Article

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HALLER, Laurence, *et al*. Influence of Freshwater Sediment Characteristics on Persistence of Fecal Indicator Bacteria. *Water, air and soil pollution*, 2009, vol. 203, no. 1-4, p. 217-227

DOI : 10.1007/s11270-009-0005-0

Water Air Soil Pollut (2009) 203:217–227
DOI 10.1007/s11270-009-0005-0

Influence of Freshwater Sediment Characteristics on Persistence of Fecal Indicator Bacteria

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Received: 17 October 2008 / Accepted: 26 January 2009 / Published online: 7 March 2009

Abstract Extended persistence of enteric bacteria in coastal sediments and potential remobilization of pathogens during natural turbulence or human activities may induce an increased risk of human infections. In this study, the effect of sediment characteristics such as particle grain size and nutrient and organic matter contents on the survival of fecal indicator bacteria (FIB) including total coliforms, *Escherichia coli*, and *Enterococcus* was investigated. The experimentation was carried out for 50 days in microcosms containing lake water and different contaminated freshwater sediments in continuous-flow and batch conditions. Results of this study revealed: (1) extended FIB survival in sediments up to 50 days, (2) higher growth and lower decay rates of FIB in sediments with high levels of organic matter and nutrients and small (mainly silt) grain size, and (3) longer survival of *Enterococcus* sp. compared to *E. coli* and total coliforms. FIB survival in sediments and possible resuspension are of considerable significance for the understanding of permanent microbial pollution in water column and therefore human risk during recreational activities.

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Keywords Fecal indicator bacteria · Sediments · Organic matter · Nutrients · Survival · Human health risk

1 Introduction

The origin of pathogenic bacteria in surface water includes municipal wastewater treatment plant (WWTP) discharges, agricultural or storm runoff, and other diffuse sources of human and animal wastes. Fecal indicator bacteria (FIB) including *Escherichia coli*, *Enterococcus* sp. (ENT), and total coliform (TC), residing in the gastrointestinal tract of humans and animals, are commonly used to assess the microbiological safety of drinking and recreational waters. Studies have shown that FIB from different sources can be accumulated and distributed in freshwater sediments (Burton et al. 1987; LaLiberte and Grimes 1982; Davies et al. 1995). The US Environmental Protection Agency (2000) and the European Union (2006) recommend the use of *E. coli* and ENT, to assess the hygienic safety of recreational waters.

In the aquatic environment, sediments may constitute a reservoir of different pollutants including inorganic and organic compounds and microorganisms. Accumulation and survival of FIB and pathogenic organisms in sediments have become a subject of increasing interest due to their negative impact on surface water quality. The survival of fecal bacteria,

once released in the aquatic environment, is determined by numerous environmental factors including temperature variations, salinity, oxygen levels, nutrient deficiencies, predation, and ultraviolet irradiation (McFeters and Singh 1991; Davies et al. 1995; Thomas et al. 1999; Hughes 2003; Craig et al. 2004).

Several field and laboratory experiments have documented FIB survival and growth in aquatic systems, especially in the presence of sediments (Gerba and McLeod 1976; LaLiberte and Grimes 1982; Craig et al. 2004; Lee et al. 2006). It has been shown that the level of FIB in aquatic compartments including water column, interstitial water, and sediment varied with seasonal period and environmental conditions (Hughes 2003; Davies et al. 1995; Goldscheider et al. 2007). There is evidence that FIB can survive and accumulate in sediments at levels of 100 to 1,000 times higher than in overlying waters (Ashbolt et al. 1993). Higher concentrations of FIB and pathogenic organisms in sediments have been attributed to the sorption of microorganisms to particles suspended in water, which then sediment out (Davies et al. 1995; An et al. 2002; Alm et al. 2003). FIB can also survive longer in sediments than in the water column since sediments provide favorable nutrient conditions (Gerba and McLeod 1976; LaLiberte and Grimes 1982), protection from sunlight inactivation (Sinton et al. 1999), and protozoan grazing (Davies and Bavor 2000). Resuspension of pathogenic organisms from sediments to the water column, due to recreational activities such as swimming in beach waters and natural turbulence such as currents, waves, or floods, may increase the risk of human infection (An et al. 2002; Evanson and Ambrose 2006), either by direct ingestion or by infiltration into the groundwater (Wildi et al. 2004).

Numerous studies relied on laboratory microcosm experiments to evaluate FIB and pathogen survival in the aquatic environment (Gerba and McLeod 1976; Burton et al. 1987; Thomas et al. 1999, Ghoul et al. 1990; Craig et al. 2004; Anderson et al. 2005). Most of these studies used bacterial strains inoculated in sediment microcosms to monitor the influence of environmental factors such as organic matter, temperature, and ultraviolet radiation on their survival. Microcosms are a very useful tool to understand the complex influence of biotic and abiotic factors on FIB persistence (Fish and Pettibone 1995). Unlike in situ experiments, the use of microcosms facilitates the investigation of fecal bacteria response to specific environmental conditions in isolation (Craig et al. 2004).

