Health Risk of Growing and Consuming Vegetables Using Greywater for Irrigation

By

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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 especially for poor communities. In line with these aims, the eThekwini municipality has introduced a multi-tier system of water supply ranging from full pressure reticulated systems along with flush toilets to standpipes and dry toilet systems. In the latter case, it was soon recognized that the disposal of greywater presented a problem. Bearing in mind that South Africa is already a water scarce region, research was initiated into finding means of using this water as a resource rather than as a waste. Initial on-site trials using the greywater to irrigate crops proved popular and it was then regarded as necessary to test the possible health effects on the communities of such a system.

A controlled field trial using pot plantings of a selected range of edible vegetables was initiated at the University of KwaZulu-Natal. Crops were tested both internally and externally for a range of indicator and potentially pathogenic organisms. Quantitative Microbial Risk Assessment (QMRA) techniques were used to assess the health risk to communities from growing and eating the greywater- irrigated vegetables. Although there was a health risk related to most of the activities, especially the handling of the greywater itself, the risks could be brought within the World Health Organisation guidelines of less than one case of disease per 10 000 people per year by the implementation of simple barrier interventions. The greywater irrigated crops themselves, did not present a statistically higher risk of infection than the crops irrigated with either hydroponic solution or tap water. These findings show the importance of applying QMRA to each case to determine health risk. This would allow the productive use of greywater and other water sources in the correct circumstances, thus providing food sustainability for people who currently do not have access to the levels of high purity water currently recommended for agriculture.

PREFACE

The experimental work described in this dissertation was carried out at the laboratories of eThekwini Water and Sanitation Scientific Services, Durban from November 2004 to December 2009, under the supervision of Dr N. Rodda and Dr D. Muir.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 – PLAGIARISM

I, Siobhan Ann Forbes Jackson declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This dissertation does not contain any other person's writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced
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5. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the Reference sections.

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DECLARATION 2 – PUBLICATIONS

DETAILS OF THE CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation

Salukazana, L., Jackson, S., Rodda, N., Smith, M., Gounden, T., Macleod, N. and Buckley, C. (2005). Plant growth and microbiological safety of plants irrigated with greywater. Paper presented at the 3rd International Conference on Ecological Sanitation, 23-26 May, Durban.

Jackson, S., Rodda, N., Salukazana, S. and Macleod, N. (2006). Microbiological assessment of food crops irrigated with domestic greywater. Paper presented at the biennial conference of the Water Institute of South Africa, 21-25 May, Durban.

Jackson, S., Rodda, N and Salukuzana, L. (2006). Microbiological assessment of food crops irrigated with domestic greywater. Water SA 32(5) pp. 700-704

Jackson, S., Muir, D. and Rodda, N. (2010). Use of domestic greywater for small-scale irrigation of food crops: health risks. 11th Waternet/WARFSA/ Symposium

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- The staff and students of eThekwini Water and Sanitation Scientific Services for all their help with obtaining the bacterial data. I am sure if you never see a blended vegetable again, it will be too soon!

DEDICATION

To the memory of my beloved brother-in-law Peter, murdered in his home on 2nd June 2008.

You lived a life of Christian goodness, sharing your immense knowledge, humour and wisdom with all you met and your worldly goods with those in need. In your retirement you dedicated yourself to teaching disadvantaged youth in Diepsloot and were a staunch member of the Saint Vincent de Paul Society in Rosebank.

Every student should have a friend and teacher like you at least once in their lives. You said you would not come to another of my graduations until my gown changed colour – I am not there yet, and I don't know if I have it in me to make it to the end, but I will do my best.

Rest in peace great man.

LIST OF ABREVIATIONS

BOD_5	Five day biological oxygen demand
cfu	Colony forming units
COD	Chemical oxygen demand
mS/m	milli-Siemens per metre
NH_3-N	Ammonia concentration as nitrogen
NO_3-N	Nitrate concentration as nitrogen
PFU	Plaque forming units
QMRA	Quantitative microbial risk assessment
TKN	Total Kjeldahl nitrogen
E. coli	*Escherichia coli*
BOD	Biological oxygen demand

1. INTRODUCTION

"The key lies in asking the right questions, challenging existing paradigms and ultimately, changing the way we think about water" (Turton 2008)

1.1 Study context

The world is becoming increasingly aware that water is a vital resource, not to be squandered or abused. In many areas, the availability of clean water is a critical issue and economic development is being delayed as a result of its lack (CSBE, 2003; Adewumi *et al.*, 2008). South Africa is not immune to these problems as its rainfall level of 497 mm/year is well below the global average of 860 mm/year (Turton 2008). Dr Kader Asmal, a previous South African Minister of Water Affairs and Forestry, in his budget speech to the National Assembly in 1997 and in his keynote address to the Stockholm Water Symposium in 1999, expressed the wish with respect to water that there would be "*some for all forever*". This seemingly simple wish is not as straightforward as it sounds when burgeoning populations, climate change and increasing levels of pollution are taken into account. South Africa is widely regarded as being a water scarce country (Adewumi *et al*, 2008; Turton, 2008). It must be recognised that this generous wish may therefore not be realised unless every drop of water is carefully husbanded, both for human use and for the environment. According to the National Water Resource Strategy report (DWAF, 2004a), around 98% of the total South African national water resource had been allocated, with over-allocation in some areas being as high as 150%. These figures were based on data from 1998 and may have worsened since: according to data from the Department of Water Affairs and Forestry quoted in Discussion Document D0405, (StatsSA, 2009), the water balance for the year 2000 was 186 million cubic meters and the prediction for 2025 is a deficit of 234 million cubic meters. It is therefore vital to change the thinking on water use and reuse.

With the change of political dispensation in 1994, it was recognized that the vast majority of South Africa's citizens did not have access to adequate services and that this would need to be rectified. The provincial statistics indicate that Gauteng has the largest share of the national population with 21.4%, closely followed by KwaZulu-Natal with 21.2% (StatsSA, 2009). The rapid urbanisation seen in South Africa and particularly in the eThekwini area (previously

Durban Metro) has resulted in informal areas of varying degrees of sophistication, from shacks built from cardboard boxes and plastic sheeting to more permanent corrugated iron and brick structures. Population growth in the eThekwini municipal area, resulting from urbanisation, natural growth and migration, has led to difficulties between socio-economic groups in terms of service provision, with many seeing the "norm" of waterborne sanitation as the only acceptable service level for all economic strata. Informal settlements, poverty, unemployment and water demands are on the increase and a culture of non-payment for and vandalisation of services threatens the economic sustainability of service provision, especially the provision of potable water (Moodliar, pers. comm. 2009)[1]. Moreover, uncontrolled wastewater disposal in informal and peri-urban settlements and rural areas on the outskirts of the city is a problem that impacts negatively on the environment and public health. In line with the guidelines set out by the Department of Water Affairs and Forestry (DWAF, 2004a), progress has been made in the supply of potable water to these areas, but often the provision of sanitation lags behind. When strategic development plans were being discussed for the Metropolitan Integrated Development Plan, a great deal of effort was put into obtaining feedback from communities with regard to what they saw as the most urgent needs in terms of service provision (Pfaff, pers. comm., 2008[2]). It was soon found that generally, the most urgent need with regard to service provision was seen as access to potable water. Originally, the provision of sanitation did not make it into the top five.

With this in mind, the eThekwini Municipality was the first municipality in the country to implement the free delivery of six kilolitres of potable water per household per month (N. Macleod pers. com[3]. with reference to eThekwini water Services Development Plan (Ethekwini, 2003). This has now been increased to nine kilolitres per month. This delivery ranged from the full pressure delivery common in the urban centres, to semi-pressure roof tanks, ground tanks and community standpipes. According to Winter *et al.,* (2008 a and b), 92% of the South African population now has access to an improved source of potable water but only 65% has access to improved sanitation. For eThekwini Municipality, the provision of water and sanitation is also affected by the topography of the region. The area rises steeply from sea level in the east and, in the western areas in particular, soon becomes very steep and inaccessible. Partially as a result of this and with available logistics in mind, a "waterborne edge" has been instituted which delineates the area to which waterborne sanitation can be provided, at least in the short term (Pfaff, pers. comm. 2009[4]). With the easing of access to

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treated potable water came the problem of disposal of sewage and greywater in areas outside the waterborne edge or in areas which did not have sewerage infrastructure. The full pressure systems within the previous urban areas had waterborne sanitation with the necessary infrastructure of sewerage pipes, pump stations and wastewater treatment plants, but in many informal settlements and rural areas there was no means of disposing of sewage and greywater other than into the latrine or onto the surrounding ground. In higher density settlements this soon led to malfunctioning latrine-type toilets or health hazards from surface pooling of discarded used water with the resulting increase in flies, mosquitoes and other vectors and the concomitant increase in risk of disease. An example of these conditions is presented in Figure 1.1, a photograph taken at an informal settlement in the eThekwini area.

Figure 1.1: Photograph taken by eThekwini staff of surface disposal of greywater leading to poor sanitary conditions.

This is not a problem peculiar to eThekwini and was investigated by Carden *et al.*, (2007 a and b) in relation to the Western Cape where conditions in the Khayelitsha settlement were discussed. Development of a better sanitation system, which would address the problems of greywater disposal, was therefore seen as a priority in eThekwini and became the focus of several Water Research Commission projects.

In any sanitary intervention, the Bellagio principles (Hurst *et al.*, 2002; WHO, 2006a) need to be kept in mind to ensure acceptability of the intervention and long term economic and environmental sustainability. These principles were drawn up in 2000 by the Environmental Sanitation Working Group of the Water Supply and Sanitation Collaborative Council and are stated as four points, covering:

- Human dignity, quality of life and environmental security at the household level
- Decision making involving all stakeholders

- Consideration of waste as a resource with holistic management
- Keeping the resolution of environmental sanitation problems to the minimum practical size

Any intervention would therefore have to address more than just the obvious one of waste removal. Interventions would need to explore possibilities which could result in closing the water loop, and at the same time improving the condition of the community. In addition, the United Nations Millennium Development Goals clearly state that extreme poverty and hunger must be eradicated, child mortality reduced, maternal health improved, HIV/AIDS, malaria and other diseases must be combated, and environmental sustainability ensured (www.un.org).

KwaZulu-Natal arguably has the highest rate of HIV infection in the world (Cullinan, 2004; Thurlow *et al.*, 2009) with a level of 36.5% antenatal prevalence, and as such its decision-makers have to be very aware of the increased health risk faced by people infected with this disease and the effect on families of the loss or incapacity of breadwinners. In many cases, the parents of a family are bedridden or have died and the household is headed either by an elder sibling or a grandparent, usually a grandmother (Biancallani, pers. comm., 2010)[5]. Disposable income is negligible and the risk of malnutrition is high. As such, food security is vital and any improvement in household access to nutritious food at low cost should be encouraged so long as the health risk can be minimized, as those suffering from the disease are extremely susceptible to other infections. The possibility of turning the problem of greywater disposal into a means of improving the nutritional situation of households was viewed with interest by both the municipality and local communities. In 2003, a pilot trial was started in a small community in eThekwini where household greywater was used to irrigate above ground crops for household consumption (T. Gounden, 2003. pers. comm.[6]). On visual inspection, the crops appeared to do well and the community was interested in extending their enterprise. It was however recognized that there might be health risks associated with growing crops in this manner and investigation was necessary before official sanction could be given to a larger project or range of crops. A joint project to investigate this was therefore initiated between eThekwini Water and Sanitation and the University of KwaZulu-Natal. This dissertation forms part of a larger project conducted under the auspices of the Water Research Commission as Project K5/1639.

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1.2 Greywater

Greywater is often regarded as untreated household effluent water from baths, showers, wash basins and laundries but not including toilet water (Otterpohl *et al.*, 1997; Dixon *et al.*, 1999; Ledin *et al*., 2001; Eriksson *et al*., 2002; Ottoson and Stenström, 2003; WHO, 2006a and b; Rodda, *et al*, 2010 in press). Some authors however, exclude kitchen wastewater from the general greywater as a result of its high concentration of oil and biodegradable compounds (Christova-Boal *et al*., 1996; Little, 2002; Al-Jayyousi, 2003; Wilderer, 2004). Because domestic greywater does not generally include wastes from toileting, except by cross-contamination, it is expected that, while it may still contain pathogens (Cassanova *et al.*, 2001; Birks and Hills, 2005), these are likely to be lower than for black water (Ottoson, 2005; WHO 2006a; Brown, 2009). Greywater will therefore present less health risk to communities using it for irrigation. The World Health Organisation guidelines for greywater re-use (WHO, 2006a and b) also present a spectrum of bacterial loading figures for greywater which show that the standard faecal indicator organisms are present at a range of levels in the various types of greywater and also within the same type from a different source. These data are presented in Table 1.1 and the use of faecal indicators is presented in Section 1.7.1. Greywater is remarkable for its variability and Eriksson *et.al* (2002) clearly present this. It is also likely to contain chemicals from household cleaning agents and medications as well as cosmetics, all of which may contribute to toxicity or produce endocrine disrupter by-products.

1.3 Drivers for re-use

Nationally, the re-use of greywater is unlikely to solve problems of water scarcity as the volumes involved are relatively small in the overall picture. In eThekwini however, approximately 60% of the potable water sold is used domestically (S. Moodliar 2009, pers. comm.[7]), so widespread re-use could have an influence on the local need for increased spending on water purchases and infrastructure such as impoundments, reservoirs and piping. For individual households and even small communities, water re-use can mean the difference between food security and malnutrition. EThekwini Municipality has increased the cost of water by approximately 10% for the 2010 – 2011 financial year and this will impact poor households in spite of the current level of 9kL free water per household (EThekwini, 2010).

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Table 1.1: Reported numbers of indicator bacteria in greywater (Adapted from Ottoson and Stenström, 2003 as used in WHO, 2006a). Different values for greywater were given by the various authors and reflect different conditions and habits

Greywater origin	Numbers of indicator bacteria (log numbers / 100 mL)			
	Total coliforms	**Thermotolerant coliforms**	***Escherichia coli***	***Enterococcus***
Bath, hand basin			4.4	1.0 – 5.4
Laundry	3.4 – 5.5	2.0 – 3.0		1.4 – 3.4
Shower, hand basin	2.7 – 7.4	2.2 – 3.5		1.9- 3.4
Greywater	7.9	5.8		2.4
Shower bath	1.8 – 3.9	0 – 3.7		0 – 4.8
Laundry, wash	1.9 – 5.9	1.0 – 4.2		1.5 – 3.9
Laundry, rinse	2.3 – 5.2	0 – 5.4		0 – 6.1
Greywater	7.2 – 8.8			
Hand basin, kitchen sink		5.0		4.6
Greywater, 79% shower	7.4	4.3 – 6.9		
Kitchen sink		7.6	7.4	7.7
Greywater		5.8	5.4	4.6

Worldwide, there is a movement towards addressing the increased demand for water while at the same time trying to cope with the decreasing availability of clean, inexpensive water sources. In the past, water was often viewed as a renewable but finite resource, but it has been suggested (Turton, 2008) that it is in fact a flux with almost infinite options for use, limited only by innovation. This means that previously, water was regarded as a stock item which was used in various ways and eventually became depleted much as the feedstock in an industrial process. What Turton (2008) suggests is that water can be used and treated for re-use in an almost endless cycle. Across the globe, advances have been made in exploiting hitherto untapped sources, developing new industrial practices which use less water in production, reducing demand through pricing structures and in recycling (Forster, 1997). In the urban environment, particularly in Africa, the demand for water can be high and where poor communities are involved, the financial burden of increased pricing can become intolerable, with such communities sinking into extreme poverty. As such, these communities need to investigate ways of using water as fully as possible and to their greatest benefit, before disposal.

According to Winter *et al.*, (2008a; 2008b) it can be assumed that greywater accounts for virtually all the water brought onto an un-sewered site and that the volumes and quality involved increase with increasing affluence. Erikson *et al.,* (2003) and Friedler and Hadari

(2006) suggest that 50 to 80% of the water going to waste is accounted for by greywater, excluding kitchen waste, while Siegrist *et al.*, (1976) assessed a level of 65% and Brown (2009) suggests a level of 60%. Re-use of such water could therefore have a marked impact on the finances of an impoverished household even though, at the levels officially supplied to such areas, it might not have an impact on water savings in the South African national context. Many countries, notably China, India and others in the east, have for centuries recycled and re-used domestic effluents successfully (Mara and Cairncross, 1989; Ahmed *et al.*, 2003). According to Adewumi *et al.*, (2006) the attitude of the public in South Africa towards water re-use still needs to be thoroughly assessed as there is resistance.

In the Chinese case, greywater has been viewed as a resource delivering both vital water and nutrients rather than as an unpleasant waste requiring removal (Mara and Cairncross, 1989). According to Redwood (2008), greywater re-use in developing countries is most often linked to improving domestic food security. Re-use also lends itself to the informal settings often encountered in the developing world.

It is now generally accepted worldwide that there is climate change which has led to more extreme weather patterns. These in turn have resulted in increasing frequencies of drought and floods and a concomitant decrease in food crop production (Stockle *et al.*, 1992; Rosenzweig *et al.*, 2002; Jones and Thornton, 2003). In addition, in many parts of the developing world, there has been a shift from subsistence farming to growing cash-crops, most recently crops for the production of bio-fuel, and this, along with prolonged droughts and severe floods, may have contributed to increased food scarcity and increasing food prices (Woods Institute, accessed July 2010).

Countries such as Sweden and Denmark have addressed the issues of water re-use nationally and there is a functioning system for delivery of greywater to commercial agriculture (Ottoson and Stenström, 2003). This greywater has been treated before reuse. This is largely not the case in the developing world, where greywater re-use is still generally restricted to use by the generating household or, at most, the immediate small community (Morel and Diener, 2006). With rising food costs worldwide and the advent of child- or elderly relative headed households as a result of the AIDS pandemic, such home or community use can however have a marked impact on communities, both financially and in terms of nutrition and health. This is especially true in South Africa where 17% of the population aged 15 to 49 lives with AIDS and there are an estimated 1.91 million AIDS orphans (StatsSA, 2009). However, for re-use to be successful in the South African setting, a change in perceptions of sanitation practices and the use of water containing human wastes needs to occur (Friedler and Hadari, 2006; Adewumi *et al.*, 2008).

1.4 Guidelines for re-use in terms of health impact

The World Health Organization recognized the need for guidelines in the use of greywater in order to protect both the health of consumers and of the environment. They therefore published guidelines for the use of greywater (WHO, 1989, 1993, 2005 and 2006a). These were further interpreted by Mara and Alabaster (2006). In general, the guidelines offered a framework for the re-use of greywater and a way of determining its suitability for use. The guideline presented three key elements:

- Health risk assessment
- Risk management guidance
- Ways of implementing the guidelines

They largely moved away from relying solely on microbial loadings towards an assessment of health risk (Mara and Alabaster, 2006). Whilst the implementation of these guidelines will probably result in a better actual health outcome for the communities using the greywater, monitoring to ensure effective control has become more complex and for managers of such schemes, the application of simple field tests is probably no longer sufficient to give the information sought. The guidelines have also recognized that there are differences both in microbial loadings and in cultural acceptance of the handling and re-use of greywater and in environmental impacts across varying communities and countries. The drive for re-use and its success will therefore vary geographically and culturally and re-education for acceptance will be necessary. The guidelines use the Stockholm Framework (WHO, 2006a) which proposes that individual countries develop their own risk-based management systems based on health outcomes. Figure 1.2 illustrates how this could be addressed. This flowchart presents a simple cyclic system for evaluating the health risk of greywater re-use and the steps that can be taken to make such a system acceptable from a human health point of view. The cycle can be entered at almost any stage, but a useful starting point is the establishment of what is regarded as a tolerable risk. Although a zero risk is generally what would be regarded by the general public as the ultimate goal, this is unachievable practically and risk is therefore often based on the World Health Organisation target for chemical carcinogens of less than 1 case per 10 000 persons per annum (WHO, 2006a). Once a target has been agreed by the stakeholders, it is possible to calculate backwards from the risk of infection to an acceptable quality of water for the project, the health target.

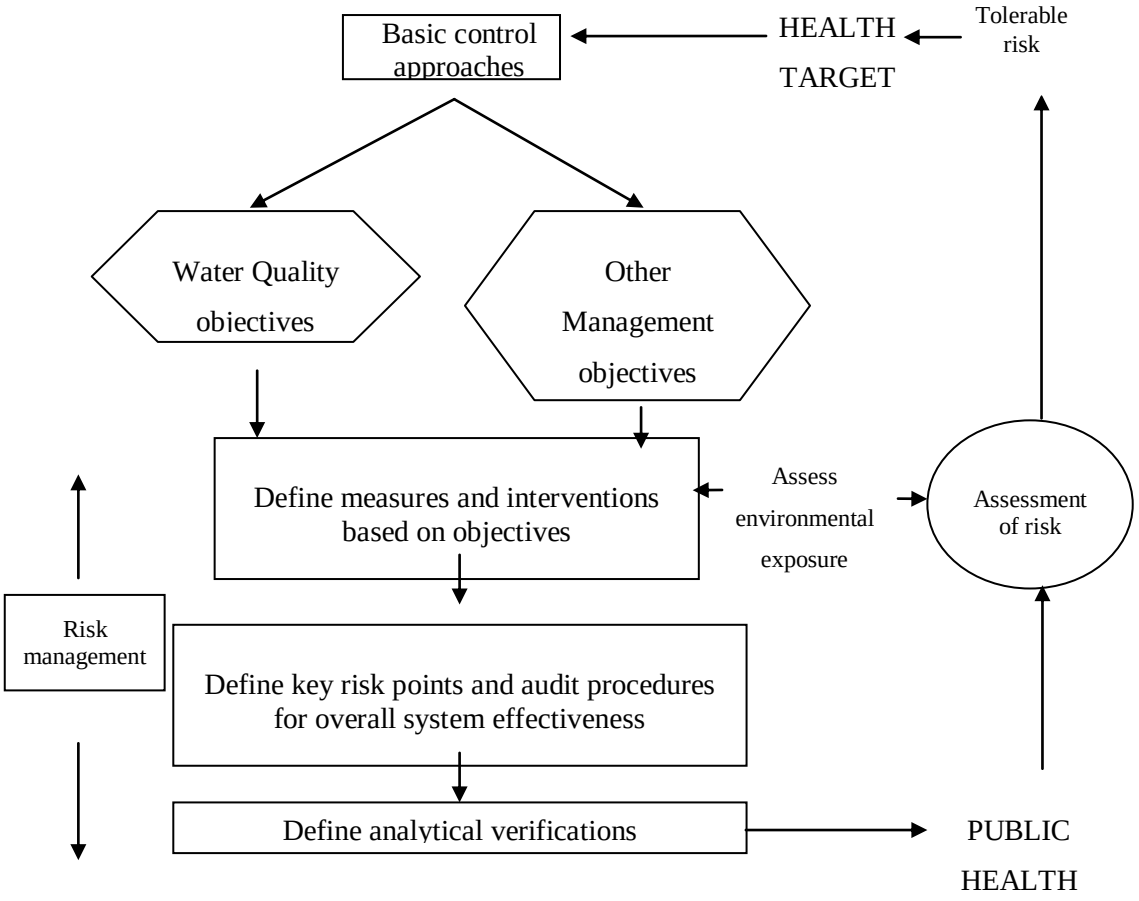


Figure 1.2: **Flowchart of risk management adapted from WHO, 2006a**

This health target must then be linked to the management objectives of volume of water available for use, crops required *etc*. The re-use system is examined for critical control points at which verification (either analytical or visual) can occur to ensure that the targets are achieved. As the users become more familiar with the system, it is then possible to reassess both the outcomes and the control points and measures, and so to tailor the system to the needs of the community applying them.

1.5 Environmental impacts

Greywater is defined, for this project, as including water from laundries and kitchens, and it is therefore expected to contain nutrients such as nitrogen and phosphorus from the detergents in use as well as from the breakdown products of proteins and lipids from skin cells and laundry stains such as are found in blood and food (Eriksson *et al.*, 2003; Winblad and Simpson-Hébert, 2004). Uncontrolled disposal of such water in a way which permits ingress into surface or

underground water sources could therefore increase nutrient loading, or even lead to eutrophication of these water sources (Morel and Diener, 2006). This in turn would lead to increased costs for the purification of water to potable standard and decrease the availability of water suitable for meeting the needs of the environment and the recreational, food and aesthetic requirements of the local community. This would run counter to the spirit of the South African National Water Act (DWAF, 1998) which requires that an environmental reserve is maintained and that a water body remains fit for use. The degradation of a water source further impacts poor communities, leading to an ever steepening decline into poverty, disease and squalor. According to Winblad . and Simpson-Hébert, (2004), greywater can also be expected to contain metals such as aluminium and compounds containing elements such as boron which are found in various washing powders and cleaning agents. The levels are not however expected to differ markedly from those in mixed household wastewater. In addition, there is a certain loading of fats and other readily biodegradable compounds from laundry, bathing and kitchen activities which could lead to soils becoming salinised or made hydrophobic when greywater is used indiscriminately (Morel and Diener, 2006). These aspects are not addressed in this dissertation as they form the basis of another research project currently being conducted under the auspices of the University of KwaZulu Natal.

1.6 Greywater re-use

Greywater has been used unofficially by communities throughout the world, either as a normal practice or during times of drought or flood. There is however debate as to whether or not this practice should be officially sanctioned. For this to happen in any particular circumstance, the benefits should outweigh the costs or risks.

1.6.1 Benefits of greywater re-use

As mentioned in Section 1.5, it is estimated that between 50 and 100% of the water brought onto an unsewered site is converted to greywater which requires disposal. When this disposal is controlled, benefits in terms of health protection, food security, protection of fresh water sources and reduction in fresh water demand can accrue. In some developing countries such as Vietnam, Jordan and Mali, greywater may be disposed of into road-side drains, and thereafter into the local surface water, impacting on community and environmental health (CSBE, 2003; Morel and Diener, 2006). According to Faruqui and Al-Jayyousi, (2002) households in Aman,

Jordan using greywater for irrigation enjoyed savings of USD 376 per annum as a result of increased crop yields and decreased water and fertiliser costs. Redwood (2004) states that a 36% saving in water bills was enjoyed by households in Cyprus which re-used greywater. Studies in New Zealand showed that an on-site greywater system recouped its initial costs in less than nine years, even before government incentives were taken into account (Brown, 2009). According to Brown (2009) no incidents of illness as a result of greywater re-use have been recorded in the literature and the incidence of indicators in greywater does not present the same health risk correlation that is seen for sewage. He goes on to say that "*Many more direct pathways for infection exist within the population, which is not significantly more exposed when greywater is recycled within the individual lot*." This lower likelihood of risk from greywater re-use is supported by the WHO (2006a), which states that pathogen levels in greywater can be over-estimated by a factor of 1 000 when indicators are used. Brown (2009) also quotes the Australian guidelines (EPHC, 2006) as stating that smaller scale greywater re-use systems present less health risk than larger scale systems.

1.6.2 Quality of greywater / wastewater irrigated produce

Microbial indicators of greywater quality are to be found alongside those for domestic wastewater (sewage) in several publications (Cassanova *et al*., 2001; Ottoson and Stenström, 2003; Winblad and Simpson-Hébert, 2004; Ottoson, 2005; Birks and Hill, 2005; Winward *et al*., 2007) and are generally found to be lower than those for sewage. This supports the premise that greywater re-use presents less risk than the re-use of untreated domestic effluent.

The health risk from vegetable crops irrigated with greywater is closely linked to the microbial standard of the water, the type of vegetable and the weather conditions. The World Health Organisation states that inactivation of pathogens is often more rapid on crops and in soils than in the stored greywater (WHO, 2006 a and b). These guidelines go on to state that pathogen inactivation is more rapid in hot, sunny conditions than in cold ones and that the greatest health risk is to be expected from salad crops, root crops and crops grown in close proximity to the ground. Some crops also appear to have a greater susceptibility to contamination than others (Armon *et al*., 2002; Solomon, *et al*, 2002; Blumenthal, 2003). According to Keller *et al*., (2008), lettuce irrigated with polished wastewater was found to comply with the regulations for food microbial quality set by the National Health Vigilance Agency of Brazil. Jacobs and van Staden (2008), reported that from nineteen surveys conducted on the re-use of greywater for irrigation, none reported a detrimental effect on vegetable growth. Shuval *et al.*, (1997) reported differences in the amount of irrigation water retained by lettuce and cucumber

respectively and Stine *et al.*, (2005) reported that cantaloupes and lettuce retained pathogens from irrigation water whereas bell peppers did not. Several references in the guidelines also stated that pathogen levels could be reduced below those found on market products through barriers such as the withholding of re-use water irrigation for one to two weeks immediately prior to harvesting (Bastos and Mara, 1995; Armon *et al.*, 2002)

1.7 Health risk assessment

For every human activity there is a degree of risk involved. Some of these, such as the use of cell phone technology, are accepted almost without thought as being well within acceptable levels. Others, such as car racing, have been accepted as high risk to those involved and are avoided by most people and discouraged by insurance companies. Perceptions of risk, especially health risk, can vary over time and what was once considered an acceptable risk becomes unacceptable as more knowledge becomes available. Public perception is also not always based on fact and emotions have to be acknowledged as having a role to play in what the public perceives as an acceptable or unacceptable risk. For most health risks, an assessment can be made either from epidemiological data or through the application of quantitative microbial risk assessment (QMRA).

Epidemiology assesses health risk of an activity by comparing the levels of disease in subjects exposed to the presumed risk with levels in those who are unexposed. Such studies are not always possible, either because of the ethics of exposing subjects to serious risks or through the scale and expense of study that would be necessary to obtain the required information (Haas, *et al.*,1999). For the information obtained from an epidemiological study to be useful, careful matching of subjects and control of extraneous factors which could confound the results has to be maintained. Bearing these difficulties in mind, it is unsurprising that epidemiological evidence is often not available for low profile activities such as greywater re-use. QMRA is a more indirect route of obtaining the risk information. Four steps are involved in the risk assessment via this route and these are presented in Table 1.2. Using these steps, predictions can be made as to how a population will react to various levels of exposure to a pathogen or toxicant. Figures on dose response can be accessed from previous studies and combined with the exposure and dose characteristics of the scenario of interest. The assessment becomes more and more accurate as figures for person-to-person transmission, immunity, duration of illness and other factors are used in the calculations. Not all of these figures are readily available for all pathogens or community susceptibilities, but enough are available to make the use of the

QMRA tools extremely useful in decision making. Even when only the basic information on level of exposure and dose-response ratios is available, QMRA can provide very useful information on the acceptability of various scenarios and the effect of alterations to steps involved in them such as the introduction of barriers to infection.

Table 1.2: Steps involved in QMRA of human health effects. (From WHO, 2006 a)

Step	Aim
Hazard identification	To describe acute and chronic human health effects associated with any particular hazard, including pathogens or toxic chemicals
Hazard characterisation	Dose-response assessment, to characterise the relationship between various doses administered and the incidence of the health effect, including underlying mechanisms and extrapolation from model systems to humans
Exposure assessment	To determine the size and nature of the population exposed and the route, amount and duration of the exposure
Risk characterisation	To integrate the information from exposure assessment, hazard characterisation and hazard identification steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty

1.7.1 Hazard identification

The pathogens expected in greywater are similar to those to be found in sewage although at a decreased loading (Cassanova *et al*., 2001; Ottoson and Stenström, 2003; Winblad and Simpson-Hébert, 2004; Ottoson, 2005; Birks and Hill 2005; Winward *et al*., 2007 and Winward *et al.*, 2007). As greywater does not contain the same faecal loading as sewage / black water (Winblad and Simpson-Hébert, 2004; WHO, 2006a), the risk of contracting disease through contact with the greywater is not expected to be as high as that for sewage. However, there could still be some risk and this should be quantified before extensive re-use is advocated by any official body such as a municipality. Table 1.3 presents the most common microorganisms to be expected in faeces, (Ottoson and Stenström , 2003; WHO, 2006a) and hence likely to be found in greywater contaminated with even a small amount of faecal matter. The actual pathogens found will vary according to those diseases endemic in the community, any current epidemics, the season of the year, microorganisms present on the skin and in body fluids, contaminants contributed by the washing and preparation of food and the general health status of the community. It must be remembered that most pathogens will only be excreted by patients exhibiting symptoms or by symptomless carriers of the disease and are therefore likely to be present in greywater at low levels (Ottoson, 2005). As pathogens may be difficult or expensive to detect, it has been common practice to use indicator organisms such as *E. coli* instead. (Gerba, 2000; Roesner *et al*., 2006).

Table 1.3: Pathogens that may be excreted in faeces along with their related diseases. Extracted from (WHO, 2006a) and (Ottoson, 2005).

Group	Pathogen	Disease and symptoms
Bacteria	*Aeromonas* sp	Enteritis
	Campylobacter jejuni/coli	Campylobacteriosis,
	Escherichia coli (EIEC, EPEC, ETEC, EHEC)	Enteritis
	Salmonella typhi/paratyphi	Typhoid/paratyphoid fever
	Salmonella spp	Salmonellosis
	Shigella spp	Shigellosis
	Vibrio cholerae	Cholera
	Yersinia spp.	Yersiniosis
Viruses	Enteric adenovirus 40 and 41	Enteritis
	Astrovirus	Enteritis
	Calicivirus (Incl norovirus)	Enteritis
	Entero virus types 68-71	Meningitis –
	Hepatitis A virus	Hepatitis
	Hepatitis E virus	Hepatitis
	Poliovirus	Poliomyelitis
	Rotavirus	Enteritis
Parasitic protozoa	*Cryptosporidium parvum*	Cryptosporidiosis
	Cyclospora cayetanensis	Often asymptomatic
	Entamoeba histolytica	Amoebiasis
	Giardia intestinalis	Giardiasis
Helminths	*Ascaris lumbricoides* (roundworm)	Ascariasis
	Taenia solium/saginata (tapeworm)	Taeniasis -
	Trichuris trichiura	Trichuriasis
	Ancylostoma duodenale /Necator americanus (hookworm)	Itch, rash. Cough, anaemia, protein deficiency
	Schistosoma spp	Schistosomiasis – Bilharziasis

A good indicator organism should always be found in high numbers where faecal contamination is present, but be absent elsewhere. It should have similar characteristics to the pathogens of interest and it should be cheap and easy to analyse (Ottoson, 2005). It has become the norm that indicators from the most likely groups *e.g.* bacteria, viruses, protozoans and helminths are included as far as possible. The most common indicators for faecal contamination in wastewater have been the coliforms and this practice has been followed for greywater as well. In addition to total and thermotolerant (previously termed faecal) coliforms and *Escherichia coli* (*E. coli*), enterococci such as *Enterococcus faecalis* and *Enterococcus faecium* and various bacteriophages have also been used as indicators (Rose *et al.*, 1986; Christova-Boal *et al.*, 1996; Ottoson and Stenström , 2003; Rose *et al.*, 2004). No single indicator can give all the information required, and using a suite composed of representatives of bacteria, viruses, protozoans and helminths provides a more complete understanding of the

possibility of the presence of pathogens. The levels of the organisms chosen as faecal indicators are likely to be far in excess of that of the pathogens described in Table 1.3. As greywater by definition contains a lower faecal loading than blackwater (sewage), it is expected that generally most of the faecally excreted pathogens will be below detection limits of the routine methods of analysis. From Table 1.3, it can also be deduced that the most likely health hazard from exposure to greywater is diarrhoea, possibly accompanied by vomiting although other disease outcomes are possible.

1.7.2 Dose response assessment

Health risks are generally assessed using epidemiological studies of disease outbreaks or the response of volunteers in controlled studies. In the latter case, healthy volunteers and less virulent strains of pathogens are generally used to determine a level at which fifty percent of the subjects become infected (ID_{50}). There is therefore no allowance for differences in infectivity of environmental strains or increased sensitivity of the host as a result of pre-existing disease. The results of these studies are not directly applicable to communities without some mathematical modelling taking place (Haas *et al.*, 1999). It is therefore necessary to integrate other scientific fields into the results obtained from epidemiology.

The discipline of Quantitative Microbial Risk assessment (QMRA) is used to enable integration of information from a variety of sources to model potential health risk. Various models have been applied to microbial risks with the beta-Poisson distribution found to fit viral as well as many bacterial infection data (Haas *et al.*, 1999). Other distributions such as the Gaussian, log-normal, negative binomial or exponential have all been used to describe the distribution of microorganisms. It is therefore necessary to have enough data available to fit a distribution for the microorganism of interest, as the assumption that all microbial data fit a log-normal distribution is not necessarily correct (Haas *et al.*, 1999). The limitation for the risk assessment models is the availability of sufficient data on specific organisms, dose/response rates and the outcome of infection in a range of human subjects. Where such data are not available, data from similar organisms or suitable indicators are used. This usually results in a worst case scenario calculation.

The discipline of Quantitative Microbial Risk Assessment is relatively young but extremely powerful. It was originally applied to chemical toxicants (Haas *et al.*, 1999) In this case, knowledge of the dose per exposure and the number of exposures was sufficient to calculate the risk of a toxic outcome. However, microbiological infection does not follow such a simple

route, with individual organisms having varying infectivity, a range of susceptibility in a community and the possibility of secondary infection all influencing the apparent overall risk. It was previously considered that, similar to many toxic chemicals, there was a threshold value for microbial pathogens below which no consequence was likely. The independent-action hypothesis, in which a single organism can cause disease, has however been found to represent the actual findings more closely (Haas *et al.*, 1999). According to this hypothesis, the presence of even one viable microorganism can lead to infection, particularly in the case of persons with low immunity such as the very young, the elderly and those with HIV/AIDS or who are otherwise immune-compromised. According to Haas *et al.*, (1999), the likelihood that one microorganism will evade the host's defences and initiate infection is however very small. For some of the indicators used in risk assessments, it is recognized that there can potentially be reproduction in the environment, particularly in the case of *E. coli* and *Enterococcus*. This is unlikely to be the case for pathogens which require a host for multiplication. In such cases, the indicator count is likely to give an over-estimation of actual risk from pathogens.

