City of
SANTA CLARITA

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July 27, 2011

Charles R. Hoppin, Chairman and Members
c/o Ms. Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
Post Office Box 100
Sacramento, CA 95812-2000

Dear Mr. Hoppin and Members of the Board:

Subject: Comment Letter - Santa Clara River Bacteria Total Maximum Daily Load (TMDL)

Thank you for the opportunity to comment on the proposed Santa Clara River Bacteria Total Maximum Daily Load (TMDL). The City of Santa Clarita (City) would like to thank the Los Angeles Regional Water Quality Control Board (Regional Board) for their efforts to acknowledge our comments from the draft version of this TMDL and amending the language and timelines to allow time for appropriate studies to be completed.

Reopener Clauses

Although the Regional Board has made considerable efforts to address the issue, the City continues to have concerns about the accuracy of the data used to make the linkage analysis. A single reopener clause has been applied four years after the effective date of this TMDL. It should be noted that four years is very little time for the submission of data and adequate studies for fires, high-flow exemptions, a background study, a land-use study, and high natural Total Suspended Solids/Fecal indicator bacteria correlation. Please include multiple periods of reopeners, perhaps at 4, 8, and 12 years, for the Regional Board to review and reconsider additional data and reports for applying this TMDL.

Bacteria Regrowth in the Storm Drain and Sediment

The California Coastal Commission funded a 2007 study on sediment and geomorphology of the Santa Clara River. (*Stillwater Sciences. 2007. Assessment of geomorphic processes for the Santa Clara River watershed*) It demonstrates the Santa Clara River has high natural sediment load. Changes in the geomorphic process started occurring prior to California becoming a state (c. 1820). The study states "Sediment supply rates to the lower Santa Clara River are high as a consequence of geological and climatic factors, but are also conditioned by significant episodic events such as landslides, earthquakes and fires." There is a significant impact to fecal indicator bacteria (FIB) growth that is central to the discussion and unique to the Santa Clara River.

FIB occur in high numbers in storm drains and sediments impacted by urban runoff, possibly due to regrowth, selective survival, or accumulation of bacteria in sediment. (*Ferguson, et al, 2005*) Studies by U.S. Department of Agriculture (USDA) scientists have confirmed that the presence of *Escherichia coli* pathogens in surface waters could result from the pathogen's ability to survive for months in underwater sediments (*Pachespsky, et al, 2010*). In addition, a 2006 Orange County Study found most bacteria in the environment grow in extracellular polymeric substances (a.k.a. EPS, or biofilm). This biofilm is found on virtually any solid surface that has contact with water and nutrients, such as storm drain pipes or sediment particles. The study concludes that if FIB grow and multiply in biofilm and are dispersed in the water column, it may account for increased bacteria levels without human or animal fecal input. Further research is needed to understand bacterial regrowth within storm drain biofilm found within the storm drain and in sediment.

Jointly and Severally Liable

The TMDL states "The cities of Santa Clarita, Fillmore, Santa Paula, and Ventura, the Counties of Los Angeles and Ventura, and the Los Angeles County Flood Control District and Ventura County Watershed Protection District are jointly responsible for meeting the WLAs assigned to MS4 discharges." The City has no jurisdictional powers over areas outside city limits. As such, the City can not regulate actions of areas upstream or downstream. Separate TMDLs for each reach is a more prudent approach that the city respectfully requests be considered.

The City also requests clarification and perhaps rewriting of the definition of "contributing" to a violation and "jointly and severally liable." If this is not feasible, then separate TMDLs for each reach of the river for this TMDL may be a prudent approach.

Once again, the City would like to acknowledge the Los Angeles Regional Water Quality Control Board for their considerable efforts to amend the draft version of this TMDL. Thank you for the opportunity to comment on this version of the Santa Clara River Bacteria TMDL. Should you have any questions, please do not hesitate to contact me at (661) 255-4337 or tlange@santa-clarita.com.

Sincerely,

Mark Pattì for Travis Lange

Travis Lange
Environmental Services Manager

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cc: Robert Newman, Director of Public Works
Heather Merenda, Sustainability Planner

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Survival of manure-borne *E. coli* in streambed sediment: Effects of temperature and sediment properties

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ABSTRACT

Escherichia coli bacteria are commonly used as indicator organisms to designate of impaired surface waters and to guide the design of management practices to prevent fecal contamination of water. Stream sediments are known to serve as a reservoir and potential source of fecal bacteria (*E. coli*) for stream water. In agricultural watersheds, substantial numbers of *E. coli* may reach surface waters, and subsequently be deposited into sediments, along with fecal material in runoff from land-applied manures, grazing lands, or wildlife excreta. The objectives of this work were (a) to test the hypothesis that *E. coli* survival in streambed sediment in the presence of manure material will be affected by sediment texture and organic carbon content and (b) to evaluate applicability of the exponential die-off equation to the *E. coli* survival data in the presence of manure material. Experiments were conducted at three temperatures (4 °C, 14 °C, and 24 °C) in flow-through chambers using sediment from three locations at the Beaverdam Creek Tributary in Beltsville, Maryland mixed with dairy manure slurry in the proportion of 1000:1. Indigenous *E. coli* populations in sediments ranged from ca. 10^1 to 10^3 MPN g^{-1} while approx 10^3 manure-borne *E. coli* MPN g^{-1} were added. *E. coli* survived in sediments much longer than in the overlaying water. The exponential inactivation model gave an excellent approximation of data after 6–16 days from the beginning of the experiment. Slower inactivation was observed with the increase in organic carbon content in sediments with identical granulometric composition. The increase in the content of fine particles and organic carbon in sediments led not only to the slower inactivation but also to lower sensitivity of the inactivation to temperature. Streambed sediment properties have to be documented to better evaluate the role of sediments as reservoirs of *E. coli* that can affect microbiological stream water quality during high flow events.

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1. Introduction

Escherichia coli are commonly used as indicators of fecal contamination, and hence public health risk, in surface waters. Although the vast majority of *E. coli* strains are non-pathogenic, a strong correlation has been observed between elevated levels of *E. coli* in water and occurrences of gastrointestinal disease (U.S. EPA, 1986). It is often assumed that elevated levels of *E. coli* in water are the result of runoff from land-applied animal manures, fecal deposition from grazing

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animals, leaching from inadequate septic fields, leaky sewage lines, and/or wastewater treatment plant outflows. However, stream, river, pond, lake, and lagoon sediments have recently begun to receive attention as important sources of waterborne *E. coli*.

Sediment has been identified as a reservoir for *E. coli* based on comparisons of bacterial concentrations in sediment with concentrations in the water column directly above the sediment layer. Many studies indicate that sediments harbor much higher populations of both fecal coliforms (FC) and *E. coli* than the overlying water (Goyal et al., 1977; Doyle et al., 1992; Buckley et al., 1998; Crabill et al., 1999; Smith et al., 2008; Rehmann and Soupir, 2009). Sediments presumably serve as a hospitable environment for bacterial survival due to the availability of soluble organic matter and nutrients (Jamieson et al., 2005a,b), protection from predators such as protozoa (Jamieson et al., 2005a,b; Decamp and Warren, 2000), and shielding from exposure to UV sunlight (Koirala et al., 2008).

The importance of the *E. coli* sediment reservoir has been documented during high flow events in streams. The concentrations of *E. coli* and FC in streams during storm events are usually 2–3 orders of magnitude higher than in the baseflow conditions (e. g., Hunter et al., 1992). Mechanical disturbance of bottom sediments can cause increased *E. coli* concentrations in the overlying waters as a result of their resuspension (e. g. Stephenson and Rychert, 1982; Seyfried and Harris, 1986; Lopez-Torres et al., 1987). Artificial flood experiments were carried out by Nagels et al. (2002) and Muirhead et al. (2004) in New Zealand. Muirhead et al. (2004) observed that *E. coli* concentrations peaked ahead of the flow peak, consistent with the entrainment of FC into the water column from underlying contaminated sediments by accelerating currents on the rising limb of the hydrograph. A two-order of magnitude increase was observed during the event. *E. coli* concentrations correlated with turbidity over the flood event ($r^2 = 0.92$) and both variables. When the turbidity returned to base levels between each flood, the *E. coli* concentrations remained elevated. Streambed *E. coli* probably can also enter water column during baseflow, although the magnitudes of this exchange are not known well.

Sediment particle size distribution and organic matter content have often been mentioned as factors affecting *E. coli* survival in sediments. However, reports on the effect of these factors are contradictory. Burton et al. (1987) found that *E. coli* survival was greater in sediments containing at least 25% clay (particles less than 2 μm in diameter), presumably due to enhanced attachment to the finer sediment particles. Other studies have shown that *E. coli* are surviving better in sediments with predominantly large particles ranging in size from 125 to 500 μm; perhaps because larger sediment particles facilitate increased porosity, permeability, and availability of nutrients (Cinotto, 2005). Similarly, diverse effects of the organic matter content on the survival were observed. Banning et al. (2003) suggested that an increase in available nutrients may have little effect on *E. coli* persistence due to competition for nutrients by other microflora. On the other hand, Lee et al. (2006) monitored *E. coli* growth in microcosms with sediments in the absence of their natural organic matter, and *E. coli* levels measured in these microcosms were below the detection limit.

Temperature is a major factor controlling *E. coli* survival in sediments, comparable to other environmental matrices. FC and *E. coli* survival in sediments are typically characterized using the exponential die-off model (Chick, 1908). However, this model has in many cases proven to be inadequate (e. g., Davies et al., 1995; Howell et al., 1996; Jamieson et al., 2004). The authors have attributed this limitation to the effects of predation and re-growth on the overall population decrease.

In agricultural watersheds, substantial numbers of *E. coli* may reach surface waters, and subsequently be deposited into sediments, along with fecal material in runoff from land-applied manures, grazing lands, or wildlife excreta. However, the data on inactivation or the survival of *E. coli* entering sediments with manure material is lacking. The purpose of this study was to assess the survival of *E. coli* in different sediments following the addition of dilute bovine manure. The objectives of this work were (a) to test the hypothesis that *E. coli* survival in streambed sediment in the presence of manure material will be affected by sediment texture and organic carbon content and (b) to evaluate applicability of the exponential die-off equation to the *E. coli* survival data in the presence of manure material. Sediments were chosen that encompassed different particle size distributions, organic carbon contents, and initial *E. coli* populations; experiments were conducted at 4 °C, 14 °C, and 24 °C to encompass a range of seasonal temperatures.