Levels of nutrients, organic matter, and grain size may vary considerably according to the type of coastal sediments and may influence microorganism persistence. There is a considerable interest in studying the influence of these parameters on FIB survival in sediments, as these microorganisms and pathogens can be remobilized to the water column, inducing bacteriological pollution and greater risks for human health during recreational activities. The objective of this study was to determine the influence of sediment characteristics including grain size and nutrient and organic matter levels in fresh water sediments on the survival of FIB. The experiment was carried out for a period of 50 days using microcosms. Microcosms were designed to simulate lake conditions and were constituted of lake water and sediments which present different characteristics. The following three sites were selected on the basis of their varying organic matter and nutrient contents: the Bay of Vidy (Lake Geneva, Switzerland, very rich in organic matter and nutrients), Lake Bret (eutrophic), and the mouth of the Versoix River in Lake Geneva (poor in nutrients). Before the start of the study, sediments were contaminated with sewage water, containing high concentrations of fecal bacteria. The experimentation was carried out in continuous-flow and batch microcosm conditions. The physicochemical parameters including water temperature, dissolved oxygen, conductivity, and pH were monitored during the whole experimentation.

2 Materials and Methods

2.1 Study Sites and Sampling

The sediments were collected from three sites: the Bay of Vidy, Lake Bret, and the mouth of the Versoix River (Fig. 1). The global positioning system locations of the sampling sites are presented in Table 1.

The Bay of Vidy is located near Lausanne city, on the northern shore of Lake Geneva. Lake Geneva is the Western Europe's largest freshwater reservoir with a volume of 89 km^3 and a maximum depth of 309 m. Lausanne, a city of 130,000 inhabitants, discharges the largest volumes of

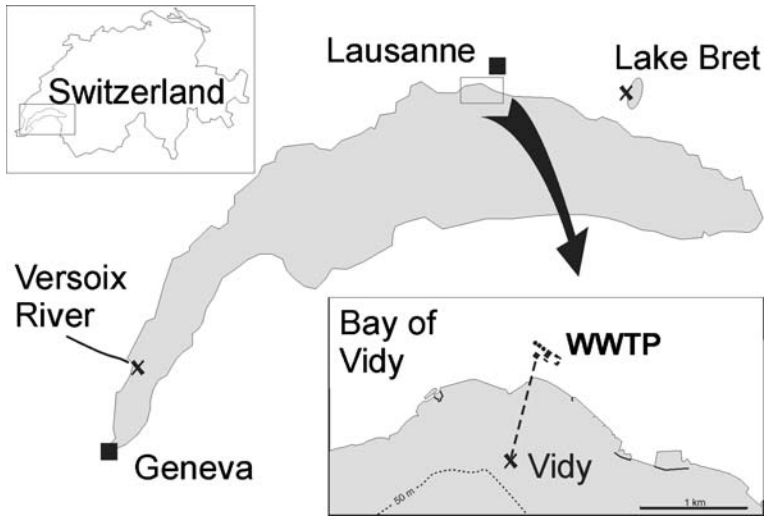

Fig. 1 Map of the study area with the positions of the three sampling points

treated domestic and industrial wastewater into the bay. The WWTP treats 1 to 3 m^3 s^{-1}. Treated wastewater is released via an underwater pipe at 700 m from the shore and 30-m water depth. As a result, this bay is the most contaminated area of Lake Geneva (Loizeau et al. 2004; Poté et al. 2008a, b) and sediments are extremely rich in organic matter and nutrients.

Lake Bret is a small and shallow reservoir lake in the surrounding area of Lausanne city. It has a volume of 3 km^3, a length of approximately 1.5 km, and a maximum depth of 20 m. It is a eutrophic lake, poor in oxygen and rich in nutrients.

The Versoix River is a small river flowing from the Jura mountains (France) into Lake Geneva, with an annual average discharge of 3.4 m^3 s^{-1}. The mouth of the river is poor in nutrients and receives no WWTP discharges.

Surface sediments (layer of 0–3-cm thickness) from the Bay of Vidy were collected in front of the WWTP outlet pipe at 40-m water depth, from a boat, using a "Ponar-type" grab sampler (SDEC, France). Sediment samples from Lake Bret and the Versoix River were taken manually at 1 m from the shore. All sediment samples were placed into sterile plastic containers and stored in a cold room at 4°C prior to analysis.

The WWTP effluent water was sampled directly from the WWTP outlet pipe. Lake water, used for the microcosms, was sampled in the area of Versoix at 5-m depth with a centrifugal pump and filtered at 1.2 μm (CUNO filter).

2.2 Microcosms

Microcosms consisted of plastic aquaria of a size of 46.5-cm length, 22-cm width, and 26-cm height with an overflow at 21 cm. Prior to the experiment, all microcosm equipment were rinsed with a solution of HCl (1 N) and with deionized water. Microcosms were designed to simulate lake conditions. They contained sediments from three different sites as mentioned above and water from Lake Geneva. The sampled sediments were first homogenized with a spatula and filled into the microcosms to a height of 2–3 cm. They were then flooded for 24 h with 5 L of treated wastewater coming from the WWTP. According to a previous study (Poté et al. 2008a, b), concentrations of TC, *E. coli*, and ENT ranging between 10^6 and 10^9 colony-forming units (CFU) 100 mL^{-1} are present in the WWTP effluent water (no microbiological treatment of water from WWTP). After 24 h, contaminated water was removed and replaced with 18 L of lake water.