Each organism has its own infection rate (Peterson and Ashbolt, 2002). This implies that, for any given population of microorganisms, the probability that each individual organism will result in infection ranges over a continuum from a zero probability to certainty of infection. For the exposed population, there are also various states that can be occupied by an individual. With regard to specific disease organisms, a person may be immune or susceptible, may be infected and exhibiting symptoms or be a silent carrier, may have recovered and have a degree of immunity or have acquired immunity through vaccination or genetics. In addition it is possible for a patient exposed to the primary source to infect others and there may already be a risk of infection in the environment other than the primary source under investigation (Roesner *et al.*, 2006). The risk calculations for microbial infections are therefore more complex than those for chemical toxicity, often requiring a dynamic risk assessment model as opposed to a static one. The concept is represented in Figure 1.3. In this figure, the various states in which a member of a community may be situated as well as the sources of infection are represented by boxes. The lines connecting the boxes indicate transition from one state to another. In each case the symbols on the connecting lines represent the rate at which conversion from one state to another occurs. The parameters β_1 and β_2 are respectively the rate of conversion of a susceptible person to an exposed one as a result of exposure to pathogens in the wastewater or the normal environment. The rate of conversion from exposed to carrier status without having shown symptoms of disease is represented by α, and P_{sym} is the rate of conversion to actual diseased status. The diseased person can in turn convert to a second carrier status at a rate δ. The conversion from either of the carrier states to a post-infection state is performed at a rate σ.

A person who has been infected and who has recovered may be protected for a period as a result of immune reactions but converts to a susceptible state again at a rate γ. All these rates are particular to the individual involved and may vary considerably (Roesner *et al.*, 2006). Standard values for these factors are available (Soller, 2006) and are frequently used when they have not been characterised for the population under review.

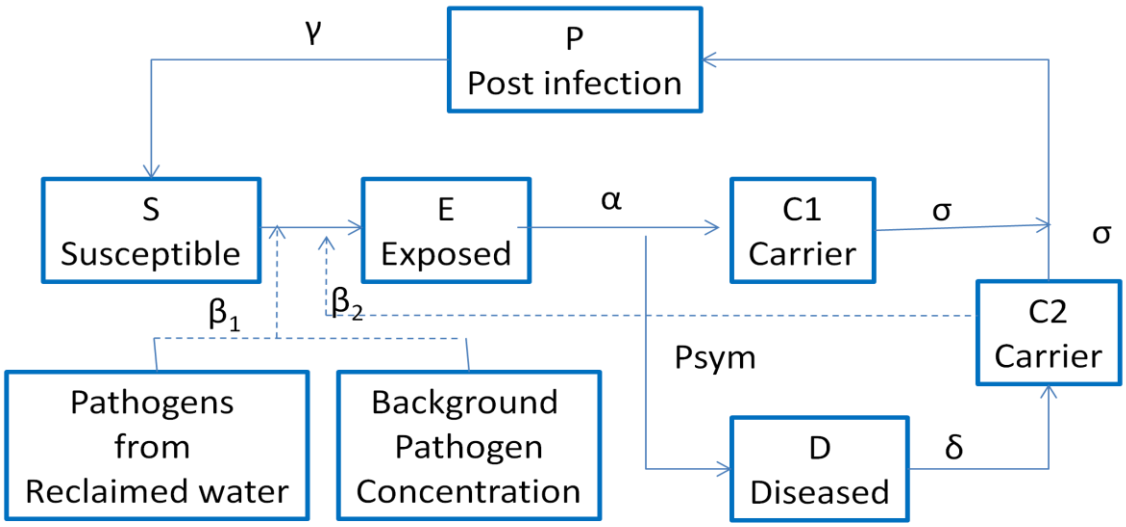


Figure 1.3: Dynamic risk assessment conceptual model from Roesner *et al.*, 2006

The dynamic model is often simplified into the static model where only the susceptible and infected states are recognized. This model is represented in Figure 1.4 where P(d) is the probability of an individual moving from the susceptible to the diseased state, controlled by the dose and infectivity of the pathogen under review.

In this model no cognisance is taken of the probability of degrees of susceptibility to infection or of the risk of secondary infection in a population. It is clear from these two figures that far more information is required to build the mathematical model for the dynamic model than for the static model. The information required for modelling the processes is given in Table 1.4**.**

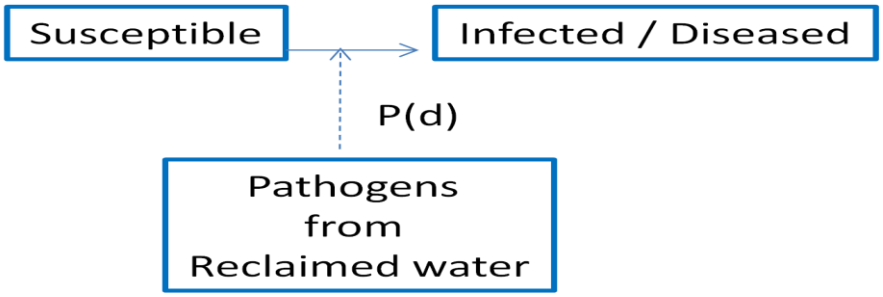


Figure 1.4: Static risk assessment model from Roesner *et al.*, 2006

Table 1.4: Parameters required for modelling either the static or dynamic disease process. From Roesner *et al.*, 2006

Class	Parameter	Static model	Dynamic model
Exposure related parameters			
	Concentration of pathogen	X	X
	Volume of water ingested	X	X
	Proportion of population exposed		X
	Frequency of exposure		X
Pathogen related parameters			
	Dose response parameter/s	X	X
	Duration of incubation		X
	Duration of infectiousness		X
	Duration of disease		X
	Duration of protection		X
	Probability of symptomatic response		X
	Person-to-person transmission potential		X
	Background concentration level		X

According to the World Health Organisation (WHO, 2006a), with reference to Blumenthal and Peasey (2002), there are very few data on epidemiological studies related to the use of greywater in agriculture. It is however, not always necessary to acquire all the information required for the dynamic model as, in some circumstances, the difference in risk calculated by the two models is insignificant. According to Soller (2006), the difference in prediction between the two models is most strongly influenced by:

- Dose of pathogen
- Exposure intensity
- Dose-response parameter β
- Dose response parameter α
- Duration of infection

It is important to be able to determine whether or not the information gained from a static model is sufficient for making sound decisions. Figure 1.5 gives one example of how this may be done. In this figure, columns three to five represent the dose levels of the pathogen being investigated, the rows present the exposure intensity, the shape, (circle, square or triangle) show the β dose-response parameter and the red colour represents the conditions resulting in a low difference between the model predictions, whilst the white is cases where the difference is not low according to the grid definition. The green indicates special requirements.

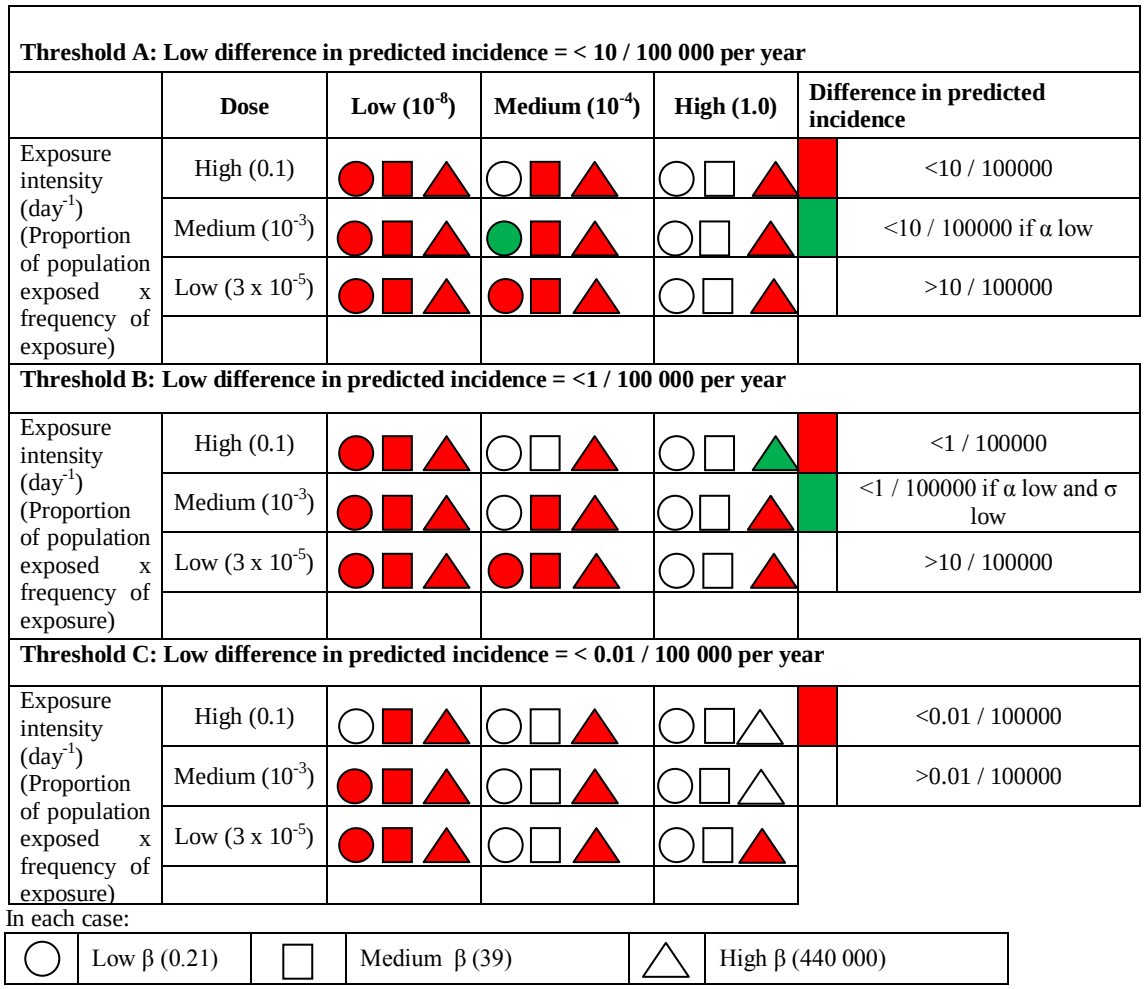


Figure 1.5: Graphical summary of model evaluation from Soller, 2006

Using this grid in Figure 1.5, decisions can be made as to whether or not it is necessary to use the dynamic model or whether the more easily calculated static model will give enough information for decisions to be made. For example, in a case where the exposure intensity is approximately 0.1, β is medium and the dose is medium, the static and dynamic models will give similar results with differences of less than 10 per 100 000 persons. However, at the level of 1 or 0.1 persons per 100 000, the differences become significant. If a difference in assessment of less than 0.001% is acceptable, the less onerous static model may therefore be

used in this case with acceptable results. This tool does not take into account the reaction when more than one infectious agent is present (Soller, 2006). In order to compare the severity of different diseases, use is often made of disability adjusted life years (DALYS). These compare the severity of the diseases by taking into account the number of days lost through premature death or disability as compared to the normal life span free of disease. To make use of this system, it is necessary to have data on the life expectancy of a community, the average duration of a disease and its severity (WHO, 2006a). This is not always available.

1.7.3 Exposure assessment

In the re-use of greywater for crop irrigation, the possibility of exposure to risk occurs when the greywater is collected, when it is used to irrigate the crop, when the soil is tilled, at harvesting of the crop, at preparation for consumption and at consumption (WHO, 2006a and b). Studies at each of these hazard points have been conducted internationally for treated wastewater and generally acknowledged pathogen reduction figures are presented in Table 1.5. Most of the research on which these figures are based has been done in temperate or cold climates and may therefore not give as high a reduction as could be expected in a warmer, sunnier climate. It is also recognized that greywater contains readily biodegradable organic compounds and as such may support the re-growth of some of the indicators generally used to estimate the faecal load (Ottoson and Stenström, 2003; WHO, 2005). The indicator loads may therefore give an inflated estimate of health risk.

Table 1.5: Reduction of pathogens on crops through use of barrier interventions. Extracted from WHO, 2006a

Control measure	Pathogen reduction (log_{10} units)	Notes
Pathogen die-off (withholding time)	0.5 – 2 day^{-1}	Dependant on temperature, sunlight intensity, humidity, crop type etc
Produce washing with water	1	Washing salad crops or fruit with fresh water
Produce disinfection	2	Washing salad crops, vegetables, or fruit with a weak disinfectant solution
Produce peeling	2	Fruits, root crops
Produce cooking	6 - 7	Immersion in boiling or close-to-boiling water until food is cooked

In order for infection to occur, subjects must be exposed to the infective agent and therefore knowledge of the route of exposure and the concentration of organisms involved is required. A

means of detecting the organisms is necessary for assessment. There are various methods for the detection of the organisms used as indicators as well as for the detection of pathogens themselves. Each has its benefits and drawbacks and these are thoroughly covered in the standard works such as the Standard Methods published by the American Water Works Association (APHA/AWWA, 2005). It is rare that pathogens are examined directly in wastewaters and it is more usual for indicators to be used (Gerba, 2000; Rose *et al.*, 2004; Roesner et al., 2006).

The most studied microorganisms are the bacteria which have been used as predictors of potential health risk for decades. The most commonly used bacterial indicators are the coliforms, with the focus gradually narrowing firstly from total coliforms to the thermotolerant coliforms and then to *Escherichia coli* itself as being the coliform most closely linked to the presence of faecal matter (APHA/AWWA, 2005). *Enterococcus* sp are now also often used (Rose *et al.*, 1986; Christova-Boal *et al.*, 1996; Ottoson and Stenström , 2003; Rose *et al.*, 2004). In classical studies, the ability of these microorganisms to grow on or in selective culture media such as agars or broths has been used. These media have become more specific and selective with time.

Originally the ability to ferment lactose in a defined period at a defined temperature formed the criterion of identification of *E. coli* (APHA/AWWA, 2005). Current cultural techniques are now often based on the expression of enzymes peculiar to the coliforms or *E. coli* itself such as β-galactosidase and β-glucuronidase respectively (APHA/AWWA, 2005) This has allowed the identification process to be accelerated from several days to less than 24 hours. Media utilising the expression of these enzymes for identification purposes are produced by several companies such as Merck and IDEXX. Such techniques are also available for enterococci and some other organisms of interest in health risk analysis, where other specific enzymes are targeted (APHA/AWWA, 2005). These methods have the advantage of being relatively inexpensive and simple enough to be performed routinely in most water microbiology laboratories. For many of the bacteriological pathogens themselves however, pre-enrichment followed by enrichment and then selective culture are still needed for the classical analyses (APHA/AWWA, 2005) and therefore results are qualitative rather than quantitative. Examples of this are *Salmonella* sp., *Shigella* sp. and *Vibrio cholerae*. This enrichment process is time consuming but many of these analyses are also performed routinely by water laboratories. For the development of the databases of exposure, this time factor is not of importance, but it could prove critical in the identification of an outbreak of illness in a community.

The culture-based techniques allow the examination of large volumes of sample when filtration, immune-capture or centrifugation is used to capture the organism of interest. The greatest drawback of the cultural techniques is that they are not available for all organisms of interest, particularly the helminths and protozoa, as well as many viruses. In some cases, the organism has not as yet been grown in culture (e.g. *Norovirus*) and in other cases the organism can go into a viable but non-culturable state in which it can still cause infection but is un-culturable by standard techniques, e.g. *Vibrio cholerae* (Hurst *et al.*, 2002).

The tissue culture technique required for the identification of human enterovirus is exacting and requires specialized tissue culture facilities unavailable to most basic laboratories. For this reason, indicator virus such as somatic or F-specific coliphage or the phages of other faecally-linked bacteria are regularly used as indicators of potential entero-viral contamination (APHA/AWWA, 2005). Other techniques such as the polymerase chain reaction (PCR), immune-capture and fluorescent *in situ* hybridization (FISH) are available at more sophisticated research laboratories. As these techniques were not available for this study, they are not discussed further here.

Whilst each of the above techniques could be used in the assessment of exposure, it is likely that classical culture techniques will continue for some time as the backbone of analysis, perhaps supported by more sophisticated techniques where possible. As indicators are generally used instead of pathogens, a relationship between the two needs to be used to estimate health risk. Brown (2009), states that indicators overestimate the presence of pathogens by approximately 1 000 times, whilst according to Hamilton *et al.* (2007b), when wastewater was used as an aerosol to irrigate crops, no increased health risk was attributed to waters with 10^4 to 10^5 total coliforms L^{-1}, implying a ratio of less than one pathogen per 10^4 indicator organisms. According to Peterson and Ashbolt (2002) bacterial and viral pathogens normally range between 10^5 and 10^{10} per gram of faeces and Mara and Horan (2003) state that *E. coli* is present at levels of 10^7 to 10^9 whilst pathogens are only intermittently present at similar levels. Ottoson (2005) presents levels of 10 to 10^4 for *Salmonella* in sewage along with levels of up to $10^{5.4}$ for *E. coli* and $10^{2.4}$ to $10^{4.6}$ for *Enterococcus* in greywater. In this dissertation a conversion factor of 10^{-2} has been used for *E. coli* and *Staphylococcus*, a factor of 10^{-4} for total coliforms, and *Enterococcus* has been considered as being present at approximately equivalent levels to pathogens in order to give a very conservative conversion to potential pathogens.

1.8 Exposure routes

According to the World Health Organisation (WHO, 2006a) there is a risk of exposure at several points in the greywater irrigation system and these are presented in Table 1.6. The consumption of the final crop is considered a major potential area of risk and vegetable consumption levels for the South African situation have been reported (Nel and Steyn, 2000) and an extract is presented in Table 1.7. Not all vegetable crops have been assessed in the latter publication and, where this is the case, for this project, data from vegetables with an apparently similar consumption rate have been used to model potential exposure.

Table 1.6: Exposure to greywater in irrigation system extracted from (Ottoson, 2005) and (WHO, 2006a)

Exposure	Health-related modelling units involved	Volume ingested
Accidental ingestion of greywater (one time exposure)	Water in retention pond	1 mL/exposure
Ingestion from a field irrigated with greywater (26 exposures)	Survival on plants	1 mL/exposure
Ingestion / inhalation of aerosols	Spray irrigation	$e^{-4.2\pm2.2}$mL

It can be expected that the health risk presented by the consumption of various crops will vary as both the actual mass and the percentage of the population consuming it on a daily basis vary markedly as can be seen in Table 1.7. These factors can be entered into the QMRA model and the effect of alterations in each can be assessed.

Table 1.7: Food and water consumption patterns in South Africa. From (Nel and Steyn, 2000)

Food/drink ingested	Food consumption in SA g/person/day	% of pop. consuming item	Comment
Water	2000	100	common assumed consumption of 2L/person/day
Cabbage	17.4	73.8	cooked
Onions	2.5	12.8	raw
Swiss chard	9.2	27.4	cooked
Carrots	3.9	30.8	cooked, flesh and skin
Potato	45.4	27.1	cooked

For a system using greywater to irrigate vegetables, the various exposure routes all have to be assessed and their influence needs to be assessed for different groups within the community. The first risk would be from handling the greywater, either in collection or in handling it during irrigation. This will only affect those members of the community involved in the practices and, through secondary infection, those with whom they come in contact. A broad outline of the

risk can be obtained by using point estimates such as the mean, mode, 95th percentile or maximum levels of indicators or pathogens in the water in the model for infection. A better estimate is obtained when distributions are fitted to the microbial data and this distribution is used. The model becomes successively more complex – and more accurate – as distributions are used for the microbial load, the probability of infection from primary exposure and secondary exposure, the probability of a range of susceptibility to infection and the rates of conversion from infection to disease. These models should be built for each route of exposure so that the final risk to an individual is a complex combination of a variety of exposure routes and distributions. Even for the first stage of exposure – that of exposure to the greywater – the probability of exposure alters dramatically depending on how the irrigation is conducted. The highest risk would be from mist spray irrigation as the contamination could be inhaled both by the workers themselves and by those within aerial exposure range. Exposure would also occur from skin contact and ingestion, each of which could have different distributions for risk of infection. A reduction of risk would occur from reducing aerial contact. This is done through preventing aerosolisation through utilising surface flow irrigation, drip or subsurface irrigation. The next area of risk would be from handling the soil during the raising of the crop. Again, the levels of the microorganisms of interest need to be modelled with distributions that take into consideration the die-off in the soils as a result of predation, temperature, water availability and other variable influences which affect microbial survival or infectivity. This route of exposure would only be applicable to the crop workers and their immediate contacts. Further exposure would occur from post-harvest handling of the crops, and crop consumption. This latter route is again varied depending on whether the crop is consumed raw, thoroughly cooked or treated in any other way such as pickling. Different distributions will apply to each of these routes.

1.8.1 Parameters required for modelling

Many of the values required for modelling are available from literature and some of these are presented in Table 1.8. In addition to these pathogen-related parameters, information about the dosage and frequency of exposure is required. For many years it was accepted that although microorganisms were particulate and therefore were not normally distributed in nature, performing a logarithm transform would give an approximately normal distribution. This is no longer always considered to be the case and other distributions such as the Poisson are considered more suitable as they consider non-continuous events as is the case with microorganisms (Haas *et al*, 1999).

Table 1.8: Pathogen dependent parameter values from Soller, 2006

	Enterovirus	**Rotavirus**	***Salmonella***	***E. coli* O157H7**	***Shigella***	**Composite**
Duration of incubation (day^{-1})	0.286 - 8	1.33- 4	1.333-16	0.33-8	0.571-4	0.067-16
Proportions of symptomatic responses	0.25-0.75	0.1-0.45	0.14-0.4	0.23-0.5	0.29-0.5	0.1-0.75
Duration of infectiousness (day^{-1})	0.133-4	0.2-0.5	0.011-0.267	0.14-0.31	0.4-0.8	0.011-4
Duration of disease	0.19-4	0.364-2	0.143-1.333	0.133-0.6	0.16-2	0.04-4
Duration of protection (day^{-1})	0.06-0.133	0.0037-0.011	0.14-96	0.052-0.11	0.005-0.1	0.0037-96
Dose response α	0.126-0.5	0.126-0.5	0.3126-0.89	0.221	0.21	0.126-0.89
Dose response β	1.26-76.16	0.21-0.84	2883.95-440000	8 722.5	42.86	0.21-440 000
Person to person transmission	0.042	0.06	0.003	0.0269	0.041	0.039
Average point prevalence	0.00016	1.4×10^{-5}	10	0.01	0.0005	0.00055

The first piece of information that is normally obtained is the dose of the organism per exposure. In the case of irrigation of crops using wastewater, this has usually been based on a mean or 95th percentile value for a sample of the entire population of the crop, assuming a log-normal distribution. Other distributions can be used and both the exponential and the inverse-Gauss may be appropriate. In most instances of risk assessment, statistical software is used to fit the most appropriate distribution to the data and the resulting information is then used for further manipulation (Havelaar *et al.*, 2003; Hamilton *et al.*, 2007a). In addition to the microbiological data, information on exposure routes and levels is also required. This is obtained from the exposure assessment.

1.8.2 Risk characterisation

According to the World Health Organisation (WHO, 2006a), risk characterisation "*integrates the information from the hazard identification, hazard characterization and exposure assessment to estimate the magnitude of the public health problem*." A probability of infection is calculated based on the data available and relating the probability either to the population as a whole or to the exposed population only. Point estimates are based on single values such as the mean or 95th percentile of the microbial concentration, but more accurate analyses are performed using values selected randomly from the distribution according to statistical probability. This is known as stochastic sampling and results in a distribution of probability of infection which allows for an understanding of the ranges and most probable values of

probability of infection under the defined circumstances. Probabilities can also be related to single exposure or annual risks. Once again various statistical distributions are used to perform this calculation depending on the organism chosen as the pathogen or indicator and the availability of data, but the exponential or β-Poisson are the most common (Soller, 2006) . Use of distribution data in the calculations allows for the range of data and therefore provides a better estimation of risk than the use of a single information value such as the mean or 95th percentile which was often used before such software packages were readily available.

1.9 Research question and hypotheses

This dissertation addresses the health risk inherent in the production and consumption of crops irrigated with greywater using a sub-soil irrigation system

The hypotheses to be tested were as follows:

Hypothesis 1: There is no difference in the microbiological quality of crops irrigated below the soil with greywater and those similarly irrigated with water of a non-waste origin.

Hypothesis 2: The health risk to handlers and consumers of crops irrigated sub-surface using greywater can be easily brought within the World Health Organisation guidelines.

1.10 Aims

Two interacting projects were undertaken by two registered students, each as research towards a Masters degree; one addressing the growth of crop plants irrigated with greywater and the other investigating the microbiological quality of the crop produced. The overall aims of the present study were:

- to investigate the occurrence of faecal indicator organisms and some selected pathogens in and on an array of vegetables irrigated sub-surface with greywater,
- to determine the differences in levels of contamination between sub-surface greywater-irrigated vegetables and those similarly irrigated with a non-wastewater source,
- to determine any differences in faecal indicator organisms and pathogen load between different types of vegetables,

- to assess if there was a health risk to community members from handling, growing or consuming these vegetables.

1.11 Delineations and limitations

The study applies only to situations similar to those found in the informal settlement investigated. In other areas of South Africa and elsewhere, where access to potable water is not as easy as was the case here, the microbiological condition of the greywater may be significantly different (Carden *et al.*, 2007 a and b) and the results obtained here will not necessarily apply. In addition, the residential sites would need to be of similar size to those investigated to ensure that greywater dispersal was adequate.

It is impossible to analyse for all the potential organisms that might be present in the greywater (Peterson and Ashbolt, 2001). A selection was therefore made based on endemic disease in similar communities, standard faecal indicators which could be analysed relatively quickly and cheaply and organisms likely to be found in greywater or on human skin which could result in infections in immune-compromised persons. In all cases, the analyses were restricted to those techniques which were readily available in the laboratory hosting the project and were based on standard culture-based methodologies. As such, no cognisance has been taken of enteric and other viruses which have the potential to cause severe disease (Ottoson and Stenström, 2003).

A small number of similar vegetables was purchased commercially and put through the same processes as the crops grown at the test site with the three different water treatments. These were intended to provide a non-statistically tested baseline of what the contamination, and hence health risk, was from current practices. A statistical evaluation was not attempted as there could be neither knowledge nor control of factors other than the irrigation waters used in the production of these crops which could have affected the results.

1.12 Outline of dissertation

The remainder of this dissertation is outlined as follows:

Chapter Two outlines the methodology used for this investigation.

Chapter Three presents the results of the investigation.

Chapter Four presents a discussion of the results in the context of the literature

Chapter Five outlines the conclusions and recommendations.

2. METHODS

2.1 Experimental setup

2.1.1 Site selection

Cato Crest is a peri-urban settlement, located in Durban, in close proximity to the University of KwaZulu-Natal, Howard College campus (Figure 2.1).

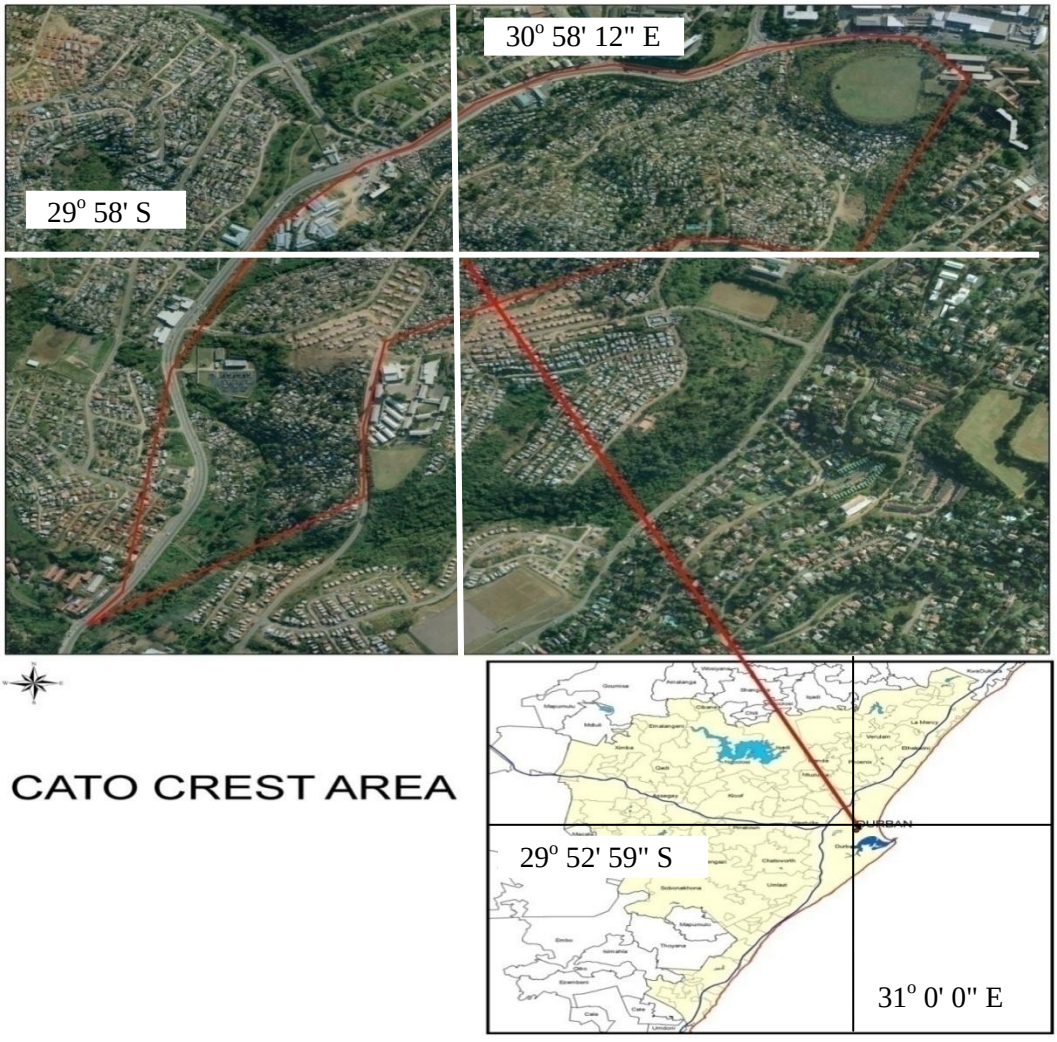


Figure 2.1: Location of the Cato Crest sampling site. (eThekwini Water and Sanitation, GIS Department)

Potable water is supplied to households in this area in 200 L ground tanks Figure 2.2) that are refilled daily with the free potable water household supply. Eight households from which

greywater was to be collected were selected by means of a questionnaire survey aimed at identifying household composition, different sources of greywater and user habits. The total number of residents in the experimental group was 53, comprising 37 adults (18-100 yr), 12 children (2-18 yr) and 4 infants (0-2yr). Selected households were supplied with 20L plastic buckets to collect their greywater. These plastic buckets were collected by eThekwini Water and Sanitation on a daily basis from Monday to Friday and delivered to the test site at the University of KwaZulu-Natal, Howard College campus.

The test site consisted of a disused tennis court, surrounded on four sides by diamond mesh wire fencing to which medium weight green shade cloth was attached. Greywater from various households was mixed in one 200 L container prior to use for irrigation in order to obtain an overall average of the greywater in the community. The test site was plumbed by eThekwini Water and Sanitation to receive both potable water and greywater for use in irrigation of the trial (Figure 2.3). Supervision of the site was done by Lumka Salukazana, a registered M.Sc. student at the University of KwaZulu-Natal.

Figure 2.2: 200L potable water ground tank

Figure 2.3: Greywater storage tanks at pilot site

2.2 Greywater characterisation

Before using greywater for irrigation, a full physico-chemical and microbiological characterisation was conducted in triplicate. Analyses were conducted at the laboratories of eThekwini Water and Sanitation according to the methods as presented in Standard Methods (APHA/AWWA, 2005) unless stated otherwise. The microbiological methods are detailed in Chapter 7, Appendix 1. Physico-chemical analysis included 5-day biological oxygen demand (BOD_5) performed using Merck Oxy-top analysis, chemical oxygen demand (COD) by microwave (Milestone) digestion using potassium dichromate, pH using a meter (Crison),

nitrate as nitrogen (NO_3-N) and ammonia as nitrogen (NH_3-N) both using sequential flow analysis (Lachat), total Kjeldahl nitrogen (TKN) using Buchi digestion and conductivity using a meter (WTW). Microbiological analysis, in triplicate, included examination for *Escherichia coli* and total coliforms using membrane filtration onto Merck™ Chromocult agar (Chapter 7, Appendix 1, Section 7.2), *Enterococcus* by membrane filtration onto *Enterococcus* selective medium (Biolab; Chapter 7, Appendix 1, Section 7.3), coliphages according to the double layer agar technique (Grabow, 1984; Chapter 7, Appendix 1, Section 7.7), *Pseudomonas* by membrane filtration onto Cetrimide agar (Biolab), (Chapter 7, Appendix 1, Section 7.4) and *Ascaris lumbricoides* according to the standard South African procedure (Archer *et al.*, 2006; Chapter 7, Appendix 1, Section 7.1). Analyses for *Shigella* and *Salmonella* were conducted using the standard enrichment procedure from Standard Methods (AWWA, 1999; Chapter 7, Appendix 1, Section 7.5).

2.3 Crop selection, setup and harvesting

Representative leafy, rooted and above-ground crops were chosen, based on the selections generally made by the community members for their domestic consumption, and plants were grown in 2 L black plastic bags at the test site.

The final crop selection was as follows:-

Above ground	Leafy -	Swiss chard (*Beta vulgaris* subsp *maritima*)
	Non-leafy	Green peppers (*Capsicum annuum*),
		Chillies (*Capsicum frutescens*)
Below ground		Carrots (*Daucus carota*),
		Beetroot (*Beta vulgaris*),
		Onion (*Allium cepa*)

Swiss chard and green peppers were chosen as representative above-ground crops. Swiss chard represents a potentially high risk crop as the entire above-ground plant is harvested for consumption and it is sometimes eaten raw as a salad vegetable. Green peppers are also often used raw in salads but are smooth-skinned with a waxy coating which repels water and only the fruit is harvested for consumption. They were therefore regarded as being of lower risk.

Beetroot and potato were considered as being low risk as they are rarely eaten raw whereas carrots and onions were considered as high risk as they are often eaten raw in salads. It was also considered that onions might present the highest health risk as their structure, a multilayered bulb, would lend itself to the entrapment of bacteria or parasites.

A simple manual drip irrigation system, initially implemented in preliminary field trials conducted by eThekwini Water and Sanitation, was adapted to a smaller scale for purposes of this study. Plastic bottles (500mL) were punctured several times through the base, and the bottles buried to approximately half to three-quarters of the length of the bottle in each plant bag (Figure 2.4 and Figure 2.5). Water was administered by pouring carefully into each bottle, ensuring that irrigation occurred below the soil surface. This differs from the method used in the field only in the size of container used. The field study typically used 20 L containers, supplying more than one plant each. Supervision of the irrigation was done by L. Salukazana, a registered MSc student at the University of KwaZulu-Natal. Three experimental treatments were investigated. Standard municipal tap water, containing no added nutrients, served as a negative control; water amended with a commercial hydroponics medium served as the positive growth control and the mixed greywater was the experimental treatment. Plants were watered daily with 500 mL of the respective treatments, with the exception of the positive control. In accordance with usage instructions, the hydroponics medium was applied once weekly and tap water was used on the remaining days for the positive control plants. The plants were set up in individual blocks per crop type, with the different treatments set side by side in columns. Supervision of the treatments was done by L. Salukazana. The trial was run over a two year period, allowing for examination over two summer and two winter seasons. In addition, vegetables of the same type were accessed from local commercial outlets for comparison purposes.

Figure 2.4: Example of the technique used to provide sub-surface irrigation of crops using a perforated 500 mL plastic bottle

Figure 2.5: Example of perforated bottle used to deliver irrigation sub-surface

2.4 Microbial analysis

2.4.1 Selection of faecal indicators / pathogens to be monitored

As explained in Chapter 1, it was anticipated that greywater would contain a lesser load of pathogens and faecal indicators than black water or combined sewage (Ottoson, 2005). As the water for this project came from relatively impoverished households, it was however expected that certain pathogens endemic to the community would probably be present. It was also expected that there would be some faecal indicator organisms as a result of full body washing, particularly of infants and sick household members, as well as washing of laundry, especially infant nappies. Although it was recognized that some members of the selected groups of organisms could re-grow in water given suitable conditions, it was considered that conclusions based on counts under such circumstances would result in even greater protection to user health as the increased numbers resulting from such re-growth would give an apparent increase in risk which would not necessarily be true. The microorganisms selected for analysis in this study are shown in Table 2.1.

Table 2.1: Microorganisms selected for analysis based on locally prevalent diseases

Organism	**Role as indicator**
Total coliforms	General bacterial load
Escherichia coli	Faecal indicator and potential pathogen
Enterococcus sp	Faecal indicator
Pseudomonas sp	Opportunisitic pathogen
Staphylococcus sp	Skin commensal / opportunistic pathogen
Ascaris lumbricoides	Endemic helminthic parasite
Somatic coliphage	Enterovirus indicator

Total coliforms are ubiquitous in the environment (Hurst *et al.*, 2002) and can be used to give an indication of whether or not there has been any die-off of microorganisms in general as a result of the handling of the greywater when post-treatment results are compared with those pre-treatment. *Escherichia coli* are a broadly accepted indicator of faecal contamination by warm blooded animals, including humans, as well as being a potential pathogen (Bitton, 2005). *Enterococcus* species, especially *Enterococcus faecalis* is also a widely used faecal indicator but tends to survive longer in water than *Escherichia coli* (Bitton, 2005). *Pseudomonas*

species are opportunistic pathogens particularly of the skin and can cause severe illness in immune-compromised persons as well as in infants. *Staphylococcus* species are causative agents of skin infections such as boils as well as being part of the normal skin flora (APHA/AWWA, 2005). *Ascaris lumbricoides* was used both as an indicator of endemic infection itself and as an indicator of potential contamination with protozoan or other helminthic parasites (Rose *et al.*, 2004). Somatic coliphage were used as indicators for enteroviruses which could be expected in the greywater contaminated with faeces through bathing and washing of faecally soiled laundry (Rose *et al*., 2004).

