The role of algae in the removal of Escherichia coli in a tropical eutrophic lake

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\textbf{A B S T R A C T}

Eutrophication and its accompanying algal development in lakes is a nuisance and may be problematic for aquatic life, but limited algal development may have some beneficial consequences. Dissolved oxygen concentration and pH increases attributed to algae in algal-based treatment ponds may occur in eutrophic lakes and can result in the inactivation of faecal coliforms in eutrophic lakes. We investigated the die-off of Escherichia coli placed in dialysis tubes in a eutrophic lake at different depths and locations. The importance of \textit{E. coli} attachment to algae and suspended matter was also assessed. Algal presence in \textit{E. coli} is directly proportional to the chlorophyll a concentration of the lake. Under laboratory conditions, as chlorophyll a concentration increases in light however, an optimum chlorophyll a concentration (0.24 mg/L) is reached after which the rate of decay of \textit{E. coli} decreases. These results show that limited algal presence representing optimum chlorophyll a concentration in restored ecosystems may have public health benefits for rural communities in developing countries that depend on raw water for domestic activities.

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1. Introduction

Nutrient enrichment of lakes and its accompanying algal development is a nuisance for water supply and recreational uses. Excessive development of algae may result in death of aquatic life but limited algal presence may have beneficial consequences that may not have been previously reported in literature. Wang \textit{et al.} (2009) showed that excessive algal presence can be controlled by the introduction of a mosaic community of macrophytes.

The effect of algae on the removal of faecal bacteria in wastewater treatment ponds and Free Water Surface (FWS) Wetlands are well documented in literature (Curtis, 1990; Curtis \textit{et al.}, 1992; Van der Steen \textit{et al.}, 2000a;b; Awuah, 2006; Garcia \textit{et al.}, 2008) and algal treatment ponds appear to be more effective in the removal of faecal bacteria than macrphyte-based ponds (Awuah, 2006). Eutrophic lakes may show a comparable effect in the removal of faecal bacteria considering the similarity in the biological and biochemical processes in these two aquatic environments. Pu \textit{et al.} (1998) observed that attached algae, submerged and dying macrophytes assisted in the removal of pollutants. Increased oxygenation in treatment ponds or lagoons attributed to algal presence has been observed to affect faecal bacteria die-off due to the production of toxic forms of oxygen (Curtis \textit{et al.}, 1992). Maturation ponds of waste stabilization ponds usually have chlorophyll a concentrations of between 500 and 2000 µg L\textsuperscript{−1} (Feachem \textit{et al.}, 1983). Lakes with chlorophyll a concentrations above 13 µg/L are considered eutrophic and these algal concentrations are enough to increase oxygen concentrations of lakes to levels that may be harmful to faecal bacteria. Oxygen concentrations above 0.5 mg L\textsuperscript{−1} have been shown to contribute to the removal of faecal bacteria (Van Buuren and Hobma, 1991). Algal presence also leads to high pH levels that tend to be bactericidal even in the absence of high oxygen concentrations (Maynard \textit{et al.}, 1999). Eutrophic lakes on the other hand may not have such high pH values as occur in maturation ponds but may experience modest fluctuations in pH due to diurnal variation in carbon dioxide concentration as a result of photosynthesis. Fluctuations in pH are known to negatively affect survival of \textit{Escherichia coli} (Awuah, 2006) and could therefore result in significant removal of faecal bacteria in eutrophic lakes.

Bacteria in the aqueous medium attaches itself to solid surfaces by secreting extracellular polysaccharides and the quantity and composition of this extracellular polysaccharides may affect the attachment properties of the bacteria (Sanin \textit{et al.}, 2003). Algal presence in eutrophic lakes as suspended matter, may also serve as surfaces for bacterial attachment resulting in the formation of aggregates which may eventually get sedimented from the water column to the lake bottom, thus improving the water quality of the
lake. A high degree of faecal bacteria attachment to algae and suspended solids in eutrophic lakes could therefore constitute a major natural means of removal of bacteria pathogens from eutrophic lakes. The importance of such a phenomenon in eutrophic lakes has not been reported in literature.

Some studies, however, reported that the release of algal organic matter as a result of algal cell lysis can enhance coliform survival and even growth (Lake et al., 2001; Bouteleux et al., 2005) because algal degradation provides the carbon and energy sources for the survival and growth of coliforms (Van der Steen et al., 2000a). This suggests that degradation of algal cells may undermine any disinfection activity that living algae may have accomplished.

