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Diversity of Algae and Potentially Toxic Cyanobacteria in a River Receiving Treated Sewage Effluent: A case of Notwane River (Gaborone, Botswana)

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Abstract

Algae and cyanobacteria are important primary producers and can be used to indicate the health status of a water body. The aim of this study was to investigate eutrophication levels of a river receiving sewage effluent, to determine the diversity of algae and cyanobacteria species and to identify species with a potential for toxicity. This study showed that the average phosphates level in Notwane River were 1.45mg/L which was slightly above the 1 mg/L recommended environmental guideline by EPA, while the average amount of nitrates in Notwane River was 5.53mg/L which was within the 10mg/L acceptable limit. 33 species of 29 genera representing four phyla; Chlorophyta, Cyanophyta, Euglenophyta and Bacillariophyta were identified. Although dense blooms of cyanobacterial species whose crude extracts were found to have toxic effects on mice were found at the wastewater stabilisation ponds dumping the sewage effluent, only a few colonies of Microcystis spp. were found at the river. These attributes gave an indication that the river, during the study period, was a relatively healthy river.

Keywords: Algae, potentially toxic cyanobacteria, sewage effluent.

Introduction

Botswana being a semi-arid and arid country with very little surface water, the few water bodies available provides the only form of surface water for humans, livestock and wildlife[1]. Notwane River is the most important river in the south-eastern Botswana, as about one-third of Botswana's population reside in its basin and depend on this river for domestic use and watering livestock[2]. This river is currently providing irrigation water for a number of horticultural farms that have arisen alongside it.

After treatment of wastewater from Glen Valley wastewater treatment plant in Gaborone, Botswana, the effluents are channelled into the old Broadhurst and Phakalane wastewater stabilization ponds before discharge into the Notwane River. These effluents are normally loaded with nutrients such as nitrates and phosphates which cause eutrophication within the stabilization ponds and the river itself. Studies done on the efficiency of Glen valley wastewater treatment plant by Nkegbe *et al*[3] showed that high amounts of phosphates and nitrates were discharged into the Broadhurst and Phakalane stabilization ponds and subsequently into Notwane River. The enrichment of the effluent accompanied with other environmental parameters such as changes in water temperature, increase in pH, low light intensity and high water retention times in the ponds promote the growth of algae and cyanobacteria[4- 8]. Cyanobacteria have a potential to reproduce into large populations in water bodies leading to formation of cyanobacterial blooms [9] which may be toxic to humans and animals[10-14]. Siame *et al*[15] observed blooms in Notwane River and Moswakgalagadi[16] observed blooms in Phakalane stabilization ponds, dominated by a cyanobacterium *Microcystis aeruginosa* and other species such as spirulina, oscillatoria and anabaena.

Since algae are important primary producers in aquatic environments, they can be used to indicate the health status of a water body. In this study, diversity of algae and cyanobacteria species was determined at the Phakalane wastewater stabilization ponds and Notwane River, and those that had the potential for toxicity identified.

Sampling Site: Glen valley (S 24^0.34′.94mins, E 25^0.58′.82mins, 967) is a peri-urban area located 15km northeast of Gaborone, Botswana with an area of around 234 hectares, land whose surroundings are primarily residential, recreational and open space. The phakalane wastewater stabilization ponds are situated in the Glen valley and consist of a series of three large ponds that receive over 70 hectares of treated wastewater from the Glen valley wastewater treatment plant. They act as the second series of maturation ponds after the Broadhurst wastewater stabilization ponds used in the tertiary treatment of the wastewater effluents.

Notwane River (S 24^0.34′.93mins, E 25^0.58′.86mins, 966) is a major tributary of the Limpopo River. The river flows adjacent to Gaborone, the capital city of Botswana. The flow of Notwane River was altered when Gaborone dam was built to supply water to Gaborone city. Therefore, this river only has water flowing during seasons of high rainfall when the dam overflows; otherwise during periods of average rainfall, flow in the river consists almost entirely of wastewater effluent from the Glen valley wastewater treatment plant [2]. All effluent from the phakalane stabilization ponds pours into the riverbed and forms Notwane River which flows through Oodi village into Matebeleland.

