

## Unialgal Blooms of Cyanobacteria in Oxidation Ponds of the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Thailand

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### Abstract

Intense phytoplankton blooms often occur in the oxidation ponds of the Laem Phak Bia Environmental Research and Development Project (LERD Project) royally initiated by His Majesty the King in Petchburi Province, Thailand. It is of concern that the bloom phytoplankton species may produce toxins that can cause illness in both humans and wildlife. Therefore, the main objective of this study was to investigate the species composition and abundance of the phytoplankton that caused the blooms. We also determined environmental variables (water and sediment) that promoted rapid development and dense growth of phytoplankton from three oxidation ponds in March 2014. The results revealed that nutrient concentrations in all three ponds were exceptionally high (total phosphorus of  $2.9 \pm 0.8$  mg/L and total nitrogen of  $5.3 \pm 2.4$  mg/L). Furthermore, the blooms of phytoplankton were regulated mainly by nitrogen as a limiting factor. Environmental variables such as a high pH ( $8.8 \pm 0.6$ ) and dissolved oxygen ( $10.1 \pm 2$  mg/L), and low transparency ( $0.2 \pm 0.0$  m) indicated that the intense blooms (chlorophyll a concentration of  $260.6 \pm 97$  µg/L) occurred during this investigation. The results of the phytoplankton study showed that cyanobacteria were the major group of phytoplankton recorded. *Spirulina platensis* was the most abundant species (866,410 cells/L) and was composed of more than 90% of all phytoplankton abundances. *S. platensis* is a non-toxic producer and in fact has high nutritional value that may be beneficial to aquatic fauna. The internal nutrient sources from the sediment played a less important role in promoting the growth of phytoplankton since the levels of organic matter and nutrient contents were low.

**Keywords:** cyanobacteria; oxidation ponds; phytoplankton; Thailand; water quality

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### 1. Introduction

Oxidation ponds are one of the popular waste water treatment systems operating in Thailand. This sewage treatment method has a low cost of investment and construction as well as low operating costs compared to other systems. Oxidation ponds are also suitable for small communities, especially in areas where land prices are not expensive. The oxidation ponds are designed specifically to treat wastewater through the decomposition processes of organic matter (aerobic, facultative and anaerobic conditions) by bacteria, together with the biological process of phytoplankton that produces oxygen for the system and absorbs nutrients (Oswald *et al.*, 1953; Wrigley and Toerien, 1990; Nurdogan and Oswald, 1995; Hoffmann, 1998; Abdel-Raouf *et al.*, 2012).

The oxidation pond treatment system of the Laem Phak Bia Environmental Research and Development Project (LERD Project) initiated by His Majesty the King was established in 1991 in Petchburi Province,

Thailand (Laem Phak Bia Environmental Research and Development Project, 2008). The main purpose of this project is to treat municipal wastewater from Petchburi Province by using a series of five shallow ponds with a total wastewater storage capacity of 20,000 m<sup>3</sup>/day. The three main steps of treatment are as follows (Ketkaew *et al.*, 2012). Initially, wastewater is pumped to a sedimentation pond to remove suspended solids and then the wastewater flows under gravity to the next three oxidation ponds. The second step is the treatment process by microbial activities under aerobic, facultative and anaerobic conditions. The last step is polishing in a stabilizing pond as phytoplankton die out due to nutrient deficiencies before treated water is discharged to the environment. This full cycle occurs over a 21-day period with the wastewater spending seven days in each type of pond (Gerrard, 2010).

