INDICATOR ORGANISM LEVELS IN EFFLUENT FROM QUEENSLAND COASTAL STPS

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Abstract

The microbiological quality of the effluent from 33 sewage treatment plants (STPs) along the Queensland coast was investigated as part of a project to develop a quantitative model to assess the health risks associated with irrigating sugar cane with treated effluent.

Initially a single sample from each of the 33 STPs was tested to provide an indication of the microbiological quality of effluent available for irrigation use. Ten of these STPs, selected to give a range of effluent quality and treatment types, were then tested 4 to 6 times over a 12 month period to investigate in-plant variation.

Microbial counts varied widely between and within STPs. Ranges for all 92 samples (with geometric means in brackets) were: thermotolerant coliforms, 0 to 24,000,000 (388) /100mL; *Clostridium perfringens*, 0 to 15,000 (90) /100mL; coliphages, 0 to 6,370 (4) /100mL; *Cryptosporidium*, 0 to 600 (<10) /L and *Giardia*, 0 to 4,840 (98) /L. All of the 10 STPs which were sampled over 12 months had *Giardia* counts above 150/L in at least one sample, and one STP had counts above 1,000/L for all 5 samples. No seasonal trends were evident in the counts of any of the microorganisms assayed, and no relationship was evident between microbial count and STP type, population size or STP throughput.

None of the statistically significant relationships between assays reported in this paper were strong enough for the reliable prediction of the results of one assay from the results of another.

Key Words

Sewage effluent, indicator organisms, pathogens, wastewater reuse, irrigation, sugar cane, thermotolerant coliforms, *Giardia*

Introduction

Use of secondary-treated effluent for spray irrigation of sugar cane is an attractive proposition for Queensland cane farmers. Effluent compares favourably in cost with other sources of irrigation water, and has some nutritive value for the cane, while cane fields may provide an environmentally safer site for disposal of the effluent compared with direct outfall to water bodies (Gardner et al, 1998). Disease-causing organisms (pathogens) persisting in the secondary-treated effluent and subsequently transferred to the cane are eliminated during processing of the sugar. However there may be an increased risk of infection to farmers and their families and neighbours who come into direct contact with the effluent and its aerosols, and to consumers of vegetable and salad crops grown near effluent-irrigated farms (Mara and Cairncross, 1989). This risk is difficult to assess, although empirical evidence and epidemiological investigations suggest the risk is small (Fattal et al, 1986, Shuval et al, 1989).

Effluent-borne pathogens which cause human infections through direct contact include bacteria such as *Salmonella*, *Shigella* and *Escherichia coli*, the protozoan parasites *Giardia* and *Cryptosporidium*, parasitic worms (helminths) and enteric viruses (Bitton, 1994). Enteroviruses, coliphages, coliforms and *Salmonella* have been demonstrated in aerosols formed during spray-irrigation of crops and recreational areas with sewage effluent (McNeill, 1985).

Microbiological monitoring of Queensland STPs is largely restricted to assaying faecal coliform levels, with only a few STPs testing for bacteriophages, enterococci, *Salmonella*, *Giardia* and/or *Cryptosporidium*. Frequency of testing, source of testing (STP, local government, state government, hospital or private laboratory) and test methods used vary markedly between STPs, and the small amount of data available is of little use in comparing effluents from different STPs, or in quantifying health risks associated with their reuse.

Most regulations and guidelines controlling the reuse of wastewater specify acceptable levels of thermotolerant coliforms for various practices, and usually do not specify levels of protozoans and viruses (Table 1). This is an acknowledgment of the difficulties involved in assaying the latter, rather than confidence in the predictive value of the indicator assays. The inability of assays for traditional indicator organisms such as coliforms, thermotolerant (faecal) coliforms and coliphages to accurately predict levels of the majority of the faecally-transmitted infectious agents is well documented (Bitton, 1994). However, in the absence of practical and specific methods for routinely assessing pathogen levels the traditional methods remain useful, primarily to monitor the disinfection process. The thermotolerant coliform count also provides an indication of the likely levels of pathogenic enterobacteriaceae present in an effluent. The coliphage count is useful as a conservative indicator of viral disinfection (Havelaar,1991).