This study aims to understand the importance of algae in the removal of E. coli from eutrophic lakes and to specifically underscore the effect of varying algal concentrations on E. coli removal. In addition, the importance of E. coli attachment to algae in a eutrophic lake is assessed in relation to its removal from the lake. Survival experiments in a eutrophic lake involving E. coli in dialysis tubes immersed at different depths and locations were therefore expected to unmask some of the possible effects of algae in eutrophic lakes. Since the variation in chlorophyll a concentration provided by field conditions were minimal and do not therefore explore possible scenarios that may exist in other eutrophic lakes, additional batch laboratory experiments using E. coli and laboratory cultured algae were carried out.

2. Materials and methods

2.1. Study area

Weija Lake, formed by the damming of the Densu River, is located 40 km west of Accra, Ghana (West Africa), and together with the Densu River drains a total area of 2072 km². The lake, located within latitude 5°30’N–6°20’N and longitude 0°10’W–0°35’W is polymictic and is mainly a source of drinking water for over 2.6 million people in the western part of Accra as well as irrigation for some 200 ha of farmland. It has an estimated fish yield of 380 metric tons per year (Ministry of Works and Housing, 1998). Algal sampling of the lake prior to the lake experiment showed Chlorella sp. and Anabaena sp. as the most dominant algal species at the experiment sites. Other algae in minimal quantities include Oscillatoria sp., Eunastus sp. and Synedra sp.

2.2. E. coli decay experiment in the lake

E. coli ATCC 25922 (Strain info, 2010) suspension of concentration (1–3) × 10⁷/mL and henceforward referred to as E. coli was washed twice in saline solution (0.85% saline) to remove nutrients and 15 mL of this suspension was placed in Spectra/Por 1 dialysis tubes in groups of 10 tubes. Saline solution (0.85% saline) was prepared by dissolving 1 tablet of Oxoid BR0053G saline tablet in 500 mL of distilled water, autoclaved at 121 °C for 15 min. Spectra/Por 1 dialysis tubes are regenerated cellulose and molecular porous dialysis tubes of molecular weight cut off (MWCO) of 6000–8000 Da. Analysis of the spectra characteristics of the dialysis tubing in a Uvicon 930 UV/Visible spectrophotometer, Kontron Instruments, Switzerland, showed that only light of wavelengths >350 nm are transmitted by the dialysis tubes (Bergstein-Ben et al., 1997). The suspension was constantly stirred during distribution into dialysis tubes to avoid settling. Dialysis tubes were suspended 0.20 m below the lake surface at two locations (1 and 2), separated by a distance of 5 km. Sites were selected based on visual inspection of algal abundance exhibited by green suspended cells of algae and it was assumed that chlorophyll a concentration will differ at these two locations. Additional dialysis tubes were suspended at a depth of 20 m below the lake surface at location 2 only. Two control setups were suspended 0.20 m below the lake surface at location 2 only, both consisting of filtered lake water containing dialysis tubes of E. coli (Fig. 1). Filtration of the lake water for the two controls entails pouring lake water through an eight-layered cotton cloth to filter off algal cells. Control A was filtered once (at the start of the experiment) allowing growth of algal spores. Control B was filtered every other day during the period of the experiment.

One hour after the immersion of the dialysis tubes into the lake, temperature, dissolved oxygen concentration, pH and conductivity inside and outside of the tubes were determined and monitored during the period of the experiment between the hours of 7:00–8:00 am at a depth of 0.2 m below the lake surface. Dissolved oxygen concentration, pH and conductivity measurements were done with a WTW Oxi 330, WTW pH 340 and WTW LF 340 meters respectively. E. coli numbers were assessed 0, 1, 3, 5 and 7 days after incubation in the lake. The experiment lasted for 7 days and made use of two duplicates, each having three subreplicates making a total of 6 replicates per treatment. E. coli counts inside dialysis tubes were measured using the spread plate technique (APHA, 2005) on chromocult agar plates incubated at 37 °C for 24 h (Byamukama et al., 2000; Wang and Fiessel, 2008). The decay rates of the E. coli in the incubations, Kd were calculated as a gradient of the regression line of the first order decay equation (Marais, 1974; Dewedar and Bahgat, 1995):

\[
\ln N_t = -K_d t + \ln N_0
\]

where \(N_t\) = E. coli count per 100 mL at a time \(t\), \(N_0\) = E. coli count per 100 mL at the start of the experiment, and \(t\) = time (days) of incubation.

