Microbial removal rate efficiencies measured for coral sand

Objective:

Two experiments to evaluate effective removal rates for model pathogens in lab columns packed with coral sand from the Pacific atoll of South Tarawa, Kiribati.

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Expt. 1: Model filter for septic tank effluent drainage field

Tracers:

E. coli J6-2,
Enterococci faecalis,
F-RNA (MS2) bacteriophage
Adeno-, Echo-, Noro-, Rota-virus

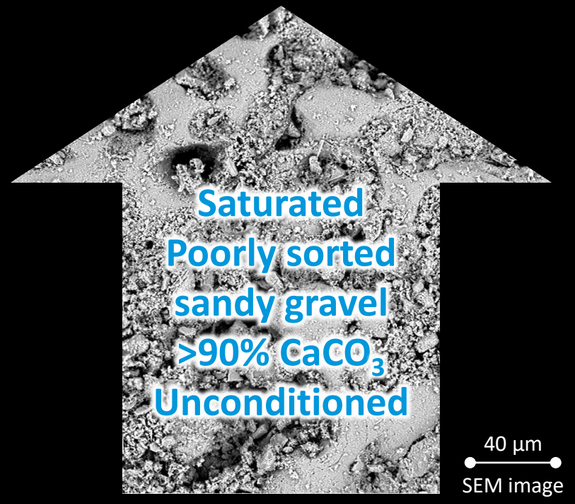
Results:

- Log-removal values >4
- Bacterial tracers *E.coli* & *Enterococci faecalis* eluted
- Viral indicators & viruses retained in column

Expt. 2: Model groundwater system - aquifer material from Bonriki Freshwater Reserve

Results:

- *E. coli* removal rate $0.02 < \lambda < 0.07$ $\log_{10}$ cm^{-1}
- *E. coli* removal more than MS2 phage



Tracers:

E. coli J6-2
F-RNA (MS2) bacteriophage

Research aims

- Freshwater and sanitation problems afflicting low-lying atolls in the Pacific are numerous and some of the most critical in the world.
- One problem is protection of the shallow and finite groundwater resource from faecal contamination.
- Another is sewage disposal.
- On South Tarawa, in the Republic of Kiribati, freshwater is sourced almost entirely from groundwater, abstracted from the freshwater lens. The Bonriki Water Reserve is the area from which groundwater is abstracted for reticulated community supply.
- Open defecation has long been the traditional sanitation practice. Recent efforts to improve sanitary practices on the atoll includes installation of toilets and septic tank systems that in many cases are not coupled to any engineered drainage field.
- The land on South Tarawa consists entirely of weathered reef deposits or coral sand. The coral sand is composed mainly of aragonite ($CaCO_3$). The beaches facing the Pacific ocean comprise well sorted sand material, which tends to have a slightly higher magnesium content than material found in land and within the lagoon.
- ESR has undertaken to experiments to evaluate the efficacy of coral sand from South Tarawa at microbial removal.
- **Experiment 1** aimed to examine coral beach sand, as it might be used as a filter in an engineered effluent drainage field.
- **Experiment 2** was an initial examination of surficial (aquifer) sediments from the Bonriki Water Reserve.

