known check standard was also analyzed to ensure accuracy of results.

Data analysis

The measurements were conducted in triplicates and the standard deviation and the mean were calculated, using Microsoft (MS) Excel (2003) spread sheet for each sampling point. The graphs were plotted using MS Excel spread sheet.

RESULTS AND DISCUSSION

Selection of sampling sites

The study area was the Luvuvhu River catchment which comprised of the Nandoni Dam (main reservoir) with a main inflowing streams, hand dug wells and outflows from the dam. A total of five river sampling sites, two shallow hand dug wells and one in-reservoir sampling site was chosen for the investigation (Figure 2). Each of the sampling site was selected to give a clear indication of the quality of water entering the reservoir, the quality of water in the reservoir prior to discharge, the quality of water in shallow hand dug wells that are located close to the reservoir and the quality of water discharged for downstream water users.

The effect of physico-chemical parameters on the production of microcystin-LR: Mvudi and Dzindi Rivers

The Mvudi River sampling points were selected to assess the quality of water before and after the Thohoyandou discharge point prior to joining Dzindi River. The physicochemical conditions measured were pH, water temperature, inorganic nitrates and soluble reactive phosphates (Figure 3).

The pH was alkaline for most of the study period and the water temperature was slightly cooler for site 2 in comparison to sites 1 and 3 (Figure 3A). The water temperature increased from July to December as expected since December is time for rains and high temperatures (Figures 3A and C). The range of water temperature changes were below 24°C. There was a slight difference in temperature change between sites. This may be attributed to the discharge of treated effluent from Thohoyandou sewage treatment plant close to site 2. Thermal increase in sampling site 3 may be attributed to the lack of vegetation (tree cover) at Dzindi Bridge. The water temperature at these sites may encourage the incipient growth of cyanobacteria which was below the 25 - 35°C range which is the optimum temperature for cyanobacteria growth (Howard and Easthope, 2002).

The pH values at the sites were alkaline (all above pH = 7.00) except in November (Figures 3A and C). The high pH at both sites can be attributed to the geological formation of Sibasa basalt in the area (SRR, 2001). The cyanobacteria usually grow within a pH range of 7.5 to 9.0 (Howard and Easthope, 2002). The pH levels in both sites were found to be within the range that promote algae cyanobacteria and remains within the South Africa water quality standard limits (6.0 to 9.0) (DWAF, 1993).

As expected, there were high levels of nutrients (soluble reactive phosphorus and nitrates) at site 2 in comparison to site 1 (Figure 3B). There was a difference in nitrate concentrations between sampling sites 1 and 2. The possible source of nitrate in site 1 was due to crop farming at "JJ" Bridge upstream of site 1. The source of nitrate in site 2 may be attributed to the discharge of

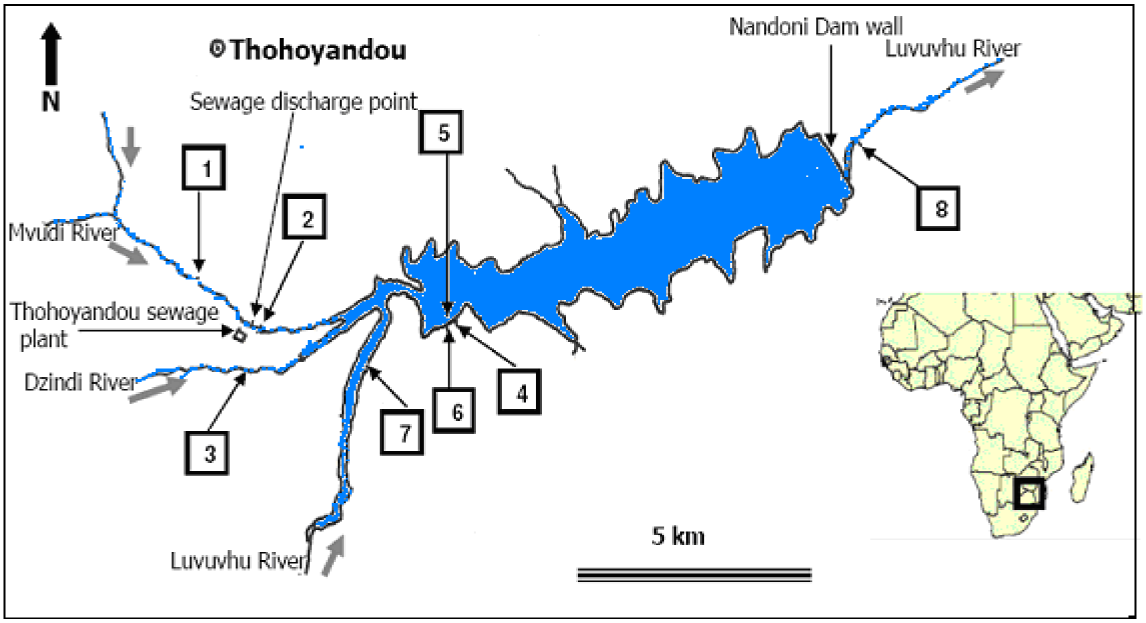

Figure 2. Location of Luvuvhu River catchment.

