

Microbial water quality of recreational lakes near Tbilisi, Georgia

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ABSTRACT

Microbial safety of recreational water is one of the major human public health issues in developing countries. Three water bodies, the Tbilisi Sea, Kumisi and Lisi lakes, in the South Caucasus region near Tbilisi, Georgia, were monitored in 2006–2009 to determine microbiological quality using standard methods. Microbial pollution indicators were determined in parallel with phytoplankton abundance and measurement of a number of physical–chemical parameters. Kumisi Lake, a brackish water body in an active agricultural area, appeared to be the most polluted, whereas the Tbilisi Sea, a freshwater reservoir was the least polluted. High values for fecal indicators in all three lakes in summer and early autumn were revealed. In our study, total enterococci counts (TEC) appeared to be a better indicator than either fecal or total coliform counts for the evaluation of fresh and brackish microbial water quality. We found significant correlation between total *Vibrio* counts and TEC for all three water bodies. Prevalence of somatic coliphages and *V. cholerae*-specific phages as additional water pollution indicator significantly correlated with abundance of the host bacteria. Particular phytoplankton groups in the lakes responded to the changes of fecal indicators; however, no correlation was observed between dominant zooplankton taxonomic groups and microbial parameters.

Key words | fecal indicators, phages, phytoplankton, recreational waters, vibrios, water quality

INTRODUCTION

Microbial contamination of aquatic ecosystems is a major public health concern in both developing and developed countries. Pathogen composition in a particular location may vary depending on the type, nature (e.g., lake, estuary), and microbial profile of effluents from surrounding areas. In waters used for recreation, organisms of concern include *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Escherichia coli*, *Clostridium* spp., and various human enteric viruses and protozoa (Golubovskaya 1978; Fleisher *et al.* 1993; Wade *et al.* 2003; Fong & Lipp 2006). In general, the most significant threats are autochthonous microorganisms that

may be triggered to multiply when conditions are favorable. In addition, reservoirs often are contaminated through external loading, e.g., runoff from agricultural and/or urban catchments via rain waters or direct discharge of effluents from farms. Enteric coliform bacteria (e.g., total coliforms or fecal coliforms), as well as *E. coli* and enterococci are the most widely used indicators of water microbial pollution (US EPA 1986; APHA 1998; WHO 2001; Wheeler *et al.* 2002). Total vibrios, including *V. cholerae*, can be considered as model organisms for the abundance of autochthonous flora, and their abundance

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has also been discussed in relation to water organic enrichment (La Rosa *et al.* 2001).

In addition, various bacteriophages may be used as indices for water fecal pollution (US EPA 1986; WHO 2001).

Along with microbial pollution indicators, the saprobic and trophic level of a reservoir can be defined by the biomass and diversity of phyto- and zooplankton (Liebman 1962; APHA 1998; Willen 2000; Ruoppa & Heinonen 2006). Phytoplankton represents a key biological element in natural aquatic environments in terms of primary production, oxygen formation systems and as an important component of the food chain. Furthermore, since the life cycle of algae is relatively short, changes in water quality impact them quickly and by tracking these changes they can serve as effective indicators of water pollution (Golubovskaya 1978; APHA 1998).

Consistent, long-term monitoring of a full spectrum of water quality indicators in aquatic areas, especially those used for recreational purposes, can be crucial for the prevention of many water-borne infections. The ecological status, especially the microbial pollution rate of inland recreational water bodies has not been sufficiently reported in Georgia after the collapse of the Soviet Union. Thus, this paper provides the first detailed data on the ecological status, with a focus on microbial pollution of the inland reservoirs in the vicinity of Tbilisi, of the Tbilisi Sea, Kumisi Lake, and Lisi Lake during the last two decades.

Brief description of the studied water bodies

The three lakes included in this study, the Tbilisi Sea, Kumisi Lake, and Lisi Lake, are located near the capital city of Tbilisi, Georgia (Figure 1). They differ from each other biogeochemically, as well as by anthropogenic load (Barach 1964; Apkhazava 1975; Tediashvili *et al.* 2002; Jaiani 2004; Janelidze 2008). Kumisi Lake was formerly a natural salt lake which was converted into a brackish water reservoir by construction of a tributary channel from the Mtkvari River in the 1960s. Currently, the lake is fed through a channel of the River Algeti. The lake is situated 35 km east of Tbilisi at an altitude of 470 m, has a total surface of 5.0 km², a depth of 4.5 m and watershed area 97 km². Lisi Lake is located northwest of Tbilisi, at an altitude of 624 m, with a surface area of 0.47 km², watershed size

16.1 km² and an average depth of 2.5 m. The Tbilisi Sea, a man-made reservoir created in the 1950s from the waters of the Yori River, is located northeast of Tbilisi at an altitude of 580 m with a total surface of approximately 11.6 km² and a maximum depth of 45 m. The reservoir has one inflow and two outflow irrigation channels. The Tbilisi Sea is characterized by weak winter and well-expressed summer direct stratification. All three bodies of water are intensively used for recreation and fishing, as well as for irrigation. In addition, the Tbilisi Sea is a source of drinking water for a number of districts of Tbilisi.

MATERIALS AND METHODS

Sampling sites

Each lake was sampled at two sites: Kumisi Lake site 1 – N 41°35.153' E 044°51.591', site 2 – N 41°34.839' E 044°51.304'; Lisi Lake site 1 – N 41°44.440' E 044°44.261', site 2 – N 41°44.483' E 044°44.326'; Tbilisi Sea site 1 – N 41°46.150' E 044°48.904', site 2 – N 41°45.765' E 044°50.308'.

Sample collection and processing

The lakes were sampled on a regular basis from June 2006 to November 2008 and an additional three sampling rounds were conducted in the summer of 2009 (July–September). More frequent sampling (twice monthly) was performed during the summer months due to elevated water temperature and increased anthropogenic load that might lead to water quality deterioration. Sampling occurred between 11:00 AM and 3:00 PM, and measurements of hydrochemical parameters, such as temperature, salinity, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were completed on-site using a portable multilog meter (YSI 556 MPS, Yellow Springs Instruments, Colorado, USA). One hundred to one hundred and twenty liters of water were filtered on-site through 200 and 64 µm plankton nets and concentrated plankton samples were fixed with 37% formalin. Plankton-free water (PFW) samples were processed to determine microbiological load within 4 h of collection, as described by Huq *et al.* (2006).