Material and Methods

Composite samples were collected from three oxidation ponds at Phakalane and at six sites chosen randomly along Notwane River. Sampling was done between the months of March and September of 2009. Surface water was skimmed using a clean beaker and poured into a 1 litre pyrex bottle. In sites containing algal blooms, surface scum was skimmed off using a beaker or a sieve and delivered into a clean pyrex bottle.

Total nitrogen analysis was carried out using the ultraviolet spectrophotometric screen method and total phosphorus analysis was carried out using the Ascorbic acid method, both described in APHA[17].

Subsamples were drawn and microscopic examination for algal identification carried out using a Zeiss compound microscope with x10, x40 and x100 oculars. A Nikon camera was fitted onto the eyepiece to take photos for identification. Identification of the algal species was performed by observation of distinguishing morphological features cited in several standard keys and textbooks which included; Freshwater Algae, Their microscopic world explored[18], Key for Identification of freshwater algae common in water supplies and polluted waters[17], A beginner's guide to freshwater algae[19], and An illustrated guide to River Phytoplankton[20].

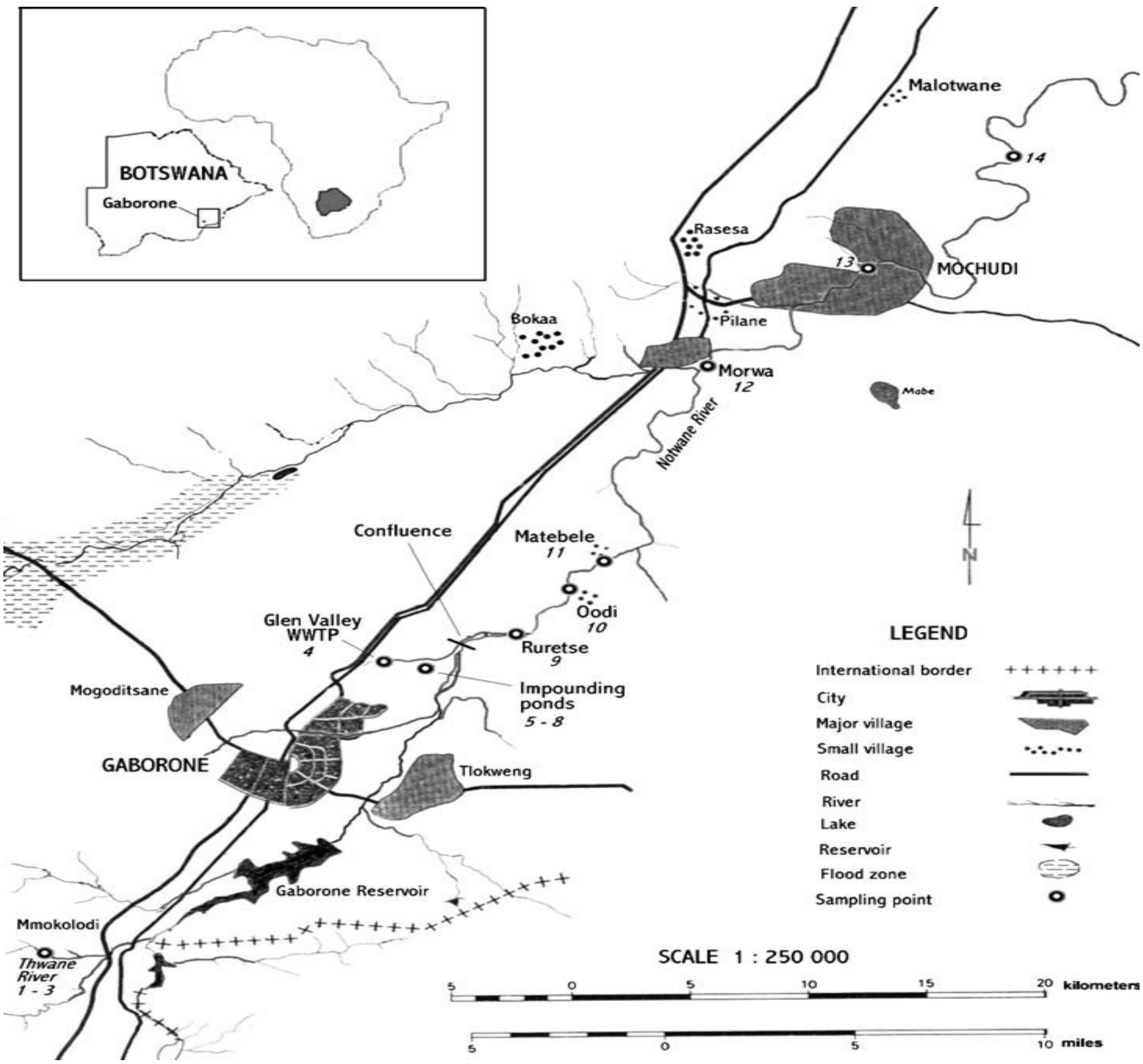


Figure-1
Map of Botswana and study site (adapted from Mladenov *et al*, 2005)

Toxicity analyses on samples found to contain species of cyanobacteria known to be potentially toxic were carried using the mouse toxicity test/mouse bioassay. The samples were freeze-thawed three times in a 24hr/6hr freeze/thaw cycle and then centrifuged at 10000rpm for 10 minutes. Thereafter, 2mL of the supernatant was intraperitoneally injected into mice (15-25g) and followed by a 24hr observation. A negative control that was injected with sterile distilled water and a positive control injected with a sample known to be hepatotoxic were used.

Results and Discussion

Table-1 shows the nutrient status of Phakalane stabilization ponds and Notwane River. At the Phakalane stabilization ponds, high levels of phosphates were recorded in March with the highest record being 3.68mg/L at PD3. The lowest levels were recorded in April with the least being 0.33mg/L at PD3. High levels of nitrates were recorded in July and August with the highest record being 10.69mg/L at PD3 in August. The lowest levels were recorded in April with the least being 1.21mg/L at PD3. Along Notwane River, high levels of phosphates were recorded in March and August. The highest recording was 3.86mg/L in August at ST1.The lowest level was 0.05mg/L and was recorded in April at ST2. High levels of nitrates were recorded in July and August with levels reaching over 10mg/L. The highest recording was 12.57mg/L in July at ST6. The lowest level was 1.24mg/L and was recorded in April at ST1. Low levels were recorded in March and April where most recordings were below 2mg/L.