Phytoplankton usually blooms in the oxidation ponds of the LERD Project due to excessive nutrient contents. The tropical climate conditions in Thailand such as high light intensity and high temperature also

promote more frequent and intense phytoplankton blooms. The blooms of some phytoplankton groups such as cyanobacteria that produce toxins may pose problems to discharged areas if not properly managed (Barrington *et al.*, 2011). In Kenya, for example, it was found that cyanobacteria (*Microcystis* sp.) were occasionally present and dominant in the oxidation pond, and microcystin concentrations of up to 551.08 mg dry weight (DW) of cyanobacteria biomass were detected (Kotut *et al.*, 2010). It is suggested that wildlife species are potentially at risk of toxic explosion because they regularly drink water from the discharged ponds (Kotut *et al.*, 2010). A study in New Zealand also revealed that microcystins were detected in oxidation ponds at concentrations from 0.05 µg/L to 100 µg/L. The major species of cyanobacteria that was recorded were *Planktothrix mougeotii*, *Microcystis aeruginosa* and *Arthrospira* sp. (Wood *et al.*, 2006).

This study aimed to investigate the species composition and abundances of phytoplankton in three oxidation ponds of the LERD Project. We also determined environmental parameters (water and sediment) that were involved in the blooms of phytoplankton. The results will lead to the proper management and monitoring of high phytoplankton densities for the safety of wildlife at the discharge point and the health of the coastal environment downstream mainly mangrove forests.

## 2. Materials and Methods

### 2.1 Study site

Established in 1991, the King's royally initiated Laem Phak Bia Environmental Research and Development Project (LERD Project) is situated in Laem Phak Bia Sub-district, Ban Laem District, Petchburi Province, Thailand (UTM 1442240-1443480 N and 0617780-061927 E). The size of the project is approx. 7,500 hectares plus a 300 meter-width of natural mangrove forest along the muddy beach at the mouth of the Petchburi River (Jitthaisong *et al.*, 2012). The LERD Project has five waste water treatment ponds as shown in Table 1. The

wastewater treatment ponds receive about 7,000 m<sup>3</sup> of wastewater and sewage daily from Petchburi municipality (population 50,000 people). In this study, we investigated the water quality of three oxidation ponds (1, 2 and 3) at five sampling points in each pond.

### 2.2 Water sample collection and analysis

Collection of water samples in oxidation ponds 1, 2 and 3 of the LERD Project was conducted in summer in March 2014. There were five sampling points in each oxidation pond (one sampling point in each of the four corners and one sampling point in the center of the pond). Six parameters of water quality were measured on-site: temperature (°C), pH, conductivity (µS/cm), total dissolved solid (mg/L), dissolved oxygen (mg/L)-using a digital multi parameter analyzer (consort C933), salinity (ppt)-using a refractometer, and transparency (m)-using a Secchi disc. Water samples of two litres were also collected at approx. one m below the water surface at each sampling point. Water samples were collected in plastic bottles, stored in a cooler (4°C) and transported to the laboratory of the Department of Environmental Technology and Management, Kasetsart University, Bangkok, Thailand for chemical analysis. The collected water samples were tested for total nitrogen (TN; mg/L), total phosphorus (TP; mg/L) and chlorophyll a (µg/L) according to the Standard Methods for the Examination of Water and Wastewater. Concentrations of chlorophyll a were investigated by filtering 100 ml water samples through Whatman glass microfibre filters (GF/C, 47 mm) and then analyzing based on a standard acetone extraction method.

Studies of phytoplankton biodiversity and abundance were conducted using a phytoplankton net with a mesh size of 20 µm. Samples of five litres of surface water from each of the five sampling points in each oxidation pond were poured through the plankton net and then fixed with 70% ethanol and sent to the laboratory of the Department of Fishery Biology, Kasetsart University, Bangkok, Thailand for species identification and cell counting.

Table 1. Physical characteristics of wastewater treatment ponds of LERD Project

Ponds	Name	Depth (m)	Water surface area (m <sup>2</sup> )	Volume (m <sup>3</sup> )
1	Sedimentation pond	2.43	10,134	21,969
2	Oxidation pond 1	2.22	28,795	60,905
3	Oxidation pond 2	1.93	32,140	59,006
4	Oxidation pond 3	1.63	33,192	54,011
5	Polishing pond	1.41	42,878	59,154

Source: Jitthaisong *et al.* (2012)

### 2.3 Sediment sample collection and analysis

Sediment samples (five replicates) were taken from each of the five locations in the oxidation ponds 1, 2 and 3 in March 2014, using a plastic bucket (approx. depth 50 cm, diameter 30 cm). Sediment samples were air dried and then analyzed for pH, electrical conductivity (dS/cm), moisture content (%), organic matter content (%), total Kjeldahl nitrogen (%), total phosphorus (%) and soil texture at the Department of Soil Science, Kasetsart University, Bangkok, Thailand.