The work reported here was done as an initial part of a project to develop a quantitative model to assess the health risks involved in spray-irrigating sugar cane with secondary-treated effluent.

Methods

To assess the microbiological quality of effluents available for sugar cane irrigation, we determined the levels of selected microorganisms in the effluents from 33 sewage treatment plants (STPs) near sugar-producing areas in Queensland in 1995/96, including some samples from stabilisation ponds receiving secondary-treated effluent. Ten of these STPs, selected to provide a range of effluent quality and STP characteristics, were then sampled on several occasions during 1996/97 in order to investigate in-plant variation and to determine whether any seasonal effects could be detected. Microorganisms assayed included thermotolerant coliforms, *Salmonella, Clostridium perfringens,* the protozoan parasites *Giardia* and *Cryptosporidium,* helminths (parasitic worms) and coliphages of *E. coli* strain K13.

For the initial, single sample survey, 33 STPs were chosen on the basis of their proximity to sugar-producing areas, the feasibility of using their effluents for irrigation and to give the widest geographical spread over the length of the Queensland coastline involved in sugar production, from 16°S to 28°S. Sites are identified in Tables 2, 3 and 4 by number only, from 1 (the most northerly) to 33 (the most southerly). Treatment processes used included oxidation ditch, biofiltration, Imhoff tank, activated sludge and nitrogen reduction. Chlorine disinfection was used by 27 of the STPs, one used ultraviolet irradiation and 5 had no final disinfection process. Equivalent person (EP) capacities ranged from 1,500 to 240,000 and flow rates from 0.4 to 200ML/day. This initial survey was done to provide an indication of the quality of effluents available for irrigating sugar cane, rather than to provide a precise estimate of the average effluent quality from these sites.

For the multiple analysis survey, 10 of the STPs were chosen for regular sampling on the basis of STP and effluent characteristics in the initial survey, to provide low to high microbial counts, 5 different STP types, a range of STP sizes and use and non-use of chlorine to disinfect the effluent before discharge from the STP.

Four of the STPs, including one of the 10 from the second survey, discharged into holding ponds before final discharge. Samples of the influents/effluents from these ponds were also tested.

Sample collection and preparation. Samples were collected from the outlet stream of STPs (before ponding) and from pond outlets into sterile polyethylene containers. One litre was collected for bacteriology (with sodium thiosulphate solution to 0.1% final concentration to remove residual chlorine) and 2 L for parasitology and virology (without thiosulphate). Samples were held on ice during transport to the laboratory. Bacterial counts were done within 24 hours of collection. Samples for parasitology and virology were held at 4°C until tested.

Bacteriology. Appropriate volumes of sample, and appropriate ten-fold dilutions were filtered through 0.45µm filter discs (Millipore). Faecal coliforms were determined on m-FC agar (Difco)

NHMRC May 1995	
USE	STANDARD
Injection to aquifer	< 1000 thermotolerant coliforms/100 mL
Unrestricted public access, urban	< 10 " " " "
Controlled public access, urban	< 1000 " " " "
Spray irrigation of crops	< 10 " " " "
Pasture irrigation	< 1000 " " " "
Dairy cattle pasture	< 200 " " " " #
	< 10 " " " " ##
Aquaculture	< 10 " " " "
Primary contact recreational	< 150 " " " "
Non contact recreational	< 1000 " " " "
Ornamental water bodies	< 10000 " " "
VICTORIAN EPA 1996	
USE	STANDARD
Unrestricted public access	< 1 thermotolerant coliforms/100 mL < 2 virus/50 L < 1 parasite*/50 L
Unrestricted access but no food crops	< 10 thermotolerant/100 mL
Restricted public access	< 1000 thermotolerant coliforms/100 mL
NSW RECYCLED WATER COMMITTEE 1993	
USE	STANDARD
Unrestricted access - urban use	< 1 thermotolerant coliforms/100 mL < 2 virus/50 L < 1 parasite* /50 L
INTERIM GUIDELINES FOR DECLARED WASTE WATER DNR 1996	
USE	STANDARD
Unrestricted public access	< 10 thermotolerant coliforms/100 mL
Urban with controlled access	< 150 " " "
Agriculture (fodder crops)	< 1000 " " "
Agriculture (unprocessed food crop)	< 10 " " "
Agriculture (processed food crop)	< 1000 " " "

