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Optimization of Secondary Wastewater Reuse to Minimize Environmental Risks

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Optimization of Secondary Wastewater Reuse to Minimize Environmental Risks

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Both participating research groups mainly based the cooperation on comparing and using similar laboratory analysis methods and conducting complementary field experiments.

Abstract

The main purpose of the research was to examine approaches and to evaluate methods for minimizing the risks during applying treated domestic wastewater for agricultural irrigation. This general purpose consisted of examining under field conditions the possibilities when implementing different application technologies for minimizing health and environmental risks. It was assumed that Subsurface Drip Irrigation (SDI) will provide adequate conditions for safe effluent reuse. Controlled field experiments were conducted in commercial fields to evaluate the alternatives. Main efforts were conducted in Israel in the grape vineyard in Arad heights, in the field crops in Kibbutz Chafets Chaim and in Arizona in fields adjacent to the University campus.

The complementary part was to examine the behavior of the various pathogens in the effluent-soil-plant system. The analysis is based on controlled experiments, primarily in greenhouse along with field experiments. Molecular biology methods were used to identify the behavior of the pathogens in the components of the system.

The project included as well examining the effluent quality in various sites, primarily those in which treated wastewater is reused for agricultural irrigation. The monitoring included conventional parameters however, also parasites such as *Giardia* and *Cryptosporidium*.

The results obtained indicate the prominent advantages of using Subsurface Drip Irrigation (SDI) method for minimizing health and environmental risks during application of secondary effluent.

A theoretical model for assessing the risks while applying treated wastewater was completed as well. The management model shows the risks during various scenarios of wastewater quality, application technology and related human exposure.

Achievements

The research results confirmed many of the hypothesized assumptions and can lead to better reuse of treated wastewater for agricultural and other purposes;

1) Effluent can be used safely and with minimal risks under Subsurface Drip Irrigation (SDI), assuming the field is adequately irrigated, namely the soil surface remains dry.

2) Better yields can be obtained under SDI as compared with conventional onsurface drip irrigation methods.

3) Under SDI most of the pathogens remain close to subsurface effluent point source, not reaching the soil surface.

4) Since the soil surface remains dry, minimal use of pesticides and herbicides is required thus adding to improved environmental control.

5) The technology of SDI has to be adapted according to the field crop: shallow roots' plants are more sensitive to the location depth of the emitters.

6) Low quality treated wastewater might have adverse effects on the quality of the agricultural products. That refers for example to the wine quality when the ammonia content in the effluent is relatively high. In general, a vineyard orchard requires between 90 to 120 Kg nitrogen per hectare per season and annual water amount is up to 4,000 m³/ha. It means that the ammonia content in the applied effluent roughly should not exceed 25 mg/l.

7) Definitely no virus penetration through the root system into the plants takes place during effluent application. Most of the pathogen contaminations of the plants and fruits is probably external or take place during damage to any part of the plants.

8) The main parasites (*Giardia* and *Cryptosporidium*) which are currently in the focus of research where detected in raw sewage, secondary effluent, effluent withdrawn from storage reservoirs, and the soils irrigated with the treated wastewater. Additional work is required to learn the linkage between the wastewater treatment technology, effluent quality, the effect of storage and survival in the soils irrigated with effluent.

9) Improved laboratory methods have to be developed and adapted for analyzing the parasites survival in the soils irrigated with wastewaters.

10) The management model developed shows the significance and inter-relationships among the various parameters evolved during wastewater reuse. These include the community exposure level and immunity, wastewater quality, application technology and timing between last irrigation and harvesting. Indirectly, it includes also the plants characteristics and is related products consumption habits and processing.

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CHAPTER I

Wastewater Reuse and Risk Assessment

Public awareness of environmental concerns coupled with increasing legislation is creating ever-widening circles of potential movement delineating towards better control of the environmental. This movement is supported strongly from the other wing by intensified processes of conventional water shortage and continuous deterioration in water quality.

Consequently intense efforts are in progress for identifying and putting into use additional sources (i.e., unconventional or marginal waters) such as deep groundwater, treated wastewater, brackish water. Run-off water varies in time, amounts and location and therefore is considered as a non-stable source. Saline water is obtained in the form of drainage water from agricultural fields or can be found in deep fossil aquifers. The use of saline water is associated with limited agricultural yields, however, with improved products quality (Pasternak and DeMalach, 1987).

The concept of treated wastewater reuse is by no means a new approach. The Greek people used sewage for irrigation and same practice has been experienced in China for many centuries. Currently the number of countries that experience an imbalance water demand is increasing due to population dynamics, conventional water quality deterioration, industrial growth, increasing demand along with standard of life elevation and the climate change global phenomena.

Recycling of inferior waters can be viewed in various directions. It includes the use of rain-water collected from roofs and flood plains, recycling of gray wastewater in industry and domestic quarters, irrigation with treated domestic wastewater, aquifers and lakes water recharge. Additional routes of reuse are probably under investigation.

Domestic treated wastewater is a relatively stable water source and can be utilized for agriculture, industry, recreation, gardening, industrial plant cooling, and recharge of groundwater (Cromer *et al.*, 1984; Oron *et al.*, 1986; Burau *et al.*, 1987; Asano and Milles, 1990). Practically, it is associated with implementation of advanced irrigation technologies and water harvesting, for reducing demands and satisfying water requirements. Furthermore, drought events, that often occur in the dry regions, amplify water management problems and require long-term measures to reduce the water systems vulnerability, and short-term measures to mitigate drought impacts. Water resources management in arid and semi-arid region is a complex, multifaceted task due to the many hydrological, environmental, economic, social and managerial factors that need to be integrated. The holistic approach is appropriate in order to provide all users of different sectors sufficient supplies of adequate water quality while ensuring the necessary level of preventing pollution and environmental control.

Major efforts are in regions where rainfall is scant or sporadic and the output of existing water sources is insufficient to meet growing demands. This water scarcity in arid and semi-arid regions has enhanced the search for additional sources currently not exploited intensely as well with improved technologies of renovation.

The use of treated wastewater for agricultural irrigation is attractive due to a series of invaluable consequences (Gamble, 1986; Chang *et al.*, 1990): (a) water shortage problems can be resolved; (b) large amounts can be disposed during the entire year with or without storage (under distinct circumstances the storage can be considered as an extra treatment phase) with minimal environmental risks (Shelef, 1991; Oron *et al.*, 1992; Juanico and Shelef, 1994); (c) there are economic benefits due to the nutrients content that increase the fertilizing properties of the effluent (Oron *et al.*, 1986; Nielsen *et al.*, 1989). Associated is the potential of utilizing the sludge, primarily for agricultural fertilization, as an alternative disposal solution.

Disposal of wastewater and sludge for agricultural utilization diminishes potential point pollution and exemplifies the constructive approach of controlling the risk of environmental pollution. Combining effluent and sludge disposal and reuse for agricultural purposes is flexible and can be adjusted according to local conditions (Sarıkaya and Eroglu, 1993). Despite the worldwide practice of effluent reuse, mainly for agricultural irrigation, there are still several conflicting opinions regarding possible adverse environmental and health impacts (Smith, 1982; Nellor *et al.*, 1985; Ward *et al.*, 1989; Rose and Gerba, 1991). These dichotomous views and related considerations stem frequently from the wastewater treatment level and quality control feasibilities, background and orientation of the decision making level regarding effluent reuse usually takes global aspects of the integrative problem into account only to a very limited extent.

The purpose of current work was to verify the hypothesis that treated domestic wastewater can be reused safely for crop irrigation under Subsurface Drip Irrigation (SDI) systems. Under SDI the soil surface is kept dry and contact with pathogens is minimized. The dryness of the upper soil layers, including the soil-surface has several agronomic, agricultural, economic, health and environmental advantages.

Extended efforts have been conducted last years with the support of BARD Fund to search for improved methods to reuse domestic wastewater for agricultural irrigation. The improved used can be viewed in two major complimentary routes: (i): high agricultural productivity, which is expressed in maximal yields (both in quantity and quality) expressed in highest return per unit area (field) and per unit amount of applied effluent and other production factors such as fertilizers and manpower, and; (ii) using the treated wastewater with minimal health and environmental risks.

The research was conducted in several complimentary routes. These include field studies in commercial fields, laboratory analysis of collected wastewater, soil and plant material, controlled experiments in green houses under very strict conditions and management modeling referring to the risks associated with effluent reuse.

The results indicate that treated wastewater can be reused safely under SDI. This was proved in the field experiments and the management analysis. Sometimes the main draw-back is with the product quality which might be due to extended amounts of nutrients (surplus of ammonia might deteriorate the quality of the wine obtained from a vineyard irrigated with effluent. In controlled experiments it was as well found that viruses cannot penetrate into the plants via the root system, hence applying effluent via is safe in all sense.

Various environmental parameters were used to confirm the hypothesis. Besides the conventional parameters such as chemical and biological constituents, several pathogens were detected. These include fecal coliforms, viruses, various phages and protozoa (*Cryptosporidium* and *Giardia*).

A management model for the assessment of risk of infection associated with wastewater irrigation of edible plants was developed. The model takes into account several criteria: (i) the quality of the applied wastewater, (ii) the irrigation method, (iii) the time between irrigation, harvest, and subsequent consumption, and (iv) consumers' behavior.

Risk assessment is a useful tool to upgrade reclamation schemes to acceptable health safety standards. An increase of the elapsed time between irrigation and consumption is the most effective operational change (one order of magnitude per five extra days). Other operational improvements are increasing the virus removal efficiency of the wastewater treatment (one order of magnitude risk reduction for each extra log of removal), and a switch from spray to DI and primarily to SDI (two to three orders of magnitude risk reduction).

The annual risks of infection shows large deviations. These broad ranges are probably due to a mix of uncertainty and variability of data. Extra data are required to increase the quality of the input parameters to decrease uncertainty. The distribution of the output parameters then represents the diversity of the populations.

There is currently a trend towards downgrading the risks associated with highly treated wastewater for irrigation of crops, particularly those to be consumed raw. The results of the model print-out that even with $\log_{10}$ reductions, if the irrigation method is such that leads to high level of contamination, without adequate lag times between watering and harvesting, then the risks are apparent. Implementing a lag time between effluent application and harvesting is an additional barrier, confirming risks reduction and should be recommended as a mandatory assessment for all food crops by state

federal agencies, thus restricting spray irrigation of reclaimed wastewater and certain crops. This approach certainly could be used by codex when examining the risks associated with imported agricultural products that are likely to be irrigated with low quality treated wastewater.

Future related research associated with pathogen risk assessment should include the followings:

- * Defining the acceptable risk levels.
- * Defining dose-response models considering the influence of local circumstances such as immunity on the human response to pathogens.
- * Obtaining additional information regarding occurrence and prevalence of pathogens in wastewater, and their removal in wastewater treatment.
- * Obtaining additional information regarding the paths of pathogens into and onto the crop through spray irrigation, on surface drip irrigation, and subsurface drip irrigation.

CHAPTER II

Field Studies in Israel

General

Field experiments are in progress in Israel in several commercial fields in order to examine the effects of treated wastewater reuse on plants, soil and environment. The experiments are conducted in the commercial Fields of Arad Height (FAH) project, in the vicinity of the city of Arad (Israel). Vine grapes (Sauvignon; Cabernet; Marlot) and almond trees are the main orchards species. In the second experimental site in Kibbutz Chafets-Chaim (KCHM)) fields crops such as corn, cotton, cabbage and red-paprika are irrigated with secondary treated domestic wastewater obtained from several adjacent communities and stored in the reservoir of KCHM.

Figure 1. Environmental characteristics in Arad Heights site, 1998

Additional work is carried out in other sites in order to examine the long range time effects on changes in the soil, and possibly on the fertility of the lands subject to

effluent application. That work is mainly associated with monitoring of commercial agricultural fields located in different regions in Israel.

Figure 2. Environmental characteristics in Arad Heights site, 1999

The Arad Heights agricultural fields encompass a total acreage of about 450 ha. The cultivated fields are located, around 525 m above sea level. Mean annual precipitation during the rainy season is around 150 mm. Mean maximal daily temperatures during the warm summer months rise to approximately 29°C. Mean minimal daily temperature during winter decrease to 6°C. The relative humidity is relatively low during the summer season, and is in the range of 15 % to 30 %. Maximal solar radiation is in the range of 1,000 Watt/m² to 1,100 Watt/m².

Figure 3. Water and effluent application in Arad vineyard orchard, 1998

The experimental layout in Arad Heights

Experimental plots were designated in the Marlot vineyard grapes orchard section. The grapes were planted on August 1991 at row spacing of 3.0 meters and inter-row plant spacing of 1.5 meters. Until 1994 the orchard was irrigated with tap water applied via an onsurface drip system. Each grape row was served by one drip lateral. Emitters with a flow rate of 3.5 l/hr were installed apart 0.75 meters on the laterals. The vineyard orchard is irrigated once a week. The amount of water applied is based on class "A" pan evaporation (Figures 1, 2 and 3). The pan coefficient for the grapes

varies according to physiological plants development and is between 0.3 and 0.5. Excess water application might have adverse effects on the wine quality. Total recommended annual amount of effluent and water to be applied is usually in the range of 400 mm to 450 mm. Water and effluent application for 1998 is described in Figure 3.

Figure 4. Schematic layout of the experimental layout of effluent irrigation of vineyard with emitters of 2.3 l/hr, 0.5 m apart on the lateral

Four treatment are examined in the experiment in the Marlot vineyard-grape section (Figure 4):

- 1) Onsurface Drip Irrigation with Tap-water (DIT).
- 2) Subsurface Drip Irrigation with Tap-water (SDIT).
- 3) Onsurface Drip Irrigation with Effluent (DIE).
- 4) Subsurface Drip Irrigation with Effluent (SDIE).

Water and effluent supply in Arad Heights fields

Water for irrigation is supplied from two sources: (i) high quality water is obtained from local wells, located around 30 km west of the fields; and, (ii) treated domestic wastewater from the adjacent City of Arad, which will be the main source in the near future. Currently the wastewater is treated in a series of stabilization ponds to a secondary level (Tables 1 and 2). During 1997 a new sewage treatment facility and four reservoirs will be completed, thus improving the effluent quality for agricultural reuse. Standard methods were commonly used (APHA, 1995).

Table 1. Quality of the treated wastewater applied for irrigation in Arad vineyard orchard, 1998

Parameter mg/L	Date								
	23 March*	5 May	21 May	28 May	25 June	7 July	8 July	16 July**	17 August
pH (-)	8.05	8.17	8.3	8.21	8.46	8.75	8.6	8.39	8.39
EC, dS/m	1.62	1.65	1.65	1.6	1.7	1.65	1.7	1.77	1.72
TSS	29	8	40	100	38	40	45	51	25
Cl	220	240	283	322	-	-	-	-	-
SO ₄	13.9	32	24	28.2	-	-	-	-	-
Br	2.85	-	3	3.8	-	-	-	-	-
HCO ₃	488	506	549	457	610	520	488	470	408
Na	172	210	226	236	188	200	194	220	210
K	32	28	37.5	44	32.5	39	38	38	35
Ca	48	61	62	62	36.2	43.4	43.6	38.5	39.7
Mg	27.5	25	29.5	29	30.5	31	31	32	30
Alkalinity	400	415	450	375	500	400	370	385	335
N-NH ₄	30.1	23.1	26.3	22.5	30.3	30.3	30.6	18.5	5.04
PO ₄	25.1	16.2	23.9	11.8	78	76	74	52.9	32.5
BOD total	11	15	-	17	11	8	7	19	9.6
BOD filtered	6	12	-	3	4.5	2	0.5	9.6	4.5
COD total	160	120	230	240	300	440	440	160	100
COD filtered	120	50	130	140	160	210	220	130	60
SAR (-)	6.93	8.09	8.37	8.78	7.88	8.01	7.77	8.97	8.66
PAR (-)	0.54	0.45	0.58	0.68	0.56	0.65	0.63	0.64	0.60

*- after stabilization pond; **- before filter (after reservoir); - Not monitored

Effluent application

According to the experimental set-up, one drip lateral is serving every vine grape row. The emitters with a flow rate of 2.3 l/hr are 0.5 meters apart on the laterals. Each of the four treatments is conducted under six replications. Every replication consists of three grapes rows, of which only the central one is monitored. Each plot consists of

approximately 26 plants namely, is around 43 meters long. This arrangement allows to have boundary plants and rows, usually required to off-set any marginal effects.