Table 1 Global positioning system location of sampling sites in Swiss coordinates

Sampling sites	*X*	*Y*
Bay of Vidy	534,676	151,543
Lake Bret	548,817	151,659
Versoix River	502,230	125,625

2.3 Survival Study

Two series of experiments were performed: a series of three continuous-flow microcosms and a series of three batch microcosms. Each microcosm from each group contained sediments from a selected site and was named as followed:

- MV1: sediments from Vidy + lake water (water renewal)
- MB1: sediments from Bret + lake water (water renewal)
- MVe1: sediments from Versoix + lake water (water renewal)
- MV2: sediments from Vidy + lake water (batch)
- MB2: sediments from Bret + lake water (batch)
- MVe2: sediments from Versoix + lake water (batch)

Continuous-flow microcosms were supplied with lake water, pumped from a reservoir with a peristaltic pump (Tygon R-3607), through silicone tubings. The renewal rate of water was about 10 L day^{-1} so the mean renewal time of a microcosm was approximately 1.8 days. Water was introduced in microcosms at mid-height close to the wall opposite to the overflow. All microcosms were thermostabilized and kept in a room with artificial light at a temperature of 20±2°C.

During experimentation, microcosm sediments were sampled on days 1, 5, 10, 20, 30, 40, and 50 for bacterial analysis. For each time of sampling and each set of conditions, all the analyses were conducted in triplicate.

2.4 Sediment and Water Characterization

The grain size distribution was measured using a particle size analyzer, Coulter® LS-100 (Beckman Coulter, Fullerton, CA, USA), following ultrasonic dispersal in deionized water (Loizeau et al. 1994). The proportions of three major size classes (clay <2 μm; silt 2–63 μm; and sand >63 μm) were determined from size distributions, as well as the median grain size.

The sediments were dried at 60°C during 48 h and the water content was calculated from weight difference. The sediment total organic matter content was measured by loss on ignition at 550°C for 1 h in a Salvis oven (AG Emmenbrücke, Luzerne, Switzerland). Total nitrogen and ammonium concentrations in the sediments were measured by using the method of Kjeldhal (1883). Total phosphorus and its different forms were determined following the fractionation scheme of Williams et al. (1976) as modified by Burrus et al. (1990). Sediment characteristics were measured before and at the end of the experimentation.

Water physicochemical parameters including conductivity, temperature, pH, and concentration of dissolved oxygen were measured using a Multi 350i (WTW, Germany) probe, each time sediment microcosms were sampled for microbiological analysis.

2.5 Bacteria Quantification

Fecal indicator bacteria including TC, *E. coli*, and ENT were quantified in the different sediment samples. Bacteria were resuspended by adding 100 g (wet weight) of sediment to 500 mL of 0.2% sodium hexametaphosphate ($Na_6(PO_3)_6$) in 1-L sterile plastic bottles and mixed for 30 min using the agitator rotary printing press Watson-Marlow 60 1 controller (modified methodology from Balkwill and Ghiorse 1985). The mixture was centrifuged at 4,000 rpm for 15 min at 15°C. FIB in the supernatant were then counted according to the Swiss standard methods for water quality determination, using the membrane filtration method (OHyg 2005). For each sample, triplicates of 20 mL of supernatant were passed through a 0.45-μm filter (47-mm diameter, Millipore, Bedford, USA), which was placed on different FIB culture media (Biolife, Italiana), supplemented with the antifungal compound Nystatin (100-μg mL^{-1} final concentration), using the following incubation conditions: TC: Endo agar medium, incubated at 35°C for 24 h; *E. coli*: tryptone soy agar medium, incubated at 30°C for 4 h and transferred to tryptone bile x-glucuronide medium at 44°C for 24 h; ENT: Slanetz Bartley agar medium, incubated at 44°C for 48 h and transferred into Bile Aesculin agar medium at 44°C for 4 h. The results are expressed as CFU per 100 g of dry sediments (CFU 100 g^{-1}).

2.6 Statistical Analysis

To facilitate comparisons between indicators and types of sediments, the decay rate constant (k) was estimated, by fitting experimental results to the following equation: $N_t = N_0 \times e^{-kt}$, where N_t is the number of bacteria at time t and N_0 is the number of

bacteria at time $t=0$ ($t=0$ corresponds to the start of the decay phase; Davies and Evison 1991). The decay rate constants measured for each indicator in every microcosm were compared by the standard statistical t test. Statistical treatment of data has been realized using SigmaStat 3.11 (Systat Software, Inc., USA).

3 Results

3.1 Water Characteristics

Table 2 shows the physical and chemical characteristics of water monitored throughout the whole experimentation period, in each microcosm. Water temperature was kept at near-constant levels at 20±2°C. pH remained between 7.3 and 8.5 in all microcosms over the sampling period. In the continuous-flow microcosms, conductivity stayed constant with an average of 285 μS cm^{-1}, but, in the batch microcosms, it gradually increased with time. The measured values (beginning–end of experimentation) were 286–404, 276–701, and 279–512 μS cm^{-1} for MV2, MB2, and MVe2, respectively. In the lake pre-filtered water, conductivity values ranged from 270 to 280 μS cm^{-1}. Dissolved oxygen dropped significantly in the microcosms with sediments from Vidy (MV1 and MV2), from 8 mg L^{-1} at the beginning of the experiment to approximately 0.6 mg L^{-1} at the end. In the microcosms with sediments from Bret and Versoix, dissolved oxygen was around 8 mg L^{-1} on day 1, decreasing slightly during the sampling period to reach values between 5.3 and 6.3 mg L^{-1} at the end of the study.

