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Survival of *Escherichia coli* in Lake Bottom Sediment†

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The survival of *Escherichia coli* in bottom sediment (Lake Onalaska, navigation pool no. 7, Mississippi River) was studied by using in situ dialysis culture of sterile (autoclaved) and unsterile sediment samples. Bags made from dialysis tubing were filled with either course sand sediment (28.8% fine) or organic, silty clay sediment (77.2% fine) and placed at the sediment-water interface. Bags representing sterile controls, unsterile uninoculated controls, autoclaved inoculated sediment, and unsterile inoculated sediment were studied during a 5-day period for each sediment type. Daily most-probable-number determinations indicated that *E. coli* populations in unsterile inoculated sediment fluctuated between 5.3×10^2 and 2.2×10^3 bacteria per g of silty clay and between 3.0×10^3 and 1.4×10^4 bacteria per g of sand. Autoclaved silty clay sediment inoculated with 1.0×10^6 bacteria per g increased to 2.2×10^8 bacteria per g in 3 days. During the same period, autoclaved sand sediment inoculated with 1.2×10^5 cells per g increased to 5.4×10^7 bacteria per g. By day 5, populations in both cultures had decreased by 1 log. The ability of *E. coli* to survive for several days in aquatic sediment in situ suggests that fecal coliforms in water may not always indicate recent fecal contamination of that water but rather resuspension of viable sediment-bound bacteria.

The survival of enteric bacteria in aquatic ecosystems has received considerable attention because of the dangers that pathogenic members of the group pose to humans (23). Although certain studies have shown that enteric bacteria die (or at least disappear) rapidly (6, 29, 30), more frequent are accounts of extended survival and growth (4, 5, 10, 14, 22, 24, 25, 36). Both field investigations and laboratory studies have documented several cases of enteric survival and growth in aquatic systems, especially in the presence of sediment (10, 14, 28, 33). However, the effect of sediment on bacterial survival in situ has not been assessed. Furthermore, most of the work done on the water column and all except one sediment study (14) have been limited to sterile culture systems. We therefore chose to investigate the in situ survival of the fecal coliform (FC) *Escherichia coli* in lake bottom sediment by using dialysis culture techniques.

MATERIALS AND METHODS

Study sites. The study sites were located in Lake Onalaska, an impoundment of lock and dam no. 7 in the Mississippi River at Dresbach, Minn. This large (surface area, 2,146 hectares), shallow (average depth, 155 cm) pool receives water from the Mississippi and Black rivers and has water chemistry characteristics similar to the Mississippi River proper (unpublished data). Lake Onalaska does not stratify, has an average annual alkalinity of 125 mg/liter (as $CaCO_3$), supports extensive growths of rooted, aquatic vegetation, has a high nutrient-trapping efficiency, and quickly is progressing toward hypereutrophic conditions (unpublished data).

Because sediment types in the lake range from medium sand to organic mud, two study sites were selected to represent the river environment. Sand and silty clay (i.e., mud) sites were established 0.77 km apart in approximately 160 cm of water. No rooted vegetation was located within 30 m of either site (Fig. 1).

Light extinction curves were determined for both sites with a submersible photoelectric cell (model 420, relative irradiance meter, Hydro Products, San Diego, Calif.). Temperature and dissolved oxygen at the sediment-water interface were monitored daily throughout the test period with a dissolved oxygen meter (YSI 54A, Yellow Springs Instrument Co., Yellow Springs, Ohio). Conductivity, turbidity, and pH values were provided by G. Jackson, Columbia National Fishery Research Laboratory, U.S. Fish and Wildlife Service, La Crosse, Wisc.