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Experiment 1: Methods

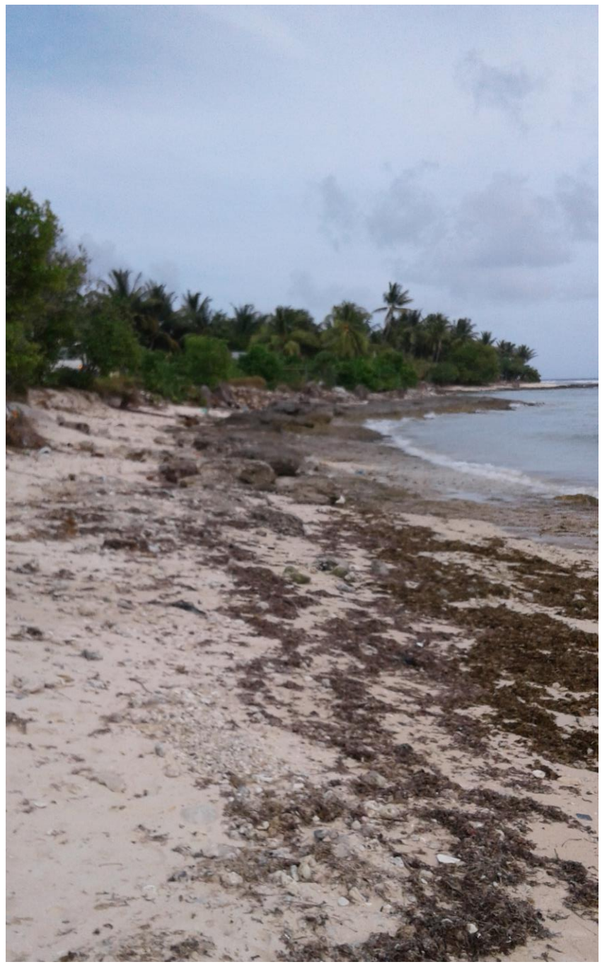

Fig. 1 Bikenibeu beach, South Tarawa, from which coral beach sand sampled.

Coral sand material:

- Coral beach sand sampled from Bikenibeu beach (Fig.1).
- Particle size distribution determine by dry-sieving.
- Mineralogical composition evaluated by XRD.

Experimental column apparatus:

- 400 mm-long, 106 mm-diam. glass columns; dry-packed; prepared in triplicate (Fig.2).
- Operated unsaturated; gravitational drainage.
- Septic tank effluent used to condition the columns: 16 mm head of effluent applied twice/day for 27 days.

Microbial tracer experiment:

- Septic tank effluent spiked with *E. coli* J62, *Enterococci faecalis*, MS2 bacteriophage, adenovirus, echovirus, norovirus and rotavirus.
- 35 mm head applied to each conditioned column.
- Any drainage collected and analysed for microbial content.
- Columns destructively sampled and target organisms enumerated.
- 25 g wet weight sand washed in 250 mL pH 8 saline solution; shaken for 2 mins; left to settle 30 seconds; 1 mL aliquot pipetted off for analysis.

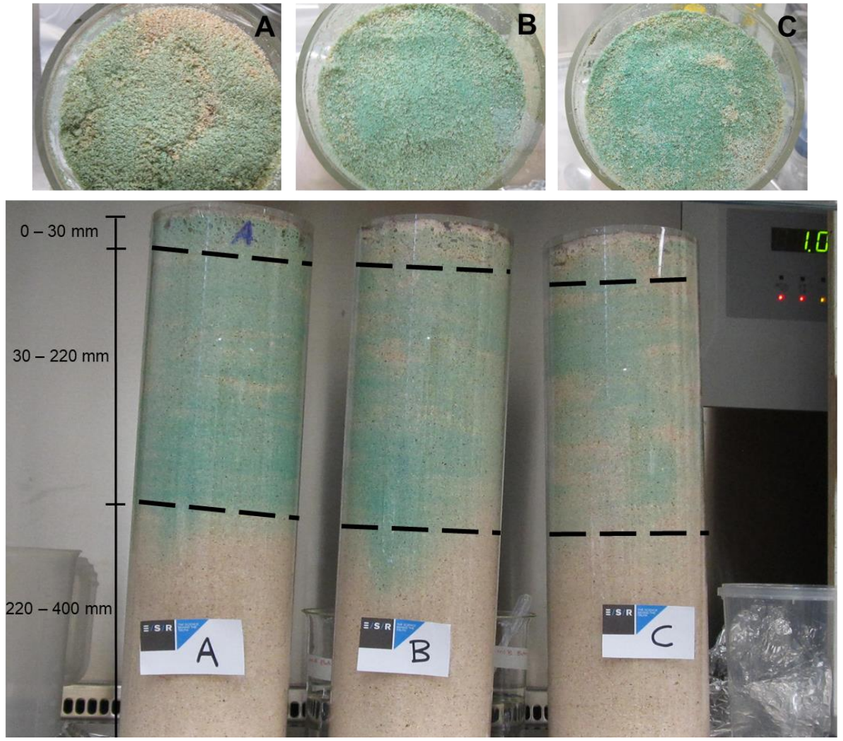

Fig. 2 Example of sand-packed glass column apparatus. Blue-stain is food-colouring, applied to examine uniformity of packing process/preferential flow. Depth sections over which sediments destructively sampled and analysed are marked.