5 days withholding

no withholding period

* "Parasite" includes *Cryptosporidium* and *Giardia* oocysts/cysts

Table 1. Extracts from National and State Guidelines

Plant Number	Plant Type[a]	Equiv. Person Capacity x100	Flow ML/d	Effluent Disinf.[b]	Thermo-tolerant Coliforms /100mL	*E.coli* /100mL	*Salmonella*[c] / 100mL	*C.perfringens* / 100mL	Phage / 100mL	*Crypto-sporidium* /L	*Giardia* /L
1	OD	80	2	Cl	0	0	0	15	0	<10[d]	20
2	OD	25	1	Cl	11	0	0	240	0	<10	300
3	BIO	500	14	Cl	2800	600	0.7	180	0	<5	190
4	OD	75	1	Cl	1	1	0	2	0	<5	<5[e]
5	BIO	550	14	No	1050000	430000	110	300	1004	5	100
6	BIO	60	15	Cl	129	30	0	2000	68	120	3080
7	IT	30	1	Cl	3	0	0	90	0	<10	1010
8	BIO	60	2	Cl	6900	0	0	120	0	<20	760
9	BIO	NA[e]	9	UV	13600	1900	2.3	500	10	<20	680
10	OD	NA	NA	No	480	390	0	70	2	10	40
11	BIO	100	3	Cl	3200	3200	0	0	49	390	1530
12	BIO	15	1	Cl	34	34	0	0	0	<10	100
13	BIO	50	2	Cl	1400	0	0	0	1	10	20
14	BIO	38	1	Cl	4	0	0	0	5	<10	30
15	BIO	550	20	Cl	3200	0	0	0	11	10	170
16	OD	55	2	Cl	600	0	0	0	30	<10	110
17	OD	20	0.6	No	80000	54	0.9	1430	6	NT[f]	NT
18	BIO	134	3	Cl	15	9	0	14000	13	NT	NT
19	Ox/BIO	380	6	Cl	4	1	0	440	5	NT	NT
20	AS	300	7	Cl	14	8	0	2900	NT	NT	NT
21	BIO	150	3	Cl	0	0	0	0	0	<10	50
22	OD	100	3	Cl	59	15	0	480	0	<5	<5
23	NR	100	2	Cl	20	0	0	40	0	85	70
24	AS	850	15	Cl	40	0	0	160	0	10	<5
25	IT	30	1	Cl	1570	50	0.3	6000	1	110	3500
26	OD	22	1	No	2120000	0	0	130	5	120	105
27	AS	150	5	Cl	0	0	0	6	1	210	320
28	AS	350	11	Cl	7	7	0	1200	1	30	30
29	AS	6000	200	No	100000	40000	3.9	NT	367	NT	NT
30	AS	2400	45	Cl	0	0	0	0	10	<10	50
31	OD	530	9	Cl	0	0	0	6	0	10	<10
32	BIO	220	5	Cl	22	15	0	50	8	NT	NT
33	AS	15	0.4	Cl	1	0	0	0	0	<10	<10

[a] OD: oxidation, BIO: biofiltration, IT: Imhoff tank, OX/BIO: combination OD and BIO, AS: activated sludge, NR: nitrogen reduction
[b] Cl: chlorination, UV: ultraviolet irradiation
[c] Most Probable Number, 0 = <0.3/100mL
[d] Limit of detection varies with turbidity of sample
[e] NA: not available
[f] NT: not tested

