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Assessing riverine sediment–pathogen dynamics: implications for the management of aquatic and human health risk

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Abstract While it is established that sediment/floc can harbour a significant quantity of pathogens, the erosion, transport and deposition dynamics of the sediment-associated pathogens is not well understood in relation to ecosystem and human health impact. In this study, annular flume experiments were run with *Pseudomonas* spp. CTO7::*gfp*-2 inoculated sediment to assess the erosion, transport and fate of indicator organisms in river systems. Correlative microscopy was used to visually assess the microbial-floc relationship and a flow-cell/shear-cell was used to assess the strength of the indigenous *E. coli* microbe-floc association. Results indicate that suspended cohesive flocs are the dominant form of pathogen delivery to the sand bed. Significant correlations were found between the indicator organisms (CTO7-gfp and *E. coli*) suspended solid concentration and shear level. It is concluded that the bed sediment can represent a significant source of pathogenic organisms to the water column, with regulatory water samples not necessarily reflecting recent microbial contamination from terrestrial sources, but also a re-suspension of previously settled pathogens from the river bed. As such, sediment–pathogen dynamics should be considered when identifying source areas, determining aquatic and public health risk, and modelling pathogen transport in river systems.

Key words floc; bacteria; pathogen; erosion; transport; fate

INTRODUCTION

It is well understood that bacteria (including pathogens) are often strongly associated with sediments/flocs (bed and suspended) (Krometis *et al.*, 2007; Tang *et al.*, 2009). This association serves three ecological/sedimentological functions: (1) bacteria use the sediment as a place for attachment, assimilation of food (DOC, POC) and protection from predation and other environmental stresses (Gerba & McLeod, 1976); (2) bacteria along with their secreted extracellular polymeric substances (EPS) (Leppard, 1997) help promote flocculation, increasing particle size by binding particles together in a complex matrix or floc (Droppo, 2001); and (3) flocculation increases the downward flux of sediments and therefore the delivery of associated pathogens to the river bed (Droppo *et al*., 2009). Once on/in the bed, the sediment and pathogens can undergo consolidation and biostabilization (biofilm development) and, therefore, may represent a reservoir of potential pathogenic organisms for downstream transport if remobilized during storm events (Droppo, *et al.*, 2009; Rehmann & Soupir, 2009; Wu *et al.*, 2009). As indicator organisms and pathogens have been shown to survive for extended periods of time in aquatic sediments (Davies *et al.*, 1995), the erosion of sediment and associated pathogens can pose potentially significant aquatic and human health risks, especially if sensitive areas such as drinking water intakes are the downstream receiving areas (Donovan *et al.*, 2008). The risk is further exacerbated by the potential for pathogen dissociation from the floc matrix into a planktonic state, where ingestion and possible disease is made more likely under increasing shear scenarios with associated deflocculation. The objective of this paper is to examine the erosion, transport and deposition of *Pseudomonas* spp. and *E. coli* (indicator organisms) for a sand bed river using a 5-m annular flume to simulate storm flow. Further, the paper examines the implications of the sediment–pathogen association for the regulatory management of aquatic and human health risks.

METHODS

Sediment was collected from Sunnyside Beach (SB), Toronto, Ontario, Canada, to simulate a sand bed river substrate for erosion experiments within a 5-m annular flume (Krishnappan, 1993). This sediment was selected as Sunnyside Beach has been closed for between 36 and 69% of the swimming season (2005–2008) (City of Toronto, 2009) due to high levels of indicator organisms. It is believed that the organisms brought into suspension are associated with the fine-grained sediment fraction eroded from the interstitial voids in the sand (similar winnowing of fines is anticipated for river erosion experiments on the same sediment).

Approximately 140 L of sediment was collected from the swash zone of the beach and this was homogenized with an inoculant of fluorescently labelled *Pseudomonas* spp. CTO7::*gfp*-2 (CTO7-gfp) at a concentration of approximately 10^7 cells mL^{-1}. The sediment/microbe mixture was then laid down in the flume forming a 2–3 cm bed and the flume was then filled with approximately 12 cm of Lake Ontario water.