2. Materials and methods

2.1. *Sediment sampling*

Streambed sediments were obtained from a first order stream in the riparian corridor bordering the OPE3 research field at the USDA-ARS, Beltsville, MD (39°2′5″N, 76°54′28″W). The site has previously been described in detail by Angier et al. (2005). Three locations along the stream were chosen based on accessibility to the location, straight flow path, and sufficient sediment in the streambed. Location A was approximately 70 m from the headwaters; location B was approximately 290 m from the headwaters and 220 m from the location A; while location C was 760 m from the headwaters and 470 m from location B.

Preliminary sampling was conducted to ensure that there were differences in sediment texture and organic carbon content between the three locations and to determine the dependence of *E. coli* concentrations on depth in sediments. A grid sampling method was applied to ensure that samples were not taken from the same place twice. Four sediment cores were taken by driving a clear plastic auger sleeve 20 cm into the sediment. The top 5-cm layer was divided into top 1 cm, 1–3 cm and 3–5 cm subsamples.

2.2. *Experimental setup*

E. coli survival experiments were conducted in Plexiglas boxes that were constructed to act as flow-through chambers with dimensions of 43 cm long, 20 cm deep, and 12 cm high. The chambers contained Styrofoam bottom holder to hold sediment samples. Each Styrofoam holder had 10 rows of 5

circular indentations to hold the 15 mL cups with cup rims level with the holder surface. Stream conditions were simulated by creating a steady flow of natural stream water over the sediment in the flow chamber. Flow was generated using a Mini-Jet 404 submersible Marineland aquarium pumps (Spectrum Brands, GA). The flow rate was between 600 and 700 mL/min for the duration of the experiment in all chambers. The pumps were placed inside a water reservoir where the natural stream water was stored and pumped through the chambers through six holes, causing the water to be evenly distributed into the chamber. Six identical holes on the opposite wall of the flow chamber collected water discharge, which was then directed back into the water reservoir. This system created a closed circuit of flowing water with the 5-cm water column above sediment.

Nine flow chambers were built, and sediment from each location was kept at three different temperatures: 4.3 ± 0.3 °C, 14.5 ± 0.5 °C, and 22.9 ± 1.2 °C (mean ± standard deviation) in triplicate. Temperature was set by placing the flow chambers in controlled environment chambers where the respective temperatures were maintained.

2.3. *Sediment preparation*

Sediment samples were taken from the same locations where the preliminary samplings were conducted in October, 2008, on the day with the maximum and minimum air temperatures of 28 °C and 14 °C, respectively. Sediment was sampled to the depth of 3 cm because preliminary data indicated that bacterial concentrations were highest in the upper 3 cm of sediment (see the Results section). A large plastic scoop was used to collect sediment samples. Sediments were drained to remove excess water and then passed through a 2 mm sieve in order to remove organic litter.

Each sediment sample was mixed with fresh manure slurry. A 50 mL fresh manure sample was collected from the USDA-ARS Dairy Research Facility in Beltsville, MD and sieved through a 2 mm sieve to remove bedding. The manure was then diluted 1000-fold by mixing 2250 mL of sediment with 2.5 mL of manure using a BOSCH model # 0-601-194-639 drill mixer for 10 min.

2.4. *Sampling and measurements during the inactivation experiment*

Sampling of sediment for bacterial analysis and water content occurred immediately before and after the manure slurry was added to sediment. Prior to the initiation of flow chamber experiments (day 0), sediments were incubated for two days at 4 °C, 14 °C, or 24 °C to allow for equilibration. Sediment sampling was then conducted on days 0, 2, 4, 6, 11, 16, 25, 33, 44, and 119, following a logarithmic scale, with some variations due to logistics. For each sampling, five subsamples (one row of cups) were taken from each of the 9 flow chambers. The two subsamples closest to the sides were used to determine water content while the inner 3 subsamples were used for bacterial analysis. Sediment water content was measured by calculating water loss after samples were dried in an oven at 40 °C for 24 h.

Water temperature measurements were taken every 15 min using temperature probes (Model 107, Campbell Scientific Inc.; Logan, UT). Air and humidity measurements were taken using a temperature probe (Model 205, Phys-Chem Scientific Corp.; New York, NY).

2.5. *Physical and chemical measurements*

A 500 mL water sample was taken from each of the 9 flow chambers for each sampling and 2 replicate Colilert-18 determinations made. The water was then equilibrated to room temperature in order to measure pH, EC, dissolved oxygen, and turbidity. The pH and dissolved oxygen were measured using the YSI 556 MPS probe (Yellow Springs, OH). Electrical conductivity (EC) was measured with the Solomat MPM 1000 probe (Norwalk, CT) while turbidity was measured with the Orbeco-Hellige Digital Direct Reading Turbidimeter (Sarasota, FL). Particle density was determined by the pycnometer method. Soil texture was found using the pipette method (Gee and Or, 2002) for each of the three sediments. Organic carbon content was determined by oven-drying two replicate 0.2 g samples weighed into capsules and combusted at 950 °C using the LECO CHN-2000 analyzer.

2.6. *Bacteria enumeration*

E. coli determinations were made using Colilert-18 (IDEXX; Westbrook, Maine). The mass of each empty tube and the wet mass of each subsample was recorded. The wet sediment and 90 mL of sterile distilled water were blended at high speed for 2 min in order to produce homogeneous slurry (Waring model 34BL97). After the slurry settled for 1 h, 5 mL of the slurry supernatant was added to IDEXX 100 mL bottles (2 bottles per subsample) containing 95 mL of sterile distilled water. One packet of Colilert-18 reagent was then added to each bottle and thoroughly shaken. After the reagent dissolved, bottle contents were poured into IDEXX trays and sealed with the IDEXX Quanti-Tray Sealer model 2X. The trays were then incubated at 37 °C for 18 h. *E. coli* concentrations were expressed in MPN g^{-1} of dry weight of sediment (gdw) using the data on sediment water contents.

3. Results and discussion

3.1. *Sediment composition*

Sediments A, B, and C varied in particle size distribution, organic carbon content, and saturated water content (Table 1). Sediments A and C belonged to the same USDA textural class of loamy sand, and sand and silt percentages were not significantly different ($p < 0.05$). Sediment C had substantially larger organic carbon content than sediment A. Sediment B samples belonged either to the sandy loam textural class or to the sandy clay loam textural class; they had 3–4-fold higher percentage organic matter and saturated water content than sediments A or C.

Indigenous *E. coli* concentrations declined by about one order of magnitude per 2 cm of sampling depth in preliminary samples taken at all locations. The dependencies of logarithms

Table 1 – Selected properties of sediment samples.

Location	USDA texture class	Percentage of textural fractions			Particle density, g cm^{-3}	Organic carbon, %	Saturated water content, cm^{3}cm^{-3}
		Sand	Silt	Clay			
A	Loamy sand	84.1 ± 0.85[a]	11.5 ± 1.06	4.45 ± 0.29	2.61 ± 0.00	1.35 ± 0.18	0.56 ± 0.07
B	Sandy loam, sandy clay loam	59.9 ± 0.45	20.7 ± 0.53	19.5 ± 0.41	2.55 ± 0.01	5.14 ± 0.80	1.99 ± 0.29
C	Loamy sand	84.5 ± 0.19	9.59 ± 0.34	5.95 ± 0.15	2.61 ± 0.01	1.78 ± 0.12	0.64 ± 0.06

a Mean values and standard deviations are separated with the "±" sign.

of *E. coli* contents on depth were linear with determination coefficients of regressions greater than 0.97. Ferguson et al. (1996) observed that FC concentrations in the top 2 cm of sediments were significantly ($p < 0.001$) higher than in the 2–10 cm layer; while Haller et al. (2009a,b) documented an order of magnitude decrease in *E. coli* concentrations per cm sediment, to a depth of 5 cm, in sediment from Lake Geneva, Switzerland. The higher concentrations of *E. coli* observed in the upper centimeter of sediment are presumably due to preferential access to nutrients and periodic replenishment of bacteria from runoff. Since the topmost layer of sediment is most affected by stream flow and disturbance, this observation has particular relevance with respect to the resuspension and transport of sediment-borne *E. coli* during storm events.

3.2. *Water parameters during inactivation experiments*

Temporal changes in water parameters in all three sediments showed similar trends that were amplified or suppressed by differences in temperature (Fig. 1). Dissolved oxygen (DO) oscillated during the first two weeks, increased during the next two weeks and declined toward the end of the experiment (Fig. 1a). The range of changes in DO at 4 °C was much less than at 14 °C or 24 °C. Changes in pH generally were opposite the changes in dissolved oxygen (Fig. 1b); increases in dissolved oxygen corresponded to decreases in pH, and vice versa, at the beginning and end of experiments. The range of pH changes at 24 °C was smaller than at the other two temperatures. The electrical conductivity (EC) in the 4 °C and 24 °C chambers remained relatively constant over time, except for a high initial value in sediment A (Fig. 1c). At 14 °C, a general increase in EC was observed in all chambers. Turbidity rapidly decreased in all flow chambers during the first week (Fig. 1d). Turbidity values at 4 °C were higher than at the two other temperatures during the second and third week, but were similar at all three temperatures by the end of the experiments (Fig. 1d).

E. coli concentrations in the water overlying all sediments initially increased from the initial value of 210 MPN 100 ml^{-1}; there was a direct correlation with temperature (Fig. 2). Subsequent decreases in *E. coli* concentrations were inversely correlated with temperature (Fig. 2). *E. coli* were the most persistent at 4 °C requiring 2 weeks to reach 10 MPN 100 mL^{-1}, while only 1 week was required at 14 °C. No *E. coli* were detected at 24 °C after 1 week.

The initial increase in *E. coli* concentrations (Fig. 2) coincidental the decrease in dissolved oxygen concentrations and

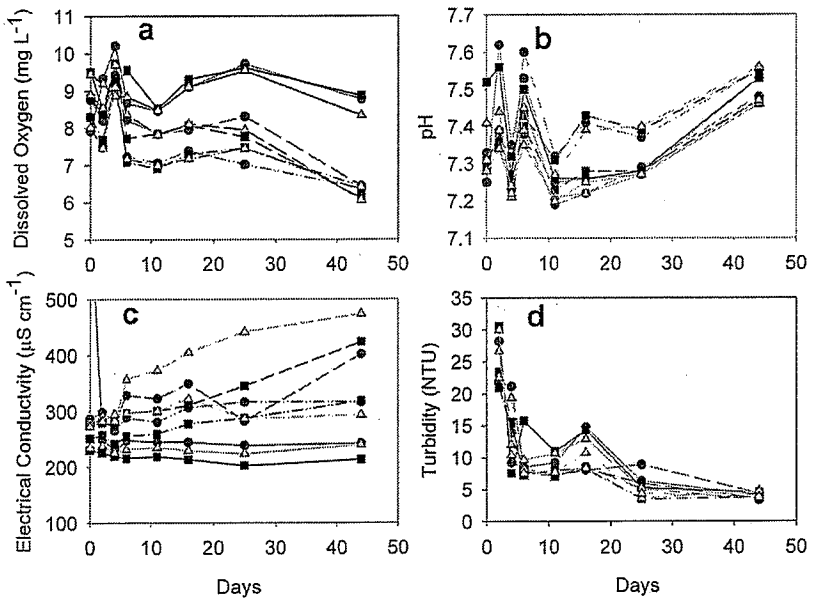

Fig. 1 – Changes in physical and chemical parameters in water column during the *E. coli* inactivation experiment. ● – sediment A, ■ – sediment B, ▲ – sediment C, solid lines – 4 °C, dashed lines – 14 °C, dashed and dotted lines – 24 °C.