Table 2. Survey of 33 sewage treatment plants on the Queensland coast

incubated at 35°C for 5h as a resuscitation step, followed by 44.5°C for 18h (APHA 1992). A representative number of colonies on each filter disc was identified using the Microbact-12 system (Medvet) which identifies *E. coli* to the species level and *Klebsiella, Enterobacter* and *Citrobacter* to the genus level, with some isolates identifiable to species level. *C. perfringens* numbers were determined by the method of Bisson and Cabelli (1979) as modified by Armon and Payment (1988). Most probable numbers of salmonellae were estimated in the initial, single sample survey by resuscitating three 100mL (concentrated by membrane filtration), three 10mL and three 1mL aliquots of the sample in 200mL, 90mL and 9mL volumes of buffered peptone water respectively, followed by selective enrichment in Rappaport-Vassiliadis broth at 42°C and Muller-Kaufmann tetrathionate broth at 37°C. These were subcultured to BGSA and XLD plates after 24h and 48h of incubation. Presumptive colonies were confirmed using Kohn's 2-tube medium (Oxoid) and agglutination with polyvalent type O antiserum (Murex). In the multiple sample analysis of 10 STPs, the presence or absence of *Salmonella* in 100mL of sample was determined by resuscitation in 900mL of buffered peptone water followed by selective enrichment and culture for *Salmonella* as described above.

Parasitology. For the detection of helminth and protozoan parasites, 1L of effluent sample to which 0.01% Tween 80 was added was centrifuged at 3000g for 10 minutes and the resultant pellet was resuspended to a volume of 1 to 2 mL, depending on the amount of residue. Helminths were detected under light microscopy by their characteristic size and shape in two 100µL aliquots. *Giardia* cysts and *Cryptosporidium* oocysts were simultaneously detected by staining two 50µL aliquots with a commercially available direct immunofluorescent test kit (Cellabs) prior to examination with an Olympus BH2 epifluorescent microscope.

Virology. Bacteriophages were analysed according to Grabow and Coubrough (1986) and Bowden (personal communication). One mL of $CaCl_2.H_2O$ (13%) was added to 100mL sewage effluent. To this sample 5mL of an overnight culture of *E. coli* K13 - ATCC 15766 (host bacterium) cultured in Tryptic Soy broth (Amyl) was added and mixed well. This mixture was then combined with 100mL of molten Phage Agar (Amyl) containing 1% triphenyltetrazolium chloride, gently mixed, poured in 50mL aliquots into petri dishes (145mm) and incubated at 37°C for 24h. The presence of phage particles was indicated by cleared zones (plaques) in an otherwise continuous bacterial lawn. Plaques were enumerated and expressed as plaque forming units (pfu) per 100mL of effluent sample. As a positive control, *E. coli* K13 was plated with a sample known to contain virulent phages.

Statistical methods. Data were combined from the initial and follow-up surveys including the ponded effluent discharge samples, giving a total of n = 92 samples for data analysis (Tables 2, 3 and 4). All test results shown as below