The flume was run at a high speed (equivalent to 0.46 Pa bed shear stress) to allow for the complete mixing of the sediment and bacteria within the flume for 30 min followed by 48 hours of settling and consolidation. The flume was then run with increasing increments of bed shear to facilitate erosion, followed by reductions in bed shear stress to facilitate particle settling as presented in Table 1. A longer duration for settling was allowed relative to erosion times. During the flume runs, suspended solid (SS) concentrations were collected every 10 min with bacteria samples collected at the end of each shear step.

Table 1 Annular flume sequence of shear steps and duration.

Shear step	Bed shear stress (Pa)	Shear duration (min)	Shear step	Bed shear stress (Pa)	Shear duration (min)
1	0.12	30	6	0.39	30
2	0.16	30	7	0.46	30
3	0.21	30	8	0.32	60
4	0.26	30	9	0.21	60
5	0.32	30	10	0.12	60

To assess the strength of the floc–microbial association, SB sediment (wet sieved at 63 μm) was also placed within a unique flow-cell/shear-cell (FCSC) (Droppo *et al.*, 2008) interfaced with a Cilas™ 930 laser particle sizer. The amount of sample used in the FCSC was determined by the obscuration percentage (21%) of the laser beam through the sample cell. Within the FCSC, the SB floc was mixed using a digital turbine at a low rate of 125 rpm ($G = 83\ s^{-1}$) for 20 hours to generate a starting equilibrium condition in floc size and microbial (indicator *E. coli*) associations. Subsequent to this, the shear rate was increased in one-hour incremental steps (200, 400 and 600 rpm) and the floc size distribution and *E. coli* concentration was determined within the last 10 min of each shear level step.

Suspended solid (SS) concentrations were determined gravimetrically by filtering a known volume of sample onto a pre-weighed 0.45-μm Millipore™ filter and reweighing the filter after drying at 100°C for 1 h to determine SS concentration ($mg\ L^{-1}$).

Numeration of *Pseudomonas* spp. CTO7::gfp-2 (CTO7-gfp) was performed by diluting a 50 mL aliquot of eroded sediment in buffered saline (VWR), and plating 0.1 mL of diluted sample onto the surface of tryptic soy agar (EMD) in duplicate. Plates were incubated at 30°C for 24 h, then screened and numerated for CTO7-gfp using a fluorescence dissection scope (Leica).

Triplicate 0.1 mL samples were spread on plates of MacConkey agar, which were used to determine *E. coli* counts during FCSC runs. Plates were incubated at 37°C for 48 h and counted according to the standard methods for the examination of water and wastewater (Water Environment Federation, 1995).

Genomic DNA from the FCSC runs was extracted from a 50 mL aliquot collected at the end of each shear level step. A 25 mL subsample was centrifuged at minimum speed to concentrate particles. The pellet was then placed in a bead tube and DNA was extracted using the Ultraclean soil DNA isolation kit (MoBio Laboratories). The genomic DNA was then quantified using PicoGreen ® dsDNA Quantitation reagent (Molecular probes).

Microscopy was used for the assessment of floc structure and floc EPS-bacteria associations. The microscopes used include conventional optical microscopy (COM), environmental scanning electron microscopy (ESEM), confocal laser scanning microscopy (CLSM) and transmission laser microscopy (TEM).

RESULTS AND DISCUSSION

The mobilization of flocculated cohesive sediments from within the sand bed matrix generally requires the movement of the sand particles via bed load to initiate re-suspension. This is in part related to the mass differences between the sediment and the biofilms growing within the interstitial voids of the sand grains, stabilizing the bed structure (Rehmann & Soupir, 2009). Such microbial integration and stabilization is illustrated in Fig. 1(a) which shows biofilm growth within a microscope flow cell containing SB sand (Sousa, unpublished data, 2009), and in Fig 1(b) showing that both live and dead bacteria were present within the eroded floc collected from flume Run 3 (Tirado, unpublished data, 2009). Figure 1(c) further illustrates the appearance of significant organic coatings around the eroded floc while Fig. 1(d) shows the internal diffuse architecture of the flocs with bacteria, EPS and clay particles making up the floc network (although the minerals dominate the mass, but not the volume). The highly biologically mediated flocs observed for SB eroded sediments are consistent with the findings from multiple freshwater environments which supports the assumption that the floc matrix has a high biological content, with the organic material (including bacteria and their associated EPS) providing much of the structural strength (Droppo, 2009).