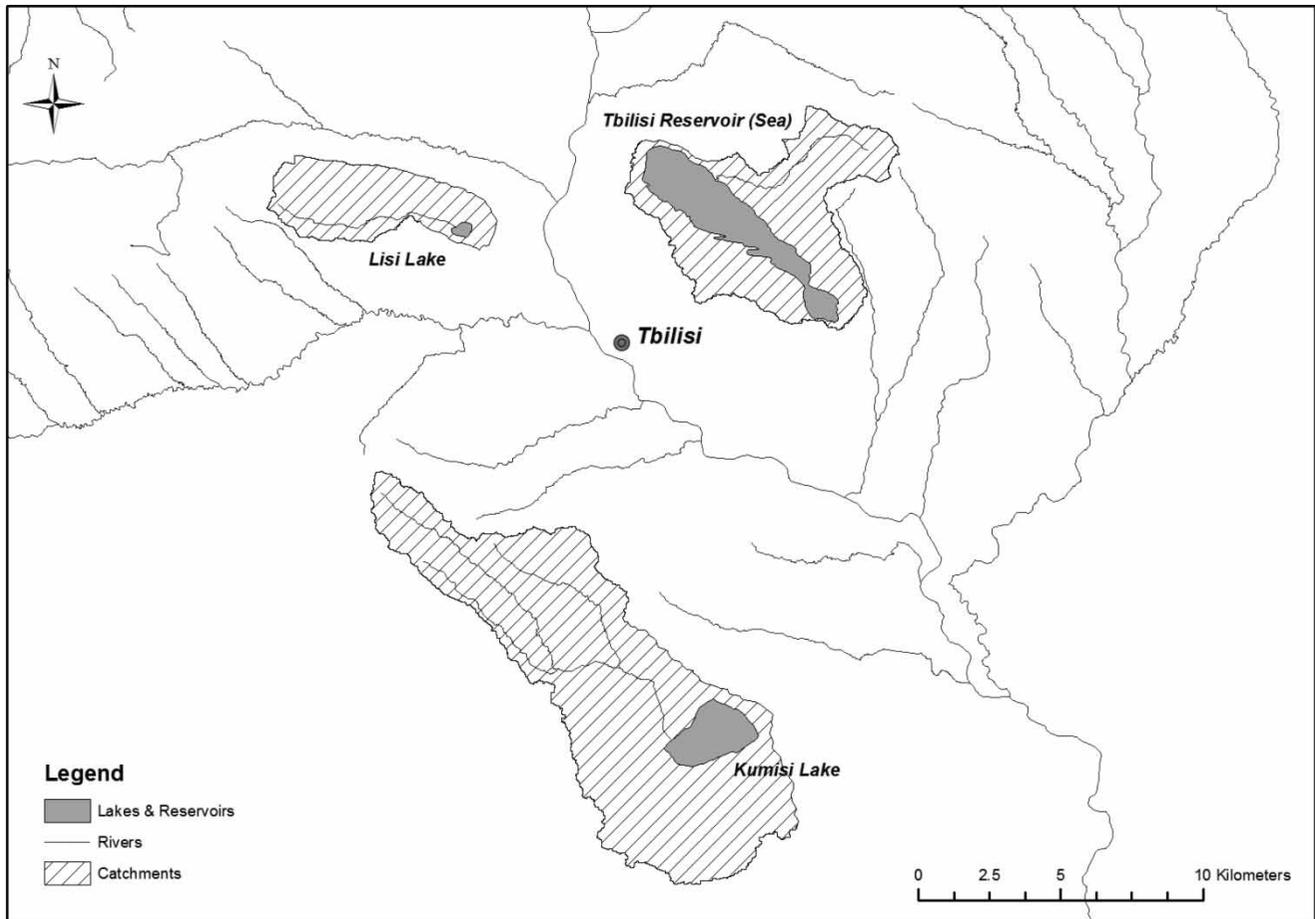


Figure 1 | Map of catchment areas for Tbilisi Sea, Lisi Lake and Kumisi Lake in the vicinity of Tbilisi, Georgia. The map presents only natural small tributaries of the studied water reservoirs. Artificial feeding sources (e.g., Iori channel for Tbilisi Sea and Algeti channel for Kumisi Lake) are not indicated since they partly flow underground. Correspondingly, watersheds of these channels are not included.

A number of microbial parameters of water quality were determined such as total culturable bacteria (TCB), total heterotrophic bacteria (THB), chlorophyll *a*, total phyto- and zooplankton, total and fecal coliforms, total enterococci, total vibrios (as model organisms for autochthonous bacteria), somatic coliphages, and *V. cholerae*-specific phages, along with the physical-chemical parameters, temperature, salinity, pH, DO, and TDS. Approximately 100 L of water samples were collected from two sites at each lake. Since no significant differences were observed between the two sampling sites in terms of microbial and physical-chemical parameters studied, we present data for only a single site throughout this paper. In our studies, measurement of some parameters (TDS, DO, total enterococci) was begun several months later due to technical reasons.

Determination of chlorophyll *a* concentration

Water samples were filtered through 0.45 μm pore-size glass fiber filters (GF/F, Whatman, UK). The filters were frozen until extraction with acetone. Extracted optical density (OD) was measured spectrophotometrically using a Helios β spectrophotometer (Thermo Spectronic, Madison, WI, USA) at optical densities of 663, 645, and 630 nm and the pigment concentration was calculated as described by Holm-Hansen & Riemann (1978).

Determination of microbial parameters

Total culturable bacterial counts (TCBC) in water samples were estimated by serial dilution and plating on trypticase

soy agar (TSA) using a pour plate technique, followed by incubation at 37 °C for 24–48 h.

Heterotrophic plate counts (HPC) in water samples were obtained by serial dilution and plating on HPC agar plates using a spread plate technique followed by incubation at 35 °C for 48 h (Birger 1982; Huq et al. 2006).

Total coliform counts (TCC) and fecal coliform counts (FCC) were obtained by a membrane filtration method: 10–100 mL of water samples were filtered through 0.45 µm cellulose nitrate membrane filters (Whatman, UK), transferred onto mFC-agar plates, and incubated at 37 and 42 °C for 24 h for determination of TCC and FCC, respectively (APHA 1998). Some colonies were selected and stored for further testing in phage isolation studies along with standard strains of *E. coli*. Petrifilms (3M, Minnesota, USA) were used for enumeration of TCC, and *E. coli* counts (ECC) according to the manufacturer's instructions.

Total enterococci counts (TEC) were obtained by membrane filtration using 0.45 µm cellulose nitrate membrane filters (Whatman, UK) on *m-Enterococcus* agar plates which were incubated for 24 h at 35 °C.

