Microbiological assessment of food crops irrigated with domestic greywater

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Abstract

Two of the challenges facing Africa in the 21st century are effective use of restricted water resources and ensuring food security. The eThekwini Municipality is not exempt from these challenges and is investigating ways in which they can be addressed in the local context. One of the aims of national government has been to ensure the supply of clean drinking water and suitable sanitation to every household. As part of its efforts in this regard, eThekwini Municipality has introduced a multi-tiered water supply programme ranging from full-pressure water systems and flush toilets to stand pipes and dry toilets. In the dry sanitation systems, there is no real provision for the disposal of domestic greywater and, bearing in mind that South Africa is a water scarce country, the municipality is investigating ways of using this water as a resource rather than viewing it as a waste. A preliminary trial was set up in 2003 using this water to irrigate vegetables to be used for home consumption. The microbiological safety of this practice needed to be examined to ensure that it would indeed help to uplift communities by the provision of healthy food as well as provide an ecologically sound use and reuse of available water.

The initial proposal was to investigate total coliforms, *E. coli* and *Enterococcus* as the normal faecal indicators for health purposes as well as *Staphylococcus* as a human skin surface micro-organism and coliphage as a viral indicator. The first controlled field trial was put in at the test site at the University of KwaZulu-Natal in 2005 using several crop types and three irrigation regimes. The vegetable samples were examined both internally and externally for the selected micro-organisms. It was found that very few *E. coli* were detected and in later crops the micro-organism list was expanded and further identification of the total coliform organisms detected is being addressed. This paper presents the results from the analyses to date.

Keywords: greywater, irrigation, health risk, domestic crops

Introduction

A previous Minister of Water Affairs and Forestry expressed the wish for water in South Africa that there would be 'Some for all, forever'. As the Southern African region is recognised as a water scarce area, action needs to be taken to ensure that this wish is fulfilled. As a leader in the water supply arena in this country, the eThekwini Municipality was the first to implement the concept of 6 kℓ of free water per household per month. At the earliest stages of implementation of community upliftment, the feeling in the communities was that one of their highest priorities was to receive potable water; the aspect of sanitation came much further down on the list. This led to the situation where communities were receiving water but had no formal means of disposing of it after use. In many instances, water was thrown outside the door of the residence resulting in pooling and unsanitary conditions including the breeding of mosquitoes. In any sanitary intervention the Bellagio Principles (Hurst, 2002) need to be kept in mind to ensure acceptability and sustainability of the intervention. In addition, KwaZulu-Natal has the highest HIV positive rate in the country and the eThekwini area is certainly not exempt. In many cases, the family bread winner is either dead or dying, households are headed by children or aged grandparents and finances are at a sub-economic level. As a result, the nutritional status of the household can be extremely poor which results in increased susceptibility to disease and a further drain on resources. An opportunity was seen for the re-use of greywater which could result in an improvement in the living conditions of these families. In 2003, a preliminary trial was set up using greywater to irrigate above-ground vegetables to be used for home consumption. The crops produced appeared to be excellent and the community involved wanted to expand operations. There was some doubt, however, about the hygiene of crops grown under such conditions and whether there needed to be some restriction in the type of crop grown. A joint project between eThekwini Water and Sanitation and the University of KwaZulu-Natal was initiated to investigate these issues.

Experimental design

A semi-field trial plot was established at the University of KwaZulu-Natal in which it was originally planned that four crops would be planted. During the course of the year additional vegetables were added and the final selection was: spinach, green peppers, madumbis, potatoes, onions, beetroot and carrots. These were grown in plastic bags filled with sterile, low nutrient Berea red sand and drip-irrigated through a plastic bottle with either normal municipal tap water, greywater or a hydroponics solution.

Irrigation through plastic bottles pierced at the bottom was to prevent splash back contamination of above surface growth as far as possible. The vegetables were watered daily with 500 mℓ of the relevant solution and harvested at maturity, after approximately three to four months. A rep-

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Figure 1
Carrots and green peppers in trial bags irrigated through plastic bottles with greywater, tap water or hydroponics solution

resentative sample of each crop was dried, the percentage dry mass of each sample was calculated and used to calculate bacterial load per dry mass. Fresh samples were weighed and placed in 100 mℓ of sterile Ringer's solution in a sterile jar and shaken on a platform shaker at 220 r/min for 1 h. Aliquots of the water were originally analysed for total coliforms and *E. coli* using Merck Chromocult medium, for faecal enterococci using Slanetz and Bartley medium and for coliphages using SABS method 221990. Enterobacteriacae, *Staphylococcus*, *Pseudomonas,* coliphages and *Ascaris* were added to the analyses as the project progressed and analysed according to standard methods.