Sediment sampling and characteristics. Sediment samples were collected with a Petite Ponar grab dredge (Wildlife Supply Co., Saginaw, Mich.), placed in new polyethylene bags, and transported to the laboratory in an ice chest. Immediately upon return to the laboratory, heterotrophic bacteria were enumerated in triplicate by the spread plate technique with plate count agar (Difco Laboratories, Detroit, Mich.). All

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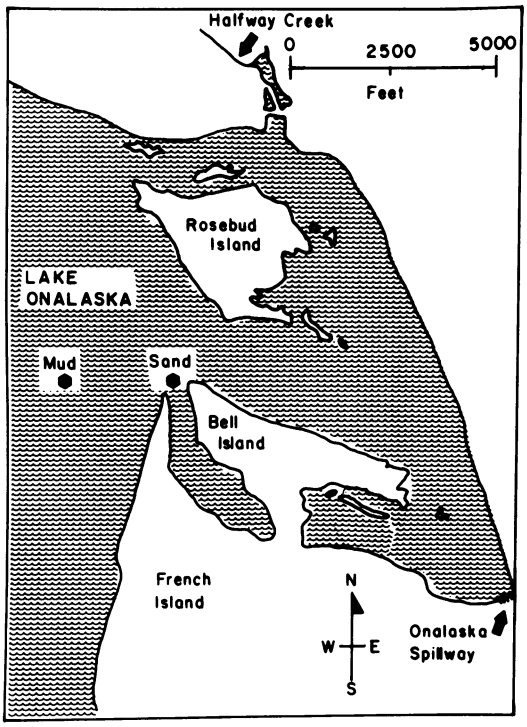

FIG. 1. Location of study sites in Lake Onalaska.

sediment dilutions were made by placing samples in sterile phosphate buffer (3) and shaking them for 1 min. Sediment moisture content was determined by drying the samples at 110°C for 24 h (7) and recording the average percent weight loss of six replicates. Organic content was measured by heating the dried sediment to 600°C for 15 min (7) and determining the average weight loss of three replicates. Sediment particle size distribution was found by the Bouyoucos hydrometer method (7).

Test organism. *E. coli* was isolated from a grab water sample taken from Halfway Creek (Fig. 1) 2.3 km downstream from the Holmen, Wisc., municipal sewage treatment plant effluent discharge flume and 3 km upstream from Lake Onalaska. The sample was tested for FC by membrane filtration (3); a typical blue colony was streaked for isolation onto plate count agar and incubated for 24 h at 35°C. An isolated colony was placed in motility test medium (11); the isolate was subsequently identified as *E. coli* on the basis of morphology, biochemistry (API 20E System, Analytab Products, Plainview, N.Y.), and cultural characteristics (11).

Survival study. A sediment sample from each of the two study sites (sand and mud) was manually homogenized (with a sterile 2-liter beaker and stirring rod) for 5 min and divided into two fractions, each approximately 500 g. One fraction was autoclaved for 15 min at 121°C. Half of each fraction was set aside for controls. The other half was inoculated with 10 ml of a twice-washed (sterile, phosphate-buffered [pH 7.0] saline), 18-h lauryl tryptose broth (Difco) culture of *E. coli* that contained approximately 1.5×10^7 bacteria per ml (McFarland barium sulfate standard, Difco) and mixed manually for 5 min.

The dialysis bags were made from sterile, cellulosic dialysis tubing (flat width, 4.5 cm), knotted at both ends, and secured with color-coded string. The bags were aseptically filled with approximately 50 g of sediment (sand or mud) representing one of the four experimental types: sterile sediment, unsterile uninoculated sediment, autoclaved inoculated sediment, and unsterile inoculated sediment. Multiple bags of each experimental sediment type were prepared and held in refrigerated lake water during transport to and from the study site.

Incubation chambers consisting of 30.5-cm^2 frames, constructed from concrete-filled, polyvinyl chloride plastic pipe (diameter, 2.5 cm), and wrapped in vinyl screen (0.95-cm^2 mesh), were used to hold the dialysis bags at the sediment surface. The chambers were marked with floats and were provided with additional weight to ensure constant contact with the top 5 cm of sediment, a location thought to be optimal for the survival and growth of bacteria (2, 20, 36).

Both the sand and the mud sediments were incubated at the same location from which they were removed. All bags were placed in chambers and lowered to the lake bottom, with the exception of the time-zero bags, which were saved for enumeration. The maximum time that elapsed from sediment removal to chamber placement was 24 h. The time-zero samples, as well as subsequent samples, were brought to the laboratory in lake water collected at the site. The bags were aseptically opened and decimal samples of sediment were transferred either directly to culture medium or to sterile phosphate buffer dilution blanks.