3.2 Sediment Characteristics

Sediment characteristics including particle grain size and organic matter and nutrient contents are given in Tables 3 and 4. These parameters were measured at the beginning and the end of the experimentation. Sites were chosen to investigate the effect of sediment type on FIB survival. Sediments from the Bay of Vidy showed a high organic matter content of about 21%. This value is higher than the values measured on other sample sites: 12.6% and 1.8% for the sediments from Lake Bret and Versoix, respectively. Concentrations of total nitrogen, ammonium, and total phosphorus measured at the start of the experiment were also higher in the sediments of Vidy, 12.6, 1.6, and 6,784 ppm, respectively, while they were considered intermediate in the sediments from Bret (4.7, 0.6, and 791 ppm, respectively) and low in the sediments from Versoix (0.5, 0.1, and 420 ppm, respectively).

Sediments varied also in terms of grain size, with a proportion of 72% of silts in Vidy to approximately 48% in the sediments of Bret and Versoix.

Between the beginning and the end of the experiment, organic matter decreased significantly in the microcosms with sediments from Vidy, from 21% at the start to a final level of 12% in MV1 and 18% in MV2, while it remained quite stable in the other microcosms. Initial concentrations of total nitrogen and ammonium in sediments from Vidy also decreased significantly by approximately 33% and 62%, respectively. In sediments from Bret, total nitrogen and ammonium levels decreased by about 21% and 25%, respectively, and, in sediments from Versoix, they stayed quite low during the whole experimentation period. Values showed an average decrease of 13% of organic phosphorus in the Vidy sediments while they remained quite constant in the other microcosms.

3.3 Survival Study

Sediments of all microcosms were contaminated for 24 h with water coming from the WWTP. FIB analysis after contamination gave initial concentrations ranging

Table 2 Some water characteristics

Microcosms	Dissolved oxygen (mg L^{-1})	Conductivity (μS cm^{-1})	pH
MV 1	0.6–8.0	278–293	7.3–7.8
MV 2	0.8–7.9	286–404	7.4–7.8
MB 1	6.3–8.2	275–292	7.9–8.5
MB 2	5.3–8.3	276–701	7.8–8.5
MVe 1	6.3–8.4	278–295	7.9–8.5
MVe 2	5.8–8.5	279–512	7.9–8.5

Range over a 50-day test period

Table 3 Sediment characteristics at day 0

Sediments	Organic matter (%)	Ntot[a] (mg kg^{-1})	NH4-N[b] (mg kg^{-1})	PTot[c] (mg kg^{-1})	PO[d] (mg kg^{-1})	NAIP[e] (mg kg^{-1})	Clay/silt/sand proportion (%)
Vidy	21.2	12.6	1.6	6,783.8	688.2	3,103.0	0/72/28
Bret	12.6	4.7	0.6	791.2	364.5	113.5	0.3/47.8/51.9
Versoix	1.8	0.5	0.1	420.1	33.8	41.5	0.1/48.4/51.5

between 1.9×10^5 and 1.2×10^6 CFU 100 g^{-1} for TC, between 1 and 8.5×10^5 CFU 100 g^{-1} for *E. coli*, and between 9.8×10^3 and 3.1×10^5 CFU 100 g^{-1} for ENT. FIB initial concentrations varied slightly between the different sediment types and may be explained by a variation in bacterial association with sediment particles.

FIB analysis showed similar patterns between the different types of sediments but differences were observed during growth and decay phases (Fig. 2a–f).

The survival curves of TC, *E. coli*, and ENT in continuous-flow microcosms with sediments from Vidy, Bret, and Versoix are illustrated in Fig. 2a–c. During the first 5 days of the experiment, TC and *E. coli* concentrations generally decreased by around one order of magnitude in all types of sediments, from 10^5 to 10^4 CFU 100 g^{-1}, but remained quite constant at 10^4 CFU 100 g^{-1} for ENT. Between days 5 and 12, microbiological analysis revealed a growth phase for almost all fecal indicators. In sediments from Vidy, TC and *E. coli* concentrations increased by around two orders of magnitude from 10^4 to 10^6 CFU 100 g^{-1}. ENT concentrations increased by one order of magnitude from 10^4 to 10^5 CFU 100 g^{-1}. In sediments from Bret and Versoix, FIB growth was not as strong as in the Vidy sediments and increased by not more than one order of magnitude. This growth was followed up by a decay phase in all microcosms. After 50 days, TC and *E. coli* were not detected anymore, but ENT still showed concentrations between 10^2 and 10^3 CFU 100 g^{-1}.

The survival curves of TC, *E. coli*, and ENT in batch microcosms, with sediments from Vidy, Bret, and Versoix, are illustrated in Fig. 2d–f. The trend was quite similar to the microcosms with water renewal. A decay phase was observed during the first 5 days of the experiment. FIB concentrations decreased by one to two orders of magnitude. This was followed by an increasing phase of 7 to 15 days. *E. coli* and ENT concentration rates generally reached 10^5 CFU 100 g^{-1} in sediments from Vidy and Bret and 10^4 CFU 100 g^{-1} in sediments from Versoix. There was not much difference between the different types of sediments for TC; they increased to levels around 10^5 CFU 100 g^{-1}. As in microcosms with water renewal, this was followed by a decreasing phase in all microcosms.

