Deposition and Survival of Enteric Microbes in Aquatic Sediments — A Brief Review

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Summary

Hawke's Bay Regional Council (HBRC) faces problems in determining the significance of sediments as contributors to loadings of enteric indicators and pathogens in local waterways. In a study conducted by HBRC, elevated levels of faecal indicator organisms were found in recreational waters during dry weather. The study raised questions as to the origins of these elevated levels, in particular, whether they came from sediment resuspension or other sources. Accordingly, the regional council obtained an Envirolink grant to enable ESR to review this topic by:

1. Providing information on the survival of pathogens and viruses in sediments, and;
2. determining whether any information exists on the correlation between indicator bacteria and pathogens counts in sediments.

Although a review of the international literature showed that occurrence and survival of indicators and pathogens in the water column has been extensively investigated, the situation is less clear in sediments. However, there is reasonable evidence that sediments can accumulate and retain enteric indicators and pathogens. Furthermore, most published evidence suggests that survival of enteric indicators and pathogens is considerably longer in sediments than it is in the overlying water column. There is also evidence that resuspension of sediments increases microbial numbers in overlying water, particularly during storm events.

The data on the relationships between indicators and pathogens in sediments are sparse and inconclusive. Overall, there is no strong evidence of correlations between indicator and pathogen levels and survival in sediments, even though continuous deposition may be expected to have a greater integrating effect than would be expected in surface waters.

Although no evidence of increased counts of indicator and pathogenic microbes in surface waters due to resuspension was found in the HBRC studies, there was nevertheless evidence of substantially elevated counts in the sediments. There are various possible approaches to better ascertain the role of sediments in Hawke's Bay rivers and estuaries, which could include adding turbidity measurements to microbial water quality surveys, and intensive monitoring of selected sites during storm events.

1. Introduction

Three principal types of water-transmissible pathogens are found in New Zealand — bacteria, such as *Salmonella* and *Campylobacter*, protozoans (mainly *Giardia* and *Cryptosporidium*), and enteric viruses, such as the caliciviruses (which includes the norovirus group) and rotaviruses. These pathogens are costly to detect and enumerate, so their possible presence is monitored by using indicator organisms, usually *Escherichia coli* (*E. coli*) and enterococci.

The presence of both indicators and pathogens in natural waters (fresh and saline) in New Zealand has been documented by regional councils throughout the country and in nationwide surveys [1-8]. However, enumeration of enteric microbes in the water column alone may underestimate the total load in a catchment, because the underlying sediments will also constitute a significant microbial reservoir. Although the presence of faecal microbes, including enteric viruses, has been reported in sediments in New Zealand coastal waters [9, 10] and rivers [11], there is generally less local information available for sediments compared to that available for the overlying water bodies. Overseas studies have demonstrated the reciprocal effects of sedimentation on removing bacteria from the water column and accumulating them in bottom strata [12-16].

Coastal lagoons have been assumed to protect coastal waters by attenuating pollutants [16]. However, there is now evidence that these coastal lagoons could act as a source of contaminants in coastal waters, because they are the recipients of and storage areas for many of the pollutants from the catchment.

In this report, we review the recent literature on the deposition and survival of key enteric indicators and pathogens in sediments. As noted above, due to a paucity of New Zealand-based information on this topic, the review relies heavily on overseas studies.

2. Settlement Processes

The extent to which indicator and pathogenic organisms are concentrated in sediments depends on the tendency of the organism to settle out of the water column, either by itself (free) or attached to particulate matter within the water.

Free bacteria can sink in a water column at rates that depend on the specific gravities of the cells, water turbulence, and their degree of motility [12, 14, 17-19]. In most rivers, estuaries and coastal waters, natural turbulence means that settling velocities of free microbes are very slow [20, 21]. This means deposition of individual cells will only account for a small proportion of their accumulation within sediments.

The protozoan pathogen, *Cryptosporidium*, as a free particle, has a slow settling velocity (0.5 $\mu m\ s^{-1}$) due to its low specific gravity [22]. In comparison, the larger protozoan, *Giardia*, has a faster settling velocity (5.5 $\mu m\ s^{-1}$).

Although there do not appear to be any published studies specifically addressing settling rates of free viruses, it is generally accepted that they can only fall out of the water column when attached to particles, as they are too small to settle naturally, even under quiescent conditions [23, 24].

Settling rates for enteric microbes attached to particles are much faster than when they are free entities, depending on the type and size of particle they are attached to [25]. For example, *Giardia* and *Cryptosporidium* readily attach to particles and attachment can be as high as 93.1% [14, 25-27].