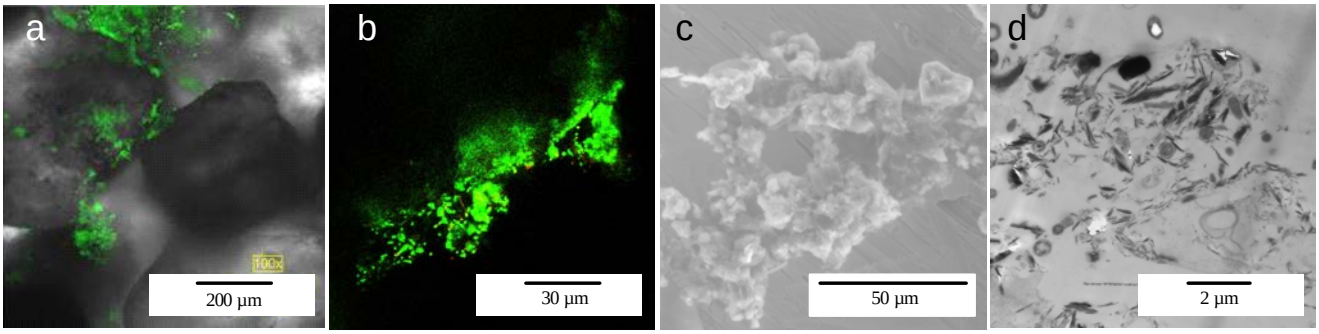

Fig. 1 SB eroded floc (natural and from flume): (a) 72 hour biofilm grown within SB sand grains in flow cell showing indigenous and CTO7-gfp bacteria in interstitial voids, (b) CSLM image of floc showing live (green) and dead bacteria (red), (c) ESEM image of floc showing organic coatings around particles, and (d) TEM image of eroded floc showing clay, bacteria and EPS matrix.

Figure 2 uses flume Run 1 results to illustrate the dynamics of sediment and microbial erosion of the SB sediment. Significant dune formation was evident within all runs. It seems likely that the bed load transport blocked the intake of the sampling nozzle during Run 2, where the SS never exceeded 20 mg L^{-1}. As such, we have disregarded this run and only focus on Runs 1 and 3. Figure 2(a) shows an initial ambient concentration at a shear of 0.12 Pa of 20 mg L^{-1} (Run 3: 10 mg L^{-1}) and a consistent increase in SS with each increment in shear to a maximum SS of 160 mg L^{-1} (Run 3: 90 mg L^{-1}). As expected, as the shear level is decreased following the highest shear (0.46 Pa), there is an immediate drop in SS over the next three 1-hour incremental steps with the concentration decreasing to 40 mg L^{-1} (Run 3: 20 mg L^{-1}).

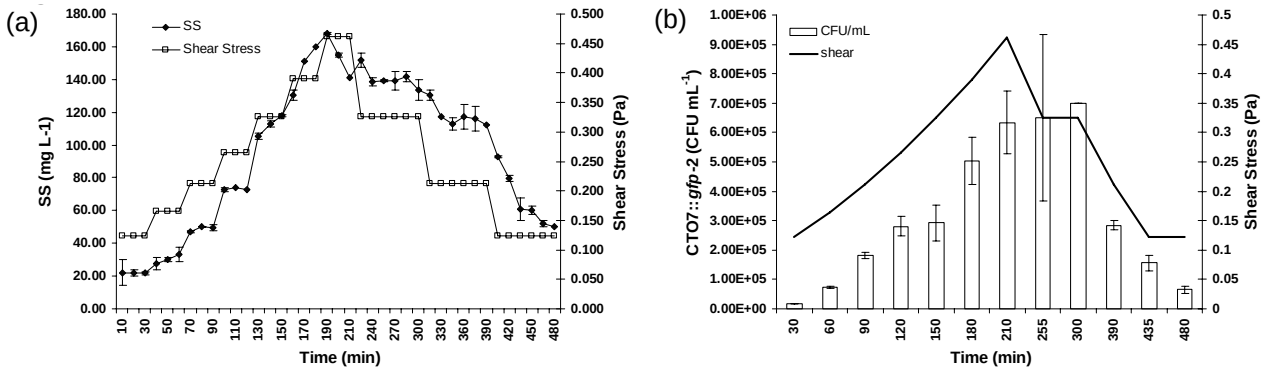

Fig. 2 Result plots from Run 1 of SS, shear stress and CTO7-gfp over time (error bars represent ± standard deviation).