2.4.2 Microbiological analyses

Throughout the time period of this project, crops were harvested as soon as the treatment with the most advanced crop reached maturity. This resulted in a range of sample sizes and numbers as in some cases, the most advanced crop was large (*e.g.* Swiss chard) or had several fruits (*e.g.* green pepper) whereas the least was small or had no fruits. All harvested vegetables were analysed for microbiological contaminants, on both the surface and the interior of the crop, in order to determine whether or not potential pathogens had penetrated the outer layers. In some cases, the organisms of interest were overrun by non-specific organisms or contaminated by fungi and these plates were rejected. Samples were randomly selected from each crop, weighed wet and then dried at 108^{o}C in a drying oven until a steady mass was obtained. A minimum of three samples per crop per treatment was assessed. The percentage moisture was calculated and used to convert all further samples to their dry mass for comparison purposes.

The extraction of microorganisms from the exterior and interior of crops was based on past eThekwini laboratory experience in the extraction of microorganisms from soil and plant samples. For analysis of external contamination of crops, fresh, weighed samples were placed into suitable volumes of sterile Ringers solution (Chapter 7, Appendix 1, Section 7.8) and shaken on a platform shaker at 220 rev/min for one hour. The solution was then drained off and utilised for microbiological analyses. All results were converted back to counts per gram dry weight of sample.

For analysis of internal contamination, the samples that had been used for external analysis were sonicated in a 20% solution of a commercial sodium hypochlorite solution (Jik) with 0.1mL of Tween 80 detergent added to ensure removal of any waxy coatings and the disinfection of residual surface microorganisms. This method had been used with success by the author to sterilise field plant tissue for tissue culture. The rinsed and drained disinfected

material was then placed in suitable volumes of sterile Ringers solution in sterile honey jars and mascerated with a sterilised domestic food blender. Aliquots of the resultant mixture were analysed as for the external samples.

At each harvesting event, a dilution series ranging from 10^0 to 10^{-8} was made for each crop/treatment set and 1 mL from each dilution was filtered through a 0.47 μm membrane filter and the filter was placed onto the specified selective medium. Total coliforms and *E. coli* were analysed using Merck™ Chromocult agar (Chapter 7, Appendix 1, Section 7.2). This is a specific-enzyme detection medium capable of enumerating total coliforms and *E. coli* by detecting β-glucuronidase and β-galactosidase activity. As a result of the chromophores in the medium, *E. coli* colonies turn purple and total coliform colonies are pink. *Enterococcus* species were enumerated similarly by membrane filtration onto *Enterococcus* selective agar (Oxoid) with positive colonies producing darkening in the agar around the colonies. Membrane filtration was also used for the analysis of *Pseudomonas* sp with positive colonies being those that produced green fluorescence on Cetrimide agar (Biolab). *Staphylococcus* sp were enumerated by membrane filtration onto Slanetz and Bartley agar (Biolab) with positive colonies being black with zones of clearing in the surrounding agar. *Ascaris* ova were enumerated using the South African recommended method which employs an ammonium bicarbonate separation and microscopic identification of ova (Archer *et al.,* 2006; Chapter 7, Appendix 1, Section 7.1). Somatic coliphage were enumerated using a double layer technique, (Grabow, 1984, Chapter 7, Appendix 1, Section7.7). All microbiological methods are given in detail in Chapter 7, Appendix 1.

2.4.3 Analysis limitations

The possibilities of re-growth or die-off of the various microorganisms in the greywater were recognized. However, as the experimental greywater was used within the same time frame as that normally found in the community setting, it was decided that the results obtained in this project would at least model what could be expected in the field.

2.5 Statistical analysis

The microbial load data were tested for normality using the Kolmogorov-Smirnov test in the statistical package Statistica 7 (StatSoft) and it was found that the majority could be fitted to a log-normal distribution although this was often not the best fit. As some data could not be

readily transformed to a normal distribution, and for others the log-normal fit was not strong, with reference to Levin (1987), the Mann-Whitney U test, a non-parametric method, was used to test pairs of data. In addition, the Kruskal-Wallis test was applied to sets of data to ensure comprehensive analysis. Mean value and 95th percentile were calculated accordingly. Box and whisker graphs of the results were prepared using Statistica 7 in order to evaluate the range of the data and the central tendency. As many of the samples resulted in nil bacterial counts, the median value was very small and often reflected as zero. This was discussed with an applied statistician (Jackson, pers. comm., 2010[8]) and it was agreed that for empirical data the median value was an acceptable, robust statistic. Best-fit distributions for use in the calculation of probability of infection were fitted using @ Risk 5.5 (Pallisade) and tested using the chi-squared test. No significant increase in contamination was found over the growing cycles so all data were used in the statistical analysis.

2.6 Health risk assessment

This project did not extend to epidemiological studies and the direct calculation of health risk in the studied community. Health risk estimates were performed using standard Quantitative Microbial Risk Assessment (QMRA) techniques. As a stochastic approach to the risk model was considered desirable, distributions were fitted to the microbial data and correction factors for the amount consumed, the percentage of the population exposed and any barrier-intervention reductions of load were applied. Monte Carlo sampling of the data for each crop / treatment-set was used with 10 000 iterations for each set. The best-fit distributions were then used to establish the dose to be used in the following equation for calculation of probability of infection as shown in Equation 2.1:

$$Pinf = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}$$

Equation 2.1: Calculation of probability of infection using a beta-Poisson distribution where d=dose of microorganisms, α andβ = the dose response parameters from Haas *et al.*, 1999 and Westrell, 2004.

Where the variation in microbiological data was insufficient to fit a distribution, for example where most results were zero but there were still a few positive results, the arithmetic mean of the untransformed results was used in the calculation as this was likely to provide a higher

[8] Jackson, Dr E.S. Applied Statistician. Cambridge, England

estimate of risk than the use of the geometric mean (Havelaar *et al.*, 2004). When only one value was available it was used in the calculations to provide maximum risk information. In accordance with WHO standards, (WHO, 2006a) a β-Poisson relationship between dose and response was used, as a range of sensitivity to infection was to be expected in the population and this technique would give conservative results better fitted to this scenario than those that would be obtained from a normal or exponential distribution (Haas *et al.*, 1999). The dose distribution obtained from @RISK was multiplied by the relevant factor per crop to convert the number of organisms per gram dry weight to number of organisms per gram wet weight so that daily average mass consumed as given in Chapter 1, Table 1.7 could be used to obtain an average daily risk. Further multiplications by the percentage of people in the population being examined likely to consume that dose, (also obtained from Chapter 1, Table 1.7 and by the conversion factor assumed to convert the number of indicator organisms to a theoretical pathogen were performed. With reference to the literature sited in Chapter 1, Section 1.7.3, levels of *E. coli* were conservatively estimated to be at least two-log units higher in value than pathogens, *Enterococcus* would give a similar load to the pathogens and total coliforms would be approximately four-log units higher than the pathogens. These levels were estimated, taking into account that pathogens are only excreted by infected members of the population, generally for short periods of time, whereas the indicators are excreted in large numbers constantly or are ubiquitous in the environment. The dose response parameters α and β were obtained from Chapter 1, Table 1.8, using the figures for *Shigella*, as this organism was most likely to approximate the risk from either enterovirus or bacteria in the greywater. Based on the observation that five out of the observed population of fifty-three people were regularly involved in the tending and harvesting of crops, the figure for the population exposed to the greywater and soil used in the calculation of risk was estimated at 9% on a daily basis and the volume of water or soil inadvertently ingested was based on the figures from the World Health Organisation presented in Chapter 1, Table 1.6. For the calculation of health risk from consumption of the vegetables, the bacterial loading for both interior and exterior was combined to provide the most conservative data for consumption of the vegetable. These values were entered into Equation 2.1so that:

d = Distribution calculated by @RISK × wet mass conversion factor × % observed population consuming dose × mass consumed × conversion factor to pathogen

$\alpha = 0.21$ and $\beta = 42.86$

It needs to be noted that the corrections mentioned occur to the dose before insertion into the equation to calculate the probability of infection as they do not necessarily have a linear impact

on the probability of infection. In addition to the basic exposure, calculations were also performed to determine the probability of infection where various risk-reducing barrier interventions were tested. These interventions and their expected reduction in microbiological load were taken from Chapter 1, Table 1.5. Again, the effect on dose was calculated before use in Equation 2.1 to calculate probability of infection.

Once the probability of infection for a single dose was calculated, it was expanded to give the yearly probability of infection using the equation (Haas *et al.*, 1999):

$$Pyearly = 1 - (1 - Pinf)^n$$

Equation 2.2: Calculation of annual probability of infection where P_{inf} is the probability of infection (from Equation 2.1) from individual exposure and n is the number of exposures per year. From Haas *et al.*, 1999 and Westrell, 2004

For the consumption of crops, n was 365 as the mass of vegetable consumed was that given for daily consumption in Chapter 1, Table 1.7, and therefore the contaminant dose was that to be expected on a daily basis in the community consuming the crop. This was regarded as a conservative estimation. Where exposure to greywater and soil were concerned, n was much lower and this is explained in Chapter 3.

The data presented here are for risk of infection, but as data for the conversion of infection to illness were not available for this population, there has been no attempt at conversion of the data to probability of illness and therefore no calculation of DALYs was possible. As there is a risk of a high incidence of immune-compromised persons in the community, to provide the greatest safety, a conversion factor of 1 could be used for the conversion from an infected to a diseased state. This would imply that anyone becoming infected would convert to illness. No inherent immunity was assumed and neither secondary infection nor protection from infection during the course of the disease would have been factored in.

As rates of conversion from an infected to a diseased or carrier state were not known for this population, P_{inf} was not manipulated further to give $P_{disease}$. With reference to Chapter 1, if the population as a whole, *i.e.* the population of South Africa, was the point of reference, the exposure per day would be extremely low as was the dose and therefore a difference in the probability of infection of less than 1 per 100 000 population could be expected from using the static as opposed to the dynamic model. For the observed population only, *i.e.* the Cato Crest community involved in this project, the exposure per day would be moderate and the dose low so a difference in probability of infection of less than 10 per 100 000, or in this case, 0.005 per

53, could be expected. It was therefore taken that the slightly expanded static model could be used here and would give sufficient information for comparison of health risk at this stage.

For each of the crops, the probability of infection as a result of crop consumption was modelled using the distribution function for each indicator organism and the values presented in Table 2.2 For each set, the 5^{th} percentile, 95^{th} percentile, maximum and mode values were determined. The data were tabulated and are presented in Chapter 8, Appendix 2, Table 8.1 to Table 8.9 and box-and-whisker plots are presented with the discussion of the results for each crop. The model was then re-run for each crop, implementing barriers to infection.

For each of the crops, the probability of infection as a result of crop consumption was modelled using the distribution function for each indicator organism and the values presented in Table 2.2. For each set, the 5^{th} percentile, 95^{th} percentile, maximum and mode values were determined. The data were tabulated and are presented in Chapter 8, Appendix 2, and box-and-whisker plots are presented with the discussion of the results for each crop. The model was then re-run for each crop implementing barriers to infection.

Table 2.2: Factors used in the calculation of probability of infection from the consumption of crops

Factor	Value and source
Produce washing with water	1-2 log reduction Chapter 1, Table 1.5
Produce disinfection	2 log reduction Chapter 1, Table 1.5
Produce peeling	2 log reduction Chapter 1, Table 1.5
Produce cooking	6-7 log reduction Chapter 1, Table 1.5
Days of irrigation per year	200
Days of consumption per year	365 Chapter 1, Table 1.7
Swiss chard	Daily consumption 9.2 g dry; % population 27.4, Chapter 1, Table 1.7
Green peppers	Daily consumption 2.5 g dry; % population 12.5, Chapter 1, Table 1.7
Chillies	Daily consumption 0.6 g dry; % population 12.8, Chapter 1, Table 1.7
Beetroot	Daily consumption 3.9 g dry; % population 12.8 Chapter 1, Table 1.7,
Potato	Daily consumption 45.4 g dry; % population 27.1, Chapter 1, Table 1.7
Carrot	Daily consumption 3.9 g dry; % population 30.8, Chapter 1, Table 1.7
Onion	Daily consumption 2.5 g dry; % population 12. Chapter 1, Table 1.78,
Conversion to potential pathogen – *E. coli*	0.001 (2-log excess)
Conversion to potential pathogen – *Enterococcus*	1 - equivalent
Conversion to potential pathogen –*Staphylococcus*	0.001 (2-log excess)
Conversion to potential pathogen – total coliforms	0.0001 (4-log excess)

For the greywater and soil exposure, the probability of infection as a result of exposure was modelled using the distribution function for each indicator organism and the values presented inTable 2.3. For each set, the 5th percentile, 95th percentile, maximum and mode values were determined. The data were tabulated and are presented in Chapter 8, Appendix 2, Table 8.1 and Table 8.2, and box-and-whisker plots are presented with the discussion of the results for each risk. The model was then re-run for each crop implementing barriers to infection.

Table 2.3: Factors used in the calculation of health risk through exposure to greywater or soil during irrigation

Factor	**Value and source**
Volume consumed per exposure to greywater	1 mL (Chapter 1, Table 1.6)
Volume consumed per exposure to soil	1g based on a similar exposure as to water
Days of irrigation per year	200 - estimated
% of Community exposed	100 – would be 9 for whole community
Conversion to potential pathogen – *E. coli*	0.01 (2-log excess)
Conversion to potential pathogen – *Enterococcus*	1 - equivalent
Conversion to potential pathogen –*Staphylococcus*	0.001 (3-log excess)
Conversion to potential pathogen – total coliforms	0.0001 (4-log excess)
Wearing gloves / washing arms	Single log reduction
Wearing boots	2-log reduction

3. RESULTS

3.1 Factors contributing to microbiological quality of crops

3.1.1 Rainfall

It was considered that rainfall might have an impact on the loading of bacteria in the soil or on above-ground crops through splash-back. Data on rainfall for the experimental period were obtained from eThekwini Parks, Recreation and Culture Department and are presented in Figure 3.1. Average monthly rainfall over the three year period showed no statistical difference among equivalent months (p=0.05), but the overall rainfall in 2007 was statistically greater (p=0.05, n=2334) than that for 2005 (n=1899) and 2006 (n=2907). The greatest rainfall occurred in the summer months (October to March) each year. It is unlikely that bacterial loadings among the months during which crops were grown would have been affected by rainfall patterns. Splash-back onto above-surface crops could have occurred during summer rainfall periods, but this was likely to be counteracted by the dilution factor and wash-off of organisms by the rainfall.

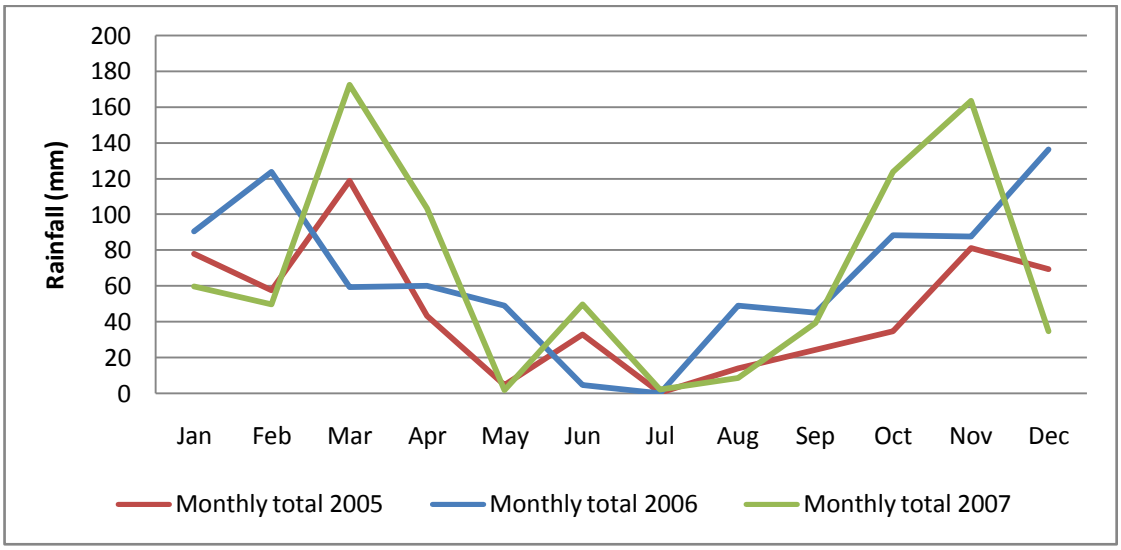


Figure 3.1: Rainfall during the period January 2005 to December 2007 measured at the Botanic Gardens, Durban. Average monthly rainfall over the three year period showed no statistical difference among equivalent months (p=0.05), but the overall rainfall in 2007 was statistically greater (p=0.05, n=2334) than that for 2005 (n=1899) and 2006 (n=2907). Seasonal variation is clearly indicated. Supplied by eThekwini Parks, Recreation and Culture Department.

3.1.2 Soil microbiology and chemistry

At the start of the trial, six samples of the Berea red soil used for the trial were analysed and at intervals throughout the trial, further samples of the soil from each of the irrigation treatments were analysed for alkalinity, free ammonia, *Ascaris* ova, COD, conductivity, *E. coli*, *Enterococcus*, *Staphylococcus* and total coliforms to detect any changes. The results are presented in Table 3.1, with all the microbiological readings presented as $\log_{10}$ values.

Table 3.1: Results from Physico-chemical and microbial analyses performed on soil samples from unwatered plants and plants irrigated with greywater, hydroponic solution or tap-water respectively. Mean, maximum, minimum and Standard deviations are provided for all parameters. No statistical significance was found between parameters measured and treatments ($p \leq 0.05$) except in the case of *Enterococcus* where the level in greywater irrigated soil was statistically higher than for the other treatments ($p \leq 0.05$).

Irrigation water type		Alkalinity	COD	Conductivity	Ammonia (free)	*Ascaris lumbricoides*		*E coli*	*Enterococcus*	*Staphylococcus*	Total coliforms
						Total	Viable				
		mg/g dry	mg/g dry	mS/m	mg/g dry	Ova/g dry	Ova/g dry	Log_{10} cfu/g dry			
Unwatered (n=4)	Mean	139	117.6	5.52		0.00	0	0			
	Min	52	86	5				0			
	Max	241	184	5.8				0	2.3	3.7	5.4
	Std Dev	100.2	40.3	0.31				0			
Greywater irrigated (n=9)	Mean	86	273.7	6.82	0.75			0	2.45	3.1	5.2
	Min	86	78	5.3	0.5			0	0	0	0
	Max	86	406	9.4	1.3	1	0	0	2.99	10.6	5.8
	Std Dev	0	89.99	1.46	0.28			0	2.5	3.2	5.2
Hydroponic solution irrigated (n=10)	Mean	86	365.4	6.47	0.95			0	1.3	3.9	5.2
	Min	86	214	4.9				0	0	0	0
	Max	86	492	8.5		1	0	0	1.9	4.7	5.5
	Std Dev	0	83.70	1.24				0	1.4	4.2	5.1
Tap water irrigated (n=11)	Mean	86	722.6	6.12	0.87	0.00	0	0	1.3	3.6	5.1
	Min	86	138	5.1	0.5			0	0	0	0
	Max	86	1553	7.6	1.8			0	2	4.5	5.5
	Std Dev	0	579.3	0.68	0.40			0	1.4	3.9	5.1

No *E. coli* or viable *Ascaris* ova were detected in the soil from any of the treatments. *Staphylococcus* and total coliforms did not show significant differences among treatments ($p \leq 0.05$), but the level of *Enterococcus* in the greywater-irrigated soil was statistically greater than that for the other two treatments ($p \leq 0.05$).

3.1.3 Greywater

At the start of the project, microbiological analyses were conducted on samples of greywater (n=32), tap water (n=20) and the hydroponic solution (n=18). The averaged results are presented in Table 3.2. No *Ascaris* ova, *Salmonella*, *Shigella* or somatic coliphage were detected in any of the water samples and *E. coli*, *Enterococcus* and *Staphylococcus* were only detected in the greywater and were present at a statistically higher level than for hydroponic solution or tap water ($p \leq 0.001$). Total coliforms were significantly higher in the greywater than in either of the other two water types used for irrigation ($p \leq 0.001$). In line with normal practice at eThekwini laboratory, a log conversion of actual bacteriological counts was used in microbiological analysis. In all cases where no microorganism was detected, the minimum detection level of 1 colony forming unit (cfu) per unit volume or mass was used. This allowed a log value of zero to be used in further computations.

Nine samples of each of the water types were subjected to chemical analysis and the data are presented in Table 3.2. The conductivity of the greywater was significantly lower ($p \leq 0.05$) than that of the hydroponic solution, indicating that the level of salts in the hydroponic solution was higher than in the greywater. The level of ammonia in the greywater was not significantly different ($p \leq 0.05$) from that in the hydroponic solution and both were significantly higher than that in the tap water ($p \leq 0.05$) where the level was extremely low. The chemical oxygen demand (COD) of the greywater was high and approximately 30% was readily biodegradable as can be seen from the results for the biological oxygen demand (BOD) and chemical oxygen demand (COD) presented in Table 3.3. This could have been as a result of kitchen and bathroom washings or organic contamination from cleansing products in use by the households.

Table 3.2: Results from physico-chemical and microbial analyses performed on greywater, hydroponic solution or tap-water respectively. Mean, maximum, minimum and standard deviations are provided for all parameters. *E. coli*, *Enterococcus* and *Staphylococcus* were only detected in the greywater and were present at a statistically higher level than for hydroponic solution or tap water ($p \leq 0.001$). Total coliforms were significantly higher in the greywater than in either of the other two water types used for irrigation ($p \leq 0.001$). The conductivity of the greywater was significantly lower ($p \leq 0.05$) than that of the hydroponic solution. The level of ammonia in the greywater was not significantly different ($p \leq 0.05$) from that in the hydroponic solution and both were significantly higher than that in the tap water ($p \leq 0.05$). The significance of the difference between the Chemical Oxygen (COD) demand in greywater and the other two water treatments could not be tested as no COD was detected in the latter.

	Alkalinity (n=9)	Ammonia (NH_3) (n=9)	Chemical oxygen demand (n=9)	Conductivity (n=9)	*Ascaris lumbricoides* (n=32,20,18 respectively)		*E. coli* (n=32,20,18 respectively)	*Enterococcus* (n=26, 20, 18 respectively)	*Staphylococcus* (n=26, 20,18 respectively)	Total coliforms (n=32,20,18 respectively)	Somatic coliphage (n=32,20,18 respectively)
					Total	Viable					
	mg/L	mg/L	mg/L	mS/m	Ova / g		Log_{10} (cfu/100 mL)				
Greywater											
Mean	179.03	19.76	584.30	69.82	0	0	4.76	0.41	1.16	6.74	0
Max	706	157	1120	230	0	0	9.00	5.34	7.53	11.11	0
Min	0	0.5	39	13	0	0	0	0	0	0	0
Std Dev	161.2	37.08	342.76	56.16			2.95	1.23	2.07	2.12	-
Hydroponic solution											
Mean	29	32.5	0	223	0	0	0	0	0.04	0.84	0
Max	29	33	0	223	0	0	0	0	0.84	4.08	0
Min	29	32	0	223	0	0	0	0	0	0	0
Std Dev	-	0.70	-	-	-	-	-	-	0.19	1.12	-
Tap water											
Mean	66	<0.5	0	30	0	0	0	0	0	0.55	0
Max	66	<0.5	0	30	0	0	0	0	0	1.81	0
Min	66	<0.5	0	30	0	0	0	0	0	0	0
Std Dev	-	-	-	-	-	-	-	-	-	0.59	-

Table 3.3: Statistical values for the measurement of biological oxygen demand (BOD) and chemical oxygen demand (COD) in greywater. Approximately 30% of the oxygen demand could be attributed to Biological Oxygen Demand (BOD) which could be as a result of washings from the kitchen and bathroom or organic contamination from cleansing products in use by the households. Levels for both BOD and COD were significantly higher than for either hydroponic solution or tap water when tested at the p=0.05 level

BOD	mg/L	COD	mg/L
Mean	180.625	Mean	584.303
Standard Deviation	84.65371	Standard Deviation	342.7572
Minimum	40	Minimum	39
Maximum	320	Maximum	1120

3.2 Microbiological quality of above-ground crops

Harvesting of crops depended largely on the growth, with harvesting of all treatments occurring when the treatment with the largest crop was ready for harvesting. Typically, this was after 6 to 8 weeks. In some cases, especially in those plants irrigated with unmodified municipal water, the crop was extremely small both in number and size. There was also some loss of crops to insects in one growth cycle.

3.2.1 Swiss chard

Visually, there was a wide range of Swiss chard leaf sizes across the three experimental treatments as shown in , Figure 3.2 A to C; further details on crop yields are available in the corresponding project by L. Salukazana. This yield variance meant that the amount of sample examined for each treatment was not always as large as had been expected when harvested at 6 to 8 weeks when the treatment with the largest crop was ready for harvesting. Some of the crop from one growth cycle was also badly affected by insect attack as shown in Figure 3.2 D. No increase in microbial levels on crops was found with successive growing cycles therefore data from all growing cycles were combined for the statistical evaluations. The three experimentally grown crops and purchased produce were analysed as described in Chapter 2. No *Ascaris* ova, *Pseudomonas* or somatic coliphages were detected in any of the samples. The remaining microorganisms varied in concentration as shown in Figure 3.2 to Figure 3.5, with many of the results being zero. In cases where the median was zero or close to zero, the 5^{th} percentile is close to the median. Median values per gram dry weight for each of the microorganisms are presented in Table 3.4.

A: Example of Swiss chard crop irrigated subsurface with greywater

B: Example of Swiss chard crop irrigated subsurface with hydroponic solution

C: Example of Swiss chard crop irrigated subsurface with tap water

D: Swiss chard crop extensively damaged by insect attack

Figure 3.2 : Examples of the Swiss chard crop from the various irrigation regimes

No increase in microbial levels on crops was found with successive growing cycles therefore data from all growing cycles were combined for the statistical evaluations. The three experimentally grown crops and purchased produce were analysed as described in Chapter 2. No *Ascaris* ova, *Pseudomonas* or somatic coliphages were detected in any of the samples. The remaining microorganisms varied in concentration as shown in Figure 3.2 to Figure 3.5, with many of the results being zero. In cases where the median was zero or close to zero, the 5th percentile is close to the median. Median values per gram dry weight for each of the microorganisms are presented in Table 3.4.

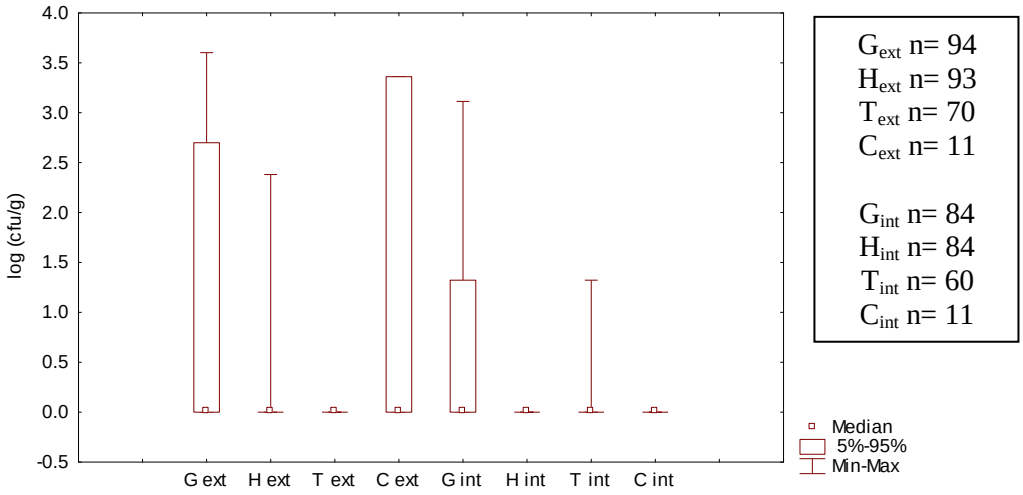


Figure 3.2: Box and whisker plot of *E. coli* counts on external surfaces (ext) or the interior (int) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

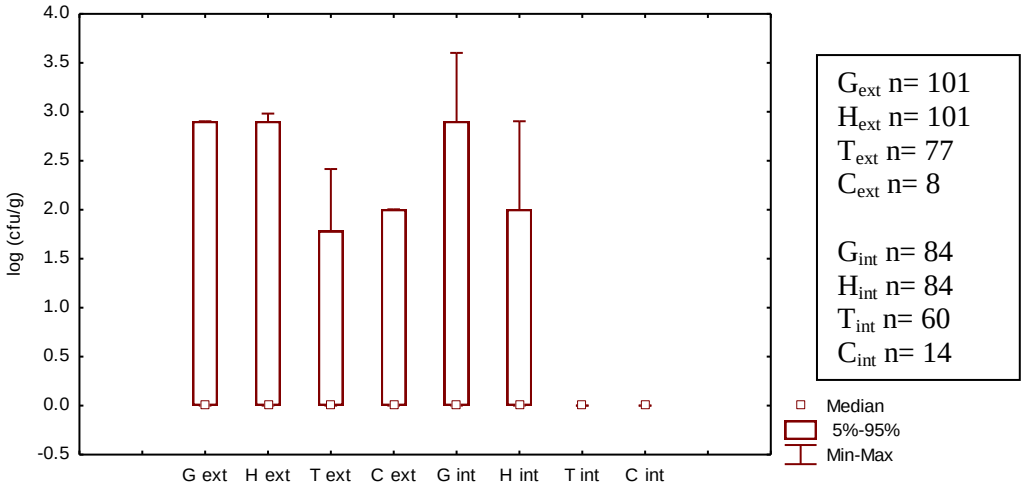


Figure 3.3: Box and whisker plot of *Enterococcus* counts on external surfaces (ext) or the interior (int) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

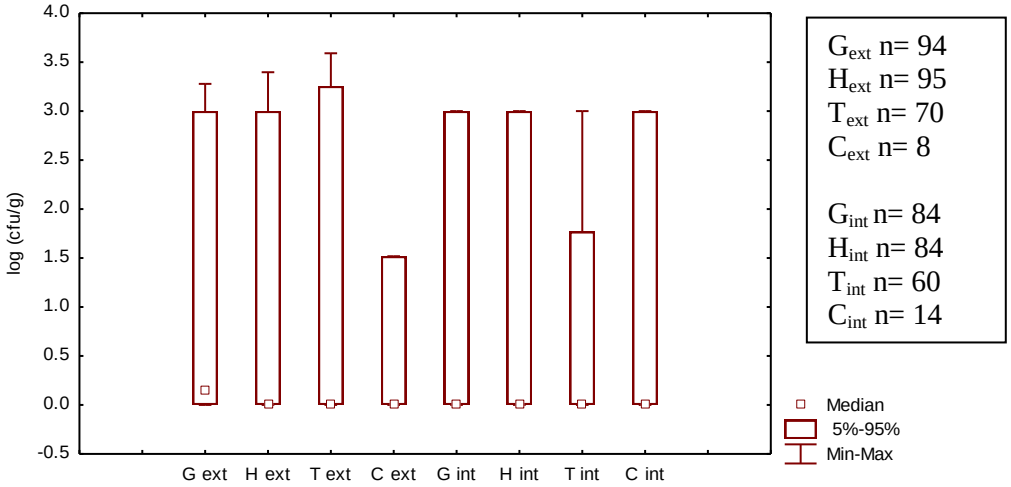


Figure 3.4: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

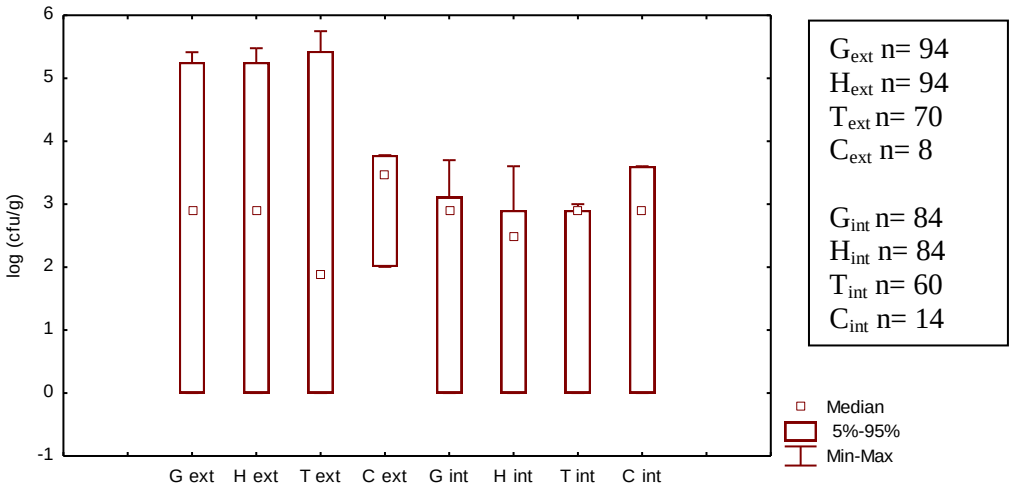


Figure 3.5: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

Table 3.4: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No *Ascaris* ova, *Pseudomonas*, somatic coliphages, *E. coli*, *Enterococcus* or *Staphylococcus* were detected internally or externally for any of the water treatments. Total coliforms were detected for all treatments.

	Gext	Hext	Text	Cext	Gint	Hint	Tint	Cint
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0
Total coliform	800	800	80	2900	800	260	800	800

The mean of the microbial loads per irrigation treatment were calculated and compared using the Kruskal-Wallis test on untransformed data to determine whether there was a difference observable in the bacterial load either internally or externally among the treatments (Table 3.5). No significant difference was found between the three water treatments at the p=0.05 level.

Table 3.5: Statistical comparison, using non-parametric (Kruskal Wallis test) methods, of means of bacterial load for the interior (int) and exterior (ext) of Swiss chard plants irrigated with greywater (G), hydroponic solution (H) or tap water (T). No significant differences were found at the p=0.05 level

		E. coli		*Enterococcus*		*Staphylococcus*		Total coliforms	
		External	Internal	External	Internal	External	Internal	External	Internal
Kruskal-Wallis	G	NS	NS	NS	NS	NS	NS	NS	NS

NS - No significant difference at p=0.05 level

3.2.2 Green peppers

The tap water-irrigated crop was stunted by comparison with the greywater-irrigated crop, as can be seen in Figure 3.6. This meant that the amount of crop available for assessment of bacterial loading on the latter crop was extremely limited and any statistics based on these results should be treated with caution. It was observed that the greywater-irrigated crop was more prolific than the hydroponic solution irrigated crop and also ripened earlier.

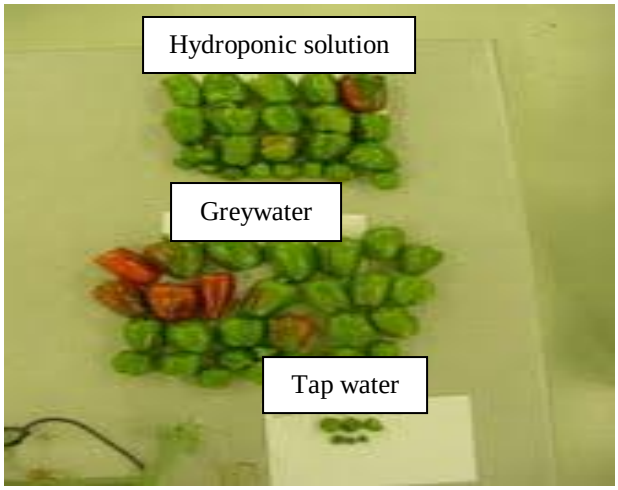


Figure 3.6: Examples of the green pepper crop showing the stunting of the tap water irrigated crop. Courtesy L. Salukazana

No *Enterococcus* was detected in or on any of the test treatment samples analysed. Counts on the commercially purchased vegetables were in general low or zero. There did however appear to be internal microbial contamination of the crops at a level higher than would have been expected from the external loadings. This was evident particularly for *E. coli* in all the treatments including the purchased produce. These positive colonies from the Chromocult™ plates were further examined using API 20E biochemical identification strips. The findings were varied, with some colonies definitely identified as *E. coli* and others being of questionable identity as is frequently the case for environmental samples. The other organisms were also detected internally, often with mean levels higher than for the external load. On discussions with an experienced botanist (S. Pillay, pers. comm. 2010)[9], it was thought that this contamination might be due to the inclusion of bacteria during the process of formation of the fruit from the flower. There was no way to confirm this conclusion with the facilities available to the project but this possibility should be further investigated.

Median values per gram dry weight for each of the microorganisms are presented in Table 3.6. Except for total coliforms, the median values for all treatments for each of the detected microorganisms were generally zero. The statistical data are presented in Figure 3.7 to Figure 3.9. No significant differences were found between the three water treatments for any of the indicators. These data are presented in Table 3.7.

[9] S. Pillay. CSIR, Durban

Table 3.6: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of green peppers irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No *Ascaris* ova, *Pseudomonas*, somatic coliphages, *E. coli*, *Enterococcus* or *Staphylococcus* were detected internally or externally for any of the water treatments. Total coliforms were detected for all treatments except externally for the commercial crop.