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Experiment 1: Results

Physiochemical properties of coral sand material:

- Moderately well-sorted, coarse sand.
- 85% calcium-magnesium carbonate ($CaMg(CO_3)_2$) + 15% aragonite (CaCO3)

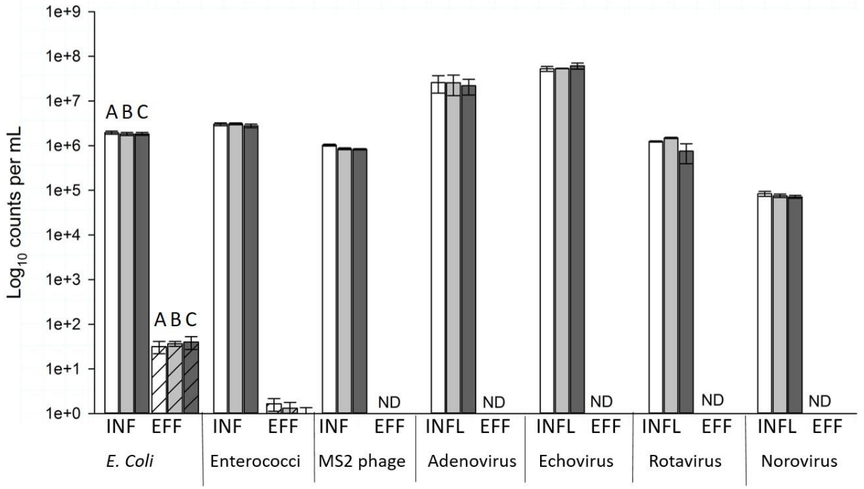

Fig. 3 Microbial counts for tracer organisms in spiked-septic tank effluent (INF) added to sand-packed columns A,B and C, also in drainage from base of columns (EFF). Note: only *E. coli* and *Enterococci* eluted from the base of the 400 mm-long columns.

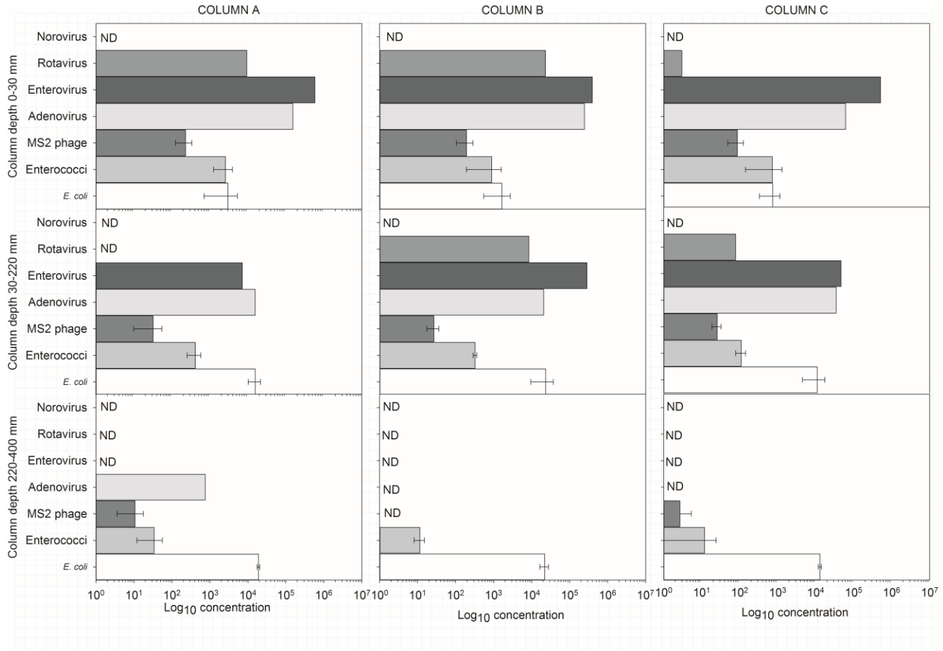

Fig. 4 Microbial counts for tracer organisms retained in various portions of the sand column, following dosing event. Note: Bacteria were enumerated as colony forming units per mL, whereas viral units were plaque-forming units per mL.