The most-probable-number (MPN) technique for FC (3) was used to enumerate sediment samples, including the sterile controls. Presumptive testing was done in lauryl tryptose broth, and several tubes of EC broth (Difco) showing gas were plated onto Levine eosin methylene blue agar (Difco) for confirmation. The integrity of the sterile controls was also monitored by spread plating sediment dilutions onto plate count agar. Field incubations began 21 August 1979 and ended 9 September 1979. Survival incubations were 5 days long, with one bag of each experimental sediment type removed for enumeration each day. The survival experiment was conducted twice.

The results were tested for significance with the paired, two-tailed Student's *t* test. Sand and mud were compared under both sterile and unsterile conditions by using the null hypothesis that either sediment supported *E. coli* with equal ability. Data were also examined for improbable results (39) that could have resulted from poor laboratory techniques.

RESULTS

Site characteristics. The sand site had sediment with 71.1% sand and 0.6% organic material; the silty clay (mud) site had only 22.7% sand, with nearly 10 times the organic content (5.8%) of the sand site (Table 1). The sediment pHs of all experimental types were similar (pH, 7.2 to 7.6), as were the numbers of naturally occurring heterotrophic bacteria (Table 1). Moisture con-

TABLE 1. Sediment characteristics

Sediment type	% Sand	% Silt	% Clay	% Organic material	% Water	pH of sterile sediment	pH of unsterile sediment	No. of heterotrophic bacteria[a]
Sand	71.1	2.3	26.5	0.6	20.8	7.6	7.5	3.8
Mud	22.7	39.5	37.7	5.8	56.9	7.6	7.2	6.0

[a] Number per gram (dry weight) of sediment $\times 10^5$.

tent, which is a function of particle size distribution, revealed substantial differences between sand and mud sites (Table 1).

Water temperature and dissolved oxygen were recorded at time zero and daily thereafter during the two 5-day survival incubations (Table 2). Temperatures and dissolved oxygen readings were very close at each recording time. Data for the other three water quality characteristics were also similar (Table 2). Light extinction studies revealed virtually identical irradiance profiles at both incubation sites, with little light (<20 mV) penetrating to the bottom.

Control studies. Autoclaved, uninoculated sediment bags showed a marked increase in the number of total heterotrophic bacteria occurring between days 4 and 5 (the numbers increased from 0 to $>10^4$/g, respectively). For this reason, all incubations were terminated on day 5. FC were not detected in any of the sterile, uninoculated control bags during the 5-day incubations. The results of MPN-FC determinations for unaltered (i.e., unsterilized and uninoculated) sand and mud fluctuated around a calculated 1 bacterium per g over the 5-day incubation period (Fig. 2).

Positive tubes of EC medium chosen at random from the highest dilutions and plated onto Levine eosin methylene blue agar developed typical *E. coli* colonies. Reliability testing of the MPN revealed no improbable results (39).

Survival studies. When additional *E. coli* were added to the unsterilized sediments, a much higher number of MPN-FC was maintained (Fig. 2). Mud had an initial population of 2.2×10^3 FC/g, which decreased only slightly over the 5-day period. The same stable population was observed in unsterile, inoculated sand, with numbers approximately 0.5 log higher (Fig. 2).

The autoclaved systems provided data that were quite different; *E. coli* inoculated into sterile sand and mud experienced marked numerical increases in 24 h (Fig. 2). The initial number of MPN-FC was several times higher in autoclaved sediment than in unsterile sediment (Fig. 2). Furthermore, the difference between the two systems became more evident with time; FC increased in the autoclaved bags but not in the unsterile ones. The rapid growth observed in autoclaved systems during the first 24 h was followed by a stationary period of 2 to 3 days and then by a decline in number.

The effect of sediment type (sand versus mud) on the number of bacteria was not the same in all of the systems studied. The total number of bacteria in unsterile sediment was 1.6 times higher in mud than in sand (Table 1). The mean FC counts in unaltered sediment were essentially the same for sand (1.6 FC/g) and mud (1.7 FC/g) (Fig. 2).

