PERSISTENCE OF *ESCHERICHIA COLI* IN RECREATIONAL COASTAL WATER AND SEDIMENT

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ABSTRACT

A laboratory based microcosm study was undertaken to determine the persistence of the faecal indicator organism *Escherichia coli* in coastal recreational water and sediment under varying environmental conditions. Intact sediment cores were taken from three distinct coastal sites in the Adelaide metropolitan area and the overlying water was inoculated with known concentrations of *E. coli*. The concentration of *E. coli* in water and sediment was determined by the membrane filtration method on days 0, 1, 2, 7, 14, and 28 following inoculation. It was demonstrated that *E. coli* could persist in coastal sediment for greater than 28 days when incubated at 10°C. Temperature was found to have an inverse relationship with *E. coli* survival in both water and sediment. In general the decay rate for *E. coli* was greater in water than in sediment. Small particle size and high organic carbon content were also found to enhance *E. coli* survival in coastal sediments.

KEYWORDS

Escherichia coli; recreational water; sediment; microcosm

INTRODUCTION

The use of the organism *Escherichia coli* as a faecal indicator organism is wide spread. It is assumed that *E. coli* behaves similarly to other bacteria of faecal origin when released into the environment. Known inadequacies exist for the use of *E. coli* as an indicator organism, but its widespread use continues in the absence of any viable alternatives (Koh, *et al* 1994, Ferguson, *et al* 1996). Organisms released into coastal or marine environments are exposed to numerous factors which cause stress including temperature change, salinity, nutrient deficiencies, predation and sunlight (Davies, *et al* 1995, Özkanca and Flint 1997, Thomas, *et al* 1999). Studies have indicated that microorganisms attached to suspended particles and sediment contribute greater numbers than those in the surface waters under both environmental and laboratory conditions (Shiaris, *et al* 1987, Davies, *et al* 1995, Crump and Baross 1996).

Microcosms of varying complexity have been used in numerous studies to investigate the survival of microorganisms in both water and sediment (Gerba and McLeod 1976, Wagner-Döbler, *et al* 1992, Bordalo 1993, Davies, *et al* 1995, Brenner, *et al* 1999, Thomas, *et al* 1999). Unlike in-situ experiments, the use of microcosms allow for investigation of specific environmental conditions. In this study a laboratory based microcosm experiment was undertaken utilising intact sediment cores taken from three different coastal areas. The decay rate of *E. coli* was determined in both overlying water and sediment from these microcosms at a variety of different temperatures.

METHODOLOGY

Microcosm design

For each microcosm run, six intact sediment cores were collected from Henley Beach, Onkaparinga River and Port Adelaide River in metropolitan South Australia which represented distinct coastal sites and sediment types (Table 1). In addition to these, six cores were filled only with overlying water to act as a control.

Perspex columns (70mm diameter, 310mm length) were inserted into sediment and overlying water at respective sites to a depth of approximately 100mm. The top of the column was capped with a rubber bung to aide the removal of the core from the sediment. The sediment core was kept in place by inserting a combination of neoprene (5mm thick) and closed-cell foam (20mm thick) bungs into the bottom of the core. This prevented the movement of both sediment and water from the column.

In the laboratory, the microcosms were placed in a water bath at constant temperature. The overlying water was removed and replaced with 500mL of water from the Onkaparinga River to maintain a constant total dissolved solids (TDS) concentration between different sediment types. This removed the potential influence of changes of salinity in overlying water caused by tidal changes at the different sites. The mean TDS concentrations were 27,766 ± 805 mg/L, 28,663 ± 1889 mg/L and 23450 ± 929 mg/L for the experiments run at 10°C, 20°C and 30°C respectively. Water pH (HACH Ltd) and conductivity (Hanna) were measured with hand held meters when microcosms were sampled.

Inoculation of microcosms

Microcosms were inoculated by adding 50μL of stock *E. coli* (ATCC 25922) suspension to overlying water giving a final concentration of approximately 1×10^7 CFU/mL. Aeration of the column provided adequate mixing of the overlying water.