Table 2. The Quality of the treated wastewater applied for irrigation in Arad vineyard orchard, 1999

Parameter mg/L	Date			
	16 June*	8 September	23 October	23 November**
pH (-)	8.21	8.7	8.35	8.83
EC, dS/m	1.74	1.9	1.86	1.8
TSS	47	30	31	92
Cl	386	465	308	317
SO ₄	***	7.9	-	41.4
Br	-	-	-	2.3
HCO ₃	427	--	433	485
N	300	-	258	232
K	39	-	52	19.6
Ca	52.5	-	60.6	59.1
Mg	37	-	37	-29.5
Alkalinity	350	-	-	-
N NH ₄	24.1	2.95	13.1	33.97
PO ₄	10.2	67.5?	75.3?	15.1
BOD total	18		8.1	16.5
BOD filtered	15		6.6	10
COD total	200	150	50	120
COD filtered	120	120	10	70
SAR (-)	8.53	-	7.09	6.78
PAR (-)	0.84	-	1.08	0.43

*- After the stabilization pond; **- Before the filter (after reservoir); *** - Not monitored

Chafets-Chaim Experiments

The Experimental Site

Field experiments are in progress in the commercial fields of Kibbutz (Combinative farm) Chafets-Chaim, Israel. Mean annual precipitation is up to 600 mm from October until March of next year. Mean minimal ambient temperature is around 8°C during January and around 17°C during August. Mean maximal temperature reaches 20°C during January and 31°C during August. Maximal class "A" evaporation is around 8 mm/day. The soil in the experimental site consists of about 36 % clay, 17 % silt and 47 % sand.

The Crop

Cultivation in the commercial fields is based on conventional crop rotation which includes cotton, corn, wheat and various vegetables. Cabbage (*Fictor sp*) was planted on the first of May, 1998 at a load of 15,000 plants per hectare. The planting was under wet soil conditions in a field which wheat was previously cultivated. The planting was at 0.5 m spacing, three rows per one bed, 1.92 m wide. The cabbage was harvested towards the beginning of September.

First experiment with vegetables was conducted during summer 1999. Paprika was cultivated under subsurface drip irrigation and effluent application. A control system with conventional onsurface drip system was installed in the field. The harvested paprika is subject to curing stages prior to marketing, thus minimizing health risks.

Figure 5. Class "A" evaporation and effluent application for cabbage irrigation in Chafets-Chaim 1998

Irrigation and Effluent Quality

The field was irrigated twice for germination by sprinkling, applying approximately 50 mm. Total amount applied by drip irrigation {both SDI and conventional onsurface Drip Irrigation (DI) was around 550 mm}. The effluent applied were obtained from the adjacent effluent reservoir (Table 3). The nutrient content in the applied effluent allowed to avoid artificial fertilization.

Table 3. Characteristics of the effluent applied for the cabbage irrigation, Chafets-Chaim, 1998 ("raw" - refers to effluent entering into the reservoir)

Parameter	5 May		3 June		11 June		16 July		23		August		31 August	
mg/L	raw	applied effluent	raw	applied effluent	raw	applied effluent	raw	applied effluent	raw	applied effluent	raw	applied effluent	raw	applied effluent
pH (-)	7.99	8.28	7.77	7.79	7.62	7.59	8.18	8.03	8.56	8.29	7.97	8.14	7.95	9.1
EC (dS/m)	1.9	1.8	1.8	1.8	1.9	1.8	2.6	1.9	2.0	1.9	1.9	1.8	2.1	1.9
TSS	142	28	61	3.7	7.1	39	127	31	128	42	136	29	242	54
BOD _i	141	15	-*	-	-	-	69	23	32	6.6	-	-	110	15
BOD _f	76	9	-	-	-	-	34	16	9	5.4	-	-	95	6.6
NH ₄	39.1	27.4	64.6	36.2	52.3	49.5	90.7	35.0	35.1	16.6	47.2	25.5	68.6	27.1
P ₄	21.2	11.9	76.9	78.5	76.2	75.0	83.9	78.8	53.4	46.5	78.7	69.3	63.0	50.2
Na	205	195	204	214	212	214	254	224	232	-	-	-	216	224
K	30.0	39.0	50.5	37.7	37.0	38.5	46.5	39.5		-	34.0	35.0	-	-
Ca	47.5	40.3	35.7	34.7	36.6	37.6	45.2	43.2	-	-	41.0	41.6	62.0	51.0
Mg	36.0	24.0	24.5	26.5	27.0	27.5	41.5	28.7	28.0	32.0	-	34.0	29.0	25.0
SAR (-)	5.46	6.01	6.43	6.66	6.48	6.47	6.58	6.48	6.79	7.24	7.28	7.13	5.68	6.2

* - Not monitored

Table 4. Characteristics of the effluent applied for the paprika irrigation, Chafets-Chaim, 1999

Parameter	Effluent Data						
	9 May	16 June	7 July	26 July	17 August	31 August	23 December
pH	7.82	8.22	8.3	8.18	8.15	7.75	8.38
EC, dS/m	2.0	2.0	1.9	2.0	1.9	2.0	2.0
TSS, mg/L	270	57	34	40	54	45	58
Cl, mg/L	329	330	350	350	-	304	292
SO ₄ , mg/L	78.6	-	-	-	-	74.1	51.6
Br, mg/L	1.0	-	-	-	-	0	0
HCO ₃ , mg/L	512	494	439	370	476	500	567
Na, mg/L	262	290	295	330	224	255	235
K, mg/L	45	35	27	38	30	56	30
Ca, mg/L	52	52	52	46	51	35	69
Mg, mg/L	32	38	39	40	25	27	28
Alkalinity, mg/L	420	405	360	305	390	410	465
N-NH ₄ , mg/L	18.0	32.0	13.8	4.9	27.9	31.6	54.2
PO ₄	34.2	14.9	28.7	16.9	50.2	31.4	24.1
BOD total, mg/L	43	32	31	38	15	33	40
BOD filtered, mg/L	3.6	13	5	18	7	18	10
COD total, mg/L	75	150	90	90	90	180	120
COD filtered, mg/L	30	80	50	42	30	100	40
SAR	9.97	10.6	10.8	12.2	9.09	11.14	8.53
PAR	1.01	0.75	0.58	0.83	0.72	1.44	0.64

Results

Arad

General Appearance

The grape vineyard plants after four years of irrigation with the SDI system looked normally, similar to the sections irrigated with the conventional onsurface DI system. During first experimental years, until the plants were adapted to the new irrigation system (SDI). Insignificant differences were observed, primarily in the color of the plants and vegetative growth.

The Grapes Yield

The grape yield is based on manual harvesting conducted during August of each year. Prior to harvesting the maturation stage and alcohol content were continuously monitored. Harvesting was conducted in each replication. Each replication consisted of the 10 designated plants. The harvested sample was further used for the wine production and quality characterization.

The grape yield was significantly higher under SDI, regardless to the water quality. Total yield was to some extent lower than during previous year (Figure 6). A similar yield pattern was obtained for the 1998 harvest.

Figure 6. The grape yield in Arad Heights, summer 1997

Grapes and Wine Quality

Usually the quality of the grapes is characterized by the final quality of the wine. The quality of the wine requires special analysis and long time storage until distributed in the market. The flavor tests require as well some time until the final product is marketable. Preliminary quality grape criteria include primarily color and acid content. Both are indicators for the expected quality of the produced wine.

Table 5. The wine quality of the August 1997 grape harvest, Arad.

Parameter	Emitter Location		Water Quality	
	Onsurface	Subsurface	Tap Water	Effluent
pH	3.93 a	3.87 a	3.78 b	4.01 a
Total Acid, gr/l	6.48 a	7.24 a	7.63 a	6.45 b
Sugar, gr/l	2.30 a	2.30 a	2.40 a	2.30 a
Alcohol, %	11.8 a	12.0 a	12.3 a	11.5 a
Volatile Acids	0.26 a	0.26 a	0.26 a	0.26 a
OD ₄₂₀ *	0.160 a	0.160 a	0.186 a	0.135 b
OD ₅₂₀	0.840 a	0.852 a	0.936 a	0.755 b
OD ₄₂₀ + OD ₅₂₀	1.001 a	1.011 a	1.122 a	0.890 b
OD ₄₂₀ / OD ₅₂₀	0.190 a	0.188 a	0.190 a	0.179 b

* OD₄₂₀ - Optical Density at wave length of 420 nm

Figure 7. Wine quality characteristics during 1998 in Arad vineyard

Figure 8. The wine quality as related to the water quality and irrigation method in Arad vineyard, 1998

Coliphages F+, PFU/gr dry soil**Coliphages CN13, PFU/gr dry soil****Onsurface Drip Irrigation** **Subsurface Drip Irrigation**

● - Emitter location

Figure 9. Distribution of the F+ and CN13 coliphages at different depths and distances from the emitter under onsurface and subsurface drip irrigation, Arad vineyard, 21 May 1998

Figure 10. A sample of soil contamination in Arad vineyard, summer 1998

The collected grapes were used for immediate production of must and later for wine, subject to the treatments. According to the results (Table 5 and Figures 7 and 8) the wine quality obtained from the plots irrigated with effluent is slightly inferior in comparison to the plots receiving tap water. The lower wine quality is probably due to the high nitrogen content in the applied effluent. Reducing the nitrogen content in the applied effluent should probably be one of the ultimate goals to improve the wine quality.

Soil and Plant Contamination

Contamination due to effluent reuse was examined in the soil and on the plants (leaves and fruits). The soil was sampled several times during the irrigation season.

Soil samples were taken 25 cm away from the drip laterals, on both sides, at the soil surface and at depths of 30, 60 and 90 cm. Monitoring of the soil contamination was similar for all examined treatments. The effect of all grouped data is summarized in Figures 9 and 10, showing the survival of fecal coliforms and coliphages F^+ and coliphages CN13 in the soil as affected by the soil moisture and organic matter content. Subject to the findings it can be concluded that for the specific soil of Arad Heights the survival of fecal coliforms and coliphages is increasing when the soil moisture content is generally above 12 percent. Additional work is in progress in order to verify these findings and to expand the pattern of pathogen detection in the soil and the plants. No virus was detected in the soil and the plants including the grapes. The absence of viruses can be partially explained by the extended retention time in the stabilization treatment ponds and the reservoir. As was found previously, moisture content is one of the dominant factors affecting pathogens content in the soil (Figures 10 and 11).

Clusters of grapes were as well subject to pathogen analysis. No grapes contamination was detected.

Figure 11. A sample of soil contamination in Arad vineyard, 25 June, 1998

Figure 12. Soil moisture and soil salinity (EC) profiles in the cabbage field, 16 July, 1998

nutrient distribution in the soil are verified by describing the changes with depth (Figure 13a). Main variations refer to the phosphorous although for SDI a real shift was also detected once for nitrogen.

Figure 13a. Nutrient profiles for onsurface and subsurface irrigation in cabbage, 16 July, 1998

Figure 13. Comparing onsurface and subsurface drip irrigation nutrient profiles under effluent application, cabbage field, 1998

Cabbage Yield

for SDI. The reason for the lower yield under SDI is probably due to the shallow root system which could not use the water efficiently like the plants under the

Chafets-Chaim

Effluent application

The field was irrigated three times per week, using data from class "A" pan (Figure 5). Total amount of effluent applied under drip irrigation was approximately 5,500 m³/ha. Although the relatively high content of suspended solids in the effluent applied, no emitter clogging problems were faced. Clogging problems were avoided by installing ring filters at the head of the irrigation distribution system. Intermittently, chlorine was injected into the effluent transportation system in order to enhance organic matter degradation processes.

Table 6. Pathogen content in the effluent applied for the cabbage irrigation, 1998 (count/100 ml)

Date	Fecal coliforms	Coliphage F+	Coliphage CN-13
3 June	2.6x10 ⁴	6.0x10 ²	6.2x10 ³
11 June	4.6x10 ⁴	2.5x10 ²	9.5x10 ²
3 July	4.4x10 ⁴	4.2x10 ³	7.4x10 ³
16 July	3.5x10 ⁴	6.2x10 ³	2.4x10 ³

Soil Moisture and Salinity

The soil moisture profile indicates that under DI the water content close to the soil surface is relatively high. Maximal water content under SDI was around the emitter. The lowest soil salinity was detected near to the emitter location, both for the DI and SDI systems. Therefore, for plants with a deep root system SDI is advantageous due to the high water content and low salinity level near the emitter. However, for plants with a shallow root system like cabbage, the increased salinity close to the soil surface might be problematic, primarily for less tolerant plants (Figure 12).

Nutrients in the Soil

The distribution of main nutrients in the soil for DI and SDI is depicted in Figure 12. Only negligible differences for nitrogen profile were detected for the two application systems. Similarly, negligible differences were encountered for potassium for the two drip systems. The profile for phosphorous differs for the two application systems. Low phosphorous can be found near the subsurface emitter and a relatively low content close to the soil surface. A high phosphorus content was detected for the DI system layout, close to the soil surface (Figure 13). The findings referring to the

conventional DI system. Although the importance of the yield in this case the main objective was to examine the effect of the irrigation technology on the soil and plant contamination. Further work is in progress to obtain higher yields.

Figure 14. Fecal coliforms profiles in the soil after applying effluent for irrigation of cabbage, 16 July, 1998

Figure 15. Fecal coliforms profiles in the soil after applying effluent for irrigation of cabbage, 16 July, 1998

Figure 16. Fecal coliforms profiles in the soil after applying effluent for irrigation of cabbage, 16 July, 1998

Figure 17. Coliphage F+ profile in the soil after applying effluent for irrigation of cabbage, 16 July, 1998

Soil and Plants Contamination

Conventional and modified methods were used to analyze the microorganisms content in the wastewater, soil and the plants (APHA, 1995; Doane, 1987). Soil and plant contamination depend to a large extent on the applied effluent quality, soil conditions, and the technology of application. The microorganisms content in the applied effluent is relatively low in comparison with other sites, probably due to the extended detention in the open-surface reservoirs (Table 6). Main factor affecting

survival of the microorganisms is the soil characteristics and actual moisture (Figures 14, 15, 16, 17 and 18). This finding holds for the fecal coliforms and the two types of coliphages. The coliphages can be used as indicators for pathogens content in the soil.

Consequently, the fruits under SDI were the not contaminated as compared with DI and spray irrigation (Table 7). This finding actually strengthen the approach that spray and sprinkler irrigation may be associated with high levels of health and environmental risks.

Figure 18. Coliphage CN 13 profiles in the soil after applying effluent for irrigation of cabbage, 16 July, 1998

Table 7. Contamination of cabbage on 31 August, 1998
(count/100 gr cabbage material)

Irrigation Method	Fecal Coliforms	Viruses	Enterococci
Subsurface Drip	ND*	ND	ND
Onsurface Drip	3.7	ND	9.2
Spray	NM**	ND	The plate is full

* NT - Not Detectable;

** NM - Not Monitored

Summary of Field Results

Field experiments are in progress in a commercial vineyard orchard and a vegetables fields. The fields are irrigated with secondary wastewater from a local stabilization pond system and temporary storage in an open surface reservoir. In both experiments the fields were irrigated by onsurface and subsurface drip systems, applying tap water (only Arad) and effluent. Emitters were installed at a depth of

approximately 40 cm. Evaluation criteria included the yields, quality of products, constituents in the soil and contamination indicators such as fecal coliforms, phages and viruses in the soil, plants and the applied effluent.

According to the results there are indications that under SDI the soil medium establishes an excellent environment for a variety of complex biodegradation processes (Chase, 1985; Taylor, 1978; Gerba *et al.*, 1981; Dizer *et al.*, 1984; Powelson *et al.*, 1990; Ho *et al.*, 1991; Young *et al.*, 1992; Farid *et al.*, 1993; Guessab *et al.*, 1993; Nasser *et al.*, 1993). These processes induce the various microorganisms to go through biodegradation processes or to be adsorbed by the soil particles (Taylor *et al.*, 1978), thus diminishing the chances of reaching the soil surface under SDI and risking the environmental and human activities. The effluent and the contained microorganisms transportation through the porous media is restrained by several complex and frequently interactive, physical, chemical and micro-biological processes (Hickman *et al.*, 1989). The efficiency of the microorganism adsorption, inactivation and binding to the soil surface particles depends on some characteristics of the porous media. Actually, these properties define the biofiltration efficiency and the potential for microorganisms removal:

No unexpected appearance or plant reaction was discovered in all the experiments. The slight reduction in the wine quality can be attributed to the high nitrogen applied due to the high content in the applied effluent (Much above secondary level standards). The cabbage plants under SDI developed less than the ones under DI. It can attributed the shallow root system of the cabbage plants and the depth of the subsurface drip laterals (approximately 40 cm) which prevented adequate water supply.