In the autoclaved, inoculated systems (Fig. 2), FC populations were usually somewhat higher in mud. However, the differences were not statistically significant. The unsterile inoculated systems demonstrated a greater difference between sediment types, with a mean FC per gram in sand which was 5.6 times higher than that in mud (Fig. 2). This is the reverse of the situation in autoclaved systems, and statistical analysis indicated that this difference was significant (at the level $\alpha = 0.05$).

DISCUSSION

The primary goal of this study was to investigate, by using dialysis culture techniques, the in situ survival of *E. coli* in lake bottom sediment. Dialysis bags were selected rather than the more popular membrane diffusion chambers (16, 29)

TABLE 2. Water characteristics

Underlying sediment type	Temp (°C)[a]	Dissolved oxygen (mg/liter)[a]	pH[b]	Conductivity (μS/cm)[b]	Turbidity (NTU)[b]
Sand	21.3 (19.0–23.8)	7.8 (6.1–10.0)	8.8	399.2	7.6
Mud	21.6 (19.5–24.0)	8.2 (6.8–10.0)	8.3	453.7	10.2

[a] Mean of 12 daily determinations made at the sediment-water interface.
[b] NTU, Nephelometric turbidity units. Mean of three determinations (data supplied by G. Jackson).

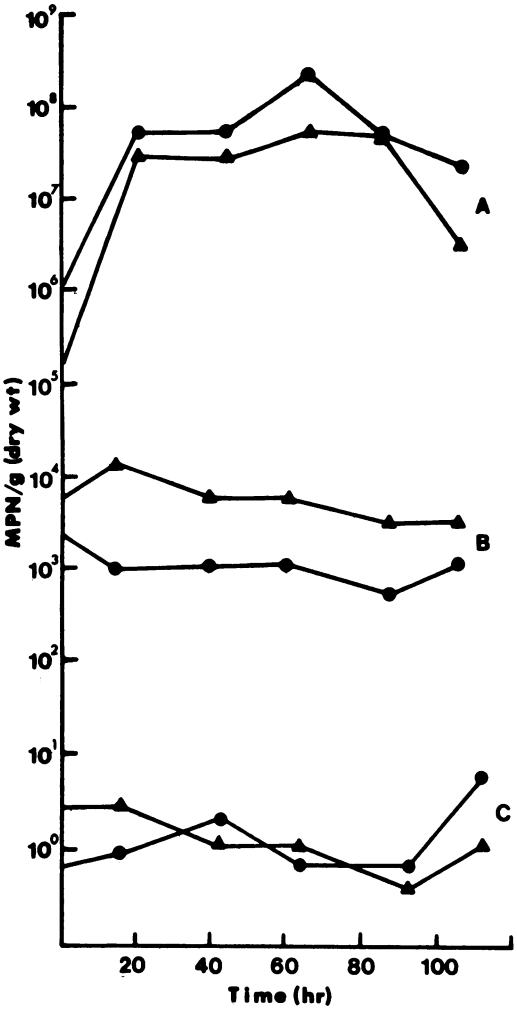

FIG. 2. FC densities in autoclaved, inoculated sediment (A); in unsterile, inoculated sediment (B); and in unsterile, uninoculated sediment (C). Symbols: ●, mud; ▲, sand.

because of the obvious inability of the latter to allow the withdrawal of sediment. In situ incubations indicated that sediment-filled bags constructed of cellulosic dialysis tubing maintained their integrity until day 5 but were visibly breaking apart by day 10. *E. coli* was chosen as the test organism because of its acceptance as an indicator of fecal pollution and because of the ease with which it can be isolated.

Survival curves for unsterile inoculated sediment were in general agreement with other studies of *E. coli* in aquatic systems. An initial lag period of 3 to 4 days followed by a sharp decline in numbers has been observed for *E. coli* in seawater (9, 30). The length of the lag period seemed to depend on temperature (31). The steady population of *E. coli* in the unsterile, inoculated bags (Fig. 2) could have been in this lag phase, as described by Carlucci and Pramer (9) and by Mitchell (30). The decline in numbers that they observed might also have been demonstrated in our study if more incubation time had been possible. The bags constructed of other material, e.g., polycarbonate, would allow for such extended incubations.