Table 1 Log removal values for target organisms, calculated from difference in influent and effluent concentrations (Fig. 3)

	E. coli	*E. faecalis*	MS2 phage	Adenovirus	Echovirus	Rotavirus	Norovirus
Column A	>4.79	>6.26	>6.97	>7.18	>7.74	>6.10	>4.92
Column B	>4.70	>6.36	>6.89	>7.12	>7.75	>6.17	>4.88
Column C	>4.66	>6.45	>5.92	>7.13	>7.75	>5.88	>4.85
Average	>4.71	>6.36	>6.59	>7.14	>7.75	>6.05	>4.89

Reference:

Humphries et al. (2019). Journal of Applied Microbiology doi: 10.1111/jam.14555

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Experiment 2: Methods

Coral sand material:

- Substrate sampled from 400 mm depth of Bonriki Water Reserve.
- Particle size distribution determine by dry-sieving.

Experimental column apparatus:

- 150 mm-long, 27 mm-diam. plastic columns prepared in triplicate.
- Packed to dry bulk density 1.50 ± 0.02 g cm^{-3}
- Operated saturated; upward flow, driven by MilliGat pumps; Q = 4.8 ± 0.28 mL min^{-1}. Temperature maintained at 28 °C.
- Artificial groundwater (AGW): Cl^- 250 mg L^{-1}, pH 8.0.

Microbial tracer experiment:

- Bromide, *E. coli* J6-2 and MS2 bacteriophage added to groundwater.
- Finite injection: 150 mL over 30 mins; subsequently flushed with clean AGW.
- Determined total mass recovery of each tracer eluted through columns from which effective microbial removal rate calculated.

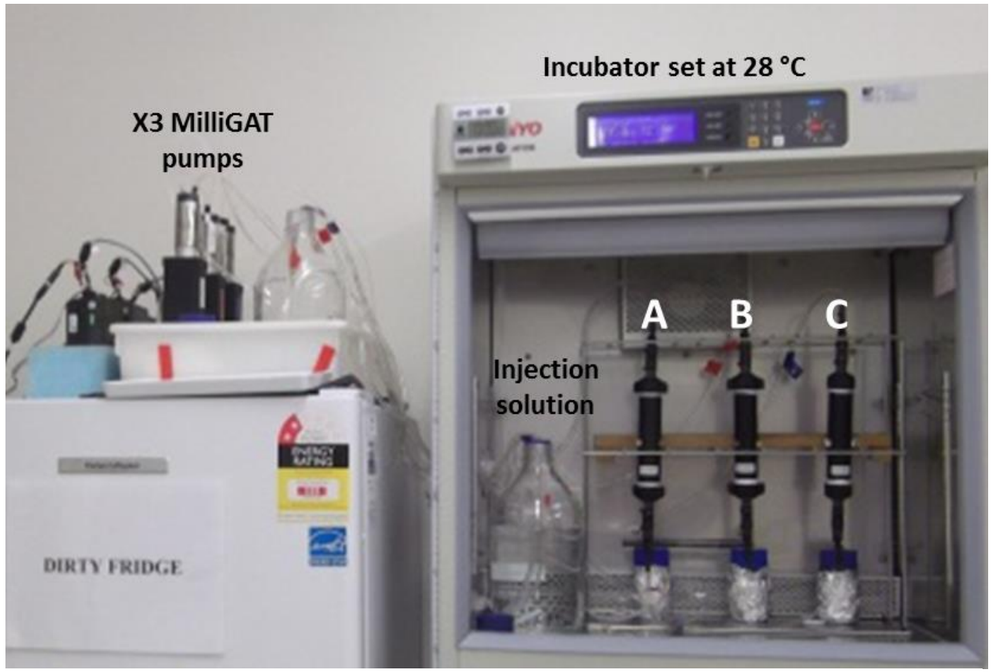

Fig. 6 Experimental set-up. Tracer experiments were run in the dark and at 28 °C - this being the average groundwater temperature on South Tarawa.