	Gext	Hext	Text	Cext	Gint	Hint	Tint	Cint
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0
Total coliforms	100	220	100	0	60	800	800	10

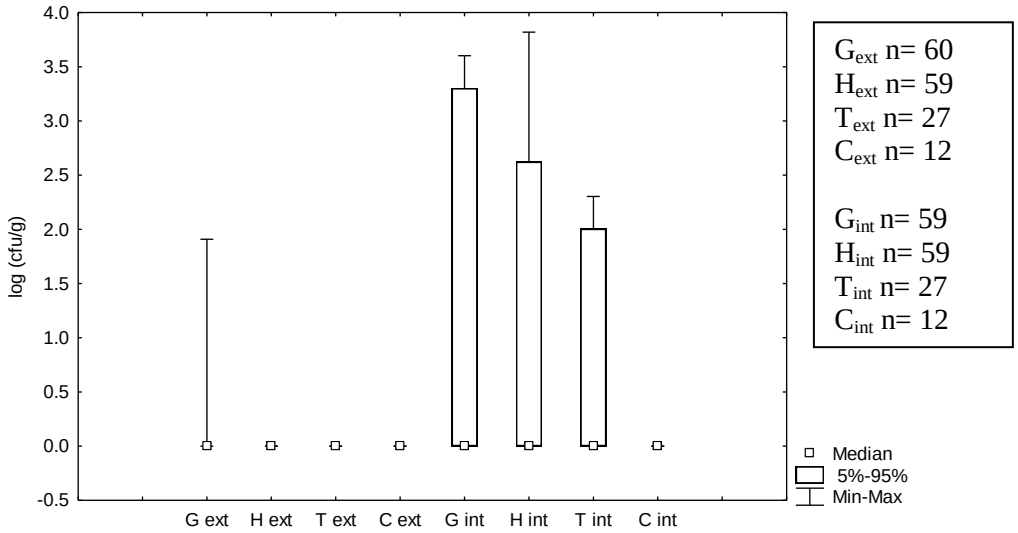


Figure 3.7: Box and whisker plot of *E. coli* counts on external surfaces (ext) or the interior (int) of green pepper fruits irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

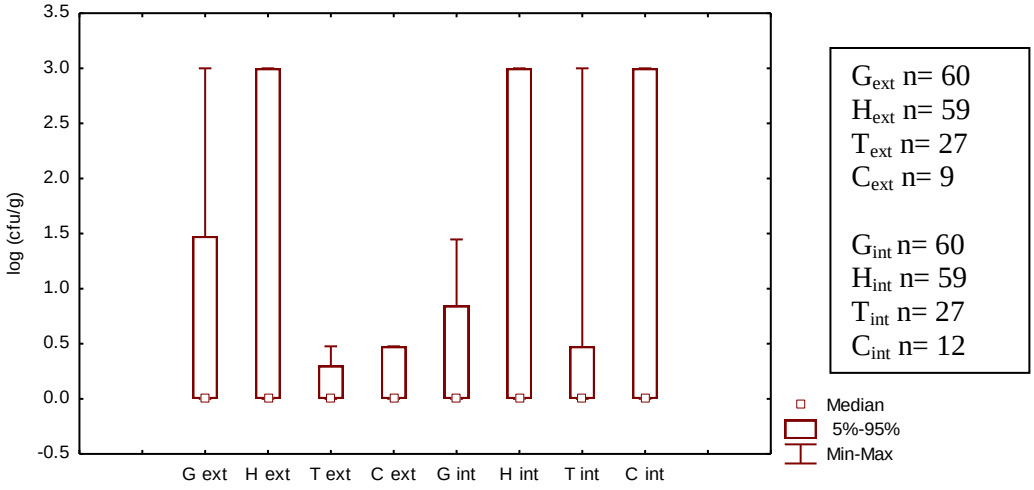


Figure 3.8: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of green pepper fruits irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

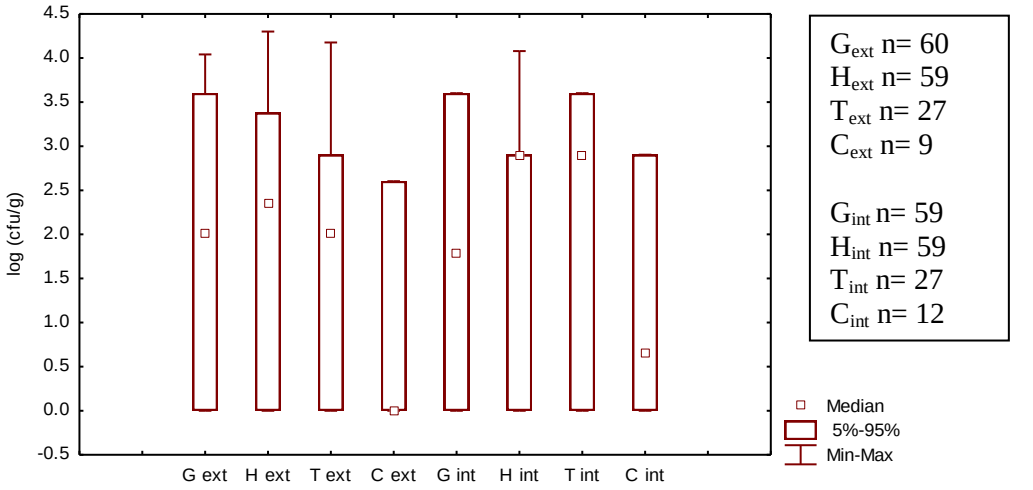


Figure 3.9: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of green pepper fruits irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the three water treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

Table 3.7: Statistical comparison, using non-parametric (Kruskal-Wallis) method, of means of bacterial load for the interior (int) and exterior (ext) of green peppers fruits irrigated with greywater (G), hydroponic solution (H or tap water (T). No significant differences were found at the p=0.05 level

		E. coli		*Enterococcus*		*Staphylococcus*		Total coliforms	
		External	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**
Kruskal-Wallis	G	-	NS	-	-	NS	NS	NS	NS

NS – no significant difference at p=0.05 level - No organisms detected

3.2.3 Chillies

After the findings of internal contamination in green peppers, it was decided to test a similar vegetable using greywater and hydroponic solution to see if the internal contamination persisted. Crops were grown for one growth cycle only. Chillies were chosen as they also form as fruit from a flower, have a waxy coating and develop fruit hanging from the plant above ground in the same way as green pepper. No *E. coli*, *Enterococcus* or *Staphylococcus* were detected either internally or externally for either of the crop treatments for this fruit. Median values per gram dry weight for each of the microorganisms are presented in Table 3.8.

Table 3.8: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of chillies irrigated with greywater (G),or hydroponic solution (H). Results are based on one growing cycle. No *Ascaris* ova, *Pseudomonas*, somatic coliphages, *E. coli, Enterococcus* or *Staphylococcus* were detected internally or externally for any of the water treatments. Total coliforms were detected externally and internally for crops irrigated with either greywater or hydroponic solution.

	Gext	**Hext**	**Gint**	**Hint**
E. coli	0	0	0	0
Enterococcus	0	0	0	0
Staphylococcus	0	0	0	0
Total coliforms	0	0	0	0

The statistical data for total coliforms are presented in Figure 3.10. As regards total coliforms, no significant difference was seen at the p=0.05 level between the three water treatments for external or internal contamination This is shown in Table 3.9, but the range of internal contamination was again slightly higher than the external.

These results show that further investigation of the routes of contamination of such fruits would be warranted at a later stage.

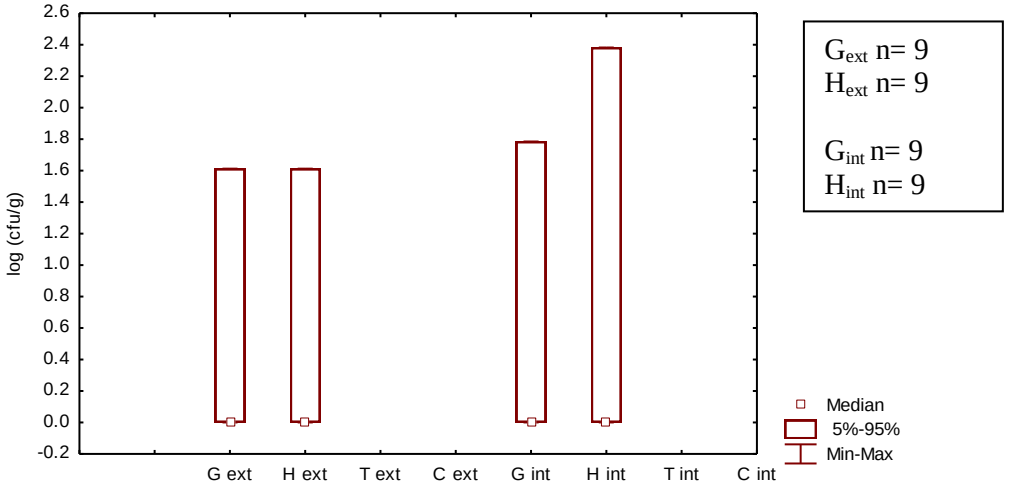


Figure 3.10: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of chillies irrigated with greywater (G), hydroponic solution (H) or purchased commercially (C). Results are based on one growing season. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

Table 3.9: Statistical comparison of means of bacterial load for the interior (int) and exterior (ext) of chillies irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C), using non-parametric (Kruskall-Wallis) methods. No significant differences were found at the p=0.05 level

	E. coli		***Enterococcus***		***Staphylococcus***		**Total coliforms**	
	External	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**
Kruskal-Wallis	-	-	-	-	-	-	NS	NS

- No organisms detected NS - no significant difference at the p=0.05 level

3.3 Microbiological quality of below-ground crops

Four crops grown below ground were investigated.

3.3.1 Beetroot

As beetroot is a root crop, it was exposed directly to greywater during sub-surface irrigation. It is however generally eaten after having been cooked thoroughly and is therefore regarded as a low risk crop. Observation of the crops did not show evidence of differences in crop size between crops irrigated with greywater and hydroponic solution, but the tap water-irrigated

crop was stunted, as can be seen in Figure 3.11. Median values per gram dry weight for each of the microorganisms are presented in Table 3.10.

Figure 3.11: Examples of tap water irrigated beetroot plants showing stunting of growth

Table 3.10: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of beetroot irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No *Ascaris* ova, *Pseudomonas*, somatic coliphages, *E. coli* was detected internally or externally for any of the water treatments. *E. coli* was detected externally for all three water treatments and internally for greywater irrigated crop. *Enterococcus* was detected internally and externally for greywater and hydroponic solution irrigated crop and externally for the commercial crop. *Staphylococcus* was detected internally and externally on crops from all three water treatments but not on the commercially bought crop. Total coliforms were detected internally and externally for all treatments.

	Gext	Hext	Text	Cext	Gint	Hint	Tint	Cint
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	60	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0
Total coliforms	1100	900	80	17550	10	200	0	30

E. coli was detected externally for all three water treatments and internally for greywater irrigated crop. *Enterococcus* was detected internally and externally for greywater and hydroponic solution irrigated crop and externally for the commercial crop. *Staphylococcus* was detected internally and externally on crops from all three water treatments but not on the commercially bought crop. Total coliforms were detected internally and externally for all treatments. The statistical data are presented in Figure 3.12 to Figure 3.15.

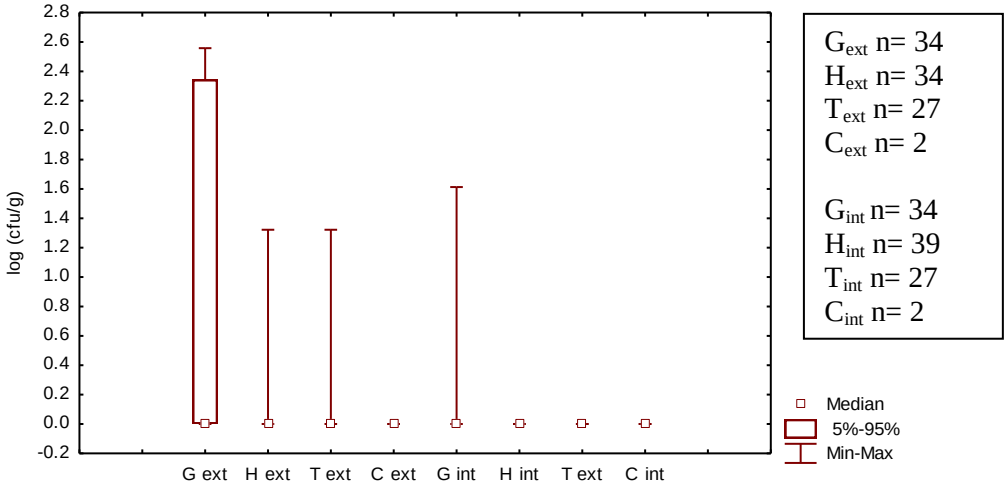


Figure 3.12: Box and whisker plot of *E. coli* counts on external surfaces (ext) or the interior (int) of beetroot irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

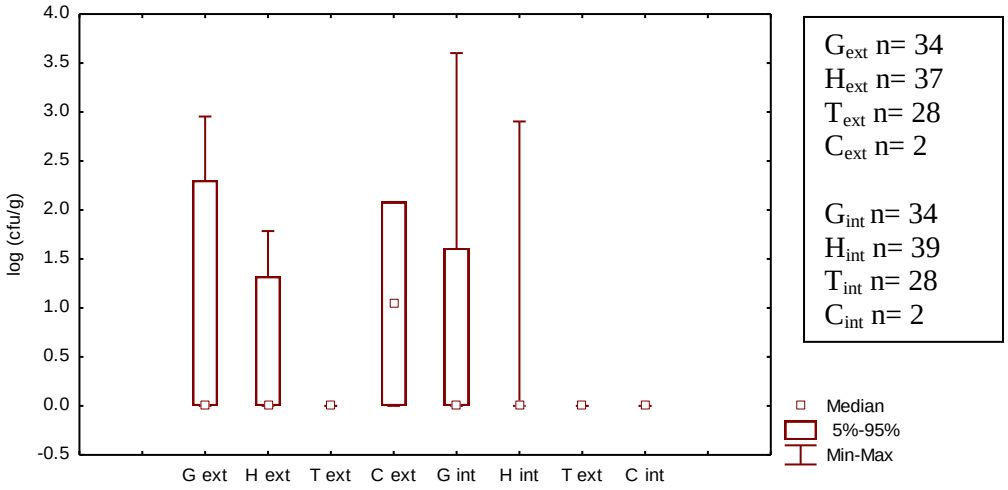


Figure 3.13: Box and whisker plot of *Enterococcus* counts on external surfaces (ext) or the interior (int) of beetroot irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

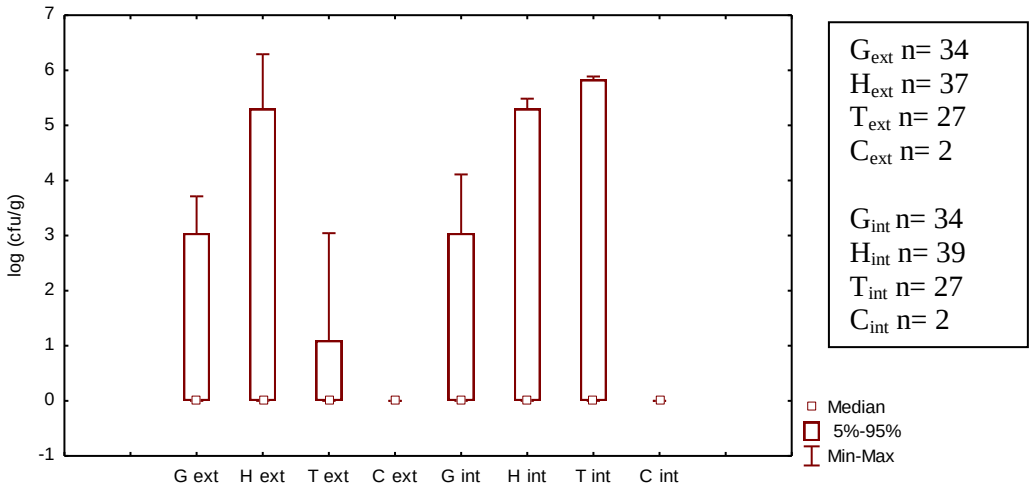


Figure 3.14: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of beetroot irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

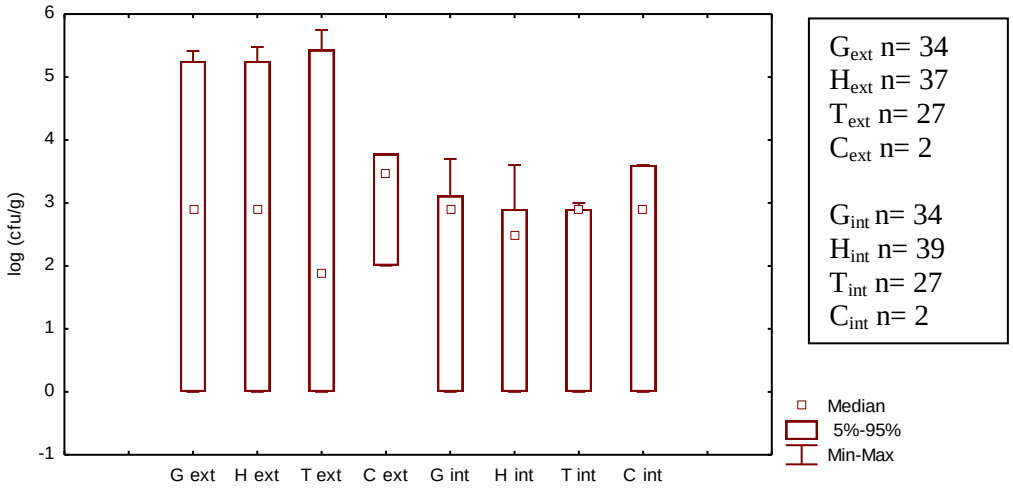


Figure 3.15: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of beetroot irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

No significant difference was apparent for any of the treatments for any of the indicators when tested with the Kruskal-Wallis test at the p=0.05 level. These results are given in Table 3.11. There was little penetration of microorganisms into the tissue of the beetroot as is shown by the

low interior counts of bacteria. No *Staphylococcus* was detected on or in the purchased produce. Except for total coliforms, the median values for all treatments for each of the detected microorganisms were generally zero. No significant increase in contamination was found over the growing cycles so all data were used in the statistical analysis.

The programme @RISK was used to fit distributions to the data for later use in determining the risk of infection. In each case the best fit was determined using the chi-squared test.

Table 3.11: Statistical comparison, using non-parametric (Kruskal-Wallis) methods, of means of bacterial load for the interior (int) and exterior (ext) of beetroot irrigated with greywater (G), hydroponic solution (H) or tap water (T). No significant differences were found at the p=0.05 level

	E. coli		***Enterococcus***		***Staphylococcus***		**Total coliforms**	
	Ext	**Int**	**Ext**	**Int**	**Ext**	**Int**	**Ext**	**Int**
Kruskal-Wallis	NS	NS	NS	NS	NS	NS	NS	NS

NS – no significant difference at p=0.05 level - No bacteria detected

3.3.2 Potato

No *E. coli* or *Enterococcus* were found for any of the samples either internally or externally. *Staphylococcus* was detected internally and externally on all crops. Total coliforms were detected externally on all crops and internally only on the commercially obtained crop. The median values for total coliforms internally and externally for all treatments were generally approximately 800 cfu/g as can be seen in Table 3.12.

Table 3.12: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of potatoes irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No Ascaris ova, Pseudomonas, somatic coliphages, E. coli or Enterococcus was detected internally or externally for any of the water treatments. Staphylococcus was detected internally and externally on crops from all three water treatments and on the commercially bought crop. Total coliforms were detected externally for all treatments and internally for the commercially bought crop.

	Gext	**Hext**	**Text**	**Cext**	**Gint**	**Hint**	**Tint**	**Cint**
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	0	0	0	0	0
Staphylococcus	4.933	4.933	4.933	2148.8	157.48	12.55	12.55	4.93
Total coliforms	800	800	800	800	800	800	800	20000

In the case of the total coliforms, the counts both internally and externally were extremely high. No differences between the trial treatments were detected. The data are presented in Figure 3.16 and Figure: 3.17.

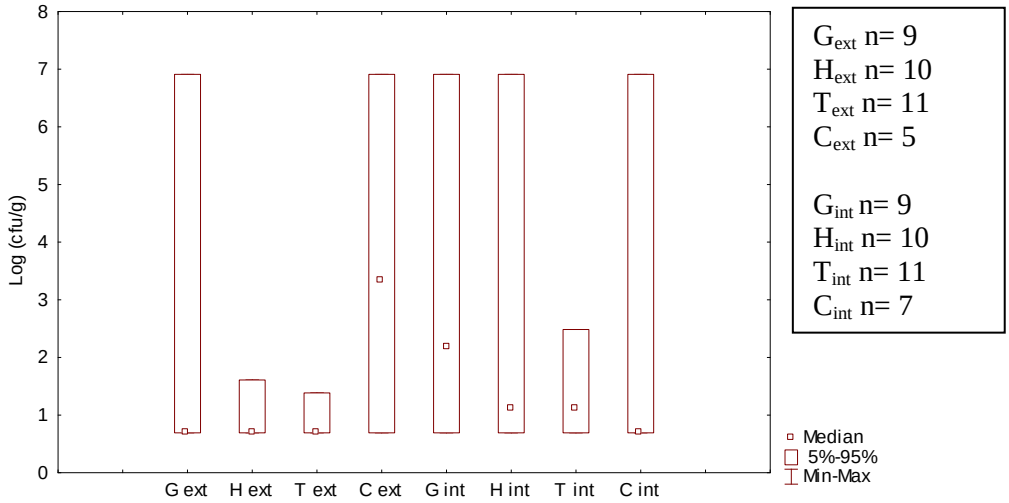


Figure 3.16: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of potatoes irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

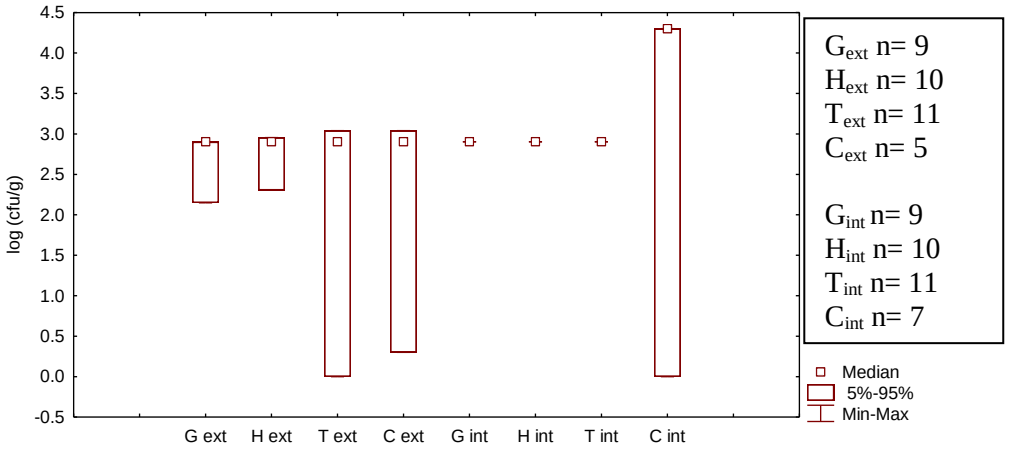


Figure: 3.17: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of potatoes irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

Little difference in contamination was seen between the external and internal results for potato. The statistical comparison of treatments is presented in Table 3.13. Means were compared using the Kruskal-Wallis test.

Table 3.13: Statistical comparison, using non-parametric (Kruskal-Wallis) methods, of means of bacterial load for the interior (int) and exterior (ext) of potatoes irrigated with greywater (G), hydroponic solution (H) or tap water (T). No significant differences were found at the p=0.05 level

	E. coli		***Enterococcus***		***Staphylococcus***		**Total coliforms**	
	External	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**
Kruskal-Wallis	-	-	-	-	NS	NS	NS	NS

NS – no significant difference at p=0.05 - No bacteria detected

3.3.3 Onion

The greywater-irrigated crop was very healthy in appearance, with little or no indication of disease and many of the bulbs grown with the greywater were of excellent size, as can be seen from the example of the crop depicted in Figure 3.18.

Figure 3.18: Example of onion grown with greywater sub-surface irrigation at the UKZN test site

Except for total coliforms, the median bacterial counts were low for both the external and internal analyses. The majority of samples were zero for *E. coli, Enterococcus* and *Staphylococcus* as can be seen in Table 3.14. The statistical data are presented in Figure 3.19 to Figure 3.22.

Table 3.14: Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. *E. coli* was detected externally on all the samples, but not internally. *Enterococcus* was detected externally on all except the tap-water irrigated crop and was not detected internally in any of the samples. *Staphylococcus* was detected externally on all samples and internally on all except the commercially purchased crop. Total coliforms were detected internally and externally on all crops.

	Gext	Hext	Text	Cext	Gint	Hint	Tint	Cint
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0
Total coliforms	4000	2900	800	150	100	40	0	0

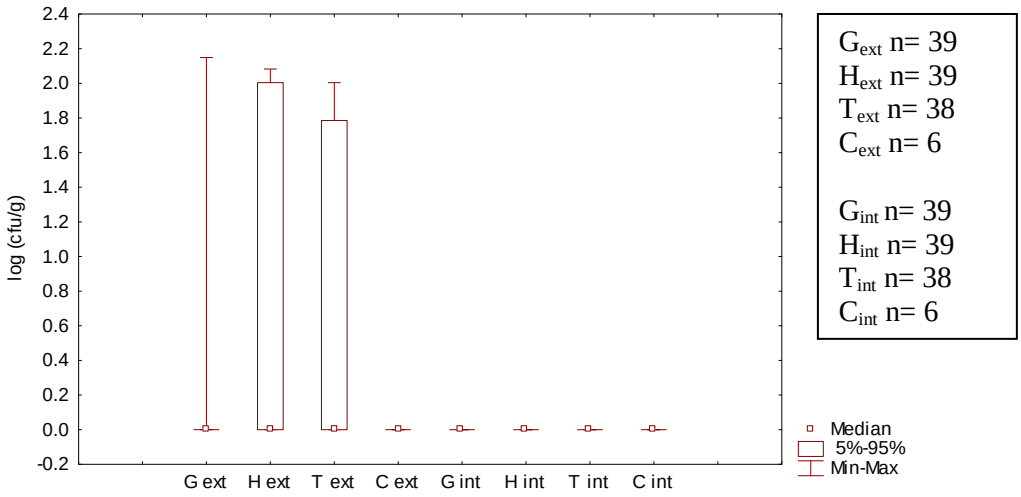


Figure 3.19: Box and whisker plot of *E. coli* counts on external surfaces (ext) or the interior (int) of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

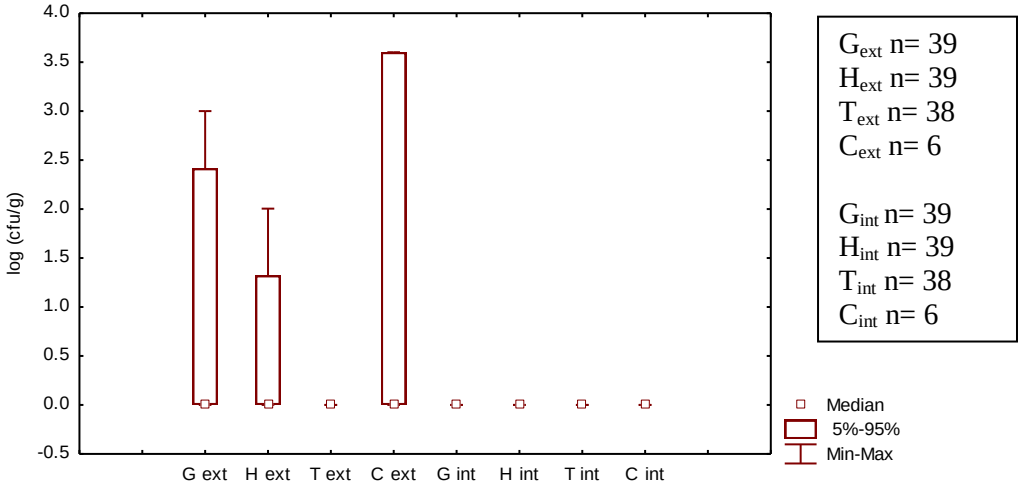


Figure 3.20: Box and whisker plot of *Enterococcus* counts on external surfaces (ext) or the interior (int) of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

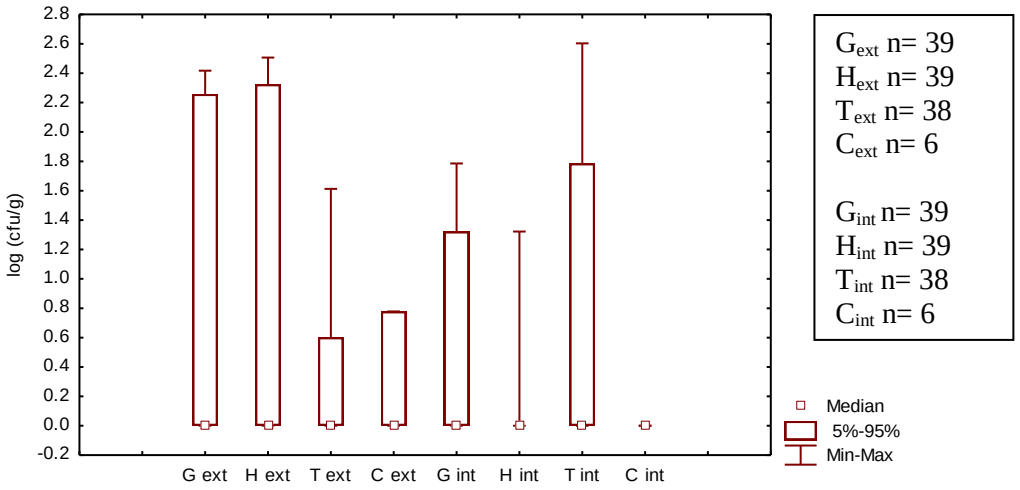


Figure 3.21: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

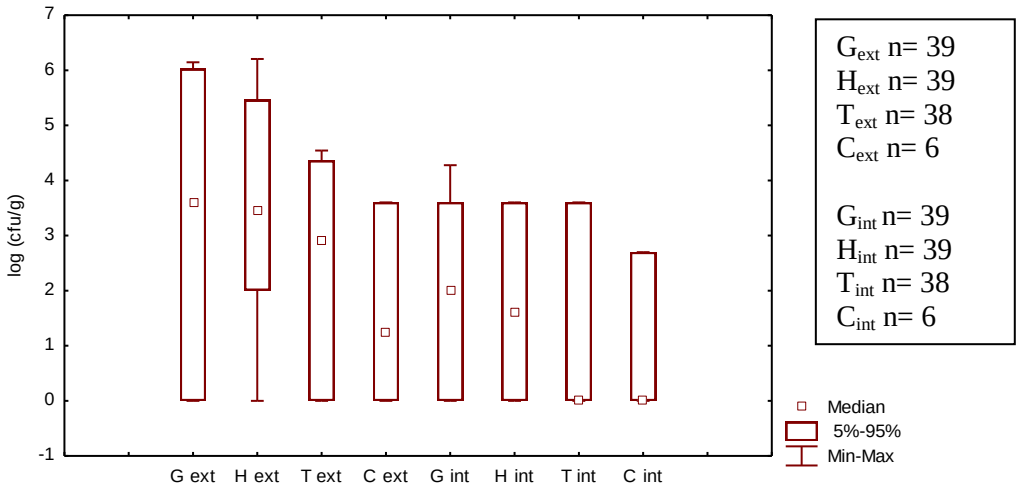


Figure 3.22: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

As can be seen in Table 3.15, no significant differences were found for any of the indicators for any of the treatments when tested with the Kruskal-Wallis test at p=0.05.

Table 3.15: Statistical comparison, using non-parametric (Kruskal-Wallis) methods, of means of bacterial load for the interior (int) and exterior (ext) of onions irrigated with greywater (G), hydroponic solution (H) or tap water (T). No significant differences were found at the p=0.05 level

	E. coli		***Enterococcus***		***Staphylococcus***		**Total coliforms**	
	External	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**
Kruskal-Wallis	NS	NS	NS	NS	NS	NS	NS	NS

NS- no significant difference at p=0.05

3.3.4 Carrots

Carrot was regarded as a high risk crop as it is a root that penetrates more deeply into the soil and, in addition, is frequently eaten raw as a salad vegetable or even directly from the ground with skin intact. No significant difference in bacterial load was seen across the growing seasons ($p \leq 0.05$). No *Enterococcus* was found internally for any of the samples. Except for total coliforms, the median bacterial loads were low for both the external and internal analyses (<10 cfu/g dry) for all treatments as is shown in Table 3.16. In some cases the range was wide (>100 cfu/g dry). The statistical data are presented in Figure 3.23 to Figure 3.26.

Table 3.16: : Median number of organisms per gram dry weight on external surfaces (ext) or the interior (int) of carrots irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. *E. coli* was detected externally for crops grown with hydroponic solution and internally and externally for crops grown with greywater. No *Enterococcus* was found internally for any of the samples but was found externally for crops grown with greywater or purchased commercially. *Staphylococcus* was detected in all crops except for those grown with tap water. Total coliforms were detected internally and externally for all treatments except externally for the commercially purchased crop

	Gext	Hext	Text	Cext	Gint	Hint	Tint	Cint
E. coli	0	0	0	0	0	0	0	0
Enterococcus	0	0	0	0	0	0	0	0
Staphylococcus	4	0	0	0	0	0	0	0
Total coliforms	2600	1100	740	0	400	0	0	580

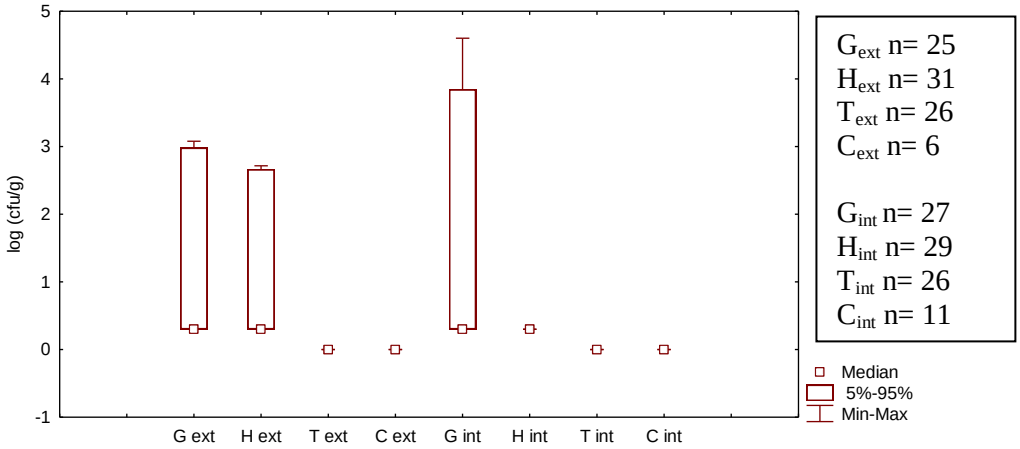


Figure 3.23: Box and whisker plot of *E. coli* counts on external surfaces (ext) or the interior (int) of carrots irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

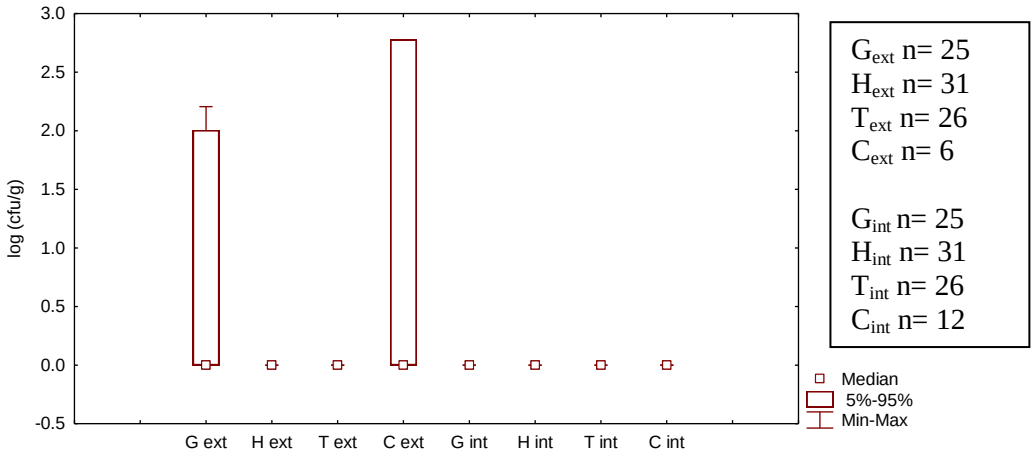


Figure 3.24: Box and whisker plot of *Enterococcus* counts on external surfaces (ext) or the interior (int) of carrots irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

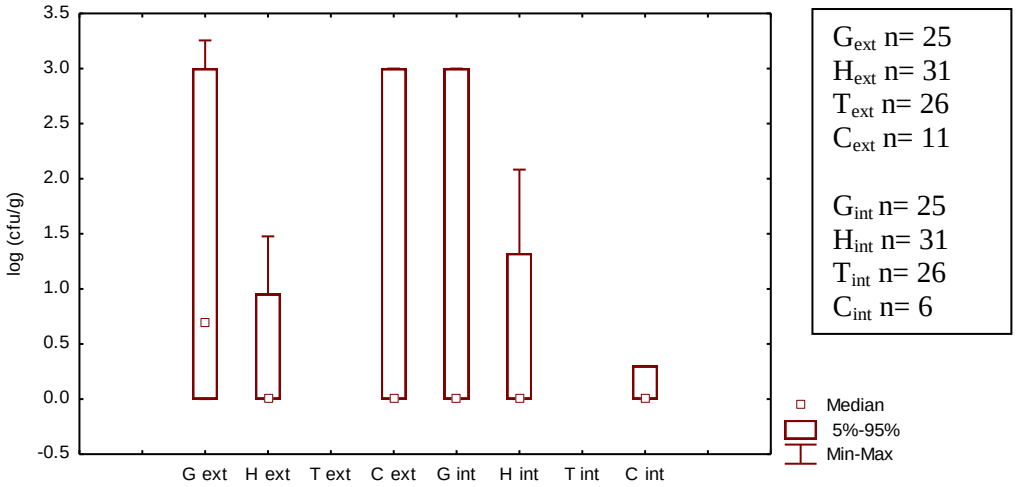


Figure 3.25: Box and whisker plot of *Staphylococcus* counts on external surfaces (ext) or the interior (int) of carrots irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

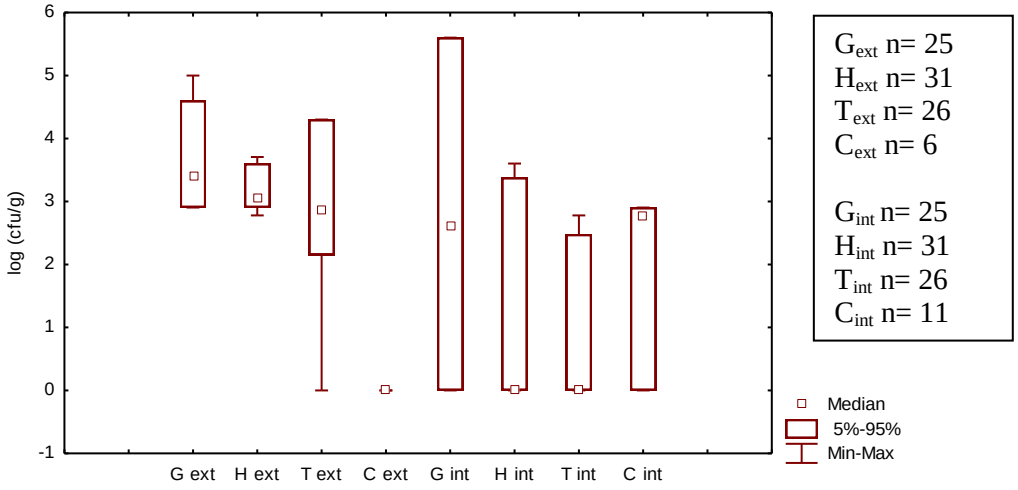


Figure 3.26: Box and whisker plot of total coliform counts on external surfaces (ext) or the interior (int) of carrots irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). Results are based on all samples taken over the complete trial period. No statistical difference was found between the treatments for either the internal or external sets when tested using the Kruskal-Wallis test (p=0.05)

No significant differences were found externally or internally for any crop for any treatment when tested at the p=0.05 level using the Kruskal-Wallis test. These data are presented in Table 3.17.