Total *Vibrio* counts (TVC) were obtained by filtering 10–100 mL of water through 0.45 µm cellulose nitrate membrane filters (Whatman, UK), which were then placed onto thiosulfate citrate bile-salts sucrose (TCBS) agar plates and incubated at 35 °C for 24 h. TVC were determined by enumeration of yellow and green/olive colonies on TCBS plates (Huq et al. 2006).

Enumeration of total phyto- and zooplankton

Formalin-fixed planktonic organisms were examined using high-resolution microscopes (Leica DMLS and Stereomicroscope M55 (Wetzlar, Germany), and the Kruss biological inverse microscope MBL 3100 (Hamburg, Germany)).

Isolation of *E. coli*- and *Vibrio*-specific phages

Isolation and enumeration of somatic *E. coli*- and *Vibrio*-specific phages were carried out through direct plating of untreated or enriched water samples into freshly isolated cultures of host *E. coli* and *V. cholerae* as well as on susceptible standard strains *E. coli* (C) 600, *E. coli* O₂₆B₆, *E. coli* O₁₁₁B₄H₁₂, *E. coli* K12F+ (Collection of the Eliava Institute),

V. cholerae classic CIP 62.13 (Collection of Institute Pasteur), *V. cholerae* El Tor 890 (M-878), and *V. cholerae* classic 145 (P-1) (Collection of the National Center for Disease Control and Public Health, Georgia). Phage densities were determined first by spot tests followed by enumeration of negative plaques using the agar overlay technique as described by Adams (1961) and Gabrilovich (1973).

Data analysis

All water tests were carried out in triplicate for each sample and the numbers were averaged to create a single value for each variable on a given sampling date. Mean value and standard error were calculated as well. For the purpose of analysis, seasons were conditionally defined based on water temperature as follows: (winter: December–March; spring: April–May; summer: June–September; autumn: October–November).

Statistical analyses were carried out using the Statistical Toolpak for Microsoft Excel 2007, in particular analysis of variance (ANOVA) and multiple regression analysis. All correlations (Pearson's *r*) listed herein were found to be significant at the 0.05 level.

RESULTS

Physical–chemical parameters

Water temperature showed seasonal variations in the studied lakes. The average summer temperature was highest in Kumisi Lake (25.3 °C) followed by Lisi Lake with (24.9 °C) and the Tbilisi Sea (23.3 °C), while the average winter temperature was highest in the Tbilisi Sea (7.5 °C), followed by Kumisi Lake (6.0 °C) and Lisi Lake (5.4 °C). The highest water temperature (29 °C) was registered for Kumisi Lake in August 2006, while the lowest water temperature (1.14 °C) was recorded for Lisi Lake in February 2008.

Results show that of the physical–chemical parameters examined, only DO and temperature had seasonal variation at a significant level ($p < 0.05$). The DO concentration was quite low in all three lakes (range: 3–5 mg/L).

In all three lakes, salinity was more or less constant during the monitoring period with the lowest values for

the Tbilisi Sea ranging from 0.085‰ (March 2007) to 0.17‰ (April 2008) and the highest values for Kumisi Lake ranging from 2.9‰ (May 2007) to 4.96‰ (September 2009). In Lisi Lake, salinity was between 1.35‰ (March 2007) and 1.98‰ (September 2009). The TDS levels, which reflect the content of inorganic and organic matter in water, did not vary significantly in the lakes and, as expected, were somewhat dependent on salinity. Consequently, the highest TDS levels were measured for Kumisi Lake (ranging between 3.55 and 5.77 g/L), followed by Lisi Lake (1.68–2.4 g/L) and the Tbilisi Sea (TDS $l < 0.2$ g/L). The pH values of the lakes were also quite constant and ranged from 6 to 8.7, which is within the range of the typical pH for natural waters (http://www.epa.gov/caddis/ssr_ph_int.html).

Chlorophyll *a* content

In our study, chlorophyll *a* concentrations in the lakes did not demonstrate seasonal variations and were not dependent on any physical–chemical (Table 1) or microbial

parameters examined, representing almost a stochastic profile. However, this parameter appeared to be indicative of the ecological status of the lakes. It is known that chlorophyll *a* content may reflect trophic status of water reservoirs suggesting intensity of algal proliferation. In the Tbilisi Sea, chlorophyll *a* content fell between 0.393 and 8.68 mg/L, which is characteristic of mesotrophic–oligotrophic water reservoirs according to the Chla content in the Carlson's classification system (Carlson & Simpson 1996). In Lisi Lake, the same parameter was higher, ranging from 1 to 18.86 mg/L, which is typical for mesotrophic lakes. The highest chlorophyll *a* values were measured in Kumisi Lake, ranging between 1.95 and 83.5 mg/L, which is characteristic of eutrophic to hypertrophic water reservoirs. Such grouping of lakes is in good agreement with the Vollenweider's classification system, based on the annual averages of Chla concentration (Vollenweider & Kerekes 1980). The Lisi Lake is an exception, which can be attributed to eutrophic lakes in 2008 having an annual average value of Chla (7.9 mg/L).

Fecal pollution indicators

In general, all three lakes had clear seasonal dynamics for the majority of the aquatic microbial parameters and fecal pollution indicators. One-way ANOVA showed significant seasonal variations ($p < 0.05$) in numbers of TCC, FCC, and TEC with highest values in summer, followed by a continuous decrease to the lowest levels in winter months (Figures 2(a)–2(c)). TEC appeared to be the most informative among fecal indicators; 100% of water samples from the Lisi Lake and 91% of the Kumisi Lake in summer months were above threshold levels for total enterococci (Table 2), while in the same period of observation, 47 and 58.8% of samples exceeded threshold levels for total and fecal coliforms in Kumisi lake, respectively (US EPA 1986; WHO 2001; Ministry of Labour, Health and Social Affairs of Georgia 2006). In all three reservoirs, high values for TCC and FCC, indicative of low water quality, were determined throughout the summer months only in 2006, while in 2007–2008, a significant ($p < 0.05$) decrease in the numbers of TCC and FCC was observed. Only a few samples collected in summer and early autumn were above recreational water standards (Figures 2(a) and 2(b)). The

Table 1 | Multiple regression analysis results showing dependence of different microbial indices on physical–chemical parameters

Indices in different lakes	Physical–chemical parameters ^a					Adjusted R ²
	Water temperature	Salinity	pH	DO	TDS	
Kumisi Lake						
FCC	–	–	–	–	–	0.47
TEC	0.002	–	–	–	–	0.47
TVC	0.005	–	–	–	–	0.54
Chla	–	–	–	–	–	0.71
Lisi Lake						
FCC	<0.001	–	–	–	–	0.55
TEC	<0.001	–	–	–	–	0.57
TVC	<0.001	–	–	–	–	0.66
Chla	–	–	–	–	–	0.1
Tbilisi Sea						
FCC	0.007	–	–	–	–	0.49
TEC	0.008	–	–	–	–	0.25
TVC	0.007	–	–	–	–	0.38
Chla	–	–	–	–	–	0.065

^aThe values with $p < 0.05$ are presented.