While the change in SS is not surprising, the significant results of this study are demonstrated by the correlation between counts of indicator organisms (CTO7-gfp and *E. coli*) and changes in shear stress and SS. For the flume runs, Fig. 2(a) and (b) illustrate that there is a significant correlation ($\alpha = 0.05$) between CTO7-gfp and shear [0.88 (Run 1), 0.60 (Run 3)], CTO7-gfp and SS [0.90 (Run 1), 0.76 (Run 3)], and shear and SS [0.88 (Run 1), 0.79 (Run 3)]. As shear increases, so do both the SS concentration and indicator organisms in suspension. Of interest is the continued increase in CTO7-gfp even after the shear was reduced for all runs (true also for Run 2 even with minimal SS sampled) followed by a sharp decline in CFUs with a rate similar to the reduction in SS. The CFUs remaining high after the reduction in shear may suggest that at the higher shear levels there is a dissociation of bacteria from the sediment into the planktonic phase. However, the subsequent rapid drop in CFUs would also suggest that the bacteria are refloc-culating with the sediment remaining in suspension, and/or are being scavenged out of suspension by the settling particles. Under either scenario, these results demonstrate that sediment dynamics are driving the microbial dynamics (erosion, transport, deposition) within the system.

In a related study, Sousa (unpublished data, 2009) found that whilst there was no significant change in attached cell counts with increasing energy levels (wave height), there were consistently 2 to 3 orders of magnitude more cells (CTO7-gfp) associated per gram of eroded SB flocs (filtered from a 50 mL sample on a 5 µm filter) than free-floating in suspension (filtrate). To assess the possible dissociation of bacteria from flocs into the planktonic phase under shear, the FCSC was employed using SB sediments with their natural microbial assemblages. Table 2 illustrates that as the shear level increased from 125 to 600 rpm ($G = 83$ to $878\ s^{-1}$), the planktonic counts of the indicator organism *E. coli* increased by two orders of magnitude in suspension (300 to 11 000 CFU mL^{-1}). When the shear level was reduced back to 125 rpm, the amount of *E. coli* in suspension decreased by 2 orders of magnitude by re-incorporation into flocs (flocculation is assumed as no settling occurred within the FCSC). The effects of shear on biological dissociation from flocs is also evident by a reduction in the extracted sediment associated genomic DNA at high shear levels compared to lower shear levels (Table 2). [It should be noted that genomic DNA

Table 2 Changes in suspended floc associated *E. coli* and genomic DNA with change in shear level within the FCSC (Tirado, unpublished data, 2009).

Shear level (rpm)	Velocity gradient G (s^{-1})	*E. coli* (CFU mL^{-1})	Genomic DNA (µg $µL^{-1}$)
125	83	3.0E+02 SD = 0.1	39.6 SD = 0.3
200	169	3.6E+03 SD = 0.1	25.0 SD = 8.0
400	478	7.0E+03 SD = 0.1	22.8 SD = 1.3
600	878	1.1E+04 SD = 0.1	10.2 SD = 0.5
125	83	9.1E+02 SD = 0.1	31.9 SD = 0.6

SD = standard deviation.

extractions are not 100% effective and represent DNA from all living organisms (future PCR analysis is planned on the extracted DNA)]. These results, in relation to changes in shear, once again demonstrate that sediment dynamics are strongly linked to microbial dynamics within river systems and that this linkage, resulting in possible microbe dissociation from flocculated sediment, may potentially cause elevated aquatic and human health risk due to waterborne pathogens.