A decline of FIB populations was observed in all microcosms after 12 to 20 days following the start of

Table 4 Sediment characteristics at day 50

Microcosms	Organic matter (%)	Ntot[a] (mg kg^{-1})	NH4-N[b] (mg kg^{-1})	PTot[c] (mg kg^{-1})	PO[d] (mg kg^{-1})	NAIP[e] (mg kg^{-1})
MV1	12.2	8.3	0.7	7,232.6	602.2	5,240.8
MV2	17.9	8.5	0.8	6,512.7	593.5	5,260.5
MB1	11.5	4.1	0.5	748.8	354.4	139.0
MB2	12.2	3.3	0.4	765.0	341.8	138.8
MVe1	2.2	0.7	0.1	483.7	46.2	55.3
MVe2	1.4	0.3	0.1	404.4	21.8	53.6

[a] Total nitrogen

[b] Ammonium

[c] Total phosphorus

[d] Organic phosphorus

[e] Nonapatitic inorganic phosphorus

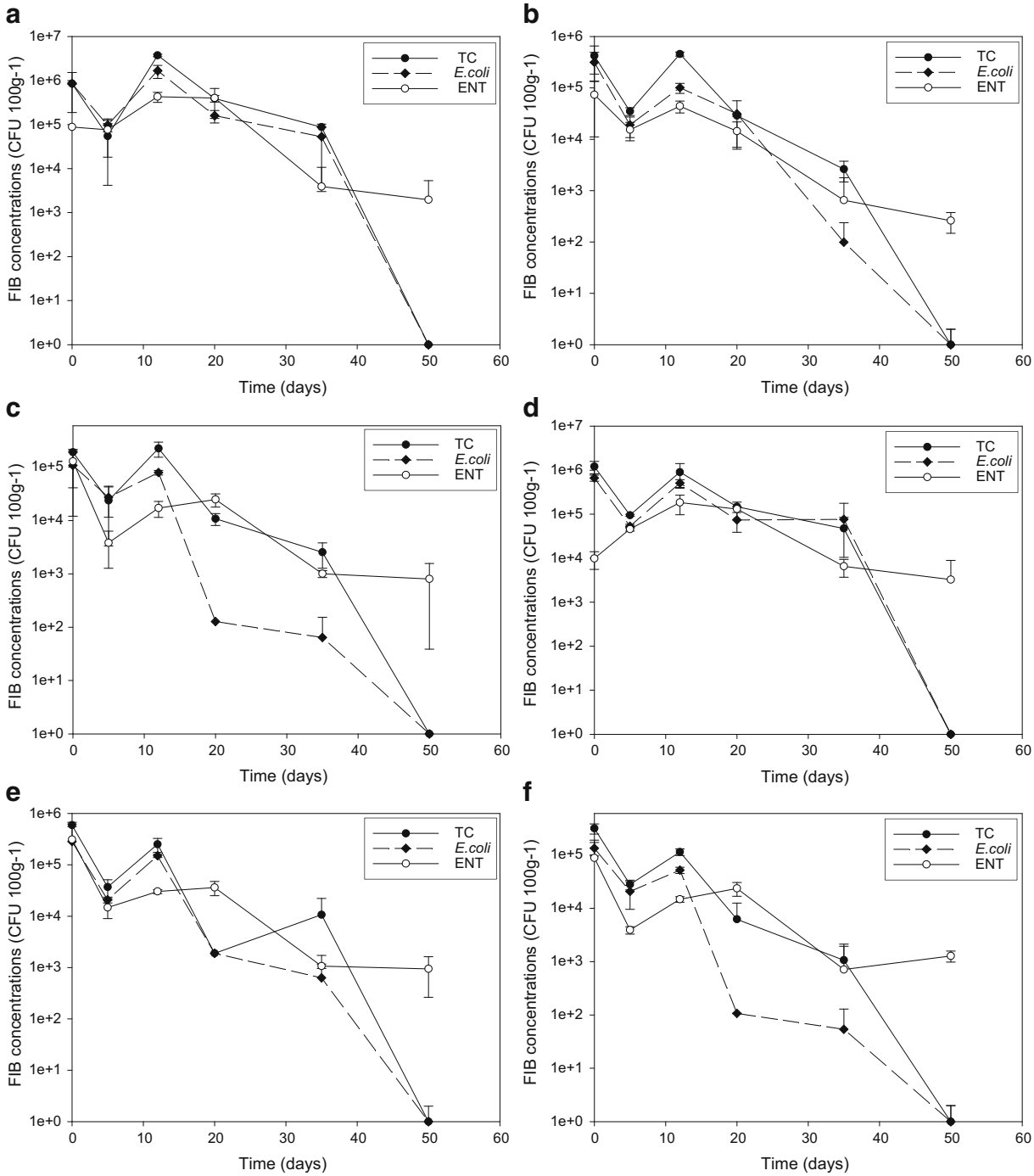

Fig. 2 Survival of fecal indicator bacteria in sediment microcosms. **a** Survival in microcosm MV1 (continuous flow with sediments from Vidy). **b** Survival in microcosm MB1 (continuous flow with sediments from Lake Bret). **c** Survival in microcosm MVe1 (continuous flow with sediments from Versoix). **d** Survival in microcosm MV2 (batch with sediments from Vidy). **e** Survival in microcosm MB2 (batch with sediments from Lake Bret). **f** Survival in microcosm MVe2 (batch with sediments from Versoix)

the experiment. The decay rate constant (k) was estimated for each microcosm by fitting experimental results to the following equation:

$N_t = N_0 \times e^{-kt}$, where N_t is the number of bacteria at time t and N_0 is the number of bacteria at time t=0 (t=0 corresponds to the start of the decay phase which is at day 12 or 20 depending on the microcosm). For all indicators, decay rates were in general lower in sediments from Vidy and Bret (higher nutrients, organic matter, and silt content) than in Versoix (Table 5).

