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HYGIENIC EVALUATION OF RECLAIMED WATER USED TO IRRIGATE FOOD CROPS – A CASE STUDY

B. Sheikh*, R. C. Cooper** and K. E. Israel***

* *Parsons Engineering Science, 2101 Webster Street, Suite 700, Oakland, CA 94612, USA*
** *BioVir Laboratories, 685 Stone Road, Unit 6, Benicia, CA 94510, USA*
*** *Monterey Regional Water Pollution Control Agency, 5 Harris Court, Monterey, CA 93940, USA*

ABSTRACT

The study was designed to test the continued validity of a field pilot project (completed in 1987) that had found irrigation of food crops with tertiary-treated reclaimed municipal wastewater to be safe. It was also designed to determine whether or not pathogenic microorganisms of concern to food safety, such as *E. coli* 0157:H7, *Cyclospora*, enteric viruses and *Salmonella* were present in disinfected tertiary recycled water. Sampling of the tertiary water was conducted at intervals over a period of three months. In addition, at the same time, samples were taken from the raw incoming wastewater, from secondary effluent, and from a control source, local well water. The results from samples of recycled water are comparable to similar tests at other well-operated, tertiary recycled water treatment plants and compare well with sources of drinking water supply. Other parasites, of lesser concern to food safety than to drinking water safety, were either absent or were detected at extremely low concentrations of empty, non-viable cysts. The Tertiary Water Food Safety Study did not detect any *Salmonella*, *Cyclospora* and *E. coli* 0157:H7 in any of the samples of tertiary recycled water from the Monterey County Water Recycling Projects (MCWRP).

KEYWORDS

Wastewater reuse; water reuse; water recycling; food crops; food safety; agricultural reuse.

INTRODUCTION

Monterey County's Salinas Valley is located on the central coastal area of California, USA, between the San Francisco Bay Area (about 200 km to the North), and the Los Angeles Region (about 400 km to the South). The Monterey County Water Recycling Projects (MCWRP) are jointly sponsored by the Monterey County Water Resources Agency (MCWRA) and the Monterey Regional Water Pollution Control Agency (MRWPCA). These projects will deliver, when operating at capacity, approximately 25 million m^3 per year (20,000 acre-ft per year) of disinfected tertiary recycled water for irrigation of about 4,700 hectares (12,000 acres) of vegetables. This tertiary recycled water (supplemented with groundwater, as necessary) is made available in a pressurized distribution network with turnouts at major access points for use in the on-farm irrigation systems. Safety of the water as related to food crops has been a paramount concern of the responsible agencies, growers and land owners in the area. Thus, the partnering agencies created the Tertiary Water Food Safety Study. They had previously relied on a long-term study (completed in 1987) of the comparative safety of irrigating vegetable crops with disinfected tertiary recycled water. That project, known as the Monterey Wastewater Reclamation Study for Agriculture (MWRSA) (Sheikh *et al*., 1990), played a significant role in establishing the projects' safety and public health protection features. Based on results obtained from this five-year field pilot study, it had been concluded that disinfected tertiary treated recycled water was safe to use for irrigation of food crops.

Water use from groundwater sources in Monterey County's Salinas Valley has resulted in overdraft of coastal aquifers. A direct result of overpumping is seawater intrusion into the aquifers, which raises the salinity of water in the wells in an ever-expanding zone along the coast of Monterey Bay. Many agricultural wells have been abandoned as the seawater intrusion front moves farther east toward Salinas. The economic implications of depletion of groundwater and salinization of an expanding volume of the aquifers for the important agricultural sector in Monterey County are ominous. A local solution to the problem is highly desirable in order to avoid loss of control over the management of the Basin groundwater. Therefore, visionary leaders and planners have looked toward other sources of water to relieve reliance on this diminishing groundwater resource. One such alternative resource is tertiary recycled water from the Regional Treatment Plant in Marina, operated by the Monterey Regional Water Pollution Control Agency (MRWPCA). Twenty years of planning, pilot studies, negotiations and lessons learned from many other California water reuse practices have led to the construction of a tertiary treatment plant and distribution system for delivery of tertiary recycled water to prime farmlands in the vicinity of Castroville. The treatment process conforms to California Title 22 requirements, which include coagulation, flocculation, filtration, and disinfection. The system was formally inaugurated on October 24, 1997. The main objective of the Tertiary Water Food Safety Study was to determine by sampling and analysis, if pathogenic organisms are present in the disinfected tertiary recycled water produced at the Regional Treatment Plant. A secondary objective was to assess, to the extent practicable, the ability of the treatment processes to remove pathogens that might be present in raw influent wastewater.