Conceptual model and management implications

Given the above discussion, demonstrating the dependence of pathogen dynamics on the erosion, transport and fate of flocculated particles in river systems, a conceptual model was developed and this is illustrated in Fig. 3. The model uses decision boxes to route pathogens between the bed sediment, suspended sediment (floc) and the planktonic phase, as influenced by the energy regimes at the sediment water interface and within the water column as a whole. The directional decision boxes of the model are related to: (1) bed shear stress relative to critical bed shear stress (dictates if pathogens will be eroded from the bed in planktonic or floc attached modes, or further consolidated and propagated in the bed sediment/biofilm); (2) fluid shear relative to floc shear strength (dictates if pathogens will be dissociated from the floc or eventually settle towards the bed in association with the floc); and (3) bed shear stress relative to floc shear strength (dictates if the floc will remain intact and deliver pathogens to the bed, or if it will break up resulting in pathogen dissociation and longer range transport). The resultant transient pathogen fate (i.e. associated with the bed, floc, or planktonic) will have implications for the potential risk to aquatic and human health. Typical assessment of human health risk from microbial pathogens in bathing waters is generally based on indicator microbial counts (e.g. *E. coli*) from whole water samples. Assessment of whole water samples, however, does not account for the sediment and energy regimes (e.g. currents), which have been shown above to possibly influence pathogen dynamics within rivers. Health related monitoring programmes, which currently neglect the sediment phase of pathogen existence, and the energy regime they preside in, may provide erroneous management decisions with possible detrimental impacts. Future policy development for the protection of aquatic and human health will require consideration of the sediment microbial phase in the decision making process.

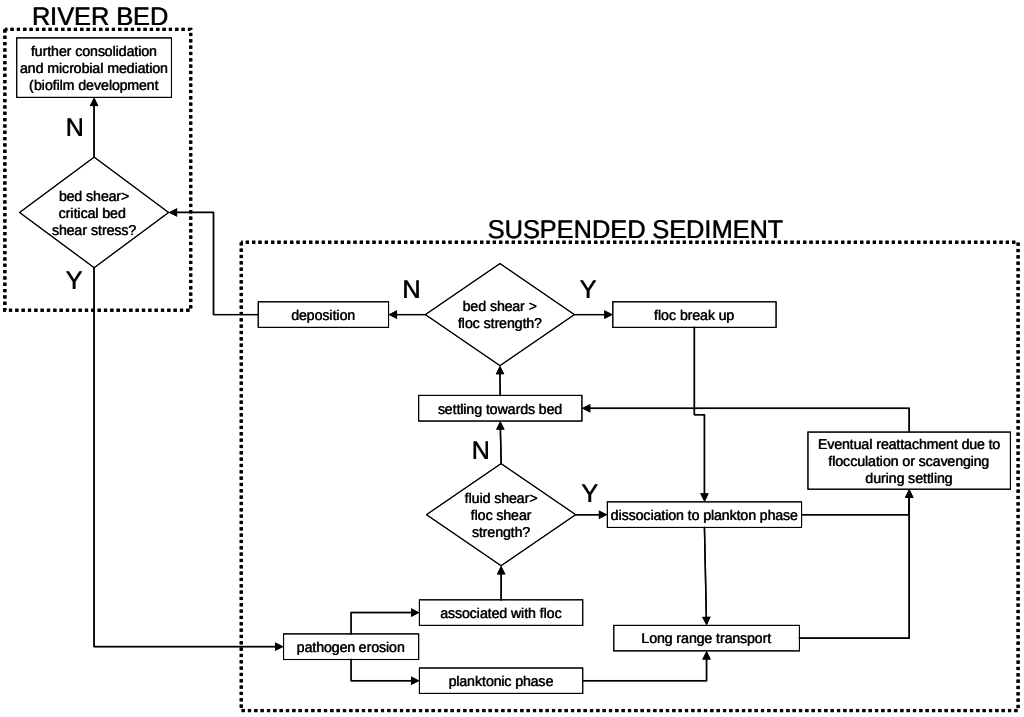

Fig. 3 Conceptual model of the linkage of pathogen and sediment dynamics within a riverine system.

CONCLUSION

The ubiquitous nature of flocculated sediment and microorganisms within natural aquatic systems and the strong association between the two substantiates the need to understand better the links between pathogen dynamics and sediment dynamics. The river bed has been shown to be a possible source of pathogens to the water column, and this suggests that microbes sampled in the water column may not necessarily represent recent contamination, but could represent a compilation of sources deposited over time and mobilized from the river bed. Indicator organisms eroded from the flume bed correlated well with both shear level and SS, but showed signs of dissociation and reflocculation during both the flume and FCSC runs. The dynamic nature of the pathogen–sediment relationship is described within a conceptual model.

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