4 Discussion

Some studies assessed the survival of FIB in both marine and freshwater sediments (Davies et al. 1995). It has been demonstrated that freshwater sediments can constitute a reservoir of FIB, which can persist according to sediment characteristics. The results of this experiment showed extended FIB survival up to 50 days and even more for ENT in freshwater sediments. Between days 5 and 12, some growth occurred in all microcosms, followed by a decay phase. FIB concentrations reached in general higher levels in the Vidy sediments compared to Bret and Versoix. The lowest rate of decay also occurred in the microcosms containing sediments from Vidy. For TC and *E. coli*, the decay rates were significantly lower in Vidy than Bret or Versoix (t test, $P<0.05$). A significant difference in *E. coli* decay rates was also observed between sediments from Bret and Versoix (t test, $P<0.05$). ENT followed the same trend as TC and *E. coli* but statistical analysis could not show any significant difference in ENT survival between sediments types. ENT decay rate constants (k) were surrounded by quite important uncertainties; therefore, the comparison between two values was quite difficult to assess. In general, higher growth rates and lower decay rate constants were measured in sediments from Vidy containing higher nutrient and organic matter levels and finer grain size.

Results of this study confirm, in agreement with previous studies, that nutrient availability may have a significant impact on microbial survival, especially *E. coli*. Gerba and McLeod (1976) attributed the longer survival of *E. coli* in estuarine sediments to the greater content of organic matter present in the sediment than in seawater. In addition to longer survival times, they also observed some growth where *E. coli* was added to samples taken from most polluted sites. In a study of pathogenic and indicator organism survival in freshwater sediments at 20°C, Burton et al. (1987) identified prolonged survival and lower decay rates of *E. coli* and *Salmonella newport* in sediments containing higher proportions of clay, organic matter, and nutrients compared to sandy sediment with low nutrient levels. Results from Craig et al. (2004) showed that in general the decay rate of *E. coli* was more important in water than in sediments. Small particle size and high organic carbon content were also enhancing *E. coli* survival in coastal sediments in the microcosms. To test the hypothesis that sediment organic content is an important determinant of FIB survival, Lee et al. (2006) monitored *E. coli* growth in microcosms with sediments in the absence of their natural organic matter. *E. coli* levels measured in these microcosms were below the detection limit.

However, no relationship was observed between the loss of nutrients and FIB decay rates. In the

Table 5 Decay constants, k (days^{-1}) for TC, *E. coli*, and ENT populations in microcosms containing different sediment types

Microcosms	TC		*E. coli*		ENT	
	Decay constant (days^{-1})	Std. error	Decay constant (days^{-1})	Std. error	Decay constant (days^{-1})	Std. error
MV1	0.28*	0.0183	0.29*	0.0281	0.07	0.0380
MV2	0.22*	0.0249	0.23	0.0819	0.08	0.0259
MB1	0.34*	0.0075	0.45*	0.0095	0.14*	0.0065
MB2	0.61	0.5	0.55*	0.0294	0.23	0.0506
MVe1	0.38*	0.0205	0.80*	0.0446	0.22	0.0569
Mve2	0.36*	0.0148	0.77*	0.0446	0.23	0.1051

*$P\leq0.05$

microcosms filled with sediments from Vidy, organic matter and nutrient levels decreased significantly in comparison to microcosms with sediments from Bret and Versoix, in which organic matter and nutrient contents did not show any substantial variation. Despite this difference in nutrient consumption, indicator bacteria evolution followed more or less the same trend in all microcosms. This indicates that sufficient nutrients were present, even in the sediments from Versoix, to support FIB limited growth and significant persistence. All types of sediments used in that experiment may not only act to extend the survival time of indicator bacteria but may also support a certain growth. These results demonstrate that indicator bacteria are capable of utilizing nutrients adsorbed to sediments or in the interstitial water from areas polluted by sewage discharges as well as from areas free of pollution. The significant decrease in organic matter, nutrients, and dissolved oxygen observed in the microcosms with Vidy sediments may be explained by consumption from native microflora naturally present in the sediments.

Our results support previous findings where in general only limited growth or no growth at all was observed in experiments with nonautoclaved sediments. Of the studies undertaken, many have used sterile sediment (Gerba and McLeod 1976; LaLiberte and Grimes 1982; Thomas et al. 1999; Lee et al. 2006). The use of sterile sediment and water removes the pressure on survival induced by the competition with and predation by naturally occurring organisms (Craig et al. 2004). In this study, FIB persistence was determined using intact nonsterile sediments, therefore retaining the effect of natural flora. This shows that predators such as protozoan and viruses may contribute greatly to FIB die-off (LaLiberte and Grimes 1982; Davies et al. 1995). FIB may also not effectively compete with native microflora for available nutrients. Long-term persistence in sediments could indicate that some growth took place but FIB were eliminated at a faster rate than growth occurred.

The survival curves of TC, *E. coli*, and ENT were not significantly different between continuous-flow and batch microcosms. The main difference, among the observed environmental factors, between continuous-flow and batch microcosms was conductivity measured during the whole experimentation period in the water column. In the continuous-flow microcosms, conductivity stayed constant with an average of 285 μS cm^{-1}, similar to the values measured in lake water, but, in the batch microcosms, it gradually increased with time certainly due to the remobilization of dissolved salts from sediments to the water column, from organic matter degradation, and also due to water evaporation. The variation of conductivity in the batch microcosms seems to have no impact on FIB survival.