Determination of coliform survival

Both sediment and water from the columns were analysed on day 0 (1 hour after inoculation), 1, 2, 7, 14 and 28. A new column was used for each sampling date. Due to there being a rapid decay of *E. coli* when incubated at 30°C, columns incubated at this temperature were analysed on day 0, 1, 2, 4 and 7 only. The number of *E. coli* present in the water was determined using the membrane filtration method for the determination of faecal coliforms (Australian Standard AS 4276.7, 1995). Results were expressed as number of colony forming units (CFU)/100mL.

Sediment samples were obtained by firstly removing the remaining overlying water. The column was then destructively sampled by placing on a coring device that extruded the sediment at controlled intervals. The top 10mm of sediment was removed and placed into a sterile beaker. Of this sediment, 25g was placed into 75mL of sterile 0.1% peptone water (Oxoid) and sonicated in a sonication bath for 10 minutes to separate the bacteria from sediment particles (Craig, *et al* 1999). Faecal coliforms were again enumerated by the membrane filtration method. The results for *E. coli* concentration in sediment were expressed as number of CFU/100g (dry weight) sediment. Dry weight was determined by drying a known quantity of sediment at 105°C for 24 hours.

The decay rate constant (k) was calculated as the slope of the line when $\log_{10}(N_t/N_0)$ was regressed against time, where N_t is the number of bacteria at time t and N_0 is the number of bacteria at time 0 (Davies and Evison 1991).

All analyses were undertaken in triplicate and expressed as the mean ± standard deviation.

RESULTS AND DICUSSION

Monitoring the overlying water for conductivity and pH demonstrated that the microcosms remained stable over the sampling period of 28 days. The stability of the microcosms over this period of time compare with other studies utilising similar designs (Wagner-Döbler, *et al* 1992). Macro-invertebrates were present in the microcosms throughout the experiment indicating that conditions were sustained similar to that of the natural environment to support in-situ organisms. Sites were chosen to investigate the effect of sediment type on survival (Table 1).

Table 1. Particle size analysis.

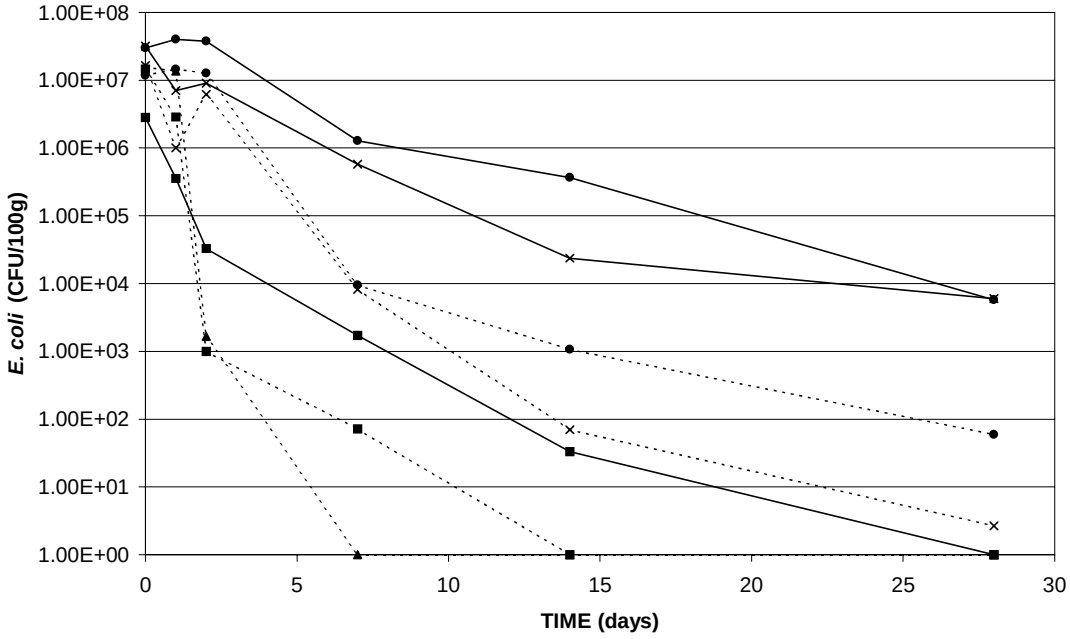
SITE	%SAND	%SILT	%CLAY	%ORGANIC C
Henley Beach	98.54	0.08	1.41	0.046
Onkaparinga	93.20	1.23	2.84	0.346
Pt Adelaide (top layer)	81.80	4.18	10.17	2.348

An example of a survival curve of *E. coli* in both sediment and water at 10°C is illustrated in Figure 1. When incubated at 10°C there was a rapid decline in the concentration of *E. coli* in the water. This was most pronounced in the microcosm containing water only, with no *E. coli* being detected after 7 days. The results

for decay rate constants at 10°C, 20°C and 30°C are given in Table 2. At all temperatures, survival was greater in sediment compared with that in water (Table 2).