Plant Number	Date	Thermotolerant Coliforms /100mL	*E.coli* /100mL	*Salmonella* +/- in 100mL	*C.perfringens* /100mL	Phage /100mL	*Crypto-sporidium* /L	*Giardia* /L
5	14/05/96	480000	220000	+	700	189	10	40
5	31/07/96	1080000	150000	+	1000	115	20	110
5	21/08/96	24000000	6000000	+	6500	6370	<30[a]	390
5	9/10/96	119000	119000	+	380	1515	<20	120
5	29/01/97	153000	99000	-	2	25	<20	460
7	26/06/96	26000	5000	-	900	0	<10	1390
7	7/08/96	1	0	-	4200	0	<20	2580
7	25/09/96	65	54	-	2400	0	<20	4840
7	27/11/96	200	200	-	940	0	40	2260
7	4/03/97	7	0	-	710	0	<10	1120
8	9/05/96	10000	5000	-	300	421	<20	720
8	24/07/96	490	90	-	970	0	<30	2850
8	3/09/96	180000	NT	+	880	218	<20	600
8	26/11/96	2070000	2070000	-	15000	940	NT[b]	NT
11	14/05/96	2	2	-	1600	0	<20	480
11	23/07/96	0	0	-	46	0	240	220
11	28/08/96	2	2	-	960	0	<10	10
11	26/11/96	150	150	-	1000	0	<20	380
11	5/03/97	61000	27000	-	8	0	<20	320
13	2/07/96	1300	600	-	2100	5	<10	10
13	27/08/96	9	5	-	1000	0	<10	70
13	8/10/96	550	60	+	570	0	<20	560
13	20/11/96	0	0	-	210	0	<20	100
13	12/03/97	9900	2700	-	630	0	<20	360
15	8/05/96	95	65	-	80	0	<20	260
15	16/07/96	4800	2400	-	2000	0	10	200
15	21/08/96	1200000	350000	+	2900	591	<10	20
15	1/10/96	540000	60000	+	220	1	<20	220
15	4/02/97	148000	92000	-	1140	1067	80	340
19	14/05/96	43	43	-	200	0	10	160
19	23/07/96	2	0	-	1500	2	<20	520
19	27/08/96	640	530	-	420	0	<10	80
19	8/10/96	6	3	-	1	0	<10	<10
19	29/01/97	0	0	-	0	0	<10	10
20	16/04/96	260	50	-	110	94	<10	140
20	2/07/96	16000	8300	-	2800	140	20	320
20	3/09/96	380	60	+	1000	5	<20	520
20	1/10/96	870	80	-	1040	96	<10	270
20	27/11/96	27000	27000	-	200	130	20	1060
20	11/02/97	150	0	-	300	0	<10	<10
24	13/05/96	65	0	-	120	0	<10	30
24	30/07/96	0	0	-	150	0	60	80
24	3/09/96	1	0	-	240	5	<10	140
24	1/10/96	21	21	-	0	0	<20	280
24	5/02/97	4	0	-	162	0	<20	60
26	26/06/96	530000	40000	-	700	29	60	180
26	6/08/96	2700	0	-	70	0	<10	80
26	9/09/96	10600	10600	-	36	1	<20	100
26	15/10/96	3300	0	-	1	0	<10	<10
26	12/02/97	73000	45000	-	0	3	<10	<10

[a] Limit of detection varies with turbidity of sample

[b] NT: not tested

Table 3. Multiple sample analysis of 10 sewage treatment plants

detectable limits were taken to be zero, while samples not tested were regarded as missing values. Simple linear correlation coefficients were calculated among assays after transformation to the logarithm_{10}(concentration + 1) scale. For the binary valued *Salmonella* assay, mean thermotolerant coliforms in samples positive for *Salmonella* were compared with negatives by t-test after transformation to logarithm_{10}(concentration + 1).

Results

Microbiological assay results for the single sample survey of 33 STPs are presented in Table 2. The results for the multiple analysis survey in which 10 of the STPs were sampled at regular intervals for 8 to 10 months are presented in Table 3. Results for ponded effluents from 3 STPs sampled once, and one STP sampled 6 times are presented in Table 4.

A high variability was seen between and within the STPs with respect to the numbers of the assayed microorganisms in the secondary-treated effluents. Ranges for all 92 samples (with geometric means in brackets) were: thermotolerant coliforms, 0 to 24,000,000 (388)/100mL; *C. perfringens*, 0 to 15,000

Plant Number & Effluent Stream	Date	Thermotolerant Coliforms /100mL	*E.coli* /100mL	*Salmonella* +/- in 100mL	*C.perfringens* /100mL	Phage /100mL	*Crypto-sporidium* /L	*Giardia* /L
26								
in	11.10.95	2120000	0	-	130	5	120	105
out	11.10.95	35000	0	-	11	0	600	850
in	26.6.96	530000	40000	-	700	29	60	180
out	26.6.96	310000	0	-	0	0	<20[a]	80
in	6.8.96	2700	0	-	70	0	<10	80
out	6.8.96	130	0	-	17	1	NT[b]	NT
in	9.9.96	10600	10600	-	36	1	<20	100
out	9.9.96	55	0	-	2	0	<20	<20[a]
in	15.10.96	3300	0	-	1	0	<10	10
out	15.10.96	9	9	-	4	239	<10	<10
in	12.2.97	73000	45000	-	0	3	<10	<10
out	12.2.97	0	0	-	0	0	NT	NT
21								
in	16.4.96	0	0	-	0	0	<10	50
out	16.4.96	87	34	-	0	0	NT	NT
17								
in	31.8.95	80000	54	+	1430	6	NT	NT
out	31.8.95	0	0	-	0	1	NT	NT
25								
in	10.10.95	1570	50	-	6000	1	110	3500
out	10.10.95	1800	1800	-	4	60	NT	NT