MATERIALS AND METHODS

The study was designed on the basis of sampling and analysis for pathogens of concern, and indicators of potential presence of pathogens originating in human or animal waste. Water samples were obtained from the following sources: (1) wastewater treatment plant influent, or raw sewage, (2) secondary effluent, (3) tertiary recycled water effluent and (4) a control water source, obtained from a well water storage tank. Laboratory personnel of MRWPCA obtained the samples in accordance with detailed procedures and training provided on-site by the principals of BioVir Laboratories. These procedures are documented in various sections of Standard Methods (APHA/AWWA/WEF, 1992) specifically cited under Methods of Analysis, below. MRWPCA personnel packaged, labeled, chilled, and shipped the samples to BioVir in Benicia, California, using the services of a designated overnight delivery company. BioVir performed analyses in accordance with the most appropriate methods applicable for each parameter, as described further, below.

Constituents and parameters analyzed

The Tertiary Water Food Safety Study was aimed toward the acquisition of information needed to respond to the questions raised regarding survivability of certain parasites and other pathogens. Collected samples were analyzed for *Salmonella, E. coli* 0157:H7, *Cyclospora Cryptosporidium*, *Giardia*, and *Legionella*. The significance of these pathogens to food safety is indirect. These are water-borne pathogens that can produce gastrointestinal diseases in the population when present in drinking water in concentrations exceeding infective doses, which can vary for different individuals. In addition to total coliform, a required monitoring analyte for compliance with the Department of Health Services Title 22 regulations, fecal coliform was also monitored during the course of this study. Samples were collected at the rate of about once per week.

Methods of analysis

Standard Methods of Analysis (APHA/AWWA/WEF, 1992) were utilized where available and applicable for specific analyses. In cases of analytes for which no standardized methods were available in the literature, the most commonly accepted procedures and methods used in the profession were selected and utilized.

RESULTS

Pathogens

Summaries of the results of assays for pathogenic agents and indicators on samples obtained from raw wastewater, undisinfected secondary effluent, disinfected tertiary recycled water, and a control (well water) are presented in Tables 1 to 4. Fecal coliform is reported because of its significance with regard to indication of potential contamination with animal and/or human waste.

Table 1. Microbial quality of raw wastewater (influent to the plant)

Sample Date	*E. Coli* 0157:H7 (CFU/ 100 mL)	*Legionella* (CFU/ mL)	*Salmonella* (CFU / 100 Ml)	*Giardia* *(No./L)*	*Cryptosporidium* *(No./L)*	*Cyclospora* *(/L)*	*Fecal Coliform* *(MPN/100 mL)*
10/29/97	N. D.*	N. D.	—**	2,000	200	N. D.	7,000,000
11/12- 11/13/97	N. D.	N. D.	N. D.	22,400	143	N. D.	17,000,000
11/17 -11/18/97	N. D.	N. D.	—	6,000	N. D.	N. D.	13,000,000
11/24/97	N. D.	N. D.	2.2	5,218	18	N. D.	30,000,000
12/2 -12/3/97	N. D.	N. D.	16	8,750	N. D.	N. D.	13,000,000
12/8 -12/9/97	N. D.	N. D.	9.2	11,000	N. D.	N. D.	11,000,000
12/15 -12/16/97	N. D.	N. D.	N. D.	17,500	160	330	17,000,000
Average* **	**N. D.**	**N. D.**	**5**	**10,400**	**74**	**47**	**15,000,000**
Range	—	—	**N. D. – 16**	**2,000 – 22,400**	**N. D. - 200**	**N. D. - 330**	**7,000,000 – 30,000,000**

* N. D. means "none detected" in the collected sample.