### 2.4 Data analysis

Values are reported throughout as mean±standard deviation and the number of samples measured. Regression analysis and coefficients of determination ( $R^2$ ) of Excel were used to determine relationships between nutrient concentrations and chlorophyll a. We also applied Primer 6 (Plymouth routines in multivariate ecological research) for multidimensional scaling analysis (MDS) of environmental variables among studied ponds together with cluster analysis that was used to determine species composition of phytoplankton. Prior to analysis, data were transformed by square root and standardized.

## 3. Results

### 3.1 Water quality

Most values of water quality measured from the three oxidation ponds (1, 2 and 3) were relatively comparable (Table 2). Overall, water quality was also homogenized within the pond as indicated by the result of cluster analysis of environmental variables (Fig. 1). The water temperature in all the oxidation ponds was rather high since measurement was carried

out from 10 am to 2 pm. Dissolved oxygen was also supersaturated with an average value from the three oxidation ponds of  $10.1\pm 2.0$  mg/L. Furthermore, all oxidation ponds had high values of pH, conductivity and total dissolved solids. Transparency varied slightly among the ponds and was only to about 0.20 m below water surface. Although the oxidation ponds are located near the shore of Petchburi Province, salinity does not appear to have any adverse effect on the water quality (salinity was not detectable). The results of nutrient concentration analysis revealed that total nitrogen and total phosphorus were exceptionally high, especially in oxidation pond 1 and lower other ponds (2 and 3). In addition, analysis of nutrient concentration corresponded well with the chlorophyll a concentration, which was highest in oxidation pond 1 with an average value of  $302.5\pm 159.5$  µg/L.

The TN/TP ratio was determined and the results revealed that all oxidation ponds were nitrogen limited to a similar level as indicated by values of the TN/TP ratio being lower than 10 ( $2.52\pm 1.07$  in oxidation pond 1,  $1.47\pm 0.62$  in oxidation pond 2 and  $1.62\pm 0.53$  in oxidation pond 3). Strong relationships between nutrients and chlorophyll a were also found. The values of coefficient of determination by regression analysis between TN and chlorophyll a and TP and chlorophyll a were 0.8677 and 0.9981, respectively.

The results of the sediment analysis showed that the pH values in all oxidation ponds were neutral. Overall, the organic matter content was low (less than 10%). Organic matter was highest in oxidation pond 1 and lower in oxidation ponds 2 and 3. Total Kjeldahl nitrogen was highest in oxidation pond 1, followed by oxidation ponds 2 and 3, respectively. Total phosphorus was relatively comparable among the 3 oxidation ponds. Sediment texture of oxidation pond 1 was clay loam, which was different from other ponds (2 and 3) that were sandy loam (Table 3).

Table 2. Water quality of oxidation ponds 1, 2 and 3 ( $n=5$ )

Parameters	Oxidation Pond 1	Oxidation Pond 2	Oxidation Pond 3
Temperature (°C)	32.2±1.8	32.9±0.8	33.0±0.5
Transparency (m)	0.23±0.06	0.23±0.03	0.22±0.03
Dissolved oxygen (mg/L)	9.7±1.8	11.0±0.9	9.6±2.9
pH	8.1±0.5	8.8±0.1	9.3±0.1
Conductivity (µS/cm)	660.6±20.4	768.6±6.5	904.8±37.6
Total dissolved solid (mg/L)	354.4±7.5	403.2±12.6	481.0±21.2
Salinity (ppt)	0	0	0
Total nitrogen (mg/L)	7.13±2.8	4.19±1.7	4.6±1.6
Total phosphorus (mg/L)	3.01±1.2	2.85±0.2	2.93±0.8
Chlorophyll a (µg/L)	302.5±159.5	219.78±43.3	259.6±36.47