treated effluent from Thohoyandou sewage treatment plant close to site 2. The nitrate in site 3 may be attributed to the use of commercial fertilizers in the subsistence farming going on around the area (Figure 3D). The nitrate level in sampling site 2 exceeded 6 mg/l DWAF guideline for domestic waters. The human consumption of such a water source may induce methaemoglobinaemia in infants (DWAF, 1996a). The nitrate level of 0.1 mg/l may promote the growth of cyanobacteria (WHO, 1999).

There were no soluble reactive phosphate levels detected at the sampling sites 1 and 3 (Figure 3B and D). Phosphates were detected at sampling site 2 (downstream of Thohoyandou sewage plant) (Figure 3B). Maybe, the discharge of treated effluent from Thohoyandou sewage plant was properly treated within those two months. Thereafter, there were high levels of phosphate and this may be attributed to the discharge of treated effluent from Thohoyandou sewage plant close to sampling site 2. The study of Oberholster et al. (2009) showed that soluble reactive phosphates of 50 µg/l were sufficient to promote the growth of cyanobacteria; but it was less than 20 ppm as detected by Munungufhala (2004) from final effluent. There were trace levels of microcystin-LR (<0.18µg/l) that were detected in the sampling sites.

The effect of physico-chemical parameters on production of microcystin-LR: Shallow hand dug wells and main reservoir water

The water quality of the main reservoir and two shallow hand dug wells was compared to determine if there was any relationship. The hand dug wells were located around 1.5 m from shoreline of the reservoir but this distance varied according to the water inflows to the reservoir. The pH range for the shallow hand dug wells and reservoir were alkaline for most part of the year (Table 2). No water sample was collected in sampling site 4 during September as the shallow hand dug well was dry and also during December as the shallow hand dug well was flooded by an overflow of Nandoni reservoir water. The mean pH values for Nandoni reservoir water ranged from 7.08 to 8.43. This pH is within the recommended limit of aquatic ecosystem (DWAF, 1996c). The cyanobacteria usually strive within a pH range of 7.5 to 9.0 (Howard and Easthope, 2002).

The water temperature for these water sources were similar and were well below 22°C. There was a slight difference in temperature change between the two hand dug wells and reservoir water. The two shallow hand dug wells (sites 4 and 6) were exposed to sunlight; may be, this contributed to high temperature in hand dug wells. The low temperature at Nandoni lake water may be due to cooler water that flowed from Mvudi River that was discharged from Thohoyandou sewage plant. The water temperature in these sampling sites did promote the incipient growth of cyanobacteria as indicated by the trace levels of microcystin-LR (<0.18 µg/l).

The levels of inorganic nitrates in sampling sites 4 and 6 were 1.77 and 0.70 mg/l and were higher than the reservoir (0.23 mg/l). We would expect the levels of nitrates to be lower in hand dug wells when compared to the reservoir water due to riverbank filtration when checking the distance between lake water and hand dug

