

# Enumeration of faecal coliforms from recreational coastal sites: evaluation of techniques for the separation of bacteria from sediments

**D.L. Craig, H.J. Fallowfield and N.J. Cromar**

*Department of Environmental Health, Flinders University, Adelaide, Australia*

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**Aims:** To identify the most efficient techniques for the separation of micro-organisms from coastal sediments and, using these techniques, to determine the concentration of faecal indicator organisms in recreational coastal water and sediment.

**Methods and Results:** Sediment samples were taken from a range of recreational coastal sites and subjected to various physical techniques to separate micro-organisms from sediment particles. Techniques investigated included manual shaking, treatment by sonication bath for 6 and 10 min, respectively, and by sonication probe for 15 s and 1 min, respectively. The use of the sonication bath for 10 min was the most successful method for removing micro-organisms from sediment particles where sediments consisted mainly of sand. When sediments contained considerable proportions of silt and clay, however, manual shaking was most successful. Faecal coliforms were then enumerated by membrane filtration in both water and sediment from three recreational coastal sites, chosen to represent different physical sediment characteristics, over a 12-month period. Faecal coliform concentrations were generally greater in sediment compared with overlying water for all samples. This was most evident in sediment consisting of greater silt/clay and organic carbon content.

**Conclusions:** This study demonstrated the importance of sediment characteristics in determining the most efficient method for the separation of micro-organisms from coastal sediments. Sediment characteristics were also found to influence the persistence of micro-organisms in coastal areas.

**Significance and Impact of the Study:** Recreational coastal sediments can act as a reservoir for faecal coliforms; therefore, sampling only overlying water may greatly underestimate the risk of exposure to potentially pathogenic micro-organisms in recreational waters.

## INTRODUCTION

The interaction of micro-organisms with sediments may enhance their survival by reducing exposure to stressors, such as sunlight and predation, or by increasing the availability of nutrients (Dale 1974; Davies *et al.* 1995). In this way, coastal sediments may act as reservoirs for pathogenic micro-organisms. The assessment of coastal recreational water quality is primarily undertaken by the

enumeration of faecal coliforms and *Escherichia coli* from the water column (NHMRC 1990). Studies have indicated, however, that the number of faecal coliforms in coastal sediments can be 10–10 000 times greater than that in the overlying water column (Shiaris *et al.* 1987). Dale (1974) demonstrated that sediment characteristics, such as particle size and organic carbon and nitrogen content, correlate with bacterial numbers. In coastal waters there may be an increased risk of infection to humans due to the re-suspension of potentially pathogenic micro-organisms from the surface sediment layer during recreational activities.

The attachment of micro-organisms to sediments poses many problems with regard to their subsequent detection

Correspondence to: N.J. Cromar, Department of Environmental Health, Flinders University, GPO Box 2100, Adelaide SA 5001, Australia (e-mail: Nancy.Cromar@flinders.edu.au).

and enumeration. If samples are viewed by microscopy, micro-organisms may be obscured by sedimentary particles or particles can effectively increase the working distance of the microscope objective causing a distortion of the image (McDaniel and Capone 1985). Alternatively, when enumerated by conventional culture techniques, such as spread plates or membrane filtration, micro-organisms attached to sediment particles may not be randomly distributed across the surface of the media.

In most circumstances it is necessary to physically separate the micro-organisms from the sediment particles. Methods other than manual shaking of samples include sonication and homogenization but there is disagreement as to which method is most effective. Dale (1974) concluded that homogenization of sandy sediment at 23 000 rev min<sup>-1</sup> for 5 min yielded greater numbers of bacteria than shaking by hand or sonication. Other studies have determined treatment by an ultrasonic bath (40–50 kHz, 100–200 W) to be more effective than homogenization (Ellery and Schleyer 1984). Epstein and Rossel (1995) concluded that, for small sediment samples ( $\leq 0.5 \text{ cm}^3$ ), optimal enumeration of bacteria was achieved using an ultrasonication probe (5-mm tip, 20 kHz, 100 W) for 180 s. As the results of these studies suggest, it is not the type of treatment alone that determines its effectiveness but also power outputs and frequencies of treatment. It is important that the separation technique is powerful enough to remove the micro-organism from the particle without harming the organism (Ellery and Schleyer 1984; Epstein and Rossel 1995).