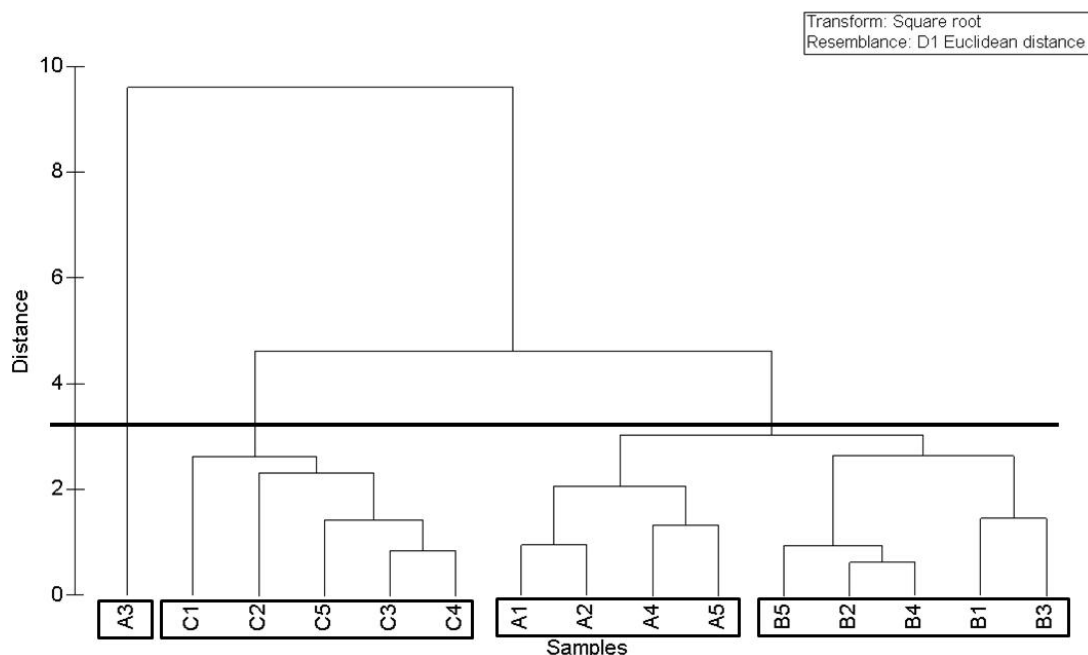


Figure 1. Dendrogram of cluster analysis using environmental variables differentiated water quality among ponds 1 (A), 2 (B) and 3 (C). Numbers (1, 2, 3, 4 and 5) in the figure represent sampling points.

The results of phytoplankton analysis showed a range of 19 to 26 species from three ponds (Table 4). The diversity of phytoplankton was highest in oxidation pond 1 (26 species) and lowest in oxidation pond 3 (19 species) (Table 4). Phytoplankton was present in large numbers. The highest density of phytoplankton was recorded in oxidation pond 3 and the phytoplankton densities were lower in oxidation ponds 2 and 1, respectively. It is also interesting that the major group (more than 90%) of phytoplankton present in all oxidation ponds was cyanobacteria and the only one dense species was *Spirulina platensis* (Table 5). In addition, the analysis of multidimensional scaling and cluster analysis indicated that groups of phytoplankton in pond 1 were rather distinct from ponds 2 and 3 (Fig. 2).