The ratio of attached to unattached microbes in waters varies markedly, depending on the particular organism, and this affects the rate of deposition in sediments. For instance, Characklis *et al.* [13] studied the portions of bacteria, protozoa and viral indicators associated with settleable particles in stormwater. *E. coli* and enterococci showed a 20-35% attachment, increasing to 30-35% during storm events. The spore-forming bacterium, *Clostridium perfringens* (used as an indicator of past contamination and may be used as a surrogate for protozoan pathogens), showed the highest attachment rate at 50-70% during storm events. The rate of attachment of bacteriophages (viruses which infect bacteria, used as indicators of viral contamination) was more variable (20-60%), but was generally found to be similar to faecal indicator bacteria. Comparable rates of attachment of *E. coli* and enterococci were reported by Fries *et al.* [28] (30–40%).

Enteric indicators and pathogens may be discharged into natural waters in effluent or farm runoff already attached to particulate matter [16, 29, 30]. Others may be discharged as free entities but attach to naturally-occurring colloids and other particles in the waterway [31]. Storm events will have important, although complex, effects on this process because there will be an increase in waterway turbulence and a decrease in sedimentation. This may increase both the number of particles bearing attached indicators and the number of particles available for attachment to free microbes. The fresh input of particles from the surrounding land, and resuspension of upstream sediments will increase the sediment load during high rainfall events (see Section 7).

3. Attachment Mechanisms

Microbes attach (adsorb) to particles by a number of complex and interrelated processes. Bacteria, viruses and most other particles tend to have a net negative charge in natural waters, and thus mutually repel each other. However, processes have been identified that can overcome this repulsion. The negative charge repulsion can be overcome by the phenomenon of a compressed electric double layer [32], allowing bacteria to attach to particle surfaces. Once attached, they are held in place by Van der Waals forces and hydrophobic interactions [33]. This effect is most marked in waters with high ionic contents, implying that particle association is higher in saline waters [34, 35]. Attachment by Van der Waals forces is sometimes termed weak reversible sorption, as the linkages are easily broken by shear forces such as wave action or turbulence [36, 37]. With this type of adsorption, bacteria are not in direct contact with the particle but are held closely to the surface by the attractant forces.

A more permanent form of adsorption can also occur, which involves physical contact between bacteria and the particulate matter. It is facilitated by cellular appendages or extracellular polymers excreted from the cell [37]. This form of attachment is not only important in removing bacteria from the water column, but may also enhance survival rates in the sediment following deposition (see Section 4).

The processes affecting virus attachment to particles have been reviewed previously [24, 38-41]. Factors generally regarded as enhancing viral adsorption, include low pH [38], high ionic strength [42], and a high cation exchange capacity [43] of the particle surfaces [44, 45].

4. Survival in Sediment

Once in the sediment, faecal indicator organisms and pathogens may remain attached to the particulate matter or they may detach. Bacteria that remain attached may survive for prolonged periods due to the associated particle offering them protection from predation and solar radiation [46]. There is also evidence that some enteric bacteria, given the right conditions, can grow in sediments [47-50]. Although the data on the survival of viruses in sediments are sparse, there is evidence that their survival is also extended in sediments [1, 3-5]. There is a paucity of research on the survival of protozoa in sediments but it may be assumed they will survive for long periods as there is evidence of extended survival in terrestrial environments [12, 51, 52].

In sediments, enteric microbes enter a very different environment to that encountered in the water column. Most importantly, when buried in bottom layers, they are protected from sunlight, which is widely recognised as the principal mechanism inactivating enteric microbes in shallow waters [6, 7, 53].

Most studies have suggested that the presence of organic matter in sediments significantly increases bacterial survival [54-57]. The organic material may provide a protective layer [54, 58] and a supply of readily available nutrients to microbes within the sediment [54, 59-62]. There have been, however, few correlations reported about organic matter levels and bacterial survival, therefore other factors may contribute to the overall survival of bacteria within sediments [59].

Predation and grazing may be significant factors affecting the survival of bacteria in sediments. Roper and Marshall [63] suggested that bacteria in sediments are protected from unicellular and multicellular predators and grazers in the interstitial spaces by two mechanisms. First, anaerobic conditions may inhibit the activities of these antagonistic organisms. Second, attachment to particles may form a physical barrier shielding bacterial cells from predation and grazing. Equally, Bauerfeind *et al.* [64] found that bacteria survived longer in sediments sterilised by autoclaving compared with natural sediment. The increase in survival, however, may also have been due to the effect of autoclaving on sediments, which may have converted nutrients into more accessible forms for utilisation by bacteria, particularly in the absence of competitors or predators. Davies *et al.* [65] showed a net die off in bacteria, except *Clostridium perfringens* (*C. perfringens*), when predators were present. They suggested that the reduced survival in natural sediment samples was due to predation or grazing by natural protozoa.