To enable the estimation of the number of micro-organisms in sediment, as distinct from the water column, it is necessary to develop methods to successfully separate these organisms from sediment particles. This research attempted to identify the most efficient technique for the separation of bacteria from different types of coastal sediment found at recreational sites in the greater metropolitan Adelaide area in an effort to more accurately determine environmental exposure in the first stage of a risk assessment of recreational coastal waters. As part of the exposure assessment, faecal coliform concentrations in overlying water and sediment were monitored at three recreational coastal sites over a 12-month period. The influence of environmental conditions, such as rainfall and temperature, was also determined.

## MATERIALS AND METHODS

### Sampling sites

Sediment and water were sampled at low tide from coastal sites situated along the greater Adelaide metropolitan area in South Australia (Fig. 1). These sites were Southport Beach, the Onkaparinga River (estuary), Glenelg North, Henley

Beach South and the Port Adelaide River. The Port Adelaide River site was approx. 100 m from a wastewater treatment plant (WTP) outlet. Water from this activated sludge plant undergoes chlorination prior to disposal. A sample was also taken from Normanville, a site that is not impacted by major development, approx. 60 km south of Adelaide on the Fleurieu Peninsula.

### Sediment characterization and separation treatments

**Sediment collection.** Samples were taken using a sterile 50-ml polypropylene syringe with the end removed. The sediment sample was placed into a sterile container prior to being transported to the laboratory. Sediment samples were stored at 4°C and analysed within 3 h of collection.

**Particle size analysis and organic carbon content.** The proportion of clay, silt and sand was determined using the pipette method (Sheldrick and Wang 1993). The dichromate method for the determination of oxidizable carbon and soil organic matter was used (Tiessen and Moir 1993).

**Techniques for the separation of micro-organisms from sediment particles.** Approximately 1 g well-mixed wet sediment was placed in 9 ml 0.1% peptone water (Difco, BD, North Ryde, Australia). The sample was then subjected to the various treatments, listed below. After treatment, the sample was left to settle for 10 min prior to aspirating the supernatant fluid. Appropriate dilutions were made and 0.1 ml of each dilution was spread plated onto vibrio artificial seawater agar and incubated for 48–72 h at 20°C (Malmcrona-Friberg *et al.* 1990). Results were expressed as cfu g<sup>-1</sup> (dry weight) of sediment.

The separation techniques investigated included hand shaking, using a sonication bath (700 W, 35 kHz; Model 895; Cooper Vision, Irvine, CA, USA) for 6 and 10 min, respectively, and a sonication probe (100 W, 20 kHz, 19-mm probe; Model B12; Branson, Danbury, CT, USA) for 15 s and 1 min, respectively. When using the sonication probe, samples were cooled in an ice slurry. Hand shaking involved vigorously shaking the sample by hand over a radius of approx. 300 mm for 1 min.

In order to determine the recovery efficiency for each treatment method, the original sedimented material (with supernatant fluid removed) from each initial treatment was resuspended in 9 ml peptone water and the separation treatment repeated.

### Dry weight determination

To determine the dry weight of sediment a known weight of sediment was placed in an oven at 105°C for 24 h and



**Fig. 1** Adelaide metropolitan coastline, South Australia

weighed. The percent dry weight was then calculated (by difference) from these results.

### Faecal coliform enumeration

**Sediment collection.** Triplicate sediment and water samples were collected from Henley Beach South, the Onkaparinga River and the Port Adelaide River over a period of approx. 12 months. These three sites were chosen as they represented distinct sediment types. Water and sediment samples were taken at low tide, at a distance from the shore where the depth of water was approximately knee-height. Grab-samples of overlying water were collected in sterile containers (1 l). Intact sediment cores were taken by inserting Perspex columns (310 × 70 mm diameter) into the

overlying water and sediment. The sediment core was kept in place by inserting neoprene (5 mm thick) and closed-cell foam (20 mm thick) bungs into the bottom of the core. This prevented the movement of both sediment and water within and from the column. Samples were analysed within 3 h of collection.

**Enumeration of faecal coliforms.** Sediment samples were prepared by removing the top 1 cm of sediment from the intact core, of which 25 g was placed into 75 ml 0.1% peptone. Using the results of the separation trial detailed subsequently in this paper, sediment was sonicated in a sonication bath for 10 min, stirred and sonicated for a further 10 min to separate bacteria from sediment particles. Faecal coliforms in sediment and water samples were enumerated

by membrane filtration (GN-6; Gellman, PaII Life Sciences, Lane cove, Australia) and incubation on membrane lauryl sulphate agar (Oxoid Australia, West Heidelberg, Australia) (Australian Standard AS4276.7 1995). Plates were incubated at 30°C for 4 h, followed by 44°C for 18 h. Presumptive faecal coliform colonies were confirmed by gas production when incubated at 44°C for 24 h in EC broth (Oxoid).