#### 4. Discussion

The results of the water quality analysis indicated that the nutrient concentrations in oxidation ponds were excessively high as has been reported in other studies (Ogunfowokan *et al.*, 2005; Kotut *et al.*, 2010). This undoubtedly promoted prolific phytoplankton blooms in all three oxidation ponds. High densities of phytoplankton resulted in very low levels of transparency (0.2 m) and saturated dissolved oxygen concentrations due to the high photosynthetic activity. Conductivity values were also high and this might have been the result of high amounts of dissolved salts coming from Petchburi municipality (Ogunfowokan *et al.*, 2005). The pH values in all ponds were basic which is indicative of high algal activity that draws down CO<sub>2</sub>

Table 3. Sediment properties of oxidation ponds (n=5)

Parameters	Oxidation Pond 1	Oxidation Pond 2	Oxidation Pond 3
pH	7.3±0.2	7.5±0.3	7.8±0.3
Electrical conductivity (dS/cm)	1.87±0.87	1.75±1.93	0.97±0.48
Water content (%)	8.6±3.6	6.1±2.6	5.1±102
Organic matter content (%)	8.62±3.60	6.01±2.60	5.13±1.0
Total Kjeldahl Nitrogen (%)	0.56±0.27	0.40±0.19	0.34±0.08
Total Phosphorus (%)	0.15±0.03	0.29±0.11	0.20±0.02
Sand (%)	30.68±2.81	14.99±4.25	8.95±0.86
Silt (%)	41.35±10.67	58.65±12.68	79.95±9.07
Clay (%)	27.97±9.70	26.36±8.93	11.1±9.32

Table 4. Species and abundances of phytoplankton in oxidation ponds (n=5)

Oxidation pond	Phytoplankton	
	Number of species	Density (cells/L)
Oxidation pond 1	26	831,330
Oxidation pond 2	24	849,460
Oxidation pond 3	19	1,002,250