[a] Limit of detection varies with turbidity of sample
[b] NT: not tested

Table 4. Analysis of ponded effluents from 4 sewage treatment plants

(90)/100mL; coliphages, 0 to 6,370 (4)/100mL; *Cryptosporidium*, 0 to 600 (<10)/L and *Giardia* 0 to 4,840 (98)/L. Helminths were not detected in any samples.

The survey was not designed to quantitatively test for the effects of location of STPs, STP types, equivalent person (EP) capacities, daily flow rates or effluent disinfection with chlorine. Other than a significant (P<0.05) effect of effluent disinfection with chlorine on thermotolerant coliform counts (geometric means 130 with chlorine, 13,370 without chlorine), none of the other STP attributes appear to be important.

In the initial survey, 11 of the 33 samples contained more than 1,000 thermotolerant coliforms/100mL and would therefore have been considered unsuitable for spray irrigation of sugar cane under most guidelines. Only 4 of the 11 contained more than 1,000 *E. coli*/100mL, with the other 7 samples containing large numbers of *Klebsiella*. *Salmonella* was detected in 6 of the 33 samples, all with thermotolerant coliform counts above 1,000/100mL and 3 with *E. coli* counts above 1,000/100mL. Of the 6 STPs not using chlorine to disinfect their effluent, 5 (83%) had thermotolerant coliform counts above 1,000/100mL, compared with 6 (22%) out of 27 STPs using chlorine. *Salmonella* was isolated from 4 of the 6 non-chlorinated effluents and 2 of the 27 chlorinated effluents.

In the second, multiple sampling survey of 10 STPs, 2 were consistently over, and 2 were consistently under, 1,000 thermotolerant coliforms/100mL. The others varied widely from sampling to sampling. Counts were consistently high in the 2 non-chlorinated STPs. However 6 of the 8 STPs using chlorine also had very high thermotolerant coliform counts intermittently. A non-chlorinated STP was positive for *Salmonella* in 4 out of 5 samplings. However *Salmonella* was isolated from 4 of the 8 chlorinated STPs on one (3 STPs) or 2 (1 STP) occasions.

Mean thermotolerant coliform counts were significantly higher (P<0.05) in samples positive for *Salmonella* compared with negative samples (geometric means 76,560 and 140, respectively). Significant correlations were also found between thermotolerant coliform counts and counts for coliphage (P<0.01), *C. perfringens* (P<0.05) and *Giardia* (P<0.05), and between *C. perfringens* counts and counts for coliphage (P<0.05) and *Giardia* (P<0.01) (Table 5).

In more than half of the samples in which thermotolerant coliforms were detected (48 of 80, 1 sample not tested for *E. coli)* *E. coli* accounted for less than half of the thermotolerant coliform count. This was most often due to high numbers of *Klebsiella* spp., particularly *K. pneumoniae*. Other non-*E. coli* thermotolerant coliforms regularly contributing to the thermotolerant coliform count included *Enterobacter agglomerans*, *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia liquefaciens*.