### Meteorological data

Daily maximum and minimum temperature (°C) and total rainfall (mm) data were obtained from a weather station located at Adelaide Airport (Bureau of Meteorology, Adelaide).

### Statistical analysis

Statistical analysis was undertaken using one-way ANOVA and significance expressed at  $P \leq 0.05$  (SPSS version 10.0.5, 1999). The Bonferroni *post hoc* test was used to compare means between test treatments to determine whether treatments were significantly different. Bivariate relationships between faecal indicator organism concentration ( $\log_{10}$  transformed) and rainfall totals over two preceding time intervals (2 and 7 d) were examined using Pearson's correlation coefficient ( $r$ ). All results were expressed as the mean  $\pm$  S.D. of three determinations.

## RESULTS

### Sediment characteristics

Particle size analysis showed that the coastal sediments could be divided into two broad categories (Table 1). Sediment samples from Normanville, Southport, Glenelg North and Henley Beach South consisted of mainly sand while the samples from the Onkaparinga River and the Port Adelaide River had higher proportions of silt and clay (Table 1). Samples from these latter sites also had a higher organic carbon content. At the Port Adelaide River the sediment was

**Table 1** Particle size analysis

Site	% Sand	% Silt	% Clay	% Organic C
Glenelg North	98.45	0.20	1.30	0.05
Henley Beach South	98.47	0.08	1.41	0.05
Southport	98.54	0.04	1.36	0.06
Normanville	98.88	0.11	0.93	0.07
Onkaparinga	95.48	1.26	2.91	0.35
Port Adelaide ( $\leq 20$ mm)	83.05	4.24	10.33	2.38
Port Adelaide ( $\geq 20$ mm)	90.43	3.20	5.57	0.80

Particle sizes: sand, 2–0.2 mm; silt, 0.2 mm–2  $\mu$ m; clay, < 2  $\mu$ m. All results normalized to 100%.

distinctly stratified, with the top 20-mm layer comprising an anaerobic horizon with a high silt, clay and organic carbon content and below that a layer consisting of a mixture of sand and silt/clay. Only the surface layer ( $\leq 20$  mm) of the sediment was evaluated as this would provide the main source of exposure to micro-organisms in any recreational activity.

### Separation of micro-organisms from sediment particles

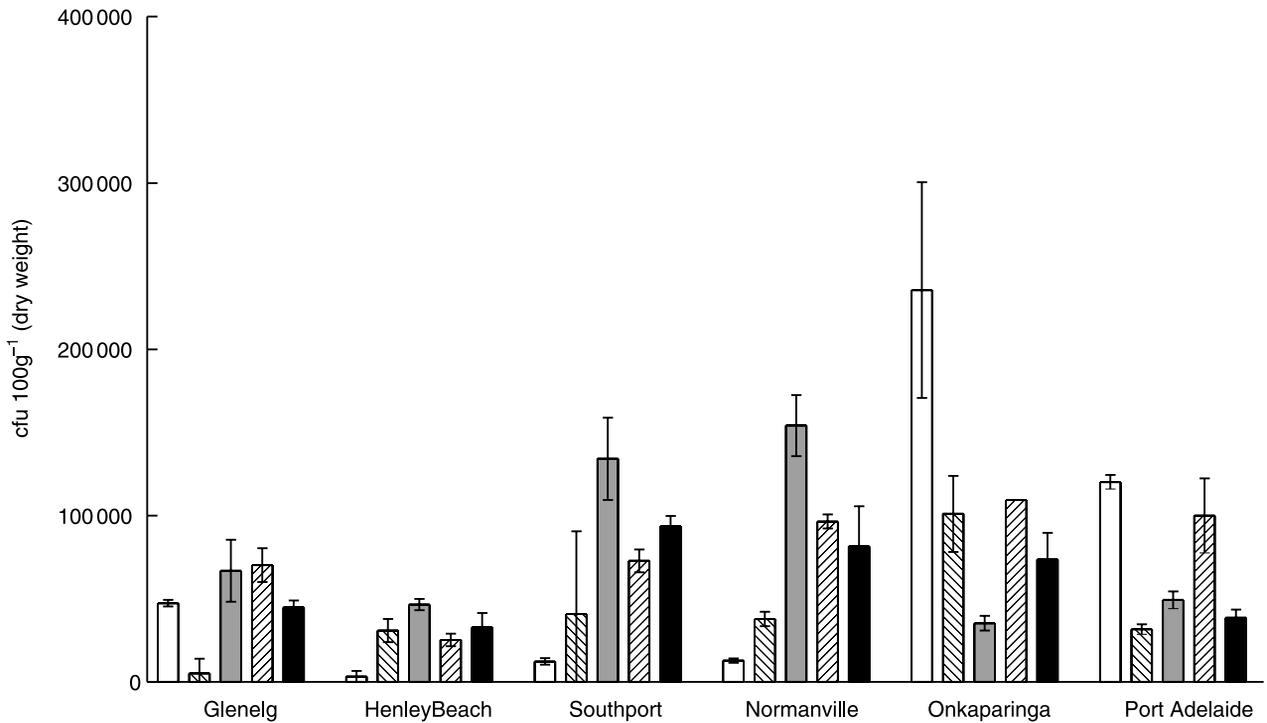
A comparison of separation techniques between sample sites revealed that, for the sandy sites, all sonication techniques resulted in significantly greater number of organisms being enumerated from sediment compared with manual shaking (Fig. 2). However, treatment of the sample by a sonication bath for 10 min provided significantly higher numbers of cfus compared with other techniques investigated ( $P < 0.05$ ). For the two sites with greater silt and clay contents (Onkaparinga River and Port Adelaide River), hand shaking was the most effective separation technique ( $P < 0.05$  for the Onkaparinga sediment).