Table 3.17: Statistical comparison, using non-parametric (Kruskal-Wallis) methods, of means of bacterial load for the interior (int) and exterior (ext) of carrots irrigated with greywater (G), hydroponic solution (H) or tap water (T). No significant differences were found at the p=0.05 level

	E. coli		***Enterococcus***		***Staphylococcus***		**Total coliforms**	
	External	**Internal**	**External**	**Internal**	**External**	**Internal**	**External**	**Internal**
Kruskal -Wallis	NS	NS	NS	-	NS	NS	NS	NS

- No organisms detected NS- no significant difference at p=0.05

3.4 Risk assessment

Although there was no statistical difference for bacterial load for the greywater-irrigated crops compared with the other treatments, quantitative microbial risk assessment was performed for each case.

As it was unlikely in many instances that the crop was peeled before consumption, the microbial concentration for both the internal and external analysis of each crop was combined

before the calculation of risk of infection for each indicator organism to ensure the most conservative outcome. The first stage of the risk assessment was fitting a distribution to the microbial data using the chi-squared test to select the best fit. An example of a typical distribution, in this case for *E. coli* on Swiss chard, is presented in Figure 3.27. From this distribution, it is clear that samples are far more likely to contain low or zero loading of *E. coli* than high loads. As risk is related to both the load of the contaminant and the likelihood of exposure to such a load occurring, the health risk to the population is likely to be low at high contaminant levels because of the infrequency of such an occurrence. The risk to the individual consuming such an infrequent high contaminant load would, however, still remain high.

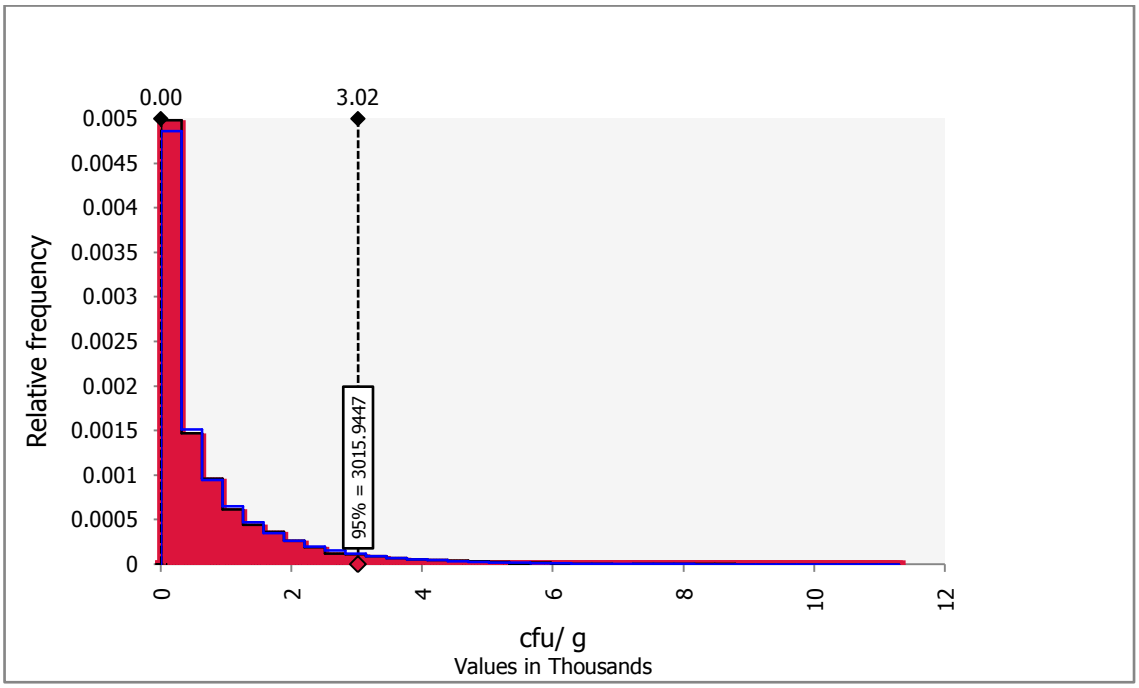


Figure 3.27: Example of a best-fit distribution fitted to the *E. coli* data for Swiss chard irrigated subsurface with greywater. All data throughout the trial was used. The 95th percentile is indicated.

The distributions fitted were then used as the range of contamination from which to calculate the ingested dose for further risk assessment. In each case, a beta-Poisson distribution was used for this calculation with the alpha and beta values for *Shigella* given in Chapter 1, Table 1.8.

An example of the calculation for *E. coli* on Swiss chard irrigated with greywater, based on Equation 2.1 and using the *E. coli* distribution given in Figure 3.27, is given in Equation 3.1.

$$Pinf = 1 - \left(1 + \frac{Distribution\ function \times\ 0.1 \times\ 9.2 \times\ 0.274 \times\ \ 0.01}{42.86}\right)^{-0.126}$$

Equation 3.1: Probability of infection based on the *E. coli* concentration detected for Swiss chard and using the beta-Poisson distribution function

The factors used in the calculation are presented in Chapter 2, Table 2.2. The model was run for 10 000 iterations, using Monte Carlo sampling, to provide the probability of infection. The distribution for this data set is presented in Figure 3.28. For the data for *E. coli* on Swiss chard irrigated with greywater it can be seen that the probability of infection drops rapidly.

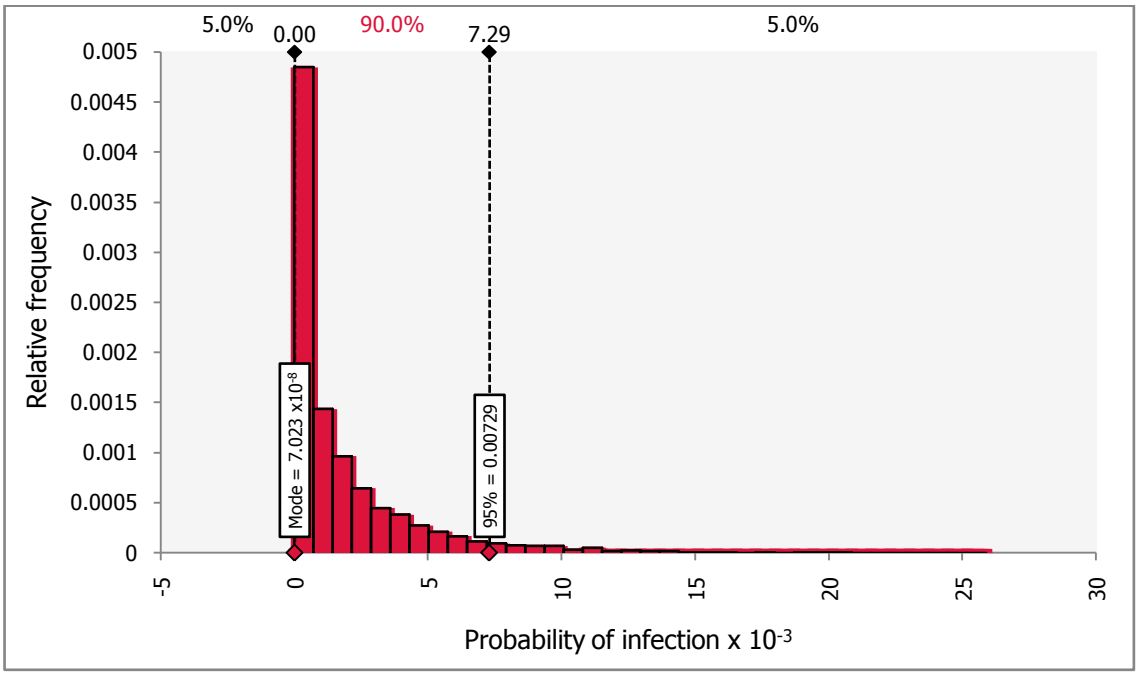


Figure 3.28: Distribution of the probability of infection based on the load of *E. coli* on Swiss chard for daily consumption of the vegetable. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4%, estimated ration of indicator to pathogen = 0.01. The mode and 95th percentiles are indicated

In order to calculate yearly risk this probability of infection was then used in Equation 2.2, where n in this case is 365, as all consumption values were regarded as the normal daily consumption for the population. The output of this calculation for infection from *E. coli* on Swiss chard, based on the distribution presented in Figure 3.28, is presented in Figure 3.29.

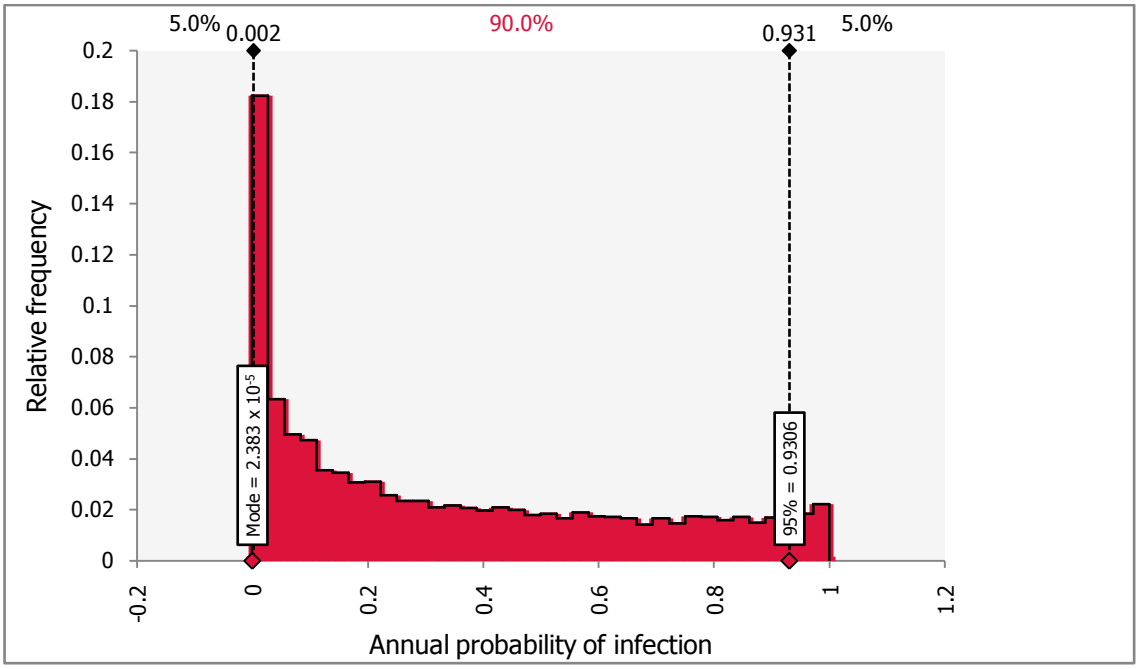


Figure 3.29: Example of the annual risk based on the loading of *E. coli* on Swiss chard irrigated subsurface with greywater. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4%, estimated ration of indicator to pathogen = 0.01 and n=365. No risk mitigating factors were modelled. The mode and 95th percentiles are indicated

All the above calculations are based on data from the crop before any risk-mitigating measures were put in place. With reference to Chapter 1, Table 1.5, the bacterial load on crops could be reduced by interventions such as washing, peeling or cooking the crop before consumption as proposed by the World Health Organisation (WHO, 2006a; Regli *et al.*, 1991). Each of these interventions would result in a reduction of risk, and interventions could be combined to increase the microbial load reduction. The impact on the annual probability of infection by reducing the microbial load by 6-log through cooking is demonstrated in Figure 3.30 where it can be seen that, under these circumstances, the 95th percentile of risk falls within the WHO guidelines of >1 excess case of disease per 10 000 persons (WHO, 2006a; Regli *et al.*, 1991).

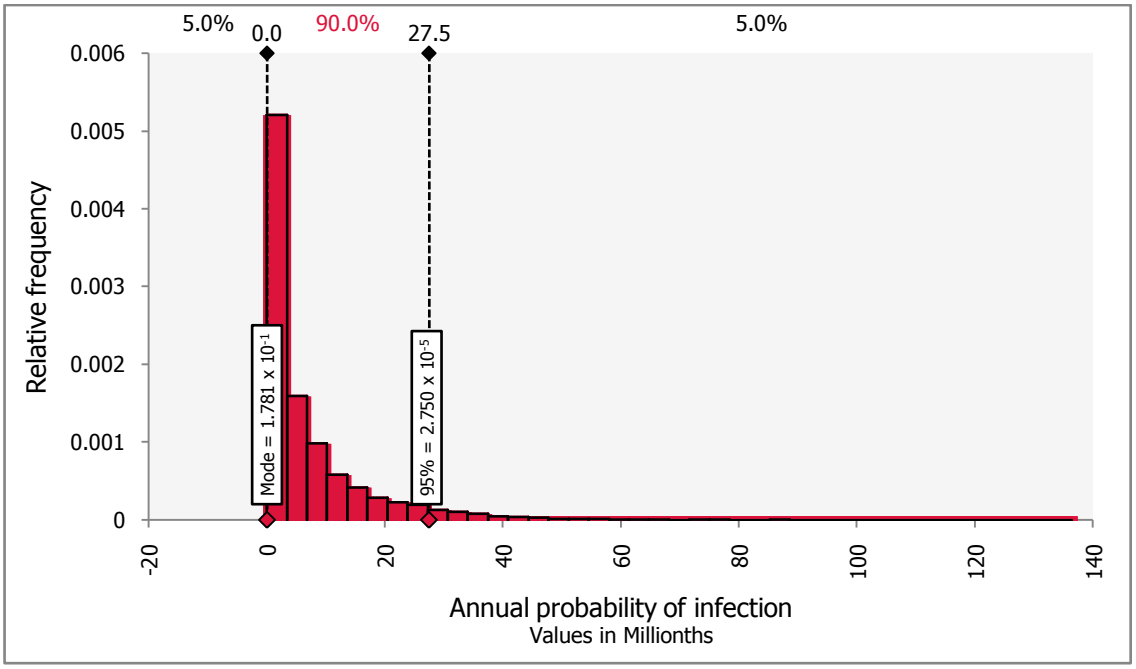


Figure 3.30: Example of the annual risk based on the loading of *E. coli* on Swiss chard irrigated subsurface with greywater and cooked before consumption. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4%, estimated ratio of indicator to pathogen = 0.01, n=365 and risk mitigation factor=6 log. The mode and 95th percentiles are indicated

3.4.1 Greywater

No *E. coli*, *Enterococcus* or *Staphylococcus* were detected in either the hydroponic solution or the tap water, so distributions could not be fitted and hence, statistical comparisons with the greywater could not be made. Distributions were fitted to the data for total coliforms obtained from all three water types. For total coliforms in greywater (n=32) the best fit was a log-normal distribution, while for hydroponic solution (n=20) and tap water (n=18) it was found to be a gamma and exponential distribution respectively. The probability of infection through ingestion of the water was modelled as described in Chapter 3, Section 3.4 using the distribution function obtained for each water type calculated using @RISK and the factors presented in Chapter 2, Section 2.5, Table 2.3. The model was then re-run instituting barriers to infection providing from 1- to 6-log reduction in microbial load. The risk to the individual agricultural worker, rather than the community as a whole, has been calculated in order to provide the highest probability of risk. Results for the three water types are presented in Chapter 8, Appendix 2, Table 8.1. The mode value of probability of infection, based on total coliform load, from annual exposure to any of the three water types is presented in Table 3.18.

As can be seen from Chapter 8, Appendix 2, Table 8.1 and Table 3.18, when no mitigation is put in place, the risk from annual exposure to the greywater at the 95th percentile level is higher than the WHO criteria (WHO, 2006a; Regli *et al.*, 1991) and there is a definite risk to the agricultural workers tending the greywater-irrigated crops over the course of a year. This risk does not exist for workers using either hydroponic solution or tap water. Introducing mitigating factors to achieve a 5-log reduction in microbial load still results in a health risk above that accepted by the World health organisation.

Table 3.18: Mode value for annual probability of infection from the accidental consumption of irrigation water by field workers based on total coliform load. The following factors were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of water = 1 mL, the percentage of the population consuming the crop = 100%, estimated ratio of indicator to pathogen = 0.0001, n=200. Risk both with a 5-log reduction of risk by the implementation of mitigating measures and with no risk abatement are presented. When no mitigation measures are implemented there is a certainty of health risk to workers using greywater whereas there is negligible risk from hydroponic solution or tap water. This risk is brought to twice the acceptable level of risk by implementing a 5-log barrier intervention

	Annual risk with no mitigation	**Annual risk with 5-log mitigation of risk**
Greywater	1	215.6×10^{-6}
Hydroponic solution	0	0
Tap water	5.88×10^{-5}	5.88×10^{-10}

3.4.2 Soil

A similar scenario to that explained for the greywater was assumed to exist for the risk of infection through the handling of soil irrigated subsurface with greywater. A consumption of 1g of soil per exposure, (based on the figures presented in Chapter 2, Section 2.5, Table 2.3 for potential volume of greywater consumed during agricultural work), has been used in the calculations and the remaining factors, except for the bacterial distribution loading, have remained the same as those given in Chapter 2, Table 2.3. No *E. coli* were detected in any of the soil samples. The model for soil was run in @RISK for the sub-set of irrigation workers, and the output is presented in chapter 8, Appendix 2, Table 8.2 with the 95th percentile values given in Table 3.19.

Table 3.19: 95th percentile value for annual probability of infection from the accidental consumption of soil irrigated with one of the three water types based on load of the various microorganisms. The following factors were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily accidental consumption of soil = 1g, the percentage of the population consuming the crop = 100%, estimated ratio of indicator to pathogen = 1 for Enterococcus, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=200. Risk both with a 5-log reduction of risk by the implementation of mitigating measures and with no risk abatement are presented. When no mitigation measures are implemented the health risk to workers using greywater is not significantly different to that from the other two treatments (p=0.05). This risk is brought within the acceptable level of risk by implementing a 5-log barrier intervention.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
Enterococcus	G	3.18335×10^{-2}	3.236×10^{-5}
	H	1	5.19×10^{-4}
	T	0.9999997	$1.81\ 9\times 10^{-4}$
Staphylococcus	G	1.4863×10^{-3}	1.487×10^{-8}
	H	4.494×10^{-3}	4.504×10^{-8}
	T	0.482845	6.646×10^{-6}
Total coliforms	G	1.14189×10^{-2}	1.149×10^{-7}
	H	0.9980694	6.739×10^{-5}
	T	0.294012	3.496×10^{-6}

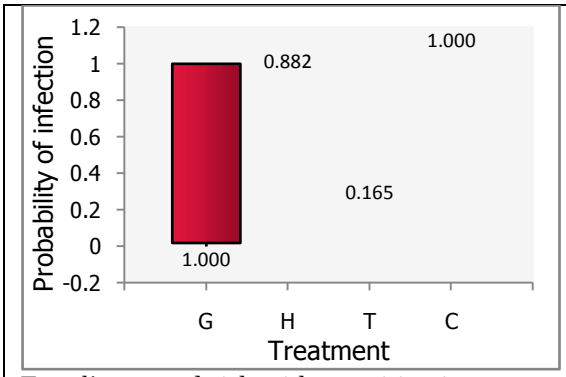
3.4.3 Risk of infection from consuming above ground crops

3.4.3.1 Swiss chard

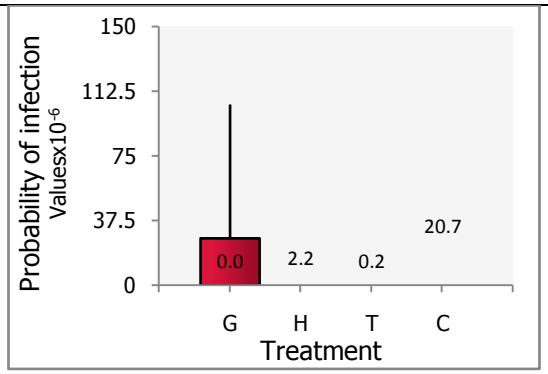
The risk of infection from the consumption of Swiss chard was calculated as outlined in Chapter 2, Section 2.6, using the figures in Table 2.2. As the majority of the results for *E. coli* were zero, except for crops irrigated with greywater, no distribution of risk could be calculated. No significant difference in health risk was found between the treatments ($p \leq 0.05$), indicating that there was a health risk involved in the consumption of Swiss chard even without using greywater irrigation. For the annual exposures, the risk from the raw crop without any barrier treatments generally rose to approximately one, *i.e.* a certainty of infection, for each indicator and for each treatment, except *E. coli* in tap water where distributions could not be calculated. However, once barriers such as withholding irrigation for 2 days immediately pre-harvest and washing of the crop took place, resulting in greater than 2-log reduction of microbial load, the risk was greatly reduced (Chapter 8, Appendix 2, Table 8.3). Values for the 95th percentile of risk are given in Table 3.20. Statistics for the annual risk of infection are presented in Figure 3.31.

Table 3.20: 95th percentile value for annual probability of infection from the consumption of Swiss chard irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for Enterococcus, 0.001 for *Staphylococcus* and 0.0001 for total coliforms 0.01, n=365. No significant difference (p=0.05) was found between the treatments. The risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
E. coli	G	1	2.742×10^{-5}
	H	0.8816289	2.164×10^{-6}
	T	0.1648252	1.803×10^{-7}
	C	1	2.074×10^{-5}
Enterococcus	G	1	0.0027167
	H	1	0.0019173
	T	1	0.0001852
	C	1	9.016×10^{-5}
Staphylococcus	G	1	0
	H	1	0
	T	1	0
	C	1	0
Total coliforms	G	0.9998433	9.282×10^{-6}
	H	0.9998397	9.256×10^{-6}
	T	0.9999998	1.707×10^{-5}
	C	0.5713752	8.519×10^{-7}

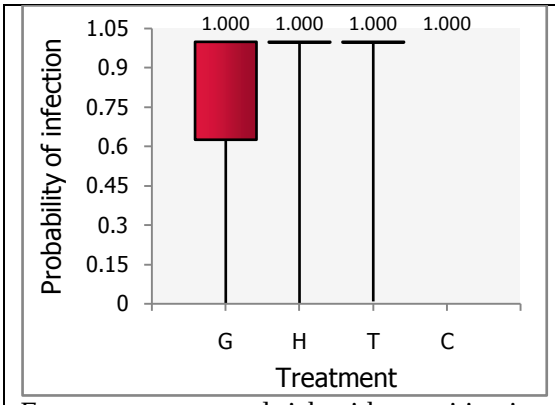


E. coli – annual risk without mitigation

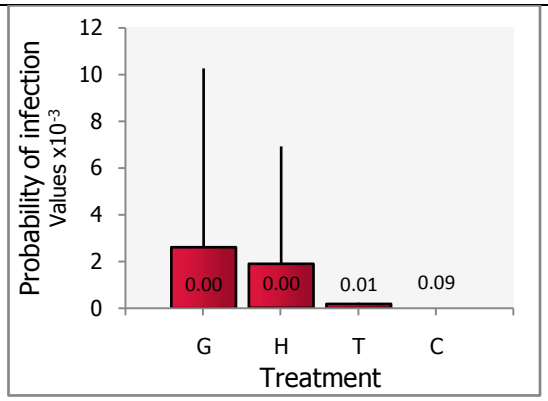


E. coli – annual risk with 6-log mitigation

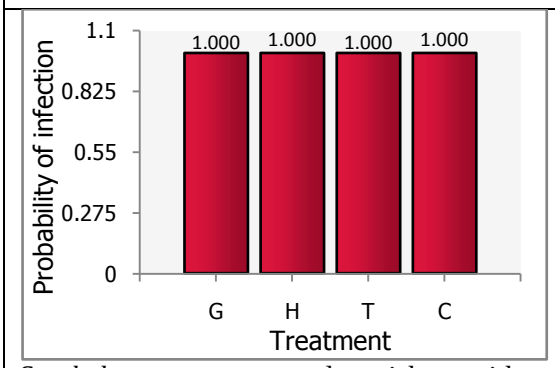
Figure 3.31: Box-and-whisker plots of the annual probability of infection from the consumption of Swiss chard irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms 0.01, n=365 . No significant difference (p=0.05) was found between the treatments. The risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value.



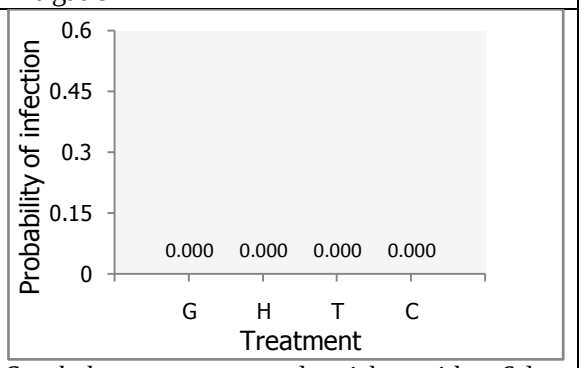
Enterococcus–annual risk without mitigation



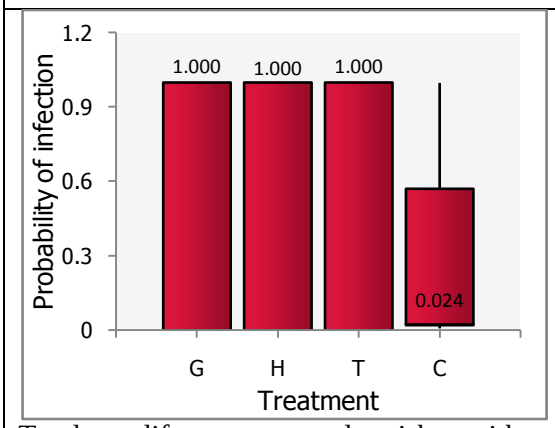
Enterococcus–annual risk with 6-log mitigation



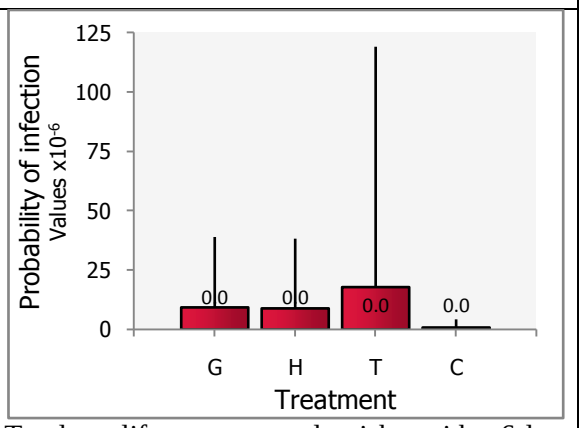
Staphylococcus- annual risk without mitigation



Staphylococcus- annual risk with 6-log mitigation



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

Figure 3.31 (Contd): Box-and-whisker plots of the annual probability of infection from the consumption of Swiss chard irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 9.2g, the percentage of the population consuming the crop = 27.4% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms 0.01, n=365 . No significant difference (p=0.05) was found between the treatments. The risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value.

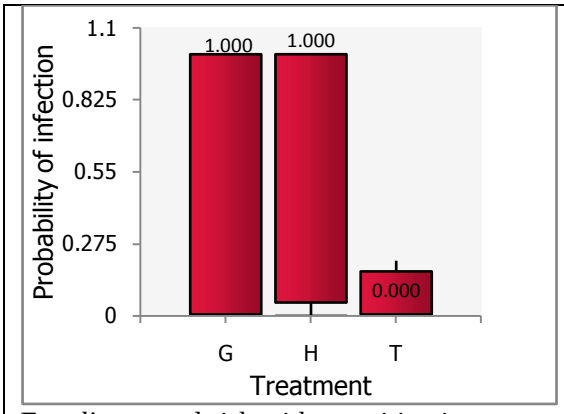
3.4.3.2 Green peppers

The risk of infection from the consumption of green peppers was calculated as outlined in Chapter 2, Section 2.6, using the figures in Table 2.2. The results for the probability of infection for each microorganism and each irrigation scheme are presented in Chapter 8, Appendix 2, Table 8.4. Values for the 95th percentile of risk are given in Table 3.20. Box-and-whisker plots of the probability of infection from annual consumption of peppers both without barrier interventions and with a 6-log reduction in load are presented in Figure 3.32.

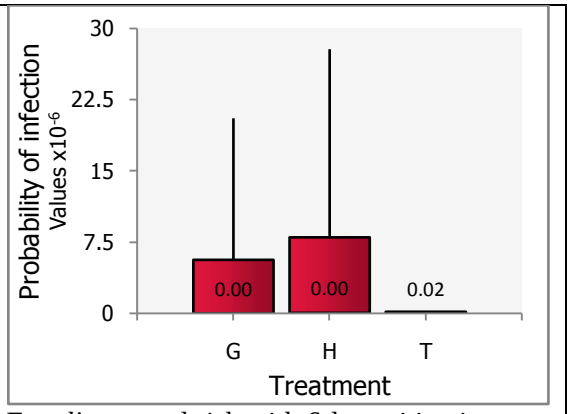
Table 3.21: 95th percentile value for annual probability of infection from the consumption of green peppers irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =2.5g, the percentage of the population consuming the crop = 12.5% (Nel and Steyn (2000), estimated ratio of indicator to pathogen =0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms 0.01, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
E. coli	G	0.9954528	5.588×10^{-6}
	H	0.9993824	7.758×10^{-6}
	T	0.1635578	1.788×10^{-7}
	C	0	0
Staphylococcus	G	0.1917181	2.131×10^{-7}
	H	0.8195605	1.732×10^{-6}
	T	0.7534859	1.413×10^{-6}
	C	0.2466788	2.838×10^{-7}
Total coliforms	G	0.0680975	7.056×10^{-8}
	H	0.0506622	5.201×10^{-8}
	T	0.0495804	5.087×10^{-8}
	C	0.0059984	6.017×10^{-9}

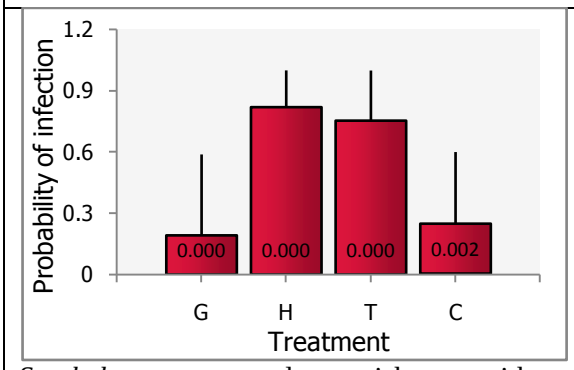
It can be seen, that as a result of the distribution of microbial load, the greywater in many instances has an apparently lower level of risk than the other treatments for the 95th percentile, although this was not found to be statistically significant ($p\leq0.05$).



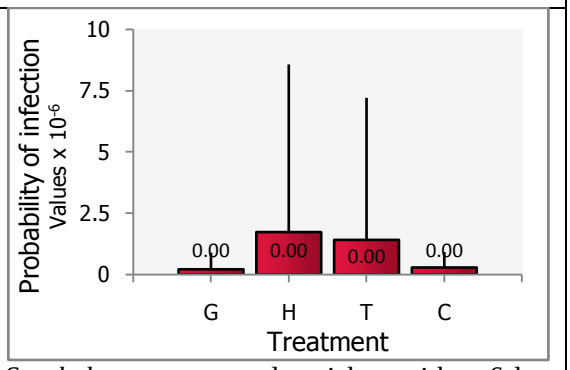
E. coli – annual risk without mitigation



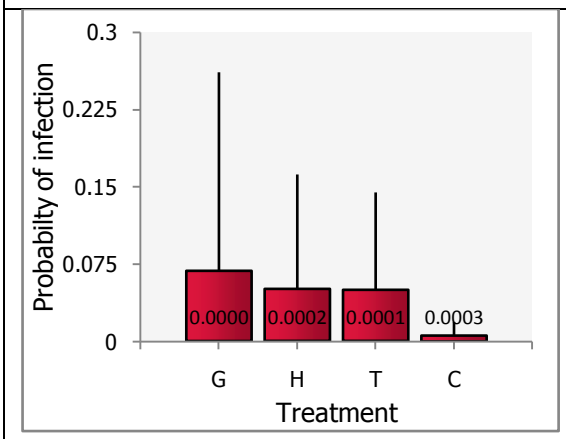
E. coli – annual risk with 6-log mitigation



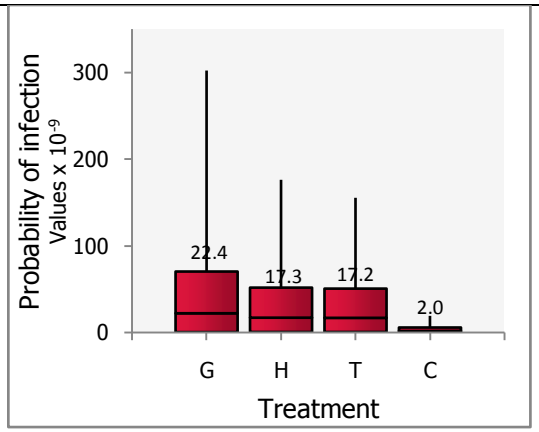
Staphylococcus–annual risk without mitigation



Staphylococcus–annual risk with 6-log mitigation



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

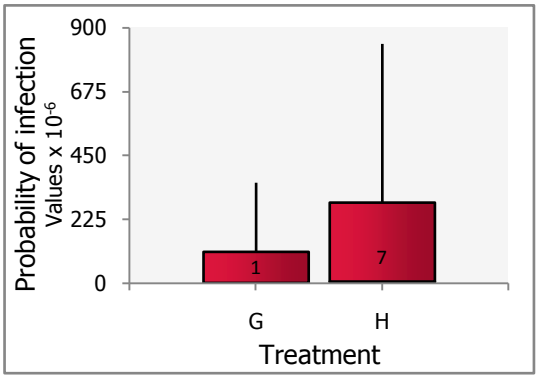
Figure 3.32:Box-and-whisker plots of the annual probability of infection from the consumption of green peppers irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop = 2.5g, the percentage of the population consuming the crop = 12.5%, estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms 0.01, n=365 and risk mitigation factor=6 log and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value.

3.4.3.3 Chillies

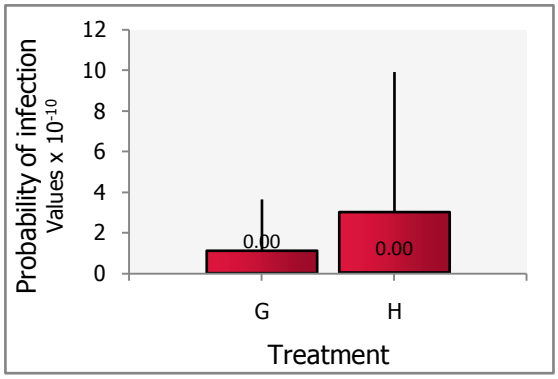
As explained in Section 3.2.3, as a result of findings of unusually high microbial contamination inside green peppers, chillies were investigated briefly only for greywater and hydroponic solution subsurface irrigation for one growth cycle. No *E. coli*, *Enterococcus* or *Staphylococcus* were detected in any of the samples. The 95th percentile of risk is presented for each combination in Table 3.22. The annual probability of infection based on the loading of total coliforms both before mitigation and with a mitigation level of 6-log, are presented in Figure 3.33. It can be seen that, even without any barrier precautions, the results for the greywater-irrigated chillies fell within the results for the hydroponic solution-irrigated crop and both complied with the WHO requirements. The full set of results is presented in Chapter 7, Appendix 2 as Table 8.5.

Table 3.22: 95th percentile value for annual probability of infection from the consumption chillies irrigated with greywater (G) or hydroponic solution (H) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =0.6 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was within the acceptable level of <1 in 10 000 even before implementation of mitigation measures.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
Total coliforms	G	1.077×10^{-4}	1.076×10^{-10}
	H	2.917×10^{-4}	2.917×10^{-10}



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

Figure 3.33: Box-and-whisker plots of the annual probability of from the consumption of chillies irrigated with greywater (G) or hydroponic solution (H). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =0.6 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was within the acceptable level of <1 in 10 000 even before implementation of mitigation measures.The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value.