Whatever the combination of factors that enhance survival, it is well known that bacteria persist longer in sediments than in overlying water columns [30, 59, 63, 64]. The extent, to which enhanced survival occurs, depends on the particular bacterium. Davies *et al*. [65] found no decrease in *C. perfringens* spores in marine and freshwater sediments over a period of 28 days. In addition, the T_{90} values (time taken for 90% inactivation) for the indicator organisms, faecal coliforms and faecal streptococci, in sediment microcosms were up to 85 days. Moore *et al.* (2003) [66] found that a strain of *Salmonella* (mr-DT-104) survived for up to 119 days in sediment, which was over twice the survival rate recorded in water (54 days). Karim *et al*. [67] reported that the die-off rate of *Salmonella typhimurium* in sediment was 0.312 log per day compared to 0.345 log per day in water samples.

In general, when enteric bacteria are released into the environment, they soon succumb to the stresses they encounter, including desiccation, low nutrient levels and sunlight. However, exceptions to this have been reported. For example, Jeng *et al.* [68] conducted a laboratory-based microcosm study on the survival of *Enterococci faecalis* in sediments from an estuary in the USA. The survival curves were characterised by a growth phase followed by a stationary phase, a logarithmic decay period, and/or finally by a tailing region. Thus, an exponential decay model could not be applied to *E. faecalis* in these estuarine sediments, which appeared to demonstrate *E. faecalis* reproduction within 10 days. In addition, the authors found no evidence that survival or growth rates were affected by the amount of organic matter present in the sediment.

A recent study on freshwater sediments in Switzerland found high levels of faecal indicator organisms: 3.1 x 10^5 *E. coli* and 1.2 x 10^5 enterococci colony forming units (CFU) per 100 gram [69]. In this survival experiment no faecal indicator growth was observed, possibly due to predation by naturally-occurring benthic organisms.

Far less is known about the survival of viruses in sediments compared to bacteria, and much of the literature contains conflicting information. Viruses are generally regarded as being too small to be sought as food by other microbes. They may, however, be ingested along with the particles to which they are attached, and thus be affected by the activities of interstitial grazers. Although the factors affecting virus survival in sediments do not appear to have been systematically investigated, sediment appears to enhance virus survival. For example, LaBelle and Gerba [31] found that virus adsorption to estuarine sediment greatly increased survival time. The T_{90} (time taken for 90% reduction in live cells) value of poliovirus varied from 1.4 days in seawater alone (protected from sunlight) to 6.0 days in estuarine sediment. Increased survival of viruses (poliovirus and rotavirus) was observed in a survey of a polluted estuary in Galveston Bay [4] where infectious viruses were isolated after 19 days when they were associated with solids, compared to up to 9 days when the viruses were freely suspended in seawater samples.

It is not possible for enteric viruses to replicate outside a host cell, so there is no possibility of growth in sediments. Bacteriophages (viruses that infect bacteria) used as surrogates for viruses were found to be more likely to adsorb to surfaces with higher total acidity and were more stable compared to free bacteriophages [5]. Although it has sometimes been suggested that enteric bacteriophages may replicate in aquatic environments [70, 71], the evidence for their replication in natural waters is questionable [72, 73]. At present it may be assumed that bacteriophages cannot replicate in sediments.

There is also little published information on the survival of pathogenic protozoans in sediments, mostly due to the costly and laborious methods required for their culture and enumeration [74, 75]. Karim *et al*. [67] investigated the survival of *Giardia* in the water and sediments of constructed wetlands. They found that, in contrast to bacteriophages (coliphages and phage PRD1), *Giardia* survived longer in the water than in the sediment. It is important to note, however, that these experiments were conducted in microcosms in the laboratory, so the relevance of the data to the natural environment could be questioned. Like viruses, protozoan parasites can only replicate inside the body of a host, so there is no possibility of their replication in the environment. Although information on survival in sediments is limited, both *Cryptosporidium* and *Giardia* exhibit prolonged survival in other environments and this, coupled with their low infective doses, means they still pose a potential risk to humans if ingested [76, 77].