CHAPTER III

Pathogens in Arizona Soils

Introduction

During the last phase of the project, the turfgrass, greenhouse, and column experiments were concluded in Tucson, Arizona. The survival of bacteriophages MS-2 and PRD-1 was assessed using sprinkle and subsurface drip irrigation (SDI). Using SDI, no turfgrass contamination by MS-2 was observed, either during the summer or winter experiments. However, sprinkle-irrigated turfgrass was readily contaminated throughout the same periods. Bacteriophage PRD-1 was detected in low numbers in turfgrass where SDI was used for irrigation in the summer, but not in the winter. However, the contamination level was substantially lower than the one observed in sprinkle-irrigated turfgrass. Strong winds (45 Km/h) during one of the experiments could have resulted in cross contamination from the sprinkle irrigated plots into the SDI plot. PRD-1 was not detected in any turfgrass sample obtained from SDI-irrigated turfgrass in the winter. In the summer, MS-2 survived in turfgrass for up to an average of 48 h, whereas in the winter season, it survived for 4 days. PRD-1 survived in turfgrass for 72 h in the summer, and in the winter, it survived for 4 days. These experiments indicate that SDI offers a safer type of irrigation of turfgrass than sprinkle irrigation. The risk of poliovirus 1 (PV1), enteric adenovirus 40 (Ead40) and bacteriophages PRD-1 and MS-2 contamination of tomato plant parts, was studied under a controlled environment in a greenhouse. Plant part, and mode of irrigation were the most significant factors associated with viral contamination. The use of surface drip irrigation resulted in higher contamination of tomato stem and aerial parts, whereas the use of SDI resulted in higher contamination of underground parts as compared to stem and aerial parts. No penetration of viruses were detected in plant tissue throughout the study. Soil type did not have a significant influence on the type or level of viral contamination of plant parts. Column experiments were designed to simulate SDI field conditions, using both unsaturated Brazito (sandy) and Pima (clay) soils, at room temperature, using the bacteriophages MS-2 and PRD-1. Virus-spiked secondary treated wastewater was dispensed by gravity through a port located in the midpoint of a one-meter long column. Viral migration was greater in Brazito soil, than

in Pima soil. The titer of both viruses peaked at very close to the injection point, and then both declined rapidly as the viruses moved away from the originating point. Downward viral migration was consistently more pronounced, than upward migration. These experiments indicate that in SDI, ascending viral migration through soil would be limited. The results obtained in these studies suggest that SDI used to irrigate crops with wastewater may reduce the risk of their contamination.

Materials and methods

Evaluation of virus recovery from soils

Soil Characteristics

Two types of soil were used throughout this study. Brazito sandy loam and Pima clay loam were collected during the hot arid summer season from two agricultural stations of the University of Arizona. Pima clay loam was obtained from a field at the Marana Agricultural Center located in the Avra valley, 20 miles northwest of Tucson, and Brazito sandy loam was collected from a field of the Campbell Ave. Farms. Soil was taken from the top 15-20 cm with a shovel. After collection, the soil was passed through a 2 mm sieve to remove large rocks and plant debris. Some characteristics of these soil types are shown in Table 15.

Production of Viruses and Tissue Culture Assay

Poliovirus type 1 (Lsc strain) (PV1), and enteric adenovirus type 40 (strain Dugan) (Ead40) were propagated in the Buffalo green monkey (BGM) kidney continuous cell line, in the human primary liver carcinoma (PLC/PRF/5) cell line, respectively (Grabow *et al.*, 1992). Hepatitis A virus (HAV) strain (HM175) was propagated also in PLC/PRF/5 cells to be used in survival experiments. Virus titration was carried out by the tissue culture infectious dose fifty (TCID₅₀) technique, on PLC/PRF/5 cells (Ead40 and HAV), or BGM cells (PV1) in 96-well (Precious and Russell, 1985). Bacteriophage MS-2 was grown and assayed in *Escherichia coli* 15597 (ATCC), and bacteriophage PRD-1 in *Salmonella typhimurium* LT2. Phage stocks were prepared by adding 5 ml of Tris-buffered saline solution to infected host bacteria petri dishes showing confluent plaque formation. Plates were kept at room temperature for 2 h to allow bacteriophage diffusion into the covering solution. Debris were eliminated by centrifugation at 10,000 × g for 15 min, and by filtration of the supernatant through a 0.22-μm pore filter. Both bacteriophages were assayed by the double agar layer

technique. PV1 and MS-2 represent a group of small RNA viruses (23-30 nm), whereas Ead40 and PRD-1 typify medium sized DNA viruses (70-80 nm).

Table 8. Soil characteristics

Parameter	Pima Clay Loam	Brazito Sandy Loam
% Sand	45.1	74.7
% Silt	30.6	14.6
% Clay	24.3	10.7
pH (saturated paste)	8.14	8.03
Conductivity (dS/m) (saturated paste)	1.6	0.82
% Total organic carbon (w/w)	0.45	0.30
% Total N (w/w)	0.052	0.034

Recovery of PV1 and Ead40 from Seeded Soil Samples

The assessment of three methods (1, 2 and 3) for the recovery of viruses from soils was carried out using both Pima clay loam, and Brazito sandy loam soils at field capacity (24 and 15 % moisture, respectively). Approximately 5×10^6 TCID₅₀ of PV1, or Ead40 were added to 40 g (wet weight) of Pima and Brazito soils in enough deionized water to achieve the corresponding field-capacity moisture. These soil preparations were then mixed thoroughly to reach homogeneity.

Method 1

In this method (Yossi Manor, personal communication), 10 g of seeded Pima and Brazito soils were suspended in 8 ml of saline solution (0.15 M NaCl), 2 ml of glycine (0.2 M) buffer, pH 9.5, and 2 ml of Freon (trichlorotrifluoroethane, Fisher Scientific, PA). The soil suspensions were stirred for 30 min, maintaining a pH of 9.5, and adjusted if necessary, with 1 M HCl or 1 M NaOH. The soil emulsion was centrifuged at $5,000 \times g$ for 30 min. The supernatant was collected, the pH adjusted to 7.2-7.4, and frozen -20°C until assayed.

Method 2

This method was adapted from a multilaboratory evaluation of methods for detecting enteric viruses in soils (Hurst et al., 1991). A volume of 10 ml of 10 % beef extract (0.1 M Na₂HPO₄, 5 mM citric acid, pH 7.0) was added to 10 g of seeded Pima

and Brazito soils. The soil suspensions were stirred for 30 min, maintaining a pH of 7.0, adjusted if necessary, with 1 M HCl or 1 M NaOH. The soil emulsion was centrifuged at 2,500 x g for 30 min. The supernatant was collected, the pH adjusted to 7.2-7.4, and frozen at -20°C until assayed.

Method 3

This procedure was developed at the University of Arizona to detect enteroviruses in sewage sludge amended soils (Straub et al., 1992). A volume of 45 ml of 3 % beef extract (0.05 M Na₂HPO₄, 5 mM citric acid, pH 9.4) was added to 5 g (1:10 ratio) of seeded Pima and Brazito soils. The soil suspensions were stirred for 30 min, maintaining a pH of 9.4, adjusted if necessary with 1 M HCl or 1 M NaOH. The soil emulsion was centrifuged at 15,300 x g for 30 min. The supernatant was collected, the pH adjusted to 7.2-7.4, and frozen at -20°C until assayed.

Recovery of viruses and bacteriophages from seeded soil samples

Method 3 was selected for the recovery of viruses and bacteriophages from seeded soil samples. This method was adapted as follows. Approximately 5×10^6 TCID₅₀ of PV1, Ead40, MS-2 or PRD-1 were added to 40 g (wet weight) of Pima and Brazito soils in enough secondary wastewater effluent to achieve the corresponding field-capacity moisture (24.0% moisture, Pima soil, and 15.2% moisture, Brazito soil). These soil preparations were then mixed thoroughly to reach homogeneity. A volume of 18 ml of 3 % Beef Extract (BE) (0.05 M Na₂HPO₄, 5 mM citric acid, pH 9.4) was added to 2 g (1:10 ratio) of seeded Pima and Brazito soils. The soil suspensions were stirred for 30 min, maintaining a pH of 9.4, adjusted if necessary with 1 M HCl or 1 M NaOH. The soil emulsion was centrifuged at 15,300 x g for 30 min. The supernatant was collected, the pH adjusted to 7.0-7.4, and assayed (Straub et al., 1992).

Polymerase Chain Reaction (PCR) and Reverse transcription-Polymerase Chain Reaction (RT-PCR) analyses of seeded soil samples

A series of Brazito and Pima soil samples were seeded with different amounts of Ead40 (1.3×10^2 to 2×10^4 TCID₅₀/2 g of soil) and PV1 (4.0×10^2 to 6.3×10^4 TCID₅₀/2 g of soil), diluted in deionized water. The Ead40 and PV1 primers used in this study and the PCR protocol have been described elsewhere (Abbaszadegan et al.,

1993) (Puig et al., 1994) (Straub et al., 1992). To reduce PCR and RT-PCR inhibitory substances, BE concentrates were further processed by size exclusion (sephadex G-100), and ion exchange chromatography (Chelex) (Abbaszadegan et al., 1993). Briefly, approximately 300 FI BE concentrates were passed by centrifugation through a 1-mL Sephadex-100/Chelex-100 spun column. The filtered BE concentrate was used for RT-PCR analysis. Table 9 shows the sequences, and size of amplified fragment of the enterovirus (Abbaszadegan et al., 1993), and adenovirus primers (Girones et al., 1993). Viral cDNA synthesis was carried out by conventional reverse transcription using reverse transcriptase and random primers. Viral cDNA amplification was carried out using a conventional PCR protocol, and specific primers. PCR products were separated by electrophoresis in agarose gels, stained with ethidium bromide, and visualized with a UV light transilluminator.

Table 9. Oligonucleotide Primers for Virus Amplification

Virus type	Primers sequences	Product size (bp)	Reference
PV1	5'-TCCGGC CCCTGAATGCGGCT-3' 5'-CCCAAAGTAGTCGGTTCCGC-3'	149	Abbaszadegan et al. (1993)
Ead 40	5'-GCCGCAGTGGTCTTACATGCACAT-3' 5'-CAGCACGCCGCGGATGTCAAAGT-3'	308	Girones et al. (1993)

Viral survival in soils

A total of 40 g of Pima soil, or Brazito soil at field capacity moisture (15% Pima soil, and 24% Brazito soil) were seeded with PV1, Ead40, MS-2 or PRD-1 as described (approximately 1×10^5 TCID₅₀/2 g of PV1 or Ead40; and 1×10^5 PFU of MS-2 or PRD-1). Soil samples were kept, tightly sealed, in 50-ml polypropylene tubes at 4, 22 and 37°C. A 2-g sample of the seeded soils was withdrawn from the seeded soil mixture at regular intervals, and viruses recovered using BE as described. BE viral extracts were used to conduct TCID₅₀ (PV1 and Ead40), or plaque forming (MS-2 and PRD-1) assays. In addition, to determine the persistence of nucleic acids in soils, heat-inactivated PV1 (1 min, 60°C) and Ead40 (5 min, 60°C) were seeded into both Brazito and Pima soils as indicated above. Samples were kept at 22°C and 37°C, withdrawn at regular intervals, and processed as described. Viral and bacteriophage survival was reported as the $\log_{10} N_t/N_0$ TCID₅₀/ml, which expresses the reduction in

microorganism numbers at each time interval, where N_0 and N_t are the initial and final viral titers respectively.

Persistence of Viral Nucleic Acids in Soils

Four preparations containing 25 g of Pima or Brazito soils, at field capacity moisture, were seeded with approximately 1×10^4 TCID₅₀ of heat-inactivated Ead40, and 1×10^3 TCID₅₀ of heat-inactivated PV1. These virus concentrations represent approximately one log₁₀ more of the detection limit of the RT-PCR (PV1) and PCR (Ead40) procedures, carried out in spiked soil samples in our laboratory. Two preparations were kept at room temperature (22°C) and the other two at 37°C. Soil samples were kept, tightly sealed, in 50-ml polypropylene tubes, as described above. The procedures for recovery of viruses, nucleic acid extraction, RT-PCR and PCR were carried out as described.

Figure 19. Diagram of a column used to determine the vertical transport of bacteriophages MS-2 and PRD-1 in Pima and Brazito soils (not drawn to scale)

Column experiments to determine the transport of viruses under SDI simulated conditions

Pima or Brazito soils were used in these experiments. Secondary sewage effluents were obtained from the Roger Road Wastewater Treatment Plant in Tucson, AZ. The test column consisted of a transparent Plexiglass cylinder of 100 cm of length, and an internal diameter of 5 cm. The interior of the column were roughened with sand paper to improve contact with soil, and reduce potential flow bypass. A series of

perforations, 5 cm apart, were drilled along the column to serve as sampling ports, and test water injection. To prevent layering, soil was loaded into the column in depth increments of approximately 10 cm, and stirred. The port used to inject the effluent was located at the middle of the column (Figure 19). To mimic SDI, a volume of 500 ml of secondary sewage effluent seeded with approximately 2×10^8 PFU/ml of MS-2, and 2×10^7 PFU/ml of PRD-1 was allowed to percolate through the column by gravity. When the water column in the feeder reached the level of the injection port, approximately 1.5 g of soil samples were collected from each port. Soil samples were processed as described above to extract and assay MS-2 and PRD-1. All column experiments were conducted at room temperature.

Turfgrass field experiments

Field experiments were conducted at the Turfgrass Research Center of the University of Arizona in Tucson. Bermudagrass (*Cynodon dactylon*) was planted at the beginning of the hot season in two plots of sandy loam, of approximately 13.5 m² each. One of the plots was irrigated by SDI and the other by sprinkle irrigation (SI). The turfgrass in the plot with SDI was established initially using SI. Once the turf was established, irrigation was switched to SDI. Tertiary treated wastewater was used to irrigate both plots. Irrigation was applied with a frequency that allowed for a relatively constant field capacity moisture (SDI 28 mm/h, and SI 37 mm/h). At the beginning of the cold season, bermudagrass was overseeded with ryegrass (*Lolium perenne*), which was used to conduct the experiments in the winter months. Experiments were conducted by injecting the bacteriophages MS-2 and PRD-1 in the irrigation water at an approximate concentration of 1×10^9 PFU/l of each organism. Three experiments were conducted in the summer and three in the winter. At the beginning of each experiment, a 10-ml sample of irrigation water, collected from a sprinkle, was used for enumeration of seeded bacteriophages. Five soil, and turf samples were collected from each plot before irrigation with spiked water, immediately after irrigation, and then every 24 h, until bacteriophages could not be detected any more. Approximately two grams of soil samples were collected with a metal auger, which was ethanol-flamed between sampling points. A similar amount of turf samples were collected by clipping leaves with ethanol-flamed scissors. Irrigation water, soil and turf samples were

transported under refrigeration to the University of Arizona for analysis, which was conducted within 4 h of sampling.

Greenhouse experiments

This study was conducted at a greenhouse at the Environmental Research Laboratory, University of Arizona, Tucson, Arizona. Ten-gallon (approximately 40 liters) buckets fitted with a drainage port at the bottom were placed on steel girders resting on 60-cm concrete blocks. There were six rows of buckets and each row consisted of eight buckets, with two tomato plants in each bucket. The distance between rows was one meter and plant to plant distance was 50 cm. The first four buckets in each row were irrigated with subsurface drippers located 18-20 cm deep in the root zone. The last four buckets in each row were surface irrigated with drippers placed on the surface under the plant canopy. All plants were irrigated with secondary treated wastewater, collected from the Roger Road wastewater treatment plant in Tucson, AZ. Another twelve buckets were placed at two meters of elevation on a fabricated steel structure. These buckets served as a reservoir for irrigation water. Each reservoir bucket was connected to four buckets containing plants.

Table 10. Recovery efficiencies of Polio 1 and Ead 40 from soils at field capacity moisture

Method	Pima clay loam soil ¹		Brazito sandy loam soil ²	
	Polio 1	Ead 40	Polio 1	Ead 40
1	3.12	<1	22.0	18.6
2	2.1	13.5	42.0	48.0
3	10.3	13.0	80.0	56.0

(1) 24.0 % moisture; (2) 15.2 % moisture

Soils

Pima and Brazito soils were used throughout this study. Both soils were pulverized, and stones and clumps were removed. Enough buckets were filled with 25-27 Kg of soil, depending on soil type, leaving a 5-8 cm lip at top of each bucket for irrigation. All the buckets were flooded twice, before transplanting, to eliminate dry pockets. Soil samples were collected randomly before the beginning of irrigation with wastewater. Samples were assayed for MS-2, PRD-1, PVI and Ead40. No background contamination was found before the start of the study.