3.4.4 Risk of infection from consuming below-ground crops

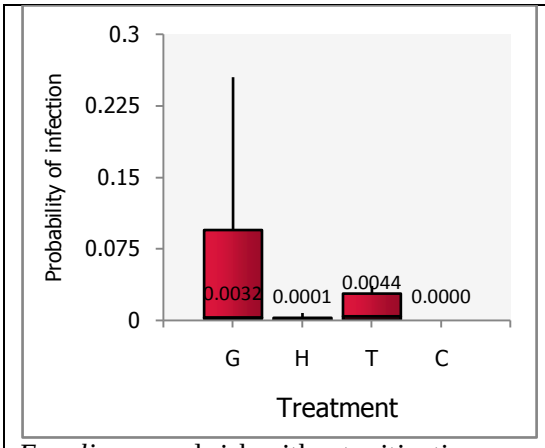
3.4.4.1 Beetroot

The data for beetroot were examined in the same way as explained previously in Chapter 2, Section 2.6, using the figures in Table 2.2. No distribution could be fitted for *Enterococcus* for tap water-irrigated beetroot as there were insufficient non-zero values. The commercially purchased beetroot also had arithmetic means used for the calculations as there was insufficient range of results to fit a distribution. It was considered highly unlikely that beetroot would be consumed either unwashed or un-peeled, therefore the result obtained for risk with the implementation of a 2-log reduction in microbial load was considered to indicate the highest level of risk. At the 95th percentile level, all indicators except *Enterococcus* showed a health risk of less than 10^{-5} for a single exposure. The 95th percentiles of risk on an annual basis are presented in Table 3.23.

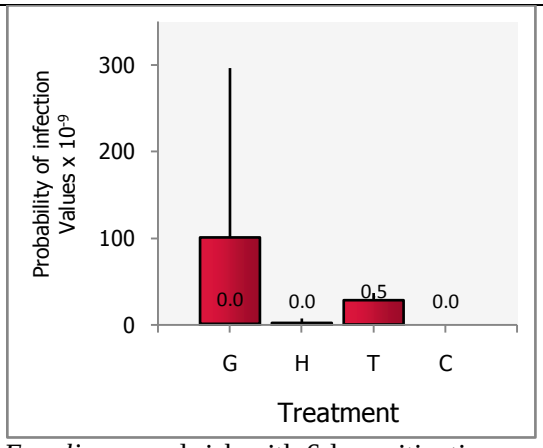
Table 3.23: 95th percentile value for annual probability of infection from the consumption of beetroot irrigated with greywater (G), hydroponic solution (H) tap water (T) or purchased commercially (C) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. No significant difference was found between the treatments for any indicator at the p=0.05 level.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
E. coli	G	0.094819	9.969×10^{-8}
	H	0.0027714	2.775×10^{-9}
	T	0.028166	2.858×10^{-8}
	C	0	0
Enterococcus	G	1	0.0004986
	H	1	0.0001912
	T	0	0
	C	1	2.143×10^{-5}
Staphylococcus	G	0.0787155	8.203×10^{-8}
	H	0.5765249	8.641×10^{-7}
	T	0.1380616	1.487×10^{-7}
	C	0	0
Total coliforms	G	0.1602551	1.749×10^{-7}
	H	0.3009514	3.589×10^{-7}
	T	0.0425956	4.354×10^{-8}
	C	0.2689353	3.139×10^{-7}

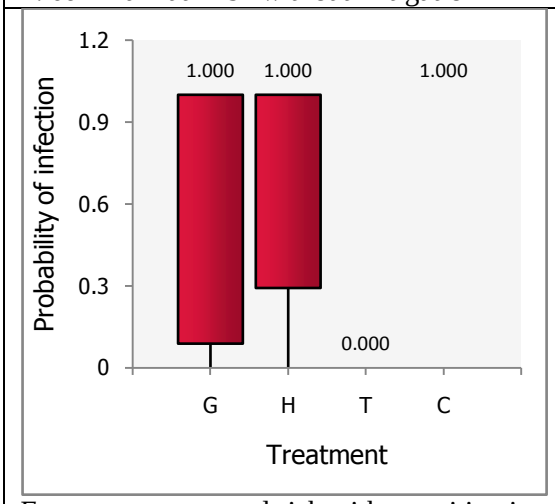
No significant difference was found for the probability of infection among the treatments ($p\leq0.05$). The annual probabilities of infection across the four treatments and for the four indicators are presented graphically in Figure 3.34. The maximum risk calculated for beetroot irrigated with hydroponic solution using total coliforms as the indicator was orders of magnitude higher than for the other treatments even though the median values were similar. There was no apparent reason for the higher concentration range of total coliforms for this treatment and further investigation would be required. From the data presented it is apparent that beetroot would be a suitable crop for growth with greywater-irrigation.



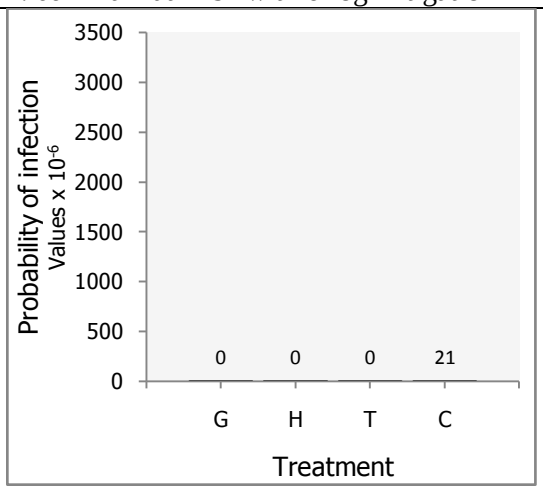
E. coli – annual risk without mitigation



E. coli – annual risk with 6-log mitigation

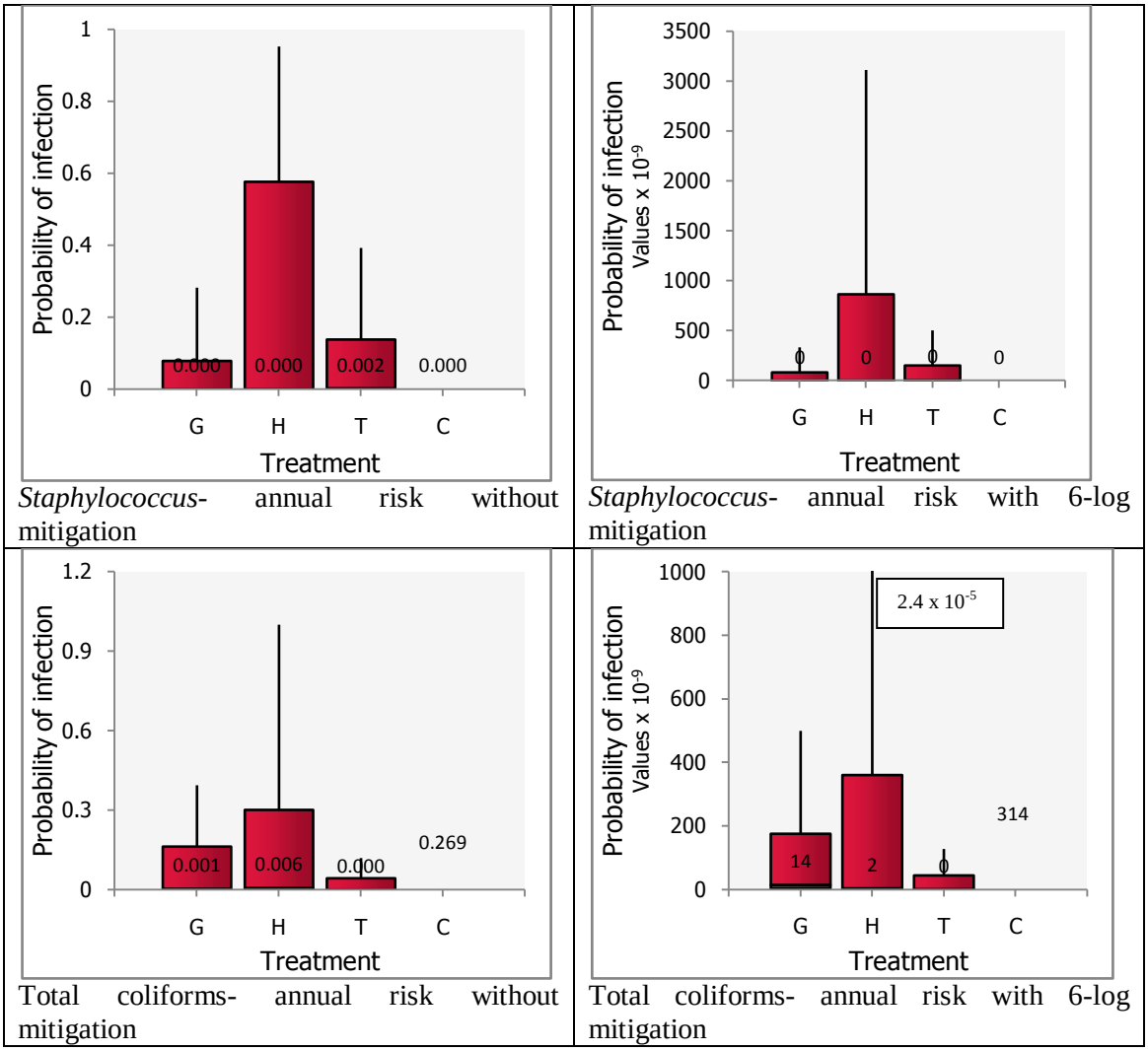


Enterococcus–annual risk without mitigation



Enterococcus–annual risk with 6-log mitigation

Figure 3.34: Box-and-whisker plots of the annual probability of from the consumption of beetroot irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value. The maximum risk based on total coliform concentration is boxed adjacent to the whisker for this treatment



Staphylococcus- annual risk without mitigation

Staphylococcus- annual risk with 6-log mitigation

Total coliforms- annual risk without mitigation

Total coliforms- annual risk with 6-log mitigation

Figure 3.34 (Contd):Box-and-whisker plots of the annual probability of from the consumption of beetroot irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value. The maximum risk based on total coliform concentration is boxed adjacent to the whisker for this treatment

3.4.4.2 Potato

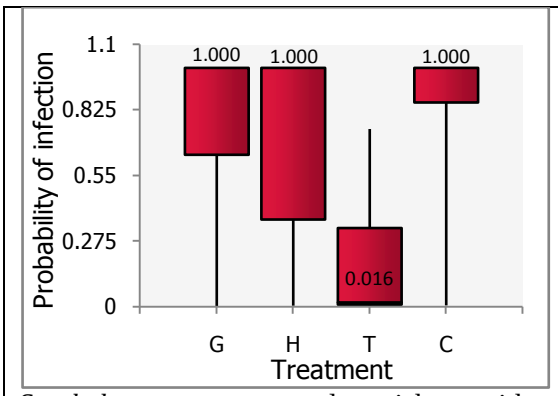
The data for potato were examined in the same way as explained previously, using the factors from Table 2.2. No *E. coli* or *Enterococcus* was detected for any of the samples from any of the irrigation systems. It is unusual for potato to be eaten uncooked, so the information for the

cooked crop, i.e. six-log removal of microorganism, was regarded as being the most relevant. The 95th percentiles of risk on an annual basis are presented in Table 3.24.

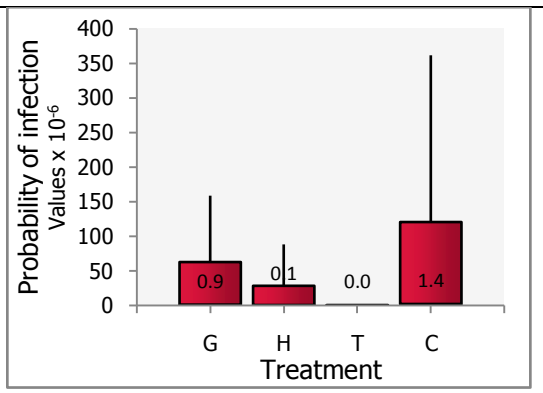
Table 3.24: 95th percentile value for annual probability of infection from the consumption of potato irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =45.4 g, the percentage of the population consuming the crop = 27.1% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
Staphylococcus	G	1	6.245×10^{-5}
	H	1	2.79×10^{-5}
	T	0.3283836	3.991×10^{-7}
	C	1	1.198×10^{-4}
Total coliforms	G	0.5864621	8.881×10^{-7}
	H	0.504534	7.055×10^{-7}
	T	0.4972232	6.907×10^{-7}
	C	0.9998772	9.555×10^{-6}

Box-and-whisker plots of the probability of infection based on annual consumption are presented in Figure 3.35 and show that the values for the greywater-irrigated crop are either similar to or less than the values for the commercially purchased crop.

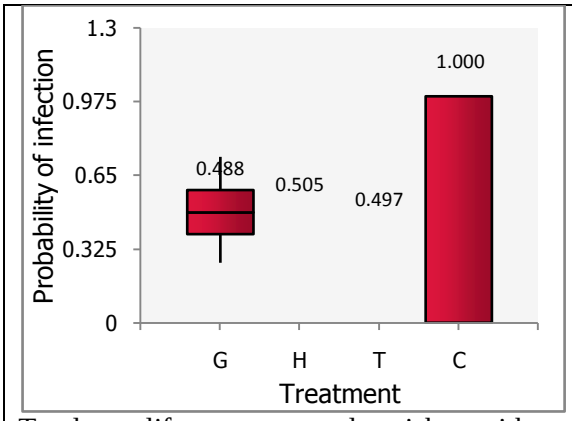


Staphylococcus- annual risk without mitigation

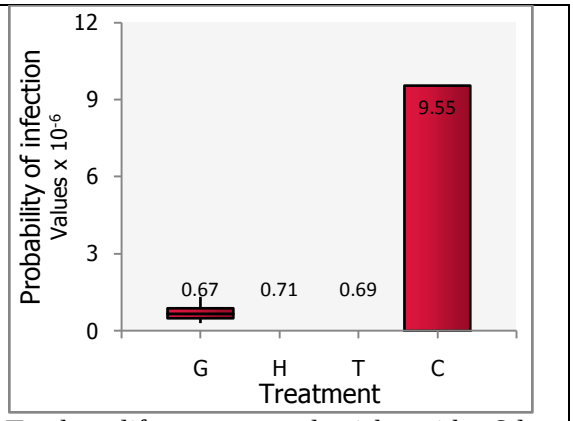


Staphylococcus- annual risk with 6-log mitigation

Figure 3.35: Box-and-whisker plots of the annual probability of from the consumption of potato irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =45.4 g, the percentage of the population consuming the crop = 27.1% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E. coli*, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

Figure 3.35 (Contd): Box-and-whisker plots of the annual probability of from the consumption of potato irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =45.4 g, the percentage of the population consuming the crop = 27.1% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was mainly brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

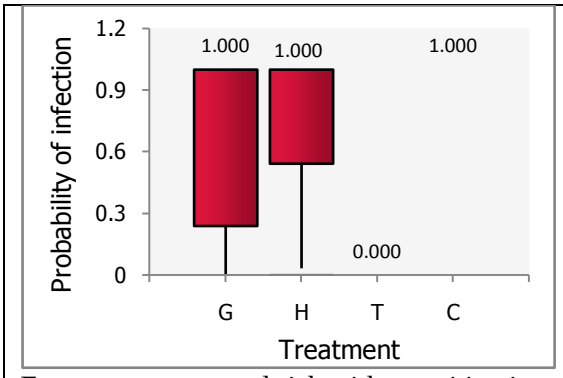
3.4.4.3 Onions

In Section 3.3.3 it was shown that the median bacterial counts in onions were low for both the external and internal analyses, with the majority of samples being zero for *E. coli*, *Enterococcus* and *Staphylococcus*. The data for onions were examined in the same way as explained previously, using the factors presented in Table 2.2. The data are presented in Chapter 8, Appendix 2, Table 8.8. Box-and-whisker plots of the probability of infection based on annual consumption are presented in Figure 3.36When the annual risk of infection was reviewed, it could be seen that if eaten raw and without any microbial load-mitigating barriers, both the greywater- and the hydroponic solution-irrigated produce presented a health risk above that considered tolerable by the WHO for all indicators (Table 3.25). When a 6-log reduction in microbial load was modelled, the risk fell within the accepted limit of 10^{-4}.

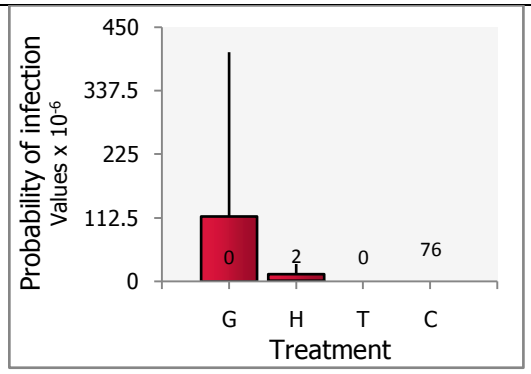
Table 3.25: 95th percentile value for annual probability of infection from the consumption of onions irrigated with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =2.5 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
Enterococcus	G	1	0.0001151
	H	0.9999955	1.336×10^{-5}
	T	0	0
	C	1	7.63×10^{-5}
Staphylococcus	G	0.2453968	2.821×10^{-7}
	H	0.3423612	4.202×10^{-7}
	T	0.2829944	3.334×10^{-7}
	C	0.0011439	1.145×10^{-9}
Total coliforms	G	0.9191817	2.557×10^{-6}
	H	0.9486512	3.027×10^{-6}
	T	0.1486227	1.611×10^{-7}
	C	0.0218821	2.213×10^{-8}

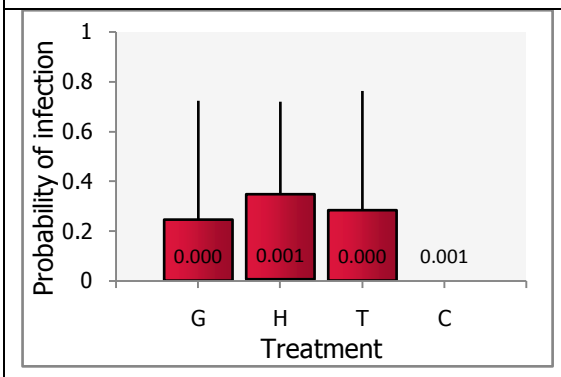
When tested statistically, no significant difference was found for any of the risks calculated from the four indicator microorganisms ($p \leq 0.05$).



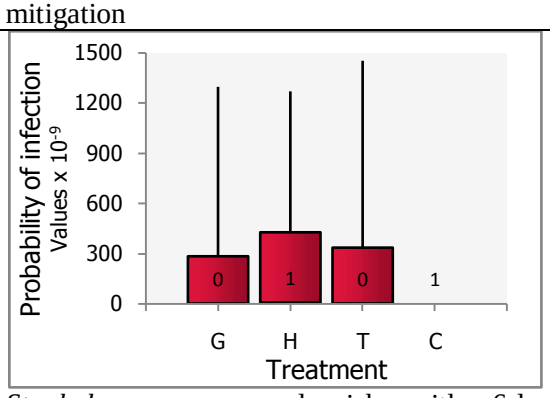
Enterococcus–annual risk without mitigation



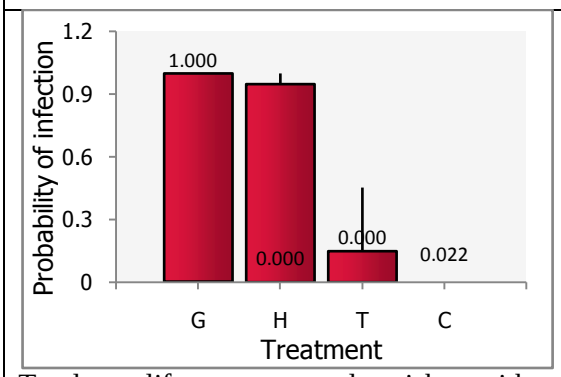
Enterococcus–annual risk with 6-log mitigation



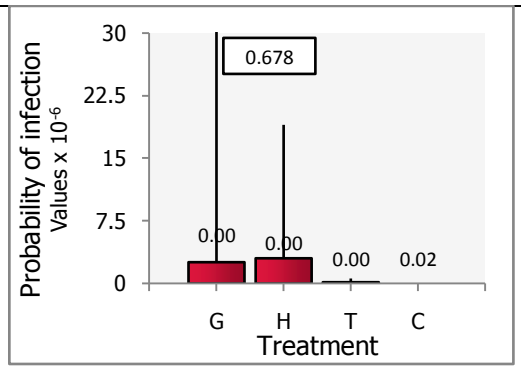
Staphylococcus- annual risk without mitigation



Staphylococcus- annual risk with 6-log mitigation



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

Figure 3.36: Box-and-whisker plots of the annual probability of from the consumption of onion irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =2.5 g, the percentage of the population consuming the crop = 12.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E*. coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value.

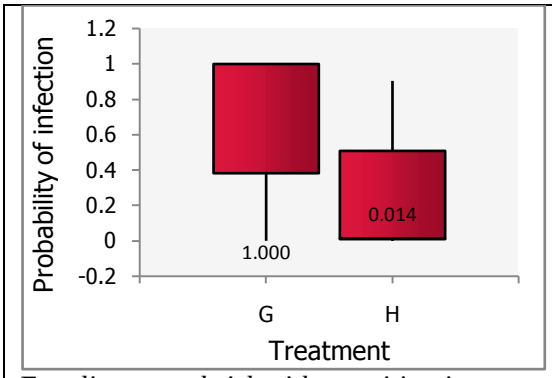
3.4.4.4 Carrots

As was stated in Section 3.3.4, no *E. coli* was detected for either the tap water-irrigated or commercially purchased crop and no *Enterococcus* was found for the hydroponic solution- or tap water-irrigated samples. No probability of infection could therefore be calculated for either of these treatments for these indicators. The data for carrots were examined in the same way as explained previously, using the factors presented in Table 2.2. The data are presented in Chapter 8, Appendix 2, Table 8.9 and Table 3.26.

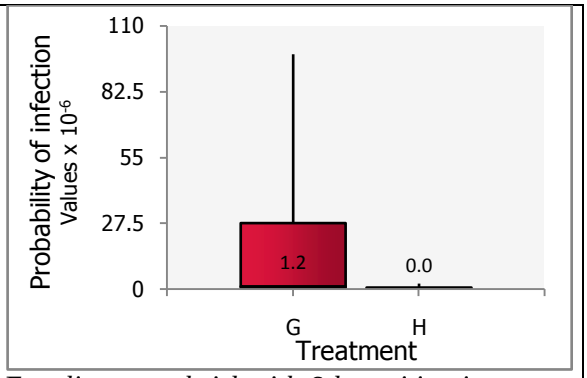
Table 3.26: 95th percentile value for annual probability of infection from the consumption of carrot irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C) based on load of the various microorganisms. The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9, the percentage of the population consuming the crop = 30.8% (Nel and Steyn (2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365 . No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load.

Microorganism	Water	Annual risk with no mitigation	Annual risk with 5-log mitigation of risk
E. coli	G	1	2.766×10^{-5}
	H	0.5088989	7.144×10^{-7}
	T	-	-
	C	-	-
Enterococcus	G	1	6.833×10^{-5}
	H	-	-
	T	-	-
	C	1	0.0002578
Staphylococcus	G	0.9999976	1.408×10^{-5}
	H	0.0815594	8.513×10^{-8}
	T	0.0316496	3.217×10^{-8}
	C	0.7100748	1.248×10^{-6}
Total coliforms	G	0.887764	2.219×10^{-6}
	H	0.2165974	2.445×10^{-7}
	T	0.3741362	4.701×10^{-7}
	C	0.058377	6.017×10^{-8}

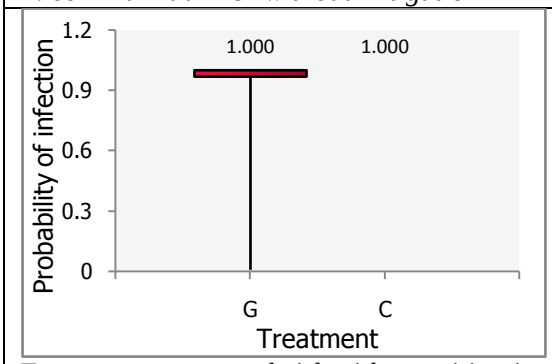
Box-and-whisker plots of the probability of infection based on annual consumption are presented in Figure 3.37. The data from all four treatments indicate that the annual risk of consumption of carrots was above the WHO specifications (WHO, 2006a) when no barriers to reduce microbial load were modelled.



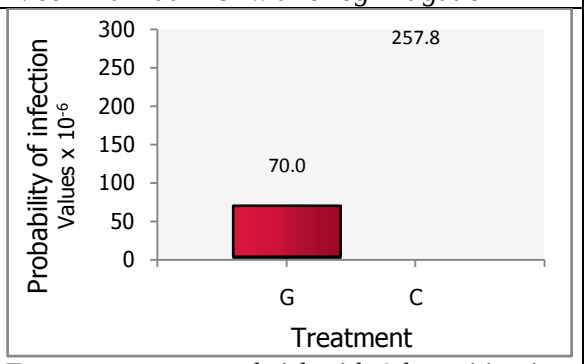
E. coli – annual risk without mitigation



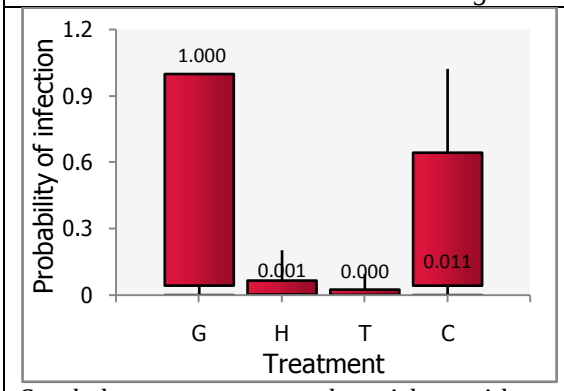
E. coli – annual risk with 6-log mitigation



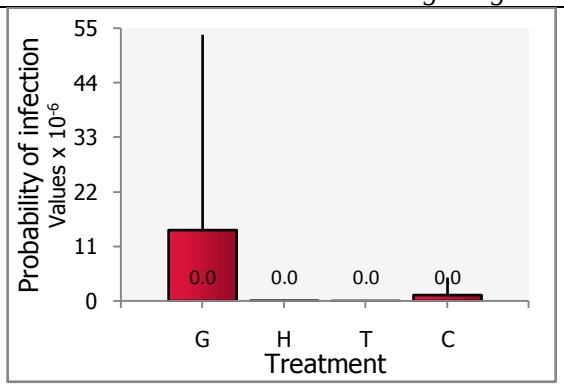
Enterococcus–annual risk without mitigation



Enterococcus–annual risk with 6-log mitigation

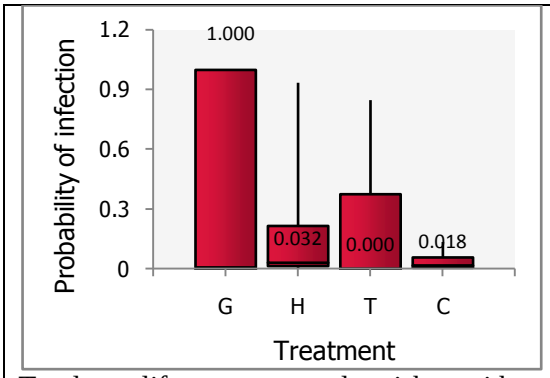


Staphylococcus- annual risk without mitigation

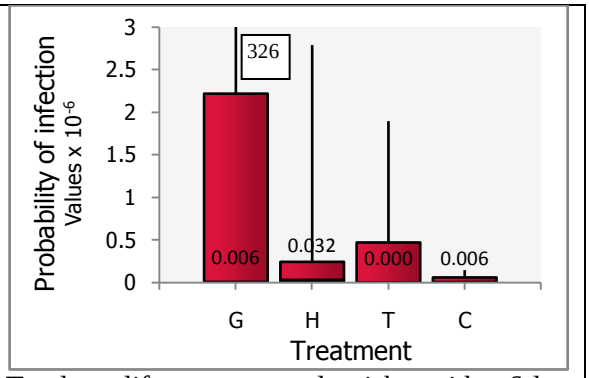


Staphylococcus- annual risk with 6-log mitigation

Figure 3.37: Box-and-whisker plots of the annual probability of from the consumption of carrots irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9 g, the percentage of the population consuming the crop = 30.8% (Nel and Steyn, 2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365. No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value. The maximum risk based on total coliform concentration for the greywater (G) treatment is boxed adjacent to the whisker for this treatment



Total coliforms- annual risk without mitigation



Total coliforms- annual risk with 6-log mitigation

Figure 3.37 (Contd): Box-and-whisker plots of the annual probability of from the consumption of carrots irrigated with greywater (G), hydroponic solution (H), or tap water (T) or purchased commercially (C). The following were used in the calculation α=-0.126, β=42.86, d=distribution fitted to the untransformed data by @RISK. This data comprised an estimated daily consumption of the crop =3.9 g, the percentage of the population consuming the crop = 30.8% (Nel and Steyn, 2000), estimated ratio of indicator to pathogen = 0.001 for *E.* coli, 1 for *Enterococcus*, 0.001 for *Staphylococcus* and 0.0001 for total coliforms, n=365. No significant difference was found (p=0.05) for the risk presented by each of the three treatments and this risk was brought within the acceptable level of <1 in 10 000 by implementing mitigation measures resulting in a 5-log reduction of microbial load. The vertical line indicates the range, the box the 5th to 95th percentile and the mode is indicated by the numeric value. The maximum risk based on total coliform concentration for the greywater (G) treatment is boxed adjacent to the whisker for this treatment

When a 6-log reduction of the microbial load was modelled, all indicators, except *Enterococcus* on the commercially purchased crop, resulted in health risks of less than 10^{-4}. The data for this treatment was biased by one sample containing 600 cfu/g dry mass whereas the remaining samples were less than 1 cfu/g dry mass. As was decided at the start of the project, this result was used as a worst case scenario to estimate potential risk. Taking the 95th percentile of risk of infection gives a conservative outcome which provides additional safety to consumers.

4. DISCUSSION

4.1 Irrigation water

In Chapter 1, Section 1.2, Table 1.1, levels of indicator bacteria found in greywater internationally were presented. When these figures are compared with the levels determined for the greywater used in this study and presented in Chapter 3, Section 3.1.3, Table 3.2, it can be seen that the concentrations are similar or that the local greywater has higher levels of microbial contamination than the reported international figures for greywater, but lower than the reported international loadings for sewage as presented in Table 4.1. This is in contrast to the findings of Carden *et al.* (2007b) who found that greywater from un-sewered informal settlements in South Africa had high levels of microbial pollution and stated that the greywater in South Africa was generally unfit for re-use. The greywater used in this study is therefore not unusual in its characteristics when related to international studies, but may be markedly different from what is found in other parts of South Africa and models used internationally could be used in this instance with a reasonable degree of accuracy.

Table 4.1: Reported number of organisms in 100 mL of greywater or sewage water respectively (Ottosson and Stenström, 2005).

Organism	**Loading $\log_{10}$ (cfu/100mL)**
Greywater	
Total coliforms	7.2 – 8.8
Thermotolerant coliforms	5.8
E. coli	5.4
Enterococcus	2.4 – 4.6
Sewage	
Salmonella	1.97 – 4.04
Enterovirus	1 - 3

As stated by the World Health Organisation (WHO, 2006a), the readily available biodegradable organics in greywater could allow the growth of indicators in the system and the levels obtained are therefore likely to over-estimate the faecal load and hence health risk. This is supported by Roesner *et al.*, (2006), who state that a high level of thermotolerant coliforms in greywater does not translate to a high pathogen risk as non-pathogenic coliforms can multiply in greywater whereas pathogens tend to die off rapidly. It is also supported by this study where none of the selected pathogens was detected during the course of the project although in some cases high levels of the selected indicators were present.

As can be seen from Chapter 8, Appendix 2, Table 8.1 and Chapter 3, Section 3.4.1, Table 3.18, the risk from annual exposure to the greywater at the 95th percentile level is higher than

the WHO criteria of less than 1 excess case of disease per 10 000 people per year (Regli *et al.*, 1991) and there is a definite risk to the agricultural workers tending the crops. This risk does not exist for workers using either hydroponic solution or tap water. Mitigating measures that could be taken to reduce the risk of infection are the use of gloves and boots and thorough washing of the face and arms after handling the greywater. According to the World Health Organisation (WHO, 2006a), these would result in a one- to two-log reduction each in exposure and a five-log reduction if all were combined. These measures are easily implemented and, if all are applied to achieve a 5-log reduction of exposure, would reduce the annual health risk levels of agricultural workers to within the range generally accepted by the World Health Organisation (WHO, 2006a; Regli *et al.*, 1991) and the United States Environment Protection Agency (USEPA, 2004).

In line with best practice in barrier protection methods, these measures should start to be introduced as far back from the end user as possible and several barriers should be in place to provide a redundancy level of protection. Interventions to reduce the microbial load in the greywater itself should be investigated as the most effective measure of reducing overall health risk to the community.

4.2 Soil

Soil from the pot trial was analysed chemically and microbiologically throughout the trial and the mean results are presented in Chapter 3, Section 3.1.2, Table 3.1. These results indicate that there is the potential for the accumulation of microorganisms in the soil with prolonged use of untreated greywater. Based on the assumptions made in the model used for field workers, the results of which are presented in Chapter 3, Section 3.4.2, Table 3.21 and Chapter 8, Appendix 2, Table 8.2, it is apparent that working with soil presents a lesser risk than working directly with greywater. There is however a similar risk from the soil for each of the water types used and the risk would be limited to those working the crop or preparing it for consumption. The use of even basic mitigation measures would bring the risk within the range considered acceptable by the World Health Organisation (WHO, 2006a; Regli *et al.*, 1991).

4.3 Above-ground crops

4.3.1 Swiss chard

When annual consumption is examined, where no barriers were applied, none of the treatments resulted in crops that complied with the WHO guidelines (WHO, 2006a; Regli *et al.*, 1991) at the 95th percentile level (Chapter 8, Appendix 2, Table 8.3). The level of risk varied among the indicators, with *Enterococcus* showing the greatest risk. No significant difference in health risk was found between the treatments ($p \leq 0.05$), indicating that there was a health risk involved in the consumption of Swiss chard even without using greywater irrigation. For the annual exposures, the risk from the raw crop without any barrier treatments generally rose to approximately one, *i.e.* a certainty of infection, for each indicator and for each treatment, except *E. coli* in tap water where distributions could not be calculated. However, once barriers such as withholding irrigation for 2 days immediately pre-harvest and washing of the crop took place, resulting in greater than 2-log reduction of microbial load, the risk was greatly reduced (Chapter 8, Appendix 2,Table 8.3).

4.3.2 Green peppers

In each case, when *E. coli* was used as an indicator, it is apparent that generally, at the 95th percentile, the risk for this crop, irrigated with either greywater or hydroponic solution, fell outside 1 excess case of disease per 1 000 for individual exposures before any barrier methods were utilised. This level of health risk is not seen for the other indicators. This could indicate re-growth of *E. coli* or that the factor used to convert the indicator counts to potentially pathogenic counts was too high. Once any of the barriers providing a load reduction of 2-log or more were instituted, the health risk became acceptable (Chapter 8, Appendix 2,Table 8.4). For annual consumption, all the treatments indicated a degree of risk at the 95th percentile level that was above the level of 1 in 10 000 (Chapter 3, Section 3.4.3.2, Table 3.21; Chapter 8, Appendix 2,Table 8.4). This could have been a result of over-estimating the mass of crop consumed on a daily basis or the percentage of the community consuming it. Further studies into dietary habits would be necessary in order to clarify this. When examined statistically, no significant differences in health risk were found between the four treatments ($p \leq 0.05$).

4.3.3 Chillies

The risk of consumption of this crop was based solely on the levels of total organisms present as no other indicators were detected. It can be seen (Chapter 3, Section 3.4.3.3, Table 3.25 and Chapter 8, Appendix 2,Table 8.5) that, even without any barrier precautions, the results for the greywater-irrigated chillies fell within the results for the hydroponic solution-irrigated crop and both complied with the WHO requirements (WHO, 2006a; Regli *et al.*, 1991). This could be attributed to the fact that this crop grows well above the ground and therefore is unlikely to be affected by any splash-back from the soil or the irrigating water. It is also consumed in much lower quantities than any of the other crops.

4.4 Below-ground crops

4.4.1 Beetroot

It was considered highly unlikely that beetroot would be consumed either unwashed or un-peeled, therefore the result obtained for risk with the implementation of a 2-log reduction in microbial load was considered to indicate the highest level of risk for either individual or annual exposure. At the 95th percentile level, all indicators except *Enterococcus* showed a health risk of less than 10^{-5} for a single exposure (Chapter 8, Appendix 2, Table 8.6). For annual consumption, only *Enterococcus* indicated a annual risk greater than 10^{-4} when a 2-log mitigation of microbial load was used (Chapter 3, Section 3.4.4.1, Table 3.27 and Chapter 8, Appendix 2, Table 8.6). These discrepancies were probably caused by one very high *Enterococcus* reading present in the greywater-irrigated set of data. This result was left in for calculation purposes as no scientific reason for discarding it could be found. No significant difference was found for the probability of infection among the treatments ($p \leq 0.05$). This indicates that, although the potential for accumulation of microorganism in the soils had been shown, this did not appear to affect the microbial quality of this crop. Although beetroot may occasionally be eaten raw in a salad, its more usual form of consumption requires boiling until well cooked. The risk from a single exposure to greywater-irrigated beetroot after boiling was generally negligible ($<10^{-10}$) and even the risk for annual consumption ($<10^{-7}$) is extremely low.