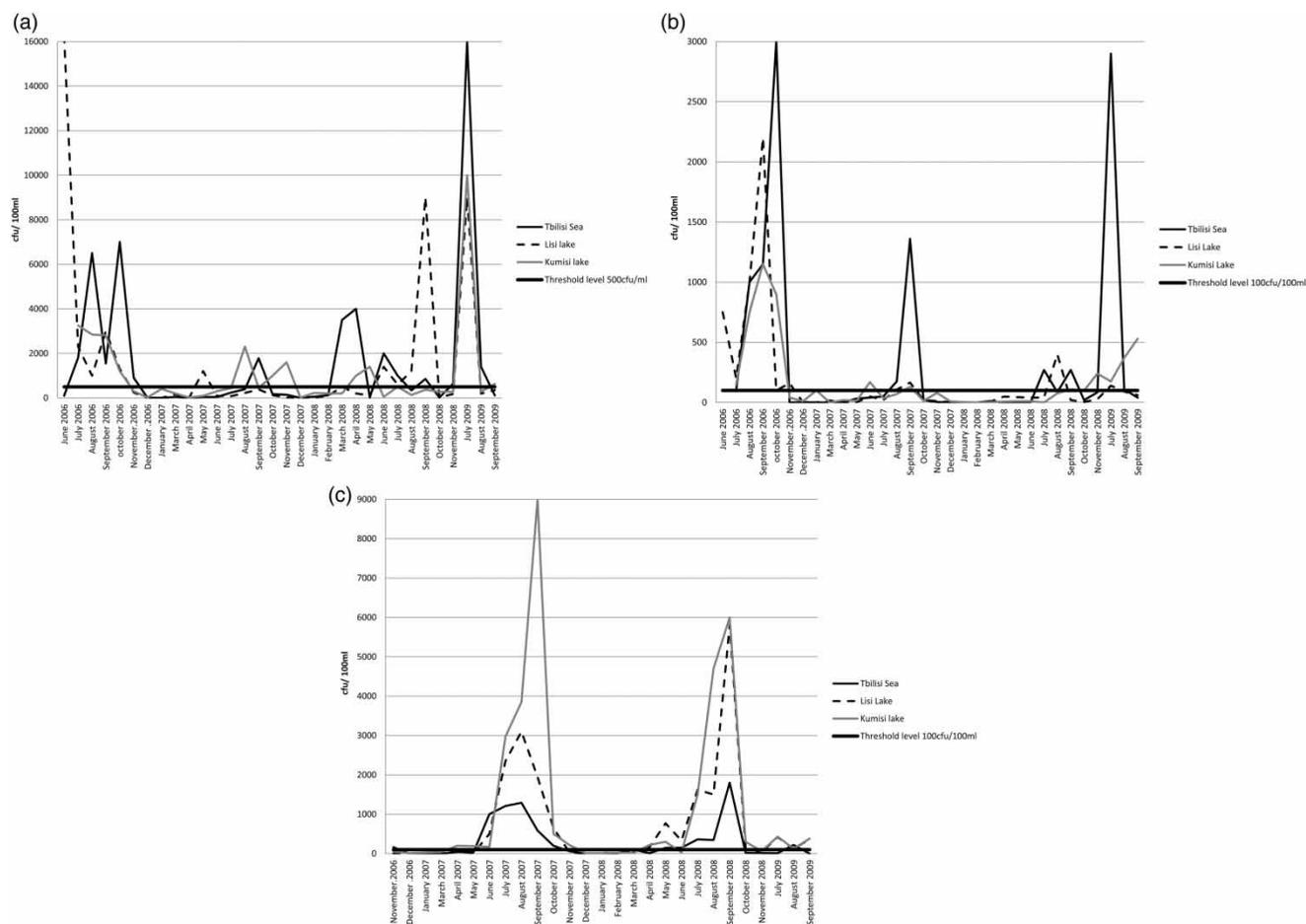


Figure 2 | Dynamics of total coliforms (a), fecal coliforms (b), and total enterococci (c) in Lakes Kumisi and Lisi and the Tbilisi Sea during 2006–2009. Numbers expressed in colony forming units (cfu) per mL.

Table 2 | Percentage of water samples exceeding the threshold levels for different index bacteria in this study

Indices	Percentage (%) of samples exceeding threshold levels					
	Tbilisi Sea		Lisi Lake		Kumisi Lake	
	Summer season	Throughout study	Summer season	Throughout study	Summer season	Throughout study
Total enterococci	81.8	48.8	100	62.2	91.0	63.0
Total coliforms	53.0	45.0	47.0	38.7	47.0	40.0
Fecal coliforms	58.8	34.4	47.0	31.3	58.8	42.0

highest TC numbers were measured in the Tbilisi Sea with 45% of total samples exceeding threshold levels and reaching its highest level in July 2009 at 16,000 cfu/100 mL (Figure 2 (a)). The highest FCC numbers were also measured in the Tbilisi Sea (October 2006 – 3,000 cfu/100 mL; July 2009 – 2,900 cfu/100 mL) (Figure 2(b)), although the percentage of

water samples exceeding threshold levels for index bacteria appeared to be lower in this water body in comparison with Kumisi Lake (Table 2). The highest enterococci counts were detected in Kumisi Lake with 63% of total samples above threshold level (Table 2) reaching 9,000 cfu/100 mL in September 2007 and 6,000 cfu/100 mL in September

2008 (Figure 2(c)). In Lisi Lake and Tbilisi Sea, 62.2 and 48.8%, respectively, of samples were above accepted standards for total enterococci (Table 2). It should be noted that the total enterococci numbers remained elevated (>100 cfu/100 mL) for longer periods in comparison with other fecal indicators in the summers of 2007 and 2008, which reconfirms the importance of enterococci in fresh and brackish water pollution monitoring. According to WHO guidelines (2001), total enterococci value >100 cfu/100 mL in the water reservoirs may account for the significant increase ($>10\%$) of cases of gastroenteritis per single exposure.