Table 11. RT-PCR and PCR detection of viral nucleic acids in soils at field capacity moisture

Soil type	Temperature	Ead 40	Polio 1
Brazito	22°C	10*	16
	37°C	6	8
Pima	22°C	16	18
	37°C	12	10

* - Days after soil was inoculated with heat-inactivated viruses

The Plants

Commercial tomato (*Lycopersicon esculentum*) varieties, Early Girl and Golden Boy were raised from seeds. Two 15-20 cm tall plants of each variety were transplanted into each bucket. After transplanting tap water was used to irrigate these plants for two weeks. Six samples were collected during the course of the experiment for each treatment. For each root and stem sample, the complete plant was removed from the container. A section of the root, 10 cm from the collar region was assayed, whereas for the stem, a section of stem 5 cm from the collar region was assayed.

Table 12. Detection of Ead 40 and pol1 in seeded Brazito soil by PCR and RT-PCR

TCID ₅₀ /g	Ead 40 PCR	TCID ₅₀ /g	Polio 1 RT-PCR
1.0×10^4	+	6.3×10^4	+
2.0×10^3	+	1.3×10^4	+
4.0×10^2	+	2.5×10^3	+
8.0×10^1	+	5.0×10^2	+
1.6×10^1	+	1.0×10^2	-
3.2×10^0	-	2.0×10^1	-

Leaves and fruits were randomly selected, and removed from the mother plant and assayed. Two 5-cm long sections of root and stem were collected from the respective part of the plant. Six average-size leaves and two average-size fruits were collected at each sampling time for analysis at each sampling point.

Table 13. Climatological conditions during the turf grass experiments

Season	Air (daily)			Soil (2-4 inches)	Relative humidity, percent	Rain precipitation mm
	Maximum	Minimum	Average			
	Summer	37.2	22.2			
Winter	22.7	1.6	12.7	10.5	32	<2.54

Source: Arizona meteorological network

Viruses

Polio1, Ead40, and bacteriophages MS-2 and PRD-1 were used in this study. All of these viruses were mixed in irrigation water at an approximate concentration of 2×10^6 infectious units of each virus for each plant, at each application time. MS-2 and PRD-1 were assayed by the double layer method. Polio1 was assayed in the Buffalo Green Monkey (BGM) kidney continuous cell line by the TCID50 method, as described. Ead40 assays were done as described, but with the addition of 1.05 mM guanidine hydrochloride to a final concentration of 1.05 mM to inhibit PV1 replication (Hurst et al., 1988). Viruses were recovered from samples by adding 3% beef extract with 0.04M sodium pyrophosphate, and then concentrated by organic flocculation. Briefly, a portion of beef extract sample was added an equal volume of Freon (1,1,2 trichloro-1,2,2-trifluoroethane, Fisher Scientific, Pittsburgh, PA), mixed thoroughly, and centrifuged at 3,000 g x for 15 min. The top aqueous layer was collected, and mixed with an equal amount of phosphate buffered saline (Badawy et al., 1985b). Antibiotics were added before assaying, to prevent bacterial growth. The numbers of viruses were expressed as Infectious Units (IU.) per leaf, per fruit, or per cm of root and stem.

Irrigation

A polyethylene trickling tube of a 2.2 cm in diameter was used as the main supply from the reservoir buckets and 4 mm diameter laterals were used to connect drippers. One lateral was used for each plant bucket. The emitters were operated under a pressure head of 2 m. Despite this low-pressure head, no significant variability in flow

between the emitters was detected. The amount of water, which the plants received, was poured into an elevated reservoir bucket. This arrangement provided a method to control both the total amount of water applied during irrigation, and the amount that each plant received.

Virus Application

Virus-containing irrigation water was applied via drippers once every ten days. Designated amounts of viruses were blended with the water in the elevated reservoir bucket, thus enabling control of the proper virus dosage. Plants were irrigated with wastewater containing viruses on days 38, 39, 48, 49, 58, 59, 68, 69, 78, 79, 88 and 89. Six samples were collected during the course of the cropping period on days 40, 50, 60, 70, 80 and 90.

Penetration of Viruses into Plant Tissue

Plant root and stem samples were assayed for penetration of viruses into plant tissues. Samples were sealed on both ends and surface disinfected twice with 70 percent ethanol. Then cortex was removed to expose vesicular bundle. Samples were processed as described above, and assayed for viruses and bacteriophages.

Results and Conclusions

Evaluation of methodology for detection of viruses in soil

Table 3 summarizes the results of virus recovery efficiencies from seeded Pima and Brazito soils. As expected, with the three tested methods, higher recoveries were recorded using Brazito sandy loam soil, in contrast to Pima clay loam soil. Overall, the most efficient method for the recovery of PV1 and Ead40, from both soil types was method number 3 (Straub et al., 1992). The recovery efficiency of PV1 from Pima soil, using method 3 was 10.3 %, whereas the recovery efficiencies of PV1 from Pima soil, using methods 1 and 2 were 3.1 and 2.1 %, respectively. Methods 2 and 3 were comparable in their ability to recover Ead40 from Pima soil, with a recovery efficiency of about 13 %. On the other hand, no Ead40 could be recovered using method 1. The recovery efficiency of PV1 from Brazito soil was 80%, followed by methods 2 and 1 with recovery efficiencies of 42 and 22 %, respectively. The recovery efficiency of Ead40 from Brazito soil, using method 3 was 56 %, while the recovery efficiencies of Ead40 from this type of soil, using methods 1 and 2 were 18.6 and 48 %, respectively. Method 1 was not further assessed because this method relies on the use of Freon as

part of the eluate. Since this chemical has been banned in the United States, its availability is gradually becoming more limited and expensive. An adaptation of method 3 was used to conduct all further experiments involving recovery of viruses from soils. It would be likely that virus recovery efficiencies as the ones reported here may be obtained in environmental soil samples. However, it is also likely that virus recovery will vary according to virus and soil types. For instance, method 1 failed to recover Ead40 from inoculated Pima clay soil samples, whereas PV1 could be recovered. The opposite was observed with method 2 in which Ead40 was recovered approximately six times more efficiently than PV1 in Pima soil. A better recovery efficiency was observed using the three methods with Brazito sandy soil. This agrees with published data (Hurst et al., 1991) in which they reported a higher recovery of indigenous enteric viruses from sandy soils than from clay soils.

Figure 20. Survival of polio 1 and Ead40 in the soil at 22°C, at field capacity moisture

Viral survival in soils

Both viruses, Ead40 and PV1, survived longer in Pima clay loam soil than in Brazito sandy loam soil. Figure 20 shows their inactivation patterns at 22°C, at field capacity moisture. In sandy loamy soil the inactivation rate of both Ead40 and PV1 was close to 1.5 log10 after 20 days. However, Ead40 survived longer. After 35 days, a 1.5 log10 reduction of the titer of Ead40 was observed, while a similar titer reduction of PV1 occurred after 25 days. At 4°C the titer of Ead40, in the two types of soils

remained unchanged for at least 60 days. During the same period, in clay loamy soil, and sandy soil PV1 lost 0.75, and 0.5 log₁₀, respectively (not shown). In clay loamy soil kept at 22°C the reduction of both Ead40 and PV1 was approximately 1.5 log₁₀ in 20 days. In sandy loam soil at the same temperature both PV1 and Ead40 survived longer than in clay loamy soil. A 1.5 log₁₀ reduction of the titer of Ead40 occurred in 32 days, while a similar titer reduction of PV1 was observed after 24 days. After 10 days at 37°C, PV1 lost 3.16 and 3 log₁₀, in sandy soil, and in clay loamy soil respectively, and Ead40 lost 2.2 and 2 log₁₀ in sandy soil, and clay loamy soil, respectively (Figure 3). Bacteriophage PRD-1 survived the longest of all tested viruses at both 22°C and 37°C. At 22°C, This bacteriophage lost slightly more than one log₁₀ in 14 days, whereas MS-2 under similar conditions lost 4.5 to 5 log₁₀ (Figure 22). At 37°C, PRD-1 was also more stable. After 6 days the titer reduction of this bacteriophage was close to 2 log₁₀, in contrast, during the same period MS-2 lost approximately 4 log₁₀ in Pima soil, and almost 6 log₁₀ in Brazito (Figure 23). Data reported earlier (Tierney et al., 1977) in the survival of PV1 in soil indicates a slightly lower rate of inactivation, with a reduction in titer of approximately 3.5 log₁₀ in contaminated plot soils during the summer season, with an average temperature of 33°C. Our experiments showed a reduction of PV1 of approximately 5.5 log₁₀ in a similar period; however, our experiments were conducted at 37°C. Since temperature is the single most important factor affecting the viral survival in the environment (Bitton, 1980), the difference in survival (Uhnnoo et al., 1986) reported earlier (Tierney et al., 1977) is not surprising. It has been suggested (Yeager and O'Brien, 1979) that the loss of PV1 infectivity in soil is the result of irreversible damage to viral RNA and protein capsid proteins. Although, the enteric adenoviruses are a major cause of viral gastroenteritis in children (Uhnnoo et al., 1986), information on their stability in soil is not available. This study demonstrated that these agents are more stable than PV1 in soils at 37°C (Figure 21), however its survival was comparable to that of PV1 in soils at 22°C. The Ead40 did not survive significantly longer than PV1 in soils held at room temperature. These results suggest a different mechanism of inactivation of Ead40 at room temperature and at 37°C. The longer survival of the enteric adenoviruses in soil at 37°C, might have been associated to DNA damage, that could have been repaired by

Figure 21. Survival of polio 1 and Ead40 in the soil at 37°C, at field capacity moisture

Figure 22. Survival of bacteriophages MS-2 and PRD-1 in the soil at 22 °C, at field capacity moisture

the host cell, while the faster inactivation in soil at room temperature, might have resulted from protein capsid damage, rendering the virions unable to enter the host cell. This has been suggested as an explanation of the different survival rate of the enteric adenoviruses 40 and 41 in tap water and sewage (Enriquez et al., 1995). Our studies showed that PV1 and Ead40 can survive in contaminated soils, especially at

lower temperatures. Therefore, SDI may offer a good alternative to surface irrigation to diminish the contamination of soil close to the surface, reducing the possibility of crop contamination.

Figure 23. Survival of bacteriophages MS-2 and PRD-1 in the soil at 37°C, at field capacity moisture

Persistence of Viral Nucleic Acids in Soils

Table 12 shows PCR results of seeded Brazito soil, with a detection sensitivity of Ead40 of about 16 TCID₅₀/g of soil by double PCR, whereas the detection of PV1 was of approximately 5.0×10^2 TCID₅₀. Table 11 summarizes the PCR (Ead40), and RT-PCR (PV1) results of nucleic acid stability in Brazito and Pima soils seeded with heat-inactivated Ead40, or PV1. Ead40 nucleic acid was detected for 10 and 6 days in seeded Brazito soil kept at 22°C and 37°C, respectively. In Pima soil, the nucleic acid of this virus was detected for longer periods, 16 days in soils kept at 22°C, and 12 days at 37°C. Generally, PV1 nucleic acids were more stable than those of Ead40. In Brazito soil inoculated with inactivated PV1, nucleic acids were detected for 16 and 8 days in soils kept at 22°C and 37°C, respectively. In Pima soil the persistence of the PV1 nucleic acids was of 18 days at 22°C and 10 days at 37°C. Our results clearly indicate that the use of PCR, or RT-PCR to determine the potential health risk associated with contaminated soils may be misleading, as false positive PCR, and

RT-PCR test can be obtained for relatively long periods in soils in which the viruses have been rendered inactive, but in which viral nucleic acids may still be present.

Figure 24. Bacteriophage MS-2 survival in the soil and turfgrass (Summer, 1999)

Figure 25. Bacteriophage PRD-1 survival in the soil and turfgrass (Summer, 1999)

Figure 26. Bacteriophage MS-2 survival in the soil and turfgrass (Winter, 1999)

Figure 27. Bacteriophage PRD-1 survival in the soil and turfgrass (Winter, 1999)

Column experiments to determine the transport of viruses under SDI simulated conditions

Column experiments were designed to simulate SDI field conditions, using both unsaturated Brazito and Pima soils, at room temperature. The MS-2 and PRD-1 spiked influents (secondary treated wastewater) were dispensed by gravity through a port located in the middle of a one-meter long column (Figure 19), and allowed to move by

capilarity and gravity through the soil. As expected, water mobility was greater in Brazito soil (sandy), than in Pima soil (clay). In Brazito soil, MS-2 could be detected above the injection point, at 15, but not at 20 cm, and its titer peaked at approximately at 10 cm from each side of the injection point. PRD-1 showed a similar, but reduced migration pattern. Its titer declined rapidly at 5 cm above and below the injection point. These findings were not surprising, as PRD-1 (60 nm) is a virus twice as large (Olsen *et al.*, 1974) as MS-2 (20-30 nm) (Powelson *et al.*, 1990). The lower mobility of PRD-1 might have been associated with hydrophobicity, as lipids are part of the structure of this bacteriophage (Olsen *et al.*, 1974). In Pima soil both viruses showed a very similar pattern of migration. PRD-1 and MS-2 migrated up to five centimeters above the injection point, but no further. PRD-1 was detected at low levels at 10 cm below the injection point, whereas MS-2 was still present, 15 cm below the injection point, but at low levels. The titer of both viruses peaked at very close to the injection point, and then both declined rapidly as the viruses moved away from the originating point. These experiments suggest that in SDI, ascending viral migration through soil would be limited, in particular in clay soils.

Turfgrass field experiments

The tertiary effluent used to irrigate the turfgrass plots had a good bacteriological quality, with less than 10 Colony Forming Units (CFU) of total or fecal coliforms per mL, this concentration is lower than that found in many raw surface waters. Heterotrophic bacteria was present in a concentration of 1×10^5 CFU/mL, which is not uncommon in raw surface waters either. Climatological characteristics recorded during the study are summarized in Table 13. During the summer season, immediately after irrigation with seeded tertiary effluent, bacteriophages MS-2 and PRD-1 were readily recovered in similar numbers from soils irrigated by either SDI or SI. Both viruses were found in large numbers in turfgrass irrigated by SI. In contrast, little PRD-1 contamination was detected in turfgrass irrigated by SDI, and no MS-2 was detected in turfgrass irrigated by SDI. Although the concentration of bacteriophages from soils irrigated by SDI, or SI were similar throughout the experiment, the highest numbers were observed in turfgrass irrigated by SI (Figures 31, 32, 33, and 34). Bacteriophages MS-2 and PRD-1 were used to determine viral survival under field conditions on turfgrass. PV1 and Ead40, could not be used in this study because of safety concerns. This study showed that SDI significantly reduces the viral contamination of turfgrass.

Only in one experiment out of three, PRD-1 was detected in SDI irrigated turfgrass samples. It is likely that the contamination of turfgrass by this bacteriophage might have been related to high winds (45 km/h) during the application of spiked irrigation water during one of the experiments.

Penetration of Viruses into the Plant Tissue

All samples were negative, and no viral penetration was observed. Penetration of PV1 in plant tissues from seeded tap has been previously reported (Oron et al., 1995). However, that work was done under outdoor field conditions, with less control of the environmental factors and unintentional contamination during sampling.

Green house experiments

Soil moisture during the experiment remained almost constant, being close to saturation. There was no significant temperature change during the course of experiment, which was 37.2°C (average of three months). The risk of contamination of both edible and non-edible parts of tomato plants irrigated with secondary wastewater was studied. Minor contamination of leaf surface under subsurface irrigation was observed in the early samples. As this experiment was conducted in a greenhouse, the possibility may exist that air currents may have caused a cross contamination of aerial parts of the plants. No leaf contamination was observed in later samples when blowers were shutoff before taking samples. Plant parts and irrigation type were the most significant factors contributing to contamination. No penetration of viruses into plant tissue was observed. It is important to note that virus concentration used in this experiment were unrealistically high. Under natural conditions pathogenic enteric viruses range in concentration from 0.1 to 10 plaque forming units per liter (Oron et al., 1995). Hence, it would seem that the risk of contamination is extremely low when applying domestic secondary wastewater through subsurface irrigation. Further research is needed to explore risk factors especially in the tuber vegetables such as radish and carrot. Subsurface irrigation requires nearly half the water needed by furrow and sprinkler system (Bernstein and Francois, 1973), which reduces the risk of viral contamination. Moreover viruses applied through subsurface drip irrigation are mostly retained by lower soil strata, thus reducing the risk of secondary contamination by field workers. Internal temperature of soil bed under subsurface irrigation is always higher than furrow or irrigation soils (unpublished data), which may help to inactivate viruses faster. Based on the results of this study subsurface drip irrigation significantly reduces

irrigation and consequently contamination risk of vegetable plants grown for consumption.