On the same days (0, 1, 3, 5 and 7 days of incubation in the lake) chlorophyll a concentrations were determined at various locations except at 20 m depth by taking water samples for chlorophyll a analysis. Light sensor readings at depth 20 m using LI-COR UWQ 4683 underwater light sensor (LI-COR, Inc., NE, USA) indicated absence of solar radiation at that depth. Chlorophyll a concentrations were determined according to NEN 6520 (1981) using four replicates per treatment. Chlorophyll a concentration, dissolved oxygen concentration and pH measurements were not done at 20 m depth due purely to logistical limitations. These parameters were inferred from absence of solar radiation at this depth. The diurnal variation of pH, temperature and dissolved oxygen concentration of the lake were monitored from 6:00 am to 6:00 pm at a depth of 0.2 m on day 0, 1 and 3 (\(n = 3\)). All experiments were carried out in the months of February and March, 2006 when algal blooms are common in the lake. Maximum and minimum day and night ambient temperatures were 33 °C and 19 °C respectively. Decay
rates of different treatments were compared statistically using an independent sample t-test of Minitab 15.0 statistical package.

2.3. Attachment of E. coli to algae in lake water

Sampling for E. coli in the lake was done by collecting 30 sub-replicates per sampling location, for three different locations in the lake using 600 mL containers. Locations within 4–8 m of shoreline were selected as sampling was based on the assumption that activities of neighbouring communities in the water may render these locations high in E. coli numbers and that E. coli may be attaching to each other and to suspended matter. Sampling was conducted once per week for five weeks at a depth of 0.2 m for E. coli presence. Water samples were pushed through a 10 mL syringe fitted with a needle to detach any attached bacteria. The syringe–needle method is able to recover a high percentage of attached bacteria, sometimes as high as 100% (Ansa et al., 2009). E. coli numbers were determined before and after syringe treatment by membrane filtration technique (APHA, 2005) on chromocult agar plates incubated at 37 °C for 24 h. E. coli numbers before and after pushing through syringes was statistically compared using paired samples t-test of Statistical Packages for Social Scientists (SPSS version 12.0). Data of wind speed on sampling days were obtained from the Surface Water Division of the CSIR Water Research Institute, Accra, Ghana.

2.4. Laboratory experiments

Algae were grown by inoculation of nutrient solution with laboratory stock of Chlorella sp. obtained from Wilson Group Inc., USA (Wilson Group, 2010) under light of wavelength 380–780 nm provided by a powerstar HQI-BT 400 lamp. Culture solution contained 13.5 mg L⁻¹ nitrogen and 2.2 mg L⁻¹ phosphorus in the form of nitrate and phosphate respectively. Resulting algae were harvested after 14 days, sieved using 250 μm and 90 μm mesh nets and concentrated by centrifugation at 1000 rpm for 30 min into a thick algal paste. Into each of four sets of sterile Erlenmeyer flasks, 250 mL of thoroughly mixed E. coli (ATCC 25922) suspensions of concentration (1.3–3.1) × 10⁸ mL⁻¹ were introduced. Each set consists of six (6) flasks. E. coli suspensions were prepared by a ratio of 1 mL stock E. coli suspension for 50 mL of sterile Oxoid CM0001 nutrient broth solution, maintained at 35–37 °C for 24 h. The E. coli suspensions were washed twice by concentrating and re-suspending in saline solution prepared as mentioned above. E. coli suspensions were vortex-mixed at optimum energy and time to avoid clustering of bacteria cells.

Harvested algae from culture setup were used to inoculate five (5) of each of six (6) flasks belonging to each set, one flask was maintained as control, having no algae. Each set of flasks was inoculated with different amounts of algae. The following mean chlorophyll a concentrations were obtained for each set: 0, 0.051, 0.24, 2.25, 5.02 and 10.5 mg L⁻¹. Two sets of flasks (serving as duplicates of each treatment), each consisting of six flasks were covered with dark polythene sheets and placed on a GFL 3019 orbital shaker together with the other two uncovered sets (also duplicates of each treatment) in a randomised block design such that each treatment of particular chlorophyll a concentration had a subreplicate of 3 samples. Each treatment of a particular chlorophyll a concentration therefore had a total of six replicates per treatment. The GFL 3019 shaker was placed 0.8 m below the HQI-BT 400 lamp in a regime of 16 h light and 8 h darkness rotating at 100 rpm. Temperature of setup varied from 20 to 25 °C. Temperature dropped to 20 °C in the night when lamp went off. E. coli was monitored at time 0, 0.25, 1, 3, 5 and 7 days using the spread plate technique (APHA, 2005) on chromocult agar plates incubated at 37 °C for 24 h (Byamukama et al., 2000; Wang and FiesSEL, 2008). Chlorophyll a concentration was measured at the beginning and end of the experiment according to NEN 6520 (1981) using four replicates per treatment. Dissolved oxygen concentration and pH of reactors were monitored at 10:00–11:00 h GMT using WTW 330 Oximeter and WTW 340 pH meter respectively.