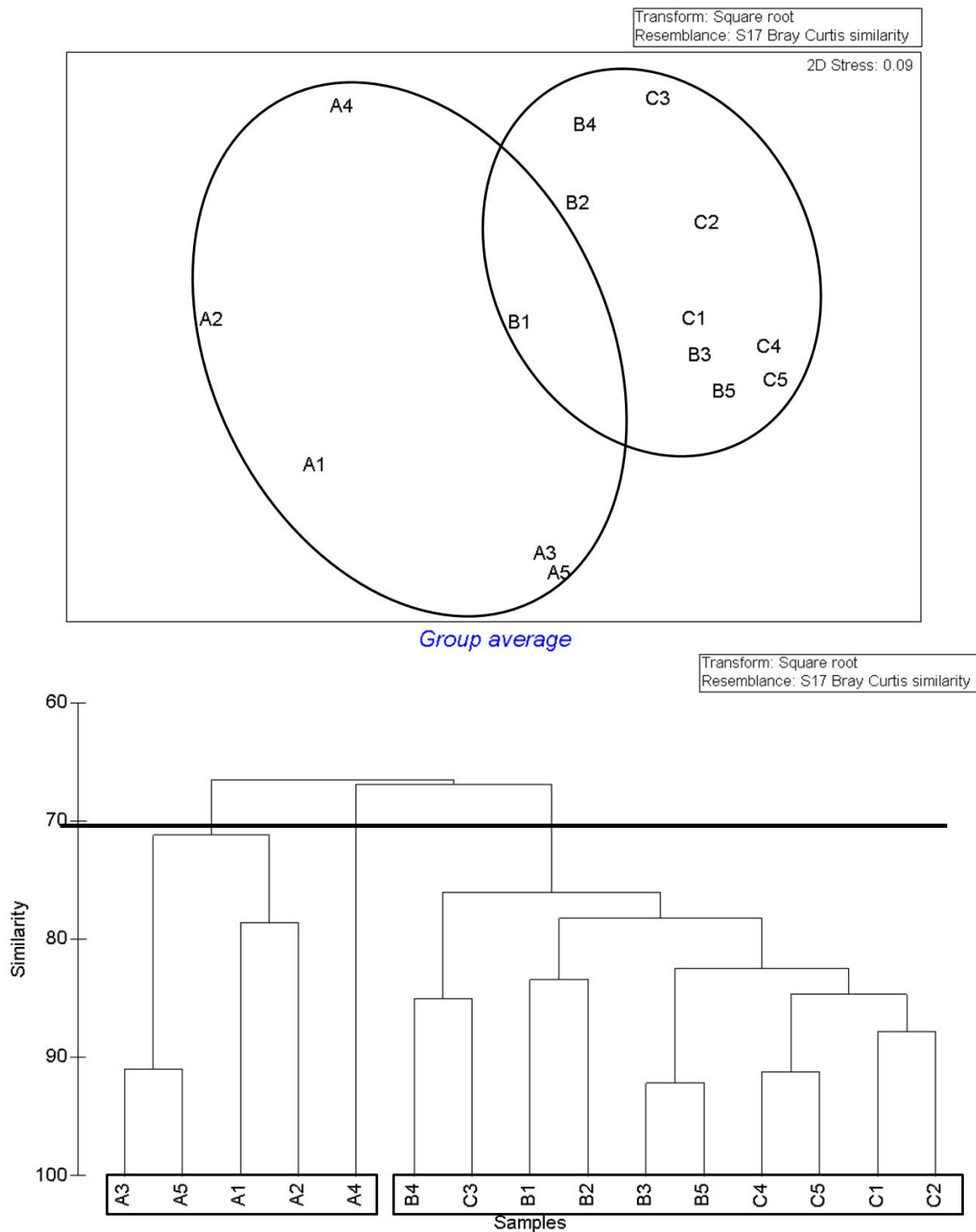


Figure 2. The results of MDS analysis (above) and cluster analysis and (below) revealed two distinct groups of phytoplankton occurring in three oxidation ponds (A, B and C are ponds 1, 2 and 3, respectively and numbers (1, 2, 3, 4 and 5) in the figure represent sampling points in each pond).

Table 5. Species list of phytoplankton in oxidation ponds

Species of phytoplankton	Oxidation pond 1	Oxidation pond 2	Oxidation pond 3
Division Cyanophyta			
Class Cyanophyceae			
Order Chroococcales			
Family Chroococcaceae			
<i>Microcystis aeruginosa</i> Kützing	3,600	3,230	18,250
Order Nostocales			
Family Oscillatoriaceae			
<i>Spirulina platensis</i> (Nordstedt) Geitler	785,110	836,660	977,460
Division Chlorophyta			
Class Chlorophyceae			
Order Volvocales			
Family Volvocaceae			
<i>Eudorina elegans</i> Ehrenberg	7,340	1,200	80
<i>Pandorina morum</i> (Müller) Bory	4,550	420	420
Order Tetrasporales			
Family Palmellaceae			
<i>Sphaerocystis schroeteri</i> Chodat	1,730	800	750
Family Coelastraceae			
<i>Coelastrum</i> sp.	370	320	320
Family Oocystaceae			
<i>Dictyosphaerium</i> sp.	320	570	2,740
<i>Oocystis parva</i> W. West		820	270
<i>Tetraedron gracile</i> (Reinsch) Hansgirg	420	60	250
<i>Tetraedron trigonum</i> (Naegeli) Hansgirg	460	60	30
Family Scenedesmaceae			
<i>Crucigenia</i> sp.	30		
<i>Micractinium pusillum</i> Fresenius	460	90	790
<i>Scenedesmus acuminatus</i> (Lagerheim) Chodat	3,900	210	
<i>Scenedesmus acutus</i> Meyen	2,040	260	170
<i>Scenedesmus ovalternus</i> Chodat	150		
<i>Scenedesmus quadricauda</i> (Turpin) Brébisson		30	40
<i>Scenedesmus</i> sp.	70	30	
Class Euglenophyceae			
Order Euglenales			
Family Euglenaceae			
<i>Euglena</i> sp.	330	570	60
<i>Lepocinclis ovum</i> (Ehrenberg) Lemmermann	400		90
<i>Lepocinclis salina</i> Fritsch	2,240	2,780	590
<i>Phacus circulatus</i> Pochmann	1,680	340	
<i>Phacus hamatus</i> Pochmann	10,060	60	
<i>Phacus helikoides</i> Pochmann	30		
<i>Phacus longicauda</i> (Ehrenberg) Dujardin	1,130	60	40
<i>Phacus pleuronectes</i> (O.F.Müller) Dujardin	30	490	40
<i>Phacus ranula</i> Pochmann	180	60	
<i>Phacus tortus</i> (Lemmermann) Skvortzov	4,670	300	
Division Chromophyta			
Class Bacillariophyceae			
Order Bacillariales			
Suborder Bacillariineae			
Family Naviculaceae			
<i>Navicula</i> sp.	30	40	160