The initial recovery efficiency using the sonication bath (10 min) was tested. The results illustrated that repeating the treatment in the sonication bath for 10 min resulted in only slightly improved recovery of micro-organisms for the sandy sites; however, it resulted in a great improvement for clay sediments (Fig. 3). The mean efficiency of initial treatment for sandy sediment types was 86% (range 81.2–90.8%). This suggests that treatment by sonication bath for 10 min is a relatively efficient method for removing bacteria from coastal sediments with high sand content. In this first instance, this method was initially not as efficient for sediments from the Port Adelaide River and the Onkaparinga River with a mean initial recovery efficiency of 62% (range 60.3–64.0%).

However, to achieve consistency for the subsequent enumeration of faecal coliforms from different recreational coastal sites, it was deemed necessary to choose one separation technique which provided the greatest recovery of micro-organisms across a wide range of sediment types. Treatment by sonication bath for 10 min on two occasions proved to be the most versatile and was, therefore, chosen as the primary separation method for subsequent analysis.

### Detection of faecal coliforms

All sites investigated complied with the NHMRC guideline for recreational water quality over the sampling period, with median concentrations of < 150 faecal coliforms 100 ml<sup>-1</sup> in the overlying water. The concentration of faecal coliforms was generally greater in sediment compared with overlying water at all sites (Figs 4–6). This was most significant at the Port Adelaide site (greatest silt/clay and organic carbon content) with a median concentration of faecal coliforms



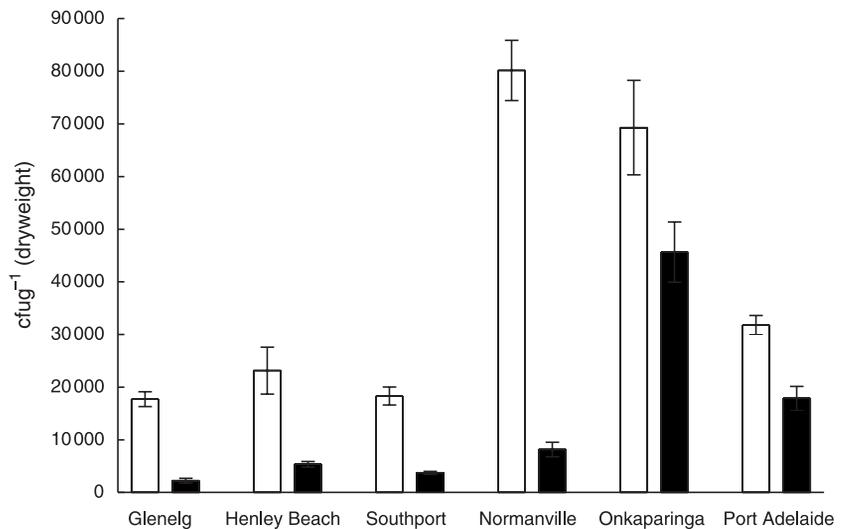
**Fig. 2** Comparison of separation techniques (mean  $\pm$  S.D.;  $n = 3$ ) for the enumeration of bacteria from coastal sediment. Sampling sites are ordered (from left to right) from greatest to least sand content.  $\square$ , Shake, 1 min;  $\text{▨}$ , sonication bath, 6 min;  $\text{■}$ , sonication bath, 10 min;  $\text{▩}$ , sonication probe, 15 s;  $\blacksquare$ , sonication probe, 1 min