Table 14. Analysis of Variance for Variety Early Girl using General Linear Model (GLM) Procedure

Source	DF	Sum of squares	Mean Square	F Value	Pr > F
soils	1	9530.6	9530.6	2.15	0.1524
irrigate	1	749.3	749.3	0.97	0.0038
organism	3	19715.6	6571.8	1.48	0.2379
parts	3	213901.9	71300.6	16.06	<.0001
soils*organism	3	20628.1	6876.0	1.55	0.2204
soils*parts	3	13124.4	4374.8	20.99	0.0216
irrigat*organism	3	2024.4	674.8	0.85	0.1277
irrigat*parts	3	271150.6	90383.5	20.36	<.0001
organism*parts	9	40953.2	4550.3	1.02	0.4412

Table 15. Analysis of Variance for Variety Golden Boy using General Linear Model (GLM) Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
soils	1	2994.4	2994.4	12.15	0.0045
irrigate	1	354.1	354.1	10.44	0.0037
organism	1	2568.8	2568.8	10.42	0.0072
parts	3	141794.8	47264.9	191.80	<.0001
soils*organism	1	741.8	741.8	3.01	0.1083
soils*parts	3	23101.9	7700.6	31.25	<.0001
irrigat*organism	1	272.4	272.4	10.11	0.1138
irrigat*parts	3	93802.7	31267.5	126.88	<.0001
organism*parts	3	5648.2	1882.7	7.64	0.0041

Contamination of Plant Tissue

The parts of the green plant which become contaminated are related to the method of irrigation. This trend was visible in both tomato varieties (Tables 14 and 15). Subsurface drip irrigation resulted in greater contamination by the enteric organisms of the roots as compared to stem and other aerial parts, whereas surface drip irrigation resulted in a higher contamination of stem and other aerial parts than roots (Figures 30, 31, 32 and 33). Under subsurface irrigation the roots of tomato variety Golden Boy, showed a much higher level of contamination compared to the roots of the variety Early Girl, the difference was not statistically significant. It can be concluded from this investigation that type of irrigation does not have a significant influence on the ability of tested organism to contaminate different plant parts. All the tested organisms behaved similarly under both the irrigation systems tested in this study (Tables 14 and 15). The type of irrigation taken as single independent variable when analyzed for variance based on their absolute values gave a significant value. It can be

concluded that under subsurface irrigation risk of contamination was significant higher for plant roots as compared all other parts. Similarly under surface irrigation contamination was significant higher for stem as compared to stem. (Figures 30,31, 32, 33 and Table 16).

Table 16 The general linear model (GLM) procedure for plant parts (variety early girl) t-Test(LSD)

t grouping	Mean	N	Parts
A	139.13	16	ROOT
B	85.31	16	STEM
C	6.25	16	LEAF
C	0.00	16	FRUIT

Figure 28. Transport of PRD-1 and MS-2 in saturated sandy soil (Brazito), Arizona

Role of Organisms and Soil

The types of viruses and bacteriophages when tested against one of the soil type, irrigation system or plant part showed a non-significant interaction (Tables 14 and 15). It can be concluded from these results that under similar soil type and irrigation conditions, the type of viruses or bacteriophages present in the irrigation water does not significantly change the risk of contamination of different plant parts. There was little impact of soil type on the behavior of the different organisms studied. Soil type

had no significant effect on plant parts contaminated under subsurface or surface irrigation, and a similar interaction between the irrigation method and different organisms was also observed (Tables 14 and 15).

Figure 29. Transport of PRD-1 and MS-2 in saturated clay loamy soil (Pima), Arizona

Figure 30. Infectious units of PV1 and Ead40 recovered from tomato plants after surface irrigation, Arizona, 1999

Figure 31. Infectious units of PV1 and Ead40 recovered from tomato plants after SDI, Arizona, 1999

Figure 32. Plaque Forming Units (PFU) recovered from tomato plants under SDI

Figure 33. Plaque Forming Units (PFU) recovered from tomato plants under surface irrigation

Contamination of Different Parts of the Plant

When we look at the contamination of plant tissue, under subsurface irrigation, risk of contamination for roots, was significantly higher in comparison to the stem, leaves and fruits, whereas under surface irrigation the stem has a significantly higher risk of contamination in comparison to roots, leaves and fruits. This trend was consistently seen in both varieties in pima clay and Brazito sandy loam soils. Leaves and fruits were not significantly different under either of the irrigation systems tested, so they belong to the same risk factor group (Tables 16, 17 and 18). This trend was observed in both the tomato varieties tested in both soil types. It signifies the importance of the type of crop being irrigated with secondary wastewater, and part of the plant used for human consumption. Based on these results, vegetable crops can be divided into three categories of potential contamination during reclaimed irrigation. According to our results, aerial fruit bearing vegetables, like tomato, are the safest if irrigated with secondary treated sewage. Leafy vegetables like lettuce intrinsically have a higher risk than aerial fruit bearing group. Finally tuber vegetables like carrot pose the highest risk amongst these groups.

**Table 17. The General Linear Model (GLM) Procedure
For Variety Golden Boy T tests (LSD)**

Parts	Comparison	Difference Between Means	95% Confidence L		
ROOT	- STEM	101.8	84.7	118.9	***
ROOT	- LEAF	162.5	145.3	179.6	***
ROOT	- FRUIT	166.2	148.5	183.9	***
STEM	- ROOT	-101.8	-118.9	-84.7	***
STEM	- LEAF	60.6	43.5	77.7	***
STEM	- FRUIT	64.3	46.6	82.0	***
LEAF	- ROOT	-162.5	-179.6	-145.3	***
LEAF	- STEM	-60.6	-77.7	-43.5	***
LEAF	- FRUIT	3.7	-13.9	21.4	
FRUIT	- ROOT	-166.2	-183.9	-148.5	***
FRUIT	- STEM	-64.3	-82.0	-46.6	***
FRUIT	- LEAF	-3.7	-21.4	13.9	

CHAPTER IV

***Cryptosporidium* and *Giardia* in Wastewater, Effluent and the Soil**

Introduction

General

Cryptosporidium and *Giardia* are common protozoan parasites which can be frequently found in surface and wastewater. The waters are often contaminated by *Cryptosporidium* oocysts from agricultural runoff due to infected stock and by *Giardia* cysts through wastewater, effluent and wildlife. The low infectious dose (approximately 30 *Cryptosporidium*) are capable of causing serious infections and waterborne outbreaks (Ferrari et al., 1999). The robust nature of these two parasites might raise serious environmental and health problems even under effluent reuse for irrigation. Therefore they became of serious concern in the water industry.

It is world wide recognized that *Giardia lamblia* and *Cryptosporidium parvum* are the two major etiologic agents which cause diarrhea diseases in humans. Although the two parasites are known since 1681 (*Giardia*) and 1895 (*Cryptosporidium*), only limited attention was paid to their effects. These parasites are endemic and sometimes epidemic throughout the world, with high prevalence in tropic and subtropic countries.

Several waterborne diseases were reported, due to the presence of *Cryptosporidium* and *Giardia* in drinking waters. The largest outbreak occurred in Milwaukee in 1993, with an estimate of 400,000 people affected and over 100 deaths (Clancy and Fricker, 1998). These two parasites are now in the focus of the regulatory agencies dealing with tap water in order to minimize the risks of additional outbreaks. *Cryptosporidium* oocysts are now known to be widely distributed via waterborne and other exposure routes (e.g. raw eaten vegetables). Conventional effluent disinfection is not very effective. However, using combined methods of UV disinfection and chemical processes might partially ease to solve the problems.

The presence of these two parasites is closely linked with effluent reuse. The outbreaks can be related to low quality wastewater treatment, improper operation of the treatment facilities, equipment failures and mixing with other low qualities waters. Since most of the effluent, primarily in arid regions, is reused for agricultural irrigation, extra work was conducted in order to detect these parasites in the effluent applied, the soil and the plants.

Etiologic agents

Giardia lamblia

Giardia lamblia is a flagellate (order Retortomonadida) with two morphological stages : cyst and trophozoite. The cyst is oval and measures 8 to 12 μm long and 7 to 10 μm wide. The mature cyst has four nuclei, curved median bodies and a linear axonemes. The trophozoite which is the vegetative stage of the parasite is 9.5 to 21 μm long and 5 to 15 μm wide. It has two nuclei, four pairs of flagellae, median bodies and axonemes which resembles a " human face ". *Giardia* cysts may survive in water for about two months, and are highly resistant to chlorination.

Cryptosporidium parvum

Cryptosporidium parvum belongs to the order Eucoccidiorida which presents merogony. The life cycle of this parasite is much more complex compared to *Giardia lamblia*. The vegetative forms (which occur in one host life cycle) are: sporozoite, trophozoite, Type I meront, merozoite, Type II meront, and macro and microgamont. The conservative form similar to *Giardia* cyst is called oocyst (3 to 6 μm) and it contains four sporozoites. The oocyst is highly resistant to most common disinfectants as well it survives in water for months. The sticky nature of *C. parvum* oocysts is one of the feature which helps them to spread and infect many hosts despite disinfection.

Materials and methods

Field sampling

Wastewater, effluent, soil and green plants material were collected in the experimental sites. The wastewater and effluent was collected in the treatment facilities, by grab samples of around 30 l each. The samples were collected from each phase of the treatment train, approximately every second month. Sampling was commonly conducted in the early morning. Theses samples were as well used for the nutrient content analysis.

The soil samples were collected at various depths, mainly at the soil surface and at depths of 30 cm and 60 cm. Usually the samples were taken around 25 cm aside from the emitter. Sampling was commonly conducted one day after last irrigation Each sample consists of two sampling points. Soil samples were taken for the different irrigation reuse methods, primarily onsurface and subsurface drip irrigation. The soil samples were stored in disinfected bags and delivered within a three hours for the analysis.

The green plant material was commonly collected towards the harvesting period. The fruits and/or leaves samples were stored in disinfected bags and under controlled cool conditions were transported to the Technion for further analysis of the pathogen

presence. Another set of samples was as well delivered to the Central Viral Laboratory in Sheba Medical Center for detection of the presence of fecal coliforms and viruses (the laboratory in Sheba Medical Center was also heavily involved in analyzing the water/effluent and plants of the controlled experiments conducted in Volcani Center (Chapter III).

Laboratory assays

The presence of *Cryptosporidium* and *Giardia* in the various components of the renovation systems was examined in the Technion laboratories, Faculty of Civil Engineering. The delivered samples from the various sites were subject to the following analysis stages:

Recovery of *Cryptosporidium* oocysts and *Giardia* cysts from wastewater, effluent and soil was performed as follows:

1) Oocysts and cysts concentration from water sources by calcium carbonate flocculation (10 L)

The concentration method was done according to Vesey et al. (1993). Briefly, 100 ml CaCl_2 (1M), 100 ml NaHCO_3 (1M) were added to 10 liters of samples, both raw wastewater or oxidation pond, in plastic containers, and mixed well. The pH was adjusted to 10 by addition of approximately 100 ml NaOH (1N). The solution was left for 4 hours to allow formed flocs to settle on the bottom of the plastic container. The supernatant was aspirated and discarded while the flocs were dissolved by addition of 100 ml 10% sulphamic acid (w/v) and centrifuged at $1,800 \times g$ for 12 minutes. The pellet was transferred to 50 ml centrifuge tubes and centrifuged at 4,000 rpm for 15 minutes. The supernatant was decanted and the final pellet was reacted with FITC-labeled specific MAb and observed under the fluorescent microscope.

2) Oocysts and cysts recovery from soil samples (experimental procedure)

- 1) 5 to 10 g of soil samples were mixed in 20 ml H_2O containing 0.1 % Tween 80 (v/v).
- 2) The sample was shaken for 10 to 15 minutes continuously.
- 3) To the above suspension 10 ml of polyethylene glycol in distilled water (50 % w/v) and 17 ml of 25 % sucrose in phosphate buffer (3M) were added.
- 4) The mixture was centrifuged for 10 minutes in a swinging bucket rotor at $700 \times g$.
- 5) The top layer and the interphase layers were transferred to new polyethylene tubes.
- 6) The suspension was vortexed for additional 1 to 2 minutes.
- 7) Distilled water was added to the centrifuge tube to obtain 50 ml and centrifuged for 10 minutes at 5600 rpm.
- 8) The upper liquid was aspirated and the pellet vortexed.

- 9) Steps 7 and 8 were repeated twice.
- 10) The final pellet volume was adjusted to a minimal volume of 5 to 10 ml.
- 11) The staining with MAb was similar to the procedure performed with water samples.

Monoclonal Antibodies

Monoclonal antibodies obtained from AquaGlo G/C fluorescent monoclonal antibodies (Waterborne Inc., New Orleans, LA, USA) against both *Giardia* and *Cryptosporidium* for simultaneous detection in water samples, or Crypt-A-Glo, against *Cryptosporidium* only, were used according to the manufacturer's instructions.

Staining with MAb in suspension

Eppendorf tubes, which contained suspensions of cysts and oocysts, were vortexed for 30 seconds. A desired portion was transferred to Eppendorf tubes and reacted with an equal amount of MAb. After incubation for 30 min. at 37°C the tubes were topped with PBS and centrifuged at 13,000 x g for 1 minute in order to remove excess antibody. This was repeated twice taking extra care to prevent losses of cysts and oocysts during the process of discarding the supernatant. The pellet was pipetted in 10 µl aliquots on well slides and incubated at 37°C for 15 min.

Table 18. *Giardia* and *Cryptosporidium* enumeration in Arad wastewater samples (26 March, 1998)

Wastewater Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Arad		
Settling pond outlet	548	48
Facultative pond outlet	167	24
Southern stabilization reservoir	0	0
Central stabilization reservoir	0	0

Table 19. *Giardia* and *Cryptosporidium* enumeration in the wastewater samples (5 May, 1998)

Wastewater Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Arad		
Raw wastewater	340	0
Settling pond outlet	0	0
Facultative pond outlet	0	0
After filter (after reservoir) in the field	0	0
Chafets-Chaim		
Inlet to reservoir	0	130
Reservoir outlet	0	0

Mounting of labeled slides

Prepared dried slides were mounted with mounting fluid (Cellabs, Australia) by pipetting it into the 12 wells. A glass coverslip (Gold Seal Cover Glass, Clay Adams) was applied and the excess fluid was absorbed with a clean paper tissue. The coverslip was sealed with transparent nail varnish and examined under a fluorescence microscope.

Microscopy

Microscopy was conducted by use of fluorescence microscope: Nikon Labphot 2 (Excitation 436 nm with B2A barrier filter). Oocysts and cysts, which were labeled with fluorescing-isothiocyanate conjugated monoclonal antibodies, were detected under the blue filter block (480 nm -excitation, 520 nm - emission).

Results

The results referring to the presence of *Cryptosporidium* and *Giardia* in the various system components are presented for all experimental sites and various plots.

Table 20. *Cryptosporidium* oocysts enumeration in soil samples from Arad vineyard and Chafets-Chaim (irrigation on 3 May, sampling on 5 May, 1998)

Soil sample	Soil-moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Vineyard			
Onsurface effluent (40 cm deep - row 47)	12.5	ND*	9
Onsurface effluent (0 cm deep- row 47)	3.26	ND	509
Onsurface effluent (40 cm deep - row 50)	14.8	ND	0
Onsurface effluent (0 cm deep- row 50)	7.7	ND	69
Subsurface (40 cm- row 47)	17.9	ND	640
Subsurface (0 cm - row 47)	8.72	ND	255
Subsurface (40 cm- row 44)	13.8	ND	Not Monitored
Subsurface (0 cm - row 44)	3.08	ND	Not Monitored
Chafets-Chaim			
Sprinkle (40 cm) (s-62)	32.8	ND	0
Sprinkle (0 cm) (s-61)	35.2	ND	0
Sprinkle (40 cm) (s-60)	31.1	ND	0
Sprinkle (0 cm) (s-59)	31.6	ND	0

* ND-Not Determined

Table 21. *Giardia* and *Cryptosporidium* enumeration in soil samples (16 July, 1998)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad- Vineyard			
Drip surface effluent (0 cm deep)	7.8	ND*	0
Drip surface effluent (30 cm deep)	12.2	ND	8
Drip surface effluent (60 cm deep)	16.5	ND	0
Drip surface effluent (90 cm deep)	18.2	ND	18
Drip subsurface effluent (0 cm deep)	8.5	ND	33
Drip subsurface effluent (30 cm deep)	12.7	ND	43
Drip subsurface effluent (60 cm deep)	15.3	ND	46
Drip subsurface effluent (90 cm deep)	17.7	ND	0
Chafets-Chaim-Cabbage			
Drip surface effluent (0 cm deep)	24.4	ND	0
Drip surface effluent (30 cm deep)	29.1	ND	0
Drip surface effluent (60 cm deep)	27.7	ND	0
Drip surface effluent (90 cm deep)	27.3	ND	0
Drip subsurface effluent (0 cm deep)	7.5	ND	0
Drip subsurface effluent (30 cm deep)	21.7	ND	0
Drip subsurface effluent (60 cm deep)	26.3	ND	0
Drip subsurface effluent (90 cm deep)	26.4	ND	0

* ND-Not Determined

Table 22. *Giardia* and *Cryptosporidium* enumeration in wastewater samples (16 July, 1998).