Multiple linear regression analysis was utilized to evaluate relationships between physical–chemical parameters (water temperature, salinity, pH, DO, TDS) and levels (lg values) of fecal coliforms and total enterococci. All three lakes gave low p -values (<0.01) only for temperature, suggesting that this parameter could substantially contribute

to the fecal coliform, total enterococci numbers in the studied water systems (Table 1).

Autochthonous microflora: total vibrios

Total vibrios, including *V. cholerae*, can be considered model organisms for abundance of autochthonous flora, and as well, abundance of vibrios has also been discussed in relation to water organic enrichment (La Rosa et al. 2001). In our studies, total vibrios were found to be temperature dependent, showing seasonal shifts and significant quantitative fluctuations in the lakes from several thousand culturable cells per milliliter in the summer months to undetectable levels in the winter months (Figure 3). As shown by multiple regression analysis, from studied physical–chemical parameters, TVC was dependent only on temperature (Table 1). A correlation between dynamic changes of TVC and enterococci was observed ($r = 0.78$ Lisi Lake, $r = 0.38$ Tbilisi Sea, and $r = 0.5$ Kumisi

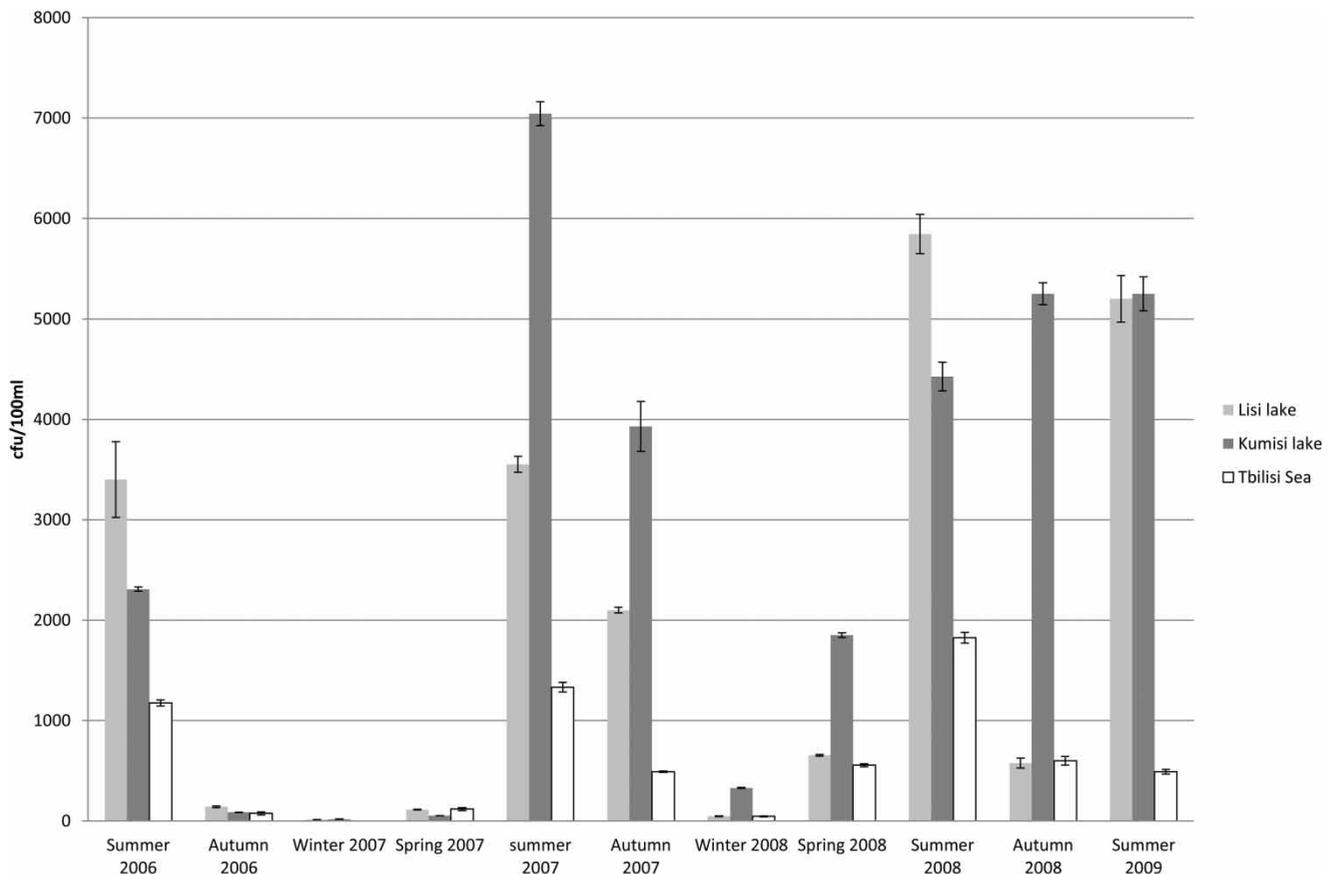


Figure 3 | Seasonal variation of total vibrios in the Tbilisi Sea, Kumisi Lake and Lisi Lake. Error bars indicate ± 1 standard error. Numbers expressed in colony forming units (cfu) per mL.

Lake). Simple linear regression model has been constructed to better evaluate TVC variations using TEC as an independent variable (Figure 4). The scatter plot (Figure 4) shows a linear relationship between these two parameters. There was no significant correlation of TVC with other aquatic microbiological parameters such as TCB, THB, or chlorophyll *a*, which showed less intense seasonal shifts than fecal indicators and TVC. TCB, THB, and Chl*a* showed a mild decrease in abundance during the winter months and more significant number variations within the summer months (data not shown).

Correlation between microbial indices and plankton groups

As phytoplankton composition can also be an important indicator of the ecological status of the water reservoirs, the relationships between the abundance of the five

dominant phytoplankton taxa (Bacillariophyta, Chlorophyta, Cyanophyta, Pyrrophyta, Chrysophyta), and the fecal pollution indicators and TVC, were analyzed. Detailed data on phytoplankton composition and diversity have been reported earlier (Kalandadze et al. 2009).

In Lisi Lake, it was found that fecal indicators, mainly TEC and TVC, showed positive correlation with several prevailing phytoplankton groups. Namely, correlation was observed between TEC and Bacillariophyta ($r = 0.46$), Chlorophyta ($r = 0.4$), and Pyrrophyta ($r = 0.38$). From five phytoplankton groups TVC was positively correlated with Bacillariophyta ($r = 0.5$) and Chlorophyta ($r = 0.368$).