This study showed that the average phosphates level in Notwane River were 1.45mg/L which was slightly above the 1 mg/L recommended environmental guideline by EPA. Mladenov *et al*[2] recorded an average of 0.93mg/L of phosphates in the Glen Valley Wastewater treatment plant effluent, 4.65mg/L in the oxidation ponds and 2.44mg/L of phosphates at Notwane River in Oodi. A weekly average of 6.82 mg/L of orthophosphates was discharged in the final effluent from Glen Valley wastewater treatment plant into the oxidation ponds and finally into the river [3]. On the other hand, Emongor and Ramolemana[1] stated an average of 3.8mg/L of total phosphorus in the Gaborone effluent.

The results of water quality monitoring from 1997 to 1999 recorded an average of 21.06mg/L of nitrates in the Glen Valley wastewater treatment plant effluent, 2.71mg/L in the oxidation ponds and 2.38mg/L along Notwane River at Oodi.[2]. In this study, the average amount of nitrates in Notwane River was 5.53mg/L. Nkegbe *et al*[3] recorded an average of 2.71mg/L in the effluent discharged from the treatment plant, while Emongor and Ramolemana[1] stated an average minimum concentration of 6.7mg/L. The proposed discharged limit to the environment is 10mg/L[21]. In Notwane River, the average amounts of nitrates were within the 10mg/L accepted limits. However, levels above 10mg/L were recorded in the months of July and August. The nitrate levels were therefore within the acceptable limits in March, April and June, but exceeded acceptable limits in July and August.

Algae are important primary producers in aquatic environments. They can also be used to indicate the health status of a water body. The species composition after microscopic examination and identification of algae and cyanobacteria is tabulated in table-2, and a pictorial representation is displayed in figure-3. Species recorded in the two study sites belonged to 33 species of 29 genera representing four phyla; Chlorophyta, Cyanophyta, Euglenophyta and Bacillariophyta. Chlorophyta or green algae were the most dominant class which accounted for 66.7% of the total number species identified. The common species were *Coelastrum* spp., *Oocystis* spp., *Scenedesmus* spp., *Chlorococcum* spp., *Chlorella* spp. and *Actinastrum* spp. The filamentous species were *Spyrogyra, Zygnema* and *Oedogonium*. These filamentous species would form blooms that would be pushed to the edges by action of wind thus forming thick scum. The green algae were mostly found in the Notwane River and a few species like *Actinastrum and Chlorella* were found in one oxidation pond.