In order to determine that decrease in E. coli numbers is a result of actual die-off and not E. coli attaching to each other and to algae, samples were taken after 1 and 3 days of incubation and samples were pushed through 10 mL clinical syringes fitted with needles to detach any attached bacteria (Ansa et al., 2009). E. coli numbers before and after pushing through syringes was statistically compared using paired samples t-test of Statistical Packages for Social Scientists (SPSS version 12.0). The decay rates of the E. coli in the incubations, Kd were calculated as a gradient of the regression line of the first order decay Eq. (1) mentioned above (Marais, 1974; Dewedar and Bahgat, 1995). Decay rates of different treatments were compared statistically using an independent sample t-test of Minitab 15.0 statistical package.

3. Results

3.1. E. coli decay experiment in the lake

3.1.1. Physico-chemical characteristics of the lake

Slightly higher pH and dissolved oxygen concentrations were observed in lake dialysis tubes compared to controls A and B (Table 2). Physico-chemical conditions of temperature, pH and DO inside the dialysis tubes were comparable to conditions in the lake. pH during the day was the highest at 14:00 h GMT (8.7) and lowest at 06:00 h GMT (8.0), while dissolved oxygen concentration peaked at 08:00 h GMT (8.8 mg L⁻¹) and reduced to the lowest value of 5.6 mg L⁻¹ by 18:00 h GMT (Fig. 2). Fig. 3 shows that there was
exchange of fluid between the contents of the dialysis tubes and the lake at all locations. Diurnal variation in conductivity was fairly constant with minimal fluctuation from 420.7 to 422.3 µS cm⁻¹. Lower conductivity was observed in the lake compared to the two controls.

3.1.2. *E. coli* decay in the absence of algae

At 20.0 m deep light penetration was absent and therefore $K_d$ measured at that depth could represent rate of decay of *E. coli* in darkness. Rates of decay of *E. coli* at 20.0 m deep (0.63 ± 0.07) did not differ significantly from rate of decay at control B (0.55 ± 0.07) where algae were filtered off every other day.

3.1.3. Effect of algae in light

Rate of *E. coli* decay, $K_d$ in the lake (1.23 ± 0.03 day⁻¹) and in control A (0.85 ± 0.08 day⁻¹) (where algae was filtered only at the start of the experiment) were both significantly higher ($p < 0.05$) than $K_d$ in control B (0.55 ± 0.07 day⁻¹) with no algae (Table 2). Locations 1 and 2 at 0.2 m below the lake surface had comparable rates of decay. A positive linear correlation was seen between decay rates and chlorophyll a concentration throughout the period of the experiment. Chlorophyll a concentration in the lake, however, did not exceed 0.08 mg L⁻¹ of chlorophyll a throughout the period of the experiment.

3.2. Attachment of *E. coli* in lake water

Sampling in the lake showed wide variations in faecal bacteria levels on the different days of sampling and so was the degree of attached bacteria (Table 3). Sampling in weeks 1, 3 and 5 showed significant bacteria numbers attached ($p < 0.05$) while the rest did not.

3.3. Laboratory experiment

3.3.1. Physico-chemical conditions

pH and dissolved oxygen concentration monitored under laboratory conditions show that pH and DO increased with increased chlorophyll a concentration till 2.25 mg L⁻¹, after which it decreased (Fig. 4).

3.3.2. Attachment or decay of *E. coli*

Detachment tests carried out on samples taken after 1 day and 3 days of incubation did not show significant increases in *E. coli* numbers after detachment except the 0.05 mg L⁻¹ incubation kept in darkness at day 1 ($t = 12.1$, $p < 0.05$) (Fig. 6).