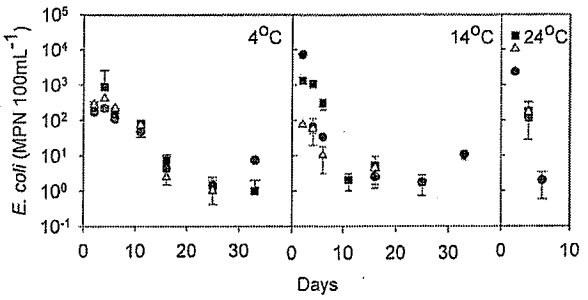

Fig. 2 – Changes in *E. coli* concentrations in water column during the *E. coli* inactivation experiment. ● – sediment A, ■ – sediment B, ▲ – sediment C. Error bars show standard deviations computed for logarithms of concentrations.

increase in pH (Fig. 1b) is consistent with many observations dating back to the work of Gale and Epps (1942). This increase in *E. coli* concentrations could be due to growth as nutrients diffused from sediments into the water, as evidenced by the increase in electrical conductivity (Fig. 1c) and/or due to the initial resuspension of sediment as indicated by an increase in turbidity (Fig. 1d). *E. coli* populations decreased more rapidly in water than in sediments (compare Figs. 2 and 3), due to rapid die-off, sedimentation, or both. Temperature was more influential than either sediment composition or initial *E. coli* concentrations in sediment in controlling water-borne *E. coli* populations (Fig. 2). Since sedimentation rates are largely independent of temperature, these data suggest that die-off was the predominant fate of water-borne *E. coli* at 24 °C.

The observed lack of correlation between *E. coli* concentrations in water vs. in underlying sediments is consistent with many previous studies. A low correlation ($r = 0.28$) was reported by Crabill et al. (1999) for concentrations of FC in water vs. in sediment along a creek in Arizona. Similarly, Byappanahalli et al. (2003) noted a relatively low correlation between concentrations of *E. coli* in stream water and in sediment ($r = 0.49$). Trends in FC populations in water could not be used to predict those in sediments and vice versa, according to a two month-long study by Doyle et al. (1992), and an attempt to correlate *E. coli* densities in water vs. in sediment was not successful in Lake Texoma, OK (An et al., 2002). Solo-Gabriele et al. (2000) suggested that *E. coli* concentrations in bank soils could affect the water column to a noticeable degree only after they increased to substantial levels. Sediments presented more hospitable environments compared with water columns in our work.

3.3. *E. coli* concentrations in sediments

Initial *E. coli* concentrations in sediment samples A, B and C used in these experiments were 17 ± 6, 923 ± 176, and 88 ± 18 MPN gdw^{-1}, respectively. Sediments were inoculated

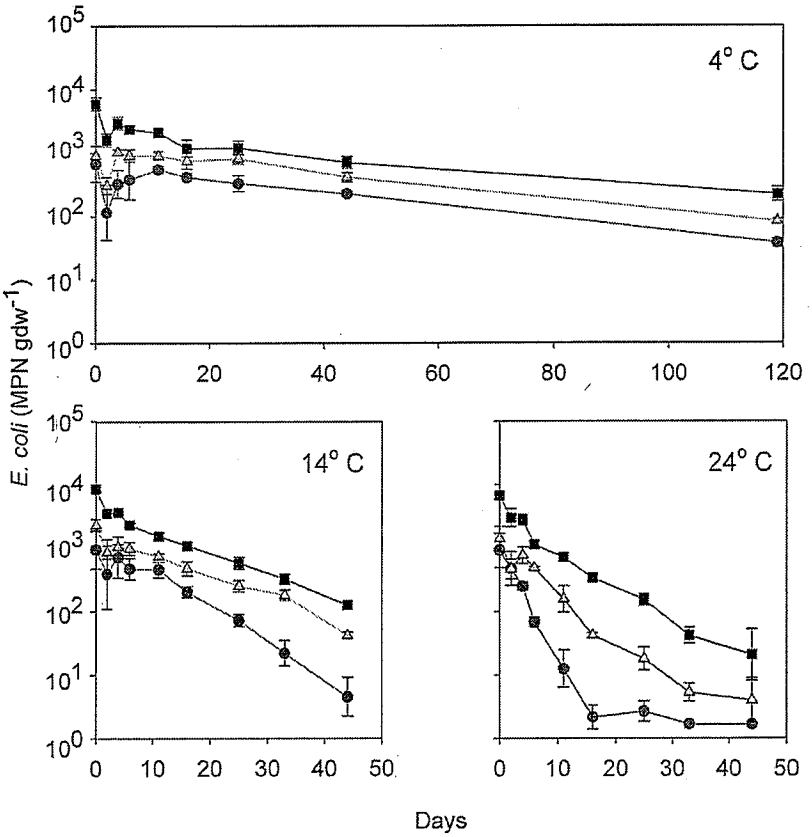

Fig. 3 – *E. coli* inactivation in sediments. ● – sediment A, ■ – sediment B, ▲ – sediment C. Error bars show standard deviations computed for logarithms of concentrations.

with approximately 2000 manure-borne *E. coli* per g sediment. Immediately after inoculation, *E. coli* concentrations in sediments A, B, and C were 435 ± 79, 2126 ± 442, and 463 ± 127 MPN gdw^{-1}, respectively.

A two- to four-fold increase in *E. coli* MPN per sediment was observed after 2 days of incubation (prior to initiation of flow chamber experiments); the largest increase was observed at 14 °C in all sediments. This is consistent with previous studies, in which 1–2 doublings is observed in fresh bovine manure, due to growth under aerobic conditions. However, the combined impact of sediment granulometric composition and organic matter in sediments on growth cannot be excluded. Perturbations associated with the collection and mixing of sediments may have resulted in enhanced availability of nutrients. It was not possible to distinguish between potential differences in growth rates of indigenous vs. added *E. coli* strains.

E. coli concentrations in sediments initially oscillated prior to onset of the exponential inactivation phase (Fig. 3); the most pronounced oscillations occurred at 4 °C. The oscillatory phase in the survival dynamics was about four days in all sediments and all temperatures. The decline in concentrations followed by the decrease or stabilization of concentrations in this period could be caused by changes in *E. coli* ribotype diversity and differences in survivability of different *E. coli* strains. Anderson et al. (2005) demonstrated differential survival of *E. coli* ribotypes in sediments in freshwater mesocosms during the four-day period after inoculation. Strain-dependent variability in growth and survival in soils has been shown by Topp et al. (2003) who demonstrated that composition of a *E. coli* community in swine manure slurry changed dramatically during incubation in soil. In both competitive and non-competitive assays, some *E. coli* strains exhibited growth during first four days of incubation whereas other strains did not. Guber et al. (2007) documented strain-dependent variation in *E. coli* associations with sand, silt and clay particles, and these differences could cause differences in *E. coli* strain survival. Yet another reason for the oscillations could be the loss of nutrients from sediment to water during first days of the incubation as indicated by the increase in electrical conductivity and turbidity (Fig. 1c and d). It could cause the increased biological activity in water as indicated by the decrease in dissolved oxygen (Fig. 1a) and increase in pH (Fig. 1b) and simultaneous decrease on *E. coli* populations in sediment (Fig. 3). As the transport of nutrients to water decreased with time, *E. coli* concentrations could stabilize (data for days 2 and 4 in Fig. 3) and the further decrease in these concentrations could be related mostly to processes in sediments. Changes in *E. coli* concentrations were substantial during first four days, and the search for explanations of those changes presents an interesting avenue for further work. The oscillatory behavior in system parameters that we have observed is not simply fluctuation, it reflects the re-equilibration in the system which has been brought to the non-equilibrium by the introduction of large population of *E. coli* and nutrients into sediment compartment.

E. coli inactivation after the first four days of the experiment resulted in die-off curves that had comparable shapes for all sediments at the same temperature (Fig. 3). Inactivation rates were directly correlated with temperature (Fig. 3). The only exceptions to exponential inactivation were in sediments A, and to a lesser extent C, at 24 °C where low steady-state *E. coli* concentrations were observed toward the end of the experimental period (Fig. 3).

Sediment B, which consistently supported the highest concentrations of *E. coli* (i.e. lowest inactivation rates), had a much finer particle size distributions, higher organic matter content, and higher indigenous *E. coli* concentrations than sediments A and C. Other researchers have observed a correlation between sediment texture and die-off. Sherer et al. (1992) and Howell et al. (1996) found that *E. coli* die-off was slower in sediments with clayey textures than in loamy textures. Slow *E. coli* die-off in clayey sediments have been interpreted by Davies and Bavor (2000) and Decamp and Warren (2000) as an indication that fine-texture sediments provide protection from predators. Another reason for the better *E. coli* survival in sediments with predominantly fine particles could be participation of *E. coli* in biofilm formation. Clay particles were shown to be conducive for the formation of biofilms in sediments. Banning et al. (2003) studied the interactions between *E. coli* inocula and biofilms of indigenous microorganisms introduced with groundwater. *E. coli* colonized all layers of the mixed population in this study. Alimova et al. (2006) demonstrated that the presence of smectite clays enhances the formation of *E. coli* biofilms; *E. coli* populations in the clay mixtures were greater than the respective populations in media without clay. Smectite-bearing clay slurries developed bacteria–clay aggregates with a substantial biofilm component within 24 h, while the exclusively bacterial suspensions did not develop any observable biofilm component.