Table-1

Amounts of Nitrates and Phosphates in mg/L at the Phakalane wastewater stabilization ponds and along Notwane River

Sample	March		April		June		July		August	
	P	N	P	N	P	N	P	N	P	N
ST1	1.99	1.58	0.06	1.24	0.64	2.97	0.7	7.09	3.86	7.01
ST2	3.01	1.6	0.05	1.32	0.54	3.5	0.8	11.28	2.27	11.02
ST3	3.14	3.9	0.37	2.94	0.17	2.64	0.27	10.87	1.91	10.29
ST4	3.68	1.64	0.63	1.41	0.63	2.97	0.91	12.47	2.82	11.23
ST5	2.91	1.62	0.78	1.51	0.62	2.75	0.77	11.26	2.29	9.42
ST6	2.64	3.06	0.76	2.01	0.62	2.88	0.44	12.57	3.25	9.92
PD1	2.14	7.59	0.37	3.94	1.5	6.99	0.66	7.09	2.92	8.3
PD2	3.48	2.63	0.38	2.21	0.66	4.72	0.66	4.89	2.91	8.44
PD3	3.68	1.76	0.33	1.21	0.65	2.61	0.78	10.69	2.25	6.97

(ST1-ST6 are sampling sites along Notwane river while PD1-PD3 are the three Phakalane stabilization ponds)

Table-2
Species of Algae identified in water samples from Phakalane oxidation ponds and Notwane River

Class	Species
Chlorophyta	*Actinastrum hantzshii* (Lag.) *Apatococcus labatus* (Chodat) *Coelastrum cambricum* (Archer) *Coelastrum microporum* (Nag.) *Chlamydomonas polypyrenoideum* (Presc.) *Chlorella* spp. *Chlorococcum humicola* (Nag.) *Closterium parvulum* (West) *Cyclotella* spp. *Eudorina elegans* (Her) *Eucapsis alpina* (Clements and Shantz) *Micractinium pusillum* *Oedogonium* spp. *Oocystis eremosphaeria* (Smith G. M) *Pandorina morum* (Bory) *Pediastrum boryanum* (Turp.) *Scenedesmus incrassatulus* (Smith G. M) *Scenedesmus quadricauda* (Turp.) *Sphaerocystis shroeteri* (Chodat) *Spyrogyra aequinoctialis* (West) *Volvox tertius* (Meyen) *Zygnema* spp.
Cyanophyta	*Merismopaedia elegans* (Smith G.M) **Microcystis aeruginosa* (Kurtz) **Microcystis flos aquae* (Kirch), *Spirulina* spp. **Oscillatoria* spp.
Euglenophyta	*Euglena* spp.
Bacillariophyta	*Navicula* spp. *Nitzshia bilobata* (Smith) *Pinnularia borealis* (Ehr) *Stauroneis parvula* (Grun) *Stephanodiscus niagarae* (Fhr)

*indicates species with a potential to produce toxins[23]

Figure-2
(a) Scum comprising of *Sphaerocystis shroeteri, Zygnema* spp., and *Oocystis* spp. (b) Bloom with *Chlorococcum humicola* and *Euglena* spp. observed at Notwane River

Cyanophyta or blue-green algae group was the second most abundant group which contributed 15.1% of all algal species recorded in this study. This group was represented by the following species; *Merismopaedia elegans, Microcystis aeruginosa, Spirulina* spp., *Microcystis flos aquae*, and *Oscillatoria* spp. The *Microcystis aeruginosa, Microcystis flos aquae* (subsequently named *Microcystis* spp.) were the most abundant as they occurred in the Phakalane oxidation ponds throughout the study period. They formed blooms that were so dense such that they formed foul smelling scum on the surfaces of the water. Although dense blooms of cyanobacterial species were found at the Phakalane ponds, only a few colonies of *Microcystis* spp. were found at Notwane River.