Of seven phytoplankton taxa detected in the Tbilisi Sea with a prevalence of Bacillariophyta (Kalandadze et al. 2009), only Cyanophyta showed correlation with fecal indicators, namely with TCC ($r = 0.67$).

Kumisi Lake appeared to contain the most abundant phytoplankton populations among the three lakes

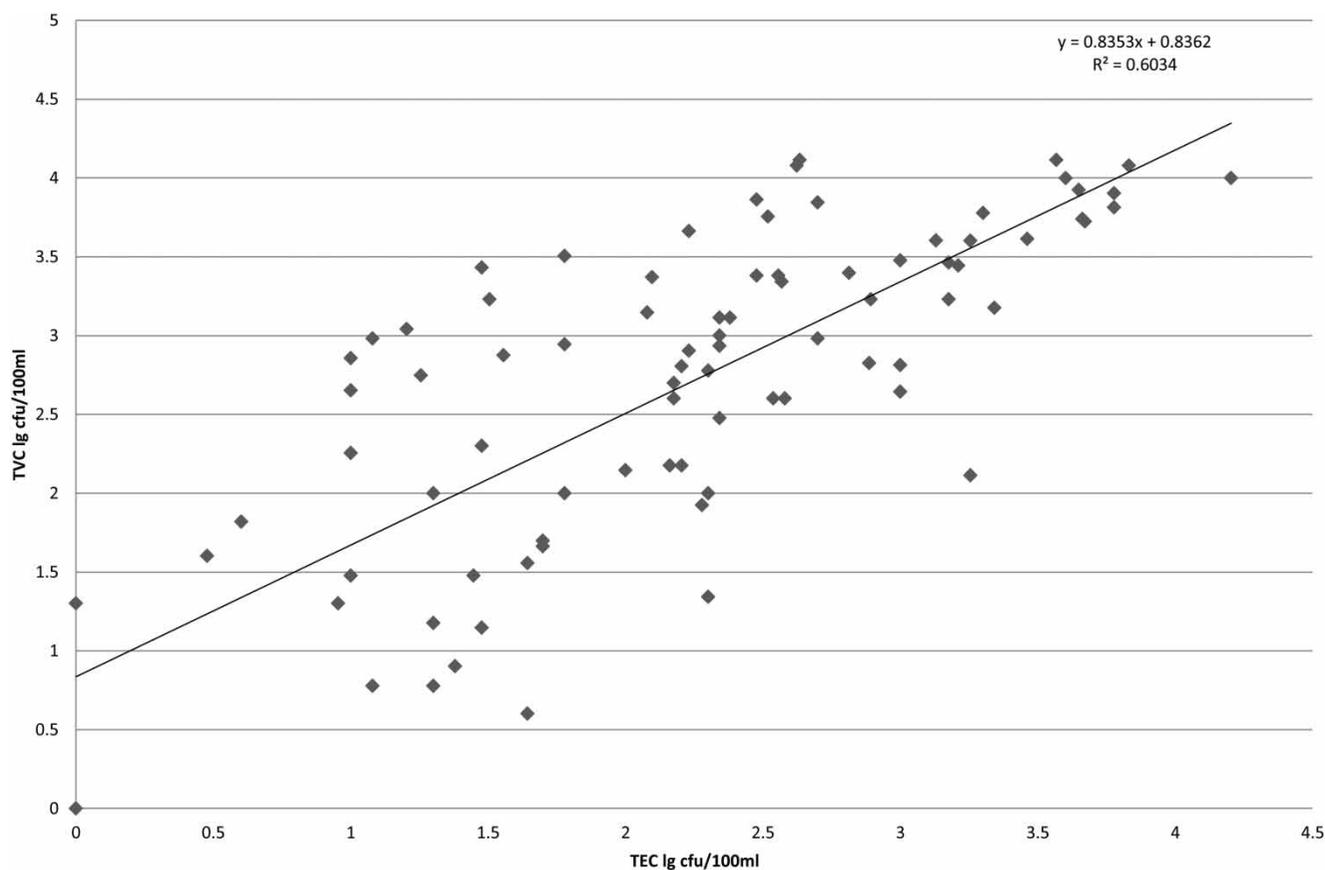


Figure 4 | Scatter plot and regression model for lg values of TEC (independent variable) and TVC (dependent variable). Combined data for three studied water reservoirs.

examined; 95% of all algae in Kumisi Lake was represented by Cyanophyta, including toxic species *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Oscillatoria planktonica*, and *O. limnetica* (Kalandadze et al. 2009). Cyanobacteria were present in high numbers (from 5×10^4 to 1×10^6 cell/mL) in Kumisi Lake water throughout the study and showed only slight seasonal shifts. Correspondingly, no correlation was found with fecal indicators; however, a correlation was observed between TCC and Chrysophyta ($r = 0.6$).

In addition to correlation analysis, multiple linear regression was used to estimate the impact of fecal pollution on phytoplankton abundance. Interestingly, Cyanophyta seem to be affected by fecal coliforms in two studied reservoirs, the Tbilisi Sea ($p < 0.001$, adjusted $R^2 = 0.49$) and Kumisi Lake ($p = 0.01$, adjusted $R^2 = 0.28$). Also two phytoplankton groups, Chrysophyta (in Kumisi Lake) and Bacillariophytain (Lisi Lake) might be influenced by TCC ($p < 0.001$, adjusted $R^2 = 0.62$) and TEC ($p = 0.02$, adjusted $R^2 = 0.18$), respectively.

No significant correlation was observed between zooplankton abundance and microbial pollution indices in the lakes examined in this study. In all three lakes studied, zooplankton was mainly represented by *Rotatoria* (dominant group), *Copepoda*, and *Cladocera* (Kalandadze et al. 2009) and fluctuations of these taxonomic groups of zooplankton did not correlate significantly with fecal indicators or abundance of vibrios (TVC) in this study.