The survival of TC, *E. coli*, and ENT has been discussed by Noble et al. (2003). In our experiments, after 50 days, TC and *E. coli* were not detected anymore. ENT die-off was also quite significant but they were still measured at day 50 at concentrations between 10^2 and 10^3 CFU 100 g^{-1}. ENT survived longer than *E. coli* and TC in all types of sediments. Hanes and Fragala (1967) also found that *E. coli* degraded more rapidly with increased sunlight intensity than did ENT, a result that was recently confirmed for bacterial samples from Southern California (Noble et al. 2003).

Results of this study revealed that TC, *E. coli*, and ENT remained cultivable for at least 40 to 50 days and thus were detectable through the culture-based method used here. Many enterobacteria, e.g., *Vibrio* sp., *E. coli*, and *Enterococcus faecalis*, can activate survival strategies including the viable but nonculturable (bacteria still viable but cannot be shown as colony-forming units by the conventional plate counts) state in response to unfavorable growth conditions and starvation (Lleò et al. 2005). They persist in the environment in conserving their viability despite the loss of their own culturability.

All these results suggest that indicator organisms released into the coastal environment can accumulate in sediment, leading to increased persistence. These findings lead to the question of whether pathogenic bacteria are also capable of extended survival in this environment. More work is needed to correlate the presence and survival of *E. coli* and other fecal indicator bacteria with that of relevant pathogens in order to assess the need for the use of additional indicators (Tallon et al. 2005).

5 Conclusion

Results of this experiment confirm extended FIB survival in freshwater sediments up to 50 days. ENT survived longer than TC and *E. coli*; they were still

present at concentrations between 10^2 and 10^3 CFU 100 g^{-1} after 50 days. This study also reveals that FIB persistence is influenced by nutrient and organic matter content in sediments. An increased growth and significantly lower decay rates were observed in sediments containing higher levels of organic matter and nutrients and smaller grain size. However, FIB followed a quite similar trend in all microcosms. All types of sediments were able to support fecal bacteria limited growth and significant persistence. These results demonstrate that indicator bacteria prove to be capable of utilizing nutrients present in sediments from areas polluted by sewage discharges as well as from areas free of pollution. Their presence and survival in coastal sediments may induce an increased risk of human infection due to the possible resuspension of other pathogenic microorganisms during natural turbulence or human activities. Extended survival of enteric bacteria in sediments and potential remobilization of pathogens may be responsible for water quality failures and are of considerable significance for the management of risk at specific recreational coastal sites.

Acknowledgement We thank Vincent Sastre for navigating the boat during the sampling phase, Benoît Ferrari and Régis Kottelat, for their precious help for the setting up and handling of the microcosms and Jean-Luc Loizeau for reviewing all the statistical analyses. Part of this study was funded by the Ernst and Lucie Schmidheiny Foundation, Geneva, Switzerland.

References

Alm, E. W., Burke, J., & Spain, A. (2003). Fecal indicator bacteria are abundant in wet sand and freshwater beaches. *Water Research*, *37*, 3978–3982. doi:10.1016/S0043-1354(03)00301-4.

An, Y., Kampbell, D. H., & Breidenbach, G. P. (2002). *Escherichia coli* and total coliforms in water and sediments at lake marinas. *Environmental Pollution*, *120*, 771–778.

Anderson, L. K., Whitlock, J. E., & Harwood, V. J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied &. Environmental Microbiology*, *71*, 3041–3048. doi:10.1128/AEM.71.6.3041-3048.2005.

Ashbolt, N. J., Grohmann, G. S., & Kueh, C. (1993). Significance of specific bacterial pathogens in the assessment of polluted receiving waters to Sydney. *Water Science & Technology (Elmsford, N.Y.)*, *27*, 449–452.

Balkwill, D. L., & Ghiorse, W. C. (1985). Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. *Applied & Environmental Microbiology*, *50*, 580–588.

Burton, G., Gunnison, D., & Lanza, G. (1987). Survival of pathogenic bacteria in various freshwater sediments. *Applied &. Environmental Microbiology*, *53*, 633–638.

Burrus, D., Thomas, R. L., Dominik, B., Vernet, J. P., & Dominik, J. (1990). Characteristics of suspended sediment in the Upper Rhone River, Switzerland, including the particulate forms of phosphorus. *Hydrological Processes*, *4*, 85–98. doi:10.1002/hyp.3360040108.

Craig, D. L., Fallowfield, H. J., & Cromar, N. J. (2004). Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. *Journal & Applied Microbiology*, *96*, 922–930. doi:10.1111/j.1365-2672.2004.02243.x.

Davies, C. M., & Evison, L. M. (1991). Sunlight and the survival of enteric bacteria in natural waters. *The Journal of Applied Bacteriology*, *70*, 265–274.

Davies, C., & Bavor, H. (2000). The fate of storm-water associated bacteria in constructed wetland and water pollution control pond systems. *Journal of Applied Microbiology*, *89*, 349–360. doi:10.1046/j.1365-2672.2000.01118.x.

Davies, C. M., Long, J. A. H., Donald, M., & Ashbolt, N. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *Applied & Environmental Microbiology*, *61*, 1888–1896.

EU (2006). European directive 2006/7/CE of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC.

Evanson, M., & Ambrose, R. F. (2006). Sources and growth dynamics of fecal indicator bacteria in a coastal wetland system and potential impacts to adjacent waters. *Water Research*, *40*, 475–486. doi:10.1016/j.watres.2005.11.027.