** — means "not performed".

*** In computing numerical averages, N. D. was set equal to zero and > was set equal to the number.

Table 2. Microbial quality of secondary effluent

Sample Date	*E. Coli* 0157:H7 (CFU/ 100 mL)	*Legionella* (CFU/ mL)	*Salmonella* (CFU / 100mL)	*Giardia* *(No./L)*	*Cryptosporidium* *(No./L)*	*Cyclospora* *(No./L)*	*Fecal Coliform* *(MPN/100 mL)*
10/29 -10/30/97	N. D.	N. D.	—	0.4	N. D.	N. D.	500,000
11/12- 11/13/97	N. D.	N. D.	—	9.3	0.18	N. D.	—
11/17 -11/18/97	N. D.	N. D.	—	0.4	N. D.	N. D.	230,000
11/24/97	N. D.	N. D.	2.2	8.5	1.8	N. D.	500,000
12/2 -12/3/97	N. D.	N. D.	2.2	5.51	0.45	N. D.	800,000
12/8 -12/9/97	N. D.	N. D.	9.2	12.2	0.10	N. D.	500,000
12/15 -12/16/97	N. D.	N. D.	2.2	6.16	0.14	N. D.	230,000
Average*	**N. D.**	**N. D.**	**4.0**	**6.1**	**0.38**	**N. D.**	**596,000**
Range	—	—	**2.2-9.2**	**0.4-12.2**	**N. D. – 1.8**	—	**230,000 – 800,000**

Table 3. Microbial, chemical quality of disinfected tertiary recycled water

Sample Date	*E. Coli* 0157:H7 (CFU/ 100 mL)	*Legion-ella* (CFU/ mL)	*Salmon-ella* (CFU/ 100mL)	*Giardia (No./L)*	*Cryptosporidium (No./L)*	*Cyclos-pora (No./L)*	*Fecal Coliform (MPN/ 100 mL)*	*Turbi-dity (NTU)**	*Chlorine Residual (mg/L)**
10/29-10/30/97	N. D.	N. D.	—	—	N. D.	—	N. D.	1.9	14
11/12-11/13/97	—	N. D.	—	—	—	—	N. D.	1.7	6.2
11/17-11/18/97	N. D.	N. D.	—	N. D.	N. D.	N. D.	N. D.	2.7	—
11/24/97	N. D.	N. D.	N. D.	0.03	N. D.	N. D.	N. D.	1.2	—
12/2-12/3/97	N. D.	N. D.	—	0.08	N. D.	N. D.	N. D.	2.3	14
12/8-12/9/97	N. D.	N. D.	N. D.	0.09	N. D.	N. D.	N. D.	1.6	12
12/15-12/16/97	N. D.	N. D.	N. D.	0.05	N. D.	N. D.	N. D.	1.5	14
Average*	**N. D.**	**N. D.**	**N. D.**	**0.06**	**N. D.**	**N. D.**	**N. D.**	**1.8**	**12**
Range	—	—	—	**N. D. – 0.09**	—	—	—	**1.2 – 2.7**	**6.2 – 14**

* Grab samples

Table 4. Microbial quality of well water (control)

Sample Date	*E. Coli* 0157:H7 (CFU/ 100 mL)	*Legionella* (CFU/ mL)	*Salmonella* (CFU/ 100mL)	*Giardia (No./L)*	*Cryptosporidium (No./L)*	*Cyclospora (No./L)*
10/29 –10/30/97	N. D.	N. D.	—	N. D.	N. D.	N. D.
11/12– 11/13/97	—	—	—	—	—	—
11/17 -11/18/97	—	—	—	N. D.	N. D.	—
11/24/97	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
12/2 –12/3/97	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
12/8 –12/9/97	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
12/15 –12/16/97	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Average	**N. D.**	**N. D.**	**N. D.**	**N. D.**	**N. D.**	**N. D.**

Removal capabilities of the tertiary process

Based on data presented in Tables 1 to 3, it is evident that *Giardia* spp. is reduced by five to six logs (100,000 to a million-fold) from influent to the plant to the finished disinfected tertiary recycled water. Because the other pathogenic agents arrive at the treatment plant at much lower concentrations, and because of their absence in the final effluent, it is not as readily possible to calculate a corresponding removal capability for them. However, based on the calculated rates of removal for *Giardia* spp., it may be possible to extrapolate similarly high removal rates for the other organisms as well. *Giardia* spp. are common in raw sources of water and in wastewaters. The *Giardia* spp. found remaining in the tertiary recycled water are empty cysts, hence they are non-viable. A discussion of the significance of non-viable cysts found in the tertiary recycled water is presented in the following paragraphs.