The role of organic matter content in *E. coli* survival could be deduced by comparison of the data for sediments A and C. Sediment C had significantly higher organic matter content and the inactivation in it was substantially slower than in A at all temperatures. Such effect of organic matter on the *E. coli* survival in sediment supports the hypothesis that the better survival of *E. coli* in B could be attributed not only to the finer texture but also to the high content of organic matter in B as compared to A and C. High organic matter content in combination with finer sediment particles could result in micro-aggregates that restrict access to pore spaces by predators. Alternatively, higher organic matter sediments could contain slow-release, polymeric nutrients that retard cell die-off. The joint positive effect of the increase in the percentage fine particles and organic matter content on the *E. coli* survival in freshwater sediments was repeatedly shown (e.g., Poté et al., 2009). We are not aware of published data of experiments in which *E. coli* inactivation was compared for sediments with the same particle size but different natural organic matter content, although the removal of the organic matter from sediment definitely speeded up the *E. coli* inactivation (Lee et al., 2006). Haller et al. (2009a,b) suggested that high levels of organic content may have allowed *E. coli* to survive for 90 days in spite of the absence of the additional input.

3.4. *Application of the exponential inactivation model*

Chick's (1908) exponential model:

$$C = C_0 e^{-\mu t} \quad (1)$$

is commonly used to simulate *E. coli* inactivation. In Eq. (1), C is the *E. coli* concentration at time t, C_0 is the initial concentration of *E. coli*, and μ is the inactivation rate constant. Transformation of Eq. (1) to the logarithmic form leads to

$$\ln C = \ln C_0 - \mu t \quad (2)$$

The logarithms of concentrations should follow straight lines at time vs. log C graphs from the very beginning of the inactivation.

Model (1) or its transform (2) could not be used directly with our data that show substantial concentration dynamics due to the re-equilibration that occurred over several days and in several stages qualitatively similar for all temperatures and sediments studied. The first stage was characterized by a substantial mass exchange between the sediment and water column. The mass exchange manifested itself in decreased *E. coli* populations in sediments concomitant with increased concentrations in the water column, along with increases in water turbidity and changes in physico-chemical water parameters. The second stage was characterized by increases in *E. coli* populations in sediments. Sediment is the environment where bacteria survive and compete with other organisms, and where populations are controlled by factors that can cause either positive changes, such as growth and/or settling from the water column, or negative changes, such as release to the water column during sediment resuspension and die-off. In our studies, growth in sediment dominated the second stage. The third stage consisted of a quasi-steady state in *E. coli* populations in sediment, prior to achieving equilibrium. The fourth stage was characterized by a log-linear decrease in *E. coli* concentrations. Results from experiments at 24 °C show that the fifth stage exists when concentrations are stabilized at a low level, and sediments provide a medium where *E. coli* populations can survive, despite competition and predation. These stable concentrations appear to be a consequence of adaptations to new nutritional constraints or protection against predation. The first three stages were relatively short; more frequent sampling, similar to that in Solo-Gabriele et al. (2000) or Desmarais et al. (2002), would be needed to quantify and model the changes in the sediment *E. coli* populations during these stages.

Because of the initial re-equilibration changes in *E. coli* concentrations, the performance of the exponential model (1), (2) varied greatly and the determination coefficients were in the range from 0.216 to 0.961. However, the dependencies of log C on times became very close to linear after the end of first three stages occurring around days 2–4, 4–6, and 12–16 at 24°, 14° and 4°, respectively (Fig. 4), and the inactivation could be described with the equation

$$\ln C = \ln C_e - \mu(t - t_e) \quad (3)$$

where C_e is the concentration at the beginning of the exponential inactivation stage and t_e is the time of the beginning of this stage. All coefficients of determination R^2 of the linear regressions (3) in Fig. 4 were greater than 0.98. We note that the exponential inactivation stage ended before the end of the experiment, and *E. coli* concentrations stabilized at low levels in sediments A and C at 24 °C (Fig. 4).

Table 2 contains values of μ obtained as the slopes of regressions in Fig 4 (multiplied by 2.303 to convert decimal logarithms into natural ones). Inactivation rates followed the sequence: $\mu_A > \mu_C > \mu_B$. at all three temperatures where the subscripts indicate sediments. The difference between the inactivation rates in sediments A and B grew with temperature: μ_A/μ_B was equal to 1.3 at 4 °C, to 1.8 at 14 °C and to 3.1 at 24 °C. A similar but less drastic difference was found between the inactivation rates in A and C: the μ_A/μ_C ratio was equal to 1.0, 1.7, and 2.1 at 4 °C, 14 °C, and 24 °C, respectively.

Inactivation rates were similar for all sediments at 4 °C. *E. coli* concentrations decreased by only one order of magnitude during four months of incubation at 4 °C. This is not surprising considering that biological activity is diminished at lower temperatures. Ishii et al. (2006), who applied the PCR-based DNA fingerprint analysis to soils of several coastal Lake Superior watersheds, documented that 92% of the *E. coli* genotypes overwintered in frozen soil.

We realize that the values of inactivation rates in Table 2 are affected by subjective decisions we have made about the beginning and end times (at 24 °C) of the exponential inactivation stage. The exponential inactivation model is not appropriate as long as bacterial growth is not negligible, e.g. during re-equilibration phase or steady states, when growth may be balanced by the predator activity, or the structure of bacterial population is adapted to physical or biological constraints as in the end of the experiment at 24 °C. The volume of measurements and amount of sediment may be prohibitive for obtaining observations frequent enough to use statistical criteria in delineation of the exponential inactivation stage. High correlation coefficients of the regressions in Fig. 4 indicate that the assumption of linearity is appropriate within the exponential inactivation time intervals that we have selected.

We note that modeling of *E. coli* fate and transport in creeks, lakes, reservoirs, etc., requires modeling interactions between the sediment and water column to complement the inactivation modeling. The release of *E. coli* from sediment into the water column during sediment resuspension, settling of bacteria associated with suspended sediment, nutrient exchange between sediment and water, and predator population dynamics have to be included in the description of *E. coli* fate and transport. Jamieson et al. (2005b) indicated that movement of sediment-borne bacteria has to be simulated along with bacteria fate and transport, because the pattern and magnitude of bacteria resuspension and deposition in streams should be related to the sediment particles to which they are attached. Modeling *E. coli* fate and transport without including resuspension and settling mechanisms has successfully captured spatial trends, but appeared to be incapable of explaining changes in *E. coli* concentrations in water during events causing sediment resuspension (e. g., McCorquodale et al., 2004; Hellweger and Masopust, 2008). Incorporating *E. coli* release into *E. coli* fate and transport models has been undertaken at different complexity levels (Wilkinson et al., 1995; Bai and Lung, 2005). Recent analysis shows that the mass exchange between freshwater sediments and water column is quite complex and includes hyporheic phenomena (Jamieson et al., 2005b; Cooley et al., 2007). The

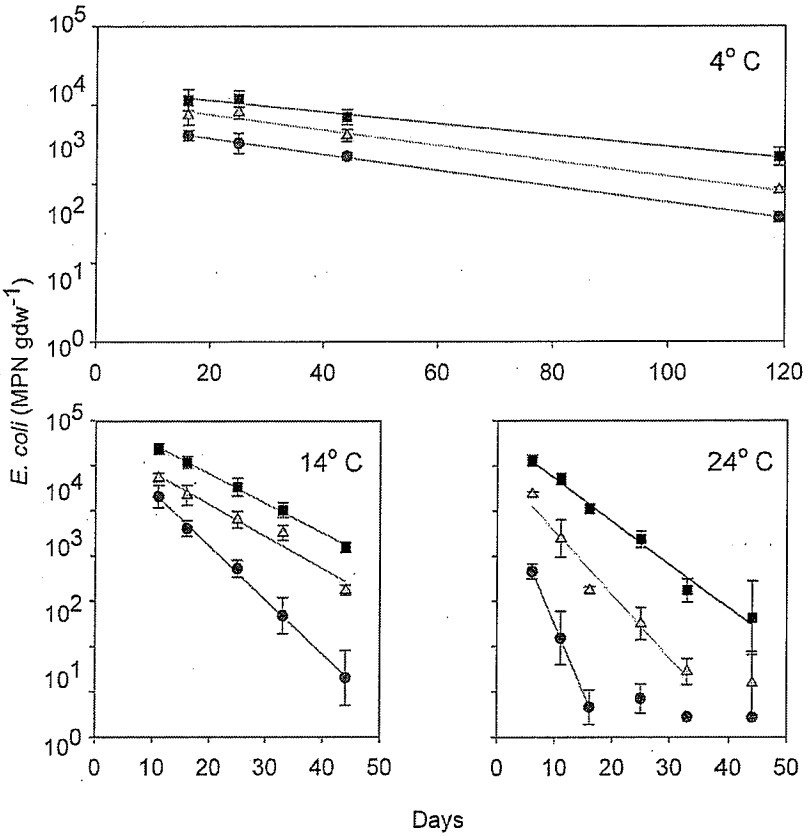

Fig. 4 – Exponential stage of *E. coli* inactivation in sediments. ● – sediment A, ■ – sediment B, ▲ – sediment C. Error bars show standard deviations computed for logarithms of concentrations.

inactivation model (3) addresses only part of the sediment bacteria dynamic. Progress in understanding mass exchange between sediment and water is necessary to develop satisfactory predictive models of *E. coli* (and other microorganisms) fate and transport in surface water sources.

3.5. *Sensitivity of inactivation rates to temperature*

Data on inactivation rates in Table 2 show that the sediment properties affect not only the absolute values of the inactivation rates during the exponential inactivation stage but also changes of those rates with temperature. *E. coli* inactivation rates were most sensitive to temperature in sediment A and least sensitive in sediment B. We quantified such sensitivity by computing ratios μ_{14}/μ_4 and μ_{24}/μ_{14} for all sediments (Table 3). An increase in temperature from 4 °C to 24 °C resulted in a 15-fold increase in μ values for sediment A but only a 7-fold increase for sediments B and C. Values of μ increased 2.5 times in A, 2 times in C and only 1.5 times in B as the temperature increased from 14 °C to 24 °C. The differences in sensitivity of the inactivation in the ranges from 4 °C to 14 °C and to 24 °C can be related to the changes in ecological interactions. In experiments on *E. coli* survival in estuarine water, McCambridge and McMeekin (1980) showed that bacterial decline depended on the presence of both bacterial and protozoan predators, the latter having a temperature

Table 2 – *E. coli* inactivation rates μ (d^{-1}) during the exponential inactivation stage.