Fish, J. T., & Pettibone, G. W. (1995). Influence of freshwater sediment on the survival of *Escherichia coli* and *Salmonella sp*. as measured by three methods of enumeration. *Letters in Applied Microbiology*, *20*, 277–281. doi:10.1111/j.1472-765X.1995.tb00445.x.

Gerba, C., & McLeod, J. S. (1976). Effect of sediment on the survival of *Escherichia coli* in marine water. *Applied & Environmental Microbiology*, *32*, 114–120.

Ghoul, M., Bernard, T., & Cormier, M. (1990). Evidence that *Escherichia coli* accumulates glycine betaine from marine sediments. *Applied & Environmental Microbiology*, *56*, 551–554.

Goldscheider, N., Haller, L., Poté, J., Wildi, W., & Zopfi, J. (2007). Characterizing water circulation and contaminant transport in Lake Geneva using bacteriophage tracer experiments and limnological methods. *Environmental Science & Technology (Elmsford, N.Y.)*, *41*, 5252–5258. doi:10.1021/es070369p.

Hanes, N. B., & Fragala, C. (1967). Effect of seawater concentration on the survival of indicator bacteria. *Journal—Water Pollution Control Federation*, *39*, 97–104.

Hughes, K. A. (2003). Influence of seasonal environmental variables on the distribution of presumptive fecal coliforms around an Antarctic research station. *Applied & Environmental Microbiology*, *69*, 4884–4891. doi:10.1128/AEM.69.8.4884-4891.2003.

Kjeldhal, J. (1883). A new method for the determination of nitrogen in organic matter. *Zeitschrift fur Analytische Chemie*, *22*, 366. doi:10.1007/BF01338151.

LaLiberte, P., & Grimes, D. J. (1982). Survival of *Escherichia coli* in lake bottom sediment. *Applied and Environmental Microbiology*, *43*, 623–628.

Lee, C. M., Lin, T. Y., Lin, C., Kohbodi, G. A., Bhatt, A., Lee, R., et al. (2006). Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. *Water Research*, *40*, 2593–2602. doi:10.1016/j.watres.2006.04.032.

Lleò, M. M., Bonato, B., Benedetti, D., & Canepari, P. (2005). Survival of enterococcal species in aquatic environments. *FEMS Microbiology Ecology*, *54*, 189–196. doi:10.1016/j.femsec.2005.03.016.

Loizeau, J.-L., Arbouillle, D., Santiago, S., & Vernet, J.-P. (1994). Evaluation of a wide range laser diffraction grain size analyser for use with sediments. *Sedimentology*, *41*, 353–361. doi:10.1111/j.1365-3091.1994.tb01410.x.

Loizeau, J.-L., Pardos, M., Monna, F., Peytremann, C., Haller, L., & Dominik, J. (2004). The impact of a sewage treatment plant's effluent on sediment quality in a small bay in Lake Geneva (Switzerland-France). Part 2: Temporal evolution of heavy metals. *Lakes and Reservoirs: Research and Management*, *9*(1), 53–63. doi:10.1111/j.1440-1770.2004.00234.x.

McFeters, G. A., & Singh, A. (1991). Effects of aquatic environmental stress on enteric bacteria. *The Journal of Applied Bacteriology*, *66*, 559–569.

Noble, R. T., Moore, D. F., Leecaster, M. K., McGee, C. D., & Welsberg, S. B. (2003). Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research*, *37*, 1637–1643. doi:10.1016/S0043-1354(02)00496-7.

OHyg (Ordonnance du DFI sur l'hygiène) (2005). *Ordonnance sur les denrées alimentaires et les objets usuels (ODIOUS)*. Berne: Le Département fédéral de l'intérieur (DFI).

Thomas, C., Hill, D. J., & Mabey, M. (1999). Evaluation of the effect of temperature and nutrients on the survival of *Campylobacter spp.* in water microcosms. *Journal of Applied Microbiology*, *86*, 1024–1032. doi:10.1046/j.1365-2672.1999.00789.x.

Poté, J., Goldscheider, N., Haller, L., Zopfi, J., Khajehnouri, F., & Wildi, W. (2008a). Origin and spatial–temporal distribution of fecal bacteria in a bay of Lake Geneva, Switzerland. *Environmental Monitoring and Assessment*-doi:10.1007/s10661-008-0401-8.

Poté, J., Haller, L., Loizeau, J.-L., Garcia Bravo, A., Sastre, V., & Wildi, W. (2008b). Effects of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Bioresource Technology*, *99*, 7122–7131. doi:10.1016/j.biortech.2007.12.075.

Sinton, L., Finlay, R., & Lynch, P. (1999). Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and Environmental Microbiology*, *66*, 230–237.

Tallon, P., Magajna, B., Lofranco, C., & Leung, K. T. (2005). Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution*, *166*, 139–166. doi:10.1007/s11270-005-7905-4.

US Environmental Protection Agency (2000). *Improved enumeration methods for the recreational water quality indicators: enterococci and Escherichia coli EPA-821/R-97/004*. Washington, D.C.: US Environmental Protection Agency.

Wildi, W., Dominik, J., Loizeau, J. L., Thomas, R. L., Favarger, P.-Y., Haller, L., et al. (2004). River, reservoir and lake sediment contamination by heavy metals downstream from urban areas of Switzerland. *Lakes & Reservoirs: Research & Management*, *9*, 75–87.

Williams, J. D. H., Jaquet, J. M., & Thomas, R. L. (1976). Forms of phosphorus in the superficial sediments of Lake Erie. *Journal of the Fisheries Research Board of Canada*, *33*, 413–429.