Viability of organisms

The analysis for *Giardia* spp. and *Cryptosporidium* spp. consists of classification of the potential viability of those organisms observed. Four criteria are evaluated when conducting this analysis: the FITC stain, and three classes of organism internal structure. The FITC stain is antibody-based and interacts specifically with *Giardia* spp. and/or *Cryptosporidium spp.* The objects would be brightly glowing candied-apple green. Next, a different type of lighting (differential contrast) is used to reveal the internal structure of a green glowing object. There are two techniques of generating this type of differential lighting. One is called Hoffman Modulation, the other Differential Interference Contrast (DIC). BioVir Laboratories employed both techniques. There are three classes of organisms based upon the status of the internal structure. The first is the absence of any internal structure and the object can be classified as "empty". It is generally believed that an empty (oo)cyst is a non-viable shell of the target organism. The next class is assigned to those organisms with no organized internal structure and these are referred to as "amorphous". Again, these may be considered non-viable. The last classification is based upon the presence of obvious internal structure, and can further be described by the degree of internal organization. Conservatively, this latter type of organism may be considered viable. *Giardia* spp. and *Cryptosporidium* spp. of all types were observed in the raw influent and to a lesser extent in the secondary effluent. In all of the tertiary effluent samples, 100 percent of the *Giardia* spp. observed were empty, non-viable cysts. It should be noted that the antibody reagent used for the detection of *Giardia* spp. does react with non-human infective forms as well and many of the *Giardia* spp. observed may have come from non-human sources.

Table 5. Occurrence and concentration of *Cryptosporidium* in different water sources

Water Type Location	Percent Positive	Average Concentration (No./ 100L)	Range	No of Samples	Notes
Recycled Water					
St. Petersburg, FL	17	0.75	N. D.-5.35	12	
MCWRP, CA*	*0*	*N. D.*	—	*6*	*See Table 3*
Groundwater					
Springs	—	4	—	7	
Well Waters	5.5	0.3	N. D.-4	12	
Well Waters	17	41	—	74	
*Monterey Well**	*0*	*N. D.*	—	*8*	*See Table 4*
High Quality Surface Waters					
Source Water	87	270	N. D.-48,400	151	66 plants in 14 states, Canada
Source Water	52	240	N. D.-6,510	262	from 72 water plants, '91-'93
NW USA	36	1.6	N. D.-5.4	52	
NYC Watersheds					
Catskill	46	1.4	N. D.-17.3		
Delaware	37	0.8	N. D.-15		
Malcolm Br'k	52	1.0	N. D.-43.4		
NYC Source	2	—	N. D.-1.38	203	
W USA Rivers	83	2	N. D.-13	6	Protected watersheds
W USA Rivers	—	8	—	3	Protected watersheds
Pristine Rivers	32	29	N. D.-24,000	59	
Pristine Lakes	53	9.3	N. D.-307	34	
Tampa Canal, FL	43	3.1	N. D.-11	7	
Drinking Water					
Filtered Water	27	1.5	N. D.-48	151	66 plants in 14 states, Canada
Treated Water	13	3.3	N. D.-57	262	from 72 water plants, '91-'93
Treated Water	17	0.1	—	36	
Filtered Water	20	0.1	—	10	Western USA
Non-Filtered	50	0.6	—	4	Western USA

Source: Data were assembled from 13 different sources, mostly by Dr. David York, Director, Florida Department of Environmental Protection, in Proceedings, Water Reuse '98, AWWA/WEF Joint Sponsored Conference, February 1-4, 1998, Orlando, Florida (with adaptation). The Florida DEP is the regulatory agency in charge of enforcing regulations on safe use of all recycled water in that state.

* Data from the Monterey County Water Recycling Projects (MCWRP) were added to the York compilation for comparison.

Table 6. Occurrence and concentration of *Giardia* in different water sources

Water Type Location	Percent Positive	Average Concentration (No./ 100L)	Range	No of Samples	Notes
Recycled Water					
St. Petersburg, FL	25	0.49	N. D.-3.3	12	
MCWRP, CA*	*80***	*6*	*N. D.-9*	*5*	*See Table 3(Note different units)*
Groundwater					
Springs	0	<0.25	—	7	
Wells	0	<0.25	—	12	
Wells	9.5	16	—	74	
*Monterey Well**	*0*	*N. D.*	—	8	*See Table 4*
High Quality Surface Waters					
Source Water	81.2	277	N. D.-	151	66 plants in 14 states + Canada
Source Water	45	200	6,600	262	from 72 water plants, '91-'93
NYC Watersheds			N. D.-		
Catskill	36	1.2	4,380		
Delaware	29	0.7			
Malcolm Br'k	46	1.3	N. D.-9.3		
NYC Source	3.0	—	N. D.-8.2	203	
Portland, OR	19	—	N. D.-23.4	Several	Protected reservoir
W USA Rivers	17	0.6	N. D.-1.38	6	
W USA Rivers	—	0.9	0.34-2.77	3	
Pristine Rivers	6.8	0.35	—	59	
Pristine Lakes	12	0.5	—	34	
3 Seattle Rivers	42	6.3	—	222	Pristine river systems in WA
Tampa Canal, FL	14	0.42	N. D.-12		
			N. D.-7		
			N. D.-520		
Drinking Water					
Filtered Water	17.1	4.45	N. D.-64	151	66 plants in 14 states + Canada
Treated Water	4.6	2.6	N. D.-9	262	from 72 water plants, '91-'93
Treated Water	0	<0.25	—	36	