Sediment	Manure-borne *E. coli*		
	4 °C	14 °C	24 °C
A	0.0233 (0.999)[a]	0.138 (0.986)	0.346 (0.986)
B	0.0169 (0.986)	0.0754 (0.997)	0.110 (0.986)
C	0.0225 (0.986)	0.0823 (0.963)	0.161 (0.968)

a Numbers in parentheses are the determination coefficients R^2 of the linear regressions of time vs. logarithm of the *E. coli* concentration.

Table 3 – Sensitivity of *E. coli* inactivation rates to temperature.

Ratio of inactivation rates[a]	Sediment A	Sediment B	Sediment C
μ_{14}/μ_4	5.92	4.46	3.66
μ_{24}/μ_{14}	2.51	1.46	1.96

a μ_4, μ_{14}, and μ_{24} – inactivation rates during the exponential stage at 4°, 14°, and 24°, respectively.

optimum of 15–20 °C and the former becoming more important as the incubation temperature increased.

4. Conclusions

E. coli inactivation under water columns in sediments amended with manure material was studied in this work using flow-through chambers. The introduction of high concentrations of *E. coli* and nutrients into sediments created a non-equilibrium state resulting in interactions between the two compartments – the water column and the sediment.

The initial resuspension of the sediment caused an immediate increase in *E. coli* content in water and the synchronous decrease in DO and pH. The die-off of *E. coli* in water was faster than in sediments at all temperatures and with all sediments, so that sediments provided a more hospitable environment for *E. coli* as compared with the water column.

E. coli inactivation in sediments was affected by sediment properties at all three temperatures. Of the two sediments with the same contents of sand and silt particles, the sediment with larger organic carbon content provided conditions for the slower inactivation. The slowest inactivation was observed in the sediment with finest particles and the largest organic carbon content.

The exponential inactivation model performed with variable degree of accuracy in simulations of the inactivation during the whole experimental period due to presence of initial oscillatory slow-inactivation stage. The steady inactivation stage that began within days 4–6 could be simulated with the exponential model in a satisfactory manner. In the nutrient-poor sandy sediment, *E. coli* concentrations stabilized at the low levels after the fast inactivation.

We observed the effect of sediment properties not only on the inactivation rates but also on the changes in the inactivation rates with temperature. The lower inactivation rates were for a particular sediment, the less they changed with temperature. The smallest temperature effect on the inactivation rates was found in the sediment with the finest particles and the largest organic carbon content.

The observed effects of sediment properties on *E. coli* inactivation have implications for the management of manure-borne *E. coli*. Information about the sediment particle size distributions has to be collected and used, since transport of manure-borne *E. coli* to the stream and reservoir areas where sediments have the finest particles will create better conditions for *E. coli* survival and possible mobilization to the water column during high flow events. The slow inactivation of *E. coli* in all three sediments at 4 °C indicates the likelihood of *E. coli* survival through winter. Therefore, prevention of the transport of manure-borne *E. coli* to water sources cannot be limited only to warm months.

Results of this work suggest that periodic inputs of cells and/or nutrients are required to maintain sediment-borne and water-borne *E. coli* populations. Monitoring of *E. coli* concentrations in water sources should not ignore sediments, because the release of organisms from the streambeds may distort conclusions about the environmental sources of this organism and required management practices. In this respect, more information about the survival and inactivation is needed both for other indicator organisms, such as enterococci, and for true pathogens.

REFERENCES

Alimova, A., Roberts, M., Katz, A., Rudolph, E., Steiner, J.C., Alfano, R.R., Gottlieb, P., 2006. Effects of smectite clay on biofilm formation by microorganisms. Biofilms 3 (1), 47–54.

An, Y.-J., Kampbell, D.H., Breidenbach, J.P., 2002. *Escherichia coli* and total coliforms in water and sediments at lake marinas. Environmental Pollution 120 (3), 771–778.

Anderson, K.L., Whitlock, J.E., Harwood, V.J., 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied and Environmental Microbiology 71 (6), 3041–3048.

Angier, J.T., McCarty, G.W., Prestegaard, K.L., 2005. Hydrology of a first-order riparian zone and stream, mid-Atlantic coastal plain, Maryland. Journal of Hydrology 309 (1–4), 149–166.

Bai, S., Lung, W.S., 2005. Modeling sediment impact on the transport of fecal bacteria. Water Research 39, 5232–5240.

Banning, N., Toze, S., Mee, B., 2003. Persistence of biofilm-associated *Escherichia coli* and *Pseudomonas aeruginosa* in groundwater and treated effluent in a laboratory model system. Microbiology 149 (1), 47–55.

Buckley, R., Clough, E., Warnken, W., Wild, C., 1998. Coliform bacteria in streambed sediment in a subtropical rainforest conservation reserve. Water research 32, 1852–1856.

Burton Jr., G.A., Gunnison, D., Lanza, G.R., 1987. Survival of pathogenic bacteria in various freshwater sediments. Applied and Environmental Microbiology 53 (4), 633–638.

Byappanahalli, M., Fowler, M., Shively, D., Whitman, R., 2003. Ubiquity and persistence of *Escherichia coli* within a Midwestern stream. Applied and Environmental Microbiology 69 (8), 4549–4555.

Chick, H., 1908. An investigation of the law of disinfection. Journal of Hygiene 8, 92–158.

Cinotto, P.J., 2005. Occurrence of fecal-indicator bacteria and protocols for identification of fecal-contamination sources in selected reaches of the West Branch Brandywine Creek, Chester County, Pennsylvania. U.S. Geological Survey Scientific Investigations Report 2005-5039, 91 p.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J., Mandrell, R.E., 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS ONE 2, 1159. doi:10.1371/journal.pone.0001159.

Crabill, C., Donald, R., Snelling, J., Foust, R., Southam, G., 1999. The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. Water Research 33 (9), 2163–2171.

Davies, C.M., Bavor, H.J., 2000. The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. Journal of Applied Microbiology 89 (2), 349–360.

Davies, C.M., Long, J.A.H., Donald, M., Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology 61 (5), 1888–1896.

Decamp, O., Warren, A., 2000. Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment. Ecological Engineering 14 (3), 293–299.

Desmarais, T.R., Solo-Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Applied and Environmental Microbiology 68, 1165–1172.

Doyle, J.D., Tunnicliff, B., Kramer, K., Kuehl, R., Brickler, S.K., 1992. Instability of fecal coliform populations in waters and bottom sediments at recreational beaches in Arizona. Water Research 26 (7), 979–988.

Ferguson, C.M., Coote, B.G., Ashbolt, N.J., Stevenson, I.M., 1996. Relationships between indicators, pathogens and water quality in an estuarine system. Water Research 30 (9), 2045–2054.

Gale, E.F., Epps, H.M.R., 1942. The effect of the pH of the medium during growth on the enzymic activities of bacteria (*Escherichia coli* and *Micrococcus lysodeikticus*) and the biological significance of the changes produced. Biochemical Journal 36 (7–9), 600–618.

Gee, G.W., Or, D., 2002. Particle size analysis. In: Dane, Topp, G.C. (Eds.), Methods of Soil Analysis. Part 4. Physical Methods. ASA and SSSA, Madison, WI, pp. 278–282.

Goyal, S.M., Gerba, C.P., Melnick, G.L., 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas Coast. Applied and Environmental Microbiology 34 (2), 139–149.

Guber, A.K., Pachepsky, Y.A., Shelton, D.R., Yu, O., 2007. Effect of bovine manure on fecal coliform attachment to soil and soil particles of different sizes. Applied and Environmental Microbiology 73 (10), 3363–3370.

Haller, L., Amedegnato, E., Poté, J., Wildi, W., 2009a. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. Water, Air, and Soil Pollution 203, 217–227.

Haller, L., Poté, J., Loizeau, J.L., Wildi, W., 2009b. Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. Ecological Indicators 9, 540–547.

Hellweger, F.L., Masopust, P., 2008. Investigating the fate and transport of *Escherichia coli* in the Charles River, Boston, using high-resolution observation and modeling. Journal of the American Water Resources Association (JAWRA) 44, 509–522.

Howell, J.M., Coyne, M.S., Cornelius, P.L., 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. Journal of Environmental Quality 25, 1216–1220.

Hunter, C., McDonald, A., Bevin, K., 1992. Input of faecal coliforms bacteria to an upland stream channel in the Yorkshire Dales. Water Resources Research 28 (7), 1869–1876.

Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Applied and Environmental Microbiology 72 (1), 612–621.

Jamieson, R., Gordon, R., Joy, D., Lee, H., 2004. Assessing microbial pollution of rural surface waters: a review of current watershed scale modeling approaches. Agricultural water management 70, 1–17.

Jamieson, R., Joy, D., Lee, H., Kostachuk, R., Gordon, R., 2005a. Resuspension of sediment-associated *Escherichia coli* in a natural stream. Journal of Environmental Quality 34 (2), 581–589.

Jamieson, R.C., Joy, D.M., Lee, H., Kostaschuk, R., Gordon, R.J., 2005b. Transport and deposition of sediment-associated *Escherichia coli* in natural streams. Water Research 39, 2665–2675.

Koirala, S., Gentry, R., Perfect, E., Schwartz, J., Sayler, G., 2008. Temporal variation and persistence of bacteria in streams. Journal of Environmental Quality 37 (4), 1559–1566.

Lee, C.M., Lin, T.Y., Lin, C., Kohbodi, G.A., Bhatt, A., Lee, R., Jay, J.A., 2006. Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. Water Research 40 (14), 2593–2602.

Lopez-Torres, A.J., Hazen, T.C., Toranzos, G.A., 1987. Distribution and in situ survival and activity of *Klebsiella pneumoniae* and *Escherichia coli* in a tropical rain forest watershed. Current Microbiology 15 (4), 213–218.

McCambridge, J., McMeekin, T.A., 1980. Effect of temperature on activity of predators of *Salmonella typhimurium* and *Escherichia coli* in estuarine water. Australian Journal of Marine and Freshwater Research 31, 851–855.

McCorquodale, J.A., Georgiou, I., Carnelos, S., Englande, A.J., 2004. Modeling coliforms in storm water plumes. Journal of Environmental Engineering and Science 3, 419–431.

Muirhead, R.W., Davies-Colley, R.J., Donnison, A.M., Nagels, J.W., 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research 38 (5), 1215–1224.

Nagels, J.W., Davies-Colley, R.J., Donnison, A.M., Muirhead, R.W., 2002. Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. Water Science and Technology 45 (12), 45–52.

Poté, J., Haller, L., Kottelat, R., Sastre, V., Arpagaus, P., Wildi, W., 2009. Persistence and growth of faecal culturable bacterial indicators in water column and sediments of Vidy Bay, Lake Geneva, Switzerland. Journal of Environmental Sciences 21 (1), 62–69.