	Thermotolerant Coliforms	*C.perfringens*	Coliphage	*Cryptosporidium*
C.perfringens	0.266* (n=91[a])			
Coliphage	0.594** (n=91)	0.268* (n=90)		
Cryptosporidium	0.122	0.118	0.124	
Giardia	0.230*	0.446**	0.144	0.298*

** Significant at the 1% probability level (P < 0.01)
* Significant at the 5% probability level (P < 0.05)
[a] n = 80 except where indicated

Table 5. Matrix of simple correlation coefficients between organism concentrations, after transformation to $\log_{10}$ (concentration + 1)

Discussion

On the basis of these results and considering current guidelines for thermotolerant coliforms in wastewater used for irrigation (Qld Dept Natural Resources, 1996), 11 (33%) of the 33 effluents in the single sample survey would have been unacceptable for spray irrigation of sugar cane. Of the 10 STPs in the multiple sample survey, 2 failed the thermotolerant coliform guidelines on all occasions they were sampled, and another 2 failed on most samplings. The variability of results between and within STPs reflects the variability in the microbial population in the incoming sewage, variation in the performance of a plant type compared with the other plant types, day-to-day variability in each STP's performance and sampling errors associated with sampling from a heterogenous product. Variability within plants is addressed in guidelines recommending "safe" levels of indicator organisms, through specification of the amount of compliance testing to be done in a given period.

The method used in this study to count thermotolerant coliforms employed a resuscitating incubation at 35°C prior to selective incubation at 44.5°C. Thus, stressed thermotolerant coliforms which would not otherwise be detected are included in the thermotolerant coliform count. The resuscitation step also favours the growth of non-*E. coli* thermotolerant coliforms and this may have contributed to the relatively low ratio of *E. coli* to non-*E. coli* seen in this study. The variable contribution of *Klebsiella* to the thermotolerant coliform count reduces its value as an indicator of enteric pathogens, particularly in the tropics where *Klebsiella* is more abundant, and for the examination of samples from holding ponds where *Klebsiella* can multiply to high concentrations (McNeill, 1985). Had the Department of Natural Resources' guidelines specified an *E. coli* count rather than a thermotolerant coliform count of $<1{,}000/100mL$, 29 of the effluents in the single sample survey would have passed for spray irrigation of sugar cane, rather than 22.

C. perfringens was assayed since it is resistant to chemical disinfection (Tyrrell et al 1995), and may be a better indicator of disinfection of viruses than is the thermotolerant coliform count. However, our results showed only weak correlation with phage concentrations. Some samples had very high counts of *C. perfringens* and the potential for build-up of spores in the soil as a result of long-term re-use of wastewater may be a cause for concern.

In the present study there was a significant correlation between *C. perfringens* counts and thermotolerant coliform counts ($r = 0.27$, $P < 0.01$). In a comparison between counts of *C. perfringens* and *E. coli* in marine water, Bonde (1975) found no distinct and constant relationship between the 2 indicators. Ferguson et al (1996) identified *C. perfringens* as the most useful indicator of faecal pollution in an urban estuary in Sydney, and found it was the only indicator significantly correlated to the presence of pathogenic *Giardia* ($r = 0.41$, $P < 0.05$). A similar correlation was obtained in the present study ($r = 0.45$, $P < 0.01$).

Giardia levels were much higher than those reported by Sykora et al (1991) for secondary-treated effluent in the USA. Three of the STPs in our multiple analysis survey were always above 150 cysts/L, and none was always below that level. While counts of *C. perfringens* were

ProMinent

significantly correlated with *Giardia* counts, this assay could not be used to predict levels of *Giardia* in individual samples. Similarly effluents with very high thermotolerant coliform counts generally had high *Giardia* counts, but one plant with a low thermotolerant coliform count had the highest recorded *Giardia* count. Hence the need for specific testing for *Giardia,* or development of a better indicator assay.

No helminths (including *Taenia* sp.) and relatively low levels of *Cryptosporidium* were detected in the survey. This is reassuring given their resistance to most disinfection processes, but probably reflects the low levels of these parasites in the population serviced by these STPs.