Wastewater Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Arad		
Raw sewage	0	0
Settling pond outlet	0	0
Facultative pond outlet	0	0
Reservoir outlet	0	0
Chafets Chaim		
Inlet to reservoir	0	0
Reservoir outlet	10	520

Table 23. *Giardia* and *Cryptosporidium* enumeration in the wastewater samples (17 August, 1998).

Water Sample	<i>Giardia</i> (cysts/L)	<i>Cryptosporidium</i> /L
Arad		
Raw sewage	0	0
Settling pond outlet	0	0
Reservoir outlet	2	0
After filter (in the field)	0	0
Chafets Chaim		
Reservoir outlet	0	1000

Table 24. *Giardia* and *Cryptosporidium* enumeration in soil samples (17 August 1998).

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Vineyard			
Drip surface effluent (0 cm deep)	10.3	ND*	93
Drip surface effluent (30 cm deep)	12.8	ND	120
Drip surface effluent (60 cm deep)	16.9	ND	81
Drip surface effluent (90 cm deep)	17.8	ND	39
Drip subsurface effluent (0 cm deep)	12.4	ND	27
Drip subsurface effluent (30 cm deep)	14.8	ND	0
Drip subsurface effluent (60 cm deep)	16.2	ND	0
Drip subsurface effluent (90 cm deep)	18.9	ND	28
Chafets-Chaim-Cabbage			
Drip surface effluent (0 cm deep)	28.3	ND	0
Drip surface effluent (30 cm deep)	26.9	ND	0
Drip surface effluent (60 cm deep)	25.8	ND	0
Drip subsurface effluent (0 cm deep)	10.6	ND	0
Drip subsurface effluent (30 cm deep)	22.5	ND	0
Drip subsurface effluent (60 cm deep)	24.6	ND	0

* ND-No Determined

Table 25. *Cryptosporidium* oocysts enumeration in the wastewater samples in Chafets-Chaim (31 August, 1998)

Water Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Inlet to wastewater)	0	0
Outlet of reservoir	5	255

Table 26. *Cryptosporidium* oocysts enumeration in soil samples (31 August, 1998)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Chafets-Chaim-Cabbage			
Drip surface effluent (0 cm deep)	19.6	ND*	0
Drip surface effluent (30 cm deep)	18.0	ND	0
Drip surface effluent (60 cm deep)	18.9	ND	0
Drip subsurface effluent (0 cm deep)	2.8	ND	0
Drip subsurface effluent (30 cm deep)	11.9	ND	0
Drip subsurface effluent (60 cm deep)	14.7	ND	0

* ND-Not Determined

Table 27. *Cryptosporidium* oocysts enumeration in wastewater (14 September, 1998)

Wastewater Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Arad		
Raw sewage	0	0
Settling pond outlet	50	300
Reservoir outlet	0	100

Table 28. *Cryptosporidium* oocysts enumeration in soil samples in Chafets-Chaim (14 September, 1998)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Chafets-Chaim-Cabbage			
Drip surface effluent (0 cm deep)	11.1	39	116
Drip surface effluent (30 cm deep)	13.5	0	28
Drip subsurface effluent (0 cm deep)	13.0	0	60
Drip subsurface effluent (30 cm deep)	14.6	44	44

Table 29. *Cryptosporidium* oocysts enumeration in Arad soil (27 October, 1998)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Vineyard			
Drip surface effluent (0 cm deep)	20.4	0	0
Drip surface effluent (30 cm deep)	21.8	0	47
Drip surface effluent (60 cm deep)	20.6	43	0
Drip subsurface effluent (0 cm deep)	8.5	39	118
Drip subsurface effluent (30 cm deep)	16.1	ND*	0
Drip subsurface effluent (60 cm deep)	27.4	52	52

ND - Not Determined

Table 30. *Cryptosporidium* oocysts enumeration in wastewater samples (27 October, 1998)

Wastewater Sample	<i>Giardia</i> (cysts/L)	<i>Cryptosporidium</i> /L
Arad		
Raw sewage	0	0
Settling pond outlet	0	0
Facultative pond outlet	0	0
Reservoir outlet	0	0
After filter (in the field)	0	0
Chafets-Chaim		
Inlet to reservoir	0	300
Reservoir outlet	0	0

Table 31. *Cryptosporidium* oocysts enumeration in soil samples (8 March, 1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Almonds			
Drip surface effluent (0 cm deep)	15.6	0	0
Drip surface effluent (30 cm deep)	16.6	37	73
Drip surface effluent (60 cm deep)	16.8	0	0
Drip surface effluent (90 cm deep)	17.6	0	37
Drip subsurface effluent (0 cm deep)	12.8	0	0
Drip subsurface effluent (30 cm deep)	18.8	0	0
Drip subsurface effluent (60 cm deep)	18.5	0	0
Drip subsurface effluent (90 cm deep)	18.6	0	0

Table 32. *Cryptosporidium* oocysts enumeration in wastewater samples (8 March, 1999)

Wastewater Sample	<i>Giardia</i> (cysts/L)	<i>Cryptosporidium</i> /L
Arad		
Raw sewage	0	0
Settling pond outlet	100	200
Facultative pond outlet	0	0
After filter	0	0
Chafets Chaim		
Inlet to reservoir	0	0
Reservoir outlet	0	0

Table 33. *Cryptosporidium* oocysts enumeration in soil samples (19 May, 1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Almonds			
Drip surface effluent (0 cm deep)	23.4	0	0
Drip surface effluent (30 cm deep)	22.6	0	0
Drip surface effluent (60 cm deep)	23.4	0	0
Chafets-Chaim-Sunflowers field			
Drip surface effluent (0 cm deep)	32.8	0	0
Drip surface effluent (30 cm deep)	30.8	0	0
Drip surface effluent (60 cm deep)	28.7	0	0
Chafets-Chaim -Paprika field			
Drip surface effluent (0 cm deep)	23.3	0	0
Drip surface effluent (30 cm deep)	30.4	0	0
Drip surface effluent (60 cm deep)	27.2	0	0

Table 34. *Cryptosporidium* oocysts enumeration in wastewater samples (19 May, 1999)

Wastewater Sample	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad		
Raw sewage	0	0
Settling pond outlet	0	0
Facultative pond outlet	0	0
After filter	0	0
Chafets Chaim		
Inlet to reservoir	0	0
Reservoir outlet	0	0

Table 35. *Cryptosporidium* oocysts enumeration in soil samples (16 June, 1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad-Almon			
Drip surface effluent (0 cm deep)	14.7	0	149
Drip surface effluent (30 cm deep)	14.4	0	199
Chafets-Chaim -Paprika field			
Sprinkler effluent (0 cm deep)	17.8	0	0
Sprinkler effluent (30 cm deep)	20.7	0	137
Chafets-Chaim Sunflower field			
Drip surface effluent (0 cm deep)	22.3	0	0
Drip surface effluent (30 cm deep)	25.6	0	0

Table 36. *Cryptosporidium* oocysts enumeration in wastewater samples (16 June, 1999)

Wastewater Sample	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad		
Raw sewage	0	0
Settling pond outlet	0	0
Facultative pond outlet	0	0
Reservoir outlet	0	0
Chafets-Chaim		
Inlet to reservoir	0	0
Reservoir outlet	0	0

Table 37. *Giardia* and *Cryptosporidium* enumeration in soil samples from Hebron (24 August, .1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Drip surface effluent (0 cm deep)	15.7	0	20390
Drip surface effluent (30 cm deep)	17.5	0	0
Drip surface effluent (60 cm deep)	6.4	0	0

Table 38. *Cryptosporidium* oocysts enumeration in wastewater samples (24 August, .1999)

Wastewater Sample	<i>Giardia</i> /L	<i>Cryptosporidium</i> /L
Chafets Chaim		
Inlet to reservoir	0	0
Reservoir outlet	0	0
Hebron		
Outflow to Wadi	0	58/g
Bricks Factory	0	0
"Badran" Garage	0	0

Table 39. *Cryptosporidium* oocysts enumeration in wastewater samples (8 September, 1999)

Wastewater Sample	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad		
Raw sewage	10 ₄	0
Settling pond outlet	10	60
Facultative pond outlet	0	0
Rocks pond outlet	0	0
2 stabilization reservoir outlet	0	0
Reservoir outlet	0	0
After filter (in the field)	0	0
Chafets-Chaim		
Inlet to reservoir	50	40
Reservoir outlet	100	0

Table 40. *Giardia* and *Cryptosporidium* enumeration in soil samples (Last irrigation on 6 September; sampling on 8 September, 1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad- Almonds			
Drip surface effluent (0 cm deep)	12.4	0	0
Drip surface effluent (30 cm deep)	16.6	0	0
Drip surface effluent (60 cm deep)	18.2	0	0
Chafets-Chaim -Paprika field			
Drip surface effluent (0 cm deep)	24.4	0	0
Drip surface effluent (30 cm deep)	24.6	0	0
Drip surface effluent (60 cm deep)	22.1	0	0
Drip subsurface effluent (0 cm deep)	2.3	0	0
Drip subsurface effluent (30 cm deep)	9.8	0	0
Drip subsurface effluent (60 cm deep)	14.6	0	0

Table 41. *Cryptosporidium* oocysts enumeration in wastewater samples (23 November, 1999)

Wastewater Sample	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad		
Raw sewage	0	0
Settling pond outlet	19000	0
Facultative pond outlet	0	0
Rocks pond outlet	0	0
Stabilization reservoir outlet	110	0
Reservoir outlet	30	0
Chafets Chaim		
Inlet to reservoir	2340	0
Reservoir outlet	190	0

Table 42. *Cryptosporidium* oocysts enumeration in soil samples (23 November, 1999)

Soil Sample	Soil moisture % by weight	<i>Giardia</i> /g	<i>Cryptosporidium</i> /g
Arad -Wheat-preparing for sowing			
Sprinkler effluent (0 cm deep)	23.5	0	0
Sprinkler effluent (30 cm deep)	25.1	0	40
Chafets-Chaim-Wheat-preparations for sowing			
Sprinkler effluent (0 cm deep)	28.6	120	160
Sprinkler effluent (30 cm deep)	23.7	80	180

Discussion

Sporadic inlet and inlet of *Giardia* and *Cryptosporidium* for the monitored wastewater treatment systems could be noticed. No typical content could be identified for the parasites inflow and outflow.

Attempts were also made to find any linkage between the content of the parasites in the effluent applied and the content in the soil. In addition attempts were made to correlate it with the irrigation method, sampling location and moisture content of the soil.

The fuzzy behavior of the parasites in the wastewater treatment and renovation systems and in the soil turn it into a challenging issue. Further work is required, probably by a much more careful monitoring program of the wastewater treatment systems.

CHAPTER V

Pathogens in the Soil, Penetration into Plants and Laboratory Assays

Introduction

The complementary part of the field experiments was to examine under strict controlled conditions pathogens behavior, primarily in the soil, and penetration into the plants. Pathogen can mainly penetrate via the root system. Only several works reported and discussed pathogen penetration into the plants. Oron et al (1995) reported that viruses entered into the tomato plants. However, it seems that although the precaution stages taken under the outdoor experiments, external contamination took place. The research team in Arizona reported that no virus entered into the plants irrigated with effluent. This point was as well verified under controlled conditions in the framework of this project in Volcani Research Center, Bet-Dagan and the Central Virology laboratory, Sheba Medical Center.

This part of the research included as well aspects of pathogens transportation and decay processes in the soil under effluent application. An activity indicators was used to asses the pathogen behavior in the soil.

Pathogens in the effluent, soil, and plant system

As wastewater moves through the soil, bacteria are removed by straining, adsorption, and die-off. Clays in soils enhance adsorption. However, the smallest particle size in clay soils also results in more straining of bacteria at the surface. Thus is difficult to assess the relative importance of straining and adsorption.

Adsorption is a major factor in both survival and transport of viruses in the subsurface environment. Factors influencing virus adsorption in the subsurface include (a) pH, organic matter content, aeration level, soil structure and texture, soil surface charge and soil porosity and (b) virus size, surface charge, hydrophobicity and aggregation (Hurst et al, 1988; Hurst et al, 1991; Taylor et al., 1990a, 1990b & 1990c).

The pathogenic bacteria can survive on contaminated fruits and vegetables for few days to a few weeks depending upon local conditions, weather, and the degree of contamination. Fecal coliforms populations on alfalfa irrigated with municipal sewage lagoon effluent were reduced rapidly during the first 24 hr after application of sewage under conditions of bright sunlight, low humidity and high temperatures. It has been suggested that viruses can persist for many days on plants. Limited information is

available concerning viruses that reach the interior of plants, however, these may have even longer survival times (Ward and Irving, 1987; Ward et al, 1989).

Materials and methods

General

The purpose of the experiment was to detect absorption of viruses through the root system of tomato plants and migration towards the leaves. The absorption of the viruses was examined under strict controlled condition in order to prevent any external contact and contamination.

Laboratory Assays

Laboratory experiments were in order to analyze the plants and the water for the existence of the various pathogens. The analyses were conducted according to Standard Methods ((APHA, 1995) and the following stages:

A. Processing plants samples for viral load assays

- 1) Plants 10-15 cm long were cut into small pieces with sterile scissors.
- 2) The small plant pieces were mixed in a beaker with 1 part of M-199 tissue culture media and 2 parts of 3% beef extract (pH -7.2).
- 3) The mixture was blended for 1 minute in a blender (Omni Mixer- Sorvall)
- 4) The blended mixture was centrifuged for 20 minutes in 5,000 rpm.(Sorvall RC-5)
- 5) The supernatant was collected and the debris was repeatedly treated, as is stage 2.
- 6) The supernatant from both stages of treatment was collected and an antibiotics was added (A conventional mixture of penicillin streptomycin and neomycin)
- 7) The treated samples were frozen in -20°C refrigerator.

B- Preparation of feeding solution samples

- 1) The samples were centrifuged (5,000 rpm) for 20 minutes.
- 2) The supernatant was collected antibiotics was added and the samples were kept in 20°C refrigerator.

C-Vaccine poliovirus titration

- 1) 10-cm petri dishes covered with a monolayer of BGM cell line were prepared.
- 2) Samples - 1 ml from decimal dilution of preparation of stage (B) or 4 ml from preparation of stage (C) - was poured in the dishes and was stored for viruses absorption for one hour.
- 3) The samples were collected and the cells were covered with M-199 -Bacto agar mixture and kept to solidify for about 20 minutes.
- 4) The plates were incubated for 24-48 hours in CO₂ incubator at 36°C.

- 5) The plates were covered with a 0.001% neutral red - M-199 solution and incubated for 1 hour.
- 6) The color was removed and the dishes were incubated for additional 24 hours.
- 7) The dishes were examined for plaques. The poliovirus titer was calculated. The results are given in Plaque Forming Units per 1 ml (PFU/ml)

Pathogen Indicators analysis (Sheba Medical Center)

Soil sample processing for bacteriological assays

- 1) Each soil sample was weighed
- 2) An amount of 150 to 200 ml of elution solution was added
- 3) The sample was shaken for 30 minutes with a Lab-Line shaker under a speed of 300 rpm
- 4) After 1 hour of settling 15-30 ml from the supernatant was tested by the membrane filtration method for fecal coliforms (BBL MFC Broth) and for fecal enterococci (BBL M Enterococcus agar) and incubated for 24 hour in 44.5°C and for 48 hour in 35°C, respectively.
- 5) The colonies were counted.

Processing of ground samples for viruses detection

- 1) Samples from stage 4 (of the bacteriological assays) were shaken again for 1 hour as described above with 30 ml Freon 116.
- 2) The supernatant was collected and centrifuge for 20 minutes under a speed of 4000 rpm.
- 3) The supernatant was collected, and 5 ml of antibiotics and concentrated M-199 (x10) was added
- 4) The supernatant was seeded on BGM tissue culture as described for the analysis of the tomato plants.
- 5) The soil was dried at 105°C for 48 hours and the dry weight was measured.
- 6) Assessment of microorganisms concentration for 100 gr. of soil was calculated as follows:

$$C = (N \times V_2 \times 100) / (V_1 \times W_d) \quad (1)$$

where

- C - microorganism concentration, #/100 gr dry soil
- N - number of counted colonies (for bacteria) or plaques (for viruses)
- V₁ - volume of sample seeded
- V₂ - total volume of sample

W_d - dry weight of the ground

7) Calculation of the soil moisture, percent by weight θ_w :

$$\theta_w = 100 \text{ (Wet weight - Dry weight)/(Dry weight)} \quad (2)$$

Analytical methods indicating pathogen activity

Several methods can be used in order to evaluate pathogen activity and survival in the soil. The related main parameter was the soil moisture, although the ions composition is an addition important factor. Total biological pathogen activity was based on using the FDA (Fluorefkein DiAcetate) substrate for bacterial activity assessment and Dehydrogenase (45 minuets test duration). FDA is assessed at an absorbency wave length of 494 nm. The soil samples were taken from the different vineyard plots from Arad. Each soil sample consists of a mixture of two sampling point.