4.4.2 Potato

This crop is seldom, if ever, eaten raw in communities such as the one involved in this study. The data show (Chapter 3, Section 3.4.4.2, Table 3.24 and Chapter 8, Appendix 2,Table 8.7) that, where no risk-mitigating interventions occurred, the risk of infection from individual exposure events is not within 1 per 1000 for any of the treatments. When the annual exposure risk is reviewed, the 95th percentile level showed that, where mitigating factors resulting in a 6-log reduction of risk were implemented, a health risk level of $\leq 10^{-4}$ was achieved. There are therefore no data from this project to support restriction of the growth of potatoes using subsurface, greywater irrigation in order to protect the health of end consumers as long as the crop is thoroughly cooked before consumption. The risk from handling soil discussed in Section 4.2 would however apply to consumers handling unwashed produce. If the crop is to be consumed other than in the immediate household providing the greywater, washing of the tubers before leaving the site would be advised in order to reduce the possibility of secondary transmission of infections.

4.4.3 Onions

When the individual risk of infection was reviewed (Chapter 3, Section 3.4.4.3, Table 3.29 and Chapter 8, Appendix 2, Table 8.8), it could be seen that, if eaten raw and without any other microbial load-mitigating barriers, this crop generally presented a health risk above 10^{-3}. The health risk of individual instances of the raw consumption of this crop could be regarded as likely to be significant under any irrigation system. This indicates an inherent risk for this crop. When a 6-log reduction in microbial load was modelled, both the single exposure and the annual health risk fell within the limit of 10^{-4} (Chapter 3, Section 3.4.4.3, Table 3.29 and Chapter 8, Appendix 2, Table 8.8). When tested statistically, no significant difference was found for any of the risks calculated from the four indicator microorganisms ($p \leq 0.05$). As the annual risk of consumption of greywater-irrigated onions was not significantly higher than that for the other treatments, this produce could be safely used in the same way as the currently available onions, although a barrier of at least peeling the produce before consumption raw should be required and cooking is recommended in all cases.

4.4.4 Carrots

The data for individual episodes of exposure show that there may be some health risk involved in the raw consumption of carrots irrigated with greywater as the probability of infection indicated by the indicators *E. coli* and *Enterococcus* is $\geq 10^{-3}$. The data from all four treatments indicate that the annual risk of consumption of carrots was above the World Health Organisation specifications (WHO, 2006a; Regli *et al.*, 1991) when no barriers to reduce microbial load were modelled. When a 6-log reduction of the microbial load was modelled, all indicators, for the three water treatments resulted in health risks of less than 10^{-4} (Chapter 3, Section 3.4.4.4, Table 3.30 and Chapter 8, Appendix 2,Table 8.9). The data for the commercial crop was biased by one sample containing 600 cfu/g dry mass whereas the remaining samples were less than 1 cfu/g dry mass. As was decided at the start of the project, this result was used as a worst case scenario to estimate potential risk even though the commercial crop was not used for statistical comparisons. The data from this project support the finding that carrots could safely be grown with greywater irrigation and that the health risk would be within WHO criteria (WHO, 2006a; Regli *et al.*, 1991) as long as the produce was cooked before consumption.

4.5 Overall assessment of health risk

From the results presented, it is apparent that, under the conditions prevailing in this study, it is possible to utilise household greywater for food crop irrigation without undue risk to the community so long as defined precautions are taken. The most hazardous areas of operation are the handling of the water itself and the tilling of the irrigated soil. These would both benefit from a reduction in the microbial load in the water, as would the crops produced. An intervention would need to reduce the load by at least 2-log in order to benefit the agricultural workers. It is apparent that the above-surface crops grown close to the ground are more likely to offer health risk than those growing suspended above the surface. This might not be true if a system other than sub-surface irrigation had been used. This is because, in this trial, risk of splashing of the surface of above-ground crops was reduced through the use of sub-surface irrigation; other types of irrigation, especially spraying, would not offer this benefit. As stated previously, according to the World Health Organisation, (WHO, 2006a; Regli *et al.*, 1991) a tolerable burden of disease for a single exposure is 1 excess case of disease in 1 000 people for drinking water (Regli *et al.*, 1991) and the same level should be achieved for greywater-

irrigated crops. The data presented in this study are for risk of infection, but as data for the conversion of infection to illness were not available for this population, there has been no rigorous attempt at conversion of the data to probability of illness and therefore no calculation of DALYs was possible. As there is a risk of a high incidence of immune-compromised persons in the community, to provide the greatest safety, a conversion factor of 1 could be used for the conversion from an infected to a diseased state. This would imply that anyone becoming infected would convert to illness. No inherent immunity was assumed and neither secondary infection nor protection from infection during the course of the disease would have been factored in. Even at this level, the risk of $\leq 10^{-3}$ was generally achieved for all the crops at the 95^{th} percentile level when the produce was cooked.

As greywater does not contain the same level of intestinal pathogens as sewage, the factors used in this study to convert indicator concentrations to potential pathogen concentrations are likely to over-estimate the presence of pathogens by at least two orders of magnitude. The risks presented in this study are therefore extremely conservative and protective of the health of the community using the greywater for irrigation purposes. This, in part, goes to mitigate the fact that only bacterial indicators were modelled in this study. The loads of pathogenic virus could be expected to be higher than for bacteria and the risk of infection would be concommitantly higher especially as the number of virus required to initiate infection is often lower than that for bacteria. The risks calculated from the greywater irrigation scheme examined in this dissertation are also relevant to the community – or actual household – implementing the scheme and direct health risks would become insignificant in the wider community.

Guidelines for water re-use exist in many countries and are well covered by Maimon *et al.,* (2010) and Marsden Jacobs Associates (2005). According to Rodda *et al.,* (2010 in press), the South African National Water Act (DWAF, 1998) does not specifically mention greywater in terms of guidelines for its re-use. However, application of reasonable judgement could put the control of greywater re-use in agriculture under "*the use of water containing waste for irrigation purposes*", a "*controlled activity*" even though the relevant section in the General Authorisation (DWAF, 2004b) is headed "*irrigation of any land with waste or water containing waste generated through any industrial activity or by a waterwork*", thus, by inference, exempting the domestic situation. As such, legally, a license would not be required before greywater could be used for irrigation if the volumes used fall within the guidelines specified in the general authorisation. Rodda *et al.* (2010 in press) also state that greywater irrigation would not benefit from the most relevant requirement (Chapter 2, Section 2.7 (iii) of the revisions of the General Authorisations, Gazette no 26187). This would not hold true for the greywater

examined in this study as the average greywater microbial quality as presented in Chapter 3, Section 3.1.3, Table 3.2 and Table 3.3 is within the limits specified for irrigation at the lowest volume, as shown in Table 4.2.

Table 4.2: Comparison of the mean greywater analysis values with the General Authorisation values for irrigation with used water or effluent

Determinand	**Greywater (Mean)**	**Irrigation volume <2 000m^3**	**Irrigation volume <500m^3**	**Irrigation volume <50m^3**
Faecal coliform (log cfu/100mL)	4.76	<3.3	<5	<5
COD (mg/L)	584.3	>75	<440	<5 000
pH		6.0 – 9.0	6.0 – 9.0	6.0 – 9.0
NH_4 (as N) (mg/L)	19.76	<3		
Electrical conductivity (mS/m)	69.82	<150	<200	<200

Although there are guidelines for the disposal of sewage and sewage sludge, there are as yet no formal guidelines for the use of greywater in South Africa although a Water Research Commission project on this topic is in preparation (Rodda *et. al*., 2010 in press). This document provides guidance for decisions on whether or not greywater in a particular instance is suitable for small-scale irrigation use. It covers chemical, physical and microbiological aspects to give tools for decisions on both human health risk and environmental risk. This goes beyond the parameters of this dissertation, but an extract of the microbiological requirements is presented in Table 4.3. When the target figures from this table are compared with the levels of *E. coli* detected in the greywater used for this project, Chapter 3, Section 3.1.3, Table 3.2 and Table 3.3, it is apparent that the greywater from this project would not have been considered acceptable for re-use except on a short-term, site-specific basis. However, the QMRA investigation has shown that, in the circumstances prevailing, the resultant health risk could be brought well within acceptable levels. The guidance document therefore provides guidance which would preclude the use of some greywater which could be used, in the right circumstances, without human health risk. This highlights the need for QMRA to be performed for each scenario before a final judgement is made as to the suitability of the water for irrigation purposes. The application of the targets from the guidance document needs to provide high protection of human health but might otherwise prevent a beneficial activity in the community and therefore errs on the side of caution to ensure safety in cases where QMRA has not been performed.

Table 4.3: Water quality guidance for use of greywater for small-scale irrigation in South Africa. From South African Water Quality Guidelines for Irrigation, 2nd edition DWAF, 1996 and Rodda *et al.* 2010 (in preparation)

Greywater constituent		**Target water quality range**	**Maximum water quality range (applicable only to well-drained, chemically stable soils)**	**Water quality suitable only for short-term use on site-specific basis.**	**Water quality not recommended for irrigation use**
	Greywater from project	Suitable for unrestricted use with minimal risk to human health, plants or soil	Increasing risk to human health, plants or soil	Significant risk to human health, plants or soil; tolerable for short-term use only	Excessive risk to human health, plants or soil
E. coli (colony-forming units, CFU/100 mL)	$>10^4$	< 1	$1 – 10^3$ (1 – 1 000)	10^3 - 10^5 (1 000 – 100 000) *Note: Only with appropriate exposure restrictions – see text. Range can be extended to 10^7 (10 000 000) if irrigation is sub-surface.*	$> 10^7$ (> 10 000 000)

Carden *et al.* (2007 a and b) conducted site surveys throughout South Africa into the generation and disposal of greywater. They showed that there is some resistance to its use for food irrigation purposes. These reports also noted that the greywater investigated was generally unfit for use for crop irrigation. The condition of the greywater before any treatment is largely a function of the availability of potable water. Where a reasonably adequate supply is readily available, such as was the case in the Cato Crest community, water is seldom used several times before being considered for disposal or agricultural use. Where water has to be carried for longer distances or is otherwise limited, the water is used many times before it is considered disposable and it is water under these conditions which would be considered unusable in lin with the conclusions of Carden *et al.* (2007 a and b).

Another very important study performed in South Africa was a project implementing dry sanitation and greywater re-use in Kimberley – the Hull Street project (Källerfelt and Nordberg, 2004). This collaboration between Sol Plaatjies Municipality and the Swedish International Development Cooperation Agency (SIDA) showed that simple treatment options were often insufficient to provide water of a satisfactory quality for agriculture (Källerfelt and Nordberg, 2004). However such treatment may be adequate in conditions such as are present in the Cato Crest community. Reference is made in report for WRC Project K5/1639 (Rodda *et al.* 2010 in press) to a simple prototype pre-treatment system which removes solids and fats from kitchen

greywater and provides for its aerobic biodegradation which may show promise for future implementation, as do the options presented by Whittington-Jones (2007). In the project presented in the current dissertation, no such pre-treatment occurred and only simple straining to remove suspended solids, such as hair, was implemented. The potential therefore exists to mitigate the risk of infection from greywater re-use even further. Ottoson and Stenström (2003) and the WHO (2006a) have proposed that human and environmental health can be protected by the use of treatment precautions and exposure barriers. The current project has supported this principle by illustrating that simple precautions in the handling of soil and greywater and the post-harvesting treatment of the crops can reduce the health risk from the re-use of greywater to acceptable levels.

In order to make health risk understandable to stake-holders, it is necessary to relate it to known levels of risk in the community. Figures drawn from Norman *et al.*, (2000), South African Police Services website (http://www.saps.gov.za/) and Statistics South Africa (StatsSA, 2001), are presented in Figure 4.1. From this figure it can be seen that, even when the community risk figures are expanded, without alteration in the percentage of population exposed, to reflect an exposed community of ten million, the risk of infection from consumption of greywater-irrigated produce is significantly less than common risks of severe illness or death. If a conversion from infection to illness of 50% is assumed, the risk from the greywater-irrigated produce is even lower. In addition, the risk of death from malnutrition is only 25% less than the risk of infection from consumption of greywater irrigated produce when the entire population of 10 million is regarded as having the same risk as the community growing the produce. This scenario presents the extreme of risk and it is also necessary to bear in mind that the most likely illnesses would be gastroenteritis. These would usually be non-life threatening in the general population but could however have severe or even fatal results in HIV-positive individuals. When the risks are calculated relative to the size of the community in relation to ten million people, they become non-significant. This does not in any way down-play the seriousness of the risk outcomes used for comparison purposes or imply that they are acceptable. The benefits of having excess vegetables either for exchange with other community members or for sale have not been addressed but it is clear from Ahmed *et al.* (2003), that these benefits are substantial. In addition, the introduction of a steady supply of fresh vegetables to individuals in poor communities or with lowered immunity would undoubtedly have some beneficial impact.

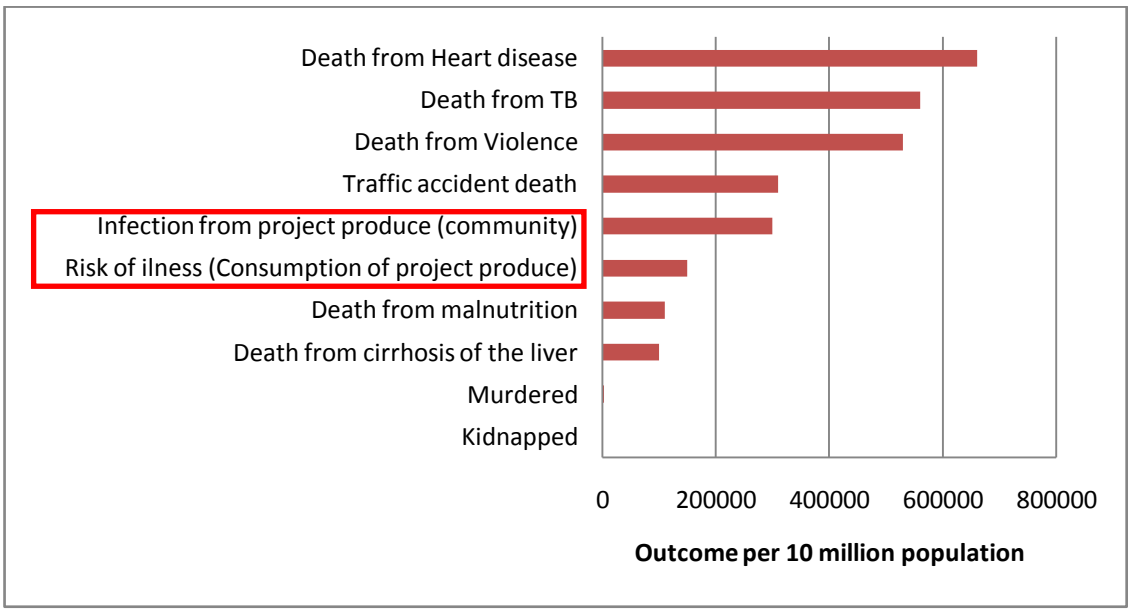


Figure 4.1: Annual outcome for various risks per 10 million persons in South Africa. (Norman et al., 2000; South African Police Service (SAPS). http://www.saps.gov.za/; StatsSA, 2001)

When the risks are calculated relative to the size of the community in relation to ten million people, they become non-significant. This does not in any way down-play the seriousness of the risk outcomes used or imply that they are acceptable. The benefits of having excess vegetables either for exchange with other community members or for sale have not been addressed but it is clear from Ahmed *et al.* (2003), that these benefits are substantial. In addition, the introduction of a steady supply of fresh vegetables to individuals in poor communities or with lowered immunity would undoubtedly have some beneficial impact.

This study has shown that greywater re-use is a feasible option for the growth of edible crops. However, as mentioned by Carden *et al.,* (2007a) and as discovered in eThekwini (MacLeod, N. 2010, *pers. com*)[10], scientific studies do not always overcome the perceptions in communities that greywater will cause disease. Greywater re-use does need to be carefully monitored and communities using it will need support from either local government or other bodies well into the future to ensure that the potential offered by this resource is used beneficially.

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5. CONCLUSIONS AND RECOMMENDATIONS

From the results reported in this study, it can be seen that the human health risk to the Cato Crest community from exposure relating to greywater irrigation of crops, can be reduced to acceptable levels by the implementation of very simple control measures. For all crops, the simple precautions of using sub-surface irrigation, basic hygiene methods and personal protective clothing would protect the health of the agricultural workers. Ensuring that all produce grown below ground is either cooked or that greywater irrigation is stopped for two days before harvesting and that the crop is peeled before being eaten raw would ensure that the community bore no additional health risk from consumption of the greywater-irrigated crops. Ensuring compliance with such restrictions would require thorough training and on-going support to the community.

At the start of this project, the following hypotheses were proposed:

Hypothesis 1: There is no difference in the microbiological quality of crops irrigated below the soil with greywater and those similarly irrigated with water of a non-waste origin.

Hypothesis 2: The health risk to handlers and consumers of crops irrigated sub-surface using greywater can be easily brought within the World Health Organisation guidelines.

The results of the project showed that for crops grown above-ground and not in contact with the surface, Hypothesis 1 can be accepted. For crops grown underground or above-ground but in contact with the soil, contrary to expectation, the greywater-irrigated crops did not show a statistically higher concentration of microorganisms than similar crops irrigated with the hydroponic solution or tap water and therefore Hypothesis 2 should also be accepted. The implementation of basic barrier precautions in the preparation of crops for consumption would however still be an advisable precaution in all cases.

It is recommended that an epidemiological study be performed utilising the expertise of the clinics servicing areas such as the Cato Crest community so that the microbiological data for rate of infection, conversion from infection to disease *etc*. could be refined and a full-scale dynamic model could be prepared for utilisation in the South African context.

The re-use of greywater should form part of sustainable water use practices and should be incorporated with other sustainable practices such as dry sanitation, composting and solar

energy utilisation. The human health risks should not be ignored, even though this research has shown that they can be easily controlled to acceptable levels. Community education is of paramount importance and has to go hand-in-hand with any re-use intervention. This dissertation has suggested that reduction in the microbial concentrations in greywater is a necessary intervention. This could be achieved by interventions such as chemo-therapy for parasite load and simple treatment of the generated greywater before use. Although using greywater for small-scale irrigation has been shown in this study to be an acceptable practice, every situation has to be examined on its own merits and where either an epidemiological study or QMRA cannot be performed, the guidance document (Rodda *et al.*, 2010, in press) should be used to determine if the water is fit for the intended use.

The results presented in Chapter 3, and Appendix 2, Table 8-1 to 8-9, show that the greatest health risk is to those members of the community who handle the greywater and the soil but that these are not significantly different from the risks inherent in growing crops with water other than greywater. These members of the community are exposed to the full range of health risk, *i.e.* from greywater itself, the soil, crop handling and crop ingestion. The greatest efforts at mitigation should therefore be guided by the risks to these people. According to Roesner *et al.* (2006), pathogens in greywater can survive for 15 days to several months whilst survival in soils or on crops is usually less than two months and is related to environmental conditions such as temperature and solar radiation. They go on to state that contact between the edible part of crops and greywater should be limited and that sprinkler systems should be avoided whereas drip-irrigation systems can be effective. By implication, contact between the agricultural worker and the greywater should therefore also be avoided and sub-surface irrigation would present less risk to the agricultural workers than spraying.

As in any mitigation regime, the interventions should be started as far removed from the final exposure as possible in order to allow additional control points as a backup in case of the failure of the major intervention. In this case, the first intervention should be concentrated at the critical point where the water enters the agricultural system in order to produce the greatest reduction in health risk, whether or not greywater is used. Additional safety could be provided by giving some treatment to the greywater before use. Disinfection with chlorine would be a reliable, simple and cheap method, but would result in an oxidising agent and probably disinfection by-products in the water which could lead to damage to the crops (Morel and Diener, 2006). A simple sand filter or solar-powered ultra-violet disinfection could also lower the microbial load without making the water less suitable for agricultural use or adding significant ongoing cost to the system (Fangyue *et al.*, 2009). Both of these should be

investigated in further studies. The barriers of gloves, boots and washing were assessed in the model and found to reduce the risk from exposure to either soil or water to acceptable levels for greywater (Chapter 3, Table 3.18 and Table 3.19).

This study addressed largely bacterial pathogens as the facility for examining human viruses was not available. As stated previously, the risk from viruses could be much higher than that from bacteria and it is recommended that investigation into the viral load of domestic greywater is done. In addition, this study did not address any of the chemical risk to human health such as those from endocrine disrupting compounds. These are becoming more widespread in the environment and, as such, should be studied ass potential risk factors for grey-water re-use.

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7. APPENDIX 1

7.1 Detection and enumeration of helminth ova

Introduction

The Department of Agriculture has suggested that sludge for use as fertiliser should be checked for viable Ascaris ova before use. Ascaris infestation is acquired by ingestion of ova containing second stage larvae. These hatch in the duodenum and the larvae penetrate the intestinal wall and enter the circulatory system where they are carried to the pulmonary system. Entering the alveoli of the lungs, the larvae moult twice to become fourth stage larvae about ten days after infection. These fourth stage larvae migrate up the respiratory tree to the pharynx where they are swallowed and pass into the stomach and hence the small intestine. Here they undergo the final moult to become adults approximately 25 – 30 days after infection. Shortly after fertilisation, the female worm begins to release embryonated ova, producing approximately 200 000 ova per female per day. Over the normal 17 month lifespan of an adult female, this approximates to 26 million ova. If these ova are deposited in a suitable environment of shaded, warm moist soil, they can develop to the infective stage in 10 – 15 days and may remain infective for months or years.

Scope/ Field of Application

This method is suitable for use on wastewater, sewage effluents, wet sludge, dry or composted sludge or urine diversion toilet waste.

Principles

This method allows the separation of parasite eggs from the rest of sludge particulate matter thus allowing concentration of most of the parasite eggs in the sludge sample.

Health, Safety and Precautions

Ascaris ova may be highly infectious, so scrupulous hygiene practices must be observed.

Sample Handling

Samples of sewage sludge must be taken before treatment with polyelectrolyte flocculating agents.
Samples must be cooled for transport.

Apparatus and Equipment

Centrifuge with swing out rotor
15 mL plastic centrifuge tubes and holders
Microscope
Vortex mixer
Large plastic funnels (± 220 mm diameter)
Filter sieves; 1 x 150 µm, 1 x 220 µm.
Retort stand
Plastic beakers: 6 x 500 mL
Plastic wash bottles: 3
Schott bottles: 1 L, 2 L, 5 L
Magnetic stirrer and stirrer bars
Glass beakers 3 x 100 mL
Plastic Pasteur pipettes
Glass slides and cover slips
Hydrometer
Balance
Drying oven
Dessicator

Reagents and Materials

Zinc sulphate – is made by dissolving 500g of chemical in 880 mL de-ionised or distilled water.
Ammonium bicarbonate (AMBIC) – this is a saturated ammoniam bicarbonate solution. This is prepared by dissolving 119g of the chemical in 1000 mL de-ionised water.
0.1% TWEEN 80 - is prepared by taking 1 ml Tween80 using a pipette and add it to 1000 mL de-ionised water.

Procedure

In all cases, mix the sample thoroughly. Weigh and empty weighing boat (DE), add a measured volume of sample and re-weigh (DW). Evaporate to dryness and weigh (DD). Repeat this last step until a steady mass is obtained. From these measurements, the dry mass of a fixed volume of sample can be calculated as given below.

Effluent / Wastewater

Support the 150 µm and 20 µm filters in two funnels, one beneath the other in a retort stand. Record the volume of sample to be used and pour it through the funnels, swirling the 20 µm filter or stirring gently with a plastic stirring rod to facilitate draining. Discard debris trapped on 150 µm filter and rinse debris from 20 µm filter into a plastic beaker ensuring that none is lost. Pour the contents of this beaker into as many centrifuge tubes as are necessary to accommodate the sample and centrifuge at 1389 g (± 3 000 rpm) for 3 minutes. Pour or suction of the supernatant and discard. Combine the pellets into enough tubes so that there is no more than 1 mL in 15 mL tube or 5 mL in a 50 mL tube. Re-suspend each pellet in a few millilitres of $ZnSO_4$ and vortex well to suspend.

Add more $ZnSO_4$ and continue mixing until tube is almost full. Centrifuge tube at 617 g (±2 000 rpm) for 3 minutes. Carefully remove tubes from centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 centrifuge tubes. Fill the tubes with reagent water to reduce the SG of the $ZnSO_4$, so as not to damage the ova and also to allow them to deposit upon centrifugation. Centrifuge the tubes at 964 g (±2 500 rpm) for 3 minutes. Remove and discard the supernatant. Combine the pellets into one tube and centrifuge again at 964 g (±2 500 rpm) for 3 minutes. Transfer the pellet to one or more microscope slides, cover with a cover slip and examine under a microscope using 10x and 40x objective. Calculate number of ova per litre of original effluent sample for each species of helminth.

Wet sludge / Sludge to land

NOTE: It is always preferable to work with small sub-samples as ova may not be easily released from a small sample to float out of the sludge during the $ZnSO_4$ flotation technique. It is preferable to increase the number of sub-samples than to try to over-load each tube in order to keep tube numbers to a minimum. The number of sub-samples will also be dependent on the helminth ova load expected.

Mix the sludge sample thoroughly by swirling and stirring with a plastic rod. Take 4 x 15 mL samples and put them into 4 x 50 mL tubes. This should be sufficient if the solids content is high, if it is low, take further sub-samples. Add either a few millilitres of 0.1% Tween80 or AmBic solution, vortex and add more wash solution. Repeat until the tubes are filled to approximately a centimetre from the top. Place the 150 µm sieve in a funnel in a retort stand with a plastic beaker underneath to catch the filtrate. Filter the well mixed tubes one at a time, rinsing each tube and washing water through the filter as well. Pour the filtrate into tubes and centrifuge at 1389 g (± 3 000 rpm) for 3 minutes, suction off the supernatant and discard it. Combine the pellets into a suitable number of tubes so that there is no more than 1 mL in a 15mL tube or 5 mL in a 50 mL tube. Resuspend the pellets in a few millilitres of $ZnSO_4$ and vortex well to mix. Keep adding more $ZnSO_4$ until the tubes are almost full. Centrifuge the tubes at 617 g (±2 000 rpm) for 3 minutes; remove and filter the supernatant through a 20 µm filter, washing well with water Collect the matter retained on the filter and wash it into two tubes. Centrifuge at 964 g (±2 500 rpm) for 3 minutes; remove and discard the supernatant. Combine the pellets into one tube using water to rinse out all the eggs. Centrifuge again at 964 g (±2 500 rpm) for 3 minutes. Remove all the pellets using a plastic Pasteur pipette and place onto one or more microscope slides as required. Cover with a coverslip and examine microscopically using the 10x and the 40x objective. Enumerate each species of helminth ova separately and report as ova per gram of sludge.

Compost – (Sludge that contains grass and sand) or UD waste

Weigh out two or more samples into 15 mL tubes. If 50 mL tubes are used, weigh out a maximum of 3 g per tube. Add a few millilitres of either 0.1% Tween 80 or Ambic and vortex well. Add more solution to about 6 mL in a 15 mL tube or 20 mL in a 50 mL tube and vortex

intermittently repeating the addition of solution until the tubes are filled to 10 mL or 40 mL respectively and have been vortexed over a period of about 30 minutes in total. Centrifuge at 1389 g (± 3 000 rpm) for 3 minutes, suction off the supernatant and discard it. Re-suspend the pellet in reagent water and vortex to wash off the Ambic or Tween80 and centrifuge again at 1389 g (± 3 000 rpm) for 3 minutes, suction off the supernatant and discard it. Re-suspend each pellet in a few millilitres of $ZnSO_4$ and vortex well to mix. Keep adding $ZnSO_4$ until each tube is almost full. Centrifuge the tubes at 617 g (±2 000 rpm). Carefully remove from the centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 tubes. Fill the tubes with reagent water to reduce the SG of the $ZnSO_4$ so as not to damage the ova and to allow them to deposit on centrifugation. Centrifuge at 964 g (±2 500 rpm) for 3 minutes; remove and discard the supernatant. Combine the pellets into one tube, using water to rinse out all the eggs and centrifuge again at 964 g (±2 500 rpm) for 3 minutes to obtain one pellet.

NOTE: If the sample contains a lot of light debris that floats with the ova, e.g. grass, filter the deposit through a 10 µm filter, collect the filtrate in tubes and centrifuge again to prepare a pellet for microscopy.

Remove the final pellet using a plastic Pasteur pipette and place it onto one or more microsope slides. Cover with a coverslip and examine microscopically using the 10 x and 40 x objective. Enumerate each species of helminth ova separately and report as ova per gram of compost or UD waste.

Calculation

The g force of the centrifuge is calculated as follows:-

G-force (org) = $(1.118 \times 10^{-5})Rs^2 = 0.00001118 \times R \times s^2$

Where:-

s = revolutions per minute

R = radius in centimeters from the centre of the rotor to the bottom of the tube bucket when in the swung-out position

Ascaris ova count is calculated as follows:-

Total ova = Count/mass = Count/ (DD – DE)

Where:-

Mass = dry mass of original sample

Reporting Results

Results are reported as ova per g dry weight of sample.

7.2 Detection and enumeration of Escherichia coli and total coliforms on Chromocult agar

Introduction

The majority of diseases carried by water are of an enteric nature and it is therefore necessary to screen water for possible faecal contamination. The search for indicator organisms such as faecal coliforms and *E. coli,* instead of for pathogens themselves, is universally accepted for the monitoring of microbial pollution of water supplies. Ideally, the finding of these indicator bacteria should denote the potential presence of intestinal pathogens. Indicator organisms should be abundant in faeces and sewage; absent or at least present in very low numbers in all other sources; capable of easy isolation, identification and numerical estimation. They should also be more resistant than pathogens to disinfectants such as chlorine, and to environmental stress. In practice, there is no organism which consistently meets all these criteria, but most of them are fulfilled by *Escherichia coli* as the essential indicator of pollution by faecal material of human or animal origin. The coliform group as a whole is used as an indicator of the hygienic status of a body of water.

The advantage of the membrane filtration technique using Chromocult™ is the speed with which results can be obtained as direct counts. For example, *Escherichia coli* counts can be available after 18 hours. This enables more rapid corrective action to be taken when required.

Scope/ Field of Application

Surface waters
Wastewater treatment works outfalls
Marine water
Swimming pool water

Potable water

Definitions

Pathogen:	Microorganism leading to disease, in this case in humans.
Indicator organism:	Microorganism which is present in large numbers whenever a pathogen is present but absent when pathogens are absent. Usually not a pathogen itself and easier to isolate and identify than pathogens.
Total Coliforms:	Gram negative bacteria possessing the enzyme β -D- galactosidase.
Escherichia coli:	As above and also possessing the enzyme β -D- glucuronidase.
Chromophore:	Chemical substrate which produces a colour on being cleaved by the specific enzyme.
Gram positive:	Organism retaining the crystal violet-iodine stain of the differential Gram stain.
Gram negative:	Organism losing the crystal violet-iodine stain of the differential Gram stain and taking up the safranin stain of the differential Gram stain.
Chromocult™	An enzyme based, chromogenic, selective agar medium suitable for the detection and enumeration of total coliforms and *E. coli*.
Tergitol™:	A detergent which helps to prevent the growth of some microorganisms.
Sub-lethally injured:	Organisms in water which have been damaged in such a way that they are slow to grow under normal culture conditions, but may be revived by the use of specialised techniques or specific media.
Masking:	Growth of non-target organisms which may interfere with the detection of target organisms.

Principles

The interaction of the constituents of the medium results in rapid growth of even sub-lethally injured coliforms. Gram negative bacteria as well as some Gram positive bacteria are inhibited by the inclusion of Tergitol®7 which does not interfere with the growth of coliforms. This helps to prevent overgrowth and masking of the target coliforms.

A chromophore-linked glucuronide is used for the identification of the enzyme .-D-glucuronidase which is characteristic for *E. coli*. Colonies producing β-D-glucuronidase release the chromophore, producing blue colonies. A second chromophore-linked substrate, Salmon-GAL, is used to detect the production of β -D-galactosidase which is an enzyme produced by total coliforms. Colonies producing the enzyme appear salmon to red. As *E. coli* produces both enzymes, the colonies appear dark blue to violet.

Health, Safety and Precautions

Interferences for this method are as follows:

Turbidity

Highly turbid samples can block membrane filters and therefore prevent proper analysis according to this method. If a sample stops going through the filter or takes an unusually long time to do so, further dilution of the sample before filtering should be considered or an MPN method should be used.

ToxicantsToxicants in the sample will interfere with the resuscitation of the organisms and therefore result in a low organisms count. This problem may occasionally be alleviated by diluting the sample.

Temperature

Too high a temperature reduces the survival of the target organisms, whilst too low a temperature permits the growth of other, non-target organisms.

Condensate

Inversion of Petri dishes for the duration of incubation prevents water droplets forming on the lid and dropping onto the surface of the medium with a resultant blurring of colonies.

Sample Handling

The bacterial load of different types of water varies and this is compensated for by using different volumes of water for analysis. The following volumes have been found to be generally appropriate.

Matrix	Volume (mL)
Potable water -	100
River water -	0.2
	0.01
Outfalls	1
	0.2
Beaches	5
	1
Pools	100

Apparatus and Equipment

Bunsen burner
Autoclave
Biohazard cabinet
Water purifier
Automatic pipette
Filter manifold and pump
Forceps
Microwave
Incubator
Plate viewer

Reagents and Materials

Chromocult® Coliform Agar
Re-hydrate 26.3 g Chromocult® Coliform agar in 1L of reagent water (See below).
Heat in a microwave until all agar is dissolved. Do **not** autoclave.
Cool small aliquot and test pH.
pH should be within the range 6.8 ± 0.2.
Adjust pH if necessary with HCl or NaOH.
Aseptically dispense medium into sterile Petri dishes and allow to solidify.
Store finished medium at 2 - 10°C in sealed plastic containers and discard unused medium after two weeks.

Record the media preparation on the appropriate form and sign.

Reagent Water - Water from the bacteriology water purifier which should have a conductivity of < 0.5 mS/m.
Sterilise in autoclave at 121^0C for 15 minutes.
Cool.
Store at $<10^0$C.

Sterile gridded 0.45 µm pore size, 47 mm diameter membrane filters. Individually packed membranes from acceptable supplier.

Calibration

Balance must have passed QC check within the last week.
pH meter must have passed QC check within the last week.
Incubator must show a steady temperature on the Laboratory Temperature Logger.
Automatic dispenser must have passed QC check within the last week.
Automatic pipettes must have passed QC check within the last week.
Medium batch must have passed QC check
Laminar flow cabinets must have passed QC check within the last week. Autoclave run must have passed QC check.

Quality Control

For each batch of media prepared, inoculate one Petri dish with positive and negative controls, namely *E. coli* and *Enterococcus faecalis*.
The *E. coli* colonies should be purple/blue and the *Enterococcus faecalis* should be minimal and grey/white in colour.

If any of the above calibrations has failed, the method should not be deemed fit for use before remedial action has been shown to be successful.

Kovacs Reagent for confirmation of *E. coli*. Suspected *E. coli* colonies can be coated with a drop of Kovacs reagent. The development of a cherry-red colour acts as confirmation. The β -

D-glucuronidase reaction is, however, sufficient in conjunction with the β -D-galactosidase reaction to confirm the presence of *E. coli* and this additional step is rarely used

Procedure

Technique

Take out of the fridge sufficient Petri dishes of medium for the expected number of samples.
Allow to warm to room temperature.
Where a 100 mL volume is used, filter directly onto the filter membrane as described in the procedure "Membrane Filtration".
Where dilutions are required, pipette 5 mL, 2 mL, 1mL, 0.2 mL or 0.01 mL volumes as necessary out of the well mixed sample.
Place in approximately 30 mL of sterile reagent water.
Filter the sample or aliquot under vacuum through a 0.45µm pore size membrane filter in a sterile filter assembly.
Open the filter assembly carefully.
Remove the membrane using sterile technique.
Roll it onto the surface of the Chromocult™ agar in a Petri dish.
Invert the Petri dishes.
Place in the 37 ± 1^0C incubator for 18 – 24 hours.
For each batch of 10 samples or less, one sample must be run in duplicate. The selected sample must have a replicate entered against it in LIMS.

Incubation

Incubate the prepared membranes in an inverted position at 37± 1^0C for 18 – 24 hours.
Incubate samples within 30 minutes of filtering.

Enumeration

E. coli colonies are dark blue to violet, total coliforms are salmon to red, and other *Enterobacteriaceae* are colourless.
Count colonies with a colony counter employing illumination and magnification.
Count *E. coli* results first and record.
Count total coliforms next.

Record actual counts in the laboratory record sheet and sign.

Enter result onto LIMS where the count per 100 mL is calculated automatically.

Note: The detection of *E. coli* is regarded as sufficient evidence of faecal pollution. However, further tests for the confirmation of *E. coli* may be carried out if considered necessary.

Disposal

Collect all counted Petri dishes and place in autoclave bag.

Autoclave at 121^0C for 15 minutes to sterilise.

Place sterilised cultures in hazardous waste disposal container for disposal by approved disposal company.

Calculation

Calculation of *E. coli*

Count all dark blue to violet colonies and multiply by the dilution factor to obtain *E. coli* per 100 mL.

Calculation of Total Coliforms

Count all salmon to red colonies and add to the number of dark blue to violet colonies to obtain the total coliform count. Multiply by the dilution factor to obtain total coliforms per 100mL.

Method Performance Assessment

This method was run in parallel with the standard mFC medium for *E. coli* detection over the course of a year. Results obtained from the Chromocult medium were found to be higher than for the standard medium, but a spot check on colonies proved the identification. In addition, this technique was run in a national ring trial comparing it to the ISO accredited Colilert method and the results were found not to be significantly different for *E. coli* determination. This method is now accredited by the American Department of Environmental Affairs and is suitable for its intended use.

Reporting Results

All total coliform and *E. coli* results are entered into LIMS where the count per 100 mL is automatically calculated. Specification limits have been set for each water type and any results outside these limits will appear on the LIMS screen in red. These results must be further investigated according to the procedure for the handling of non-conforming results.