3.3.3. Effect of algae in light under laboratory conditions

Similar observations were made under laboratory conditions, where decay rates increased with increasing chlorophyll a concentration but only up to 0.24 mg L⁻¹ chlorophyll a concentration. Beyond this chlorophyll a concentration, decay rates decreased with increasing chlorophyll a concentration. Comparable algal densities had higher rates of decay under laboratory conditions than in the lake. Significantly higher decay rates were observed in algae exposed to light, compared to its corresponding algal densities kept in darkness ($p < 0.001$, Fig. 5). The extent of the difference (mean separation $t$), varied as follows: $t = 119.1, 196.8, 130.4, 65.9$ and 67.3 respectively for setups with algal densities 0.05, 0.24, 2.25, 5.02
and 10.50 mg L\(^{-1}\). The mean difference increased with increasing chlorophyll a concentration till the optimum chlorophyll a concentration of 0.24 mg L\(^{-1}\), after which the mean separation begins to decrease. The chlorophyll a concentration with the highest decay rates had the highest survival in darkness.

### 3.3.4. Effect of algae in darkness under laboratory conditions

The effect of algae on \(E.\ coli\) decay rate in darkness was assessed by comparing the control in darkness with all the other algal densities in darkness. Significantly higher decay rates were observed in the dark control compared to all the other algal densities. Survival of \(E.\ coli\) increased with increased chlorophyll a concentration till a certain optimum chlorophyll a concentration (0.24 mg L\(^{-1}\)), after which survival decreased.

### 4. Discussion

#### 4.1. Decay of \(E.\ coli\) in the lake in the absence of algae

There was development of algae over time in control A, where algae were filtered off only at the start of the experiment. This explains why chlorophyll a concentration in this incubation was four times higher than in control B, where algae were filtered off every other day (Table 2). Control with algae filtered off every other day therefore represents oligotrophic conditions with negligible algal productivity as waters with chlorophyll a concentration below 0.013 mg L\(^{-1}\) is considered oligotrophic. Rates of decay of \(E.\ coli\) in control with algae filtered every other day (0.2 m below the lake surface) were comparable with rates of decay at 20.0 m deep in the lake. At both locations algal presence was negligible with sunlight occurring at the depth of 0.2 m but not at 20.0 m. Absence of light at 20.0 m below the lake surface suggests an absence or limited presence of primary producers which cause elevation of pH and DO concentration. This may explain the much lower decay rates (0.63 day\(^{-1}\)) at 20.0 m deep. In addition, as lake is polymictic and therefore not thermally stratified, temperatures at 0.2 m and 20.0 m deep would not differ much. Light was present in control B and major mechanisms of \(E.\ coli\) destruction therefore did not include a direct effect of light. Spectral characteristics of dialysis tubes shows that only solar radiation wavelengths >350 nm were transmitted by the dialysis tubes (Bergstein-Ben et al., 1997). At wavelengths >329 nm, important mechanisms of faecal bacteria destruction are those that act through photo-sensitizers such as dissolved organic matter and photosynthetic pigments such as algae (Curtis, 1990; Sinton et al., 2002). Low and comparable \(K_d\) observed in control with algae filtered off every other day and 20.0 m deep in the lake could be due to the low concentration of dissolved organic matter and photosynthetic pigments at both locations. The lake BOD usually varies between 2 and 10 mg L\(^{-1}\) (Table 1).

#### 4.2. Effect of algae in light under field and laboratory conditions

#### 4.2.1. Dissolved oxygen and pH effect

Algae affected the decay of \(E.\ coli\) through altering of the chemistry of the water. Fig. 3 shows that osmotic equilibrium was established by the movement of water into the dialysis tubes as solute concentration inside the dialysis tubes was higher initially. Chemical conditions inside dialysis tubes were therefore comparable to that outside the dialysis tubes. Greater conductivity values observed in controls compared to the lake may be due to the effect of evaporation in a smaller volume of lake water in the control basins and perhaps movement of ions across the dialysis tube membranes into the surrounding water. In control with algae filtered only at the start of the experiment \(pH\) and dissolved oxygen concentrations (7.8 ± 0.1, 4.4 ± 0.5 mg L\(^{-1}\)) were comparable to that observed in the lake dialysis tubes (8.1 ± 0.1, 5.1 ± 0.8 mg L\(^{-1}\)). The lake \(pH\) and dissolved oxygen concentration however were higher than that of control with algae filtered every other day (7.7 ± 0.3, 3.6 ± 1.3 mg L\(^{-1}\)), Table 2. This may have resulted in the higher rate of decay of \(E.\ coli\) in the lake as greater concentration of toxic oxygen radicals may have been produced (Curtis et al., 1992). Algal photosynthetic activity results in \(pH\) elevation and increased oxygenation but respiration and organic matter oxidation in the lake may also affect the dissolved oxygen concentration, hence its diurnal variation (Fig. 2). Maximum \(pH\) occurring in the lake was 8.7 at