Rehmann, C.R., Soupir, M.R., 2009. Importance of interactions between the water column and the sediment for microbial concentrations in streams. Water Research 43 (18), 4579–4589.

Seyfried, P.L., Harris, E.M., 1986. Detailed bacteriological water quality study examining the impact of sediment and survival times. Technology Transfer Conference. Part B: Water Quality Research (Toronto, Ontario), 347–391.

Sherer, B.M., Miner, J.R., Moore, J.A., Buckhouse, J.C., 1992. Indicator bacterial survival in stream sediments. Journal of Environmental Quality 21 (4), 591–595.

Smith, J., Edwards, J., Hilger, H., Steck, T.R., 2008. Sediment can be a reservoir for coliform bacteria released into streams. Journal of General and Applied Microbiology 54 (3), 173–179.

Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., Palmer, C.J., 2000. Sources of *Escherichia coli* in a coastal subtropical environment. Applied and Environmental Microbiology 66, 230–237.

Stephenson, G.R., Rychert, R.C., 1982. Bottom sediment: a reservoir of *Escherichia coli* in rangeland streams. Journal of Range Management 35 (1), 119–123.

Topp, E., Welsh, M., Tien, Y.-C., Dang, A., Lazarovits, G., Conn, K., Zhu, H., 2003. Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. FEMS Microbiology Ecology 44 (3), 303–308.

U.S. EPA, 1986. Ambient Water Quality Criteria for Bacteria. EPA 440/5-84-002. U.S. Environmental Protection Agency, Washington, D.C.

Wilkinson, R.J., Jenkins, A., Wyer, M., Kay, D., 1995. Modelling faecal coliform dynamics in streams and rivers. Water Research 29, 847–855.

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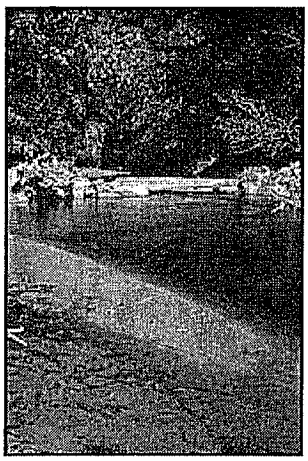
E. coli Can Survive in Streambed Sediments for Months

By Ann Perry
July 1, 2011



ARS researchers have discovered that *E. coli* pathogens can survive for months in underwater sediments, which could improve computer modeling for the bacterial contamination of surface waters. *Photo courtesy of NRCS-USDA.*

Studies by U.S. Department of Agriculture (USDA) scientists have confirmed that the presence of *Escherichia coli* pathogens in surface waters could result from the pathogen's ability to survive for months in underwater sediments. Most *E. coli* strains don't cause illness, but they are indicator organisms used by water quality managers to estimate fecal contamination.

These findings, which can help pinpoint potential sources of water contamination, support the USDA priorities of promoting sustainable agriculture and food safety.

Soil scientist Yakov Pachepsky works at the Agricultural Research Service (ARS) Environmental Microbial and Food Safety Laboratory in Beltsville, Md. He is conducting studies to learn more about where the *E. coli* pathogens in streambeds come from, where they end up, and how long they can survive. ARS is USDA's chief intramural scientific research agency.

Lab studies conducted by Pachepsky and his colleagues suggested that non-pathogenic strains of *E. coli* can survive much longer in underwater sediments than in the water column itself, and provided the first published evidence that *E. coli* can overwinter in the sediment.

The results also indicated that the pathogens lived longer when levels of organic carbon and fine sediment particles in the sediment were higher. In addition, when organic carbon levels were higher, water temperatures were less likely to affect the pathogens' survival rates.

The researchers also collected three years of data on stream flow, weather, and *E. coli* levels in water and sediments from a stream in Pennsylvania that was fed by several smaller tributaries. Then they used the information to calibrate the Soil and Water Assessment Tool (SWAT), a model developed by ARS scientists that predicts how farming practices affect water quality on watershed scale.

The resulting simulations indicated pasture runoff contributed to *E. coli* levels in nearby streams only during temporary interludes of high-water flows. Since the SWAT model currently does not include data on *E. coli* levels in streambed sediments, this research indicates that SWAT simulations would overestimate how much *E. coli* contamination in surface waters is due to pasture runoff.

Results from this work were published in *Water Research*, *Ecological Modeling*, *Critical Reviews in Environmental Science and Technology*, and *Journal of Hydrology*.

Read more about this work in the July 2011 issue of *Agricultural Research* magazine.

For further reading

- Lesser known *Escherichia coli* types targeted in food safety research
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Sediment-Associated Bacteria Release and Settling in SWAT bacteria routing

Model and SWAT add-on manual

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USDA-ARS

August, 2009

Abstract

Streambed sediment has been attracting attention as a reservoir for bacteria, including pathogenic strains. Soil and Water Assessment Tool (SWAT2005) includes bacteria transport subroutine in which bacteria die-off is the only in-stream process. The purpose of this technical bulletin was to describe SWAT bacteria routing module by including sediment-associated bacteria release and settling in streams. Streambed bacteria release and settling were computed based on the sediment resuspension and deposition modules in SWAT. The model and the necessary modifications of the SWAT 2005 code are presented.

Disclaimer

Although the code has been tested by its developers, no warranty, expressed or implied, is made as to the accuracy and functioning of the program modifications and related program material, nor shall the fact of distribution constitute any such warranty, and no responsibility is assumed by the developers in connection therewith.

Contents

Abbreviations

cfu – colony forming units

List of symbols

$bact_{ch}$	the amount of bacteria in the stream water in the reach segment (# cfu)
$bact_{ch,i}$	the amount of bacteria in the stream water in the reach segment at the beginning of the time period (# cfu)
$bact_{deg}$	the amount of bacteria released from streambed in the reach segment (# cfu)
$bact_{dep}$	the amount of bacteria attached on the deposited sediments in stream water in the reach segment (# cfu)
$bact_{free}$	the amount of free-floating bacteria in the stream water in the reach segment (# cfu)
$bact_{sus}$	the amount of bacteria attached on the suspended sediments in the stream water in the reach segment (# cfu)
bsc_1 - bsc_4	the regression coefficients in streambed bacteria concentration equation
clay	the percentage of clay in suspended sediment in stream water in the reach segment
$conc_{bact,ch}$	the concentration of bacteria in the stream water in the reach segment (# cfu/100 mL)
$conc_{bact,sed}$	the concentration of bacteria in the upper layer of streambed in the reach segment (# cfu/ton sediment)
$conc_{sed,ch,i}$	the initial sediment concentration in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O)
$conc_{sed,ch,mx}$	the maximum concentration of sediment that can be transported by the water (ton sediment/m^3 H_2O or kg sediment/L H_2O)

$conc_{sed,dep}$	the concentration of sediment deposited in water in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O)
$conc_{sed,sus}$	the concentration of sediments suspended in water in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O)
day	the day of year
sed_{deg}	the amount of sediment reentrained in the reach segment (metric tons)
sed_{dep}	the amount of sediment deposited in the reach segment (metric tons)
C_{ch}	the channel cover factor which is defined as the ratio of degradation from a channel with a specified vegetative cover to the corresponding degradation from a channel with no vegetative cover
K_{ch}	the channel or soil erodibility factor (cm/hr/Pa) which is a function of properties of the bed or bank materials
K_p	the linear partitioning coefficient of bacteria between the sediments and water (m^3 H_2O/ton sediment or L H_2O/kg sediment)
V_{ch}	the volume of water in the reach segment (m^3 H_2O)

1. Background

Streambed sediment has been increasingly attracting attention as a reservoir of bacteria, including pathogenic strains. Streambed microorganisms can be released to water in substantial amounts as sediments resuspend (Byappanahalli et al., 2003; Muirhead et al., 2004; Giddings and Oblinger, 2004; Cinotto, 2005). Streambed sediment provides a favorable chemical and biological environment for bacteria (Gannon et al., 1983), and can protect bacteria from protozoan predators (Davies et al., 1995). Therefore, sediment-associated bacteria release and settling model was developed and the code was added to the current SWAT bacteria module.

2. Model development

In sediment channel routing, the maximum concentration of sediment that can be transported by the water, $conc_{sed,ch,mx}$, (ton/m^3 or kg/L) is compared to the concentration of sediment in the reach at the beginning of the time step, $conc_{sed,ch,i}$ (Neitsch et al., 2005).

If $conc_{sed,ch,i} < conc_{sed,ch,mx}$, resuspension is the dominant process in the reach segment and the net amount of sediment reentrained is calculated:

$$sed_{deg} = \left(conc_{sed,ch,mx} - conc_{sed,ch,i}\right) \cdot V_{ch} \cdot K_{ch} \cdot C_{ch} \qquad [1]$$

where sed_{deg} is the amount of sediment reentrained in the reach segment (metric tons), $conc_{sed,ch,mx}$ is the maximum concentration of sediment that can be transported by the water (ton sediment/m^3 H_2O or kg sediment/L H_2O), $conc_{sed,ch,i}$ is the initial sediment concentration in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O), V_{ch} is the volume of water in the reach segment (m^3 H_2O), K_{ch} is the channel or soil erodibility factor (cm/hr/Pa) which is a function of properties of the bed or bank materials, and C_{ch} is the channel cover factor which is defined as the ratio of degradation from a

channel with a specified vegetative cover to the corresponding degradation from a channel with no vegetative cover. When sediment resuspends, both bacteria in sediment solution and on sediment particles are released, and the net amount of bacteria released from streambed is calculated:

$$bact_{deg} = sed_{deg} \cdot conc_{bact,sed} \quad [2]$$

where $bact_{deg}$ is the amount of bacteria released from streambed in the reach segment (# cfu), sed_{deg} is the amount of sediment reentrained in the reach segment (metric tons), and $conc_{bact,sed}$ is the concentration of bacteria in the upper layer of streambed in the reach segment (# cfu/ton sediment). Bacteria concentration in streambed is calculated by the empirical regression equation, logarithmic sine function of the days of year:

$$\log(conc_{bac,sed}) = bsc_1 \cdot \sin\left(bsc_2 \cdot \frac{day - bsc_3}{366} \cdot \pi\right) + bsc_4 \quad [3]$$

where $conc_{bac,sed}$ is the concentration of bacteria in the upper layer of streambed (# cfu/ton sediment), day is the day of year, and bsc_1 through bsc_4 are the regression coefficients in streambed bacteria concentration equation.