Vegetables for interior analysis were weighed, then washed and sonicated for 5 min in a 20% Jik and Tween solution to sterilise the exterior surface. They were then placed in sterile honey jars to which 100 mℓ of sterile Ringer's solution was added and were macerated using a food blender. Aliquots of this mixture were subjected to the same analyses as samples from the surface of the crops. Gram stains were done on individual isolated colonies for both internal and external analyses, followed by biochemical testing using API kits. Samples of the same vegetable types were obtained from local supermarkets and from a market and put through the same analytical procedures. Samples of the greywater and of the soil irrigated with greywater were analysed at the start and end of the first stage of the project.

Results

Average values of the chemical and microbiological analyses performed on the tap water, greywater and hydroponics solution at the start of the project are given below.

The nitrate levels were surprisingly low, but as they were accompanied by high ammonia levels, some reduction of nitrate to nitrite and then ammonia had probably already begun. The amount of time spent in storage tanks before use in the irrigation system is therefore important. Chloride levels were also markedly higher in the greywater than in either the tap water or the hydroponic solution. This could be expected to have an adverse effect on growth in the long term as soils might become salinised. The

TABLE 1
Mean chemical and microbiological analysis of irrigation solutions

Test	Unit	Tap water	Greywater	Hydroponic
Alkalinity	mg/ℓ $CaCO_3$	66	0	29
Ammonia (free)	mg/ℓ N	<0.50	157	32
Boron	mg/ℓ	<0.25	3.4	0.5
Cadmium	mg/ℓ	<0.05	<0.05	<0.05
Calcium	mg/ℓ	16	7.5	115
Chloride	mg/ℓ	35	220	12
Chrome	mg/ℓ	<0.10	0.14	0.1
Conductivity	mS/m	30	267	223
Copper	mg/ℓ	<0.10	0.1	<0.10
Lead	mg/ℓ	<0.05	<0.05	0.05
Magnesium	mg/ℓ	7.5	7.1	51
Nickel	mg/ℓ	<0.10	<0.10	<0.10
Nitrate + nitrite	mg/ℓ N	0.91	<0.1	88
Ortho phosphate	mg/ℓP	0.02	40	38
pH		7.4	8.1	6.3
Potassium	mg/ℓ	3.6	31	250
Selenium	mg/ℓ	<0.05	<0.05	0.08
Sulphate	mg/ℓ	15	137	576
TKN	mg/ℓN	<0.50	206	37
Total nitrogen	mg/ℓ N	0.84	206	125
Total phosphate	mg/ℓ P	0.05	69	49
E. coli	CFU/100 mℓ	0	35	0
Enterococcus	CFU/100 mℓ	0	>999	0
Phage	pfu/100 mℓ	0	0	0
Staphylococcus	CFU/100 mℓ	0	0	0
T. coli	CFU/100 mℓ	0	400 000 000	0

TABLE 2
Statistical significance of microbiological results from vegetables

	External				Internal			
	E. coli	**Entero**	**Staph**	**Coliform**	***E. coli***	**Entero**	**Staph**	**Coliform**
Carrots		ND	Tap irrigated sample significantly higher	ND	All 0	All 0	ND	Tap irrigated sample significantly lower
Spinach	ND	ND	ND	Tap irrigated sample significantly lower	All 0	Tap irrigated sample significantly lower	Hydroponicsolution significantly higher	Tap irrigated sample significantly lower
Madumbe	ND	Tap irrigated sample significantly lower	Greywater irrigated samples significantly higher	Hydroponic solution irrigated samples significantly lower	All 0	All 0	Greywater irrigated samples significantly higher	ND
Peppers	All 0	All 0	Greywater irrigated samples significantly higher	ND	All 0	All trial samples 0. Purchased samples significantly higher	ND	ND
Beetroot	All 0	Greywater irrigated samples significantly higher	Greywater irrigated samples significantly higher	ND	All 0	ND	ND	ND
Onions	All 0	ND	Greywater irrigated samples significantly higher	ND	All 0	All 0	All 0	ND

ND = no statistical difference in the results from each of the treatments.

phosphate levels were also high which indicated a potential risk of eutrophication of any water body into which the greywater might seep unless the nitrogen present was utilised. Boron levels were also high, which could result in toxicity to plants. Microbiologically, *Enterococcus* levels were markedly higher in the greywater than in either of the other solutions. This may be partially owing to the ability of this organism to survive for longer in the environment and also its ability to tolerate a more saline environment. The total coliforms were orders of magnitude higher in the greywater than in either of the irrigation controls. The soil used for potting was tested microbiologically at the start of the trial and was found to contain a high level of total coliforms but very low or non-detectable levels of the other organisms selected.

Control vegetable samples were purchased from commercial outlets targeting the high, middle and low sections of the economic market. These were treated in the same way as the samples grown in the experimental plot. All microbiological results were log-transformed before being analysed using ANOVA. The results obtained are presented in Table 2.