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#### Table 1

Characteristics of Weija Lake (unfiltered) over the period 2005–2008 (Darko and Ansa-Asare, 2009).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>pH</th>
<th>Dissolved oxygen (mg L(^{-1}))</th>
<th>Conductivity (μS cm(^{-1}))</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.5–8.7</td>
<td>3.5–10.5</td>
<td>247–527</td>
<td>2.8–15.9</td>
</tr>
</tbody>
</table>

#### Table 2

Physico-chemical conditions inside and outside dialysis tubes in controls and in the lake and \(E.\ coli\) decay rates \(K_d\) at these locations. Temperature, \(pH\) and DO are averages of 10 values ± standard deviation of measurements taken 0.2 m above the lake surface. Chlorophyll a concentrations represent averages of 20 values ± standard deviation taken at 0.2 m below the lake surface. Control A: algae filtered only at the start of experiment and control B: algae filtered every other day.

<table>
<thead>
<tr>
<th>Dialysis tubes</th>
<th>Temp. (°C)</th>
<th>(pH)</th>
<th>DO (mg L(^{-1}))</th>
<th>(^{a})Chlorophyll a (mg L(^{-1}))</th>
<th>(K_d) (day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Location 1 (0.2 m)</td>
<td>Location 2 (0.2 m)</td>
<td>Location 2 (20.0 m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control A</td>
<td>Inside 28.8 ± 0.9</td>
<td>7.8 ± 0.1</td>
<td>4.4 ± 0.5</td>
<td>0.041 ± 0.01e</td>
<td>0.85 ± 0.08h</td>
</tr>
<tr>
<td></td>
<td>Outside 29.3 ± 0.6</td>
<td>7.6 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>0.011 ± 0.02f</td>
<td></td>
</tr>
<tr>
<td>Control B</td>
<td>Inside 28.8 ± 0.8</td>
<td>7.7 ± 0.3</td>
<td>3.6 ± 1.3</td>
<td>0.047 ± 0.01e</td>
<td>1.20 ± 0.07g</td>
</tr>
<tr>
<td></td>
<td>Outside 29.4 ± 0.7</td>
<td>7.7 ± 0.3</td>
<td>3.0 ± 1.7</td>
<td>0.048 ± 0.01e</td>
<td></td>
</tr>
<tr>
<td>Lake</td>
<td>Inside 28.5 ± 0.7</td>
<td>8.1 ± 0.1</td>
<td>5.1 ± 0.8</td>
<td>0.23 ± 0.04g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outside 30.0 ± 0.7</td>
<td>8.2 ± 0.1</td>
<td>4.3 ± 1.0</td>
<td>0.013 ± 0.00e</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Mean chlorophyll a concentration ± standard deviation at location 1, 0.2 m deep (K) and location 2, 0.2 m deep (L). Same small letters refer to statistically comparable values while different letters are significantly different.
2:00 pm. In the lake, pH therefore did not reach the critical 9.5 level where pH has shown to inactivate faecal bacteria single-handedly (Parhad and Rao, 1974; Awuah, 2006). Long wavelengths such as that transmitted by the dialysis tubes are not able to damage \( E. coli \) at pH values below 8 (Curtis, 1990) and are highly sensitive to and completely dependent on the oxygen concentration, the rate of damage being proportional to the oxygen radicals’ concentration (Curtis et al., 1992). Craggs et al. (2004) noted that in the presence of sunlight and oxygen concentration range of 0–22 mg L\(^{-1}\), pH range of 8.0–9.2, pH appeared to have little influence on the inactivation of \( E. coli \).