In summary, a complex range of interacting factors appear to be involved in the survival of indicators and pathogens (and the possible growth of bacteria) in sediments. These factors are still poorly understood and more research is needed in this area.

5. Relationships Between Indicator and Pathogen Numbers in Sediments

For practical reasons, microbial water quality monitoring relies on the use of indicator organisms, such as *E. coli* and enterococci, to predict the behaviour and survival of pathogens in waterways. Although this approach can provide useful information on pathogen behaviour, recent research has highlighted problems in relying too heavily on only one or two indicator organisms to predict the behaviour of a wide range of different types of pathogens (bacterial, viral and protozoan). For example, there is now evidence that enterococci are not always reliable indicators in marine waters and waste stabilisation pond effluent [2, 6, 7, 72], and that they may also grow in some aquatic environments [47, 50]. In response to this issue, a number of studies have been published on the relative survival of indicator organisms and selected pathogens in fresh and saline waters [3, 50, 78-82].

The relative survival rate of indicator organisms and pathogens in sediments has been investigated previously. For instance, Burton *et al.* [83] compared survival of the faecal indicator organism, *E. coli*, with pathogens; *Klebseilla pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella newport* in freshwater sediments. All of the bacteria tested had prolonged survival in sediments compared to the overlying water in the microcosm used. *P. aeruginosa* and *K. pneumoniae* survived consistently longer than *E. coli* and *S. newport*. The survival of *E. coli* was comparable with *S. newport* and both survived longer in sediments with higher clay content. The authors concluded that *E. coli* was an adequate indicator of *S. newport* in several types of freshwater sediments.

Garrido-Perrez *et al.* [78] suggest that *C. perfringens* may be a good alternative to the conventional indicator organisms (namely *E. coli* and enterococci) as an indicator of faecal contamination in water and sediments as it was present in most samples and did not show any growth characteristics. In a study of an Australian estuary (water and sediment samples), *C. perfringens* was found to be the most useful indicator of faecal pollution and was the only indicator significantly correlated with the presence of pathogenic *Giardia* and the opportunistic bacterial genus *Aeromonas* [3]. This study also showed that F-RNA bacteriophage was not significantly correlated with any of the pathogens examined.

Hartz *et al.* [50] investigated the survival of the faecal indicator organisms (*E. coli* and enterococci) in beach sand and experimental microcosms. Their results showed prolonged survival of faecal indicators in sediments compared with water, and there was evidence of growth in the absence of predators. The authors postulated that this may cause problems when using these organisms as faecal indicators as the high numbers present may be resuspended in the overlying water, but it is not clear that pathogens would follow the same pattern or have the ability to grow in sediments.

A study of intertidal sediments in the UK showed the absence of the pathogenic strains of *C. jejuni*, *C. coli* and *Salmonella* [81]. Faecal coliforms and faecal streptococci were isolated throughout the year with no obvious seasonal trends in their numbers but thermophilic *Campylobacter* spp. (not *C. jejuni* or *C. coli*) showed a strong seasonality, with greater increases in numbers occurring mostly in the summer months. *In situ* deposition of bacteria from overlying water during tidal cover showed deposition rates of approximately 0.1% of the total population of faecal coliforms, 0.01% of the faecal streptococci and 1% of the *Campylobacter* spp. in the sediments. They concluded that sediment could act as a reservoir for bacteria, especially faecal indicator organisms. However, they reported an absence of *Salmonella* and thermophilic *Campylobacter* of non-human origin present in sediments, suggesting that sediment does not act as a reservoir for these organisms.

Finally, it should be noted that it is not surprising that indicator and pathogen counts are frequently poorly correlated in the water column. Indicators, by definition, are consistently present in water polluted by faecal material; pathogens tend to be present intermittently, depending on disease incidence in the upstream communities, or carriage rates in livestock in the catchment. Furthermore, there is such a wide range of pathogen types (bacterial, viral, protozoan), that one indicator alone could not be expected to simulate the behaviour of all pathogens. Rather, there is increasing investigation into the use of “model” organisms to study the incidence and survival of pathogens, *e.g.* phage PRD1 as a model of adenoviruses. Due to the fact that sediments tend to integrate microbial pollutants, closer relationships between “similar” indicators and pathogens may possibly occur. This issue requires a lot more research before conclusions can be drawn about the extent of similarities between pathogens and indicator organisms in sediments and their potential for resuspension.