Bacillariophyta group, also known as the diatoms was third with 15.1% of the total number of species. Species that were identified that representing this group included: *Navicula* spp., *Nitzshia bilobata*, *Pinnularia borealis*, *Stauroneis parvula*, and *Stephanodiscus niagarae.* The most common were *Nitzshia bilobata, Navicula* spp. and *Stauroneis parvula*, which occurred in almost all the sampling points along Notwane River throughout the study period. However, the members of this group were found in only one of the oxidation ponds.

Species with a potential to produce toxins were also identified. These included *Microcystis aeruginosa* (Kurtz), *Microcystis flos aquae* (Kirch) and *Oscillatoria* spp. However, only a few cells of *Oscillatoria* spp. were observed and therefore only samples dominated by *Microcystis* species were used for toxicity screening using mice. Cyanobacterial blooms were found at the Phakalane wastewater stabilization ponds throughout the study period but none were found along Notwane River. The bloom formation could be as a result of the nutrient-rich effluent from the Glen Valley Wastewater treatment plant that is received by the oxidation ponds[3,17]. Municipal and industrial wastewater effluents have been known to be ‘point’ sources that cause eutrophication of water bodies[22].

This study showed that there was a high diversity of algal species along Notwane River as compared to the Phakalane wastewater stabilization ponds. Although dense blooms of cyanobacterial species, most of which are known to be toxigenic, were found at the Phakalane ponds, only a few colonies of *Microcystis* spp. were found at Notwane River. These attributes gave an indication that Notwane River, during the study period, was a relatively healthy river.

Crude extracts obtained from six samples of microcystis dominated blooms collected during this study showed hepatotoxic effects on mice and subsequent death between 4 and 24 hours after intraperitoneal injection. Signs of hepatotoxicosis included difficulties in breathing and loss of balance.

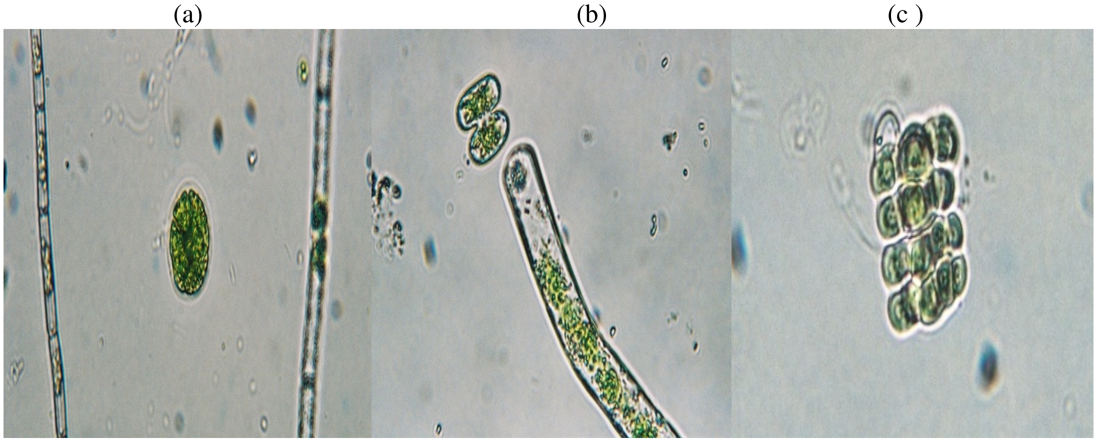
This study therefore gave an important contribution with respect to identity of cyanobacterial species that occur in Botswana that produce toxins and the tentative nature of the toxins. The toxic blooms posed a potential risk of cyanotoxin-poisoning both to humans and animals either through direct use of the water or through consumption of vegetables irrigated with water containing the blooms. However, more studies on Notwane River should be conducted to monitor the occurrence of harmful cyanobacterial blooms.