The results of our study were also similar to the laboratory studies of Tate (34) and Gerba and McLeod (14). Tate found that *E. coli* inoculated into unsterile, flooded soil lagged for 4 days before declining in numbers. Gerba and McLeod showed a slow decline in numbers of *E. coli* in marine sediment during the first 5 days, with intermittent periods of growth detected. Both of these laboratory studies, as well as our data (Fig. 2), documented a 4- to 5-day lag phase in unsterile sediment.

The results of the autoclaved, inoculated sediments (Fig. 2) also confirmed previous laboratory studies. Both Tate (34) and Gerba and McLeod (14) found that *E. coli* numbers increased rapidly, without lag, to approximately 10^7 bacteria per g (dry weight) when inoculated into sterile, flooded soil. Our in situ studies also demonstrated growth of *E. coli* to approximately 10^7 bacteria per g (dry weight).

The growth of *E. coli* in autoclaved sediment (Fig. 2) was attributed to several factors. Orlab (32) found that by filter sterilizing seawater, the 99% mortality time of *E. coli* was extended by 12%. Autoclaving does more than eliminate competitive and predatory organisms; it appears to chelate toxic metals (30) and increase the availability of nutrients (14, 21).

The results of the sediment analyses (Table 1) demonstrated distinct physical differences between sand and mud. However, the effect of sediment type on *E. coli* survival was difficult to discern. In autoclaved systems, mud supported more *E. coli*, whereas in unsterile systems, more *E. coli* were found in sand (Fig. 2). During most of the study, *E. coli* populations in sand and mud were parallel in both autoclaved and unautoclaved sediment. The only significant difference between sediment types was in the unsterile, inoculated systems. Once autoclaved, the two inoculated sediment types maintained comparable FC numbers.

On many occasions, the presence of fine soil particles and high organic content has been shown to enhance *E. coli* survival (15, 33, 34). Tate (34) suggested that *E. coli* can catabolize organic soil constituents, and he found that such substrates maintained populations three times greater than sand. This relationship was illustrated by our data for the sterile, inoculated

systems but not by the unsterile systems. The disagreement of the two systems might be used to support the hypothesis of Allen et al. (1), who feel that the amount of organic matter in sediment is not a good index of its ability to support pollution indicator bacteria. However, the observed effects of organics and competing bacteria vary a great deal (8, 32, 37, 38), apparently depending on the aquatic system being investigated.

In light of the literature indicating that aquatic sediment can serve as a reservoir for indicator bacteria (17, 18, 19, 28, 33) and the results of the present study, the practice of FC enumeration of sediments for public health purposes should be encouraged. However, the usefulness of sediment enumeration certainly depends on the sedimentation rate. Matsen et al. (28) have found that gradual decreases in indicator densities in water are inversely related to river discharge since waterborne indicators either settle into the sediments or are resuspended into the water column. They suggest that the physical characteristics at each sampling station regulate the sediment-water equilibrium of indicators and that river velocity is an important factor. Thus, where sedimentation occurs, the accumulation of bacteria in the sediment is possible. Once sedimentation occurs, the fate of the bacteria is regulated by their ability to metabolize benthic nutrients, withstand predatory pressure, and metabolically compete with other microbes (15).

Van Donsel and Geldreich (35) have suggested that the survival of salmonellae closely parallels that of FCs in sediment; therefore, extended survival in sediment and the potential for resuspension probably occurs for pathogenic, enteric bacteria as well as for FCs. Furthermore, both bacteria (14) and viruses (27) have been found to accumulate in marine sediment. When adsorbed, they exhibited greatly increased survival time (14, 26), and the number of viruses in sediment has been positively correlated with the number of FCs in sediment (27). Similar attempts to correlate numbers of indicator bacteria and viruses in the water column have been unsuccessful (13, 27).

This study has demonstrated the extended survival of sediment-bound *E. coli* in situ, and the resuspension of sediment-bound indicator bacteria has already been documented (12, 15, 18, 19). If enteric pathogens (bacteria and viruses) behave similarly, significant public health hazards could arise. Therefore, the enumeration of sediment-bound fecal coliforms in high-use recreational areas and in food production waters (i.e., shellfish and irrigation waters) should be considered of equal importance to bacterial density determinations in the water column; the two are closely related.

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