Source: Data were assembled from 13 different sources, mostly by Dr. David York, Director, Florida Department of Environmental Protection, in Proceedings, Water Reuse '98, AWWA/WEF Joint Sponsored Conference, February 1-4, 1998, Orlando, Florida (with adaptation).

* Data from the Monterey County Water Recycling Projects (MCWRP), were added to the York compilation for comparison.

** All positives were non-viable: they were either empty cycts or contained no organized internal structure (amorphous).

DISCUSSION

The results presented above can best be viewed in the context of water quality data for a variety of sources commonly used for intimate human contact. Because of the recent concerns about *E. coli* 0157:H7, *Cyclospora*, *Legionella*, and other pathogens in the food supply, there is not an extensive body of information for comparison. York (1998) reported a recent compilation of the occurrence of two key protozoa in several water sources in the United States and Canada. The results are adapted and reproduced in Tables 5 and 6, including a summary of the corresponding results from this study.

Similar comparative data for *Giardia* spp. are shown in Table 6.

CONCLUSIONS

Absence of viable microorganisms of concern for food safety in disinfected tertiary recycled water was demonstrated in this study. This finding corroborates and strengthens the results of the five-year field pilot study near Castroville, California USA, completed in 1987, which concluded that the tertiary water was safe for irrigation of raw-eaten food crops. The significance of the current quest for absence of pathogens in irrigation water is to assure a safety level for tertiary recycled water that is beyond question. Because the cell walls of plant roots and leaves effectively filter the irrigation water, microorganisms cannot pass through and into the edible tissues of the crops, unless the cell walls are intentionally injured. There is no significant data in the literature indicating that microorganisms, including animal viruses, can be translocated into plant tissues. A classic work (Murphy and Syverton, 1958) concluded that plant contamination via this route was unlikely and not a major public health concern. No recent work on this question has been reported. Drying and solar radiation further prevent any organisms remaining in irrigation water from continuing to be viable on plant surfaces after harvest. These mechanisms normally provide a high level of natural protection against contamination of food crops from any pathogens that might be present in the various sources of irrigation water.

Comparison of results obtained from the MRWPCA disinfected tertiary recycled water with those obtained from raw and treated drinking water sources, both for *Cryprosporidium* spp. and for *Giardia* spp. provide an additional indication of safety of the tertiary recycled water. Occurrence and concentration of cysts of these protozoa in tertiary recycled water is comparable with or lower than in the other waters, some of which are sources of drinking water supply for communities in the United States and Canada. Extension of this comparison to most irrigation water supplies would give tertiary recycled water an even safer ranking.

To summarize, the tertiary Water Food Safety Study did not detect any *Salmonella, Cyclospora, E. coli* 0157:H7, *Cryptosporidium*, or viable *Giardia*. This study augments the Monterey Wastewater Reclamation Study for Agriculture completed in 1987, which detected no natural, in situ virus and demonstrated over five-log removal of viruses. Together, these studies assure the safety of tertiary recycled water for irrigation of food crops that are consumed without cooking.

REFERENCES

APHA/AWWA/WEF (1992). *Standard Methods*, 18th ed., APHA/AWWA/WEF Various Sections.

Murphy, W. H. and Syverton, J. T. (1958). Absorption and translocation of mammalian viruses by plants. *Virology* **6**, 623-636.

Sheikh, B., Cort, R., Kirkpatrick, W., Jaques, R. and Asano, T. (1990). Monterey wastewater reclamation study for agriculture. *Research Journal, Water Pollution Control Federation*, **26**(3), 216-226, Alexandria, Virginia, May/June.

York, D. W. (1998). Protozoan pathogens: a comparison of reclaimed water and other irrigation waters. In: *Conference Proceedings of Water Reuse '98*, Joint AWWA-WEF sponsored conference in Orlando, Florida, February 1-4.