over the sampling period in the sediment of  $2.1 \times 10^4$  cfu  $100 \text{ g}^{-1}$  (range  $2.3 \times 10^3$ – $2.2 \times 10^5$  cfu  $100 \text{ g}^{-1}$ ) compared with 61 cfu  $100 \text{ ml}^{-1}$  (range  $< 1$ –695 cfu  $100 \text{ ml}^{-1}$ ) in the overlying water. No correlation was identified between the number of faecal coliforms in the sediment and that in the overlying water for any site.

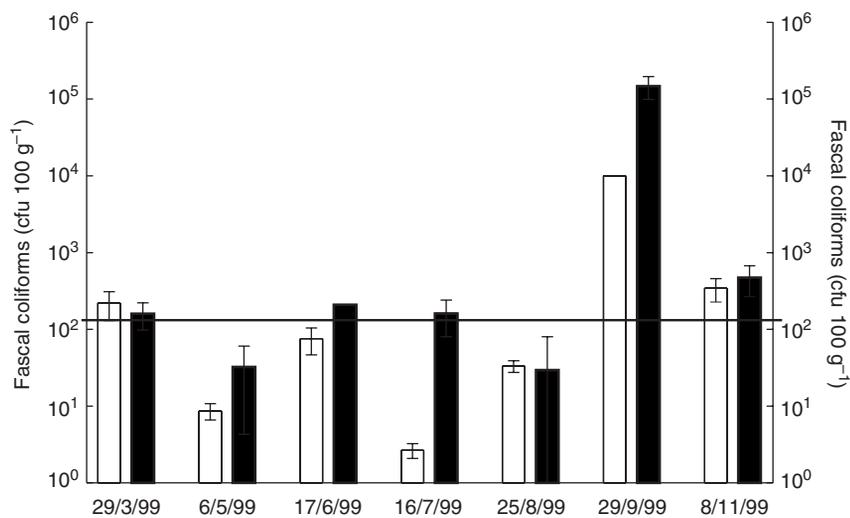
Seasonal variation of faecal coliform concentrations was only apparent at the Port Adelaide River, with slightly

elevated levels in both overlying water and sediment during the winter months. At Henley Beach and the Onkaparinga River, faecal coliform concentrations in both overlying water and sediment varied regardless of season, although more samples need to be taken during the summer months to confirm this.

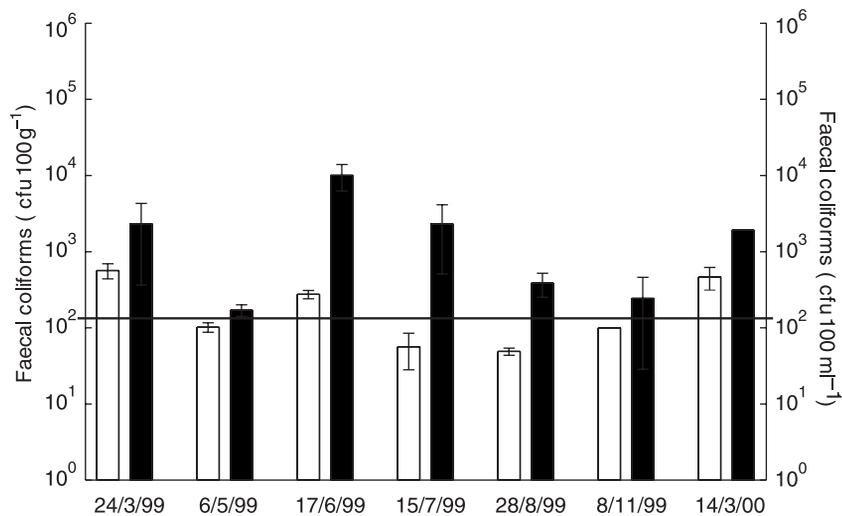
A significant correlation between faecal coliform concentration in the overlying water and sediment and rainfall in