Under laboratory conditions, glassware, particularly Pyrex glassware filter off UV light and visible light below 500 nm (Thermo Fisher Scientific, 2010). Lights of wavelengths greater than 500 nm inactivate \( E. coli \) mainly through photo-oxidation which is entirely oxygen dependent (Curtis et al., 1992) and possible effects of UVA and UVB lights may either be absent or minimal. Oxygen concentration increased with increased chlorophyll a concentration till a certain optimum (2.3 mg L\(^{-1}\)) after which oxygen concentration decreased with increased chlorophyll a concentration (Fig. 4). Decreased oxygen concentration may be due to oxidation of dissolved organic matter released by lysis of algal cells that occurs at higher algal concentrations (Wetzel, 2001). A similar trend as dissolved oxygen concentration was shown by pH changes though not as pronounced.

### 4.2.2. Chlorophyll a concentration of algae

Chlorophyll a concentration of the lake was directly proportional to the rate of decay of \( E. coli \) in the lake, suggesting that algal presence in eutrophic lakes can be an important means of \( E. coli \) removal. Chlorophyll a concentration however did not exceed 0.08 mg L\(^{-1}\). Increased chlorophyll a or algal concentration leads to increased oxygen and pH elevation leading to increased rate of decay of \( E. coli \). Expectedly locations 1 and 2 had comparable decay rates as their chlorophyll a concentration of 0.0471 ± 0.01 and 0.0481 ± 0.01 mg L\(^{-1}\) respectively were comparable (Table 2). Laboratory experiments showed that very high algal densities depicted by high chlorophyll a concentrations may be a limiting factor in the removal of \( E. coli \). Beyond chlorophyll a a concentration of 0.24 mg L\(^{-1}\) decay rate of \( E. coli \) decreases (Fig. 5). Increased chlorophyll a concentration results in increased disintegration of algal cells releasing dissolved organic matter from its cytoplasm (Bouteleux et al., 2005). Oxidation of this organic matter may lower oxygen concentration (Fig. 4). Long wavelengths of light inactivate \( E. coli \) by creating toxic forms of oxygen molecules which are injurious to bacteria cells (Curtis et al., 1992). Increased chlorophyll a concentration also increasingly filters off the effect of short wavelengths of light that can directly damage \( E. coli \) (Van der Steen et al., 2000a).

Most eutrophic lakes do not have algal densities exceeding 0.3 mg L\(^{-1}\) and this makes eutrophic lakes particularly important as capable systems of self purification. Weija Lake is a reservoir for supplying drinking water to the Western part of Accra and 20 m from the banks of this lake are settlement communities who depend on the raw water of the lake for domestic as well as recreational activities. Natural disinfection of this water body may therefore have significant public health benefits to these communities. Highest \( E. coli \) numbers observed in this lake was 1200 cfu 100 mL\(^{-1}\) (Table 3).

### 4.3. Effect of algae in darkness under laboratory conditions

Survival of \( E. coli \) in darkness increased with increased chlorophyll a concentration till a certain optimum (0.24 mg L\(^{-1}\)), after which survival decreased (Fig. 5). As pH and dissolved oxygen values were comparable in all incubations in darkness, and differences in decay rates cannot be attributed to natural decay, inactivation of \( E. coli \) in darkness may be due to another factor. Klein and Alexander (1986) attributed the inactivation of bacteria in freshwater to the presence of inhibitory substances. Others have also suggested the production of algal toxins that are detrimental to the survival of \( E. coli \) in darkness (Maynard et al., 1999).

Our work supports this later assertion as the variation in decay rate in darkness could be explained by a counteracting effect of both increasing algal organic matter and algal toxin. It is possible that enough quantities of algal toxins capable of inactivating \( E. coli \) are probably released beyond certain concentrations of algae. Further investigations are needed to ascertain this. \( Chlorella \) sp., a common alga in wastewater treatment systems was reported to produce substances toxic to \( Vibrio cholerae \) (Maynard et al., 1999). Our experiment made use of algae grown by natural colonization, having \( Chlorella \) as the dominant algal species and therefore may have produced some toxic substances. Not much however, is known about the kind of toxin produced by these algae (Maynard et al., 1999).