If $conc_{sed,ch,i} > conc_{sed,ch,mx}$, deposition is the dominant process in the reach segment and the net amount of sediment deposited is calculated:

$$sed_{dep} = (conc_{sed,ch,i} - conc_{sed,ch,mx}) \cdot V_{ch} \quad [4]$$

where sed_{dep} is the amount of sediment deposited in the reach segment (metric tons), $conc_{sed,ch,i}$ is the initial sediment concentration in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O), $conc_{sed,ch,mx}$ is the maximum concentration of sediment that can be transported by the water (ton sediment/m^3 H_2O or kg sediment/L H_2O), and V_{ch} is the volume of water in the reach segment (m^3 H_2O). When suspended sediment deposits, bacteria on settling sediment particles are deposited, and

the bacteria in the stream water are partitioned into 3 phases based on the Bai and Lung's (2005) linear adsorption assumption:

$$\frac{bact_{free} + bact_{sus} + bact_{dep}}{bact_{ch,i}} = \frac{1 + K_p \cdot conc_{sed,sus} + K_p \cdot conc_{sed,dep}}{1 + K_p \cdot conc_{sed,ch,i}} \quad [5]$$

where $bact_{ch,i}$ is the amount of bacteria in the stream water in the reach segment at the beginning of the time period (# cfu), $bact_{free}$ is the amount of free-floating bacteria in the stream water in the reach segment (# cfu), $bact_{sus}$ is the amount of bacteria attached on the suspended sediments in the stream water in the reach segment (# cfu), $bact_{dep}$ is the amount of bacteria attached on the deposited sediments in the stream water in the reach segment (# cfu), K_p is the linear partitioning coefficient of bacteria between the sediments and water (m^3 H_2O/ton sediment or L H_2O/kg sediment), and $conc_{sed,ch,i}$ is the initial sediment concentration in the reach (ton sediment/m^3 H_2O or kg sediment/L H_2O). Here, $conc_{sed,ch,i}$ is the sum of $conc_{sed,sus}$ which is the concentration of sediments suspended in water in the reach and $conc_{sed,dep}$ which is the concentration of sediment deposited in water in the reach.

Therefore, using the variables used in the sediment computation, the net amount of bacteria settled from stream water is calculated as:

$$bact_{dep} = bact_{ch,i} \cdot \frac{K_p \cdot sed_{dep}}{V_{ch} + K_p \cdot \left(conc_{sed,ch,i} \cdot V_{ch}\right)} \quad [6]$$

where $bact_{dep}$ is the amount of bacteria settled from stream water in the reach segment (# cfu), $bact_{ch,i}$ is the amount of bacteria in the stream water in the reach segment at the beginning of the time period (# cfu), K_p is the linear partitioning coefficient of bacteria between the sediments and water (m^3 H_2O/ton sediment or L H_2O/kg sediment), sed_{dep} is the amount of sediment deposited in the reach segment (metric tons), V_{ch} is the volume of water in the reach segment (m^3 H_2O), and $conc_{sed,ch,i}$ is the initial sediment concentration in the reach (ton sediment/m^3 H_2O or kg sediment/L

H_2O). The linear partitioning coefficient is calculated from the empirical regression equation (Pachepsky et al., 2006):

$$K_p = 10^{-1.6} \cdot clay^{1.98} \quad [7]$$

where K_p is the linear partitioning coefficient of bacteria between the sediments and water (m^3 H_2O/ton sediment or L H_2O/kg sediment) and *clay* is the percentage of clay in suspended sediment in stream water in the reach segment (%). *clay* normally varies between 2 and 50%.

Once the amount of bacteria released and settled has been calculated, the final amount of sediment in the reach is determined:

$$bact_{ch} = bact_{ch,i} + bact_{deg} - bact_{dep} \quad [8]$$

where $bact_{ch}$ is the amount of bacteria in the stream water in the reach segment (# cfu), $bact_{ch,i}$ is the amount of bacteria in the stream water in the reach segment at the beginning of the time period (# cfu), $bact_{deg}$ is the amount of bacteria released from streambed in the reach segment (# cfu), and $bact_{dep}$ is the amount of bacteria settled from stream water in the reach segment (# cfu).

The final bacteria concentration in the reach is calculated:

$$conc_{bact,ch} = \frac{bact_{ch}}{V_{ch}} \cdot 10^{-4} \quad [9]$$

where $conc_{bact,ch}$ is the concentration of bacteria in the stream water in the reach segment (# cfu/100 mL), $bact_{ch}$ is the amount of bacteria in the stream water in the reach segment (# cfu), and V_{ch} is the volume of water in the reach segment (m^3 H_2O).

3. SWAT input variables that pertain to bacteria release and settling in the stream

Variable Name	Definition	SWAT Input File

CLAY	*clay* : the percentage of clay in suspended sediment in stream water in the reach segment (%)	.bsn
BSC1 through BSC4	bsc_1 through bsc_4 : the regression coefficients in streambed bacteria concentration equation	.bsn

4. Model limitations and further developments

The developed model uses the measured dynamics of bacteria in streambed that is approximated with Eq. [3]. Also the model does not take into account the distribution of bacteria in sediment with depth. Further work will be performed to overcome these limitations.

5. References

Bai, S.; Lung, W.S., 2005. Modeling sediment impact on the transport of fecal bacteria. Water Research, 39(20): 5232-5240.

Byappanahalli, M.; Fowler, M.; Shively, D.; Whitman, R., 2003. Ubiquity and persistence of *Escherichia coli* in a midwestern coastal stream. Applied and Environmental Microbiology, 69(8): 4549-4555.

Cinotto, P.J., 2005. Occurrence of fecal-indicator bacteria and protocols for identification of fecal-contamination sources in selected reaches of the West Branch Brandywine Creek, Chester County, Pennsylvania: U.S. Geological Survey Scientific Investigations Report 2005-5039, pp 91.

Davies, C.M.; Long, J.A.H.; Donald, M.; Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and fresh-water sediments. Applied and Environmental Microbiology, 61(5): 1888-1896.

Gannon, J.J.; Busse, M.K.; Schillinger, J.E., 1983. Fecal coliform disappearance in a river impoundment. Water Research, 17(11): 1595-1601.

Giddings, E.M.; Oblinger, C.J., 2004. Fecal-indicator bacteria in the Newfound Creek watershed, western North Carolina, during a high and low streamflow condition, 2003: U.S. Geological Survey Scientific Investigations Report 2004–5257, accessed January 18, 2005, at http://pubs.water.usgs.gov/sir2004-5257.

Muirhead, R.W.; Davies-Colley, R.J.; Donnison, A.M.; Nagels, J.W., 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research, 38: 1215-1224.

Neitsch, S.L.; Arnold, J.G.; Kiniry, J.R.; Williams, J.R., 2005. Soil and Water Assessment Tool Theoretical Documentation (Version 2005).

Pachepsky, Y.A.; Sadeghi, A.M.; Bradford, S.A.; Shelton, D.R.; Guber, A.K.; Dao, T., 2006. Transport and fate of manure-borne pathogens: Modeling perspective. Agricultural Water Management, 86: 81-92.

6. Algorithm implementation and computer code listing

The SWAT model was compiled with COMPAQ VISUAL FORTRAN 6.0 and run using the input files (watershed topography, soils, landuse, etc.) generated from AVSWATX interface.

The new model is embedded in SWAT's bacteria routing module ('rtbact.f'). In the following Table 1, the new variables/parameters used in this module are related to variables in

equations used in the model description. Sediment routing-related variables are taken from the SWAT Fortran common field.

Table. Parameter synchronization

in codes	in equations
con_bact_sed	$conc_{bact,sed}$
kp	K_p
clay	$clay$
bsc1 through bsc4	bsc_1 through bsc_4
sedin	$conc_{sed,ch,i} \cdot V_{ch}$
deg	sed_{deg}
dep	sed_{dep}

6.1. Project configuration settings

In the FORTRAN workspace,

Click on **Project / Settings / Fortran** tab

Click on the **Category** drop down menu and set the following:

Floating point – Floating Point Exception: 0

Optimizations – Math Library: Fast

Run time – check the box next to the following:

Array & String Bounds

Floating Point Underflow

Integer Overflow

Flawed Pentium

Click **OK**.

Click on **Project / Settings / Debug** tab

Set the **Working directory** as the directory which includes the SWAT input files.

Click **OK.**

Click **File / Save Workspace**

6.2. Modification of source codes (based on SWAT2005)

6.2.1. 'modparm.f' (external dependency file)

Add new parameters and variables used in common. To compile the SWAT code, delete the 'modfparm.f' file from the project list in the FORTRAN workspace.

```
==========================================================================================
       module parm

!!     sediment-associated bacteria (Kim et al., 2009)
       real :: clay, bsc1, bsc2, bsc3, bsc4
       real :: sedin, deg, dep

       real :: wshd_sw, wshd_snob, wshd_pndfr, wshd_pndv, wshd_pndsed
       real :: wshd_wetfr, wshd_resfr, wshd_resha, wshd_pndha, percop

...
==========================================================================================
```

6.2.2. 'readbsn.f'

Add statements to read new parameters.

```
==========================================================================================
...

       read (103,*,iostat=eof) dorm_hr
       if (eof < 0) exit

!!     sediment-associated bacteria (Kim et al., 2009)
       read (103,*,iostat=eof) clay
       if (eof < 0) exit
       read (103,*,iostat=eof) bsc1
       if (eof < 0) exit
       read (103,*,iostat=eof) bsc2
       if (eof < 0) exit
       read (103,*,iostat=eof) bsc3
       if (eof < 0) exit
       read (103,*,iostat=eof) bsc4
       if (eof < 0) exit

       exit
       end do

...
==========================================================================================
```

Optionally, set the default values of the parameters.

```
================================================================================================
...

      if (bactminp <= 0.) bactminp = 0.
      if (cn_froz <= 0.) cn_froz = .000862

!!    sediment-associated bacteria (Kim et al., 2009)
      if (clay <= 0.) clay = 20.
      if (bsc1 <= 0.) bsc1 = 1.543
      if (bsc2 <= 0.) bsc2 = 2.194
      if (bsc3 <= 0.) bsc3 = 187.
      if (bsc4 <= 0.) bsc4 = 3.870

      call caps(petfile)
      call caps(wwqfile)

...
================================================================================================
================
```

6.2.3. 'rtsed.f' (in-stream sediment routing module)

Remove the variables set in 'modparm.f' as common variables in the specification statement.

```
================================================================================================
...

      use parm

      integer :: jrch
!     real :: qdin, sedin, vc, cyin, cych, depnet, deg, dep
      real :: qdin, vc, cyin, cych, depnet
      real :: depdeg, dot

...
================================================================================================
```