6. Stratification in Sediment

In a water column, enteric microbes are free to circulate with water currents and turbulence. Most, however, appear to become stratified in sediments, either due to the sequence in which the particles to which they are attached are laid down, or in response to depth-related favourable and unfavourable environmental conditions. In addition, at least in freshwater sediments, some motile enteric bacteria may be able to migrate small vertical distances.

The stratification of enteric microbes in sediments does not appear to have been extensively investigated. However, in the above study of *Campylobacter* in sediments [81], it was found that the faecal indicator organisms were isolated predominantly from the surface layers of the sediments and declined in number with depth, whereas *Campylobacter* spp were restricted to the surface layer. In another study, the survival of the faecal indicator organisms, *E. coli* and enterococci, was assessed in a 90-day laboratory experiment, where regular sampling was undertaken in sediment cores at different depths [69]. Both *E. coli* and enterococci survived for the length of the experiment (90 days) in sediment samples close to the surface (0-6 cm depth). *E. coli* survived at all depths (0-10 cm) for up to 30 days, whereas enterococci disappeared in the deepest samples (8-10 cm) taken after 30 days.

7. Resuspension

The accumulation and enhanced survival of pathogenic bacteria and viruses in sediments (and the possible growth of some bacteria) are only important if these microbes are brought into contact with the public via direct contact or resuspension of sediments. The large number of microbes present in sediments means that even if only a small proportion of them are resuspended it may be cause for concern, for example when they come into contact with recreational water users in high enough doses to cause infection.

Enteric microbes can re-enter the water column by two mechanisms - detachment from particles, or resuspension of the particles to which they are attached. The first mechanism is probably far less important due to the fact that rapid re-attachment is likely to occur, although there is evidence that viruses can be washed from particulate matter in saline environments [38].

Most of the resuspension of microbes is likely to result from sediment particle resuspension, and this is in turn, most likely to result from various forms of disturbance. It has been demonstrated that tides, boats and human activity can resuspend bacteria, and to a lesser extent viruses, in the water column [79, 84]. Even if the particles subsequently resettle, this disturbance may act to reduce microbial survival due to exposure to solar radiation in the surface waters [85]. In a study of beaches in Europe, resuspension of indicator organisms only occurred in rough water, particularly during windy weather, suggesting that only sporadic resuspension was occurring [81]. In a survey of sediments in Switzerland, the levels of enteric microbes were found to be high, but they were not deemed high enough to cause a decline in the water quality according to EU (European Union) bathing water directives, if they were resuspended. The authors suggested that levels of 10^6 CFU per 100 g would be needed for such exceedances to occur. The same study investigated the survival times of *E. coli* in sediments in laboratory trials. Die-off of bacteria in sediments was reported to be slow, with only 10% occurring within 85 days [65]. As the same proportion of *E. coli* remained culturable during the experimental period, the authors proposed that sediments offer a stable and favourable environment, which, if resuspension occurred, could provide a long-term risk of contamination. Fries *et al.* [48] reported high persistence rates in sediments of several bacteria (*E. coli*, enterococci and *Vibrio*), and that the numbers recovered significantly increased following a large storm event (Hurricane Ophelia) in the US. This research demonstrated that during heavy storms, resuspension of microbes occurred into the overlying water. Resuspension of indicator organisms and pathogens in canal sediments after heavy rainfall has also been reported in the USA [79].

8. Conclusions

Adherence to New Zealand recreational water quality guidelines only requires that *E. coli* levels in the water column are monitored. However, this may underestimate the microbial load in the catchment, because it does not account for the microbes in the sediments.

To answer the questions asked by HBRC, namely:

1. What information is available on the survival of pathogens and viruses in sediments?

A review of the international literature showed that there is strong evidence to suggest that sediments accumulate and retain enteric indicators and pathogens. Furthermore, most published evidence suggests that survival rates of microbes in sediments are considerably higher than in the overlying water column. Although the mechanisms are not fully understood, an increased level of organic matter appears to be the principal reason for the enhanced survival rates from the literature available.

2. Is there any information on the correlation between sediment resuspended indicator bacteria and pathogens/viruses?

Research in surface waters has shown that, although faecal indicator organisms fulfil a useful function in demonstrating the possible presence of pathogens and, in some cases they broadly mimic pathogen behaviour, there is a risk of over-reliance on one or two bacterial indicators.

For sediments, the data on the relationships between indicators and pathogens are sparse and inconclusive. Overall, there is no strong evidence of correlations between indicator and pathogens levels and survival in sediments, even though continuous deposition may be expected to have a greater integrating effect than would be expected in surface waters. This is an issue which requires further research before conclusions can be made.

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