The Experimental Layout

Rinsed two weeks old tomato plants (*Lycopersicon esculentum Mill.*) were installed in controlled bottles. The bottles were isolated and cleaned and contained a 250 ml hydroponic nutrient solution. The nutrient solution consisted of 15 ppm phosphate, 34 ppm nitrogen and 28 ppm potassium. The nutrient solution was enriched with 9×10^5 PFU/ml of Vaccine Polio virus type II per each bottle with a plant. The experiment lasted three days during which virus absorption and movement in the plants was detected and monitored.

Virus detection in the plants

The nutrient solution which was enriched with the virus vaccine was also colored with two colors in order to verify water, nutrient and eventually the viruses absorption and movement within the plants. Two colors were used: (i) Calofuor color commonly used in plants analysis, and; (ii) Brightener 28 fluorescent color. The colors were detected using a fluorescent microscope.

Plant sampling

Virus content in the plants was detected three times during the experimental period: (i) four hours after the color adding (leaf samples of the plants); (ii) 24 hours after the color adding, and; 48 hours after the addition of the colors.

The treatments

The experiment was based on three major treatments, with three replications each:

1) Control treatments without color addition and no viruses addition:

- 1a) Regular tomato plants (treatments 1 and 2)
 1b) Tomato plants with partial lopped-off roots (treatments 3 and 4)
 2) Control treatments with plants with color addition
 2a) Regular tomato plants (treatments 5 and 6)
 2b) Tomato plants with partial lopped-off roots (treatments 7 and 8)
 3) Plants in a nutrient solution contaminated with vaccine poliovirus type 2 (treatment 9 to 26). No color was added in this series of experiments.
 3a) Regular tomato plants
 3b) Tomato plants with partial lopped-off roots

Table 43. Controlled experiments with nutrient solution enriched with polio viruses and colors

No.	Treatments						Elapsed time, hours	Virus detcction (out)			Comments
	Virus in feed solution		Color added		Lopped off roots			Viruses in leaves	Color in leaves	Viruses in solution	
	Yes	No	Yes	No	Yes	No					
1		N		N		N	4	n*	n*		Control
2		N		N		N	4	n	n		Control
3		N		N	Y		4	n	n		Control
4		N		N	Y		4	n	n		Control
5		N	Y		Y		24	n**	y**		No virolo analysis
6		N	Y		Y		24	n	y		No virolo analysis
7		N	Y			N	24	n	y		No virolo analysis
8		N	Y			N	24	n	y		No virolo analysis
9	Y***		Y			N	24	-	Y	2.4x10 ³	
10	Y		Y			N	24	-	Y	1.1x10 ³	
11	Y		Y			N	24	-	Y	3.1x10 ³	
12	Y		Y		Y		24	-	Y	3.0x10 ³	
13	Y		Y		Y		24	-	Y	???	
14	Y		Y		Y		24	-	Y	5.0x10 ³	
15	Y		Y			N***	48	-	Y	1.5x10 ³	
16	Y		Y			N	48	-	Y	????	
17	Y		Y			N	48	-	Y	5.0x10 ³	
18	Y		Y			N	48	-	Y	2.5 x10 ³	
19	Y		Y			N	48	-	Y	1.0 x10 ³	
20	Y		Y			N	48	-	Y	1.0 x10 ³	
21	Y		Y			N	72	-	N	1.4 x10 ³	
22	Y		Y			N	72	-	N	1.0 x10 ³	
23	Y		Y			N	72	-	N	1.0 x10 ³	
24	Y		Y		Y		72	-	N	2.0 x10 ³	
25	Y		Y		Y		72	-	N	????	
26	Y		Y		Y		72	-	N	???	

* n - No color/viruses were detected in the leaves after 4 hours

** y and n - Color was detected however, no viruses in the leaves after 24 hours, respectively

*** Y and N - adding color/viruses or without adding color/viruses, respectively

Results

Tomato plants experiments

According to the experimental program both the color and virus penetration into the plants was examined and detected as described. The color was detected 4 hours after adding to the solution. The virus penetration was detected in the plants and the solution 24 hr, 48 hr and 72 hr after adding to the solution. The detailed treatments and related results are given in Table 43.

Figure 34. Dependence of dehydrogenase content on depth, distance from drip lateral, drip method, and water quality in Arad vineyard, 10 November, 1998 [(a) tap water, ODI; (b) Tap water, SDI; (c) Effluent, ODI; (d) Effluent SDI]

Pathogen activity experiments

According to the indicators used the pathogen activity varies considerably, subject to the existing conditions. These conditions are reflected by the soil moisture, depth

and distance from the laterals (influences soil moisture) (Figures 34 and 35). The results confirm previous year (1998) findings that main activity is in the upper soil layers.

Figure 35. Dependence of FDA fluorescence on depth, distance from drip lateral, drip method, and water quality in Arad vineyard, 10 November, 1998 [(a) tap water, ODI; (b) Tap water, SDI; (c) Effluent, ODI; (d) Effluent SDI]

Figure 36. Dependence of FDA fluorescence on depth, soil moisture and emitters' in Arad vineyard, 10 November, 1998 [(a) tap water, ODI; (b) Tap water, SDI; (c) Effluent, ODI; (d) Effluent SDI]

CHAPTER VI

Risk Assessment of Consuming Agricultural Products Irrigated with Effluent

Introduction

Treated wastewater utilization

Water scarcity in arid and semi-arid regions has encouraged the search for additional sources currently not exploited intensively, such as treated domestic sewage. Treated wastewater is a relatively stable water source and can be utilized, mainly for agricultural irrigation. Disposal of treated wastewater for agricultural irrigation, simultaneously solves water shortage problems and reduces potential environmental contamination. However, subject to distribution of waterborne disease, associated with treated wastewater reuse, a risk assessment of pathogen hazards, subject to diverse wastewater qualities utilization, as well as different irrigation technologies, is required.

Wastewater reclamation is the process of treating wastewater for beneficial uses, its transportation to demand sites, and its actual reuse (Pettygrove and Asano, 1985). Reclamation of wastewater allows depletion of groundwater to be minimized and helps to prevent contamination of natural water sources, by reintroducing wastewater (even if only partly treated) as an alternative water source. Shortage of water has driven consumers in arid and semi-arid areas around the world to reclaim municipal wastewater for diverse purposes, of which agricultural is the main one.

Human communities consuming raw agricultural products (vegetables and fruits) irrigated with reclaimed wastewater ingest microbial pathogens (bacteria, viruses, and parasites). The ingestion of these pathogens can cause infection and illness. Health risk assessment allows the amount of ingested pathogens and the subsequent health effects of wastewater irrigation to be quantified, and indicates the precaution phases that should be undertaken.

Consequently, one of the most important issues during wastewater reclamation is the protection of public health. Current health regulations governing wastewater reuse impose strict limitations, based on a “zero risk” alternative approach (WHO, 1989; USEPA/USAID, 1992). This approach can hardly be expected to emphasize economic benefits rather than health risks. Even so, it is now widely regarded over-cautious. Currently, almost no country or international body has yet set an acceptable risk criterion for wastewater reclamation. The USEPA drinking water guideline for enteric viruses sets a limit of less than one infection per 10,000 population per year ($<10^{-4}$).

This drinking water guideline has been used for the evaluation of wastewater reclamation projects in the absence of a common acceptable risk criterion (Asano et al., 1992; Rose et al., 1996; Shuval et al., 1997; Tanaka et al., 1998). The appropriateness level of the 10^{-4} risk standard as a measure of safety therefore needs further discussion.

The risks associated with effluent reuse

Health risk assessment is the process through which toxicological data is combined with information concerning the degree of exposure to external risks. It is performed in order to quantitatively predict the likelihood that a particular adverse response will arise in a specific human population (Paustenbach, 1997). Quantitative microbial risk assessment has recently been applied to estimate the risk of infection and illness from enteric pathogens in water and food. Several studies focus on health risk assessment of wastewater reuse in agriculture (Asano and Sakaji, 1990; Asano et al., 1992; Rose et al., 1996; Shuval et al., 1997; Tanaka et al., 1998).

Limited attention has been focused on exposure assessment and health risk analysis due to consumption of agricultural products irrigated with reclaimed wastewater. The exposure of consumers to contaminants due to wastewater irrigation depends on several factors: (i) the quality of the applied wastewater; (ii) the irrigation method, (iii) the elapse time between irrigation, harvest, and subsequent product consumption, and; (iv) consumers' habits. Previous works deal only roughly with the effect of the irrigation method, which is, in practice, one of the key issues for their exposure estimate (Shuval et al., 1997; Tanaka et al., 1998). Other sources estimate an accidental ingestion of 100 ml of irrigation water per year, without specifying whether this concerns consumers or workers (Rose et al., 1996).

The present study focuses on the risk to consumers of using agricultural products irrigated with reclaimed domestic wastewater. Risks for farmers and workers are not in the scope of this work. The risks associated with microbial aerosol dispersion and the related impact on adjacent living communities have been reported elsewhere (Applebaum et al., 1984; Ward et al., 1989).

Implication of the Risk Assessment Approach

The exposure model features

Exposure consists of a series of events in which a person (or a community) is in a close contact with a biological, chemical or physical agent (Hammad and Manocha, 1995). The prevailing route of exposure to reclaimed wastewater for human consumers is primarily through ingestion. When modeling exposure of a community to a specific phenomenon, regarding wastewater, the following should be considered:

- a) Wastewater treatment characteristics.

- b) The route of virus migration from the irrigation wastewater into and within the plant.
- c) Virus die-off during the period between last irrigation and agricultural raw products consumption.
- d) The consumption pattern of the population.

The exposure route is commonly based on an human adult whose dietary intake of fruits and vegetables is based entirely on crops irrigated with effluent. The corresponding assumptions are as follows:

* Only exposure through ingestion is considered.

Figure 37. The procedure for determining the expected annual infection risk due to consumption of agricultural products irrigated with effluent

- * The virus concentration in raw sewage is log-normally distributed. The arithmetic mean is 1,000 CFU/l and the standard deviation is 300 CFU/l (Rose et al., 1996; Tanaka et al., 1998).
- * The decay of pathogens during storage of effluent before irrigation is part of the treatment system.
- * The total period between final irrigation and consumption equals the time between final irrigation and harvest, and the period that the fruits and vegetables are stored between harvest and consumption.
- * No cross-contamination of fruits and vegetables after harvesting is considered.
- * Consumers eat 50% of their diet uncooked, unpeeled, and unwashed. A triangular distribution (minimum is 25%; maximum is 75%) is used to express the uncertainty of this estimate (Figure 39).

An Exposure Model (EM) for assessing the risk associated by consuming agricultural products irrigated with wastewater was developed. The developed EM is based on previous literature data and various field measurements. The EM quantifies the relationship between irrigation wastewater quality and the daily virus dose that consumers ingest. The expected annual risk of infection is estimated stochastically by numerical simulation using the Monte-Carlo Simulation Method (MCSM) and the developed EM. The outcomes of the numerical computations are compared, including the given and obtained risks.

Numerical simulation of exposure scenarios provides the means to express uncertainty and variability of the model input parameters by characterizing them with a distribution pattern. Variability is the impreciseness that occurs due to actual differences among segments of a population. Although the variability is not reduced, it provides additional data, thus increasing the accuracy of the analysis. Uncertainty stems from the limitation in the thoroughness of the measurement of the specific factor (Finley et al., 1994).

The numerical simulation combines treatment characteristics and effluent quality thus yielding the irrigation quality. The irrigation wastewater quality and the exposure model lead concurrently to the daily virus dose that consumers ingest. The daily risk of infection is then calculated using a dose response model implementing data from literature. Finally, the daily rate of infection is converted into an annual risk of infection taking into account the exposure frequency. The general modeling layout and data processing procedure is described in Figure 37.

The Monte-Carlo simulation method

The Monte-Carlo Simulation Method (MCSM) builds up successive scenarios using input values that are randomly selected from probability distributions, commonly utilizing the pertinent computer software. For each run, the software draws one

random variable from the distribution for each of the model input variables and computes a single result. A large number of repeated computations produce a complete distribution of the modeled results (Thompson et al., 1992).

The MCSM produces a complete distribution of output variables, of which the arithmetic mean, the standard deviation, the 95% Lower Confidence Limit (LCL) and the 95% Upper Confidence Limit (UCL) are calculated. The expected value is a probability-weighted average of the results generated during a simulation run, and equals the average (arithmetic mean) of the generated results. The calculations can be carried out using appropriate software such as the "RiskMaster for Windows" program (V.1.0C) as an add on to a Microsoft ExcelTM spreadsheet (RiskMaster, 1995).

Each simulation is carried out for approximately 12,000 computer runs to guarantee convergence and to make-up a representative sample of the near infinite number of combinations of possible input variables. The stability and convergence of the simulation are tested by running two independent simulations of 12,000 runs. The estimated mean, LCL and UCL agree within 1%. From this it was concluded that 12,000 runs are sufficient to ensure convergence and stability of the output distribution (Thompson et al., 1992).

Table 44. Summary of input data for Monte-Carlo simulation

Variable	Symbol	Dimensions	Mean value	Distribution
Virus concentration in raw sewage	C_0	CFU/l*	1000	LogNormal; $\sigma = 300$
Kinetic decay constant	k	d^{-1}	0.69	-
Daily vegetables & fruits consumption	M_i	$g/(kg \cdot ca \cdot d)$	7.7	Step; Figure 40
Human body weight	M_{body}	kg	71	Normal; Figure 40
Percent of vegetables & fruits consumed raw	f_{raw}	-	50	Triangular; Figure 40
Model: parameter Beta-Poisson model	α	-	0.247	-
Model: parameter Beta-Poisson model	β	-	0.232	-
Equivalent volume of spray irrigation	$V_{eq,si}$	l/g	$1.6 \cdot 10^{-4}$	-
Equivalent volume - drip irrigation	$V_{eq,di}$	l/g	$1.6 \cdot 10^{-6}$	Triangular; Figure 39
Equivalent volume - subsurface drip irrigation	$V_{eq,sdi}$	l/g	$1.6 \cdot 10^{-7}$	Triangular; Figure 39
$\log_{10}$ removal wastewater treatment	E_r	$\log_{10}$	Range: 0 to 6 $\log_{10}$	
Period irrigation - consumption	t_d	d	Range: 0 to 30 days	

* CFU -Colony Forming Unit

Examined scenarios

The nine initially examined scenarios combine treatment options (primary treatment, secondary treatment, or complete treatment), and a specific irrigation technique [Spray Irrigation (SI), Drip Irrigation (DI), or Subsurface Drip Irrigation (SDI)]. The elapsed time between irrigation and harvest is assumed to be constant at 15 days.

The combined simulations of the initial nine scenarios with a range of exposure scenarios was conducted in order to analyze the influence of wastewater treatment efficiency, irrigation method, and elapsed time between irrigation and agricultural products consumption on the resulting human health risk. Each scenario is a combination of wastewater treatment efficiency (between 0 log10 and 6 log10), the irrigation method (SI, DI, SDI), and the elapsed time between irrigation and consumption (between 0 and 30 days). The detailed input for the various scenarios is given in Table 44.

The dose response model

A Dose-Response Model (DRM) was developed in order to examine the effect of consumption of agricultural products irrigated by effluent on human health. The DRM shows the relationship between the rate of exposure (virus dose) and the rate of effect on the consuming human community (response). Commonly, DRMs are based on animal behavior or data obtained from controlled experiments in which adult healthy humans consume pathogens. Consequently, the data related to DRMs regarding humans reaction is limited and they are generally considered to be among the most uncertain health risk analysis models (McNab, 1997; Paustenbach, 1997).

The β Poisson Model (β PM) for rotaviruses is used dominantly as a DRM. Rotaviruses are the type of enteric viruses with the lowest infectious dose. The hypothesis of an independent action of single organisms forms the base for the β PM. The β PM was first used for drinking water and food technology, and is considered an appropriate model for virus ingestion and infection probability assessment (Rose and Gerba, 1991a , 1991b; Rose et al., 1996; McNab, 1997; Tanaka et al., 1998).

$$P_1 = 1 - (1 - D_i / \beta)^{-\alpha} \quad (3)$$

where

P_1 - Daily probability of infection by ingesting pathogens.