Bacteriophages

The content/abundance of *E. coli*- and *V. cholerae*-specific phages in all three water bodies in different seasons were evaluated through enumeration of water samples positive for these phages (Figures 5(a) and 5(b)). *E. coli*-specific somatic indicator phages and *V. cholerae* specific phages showed seasonality with peaks measured during summer months and mostly proportional to host bacterial numbers. In all three lakes, positive correlation between coliphages and FCC ($r = 0.34$ for Lisi Lake, $r = 0.36$ for Kumisi Lake, and $r = 0.36$ for Tbilisi Sea) was determined as well as between *V. cholerae* phages and TVC ($r = 0.47$ for Lisi Lake, $r = 0.57$ for Kumisi Lake, and $r = 0.36$ for Tbilisi Sea). Coliphages were also detected in a few samples

collected in winter months. The frequency of phage isolation was lower in the Tbilisi Sea in comparison with the Lisi and Kumisi Lakes (with highest values for Kumisi Lake) and can be considered an additional demonstration of the better water quality status of the Tbilisi Sea.

DISCUSSION

Our studies revealed high values of fecal indicators in the summer and early autumn in three inland lakes near the Georgian capital city of Tbilisi. Based on phytoplankton counts, physical-chemical and microbial parameters, Kumisi Lake appeared to be the most polluted of the three lakes studied. This may be due to intense agricultural activities carried out in the catchment area. The catchment includes Kumisi village with a population of up to 2,500 people, along with small cattle farms and a large poultry farm 'Kumisi' (with more than 200,000 chickens). Untreated sewage from villages and runoff from farms and pastures are important sources of pathogens, as well as a source of nitrogen and phosphorus, which promote growth and proliferation of algae, including toxic cyanobacteria. The high abundance of Cyanophyta in Kumisi Lake ($>100,000$ cells/mL) is representative of the WHO guidelines (2001) value of moderate to high health alert for human and livestock in relation to its ability to produce cyanotoxins. A lower guideline of 40,000 cells/mL is estimated to correlate with the production of 8 $\mu\text{g/L}$ of microcystin (Chorus & Bartram 1999). Thus, it can be assumed that the periodic mass die-offs of fish in Kumisi Lake may be due to toxic cyanophyta blooms and related hypoxia. Also high chlorophyll *a* concentrations in Kumisi Lake in comparison with the other two lakes studied may be linked to the massive proliferation of the cyanobacteria (Kalandadze et al. 2009).

According to our results, the Tbilisi Sea was the least polluted body of water examined. This may be due to the less intense load of pollutants from the catchment area (i.e., less-developed agriculture), as well as the nature of the reservoir itself. The Tbilisi Sea is a relatively new artificial water reservoir, while Lisi and Kumisi lakes are natural water reservoirs in which natural eutrophication began long ago. Furthermore, the Tbilisi Sea has a much larger surface area and depth in comparison to Kumisi

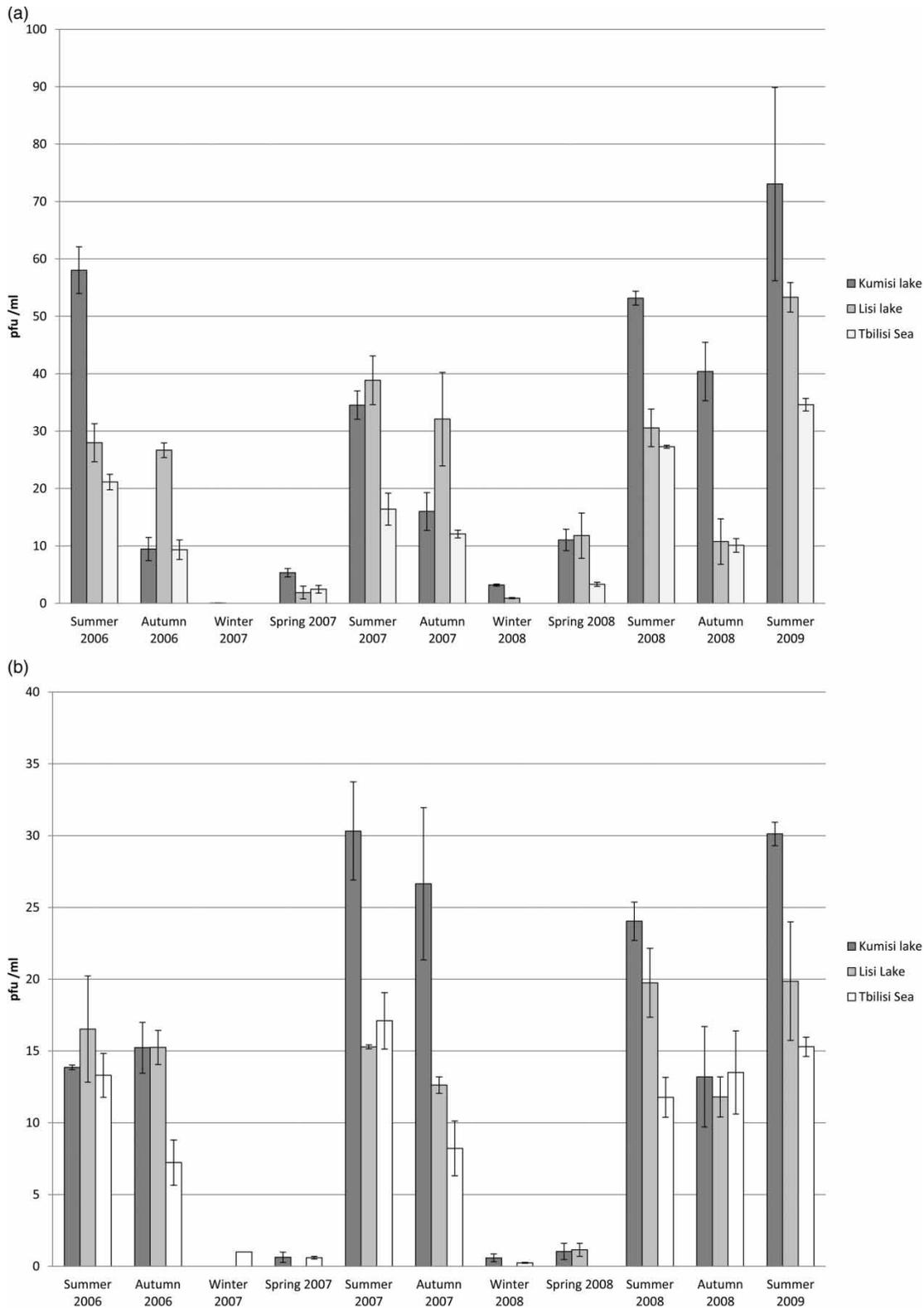


Figure 5 | Seasonal variations in isolation rate of coliphages (a) and *V. cholerae* phages (b) in the studied lakes. Error bars indicate ± 1 standard error. Phage numbers expressed in plaque forming units (pfu) per mL.

and Lisi lakes. In addition, the Tbilisi Sea is a 'running-water reservoir' with inflow and outflow channels, which contribute to higher flushing rates and correspondingly dilution of incoming pollutants from the catchment area and recreational activities. Kumisi Lake is characterized by a high fluctuation of water level depending on the current of the tributary channel from the River Algeti and also on the intensity of the lake water used for irrigation of adjacent agricultural areas. In the summer to autumn months, the shallow water ultimately favors the accumulation of microbial, organic, and inorganic pollutants.