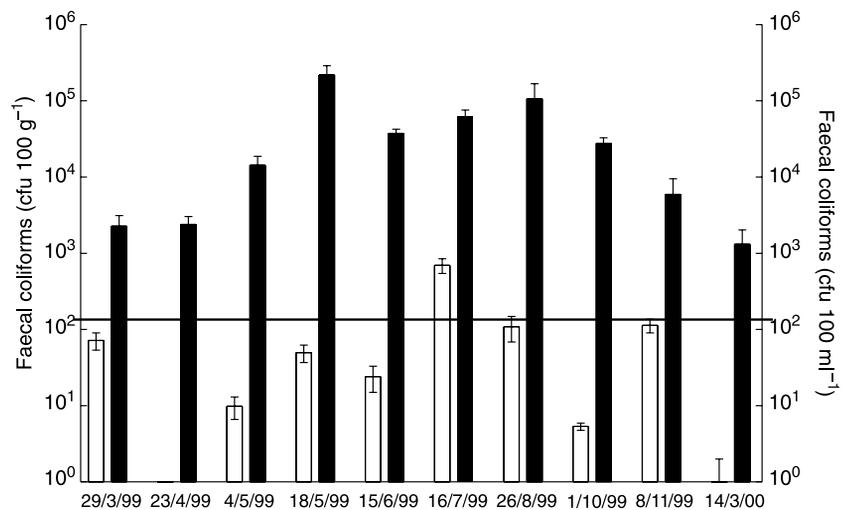
**Fig. 3** Recovery efficiencies (mean  $\pm$  S.D.;  $n = 3$ ) using the sonication bath for 10 min. Sediment from the original sonication treatment ( $\square$ ) was resuspended and the treatment repeated ( $\blacksquare$ )



**Fig. 4** Concentration of faecal coliforms (mean  $\pm$  S.D.;  $n = 3$ ) in overlying water ( $\square$ ) and sediment ( $\blacksquare$ ) from Henley Beach South. Line represents NHMRC guideline for faecal coliform concentration in recreational coastal water of  $< 150$  cfu 100 ml<sup>-1</sup>



**Fig. 5** Concentration of faecal coliforms (mean  $\pm$  S.D.;  $n = 3$ ) in overlying water ( $\square$ ) and sediment ( $\blacksquare$ ) from the Onkaparinga River. Line represents NHMRC guideline for faecal coliform concentration in recreational coastal water of  $< 150$  cfu 100 ml<sup>-1</sup>

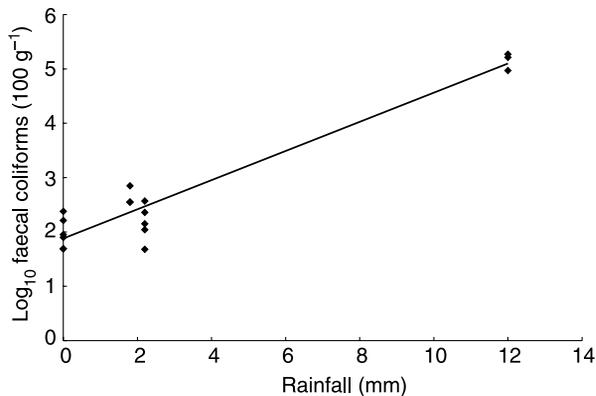


**Fig. 6** Concentration of faecal coliforms (mean  $\pm$  S.D.;  $n = 3$ ) in overlying water ( $\square$ ) and sediment ( $\blacksquare$ ) from the Port Adelaide River. Line represents NHMRC guideline for faecal coliform concentration in recreational coastal water of  $< 150$  cfu 100 ml<sup>-1</sup>

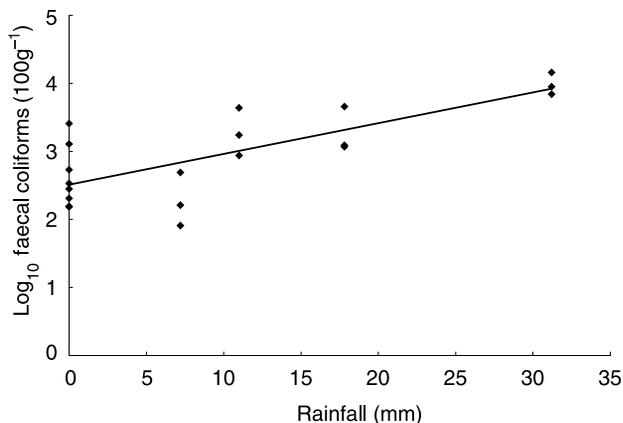
the previous 2 d was identified at Henley Beach ( $r^2 = 0.77$ ;  $P < 0.001$ ). Correlations between sediment faecal coliform concentrations at the Onkaparinga River and Port Adelaide and rainfall were noted in the previous 7 d ( $P < 0.001$  for the Onkaparinga River) (Figs 7 and 8).