D_i - Daily consumed dose of contaminant, CFU/d.

β - The β -Poisson distribution coefficient.

α - A model parameter ($\alpha = 0.232$ and $\alpha = 0.247$; Haas et al., 1983).

Successively, the annual risk can be assessed from the daily risk, namely:

$$P_a = 1 - (1 - P_1)^{365} \quad (4)$$

where P_a is the annual probability of infection by ingesting pathogens.

Wastewater treatment characteristics

Wastewater treatment systems consist customarily of a primary stage, secondary treatment, and tertiary phases. Usually BOD₅ and the suspended matter are common treatment efficiency parameters. In this analysis the virus content was used as the main removal efficiency measure:

$$E_R = 100 (C_o - C_{iw})/C_{iw} \quad (5)$$

where

E_R - Removal efficiency, percent.

C_o - Virus concentration in the raw sewage, CFU/l.

C_{iw} - Virus concentration in the irrigation reclaimed wastewater, CFU/l.

Primary treatment includes basic treatment such as screening of coarse solids and grit removal. The virus removal efficiency at the primary treatment stage is relatively low and is around 50% or 0.32 log₁₀ (Feachem et al., 1983). In the secondary treatment stage (biological treatment process such as activated sludge), a higher removal is attained. The inclusive treatment removal efficiency of a primary and secondary stages is estimated at 95% or 1.3 log₁₀ (Feachem et al., 1983).

Complete treatment consists of primary, secondary, and tertiary treatment phases. Tertiary treatment includes commonly chemical coagulation, sedimentation, and filtration or equivalent phases. The virus removal efficiency of a complete treatment is estimated at 99.9994 % or 5.2 log₁₀ (Asano and Sakaji, 1990).

The irrigation method, wastewater quality and the crop

Agricultural crops and their products are commonly contaminated by two major routes: (i) direct external contact of the applied wastewater with the surface of the plants' parts (ii) penetration of microorganisms through the roots system into the plants internal parts.

Direct contact of the fruits and vegetables with the reclaimed wastewater is associated with contamination by pathogens which stick to the surface area of the plants parts. Additional contamination is due to pathogens penetrating into the plant through injured surfaces. Contact contamination depends largely on the applied irrigation method. Relatively large amounts of wastewater and aerosols are in contact with the crop surface during SI causing high contamination levels. Common DI provides wastewater to plants through on surface laterals. Under DI, contamination of plants by direct contact with the irrigation wastewater, can take place only if the foliage and fruits are almost entirely attached to the emitters. It includes mainly low

spreading plants like cucumber and tomato. However, the extent of the contamination is lower than in the case of SI, since no aerosols are distributed. Data regarding pathogen level in crops under DI are scarce and frequently inconsistent. Measurements of soil and plants which were carried out in the past demonstrate contamination levels that are at least two orders of magnitude lower than under SI (Shuval, 1980; Oron et al., 1990).

In high erecting plants like corn, grapes and most deciduous orchards, there is a low probability that their loparts will be contaminated. Under SDI the direct surface contamination is eliminated entirely.

Previous works made it evident that contamination through direct uptake by the roots is almost impossible and can be considered negligible in comparison with contamination by direct contact (Sadovski et al., 1978; Shuval et al., 1980; Katzenelson and Mills, 1984; Oron et al., 1991; Oron, 1995). The soil surrounding the subsurface emitters and plant roots performs as a complementary removal phase for viruses (Oron, 1996).

Figure 38. Expected crop contamination by pathogens, based on various on lumbricious, Fecal Coliforms (FC), salmonella and Total Coliforms (TC), when 16 ml of reclaimed effluent per 100 g of crop remains on the crop

The main mechanism for contamination through SI is by direct contact of the effluent with the plant foliage and fruits. Measurements of different pathogens' presence on various plants show that a constant volume of wastewater and its microorganisms remain attached to the plant after irrigation. Shuval et al. (1997) found in their studies that only an amount of 10.8 ml of wastewater remained on every 100g of lettuce plants. This is lower by about 40 % than findings in other works. Taking data from various works reveal a linear relationship between the content of pathogens in the irrigation wastewater and their related concentration on the plant (Figure 38). The linearity verifies that contamination by direct contact is a physical process and does not depend on the type of microorganism..

In order to be able to compare the SI method and the DI and SDI methods, the Effluent Equivalent Volume (EEV) concept was adapted. The EEV approach is used for DI, with a triangular distribution to express variability and uncertainty. The triangular distribution is a conservative characterization of a normal distribution, and takes into account high level of uncertainty (Finley et al., 1994). Figure 39 shows the distribution diagram of the equivalent volume which has an average virus content of 0.16 ml in the irrigation wastewater penetrating into 100 g of plant matter (minimum is 0.016 ml/100g; maximum is 1.6 ml/100g).

Figure 39. The distribution of the Equivalent Effluent Volume for Drip Irrigation (DI) and Subsurface Drip Irrigation (SDI) which used in the Monte-Carlo Simulation (The x-axis is logarithmic); The mean value for DI is 0.16 ml/100g, the for SDI 0.016 ml/100 g

Under SDI, the risk of crop contamination is further reduced by minimizing the direct contact between the upper parts of the plant and contaminated wastewater or the soil. The two main mechanisms of contamination under SDI are either by over irrigation, causing the effluent to reach the soil surface, or through penetration via the root system and internal migration to the upper parts of plants. The limited data regarding SDI revealed that very small amounts or no viruses can penetrate into the plants (Shuval et al., 1980; Katzenelson and Mills 1984; Oron, 1995; Oron et al., 1997). The EEV concept was as well applied for SDI and a triangular distribution (Figure 39). Under SDI and for a triangular distribution, the average EEV was 0.016 ml/100 ml, the minimum 0.0016 ml/100g and the maximum 0.16 ml/100 g. Comparisons with on surface conventional DI irrigation demonstrates that at least two orders of magnitude reduction in pathogen levels in soil and crops can be attained under SDI (Campos et al., 1998).

Effect of irrigation, harvesting and consumption timing on virus die-off

Under adequate environmental conditions the viruses can survive for extended periods of several months (Feachem et al., 1983). Virus survival depends on surrounding conditions, however, and their multiplication needs a suitable host. Natural decay processes of viruses depend on moisture, salinity, temperature, pH, and radiation intensity. The fate of pathogens in the environment is usually represented by a first-order rate die-off kinetics {Equation (6)}. A decay constant in the range of 0.65 d⁻¹ to 0.73 d⁻¹ is often used for viruses (Asano and Sakaji, 1990; Shuval et al., 1997; Tanaka et al., 1998):

$$C_c = C_{iw} [\exp (- k t_d)] \quad (6)$$

where,

C_c - virus concentration at elapsed time t_d after irrigation or at consumption, CFU/l.

C_{iw} - initial virus concentration of irrigation water, CFU/l.

k - kinetic decay constant, d⁻¹.

t_d - elapsed time between final irrigation and consumption, d.

Agricultural products consumption pattern

Fruits and vegetables are the major parts of the human diet which are affected by irrigation with reclaimed wastewater. The USEPA (1977) investigated the daily intake of fruits and vegetables per body weight in the United States. The USEPA analysis is based on a mean common body weight of approximately 71 kg (Finley et al., 1994). The fraction of fruits and vegetables that is consumed uncooked, unpeeled, and unwashed, can be described by a triangular distribution pattern (average 50%;

minimum 25%; maximum 75%). The combination of these data leads to a daily per capita consumption of raw fruits and vegetables, that is affected by wastewater application (Figure 40).

Figure 40. The daily per capita consumption distribution of raw fruits and vegetables as a combined function of body weight, fruits and vegetable consumption pattern (sources: USEPA, 1977; Finley et al., 1994)

The Exposure Model (EM)

Subject to the above considerations the EM was defined and examined. The exposure due to ingestion of contaminated food can be estimated as the product of contaminant concentration in the consumed food and the amount of food consumed per day (Hammad and Manocha, 1995):

$$D_i = f_{raw} * M_{body} * M_i * c_{iw} * V_{eq} * \exp(-kt) \quad (7)$$

where

- D_i - daily consumed dose of contaminant, CFU/(ca*d).
- f_{raw} - fraction of fruits and vegetables eaten raw.
- M_{body} - human body weight, kg.
- M_i - daily consumption per capita per kg of body weight, g/(kg*ca*d).
- c_{iw} - virus concentration of irrigation water, CFU/l.
- V_{eq} - equivalent volume of irrigation water present in the crop g^{-1} .

The above expression allows to examine the combined effects of human consumption habits for vegetables and fruits as related to the applied wastewater quality and application method.

Figure 41. Distribution frequency of the annual infection risk for spray irrigation with complete effluent and 15 days time between harvest and consumption (C/si/15 scenario, based on 12,000 computer runs)

Results

Results of Monte-Carlo simulation

The EM was examined for various situations and conditions. A sample result for a one scenario C/si/15 (Table 45 - complete treatment effluent applied through SI, with 15 days elapsed time between irrigation and consumption) is presented in Figure 41. The results (Figure 41) exemplify the outcome of one numerical simulation of 12,000 computer runs. The frequency distribution diagram shows the number of computer runs that result in an annual risk of infection for various intervals. That preliminary stage is required primarily to assess the approximate number of computer runs which are employed for the entire examination of the EM for all other scenarios.

Scenarios of risks

The EM was further examined for various hypothetical situations which might be faced in practice in agricultural fields. These scenarios are combinations of irrigation methods, effluent qualities and various elapsed times. The sample results include the mean annual risk and related standard deviation, lower and upper confidence limits (Table 45). Consequently, some conclusions can be drawn:

Table 45: Annual risk of infection for nine scenarios based on 12,000 computer runs. All scenarios are based on an elapsed time between irrigation and consumption of 15 days

Scenario			Annual risk of infection			
Code*	Effluent type	Irrigation method	Average	Standard deviation	LCL	UCL
P/si/15	primary	spray	10^{-1}	10^{-1}	10^{-2}	10^{-1}
S/si/15	secondary	spray	10^{-2}	10^{-2}	10^{-3}	10^{-2}
C/si/15	complete	spray	10^{-6}	10^{-6}	10^{-7}	10^{-5}
P/di/15	primary	on surface drip	10^{-3}	10^{-2}	10^{-5}	10^{-2}
S/di/15	secondary	on surface drip	10^{-4}	10^{-3}	10^{-6}	10^{-3}
C/di/15	complete	on surface drip	10^{-8}	10^{-7}	10^{-10}	10^{-7}
P/sdi/15	primary	subsurface drip	10^{-4}	10^{-3}	10^{-6}	10^{-3}
S/sdi/15	secondary	subsurface drip	10^{-5}	10^{-4}	10^{-7}	10^{-4}
C/sdi/15	complete	subsurface drip	10^{-9}	10^{-8}	10^{-10}	10^{-8}

* - P - Primary; S - Secondary; C - Complete

* The range between the LCL and UCL is two orders of magnitude for SI, and three orders of magnitude for DI and SDI.

* The UCL for all scenarios with complete wastewater treatment is well below the USEPA drinking water guideline. The only other scenario meeting the guideline is the SDI with secondary effluent.

* Secondary treatment prior to the application for irrigation reduces the health risk of wastewater reuse by at least one order of magnitude.

* Complete treatment of effluent prior to the application for irrigation reduces the health risk of wastewater reuse by nearly four orders of magnitude.

* Drip irrigation involves a health risk lower by nearly two orders of magnitude in comparison with SI.

* Subsurface irrigation further reduces health risks by at least one order of magnitude compared to conventional drip irrigation.

Figure 42. The relationship between annual risk of infection (UCL) and wastewater treatment efficiency (based on 12,000 computer runs). The elapsed time between irrigation and consumption is for all cases is 15 days

The results obtained from this model show that several practical operational measures should be changed in order to reduce the annual risk of infection in wastewater reclamation project. Risk analysis only quantifies the health benefits of every operational change. The decision to take one operational measure can also be based on a socio-economic analysis (Haruvy, 1997).

Virus removal efficiency of the wastewater treatment system

Several simulation scenarios were examined for varying virus removal treatment efficiencies. Increasing the virus removal efficiency decreases the annual risk (UCL) of

infection (Figure 42). For an elapsed time of 15 days between irrigation and consumption, the virus removal efficiency must be 4 log₁₀ for SI, 3 log₁₀ for DI, and 2 log₁₀ or less for SDI in order to comply with the USEPA guideline ($< 10^{-4}$).

Figure 43. The relationship between annual risk of infection (UCL) and elapsed time between irrigation and agricultural products consumption (based on 12,000 computer runs). The wastewater treatment efficiency is constant at 2 log₁₀.

Elapsed time between final irrigation and agricultural product consumption

A range of scenarios was examined for various elapsed periods between irrigation and consumption, while virus removal efficiency in the wastewater treatment system remained constant at 2 log₁₀. By augmenting the time between the last irrigation and consumption it is possible to decrease the UCL of the annual risk of infection (Figure 43). For a set treatment efficiency of 2 log₁₀ and the conditions set, the elapsed time must be 20 days for SI, 13 days for DI, and approximately 11 days for SDI, according to the calculations, in order to comply with the USEPA guideline ($< 10^{-4}$).

Combining treatment efficiencies and elapsed times

The concluding modeling was used to examine a broad range of combinations of removal efficiencies in the treatment facilities and elapsed times. These were examined for the three main irrigation methods (Figure 44). One simulation was run for each combination of a virus removal efficiency between 0 log₁₀ and 6 log₁₀ (intervals of 1 log₁₀), and an elapsed time between 0 days and 30 days (intervals of 5 days). The results of the numerous simulation show the UCL of the annual risk of infection for SI, DI, and for SDI.

Figure 44. Relationship between annual risk, elapsed time between irrigation and consumption and virus removal efficiency of the wastewater treatment system for three irrigation methods (based on 12,000 computer runs)

Discussion and Conclusions

This study assesses the consumers' health risks due to use of agricultural products irrigated with reclaimed wastewater by numerical simulation with the MCSM. It focuses on the influence of wastewater treatment level, application method, irrigation timing, and consumers' behavior, on the exposure to enteric viruses.

A model for the assessment of risk of infection associated with wastewater irrigation of edible plants was developed. The model takes into account several criteria: (i) the quality of the applied wastewater, (ii) the irrigation method, (ii) the time between irrigation, harvest, and subsequent consumption, and (iv) consumers' behavior.

Risk assessment is a useful tool to upgrade reclamation schemes to acceptable health safety standards. An increase of the elapsed time between irrigation and consumption is the most effective operational change (one order of magnitude per five extra days). Other operational improvements are increasing the virus removal efficiency of the wastewater treatment (one order of magnitude risk reduction for each extra log of removal), and a switch from spray to DI and primarily to SDI (two to three orders of magnitude risk reduction).

There is a ten order of magnitude difference in the risk of infection between the different exposure scenarios with the same water quality. The large differences in risk of infection between exposure scenarios indicate the need for setting risk-based criteria for wastewater reclamation, rather than a single water quality guideline. Operational practices plays an important role in reducing risk by limiting exposure to pathogens.

The risk comparison in this study provides information regarding the relative risks. However, it does not provide a standard for risk acceptability. Setting acceptable risk limits that will ensure adequate public health protection is a much more complex task that also includes socio-economic considerations.

The annual risks of infection shows large deviations. These broad ranges are probably due to a mix of uncertainty and variability of data. Extra data are required to increase the quality of the input parameters to decrease uncertainty. The distribution of the output parameters then represents the diversity of the populations.

The application of sophisticated mathematical modeling frequently provides some levels of uncertainty. The outcome of a simulation can provide guidelines which, however, can only seldom be more accurate than the input data. Virus concentrations in wastewater vary from one site to another. The EEV approach for the uptake of viruses tries to by-pass this limitation along with the scarce available data. In addition, the information related to human behavioral and immunity is also scarce.

The exposure assessment contains less uncertainty than other phases of the risk assessment, especially dose-response modeling. Frequently, the dose-response models

may not be very representative for less advanced communities, such as infants and elderly. These models, however, can be transferable to different private locations after adequate modifications, taking into account immunity and disease tolerance characteristics of the community.

Future related research associated with pathogen risk assessment should include the followings:

- * Defining the acceptable risk levels.
- * Defining dose-response models considering the influence of local circumstances such as immunity on the human response to pathogens.
- * Obtaining additional information regarding occurrence and prevalence of pathogens in wastewater, and their removal in wastewater treatment.
- * Obtaining additional information regarding the paths of pathogens into and onto the crop through spray irrigation, on surface drip irrigation, and subsurface drip irrigation.

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