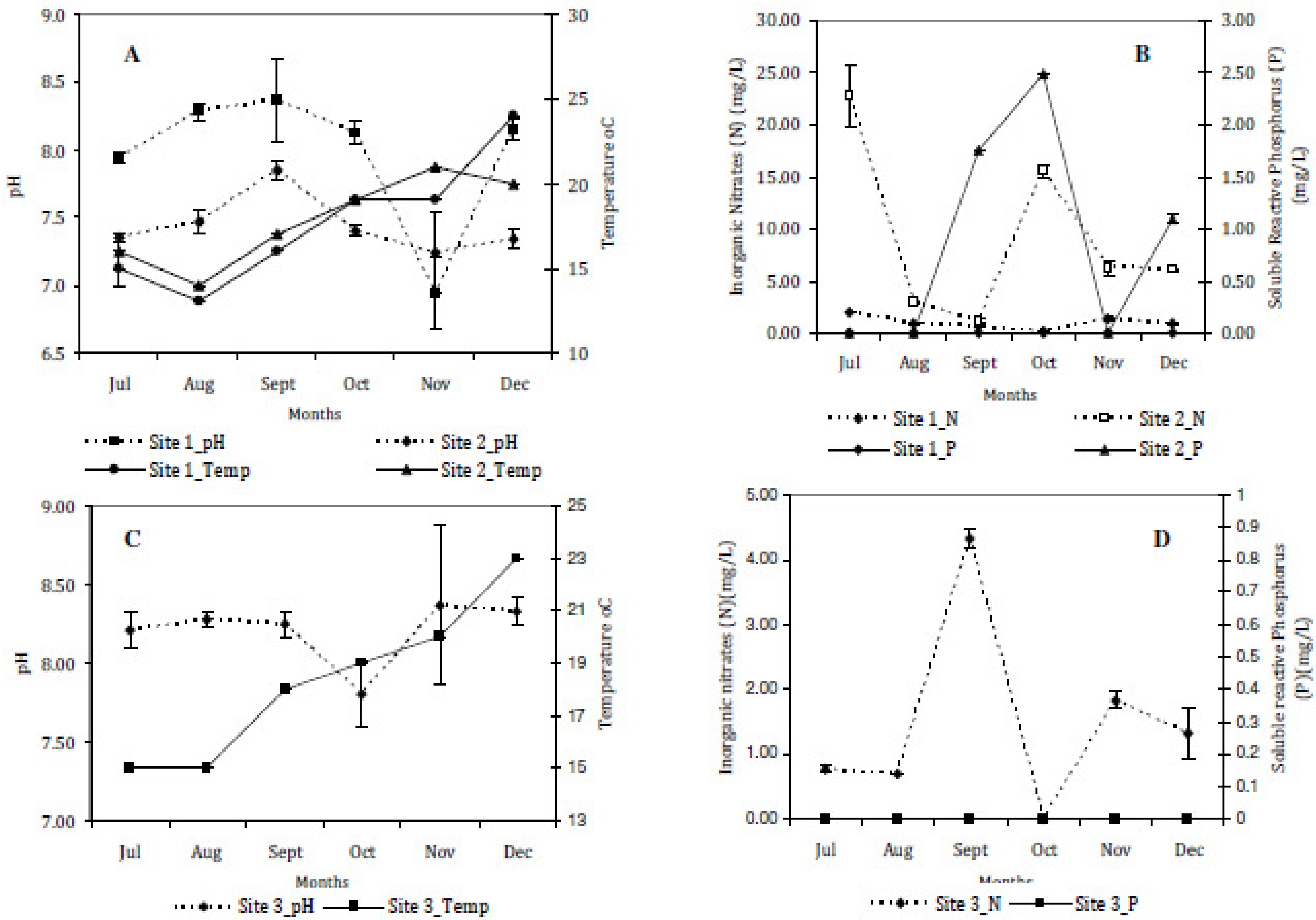

Figure 3. Physico-chemical parameters for sites 1 to 3: (A) Site 1 and 2: pH and temperature; (B) site 1 and 2: inorganic nitrates and soluble reactive phosphates; (C) site 3: pH and temperature and (D) site 3: inorganic nitrates and soluble reactive phosphates.

Table 2. Physicochemical characteristics and levels of microcystin-LR in the main reservoir and shallow hand dug wells.

Physicochemical parameter	Sampling site 4	Sampling site 5	Sampling site 6
pH	6.70 - 8.49	7.08 - 8.43	7.38 - 8.19
Water temperature (℃)	19 - 21	19 - 20	15 - 22
Inorganic nitrates (mg/l)	0.00 - 1.77	0.00 - 0.26	0.00 - 1.17
Soluble phosphates (mg/l)	0.00	0.00	0.00
Microcystin-LR (µg/l)	<0.18	<0.18	<0.18

Figure 4. Hand dug well.

wells (Figure 4). But since the results are indicating otherwise (vise-versa), the assumption is that the area or the surrounding area might have been used for agricultural purposes before brick making started, hence the relatively high level of nitrates in the hand dug wells. There were remarkable differences in nitrate values between two hand dug wells and reservoir water. The possible explanation to the low level of nitrates in sampling site 5 may be attributed to nitrate being adsorbed onto sediments and dilution effect as water was shallow and stored (Zamxaka et al., 2004). There were no phosphates and microcystin-LR detected in the hand dug wells and reservoir water. Since the nitrate levels for the sampling sites were above 0.1 mg/l, this suggests that the nitrates levels in the two sampling sites can sustain the growth of cyanobacteria (WHO, 1999).