Water temperature is another important abiotic factor governing various parameters in aquatic environments, including microbial activity and growth (Golubovskaya 1978). The Tbilisi Sea is characterized by the lowest water temperatures in the summer months and the highest water temperatures during the winter months among the three lakes. This is likely due to the large volume of the Tbilisi Sea, and its vertical stratification. Since both Kumisi Lake and Lisi Lake are relatively shallow, the water should be well mixed most of the time. The highest water temperature was measured in Kumisi Lake in summer months, which likely contributes to increased microbial pollution and eutrophication of this reservoir. As shown by multiple regression analysis, the water temperature seems to significantly influence the water microbial pollution indicator levels in this study. Therefore, the difference in water microbial quality between the studied water reservoirs can also be explained by differences in water temperatures along with other (anthropogenic) factors.

Water quality monitoring protocols (US EPA 1986; WHO guidelines 2001) recommend testing of fresh water lakes for enterococci counts, in addition to other indicators, such as total and fecal coliforms. During the study, enterococci content exceeded the threshold values in all three lakes where low values of fecal and total coliforms were measured. Moreover, enterococci indicated low microbial water quality of the studied water reservoirs for longer periods than fecal coliforms. Thus, our findings reinforce the importance of enterococci in monitoring of fresh and brackish water reservoirs.

Similar to fecal indicators, higher TVC values were seen in Kumisi Lake, although in some cases, levels in Lisi Lake occasionally exceeded those seen in Kumisi Lake. The lowest TVC values were observed in the Tbilisi Sea. These

differences may be due to different pollution levels in the lakes as well as differences in water temperature (see above) and salinity. Since Kumisi and Lisi lakes are both brackish water lakes, vibrios are supposed to grow well (Pruzzo *et al.* 2005). In fact, in our previous report, we demonstrated the endemicity of toxigenic *V. cholerae* in these lakes (Grim *et al.* 2010). This indicates the necessity to carry out surveillance, not only for fecal indicators of pollution, but for pathogenic vibrios, including toxigenic *V. cholerae*, as a preventive measure for the potential outbreak of cholera.

The detection of certain bacteriophages can also be used as a measure of bacterial pollution. In our study, we found both *E. coli*- and *V. cholerae*-specific phages in all three lakes, with the highest abundance found in Kumisi Lake. These results likely reflect overall water quality of these lakes as they correlate well with total enterococci counts and other indicators of pollution (i.e., total and FCCs). Significant correlation between abundance of phages and their hosts is not surprising, since environmental conditions which promote the proliferation of host bacteria may also permit the replication of their phages (Jiang *et al.* 2007). In our study there were cases of phage detection, especially in winter months, without isolation of the host bacteria, probably due to better survival of the phages in the water environment, demonstrating the importance of phages as pollution indicators in water quality monitoring in regions with temperate climates.

The correlation between certain phytoplankton taxa and microbial parameters may indicate a possible indirect influence of microbial pollution on phytoplankton abundance since organic and inorganic load may promote both algal and bacterial proliferation.

Recent studies have demonstrated a correlation between sulfates, nitrates, and total and fecal coliforms (Nirmal Kumar 2009). Furthermore, fecal indicators may originate from different sources, namely total coliforms from soil, vegetation, and animals, while fecal coliforms and enterococci are found mostly in warm-blooded animals and thus may enter the water with their specific complexes of organic or inorganic compounds. This may explain the different response of particular phytoplankton taxa to specific fecal indicators and TVC.

In our study, there was no correlation between zooplankton abundance and microbial pollution indices in the

lakes examined. While this finding is not in agreement with previous observations of other investigators (Simidu *et al.* 1977; Huq & Colwell 1995) demonstrating association of aquatic bacteria including *V. cholerae* with copepods, the absence of correlation could be explained by non-temperature dependent profile in zooplankton dynamics in our study while vibrios are known to be strictly temperature dependent.

Based on our data, during the summer to autumn period, Lisi Lake has an intermediate state between moderately and polluted water according to Georgian water quality classification standards (Ministry of Labour, Health, and Social Affairs of Georgia 2006). Likewise, it can be classified between sufficient and poor quality water bodies according to Directive 2006/7/EC of the European Parliament and of the Council (2006). It should be noted that in our earlier assessments (Kalandadze *et al.* 2009), Lisi Lake was classified as a β -oligosaprobic- β -mesosaprobic water reservoir based on the presence of indicator phytoplankton species (Liebman 1962; Willen 2000). In the same study (Kalandadze *et al.* 2009), the Tbilisi Sea was classified as an oligosaprobic- β -oligosaprobic water reservoir. According to European criteria, in the summer and autumn months, water quality in the Tbilisi Sea can be sufficient to poor, while by Georgian standards (Ministry of Labour, Health, and Social Affairs of Georgia 2006), it is classified as a moderately polluted reservoir in the warm season. The fact that classification by microbial parameters shows higher pollution status in comparison with the saprobic level may be due to lower organic and inorganic loads, but increased fecal pollution.

The Kumisi Lake in the summer and autumn months can be classified as a poor water quality reservoir (Euro Commission Directive 2006), while by Georgian standards (Ministry of Labour, Health, and Social Affairs of Georgia 2006), it corresponds to a moderately polluted to a polluted water reservoir. In our recent paper (Kalandadze *et al.* 2009), Kumisi Lake was classified as α , β -mesosaprobic water reservoir.

CONCLUSIONS

Based on our results, we conclude that the water quality of all three lakes during the summer and early autumn did

not comply with the Georgian and international water quality criteria for recreational waters.

Enterococci numbers indicated low water quality for longer periods than total and fecal coliforms. This confirms the importance of enterococci in water pollution estimation in fresh and brackish water bodies.

Total vibrios, used as model organisms for the abundance of autochthonous flora, positively correlated with numbers of total enterococci.

Particular phytoplankton groups responded differently to changes in numbers of fecal indicators.

Detection of *E. coli*- and *V. cholerae*-specific phages was proportional to recovery of host bacteria from water samples with the exception of some winter months.

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