### 4.4. Attachment of \( E. coli \) in lake water

The importance of \( E. coli \) attachment to algae and suspended particles in Weija Lake as a possible mechanism of bacteria pathogen removal in the lake was assessed with the assumption that \( E. coli \) may be attached to each other, algae and suspended particles at the water contact sites of the lake where human activity is high. Electron microscopy of a colonized algae–bacteria mixture had shown that a slime matrix engulfs both the bacteria and the algae during attachment (Holmes, 1986), forming fast sinking aggregates (Grossart and Simon, 1998) which may eventually get sedimented faster to the lake bottom. Sampling in the lake showed wide variations in \( E. coli \) numbers on the different times of sampling and so was the degree of attached \( E. coli \) (Table 3). Variations in \( E. coli \) numbers could be attributed to inflow of water from upstream and also to defaecation along the banks by settlers or free range cattle or both. Sampling on weeks 1, 3 and 5 showed significant \( E. coli \) attached (p < 0.05) while weeks 2 and 4 did not. This suggests that bacteria pathogen attachment to algae and suspended solids could be an important mechanism of pathogen removal in

### Table 3

<table>
<thead>
<tr>
<th>Week</th>
<th>Wind speed (ms(^{-1}))</th>
<th>( E. coli ) count 100 mL(^{-1}) Before detachment</th>
<th>Attached (%)</th>
<th>t-Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2</td>
<td>849 ± 6.1</td>
<td>251.6 ± 26.1</td>
<td>196.3</td>
<td>6.13</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>69.1 ± 5.0</td>
<td>64.1 ± 3.9</td>
<td>Insignificant</td>
<td>0.74</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>933.6 ± 27.4</td>
<td>1170.9 ± 71.4</td>
<td>25.4</td>
<td>3.35</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>180.8 ± 7.7</td>
<td>209.6 ± 13.4</td>
<td>Insignificant</td>
<td>1.64</td>
</tr>
<tr>
<td>5</td>
<td>2.1</td>
<td>272.8 ± 15.0</td>
<td>357.2 ± 24.4</td>
<td>30.9</td>
<td>2.80</td>
</tr>
</tbody>
</table>

* Data obtained from Surface Water Division, CSIR Water Research Institute.
eutrophic lakes as attached E. coli could eventually be removed from the water column through sedimentation. Wind speed and direction can affect E. coli distribution in a lake (Whitman et al., 2004) by re-suspending the E. coli in the lake (Table 3). Increased suspended matter could thus constitute an increased surface area for E. coli attachment.

4.5. Attachment of E. coli in laboratory incubations

The purpose of performing detachment tests on days 1 and 3 laboratory incubations was to ensure that E. coli attached to each other and to suspended particles and algae is detached in order to avoid underestimation of E. coli numbers. Decreased numbers of E. coli resulting from E. coli attachment could be wrongly interpreted as decay. Bacteria cells sometimes attach to each other, appearing as a single colony on agar plates, a phenomenon that can lead to severe underestimation of the actual bacteria numbers present. In washing E. coli suspension in normal saline, the process of centrifugation is employed and this can lead to attachment of E. coli to each other. These attached E. coli could subsequently detach and the increased E. coli number could be erroneously interpreted as growth (Dewedar and Bahgat, 1995; George et al., 2002). The use of the syringe and needle method can completely recover all attached bacteria by the application of shear stress on the attached bacteria, thus dislodging it (Ansa et al., 2009). This method is however limited by the subjective nature of the force applied on the syringe which could introduce a wide variation in performance. Attachment of bacteria to objects may occur within 24 h (Awuah, 2006) to 3 days (Leff and Leff, 2000). Detachment tests done on samples taken after 1 day and 3 days of incubation did not show significant increases in E. coli numbers after detachment except the 0.05 mg L⁻¹ incubation kept in darkness at day 1. This suggests that the E. coli counts were not underestimated as a result of attachment to each other and to algal cells (Fig. 6) and that decreased E. coli numbers is as a result of decay.

5. Conclusions

- Algae are significantly reducing E. coli contamination in eutrophic lakes through increased oxygenation and pH elevation.
- At chlorophyll a concentration ≤0.08 mg L⁻¹ in Weija Lake, decay rate of E. coli is directly proportional to chlorophyll a concentration of the lake. Under laboratory conditions, as chlorophyll a concentration increases in light, an optimum chlorophyll a concentration of 0.24 mg/L is reached after which rate of decay of E. coli decreases.
- E. coli decay in darkness was affected by chlorophyll a concentration. Further investigations are necessary to ascertain whether other factors such as algal toxins are controlling the decay rates of E. coli through algal density.
- Limited algal development representing optimum chlorophyll a concentration for maximum E. coli decay can be encouraged in restored ecosystems or wetlands in order to achieve significant reductions in E. coli numbers. This may have huge public health benefits for communities in developing countries in particular who use raw untreated water from lakes and other freshwater sources. As this study was done with indicator bacteria (E. coli), any parallel conclusions for pathogens need to be verified by further experiments.
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