Table-3
Results of mouse toxicity testing

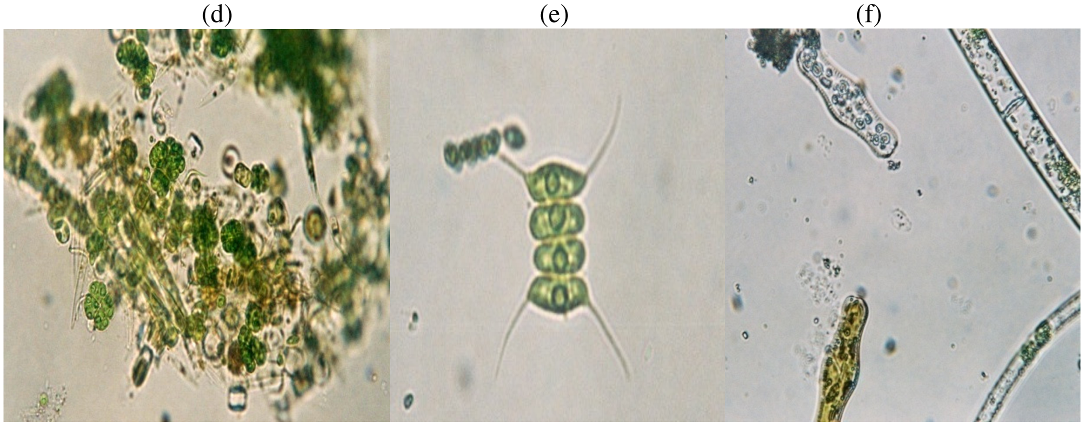
Sample	Weight of dry cells from crude extract	Average Weight of mouse in (g)	Time of Injection (2 mice per treatment)	Time of Death (before)	Average time to Death (after)
*A	0.03g/mL	17.9	0715hrs 0720hrs	0000hrs 0000hrs	16hrs
*B	0.032g/mL	16.7	0726hrs 0729hrs	0100hrs 0100hrs	18hrs
*C	0.028g/mL	22.1	0737hrs 0741hrs	0100hrs 0100hrs	18hrs
*D	0.035g/mL	15.1	0747hrs 0751hrs	1200hrs 1200hrs	4hrs
*E	0.029g/mL	15.2	0800hrs 0803hrs	0830hrs 0830hrs	24hrs
*F	0.13g/mL	24.5	0811hrs 0815hrs	0830hrs 0830hrs	24hrs
P-Control *G	0.03g/mL	18.1	0819hrs 0822hrs	0830hrs 0830hrs	24hrs
N-Control DH_20	-	25.1	0710hrs	Did not die	Did not die

*A(April-Pond1), B(June-Pond1), C(June-Pond2), D(July-Pond1), E(July-Pond2), and F(Aug-Pond1), G (2009-Broadhurst WWSP)

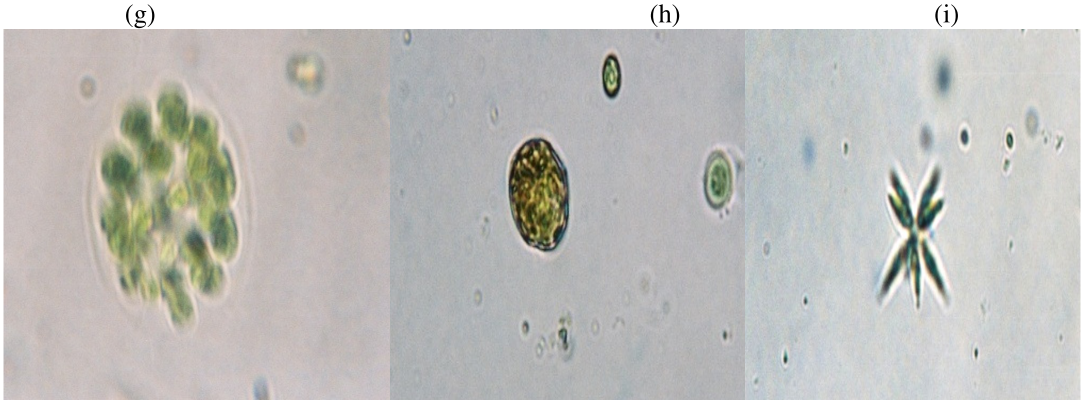
(a) (b) (c)