and thus elevating the pH values (Hinga, 1992; Kotut et al., 2010). We also found a relationship between nutrient concentrations and chlorophyll a. In particular, nitrogen is a limiting factor that regulates the blooms of phytoplankton in all studied ponds. In general, municipal wastewater and sewage contain higher concentrations of phosphorus (washing powder and liquid) than nitrogen (mainly from diffuse agricultural sources).

The results of phytoplankton species and abundance were interesting. The major group of common phytoplankton bloom was cyanobacteria. Several studies have shown that blue green algae are generally dominant in oxidation ponds and therefore it is of concern that toxins produced by blue green algae may be harmful to domestic and wildlife animals as well as people using water from the receiving lakes and rivers (Ogunfowokan et al., 2005; Kotut et al., 2010). In the Nakuru town, for example, *Microcystis* sp. grew prolifically on some occasions and cyanotoxin concentrations were found in the sewage treatment plant. However, the results from the current study disclosed that *Spirulina platensis* was the main bloom-forming cyanobacteria and it is a non-toxic producing species.

The unialgal blooms of *S. platensis* in the oxidations ponds of the LERD Project could be the result of high alkalinity or high pH as also occurred in a soda equatorial lake in Kenya (Melack, 1979). *S. platensis* is an alkalophilic cyanobacterium and exhibits optimal growth at pH 9.0 to 10.0 (Belkin and Boussiba, 1991). *S. platensis* can also use ammonia at high pH values as well as having resistance to the ammonia-mediated uncoupling of photosynthesis (Belkin and Boussiba, 1991). In fact, this species has high nutritional values such as good quality protein (Boussiba and Richmond, 1980; Anusuya et al., 1981) and thus supporting and enriching aquatic organisms that feed on them. The study of Khowhit et al. (2015) demonstrated that discharged water from the LERD Project promoted an abundance of filter feeders, especially *Meretrix casta* that was present in high numbers along the shore of the LERD Project.

Pond sediment played a less important role in promoting eutrophication in the oxidation ponds. The organic and nutrient contents were rather low and thus could not be an important internal source of nutrients. This could be explained that the high release of phosphorus from the sediment, due to mineralization, is most likely to be found in sediment where the organic phosphorus content was high (Boström and Pettersson, 1982; Phillips et al., 1994). Low sediment accumulation firstly could be the result of sediment being removed in the sedimentation pond (the first

pond of wastewater treatment system). Secondly, sediments in oxidation ponds are dredged regularly to maintain high water storage capacity and high wastewater treatment efficiency.

## 5. Conclusions

Cyanobacteria blooms occurred in all oxidation ponds during sampling due to excessive nutrient concentrations and the dominant species was *Spirulina platensis*, a non-toxic producer. Values of some environmental parameters were also indicative of intense eutrophication. High phosphorus concentrations in the waste water indicated the source of municipality generated mainly by household sewage and markets. Nitrogen was a limiting nutrient of the blooms of phytoplankton. Internal source of nutrients was not significant due to low accumulation of organic matter. Regular examination of any contaminated toxins that may be produced by cyanobacteria from discharged effluent is still important. This is because toxic cyanobacteria such as *Microcystis* may make an appearance and dominate phytoplankton blooms that could pose negative impacts on discharged environment.

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