Coliphage counts were correlated with thermotolerant coliform counts in the present study ($r = 0.59$, $P < 0.01$). The enteric viruses were not assayed. The relevance of bacteriophages as surrogates of other viruses has been reviewed (Havelaar 1991) and they are regarded as useful indicators of the efficiency of the disinfection process in eliminating viruses. Coliphage levels in this study were generally low in chlorinated effluents, so it is likely that enterovirus numbers in these effluents were also low. Methodologies for phage assays are becoming more standardised than they have been, and there may be a case for specifying acceptable phage levels in water for agricultural reuse, using a phage with similar disinfection resistance to enteroviruses. MS2 phage appears a reasonable candidate as it has received considerable study as a possible indicator of enteroviruses, and its characteristics comparative to enteroviruses are well documented (Havelaar 1991).

This study was not able to quantitatively compare treatment types and STP sizes with respect to effluent quality, beyond the effect of chlorination on the thermotolerant coliform count and on *Salmonella* isolation rate. The apparent link between non-use of chlorine disinfection and high thermotolerant coliform counts and high *Salmonella* isolation rates is noteworthy considering the pressure to phase-out chlorination as the final disinfection process.

Data in Table 4 comparing effluent quality at the inlet and outlet of holding ponds at 4 STPs indicate a general fall in indicator organism levels after ponding. However higher levels were seen in pond outflows compared with inflows on at least one sampling, for all organisms except *Salmonella*. Multiplication of *E. coli* can be expected in nutrient rich water in tropical and sub-tropical temperatures (Hazen 1988, McNeill 1985, Rivera et al 1988) and excreta from ducks and geese have increased *E. coli* levels in wetland systems in Queensland (Greenway and Simpson 1996) so the value of assays for thermotolerant coliforms and coliphages in these ponds is limited. Also little was known of the conditions under which the holding ponds were operating, and it is possible that detention times were too short to effect consistent reductions in the indicator bacteria and protozoans.

HEALTH RISKS

This study complements two other studies on health risks from effluent reuse, which have been published by Fegan et al. (1998) and Rynne and Dart (1998).

The Fegan study reviewed much of the historic data on effluent irrigation and concluded that "the published literature provides very little epidemiological evidence that indicates a significant risk to health exists from the reuse of wastewater".

However financially realistic epidemiological studies are a relatively blunt instrument to use for health studies on reuse, especially when acceptable risk can be as low as one extra infection per 10,000 persons per year (USEPA 1989).

Rynne and Dart (1998) argued for the use of Quantitative Microbial Risk Assessment techniques, but acknowledged the difficulty of enumerating the concentration of the organisms (enteric virus or protozoan) causing infection, and the computational difficulty in doing the risk assessment on a routine basis.

As an alternative, Rynne and Dart presented Decision Trees of quantitative health risk based on the degree of effluent treatment, disinfection method, irrigation method and withholding period after irrigation.

In a companion study of disinfected effluent in south east Queensland, Rynne and Dart identified enteric virus levels in excess of 10^4 pfu/L, with the effluent often being used for golf course irrigation. The absence of frequent outbreaks of gastro intestinal illnesses by golfers suggest to us either very high levels of personal hygiene, or the considerable difficulty in associating effluent quality with health response.

Rynne, F. and Dart, P. (1998). Microbial health risks associated with effluent reuse. Research Report No. 144, Urban Water Research Association of Australia.

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Fegan, N., Gardner, T. and Blackall, P. (1998). Health Risks associated with the Reuse of Effluent for Irrigation. Queensland Department of Natural Resources, DNRQ 980130 62pp.

Conclusions

This work has provided data on the levels of indicator organisms and pathogens in effluents from Queensland STPs, and this data will be used in developing a quantitative model to assess the health risks associated with the reuse of effluent to irrigate sugar cane. Further data are required on the numbers and survival of potentially pathogenic viruses in the effluents, and on the numbers, survival and spread of viruses and other pathogens in aerosols generated by spray-irrigation.

The variability of the levels of organisms in effluents, and the lack of reliable indicators for the presence of many pathogens indicate a need for caution in managing effluent-irrigation, beyond satisfying guidelines listing acceptable levels of indicator organisms. Regular monitoring of treatment processes, and the application of a well-constructed and relevant risk assessment model are also important facets in ensuring public health risks associated with effluent reuse for irrigation are acceptably low.

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