## DISCUSSION

Characteristics of the coastal sediments investigated demonstrated significant variability in both particle size and organic carbon content. The influence of sediment characteristics on the effectiveness of separation techniques suggests that the binding properties between the bacteria and particle are highly dependent on the size and composition of the particle. This confirms a study by McDaniel and Capone (1985) who found that there were significant differences in the effectiveness of separation techniques with



**Fig. 7** Correlation between faecal coliform concentration in Henley Beach sediment and rainfall in the previous 2 d ( $y = 0.268x + 1.881$ ;  $r^2 = 0.93$ )



**Fig. 8** Correlation between faecal coliform concentration in the Onkaparinga River sediment and rainfall in the previous 7 d ( $y = 0.045x + 2.510$ ;  $r^2 = 0.58$ )

the type of sediment being studied. Their results showed that, when determining the most efficient treatment method, it is also necessary to establish the optimal treatment time. Cell death or injury may result due to vibration or heating of the sample over the course of the sonication treatment (McDaniel and Capone 1985). In the current study it has been demonstrated that treatment time significantly influenced the recovery of bacteria from coastal sediment.

The relative efficiency of treatment by sonication bath for 10 min was greater for sediments with a high sand content. For sediments containing greater proportions of silt, clay and organic carbon the recovery efficiency was significantly less (62% compared with 86% for sandy sediments), indicating that a second treatment was required when this method was subsequently used. A study by Epstein *et al.* (1997) utilized radioisotope labelling of bacteria to quantitatively determine the efficiency of sonication treatment (using a sonication probe) in enumerating bacteria from sandy sediments. The radioactive labels used were [<sup>3</sup>H]thymidine and [<sup>14</sup>C]leucine which are incorporated into bacterial DNA or protein, respectively. The efficiency of treatment was determined by comparing the amount of radioactive label in the supernatant fluid (dislodged bacteria) compared with that left in the sediment (bacteria still particle bound). Comparisons between treatment methods were, therefore, considered quantitative rather than relative. That study demonstrated that treatment by sonication probe for 80–160 s resulted in 88–98% of all bacteria present in the sediment being enumerated, which was similar to the recovery efficiencies observed in this current study.

For this study, no attempt was made to use chemical treatment in combination with the physical dispersion techniques. A previous study by this group (Flint, unpublished), using sodium pyrophosphate, had been shown to have no significant effect. This finding confirms that of other studies investigating the use of chemical dispersants (0.01 mol l<sup>-1</sup> sodium pyrophosphate) in combination with physical treatment (sonication) to remove bacteria from marine sediment (McDaniel and Capone 1985; Epstein and Rossel 1995). These studies found that the mean number of bacteria recovered from identical samples treated by a combination of chemical dispersion and sonication and sonication alone were not significantly different. In comparison with untreated samples (no sonication), however, treatment by these methods resulted in significantly higher numbers of bacteria being recovered. Treatment by sonication also resulted in a lower coefficient of variation in comparison with untreated samples and a more even dispersion of bacteria over the filter surface when viewed microscopically (McDaniel and Capone 1985).

It is likely that the number of bacteria enumerated in this study was an under-estimate of the total present, with the

possibility of some bacteria being damaged by sonication and others not being removed from sediment particles. It was demonstrated, however, that the number of organisms enumerated was significantly related to the type of treatment applied. Treatment of sandy sediment by sonication bath for 10 min resulted in significantly greater numbers of bacteria compared with shaking by hand. In addition, the number of organisms enumerated from sandy sediment after a second treatment by sonication bath for 10 min was proportionally low compared with the initial treatment, suggesting that the technique is relatively effective in separating bacteria from sediment particles. If, for reasons of comparability, it is desirable to employ only one separation technique, the most effective separation technique investigated overall was treatment by a sonication bath for 10 min. It is recommended that, for silt/clay sediment types, the sample be treated twice in an effort to improve recovery. Indeed, for consistency, this method (two treatments) was subsequently used by the authors in the seasonal study of faecal coliform concentrations at three recreational coastal sites.