The effect of physico-chemical parameters on production of microcystin-LR: Luvuvhu River and main reservoir

The water quality of Luvuvhu River (upstream and downstream of the dam wall) and the main reservoir was compared to determine if there was any relationship. The pH values at sampling sites were alkaline and were all above pH = 7.00 (Table 3). The cyanobacteria usually form within a pH range of 7.5 to 9.0 (Howard and Easthope, 2002). This pH is within the recommended limit for aquatic ecosystem (DWAF, 1996c). There were similar ranges in water temperatures between sampling sites (Table 3).

The level of inorganic nitrates in all the sampling sites varied with low levels being detected in the in-reservoir water and close to 2 mg/l for the outflows from the reservoir (Table 3). The source of nitrates in the Luvuvhu River (site 7) may be attributed to the agricultural activities that are occurring upstream such as the Levubu commercial farms and subsistence farming at Tshakhuma communal areas (drained by the Latonyanda River). In the upstream of the Levubu commercial areas, there is the Albasini Dam which provide raw water supply to the Levubu Irrigation Scheme (eWisa, 2008). Over the years, the dam has received considerable amounts of agricultural runoffs rich in nutrients: nitrates (range from 1.16 to 6.65 mg/l) and nitrites (0.14 to 0.17 mg/l) and traces of

Table 3. Physicochemical characteristics and levels of microcystin-LR in Luvuvhu River and main reservoir.

Physicochemical parameter	Sampling site 7 (upstream)	Sampling site 5 (in reservoir)	Sampling site 8 (downstream)
pH	7.14 - 8.68	7.08 - 8.43	7.85 - 8.43
Water temperature (℃)	19 - 21	19 - 20	19 - 21
Inorganic nitrates (mg/l)	0.00 - 1.73	0.00 - 0.26	0.00 - 1.93
Soluble phosphates (mg/l)	0.00	0.00	0.00
Microcystin-LR (µg/l)	2.00	<0.18	<0.18

phosphates (Mamali, 2006; Alukhwathi, 2007). There were no phosphates detected in the Luvuvhu River and reservoir water (Table 3). The research findings are in agreement with the study of Alukhwathi (2007) who found traces of soluble reactive phosphates. The study of Vézie al. (2002) showed that there was nutrient limitation and the environmental conditions were favourable to the proliferation of non-toxic *Microcystis* strains over their toxic cousins. This may help explain the near absence of high levels of microcystin-LR in most of the surface water sources that were studied. The cyanobacteria in particular, *Microcystis* generally strive well in water temperatures between 15 and 30℃ (Van der Westhuizen and Eloff, 1985; Robarts and Zohary, 1987) but the water temperatures of 20℃ resulted in highest production and release of microcystins (Van der Westhuizen and Eloff, 1985).

However in August 2009, there were high levels of microcystin-LR detected at Luvuvhu River (upstream) in comparison to that in reservoir water and outflows (Table 3). This sample point is close to the confluence of Dzindi and Mvudi Rivers which bear high loads of nutrients (nitrates and phosphates). The water temperatures and the available nutrients of varying levels may promote the incipient growth of cyanobacteria as indicated by trace levels of microcystin-LR with the exception of sampling site 7.

The 1 µg/l is the safe limit suggested by the Word Health Organization for drinking and recreational water (WHO, 2004). Ueno et al. (1996) had advocated for even stricter guideline value for microcystin-LR of 0.1 µg/l due to potential liver damage since rural communities maybe consuming surface water containing the cyanotoxins at low levels over a period of time. The studies of Foss-Kankeu et al. (2008) also found that rural communities in the Limpopo region were exposed to low levels of these cyanotoxins such that the potential for long term liver damage was great (Falconer et al., 1983). The assumption is that the microcystin-LR were transported from the upstream of the river where there is intensive farming activity and a possibility of higher loads of nutrients supporting the growth of cyanobacteria.

Conclusion

Although low levels of microcystin-LR were detected in the Luvuvhu River catchment, we can conclude that there is a health risk from cyanotoxins posed to rural communities that may be consuming the surface water without any form of treatment.

The research findings indicated that even though water temperature, pH and inorganic nitrate levels were within the range that promotes the growth of cyanobacteria, the near absence of soluble reactive phosphates maybe a limiting factor. Furthermore, there may be biodegradation of algal toxins either by bacteria and or by aquatic macrophytes. It is the latter that may be of interest in further research since Luvuvhu River catchment is home to some of the aquatic macrophytes. Also, the low levels of soluble reactive phosphates in the outflows may indicate that some of them might be going into sediments in the reservoir, thus requiring further research to verify the status quo.

It is also strongly recommended that there should be a regular monitoring of cyanotoxins including identifying the cyanobacteria that is responsible since Nandoni dam is a raw water supply for Vhembe and Mopani districts.

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