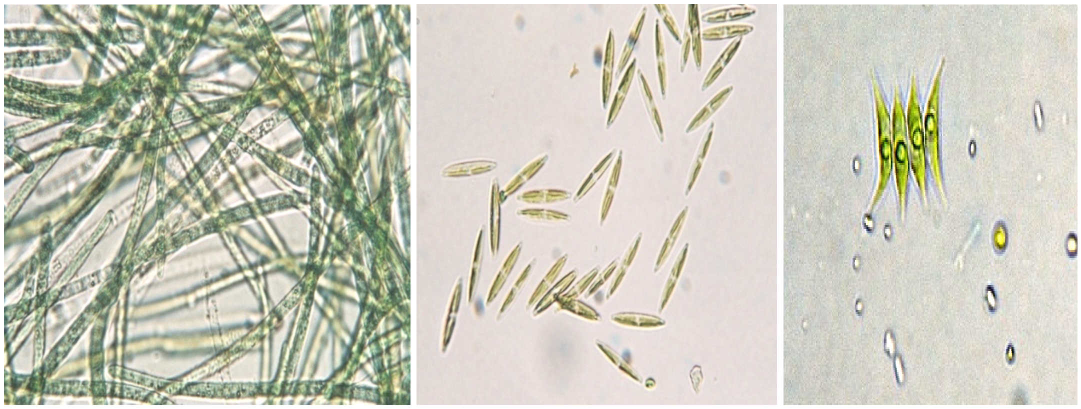
(d) (e) (f)



(g) (h) (i)



(j) (k) (l)



(m) (n) (o)

Figure-3

(a) *Microcystis aeruginosa* colonies (Kurtz), (b) *Spirulina* spp., (c) Filament of Zygnema, (d) *Pandorina morum* (Bory), (e) *Zygnema* spp., (f) *Eucapsis alpina* (Clements and Shantz), (g) Cells of *Apatococcus lobatus* (Chodat) (h) *Scenedesmus quadricauda,* (i) *Pinnularia borealis* (Ehr), (j) *Eudorina elegans* (Her), (k), (l) *Actinastrum hantzshii* (Lag), (m) Filaments of Oscillatoria, (n) *Nitzshia bilobata* (Smith), (o) *Scenedesmus incrassatulus* (Smith)

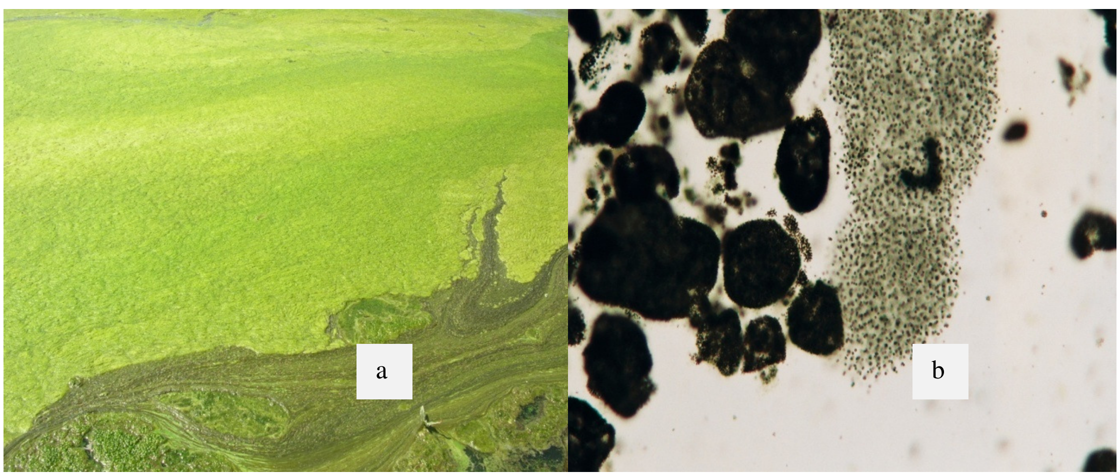


Figure-4

(a) A bloom of *Microcystis* spp. as observed in the oxidation ponds and (b) *Microcystis* colonies as viewed under the microscope

Conclusion

Notwane River is an important source of water in Botswana and therefore the quality of its water is significant. This study showed that there was a high diversity of algal species along Notwane River as compared to the Phakalane wastewater stabilization ponds. The average phosphates level in Notwane River was slightly above the recommended environmental guideline while the average nitrates level was within the acceptable limit.

Cyanobacterial blooms were found at the Phakalane wastewater stabilization ponds throughout the study period but none were found along Notwane River. Only a few colonies of *Microcystis* spp. were collected at the beginning of the study period. These attributes gave an indication that Notwane River, during the study period, was a relatively healthy river.

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