The year-long investigation of three sites demonstrated that the concentration of faecal coliforms was generally greater in the sediment compared with overlying water at the three sites investigated. This was most evident at the Port Adelaide River and the Onkaparinga River sites, suggesting that sediment characteristics (particle size and organic carbon content) may have an influence on the persistence of faecal coliforms at recreational coastal sites. The concentration of faecal coliforms in the Port Adelaide River sediment was often 1000-fold higher than in the overlying water ( $3.7 \times 10^5$  cfu  $100 \text{ g}^{-1}$  compared with  $2.0 \times 10^1$  cfu  $100 \text{ ml}^{-1}$ , respectively, on 15 June 1999). On two occasions, no faecal coliforms were detected in the overlying water; however,  $> 1 \times 10^3$  cfu  $100 \text{ g}^{-1}$  were detected in the sediment. These results confirm those of other studies which have identified greater survival of faecal coliforms (and pathogenic micro-organisms) in coastal sediment compared with water (Goulder 1977; Shiaris *et al.* 1987; Lipp *et al.* 2001; Valiela *et al.* 1991; Davies *et al.* 1995; Fish and Pettibone 1995; Obiri-Danso and Jones 2000). Unlike other studies, however, the number of faecal coliforms enumerated from the sediment did not correlate with those enumerated from the water column (Valiela *et al.* 1991).

Seasonal variation in faecal coliform concentration was only observed at Port Adelaide, with higher concentrations in both overlying water and sediment during the colder months. A study by Obiri-Danso and Jones (2000) found that the concentration of faecal coliforms and faecal streptococci in coastal sediment did not vary seasonally; however, in contrast, *Campylobacter* spp. were only enumerated during the colder months. In earlier studies, the same authors identified higher concentrations of faecal

coliforms in overlying water at a recreational coastal site during the non-bathing season (Jones and Obiri-Danso 1999; Obiri-Danso and Jones 1999a). The increased concentration of faecal coliforms in both sediment and overlying water at the Port Adelaide River site during winter may highlight the effects of temperature and sunlight exposure on microbial survival. Obiri-Danso and Jones (1999b) demonstrated statistically significant correlations between exposure to u.v.b. and temperature with reduced faecal indicator concentrations in water samples taken from a coastal site on the same day (higher concentrations in the morning compared with the afternoon). Other laboratory-based studies have also identified decreased survival of faecal coliforms on exposure to u.v. light (Fujioka *et al.* 1981; Davies and Evison 1991) although Sinton *et al.* (1999) concluded that longer wavelength light associated with sunlight had a greater effect on faecal coliform inactivation than shorter wavelength light. It has also been determined that the survival of faecal coliforms in water is extended at lower temperatures (Vasconcelos and Swartz 1976; Flint 1987; Terzieva and McFeters 1991). The most likely source of faecal indicator organisms at this site is a nearby WTP outlet, with little immediate impact of stormwater associated with rainfall. The site is also comparatively shallow and not affected by large flow or turbulence caused by wave action. Therefore, the lower temperature and sunlight associated with winter may lead to increased survival of faecal coliforms in both water and sediment. The Port Adelaide River is used throughout the year for recreation, primarily fishing. The high numbers of faecal coliforms, especially in the sediments during the winter, may, therefore, pose a significant risk of exposure to faecal contamination due to secondary contact recreation.

Faecal coliform concentrations at Henley Beach were influenced by rainfall. The peak concentration of faecal coliforms which occurred on 29 September coincided with high rainfall (daily rainfall total 12 mm; Bureau of Meteorology). This high rainfall resulted in significant flow of the Torrens River, the outlet of which is situated at Henley Beach. Rainfall also resulted in the concentration of faecal coliforms detected in the water column being similar to that in the sediment. The influence of high rainfall on faecal coliform concentrations at recreational coastal sites has also been identified by other studies (Crowther *et al.* 2001; Lipp *et al.* 2001; Rees *et al.* 1998). This suggests that the detection of faecal coliforms in the water column is an indication of recent faecal contamination. The ability of sediments to act as a reservoir for faecal coliforms suggests that sediments may provide a more stable indicator of long-term faecal contamination.

The results of this study indicated the importance of determining the most effective separation technique for each individual sediment type. The identification of greater

numbers of faecal coliforms in sediment compared with overlying water suggests an increased risk of exposure to potentially pathogenic micro-organisms due to possible resuspension during recreational activity. Further studies are ongoing to quantify this potential resuspension of pathogenic organisms from sediment into the overlying water to provide a more accurate estimate of exposure in the first stage of a health risk assessment for recreational coastal water.

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