No coliphage or *Ascaris* ova were detected in any of the samples analysed.

As was expected, the coliform load was the highest in all the vegetables and madumbes showed the highest loading on their exterior. This is possibly because of the roughness of the external skin which provides and ideal niche for bacterial growth. It was unexpected to find relatively high levels of micro-organisms in the interior of the vegetables. This could have been due to insufficient disinfection of the exterior surface before homogenisation or to genuine uptake of organisms. This could not be clarified at this stage of the experiment but will be investigated as the project progresses.

Discussion

Of the vegetables examined, carrots, spinach, onions and peppers are frequently eaten raw. The risk to humans is therefore highest for consumption of these vegetables. In a first world environment, these vegetables would be thoroughly washed first, but in economically impoverished households, water for washing is scarce and the final result might not be as good as it should be. Onions and carrots are both usually peeled before eating, so the risk for these is reduced and only the internal contamination would be effective. The risk analysis has been worked using the *Enterococcus* and *Staphylococcus* results for each of the four vegetables mentioned and using a probability of infection per ingested organism of 0.00001 which is an overall estimated value for bacteria (Schertenlieb, 2005). In each case it was estimated that 100 g wet weight of the vegetable might be eaten raw on average once a week. These would give a worst case scenario as no pathogen is likely to be present at as high a level as either of the selected micro-organisms. The calculations are shown in Tables 3 to 9.

When the risks for the various treatments were compared using the ANOVA analysis, it was found that there was no significant difference between the treatments. Use of the greywater irrigated vegetable would therefore not appear likely to cause any additional disease within the community. Further work will be done to clarify the issue of organisms detected inside the vegetables and identify some of the numerous coliforms. Some estimate of actual pathogens may also need to be addressed. At this stage of the project it would appear that the reuse of greywater for the irrigation of vegetables would be of benefit to communities both nutritionally and economically. Environmental effects will however also need to be considered.

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TABLE 3 Estimation of risk in relation to *Enterococci* on carrots				
	Greywater	**Hydroponic**	**TAP**	**Bought vegetable**
Concentration of organisms per gram of vegetable (mean)	9	1	1	1
Mass ingested per day (dry mass)	35	35	35	35
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.08	0.009	0.009	0.009

TABLE 4 Estimation of risk in relation to *Staphylococcus* on carrots				
	Greywater	**Hydroponic**	**TAP**	**Bought vegetable**
Concentration of organisms per gram of vegetable (mean)	1	2	2	1
Mass ingested per day	35	35	35	35
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.009	0.018	0.018	0.009

TABLE 5A Estimation of risk in relation to *Enterococcus* on peppers				
	Greywater	**Hydroponic**	**TAP**	**Bought vegetable**
Concentration of organisms per gram of vegetable (mean)	5	1	1	1
Mass ingested per day	27.6	27.6	27.6	27.6
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.035	0.007	0.007	0.007

TABLE 5B Estimation of risk in relation to *Staphylococcus* on peppers				
	Greywater	**Hydroponic**	**TAP**	**Bought vegetable**
Concentration of organisms per gram of vegetable (mean)	4	2	2	1
Mass ingested per day	27.6	27.6	27.6	27.6
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.028	0.014	0.014	0.014

TABLE 6 Estimation of risk in relation to *Enterococci* on onions				
	Greywater	**Hydroponic**	**TAP**	**Bought vegetable**
Concentration of organisms per gram of vegetable (mean)	16	1	1	1
Mass ingested per day	28.64	28.64	28.64	28.64
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.117	0.0073	0.0073	0.0073

TABLE 7
Estimation of risk in relation to *Staphylococcus* on onions

	Greywater	Hydroponic	TAP	Bought vegetable
Concentration of organisms per gram of vegetable (mean)	1	16	1	2
Mass ingested per day	28.64	28.64	28.64	28.64
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.0073	0.117	0.0073	0.014

TABLE 8
Estimation of risk in relation to *Enterococci* on spinach

	Greywater	Hydroponic	TAP	Bought vegetable
Concentration of organisms per gram of vegetable (mean)	3	1	2	1
Mass ingested per day	14.02	14.02	14.02	14.02
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.011	0.0036	0.0071	0.0036

TABLE 9
Estimation of risk in relation to *Staphylococcus* on spinach

	Greywater	Hydroponic	TAP	Bought vegetable
Concentration of organisms per gram of vegetable (mean)	3	1	2	2
Mass ingested per day	14.02	14.02	14.02	14.02
Number of days ingested per year (mean)	52	52	52	52
Probability of infection	0.00001	0.00001	0.00001	0.00001
Probability of illness	0.49	0.49	0.49	0.49
Estimated annual risk of illness	0.011	0.0036	0.0071	0.0071

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