Persistence of *E. coli* was greatly reduced when samples were incubated at 20°C and 30°C respectively compared with 10°C. For all samples there was a significant increase in decay rates in the water compared to sediment (graphs not shown). The most pronounced increase in decay rates of *E. coli* occurred when microcosms were incubated at 30°C. There was little difference in the decay rate of *E. coli* in sediment from Henley Beach at 20°C compared with 10°C. There was, however, an increased decay rate in sediment from the other two sites. These results compare with other studies, demonstrating prolonged survival of *E. coli* in water at lower temperatures (Flint 1987, Özkanca and Flint 1997).

Figure 1. Survival of *E. coli* incubated at 10ºC in sediment (—) and water (----) from Henley Beach (■), Onkaparinga (×), Pt. Adelaide (●) and column containing water only (▲).



Results illustrated that survival was influenced by sediment type. Sediment from Henley Beach (mainly sand) was less suitable for the survival of *E. coli* (represented by a greater decay rate compared with other sediment types in Table 2). This reflects intrinsic differences between the sediment types such as particle size and nutrient availability. There have been limited investigations into the effect of sediment type on the survival of *E. coli* (or other pathogenic microorganisms). Of many studies undertaken, sterile sediment and water has been used (Brenner, *et al* 1999, Thomas, *et al* 1999). As illustrated by Gerba and McLeod (1976), autoclaving has the effect of eluting nutrients from sediment, therefore prolonging survival of microorganisms due to the increased availability of nutrients. The use of sterile sediment and water also removes the added pressure on survival by competition with natural organisms. For this study, the persistence of *E. coli* was determined using intact sediment cores. Conditions of the study represents that of the natural coastal environment as closely as possible. Unlike other studies investigating the survival of microorganisms in sediments, this experiment retains the effect of natural flora.

Table 2. Decay rate constants (k; days^{-1}) for *E. coli* in water and sediment.

	Water			**Sediment**		
	10ºC	20ºC	30ºC	10ºC	20ºC	30ºC
Henley Beach	-0.45	-0.89	-2.40	-0.32	-0.32	-1.36
Onkaparinga	-0.24	-0.52	-2.09	-0.13	-0.22	-0.91
Port Adelaide	-0.21	-0.45	-2.10	-0.14	-0.49	-0.58
Water only	-1.04	-1.03	-2.13			

For both microcosms containing Henley Beach sediment (sand) and water only, there was a rapid decline in the number of *E. coli*, with none being detected after seven days. These findings of high decay rates is

comparable with other studies investigating the survival of *E. coli* in non-sterile water (Flint 1987). The problem with comparing results between studies is that sediment and water characteristics are not often described in detail. As indicated by the results of this study, differences were detected in the survival of *E. coli* in the water column depending on sediment type. A study by Gerba and McLeod (1976) suggested that microorganisms survived in sediment for prolonged periods of time due to the increased availability of nutrients. Results from this current study confirm that nutrient availability may have a significant effect on microbial survival. Under most experimental conditions, *E. coli* persisted in sediment from Pt. Adelaide (highest organic carbon content) longer than sediment from the Onkaparinga and Henley Beach (lower organic carbon contents respectively).

CONCLUSION

Results of this study demonstrate the prolonged survival of the faecal indicator organism *E. coli* in coastal sediments. The decay rate of *E. coli* was found to be influenced by sediment type with greatest survival in sediment with high organic carbon content. These findings have significant implications in regard to the estimation of risk of infection during recreational activities. The presence of *E. coli* in coastal sediments may indicate an increased risk of infection due to the possible resuspension of other pathogenic microorganisms during natural turbulence or human activity. Further studies are required to estimate this likely risk, as well as determining the survival of other pathogenic microorganisms under these conditions.

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