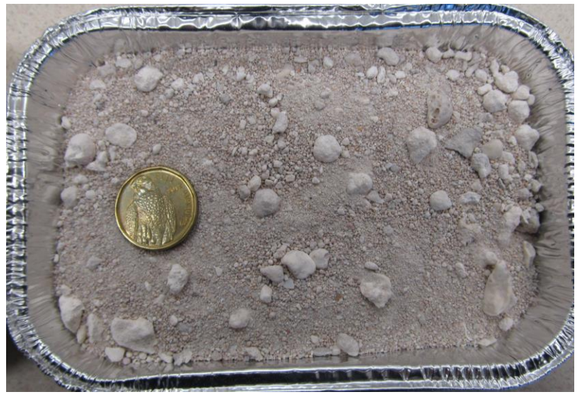

Fig. 5 Coral sand substrate sampled from Bonriki Water Reserve and used to pack columns.

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Experiment 2: Results

Physical properties of substrate tested from Bonriki Water Reserve:

- Very poorly sorted sandy medium gravel.
- Total porosity of packed columns was 0.41 ± 0.02.

Removal rates

- MS2 phage removal found to be caused by plastic column apparatus.
- Notwithstanding this error, more MS2 eluted than *E. coli.*
- Removal rates for sand-packed columns compensated for such bias.

Table 2 Tracer mass balance and effective removal rates calculated for saturated sand-packed columns. Note: removal rates are corrected for losses measured in empty control column tests.

		Column ID			
		A	B	C	average
Tracer mass recovered (empty control columns)					
Bromide	%	98.7	99.6	103.2	100.5
E.coli J6-2	%	116.1	86.1	88.6	97.0
MS2 phage	%	52.1	60.8	30.3	47.7
Tracer mass recovered (sand-packed columns)					
Bromide	%	98.6	99.5	101.6	99.9
E.coli J6-2	%	16.8	6.5	0.06	7.8
MS2 phage	%	27.7	31.6	36.8	32.0
Calculated microbial removal rates					
E.coli J6-2 log-removal rate (sand), $\lambda_{E.coli}$	cm^{-1}	0.05	0.02	0.07	0.05
MS2 phage log-removal rate (sand), λ_{MS2}	cm^{-1}	0.04	0.04	-0.01	0.02

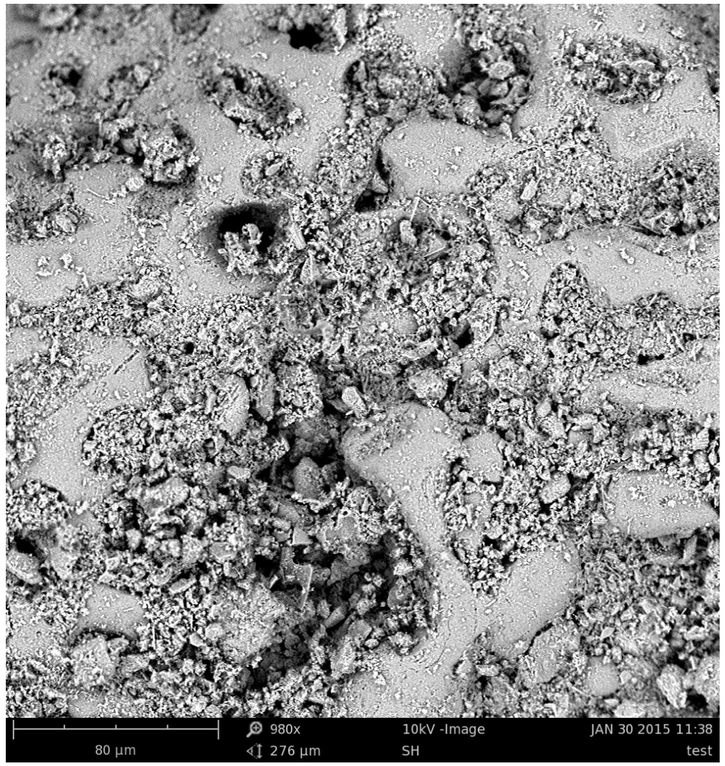

Fig. 7 Scanning electron microscope image of sand analysed from Bonriki Water Reserve. Note the high secondary porosity.

Reference:

Burbery et al. (2015). Journal of Environmental Quality doi: 10.2134/jeq2015.01.0044

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