6.2.4. 'rtbact.f' (in-stream bacteria routing module)

Specify new variables and parameter, and add new sediment-associated bacteria module after computing bacteria die-off (applied for only less-persistent bacteria).

```
================================================================================================
...

      use parm
      implicit none

!!    sediment-associated bacteria (Kim et al., 2009)
      real :: kp, con_bact_sed
      real, parameter :: pi = 3.1416

      real, external :: Theta

...

      totbactlp = totbactlp * Exp(-Theta(wdlprch,thbact,wtmp)*tday)
      totbactlp = Max(0., totbactlp)

!!    sediment-associated bacteria (Kim et al., 2009)
!!    considering only less persistent bacteria(totbactlp)
```

```
        con_bact_sed = 1e6 * 10**(bsc1*sin(bsc2*pi*(tday-bsc3)/366)+bsc4)   !! cfu/ton
        totbactlp = totbactlp + con_bact_sed * deg / 1e4   !! resuspension

        kp = (10**(-1.6)) * (clay**(1.98))
        totbactlp = totbactlp * (1 - (kp*dep)/(netwtr+kp*sedin))   !! deposition

!!      new concentration
        netwtr = 0.

...
==========================================================================================================
```

6.3. Modification of input data file (based on SWAT2005)

6.3.1. 'basins.bsn'

Add the values of new parameters.

```
==========================================================================================================
Basin DATA           .bsn file Tue Feb 17 17:49:00 2009 AVSWATX2003 - SWAT interface MDL
Modeling Options: Land Area
Water Balance:
          1.000     | SFTMP : Snowfall temperature [°C]
          0.500     | SMTMP : Snow melt base temperature [°C]
          4.500     | SMFMX : Melt factor for snow on June 21 [mm H2O/°C -day]

...

          0.000     | GDRAIN_BSN
          0.000     | CN_FROZ
          0.000     | DORM_HR
         20.000     | CLAY(%)
          1.543     | BSC1
          2.194     | BSC2
        187.000     | BSC3
          3.870     | BSC4
==========================================================================================================
```

Growth of *E. coli* and Enterococci in Storm Drain Biofilm

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County of Orange, Health Care Agency Public Health Laboratory

October 13, 2006

The Issues that Need to be Addressed

- In Southern California, over $50 million is spent on reducing fecal indicator bacteria levels in urban runoff and beaches; yet some beaches appear to have persistent levels of indicator bacteria
- Enterococci and *E. coli* can be found in high numbers in most storm drains & creeks.
- Is every one of these bacteria coming from human or animal fecal sources?
- Is it possible that these bacteria are able to survive and grow in the environment?

Recent Studies

- Fecal indicator bacteria can regrow in aquatic environments in tropical areas (Roll & Fujioka, 1997; Solo-Gabriele, et.al., 2000)
- Fecal indicator bacteria occur in high numbers in storm drains & sediments impacted by urban runoff, possibly due to regrowth, selective survival or accumulation of bacteria in sediment (Ferguson, et al, 2005)
- *Enterococci faecalis* from beach shoreline water and river sediments were tested by Pulsed-field gel electrophoreisis (PFGE), a DNA finger-printing method.

Results of PFGE Analysis of Enterococci

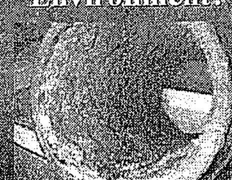
- *Clonal* strains found in water and sediment over a 2 month period indicated survival & regrowth, in addition to contamination.

Can *E. coli* and Enterococci Grow in the Environment?

Most bacteria in the environment grow in **BIOFILM**: a community of microorganisms surrounded by extracellular polymeric substances (EPS).

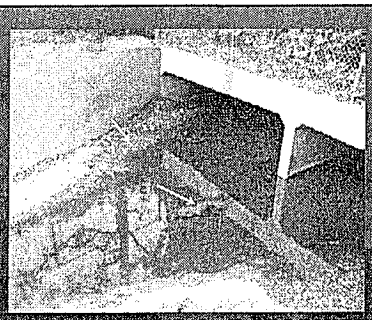
i.e. **A STICKY SUBSTANCE aka SLIME**

Where Is Biofilm Found in the Environment?



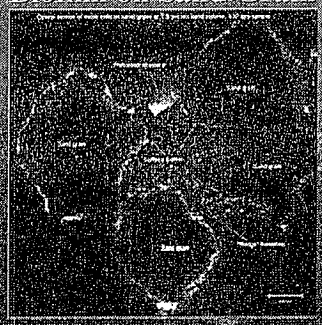
www.esemag.com/0105/0105ed.html

On virtually any solid surface that has contact with water and nutrients. . .as large as storm drain pipes or as small as sediment particles.



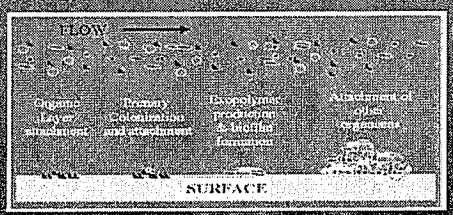
Biofilm occurs on concrete surfaces, sediment, debris, particulates in water, plants, leaves, grass…etc. It takes on many forms, from the "slippery layer" to "strands" attached to rocks and plants.

Biofilm on Sand Grain



Confocal Scanning Laser Imaging – Dr. Paul L. Bishop, Purdue University

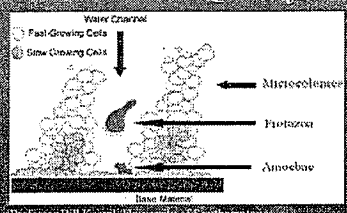
How are Biofilms Formed?



http://www.jimengineer.com/CDA/ArticleInformation/features/BNP_Features_Item/0,2752,2497,00.html

Absorption of bacteria (sec); attachment (sec – min); growth of bacteria (hrs – days); EPS formation (hrs – days); attachment of other organisms (days – months)

How Could Bacteria in Biofilms Affect Water Quality?



(Professor Bill Keevil, University of Southampton)

Biofilm provides a safe environment for bacterial replication, access to water & nutrients and protection against microbial predators, UV, drying and disinfectants. Cells detach from the biofilm & enter water column which may lead to "false" postings! Detachment is related to factors such as water flow velocity & shear forces, nutrient availability, and aging of biofilm.

Can *E. coli* and Enterococci Form Biofilm?

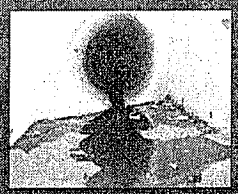
- Many environmental bacteria reside in biofilm.
- *E. coli*, some coliform bacteria and enterococci are known biofilm producers.
- To date, only a few studies have been done on the occurrence of fecal indicator bacteria in biofilms in the environment.

Preliminary Studies Conducted by OCPHL

- **Hypothesis**: Since bacteria in the environment grow in biofilm, does *E. coli* and enterococci also survive and grow in biofilm associated with storm drain water?

Study Location & Methods

Biofilm, overlying water and underlying sediment from the Costa Mesa Channel were collected and analyzed for *E. coli* and enterococci



Costa Mesa Channel (CMC)

Sample collection

Results

- Up to 4.6 million enterococci per 100g* (or 100 ml) biofilm found compared to the enterococci single sample standard for ocean water/contact sports of 104 CFU/100 ml
- Up to 1.8 million *E. coli* per 100g* (or 100 ml) biofilm found compared to fecal coliform/*E. coli* single sample standard of 400 CFU/100 ml

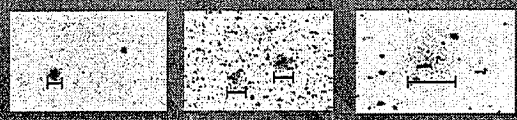
*assuming 1g is equivalent to 1 ml

Laboratory Experiments to Assess Biofilm Formation/Bacterial Growth

- *E. coli* and enterococci were inoculated into containers with storm drain water overlying microscope glass slides in the lab under conditions similar to the environment.
- The microscope slides were removed daily and visualized for biofilm development and bacterial growth.

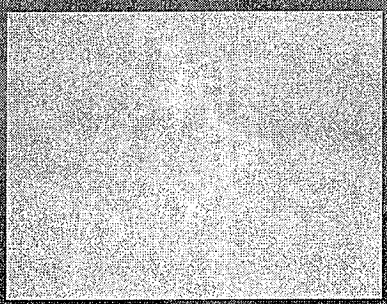
Results

Enterococci cells attached to microscope slides. Cells divided and formed clusters/microcolonies that increased in size with time.
Growth was dose dependent and may be related to quality of storm water.



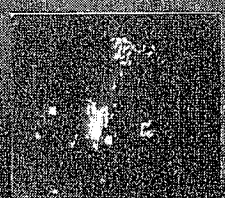
24 hours 48 hours 72 hours

Crystal violet stain (10x)

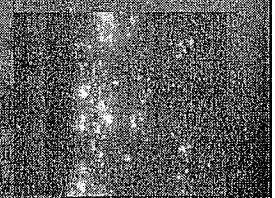


Biofilm extracellular polymeric substance (EPS), i.e. biofilm "sticky substance", on microscope slide incubated in storm water inoculated with *Enterococci faecium* (Calcofluor stain)

Increasing numbers of attached bacterial cells on microscope slides were also visualized using PNA FISH (peptide nucleic acid probes and fluorescence in situ hybridization). Live cells fluoresce (green or red).



Escherichia coli



Enterococci faecium

Biofilm Formation & Detachment on PVC Pipe & Concrete

- A PVC pipe & concrete pieces were placed in storm drains for up to 15 days to allow for biofilm formation.
- Pipe & concrete were rinsed & flushed vigorously to remove unattached bacteria & then sonicated to detach bacteria embedded in biofilm.
- Higher levels of *E. coli* and enterococci were found after sonication suggesting biofilm formation.

Conclusion

- *E. coli* and enterococci can growin biofilm in conditions similar to a storm drain environment.
- The results of this study are preliminary, further research is needed on bacterial biofilm and the impact it could have on public health and bacterial standards.
- Understanding the bacterial growth in biofilm found in storm drains, rivers and estuaries could have a profound impact on finding solutions to bacterial pollution in water.

Conclusion (cont'd)

- If fecal indicator bacteria grow and multiply in biofilm and are dispersed into the water column, it may account for increased bacterial levels without human or animal fecal input.
- This would confound assessment of water quality standards and the relationship between bacterial indicator levels and swimmer illness.
- The public health significance of indicator bacteria from sources other than sewage should be investigated in epidemiological studies.

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