7.3 Detection and enumeration of Enterococcus on Enterococcus selective agar

Introduction

The search for organisms indicative of faecal pollution instead of for pathogens directly is universally accepted for the monitoring of microbial pollution of water supplies. Ideally, finding indicator bacteria should denote the potential presence of intestinal pathogens. Indicator bacteria should be abundant in faeces and sewage. They are usually absent or at least present in very low numbers in all other sources and are capable of easy isolation, identification and numerical estimation. They should also be more resistant than pathogens to disinfectants such as chlorine and to environmental stress. In practice, there is no bacterium which consistently meets all these criteria. Enterococci have however been found useful in this regard as, although present in faeces in lower numbers than *E. coli*, it has been shown that the ratio of faecal coliforms to enterococci is a useful indicator of whether water has been polluted predominantly by human or animal faeces. The method is not however exact and should only be used as an indicator. Studies at marine and fresh water bathing beaches also indicate that swimming-associated gastroenteritis is directly related to the water quality and that enterococci are the most efficient indicator of this.

Scope/ Field of Application

Fresh water.
Marine recreational water.
Potable water
Swimming pools

Definitions

Enterococcus: This group is a subgroup of the faecal streptococci and includes *E. faecalis*, *S. gallinarum* and *S. avium.* All give a positive reaction with Lancefield's Group D antisera and have been isolated from the faeces of warm blooded animals. For this method, enterococci are those bacteria which produce black colonies on Enterococcus selective agar and are catalase negative and Gram positive.

Principles

Sodium azide is a selective agent which suppresses the growth of Gram positive organisms. Esculin hydrolysis and bile tolerance are regarded as being reliable, constant characteristics of faecal streptococcii. The microorganisms hydrolyse the glycoside esculin to dextrose and esculin which forms an olive green to black complex with iron (III) ions.

Health, Safety and Precautions

Temperature Incubation temperature is critical. The incubator must hold the 37±1^{o}C temperature throughout the chamber over a 48 hour period.

Turbidity Membranes are unsuitable for use with waters of high turbidity as the membrane will block before sufficient water can be filtered. In such a case either smaller volumes of the sample could be used or a pre-filter may be fitted ahead of the membrane filter to trap the non-microbial suspended material.

Toxicants Toxic metals
Toxic organics such as phenols.

Bacteria *Staphylococcus aureus* may cause a false positive result.

Sample Handling

Keep samples chilled from the time they are sampled till delivery to the Laboratory.
Use insulated boxes and freezer bricks to keep the samples cold.

Analyse samples as soon as possible.

Do not hold samples for more than eight (8) hours before analysis.

Use volumes yielding 20 to 50 colonies per membrane where possible.

When the bacterial density is unknown, filter several decimal volumes to establish the density. Estimate the volume expected to yield a suitable membrane colony count and select two additional volumes representing approximately one tenth and ten times this volume respectively.

The bacterial load of different types of water varies and this is compensated for by using different volumes of water for analysis. The following volumes have been found to be generally appropriate.

Matrix	Volume (mL)
Potable water -	100
River water -	0.2
	0.01
Outfalls	1
	0.2
Beaches	5
	1
Pools	100

Apparatus and Equipment

Electronic colony counter.

Automatic pipettes of suitable volume.

Membrane filtration unit and manifold.

Line vacuum, electric pump and filter flask.

Flask for safety trap containing silica gel.

Forceps with smooth tips to handle filters.

Bunsen burner for sterilisation.

Petri dishes, sterile plastic.

Tubes, glass for dilutions.

Membrane filters, white, gridded, 47 mm diameter.

Incubator.

Reagents and Materials

Enterococcus Selective Agar

Suspend 55g Enterococcus Selective Agar in 1 lire reagent water. Boil whilst stirring until completely dissolved. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C. Mix well and pour into plates.

pH 7.1 ± 0.2 pH units.

Composition	g/L
Esculin	1.0
Ferric Ammonium Citrate	0.5
Ox Bile	10.0
Peptone	3.0
Sodium Azide	0.5
Sodium Citrate	1.0
Tryptone	17.0
Yeast Extract	5.0
Agar	12.0

Calibration

Balance must have passed QC check within the last week.

pH meter must have passed QC check within the last week.

Incubator must show a steady temperature on the Laboratory Temperature Logger.

Automatic pipettes must have passed QC check within the last week.

Media batches must have passed QC check.

Laminar flow cabinets must have passed QC check within the last week.

Autoclave run must have passed QC check.

If any of the above calibrations has failed, the method should not be deemed fit for use before remedial action has been shown to be successful.

Quality Control

Perform positive and negative controls on each batch of media.
Positive control is Entero*coccus faecalis*
Negative control is *Escherichia coli.*

Procedure

Technique

Take out of the fridge sufficient Petri dishes of Enterococcus medium for the expected number of samples and allow to warm to room temperature.
Where a 100 mL volume is used, filter directly onto the filter membrane.
Where dilutions are required, pipette 5 mL, 2 mL, 1mL, 0.2 mL or 0.01 mL volumes as necessary out of the well mixed sample and place in approximately 30 mL of sterile reagent water.
Filter the sample or aliquot under vacuum through a 0.45µm pore size membrane filter in a sterile filter assembly.
Open the filter assembly and remove the membrane using sterile technique.
Roll it onto the surface of the agar in a Petri dish.
For each batch of 10 samples or less, one sample must be run in duplicate. The selected sample must have a replicate entered against it in LIMS.

Incubation

Invert the Petri dishes.
Incubate the prepared membranes at 37±1^{o}C for 48 hours.
Incubate samples within 30 minutes of filtering.

Enumeration

Enterococci form dark brown to black colonies with esculin production
Count colonies with a colony counter employing illumination and magnification.

Verification

Pick selected colonies and streak for purity onto brain heart infusion agar.

Incubate at 37±1°C for 24-48 hours.

Transfer a loop full to each of two microscope slides.

Add a few drops of fresh 3% hydrogen peroxide to one slide.

If no bubbles appear, the culture is catalase negative.

Make a Gram stain of the other slide.

Enterococci are catalase negative, Gram-positive, ovoid cells, 0.5 - 1.0µm in diameter, mostly in pairs or short chains.

OR

API Strept.

Calculation

Compute the density from the sample quantities which produce counts within the desired range of 20 - 50 colonies.

$$\text{Enterococcus / 100 ml} = \frac{\text{Enterococcus x 100}}{\text{mL of sample filtered}}$$

Method Performance Assessment

This is the standard method for enumeration of faecal streptococci and enterococci – Standard Methods no 9230 C and is deemed suitable for use in water analysis.

Reporting Results

Results are expressed as colony forming units per 100 mL.

7.4 Detection and enumeration of Pseudomonas species

Introduction

Pseudomonas aeruginosa is the type organism for the Pseudomonadaceae and is found widely distributed in nature. It is the ultimate opportunistic pathogen, capable of infecting almost any tissue when the immunity shield is damaged. Several pseudomonads can grow in water containing minimal nutrients and therefore may be of importance as water borne pathogens, particularly in hospital environments or any environment where immuno-compromised people may come into contact with them. Pseudomonads are widely distributed in soil and water. Several species are pathogenic for man or animals, others cause spoilage of meats and other foods. *P. aeruginosa* produces a water soluble blue pigment (pyocyanin) and a water soluble fluorescent pigment (pyoveridin). It is mainly a soil and water saprophyte but it is frequently an opportunistic pathogen and can often be isolated from wounds, burns and urinary tract infections.

Scope/ Field of Application

Swimming pools
Potable water from distribution lines.

Definitions

Pseudomonas aeruginosa: Straight or curved Gram-negative, strictly aerobic rod, member of the Pseudomonadaceae which produces green, fluorescent pigment.

Principles

The use of cetrimide largely inhibits the growth of accompanying microbial flora.
Pigment production by *Pseudomonas aeruginosa* is not affected.
P. aeruginosa colonies produce a blue-green pigment and fluoresce under UV light.

Health, Safety and Precautions

Interferences in this method are as follows:

Temperature

Incubate plates at 42 ± 1 ^{0}C.

Turbidity

Membranes are unsuitable for use with waters of high turbidity as the membrane will block before sufficient water can be filtered.

In such a case either smaller volumes of the sample could be used or a pre-filter may be fitted ahead of the membrane filter to trap the non-microbial suspended material.

ToxicantsToxicants in the sample will interfere with the resuscitation of the organisms and therefore result in a low organisms count. This problem may occasionally be alleviated by diluting the sample.

Temperature

Too high a temperature reduces the survival of the target organisms, whilst too low a temperature permits the growth of other, non-target organisms.

Condensate

Inversion of Petri dishes for the duration of incubation prevents water droplets forming on the lid and dropping onto the surface of the medium with a resultant blurring of colonies.

Bacterial competition

If heavy contamination with other microorganisms is expected, 15µg/L of nalidixic acid may be added.

Any used or unused media is to be autoclaved and disposed of in the medical waste.

Sample Handling

Filter sufficient sample to provide plates with approximately 20 - 80 colonies.
For natural waters this is usually about 100 mL.
For chlorinated waters up to 500 mL depending on the source.

Apparatus and Equipment

Electronic colony counter
Automatic pipettes of suitable volume
Membrane filtration unit and manifold
Line vacuum, electric pump and filter flask
Flask for safety trap containing silica gel
Forceps with smooth tips to handle filters
Bunsen burner for sterilisation
Petri dishes, sterile plastic
Tubes, glass for dilutions
Membrane filters, white, gridded, 47 mm diameter.
Incubator
UV light

Reagents and Materials

Cetrimide Agar

Prepare according to manufacturer's instructions
Cool and pour into Petri dishes.
Store in plastic containers at 4 -10^{0}C for a maximum of four weeks.

Milk Agar

Yeast extract	3g/L
Peptone	5g/L
Milk solids (equivalent to 10ml fresh milk)	1g/L
Agar	15g/L

Final pH 7.2 +/- 0.2

Quality Control

Balance must have passed QC check within the last week.
pH meter must have passed QC check within the last week.
Incubator must show a steady temperature on the Laboratory Temperature Logger.
Automatic dispenser must have passed QC check within the last week.
Automatic pipettes must have passed QC check within the last week.
Medium batch must have passed QC check.
Laminar flow cabinets must have passed QC check within the last week.
Autoclave run must have passed QC check.
Positive control is *Pseudomonas aeruginosa (ATCC 9027)*. The colonies should be blue/green and fluorescent.
Negative control is E*nterococcus faecalis (ATCC 7080)*

If any of the above calibrations has failed, the method should not be deemed fit for use before remedial action has been shown to be successful.

Procedure

For each batch of 10 samples or less, one sample must be run in duplicate. The selected sample must have a replicate entered against it in LIMS.

Incubation

Incubate the prepared membranes in an inverted position at 42 ± 1^0C for up to 48 hours.
Incubate samples within 30 minutes of filtering.
Examine colonies after 48 hours.

Enumeration

Colonies producing a blue-green pigment and fluorescing under UV light are considered presumptive *P. aeruginosa*.

Verification

Identification may be confirmed by streaking onto milk agar.

Pseudomonas aeruginosa hydrolyses casein and produces a yellow to green diffusible pigment.

API test strips may also be used.

Calculation

Compute the density from the sample quantities which produce counts within the desired range of 20-60 colonies.

$$\text{Presumptive } P.\ aeruginosa \text{ / 100 mL} = \frac{Pseudomonas \times 100}{\text{mL of sample filtered}}$$

Presumptive *P. aeruginosa* are expressed as the number of colonies counted per 100 mL of sample.

Reporting Results

The results are expressed as colony forming units (CFU) per 100 mL.

7.5 Detection of Salmonella or Shigella sp.

Introduction

Salmonella sp. are generally associated with incidents of food poisoning but have also been detected in surface waters, particularly those exposed to poultry. The organism is generally only present at very low levels and it is therefore necessary to concentrate or enrich the sample before analysis to enable detection.

Scope/ Field of Application

Waters from a variety of sources except raw sewage.

Definitions

Salmonella A Gram-negative, oxidase negative, facultatively anaerobic, non-spore forming, rod-shaped bacterium, capable of utilising citrate and producing gas from glucose.

Principles

Pre-enrichment

Pathogenic organisms are only likely to be present at low concentrations in a water sample. It is therefore necessary to pre-enrich in a non-selective medium.

Selective enrichment

Selective enrichment is necessary to increase the proportion of *Salmonella* species in relation to other organisms in the pre-enrichment culture.

Selection on solid media

In order to increase the probability of detecting *Salmonella* organisms, two solid media are used to detect the target organism. XLD is only slightly toxic to fastidious *Salmonella*. *Proteus* and other coliforms produce yellow colonies in contrast to the black centred clourless (but red in appearance) colonies formed by *Salmonella*. Incubation in excess of 24 hours is not recommended. Brilliant green/phenol red lactose medium produces colonies which are red or slightly pink-white and opaque with red surroundings.

Confirmation

Presumptive colonies are confirmed using biochemical and/or serological tests.

Health, Safety and Precautions

Salmonella is a pathogenic organism and strict hygiene and sterility practices must be followed at all times in order to prevent infection.

Sample Handling

Collect a sample volume of at least 1L for concentration.
Keep samples cool between sampling and delivery to laboratory.

Apparatus and Equipment

Autoclave
Water bath at 70^0C and 42^0C
pH meter P-M-011
Inoculating loops
Petri dishes
Culture tubes with caps
Filter manifold and pump
Filter holders
0.47µm membranes
Schott bottles
Incubator
Electronic balance

Reagents and Materials

Buffered peptone water

Obtained from commercial supplier.

Peptone	10g	
Sodium chloride	5g	
$K_2HPO_4.10H_2O$	3.5 g	(di-potassium hydrogen phosphate)
KH_2PO_4	1.5g	(potassium di-hydrogen phosphate)
Water	to 1 000mL	

Dissolve all ingredients, heating gently.
Adjust pH to 7.2± 0.1.

Dispense into bottles/tubes as required.

Sterilise at 121^0C for 15 minutes.

Store in refrigerator for up to 3 months.

Malachite green / Magnesium chloride

Obtained from commercial supplier.

Peptone (Animal tissues)	4g
Peptone (Soya)	1g
NaCl	8g
$K_2HPO_4.3H_2O$	0.4g
Water	to 1 000 mL

Supplement 1

$MgCl_2.6H_2O$	31.7g
Water	to 100 mL

Store sealed.

Supplement 2

Malachite green oxalate 0.4g

Water to 100 mL

Dissolve constituents of basic medium, heating gently.

Add supplement 1.

Add 10 mL of supplement 2.

Dispense about 10 mL of medium into each culture tube.

Sterilise at 115^0C for 15 minutes.

Brilliant green/phenol red lactose agar

Obtained from commercial supplier.

Meat extract powder	5g
Peptone (animal)	5 g
Na_2HPO_4	1 g
NaH_2PO_4	0.6 g

Agar	About 15 g
Water	to 900 mL

Supplement 1	
Lactose	10 g
Sucrose	10 g
Phenol red	0.09 g
Water	to 100 mL

Supplement 2	
Brilliant green	0.5 g
Water	to 100 mL

Dissolve constituents of basic medium.
Autoclave at 121^0C for 15 minutes.
Dissolve constituents of supplement 1 in sterile water.
Heat in water bath at 70^0C for 20 minutes.
Cool to 55 ± 1^0C and use immediately.
Add the prepared supplement 1 and 1 mL of brilliant green solution to the agar before distribution.
Ensure pH is 7.0 ± 0.1.

Dry agar plates immediately before use.

XLD agar

Obtained from commercial supplier.

D(+)-xylose	3.5 g
L(+)-lysine	5 g
Sodium deoxycholate	2.5 g
Yeast extract	3 g
Saccharose	7.5 g
Lactose	7.5 g
Sodium chloride	5 g
Sodium thiosulfate	6.8g

Iron (III) ammonium citrate	0.8 g
Agar	13 g
Water	To 1 000 mL

Supplement	
Phenol red	0.4 g
Water	To 100 mL

Dissolve all ingredients including 20 mL of the supplement by heating to boiling.
Adjust pH to 7.4 ± 0.1.
DO NOT AUTOCLAVE.
Cool to 50^0C.
Pour into Petri dishes.

Nutrient agar

Obtained from commercial supplier.

Meat extract	3g
Peptone	5g
Agar	15g
Water	to 1000 mL
Sodium chloride	5g

Dissolve all constituents by boiling.
Adjust pH to 7.0 ± 0.1.
Sterilise in autoclave at 121^0C for 15 minutes.
Dispense into Petri dishes.

Iron/two-sugar agar (Kligler)

Obtained from commercial supplier.

Meat extract	3g
Yeast extract	3g
Peptone	20g
Lactose	10g

D(+)-Glucose	1g
Iron(III) citrate	0.2 g
Sodium chloride	5g
Sodium thiosulphate	0.5 g
Agar	12g
Phenol Red	0.025g
Water	to 1000 mL

Heat gently to dissolve all constituents, including 6 mL of supplement.
Adjust pH to 7.4 ± 0.2
Autoclave at 121^0C for 15 minutes.
Pour about 6mL into each tube.
Allow medium to set at a slant to give a butt approximately 2.5 cm long.

Urea Agar

Obtained from commercial supplier.

Peptone	1g
D(+(-Glucose	1g
Sodium chloride	5g
Potassium dihydrogen phosphate	2g
Agar	12 g
Phenol Red	0.012g
Water	To 1000 mL
Supplement 2	
Urea crystals	40g
Water	To 100mL

Heat gently to dissolve all constituents including 3mL of supplement 1 in 950 mL of water.
Autoclave at 121^0C for 15 minutes.
Cool to about 50^0C
Sterilize supplement 2 by filtration.
Aseptically add 50 mL of solution 2 to the autoclaved medium.

Mix well.

Distribute aseptically in tubes.

Allow medium to set at a slant to give a butt approximately 2.5 cm long.

L-Lysine Iron Agar

Obtained from commercial supplier.

Meat peptone	5g/L
Yeast extract	3g/L
D(+)-Glucose	1g/L
L-Lysine monochloride	10g/L
Sodium thiosulphate	0.04g/L
Ammonium ferric citrate	0.5g/L
Bromocresol purple	0.02g/L
Agar	12.5g/L

Dissolve 32g in I litre reagent water and pur into tubes. Adjust to pH 6.8±1.

Autoclave at 121^0C for 15 minutes and let set as slants.

Calibration

Ensure balance has been calibrated within the last week.

Ensure pH meter has been calibrated within the last week**.**

Incubator must show a steady temperature on the Laboratory Temperature Logger.

Automatic pipettes must have passed QC check within the last week.

Medium batch must have passed QC check.

Laminar flow cabinets must have passed QC check within the last week.

Autoclave run must have passed QC check.

If any of the above calibrations have failed, the method should not been deemed fit for use before remedial action has been shown to be successful.

Quality Control

Include a positive *S. Typhimurium* and a negative *E. faecalis* sample with each sample run.

Procedure

Filter a minimum of 1L of sample through 0.45µm membrane filters.

Place filters in 50 mL of buffered peptone.

Incubate at 37± 1^0C for 16-20 hours.

Pre-warm malachite green/magnesium chloride medium to 42± 0.5^0C.

Transfer 0.1 mL loopfuls of the culture to 10mL malachite green/magnesium chloride medium.

Incubate at 42± 0.5^0C for 18-24 hours.

Plate a loopful of the enrichment culture onto plates of each of brilliant green/phenol red lactose agar and XLD agar.

Incubate at 37± 1^0C for 24 hours.

Black centered, colourless (appear red) colonies on XLD are presumptive *Salmonella*.

Colourless colonies are presumptive *Shigella*.

Pinkish white opaque colonies with a red background on brilliant green broth are presumptive *Salmonella*.

Pick at least five typical colonies from each positive agar medium onto pre-dried nutrient agar, streaking for isolated colonies.

Incubate at 37± 1^0C for 18 - 24 hours.

Streak and stab butt of tube of iron/two sugar agar.

Incubate at 37± 1^0C for 24 hours. Typical *Salmonella* on iron/two sugar slants show red slants with gas formation and yellow butts with blackening of the agar.

Inoculate tube of urea agar.

Incubate at 37± 1^0C for 24 hours. Typical *Salmonella* on urea slants show a negative reaction i.e. no rose-pink colour followed by deep cerise.

Inoculate a colony just below the surface of the liquid L-Lysine decarboxylase medium.

Overlay with sterile liquid paraffin or oil.

Incubate at 37± 1^0C for 18 - 24 hours. Typical *Salmonella* cultures show a purple colour.

Confirm identification using serological techniques or API test strips if necessary. The sample may also be sent to a specialist serological laboratory for confirmation if this is required.

<u>Method Performance Assessment</u>

This method is the same as ISO. 6340:1995(E).
Specificity 100%
Selectivity 97.5%

Reporting Results

The results are reported as the presence or absence of *Salmonella* or *Shigella* in the sample volume.

7.6 Detection and enumeration of Staphylococcus

Introduction

Staphylococci are Gram-positive coccoid organisms primarily associated with the skin and mucous membranes. Their detection in recreational water is indicative of poor disinfection and hygiene practices in the running of the pool.

Scope/ Field of Application

Swimming pool water
Salt water
Fresh water

Definitions

Pathogen:	Microorganism leading to disease, in this case in humans.
Indicator organism:	Microorganism which is present in large numbers whenever a pathogen is present but absent when pathogens are absent. Usually not a pathogen itself and easier to isolate and identify than pathogens.
Staphylococcus:	A Gram-positive coccus associated with the skin of humans.

Gram positive: Organism retaining the crystal violet-iodine stain of the differential Gram stain and taking up the safranin stain of the differential Gram stain.

Masking: Growth of non-target organisms which may interfere with the detection of target organisms.

Principles

Baird Parker medium contains lithium chloride and tellurite to inhibit the growth of accompanying microbial flora. It also contains pyruvate and glycine which selectively stimulate the growth of staphylococci. Staphylococci form black colonies on the medium surrounded by zones of clearing in the surrounding agar.

Health, Safety and Precautions

Interferences for this method are as follows:

Turbidity

Highly turbid samples can block membrane filters and therefore prevent proper analysis according to this method. If a sample stops going through the filter or takes an unusually long time to do so, further dilution of the sample before filtering should be considered or an MPN method should be used.

ToxicantsTocicants in the sample will interfere with the resuscitation of the organisms and therefore result in a low organisms count. This problem may occasionally be alleviated by diluting the sample.

Temperature

Too high a temperature reduces the survival of the target organisms, whilst too low a temperature permits the growth of other, non-target organisms.

Condensate

Inversion of Petri dishes for the duration of incubation prevents water droplets forming on the lid and dropping onto the surface of the medium with a resultant blurring of colonies.

Staphylococci can cause boils and other skin infections so should be handled with appropriate care.

Sample Handling

Choice of Sample Size

The bacterial load of different types of water varies and this is compensated for by using different volumes of water for analysis. The following volumes have been found to be generally appropriate.

Potable water	-	100 mL
River water	-	0.2 and 0.01 mL
Outfalls	-	1 and 0.2 mL
Beaches	-	5 and 1 mL
Pools	-	100 mL

Apparatus and Equipment

Bunsen burner
Autoclave
Biohazard cabinet
Water purifier
Automatic pipette
Filter manifold and pump
Forceps
Microwave
Incubator
Plate viewer

Reagents and Materials

Baird-Parker Agar
API Staphylococcus test strips
Brain-heart infusion broth (BHI)

Reagent Water

Water from the bacteriology water purifier which should have a conductivity of <0.5mS/m.

Sterile water

Sterilise reagent water in autoclave at 121^0C for 15 minutes.

Cool.

Store at $<10^0$C.

Sterile gridded 0.45 µm pore size, 47 mm diameter membrane filters. Individually packed membranes from acceptable supplier.

Calibration

Balance must have passed QC check within the last week.
pH meter must have passed QC check within the last week.
Incubator must show a steady temperature on the Laboratory Temperature Logger.
Automatic dispenser must have passed QC check within the last week.
Automatic pipettes must have passed QC check within the last week.
Medium batch must have passed QC check.
Laminar flow cabinets must have passed QC check within the last week.
Autoclave run must have passed QC check.
For each batch of media prepared, inoculate one Petri dish with positive and negative controls, namely *E. coli* and *Enterococcus faecalis*.
The *E. coli* colonies should be purple/blue and the *Enterococcus faecalis*. should be minimal and grey/white in colour.
If any of the above calibrations has failed, the method should not be deemed fit for use before remedial action has been shown to be successful.

Quality Control

Negative control *E. coli*

Procedure

Filter required volume of sample through a membrane filter.

Roll membrane onto surface of plate and incubate inverted for 45-48h at 35□ C.

Select plates containing 20-200 colonies for counting .

Colonies of *S. aureus* are circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-coloured (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle.

If confirmation is necessary, transfer suspect *S. aureus* colonies into small tubes containing 0.2-0.3 mL BHI broth and emulsify thoroughly.

Inoculate agar slant of suitable maintenance medium, e.g., TSA or nutrient, with a loopful of BHI suspension.

Incubate BHI culture suspension and slants 18-24 h at 35°C.

Retain slant cultures at room temperature for ancillary or repeat tests in case coagulase test results are questionable.

Add 0.5 mL reconstituted coagulase plasma with EDTA to the BHI culture and mix thoroughly.

Incubate at 35°C and examine periodically over 6h period for clot formation. Only a firm and complete clot that stays in place when the tube is tilted or inverted is considered positive for *S. aureus*. Partial clotting, formerly 2+ and 3+ coagulase reactions, must be tested further.

Test known positive and negative cultures simultaneously with suspect cultures of unknown coagulase activity.

Stain all suspect cultures with Gram reagent and observe microscopically. A latex agglutination test may be substituted for the coagulase test if a more rapid procedure is desired.

Incubation

Incubate the prepared membranes in an inverted position at 35 - 37^{o}C for 24 hours. Incubate samples within 30 minutes of filtering.

Enumeration

Count gray to jet black colonies using an illuminated plate counter.

Disposal

Collect all counted Petri dishes and place in autoclave bag.
Autoclave at 121^{0}C for 15 minutes to sterilise.
Place sterilised cultures in hazardous waste disposal container for disposal.

Calculation

If a dilution has been performed, it is necessary to multiply the number of colonies counted by the inverse of the dilution factor to obtain number of colonies per 100 mL.

Reporting Results

All results are entered into LIMS where the count per 100 mL is automatically calculated. Specification limits have been set for each water type and any results outside these limits will appear on the LIMS screen in red. These results must be further investigated according to the procedure for the handling of non-conforming results.

7.7 Detection and enumeration of somatic coliphage

Introduction

Coliphages are bacteriophages (bacterial virus) that infect and replicate in coliform bacteria and appear to be present wherever total and faecal coliforms are found. They are utilised as indicator organisms to represent the human enteroviruses as they are of similar size and react in a similar fashion to environmental conditions.

Scope/ Field of Application

Marine water.
Other water sources.
Sediments.
Sludges.
Shellfish extracts.

Definitions

Somatic coliphage	A virus having a selected strain of *E. coli* as its host, but not requiring the sex pili for infection.
Plaque	A zone of clearing in the bacterial lawn caused by cell lysis.

Principles

The sample is mixed with a small volume of semi-solid nutrient medium, a culture of host strain is added and the mixture is plated onto a solid nutrient medium. The phage infect the bacterial cells causing lysis. This results in a clearing of the bacterial lawn. Plates are incubated and read once visible plaques appear.

Health, Safety and Precautions

Coliphage are not considered to be pathogenic to humans.

Sample Handling

Select the size of sample dependent on the expected number of phage.
Perform serial dilutions on heavily contaminated samples before use.

Apparatus and Equipment

Petri dishes
Autoclave
Incubator at 37 ± 1^{0}C

Water bath at 48±2^{0}C

Microwave for melting media

pH meter

Plate counting apparatus

Deep freeze at (-20±5)^{0}C

Pipettes

Glass bottles

Refrigerator

Reagents and Materials

Nutrient agar plus Nalidixic acid (0.5g /20 mL)

0.5 g Nalidixic acid.

Dissolve in 4 ml NaOH (1M) add 16 mL water.

Filter sterilise into a sterile bottle.

Keep for not longer than 4 weeks.

Standard culture *E. coli* strain

Nutrient broth

Phage base agar

11g Agar

13g Tryptone

8g NaCl

1.5g Glucose

1L reagent water

Phage top agar

6g Agar

10g Tryptone

8g NaCl

3g Glucose

Nalidixic acid (1mL stock /100mL agar)

1L reagent water

Calibration

Balance must have passed QC check within the last week
pH meter must have passed QC check within the last week
Incubator must show a steady temperature on the Laboratory Temperature Logger Automatic pipettes must have passed QC check within the last week
Medium batch must have passed QC check
Laminar flow cabinets must have passed QC check within the last week Autoclave run must have passed QC check
If any of the above calibrations has failed, the method should not be deemed fit for use before remedial action has been shown to be successful.

Quality Control

For each batch of media prepared, inoculate one Petri dish each with positive and negative controls.

Procedure

Take one loopful of host culture from stock.
Subculture in 50 mL nutrient broth at 37 ± 1^0C for 4 hours.

On day of analysis

Pour phage agar base into 10 x 90 mm Petri dishes.
Allow to set.
Prepare phage top agar.
Maintain at 48 ± 2^0C in water bath.
Warm 50 mL of sample in water bath.
Add 125 mL of phage top agar and 10 mL of *E. coli* host culture.
Swirl gently to mix, avoiding bubbles.
Dispense evenly onto prepared agar base.
Allow to set.
Incubate at 37±1^0C for 18-24 hours.
Count plaques.

Calculation

2 x Total number of plaques in all the Petri dishes = phage per 100mL of sample.

Method Performance Assessment

The laboratory participates in inter-laboratory proficiency exercises, when available.

Reporting Results

Results are reported as plaque forming units (pfu) per 100 mL.

7.8 Ringers Solution

NaCL	6 g
KCL	0,075 g
CaCl2	0,1 g
NaHCO3	0,1 g
Reagent water	1 L

Dissolve the salts in reagent water and make up to 1L with reagent water. Autoclave before use.

8. APPENDIX 2

Table 8.1 Probability of infection from single exposure and annual probability of infection for an individual agricultural worker irrigating crops subsurface.

			Single exposure					Annual exposure				
Organism	**Irrigation**	**Mitigation**	**Min**	**Max**	**Mode**	**5%**	**95%**	**Min**	**Max**	**Mode**	**5%**	**95%**
Total coliform	G	None	0.083324	0.083324	0.083324	0.083324	0.083324	1	1	1	1	1
		-1	0.010456	0.010456	0.010456	0.010456	0.010456	0.877831	0.877831	0.877831	0.877831	0.877831
		-2	0.001075	0.001075	0.001075	0.001075	0.001075	0.193486	0.193486	0.193486	0.193486	0.193486
		-3	0.000108	0.000108	0.000108	0.000108	0.000108	0.021322	0.021322	0.021322	0.021322	0.021322
		-4	1.078×10^{-6}	1.078×10^{-6}	1.078×10^{-6}	1.078×10^{-6}	1.078×10^{-6}	0.0002156	0.0002156	0.0002156	0.0002156	0.0002156
	H	None	0	0	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0	0	0
		-4	0	0	0	0	0	0	0	0	0	0
	T	None	2.94×10^{-7}	2.94×10^{-7}	2.94×10^{-7}	2.94×10^{-7}	2.94×10^{-7}	5.879×10^{-5}	5.879×10^{-5}	5.88×10^{-5}	5.88×10^{-5}	5.88×10^{-5}
		-1	2.94×10^{-8}	2.94×10^{-8}	2.94×10^{-8}	2.94×10^{-8}	2.94×10^{-8}	5.88×10^{-6}	5.88×10^{-6}	5.88×10^{-6}	5.88×10^{-6}	5.88×10^{-6}
		-2	2.94×10^{-9}	2.94×10^{-9}	2.94×10^{-9}	2.94×10^{-9}	2.94×10^{-9}	5.88×10^{-7}	5.88×10^{-7}	5.88×10^{-7}	5.88×10^{-7}	5.88×10^{-7}
		-3	2.94×10^{-10}	2.94×10^{-10}	2.94×10^{-10}	2.94×10^{-10}	2.94×10^{-10}	5.88×10^{-8}	5.88×10^{-8}	5.88×10^{-8}	5.88×10^{-8}	5.88×10^{-8}
		-4	2.94×10^{-12}	2.94×10^{-12}	2.94×10^{-12}	2.94×10^{-12}	2.94×10^{-12}	5.88×10^{-10}	5.88×10^{-10}	5.88×10^{-10}	5.88×10^{-10}	5.88×10^{-10}

Table 8.2: Probability of infection from single exposure and annual probability of infection for an individual working with sub-surface irrigated soil

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
Enterococcus	G	None	0.0307664	0.0307664	0.0307664	0.0307664	0.0318335	0.0318335	0.0318335	0.0318335
		-1	0.0033371	0.0033371	0.0033371	0.0033371	0.275589	0.275589	0.275589	0.275589
		-2	0.0003366	0.0003366	0.0003366	0.0003366	0.0318335	0.0318335	0.0318335	0.0318335
		-3	3.369×10^{-5}	3.369×10^{-5}	3.369×10^{-5}	3.369×10^{-5}	0.003231	0.003231	0.003231	0.003231
		-5	3.369×10^{-7}	3.369×10^{-7}	3.369×10^{-7}	3.369×10^{-7}	3.236×10^{-5}	3.236×10^{-5}	3.236×10^{-5}	3.236×10^{-5}
	H	None	0.0017393	0.0017393	0.0017393	0.0017393	1	1	1	1
		-1	0.0001747	0.0001747	0.0001747	0.0001747	0.9925162	0.9925162	0.9925162	0.9925162
		-2	1.748×10^{-5}	1.748×10^{-5}	1.748×10^{-5}	1.748×10^{-5}	0.4030877	0.4030877	0.4030877	0.4030877
		-3	1.748×10^{-6}	1.748×10^{-6}	1.748×10^{-6}	1.748×10^{-6}	0.0505617	0.0505617	0.0505617	0.0505617
		-5	1.748×10^{-8}	1.748×10^{-8}	1.748×10^{-8}	1.748×10^{-8}	0.000519	0.000519	0.000519	0.000519
	T	None	0.0032916	0.0032916	0.0032916	0.0032916	0.9999997	0.9999997	0.9999997	0.9999997
		-1	0.000332	0.000332	0.000332	0.000332	0.8315227	0.8315227	0.8315227	0.8315227
		-2	3.323×10^{-5}	3.323×10^{-5}	3.323×10^{-5}	3.323×10^{-5}	0.1660088	0.1660088	0.1660088	0.1660088
		-3	3.323×10^{-6}	3.323×10^{-6}	3.323×10^{-6}	3.323×10^{-6}	0.0180242	0.0180242	0.0180242	0.0180242
		-5	3.323×10^{-8}	3.323×10^{-8}	3.323×10^{-8}	3.323×10^{-8}	0.0001819	0.0001819	0.0001819	0.0001819
Staphylococcus	G	None	0.0001617	0.0001617	0.0001617	0.0001617	0.0014863	0.0014863	0.0014863	0.0014863
		-1	0.0016107	0.0016107	0.0016107	0.0016107	0.0001487	0.0001487	0.0001487	0.0001487
		-2	0.0001617	0.0001617	0.0001617	0.0001617	1.487×10^{-5}	1.487×10^{-5}	1.487×10^{-5}	1.487×10^{-5}
		-3	1.618×10^{-5}	1.618×10^{-5}	1.618×10^{-5}	1.618×10^{-5}	1.487×10^{-6}	1.487×10^{-6}	1.487×10^{-6}	1.487×10^{-6}
		-5	1.618×10^{-7}	1.618×10^{-7}	1.618×10^{-7}	1.618×10^{-7}	1.487×10^{-8}	1.487×10^{-8}	1.487×10^{-8}	1.487×10^{-8}
	H	None	0.1554871	0.1554871	0.1554871	0.1554871	0.004494	0.004494	0.004494	0.004494
		-1	0.024178	0.024178	0.024178	0.024178	0.0004503	0.0004503	0.0004503	0.0004503
		-2	0.0025766	0.0025766	0.0025766	0.0025766	4.504×10^{-5}	4.504×10^{-5}	4.504×10^{-5}	4.504×10^{-5}
		-3	0.0002594	0.0002594	0.0002594	0.0002594	4.504×10^{-6}	4.504×10^{-6}	4.504×10^{-6}	4.504×10^{-6}
		-5	2.596×10^{-6}	2.596×10^{-6}	2.596×10^{-6}	2.60×10^{-6}	4.504×10^{-8}	4.504×10^{-8}	4.504×10^{-8}	4.504×10^{-8}

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
	T	None	0.0727894	0.0727894	0.0727894	0.0727894	0.482845	0.482845	0.482845	0.482845
		-1	0.0088652	0.0088652	0.0088652	0.0088652	0.064252	0.064252	0.064252	0.064252
		-2	0.0009073	0.0009073	0.0009073	0.0009073	0.0066236	0.0066236	0.0066236	0.0066236
		-3	9.094×10^{-5}	9.094×10^{-5}	9.094×10^{-5}	9.094×10^{-5}	0.0006644	0.0006644	0.0006644	0.0006644
		-5	9.096×10^{-7}	9.096×10^{-7}	9.096×10^{-7}	9.096×10^{-7}	6.646×10^{-6}	6.646×10^{-6}	6.646×10^{-6}	6.646×10^{-6}
Total coliforms	G	None	5.742×10^{-5}	5.742×10^{-5}	5.742×10^{-5}	5.742×10^{-5}	0.0114189	0.0114189	0.0114189	0.0114189
		-1	5.743×10^{-6}	5.743×10^{-6}	5.743×10^{-6}	5.743×10^{-6}	0.0011479	0.0011479	0.0011479	0.0011479
		-2	5.743×10^{-7}	5.743×10^{-7}	5.743×10^{-7}	5.743×10^{-7}	0.0001149	0.0001149	0.0001149	0.0001149
		-3	5.743×10^{-8}	5.743×10^{-8}	5.743×10^{-8}	5.743×10^{-8}	1.149×10^{-5}	1.149×10^{-5}	1.149×10^{-5}	1.149×10^{-5}
		-5	5.743×10^{-10}	5.743×10^{-10}	5.743×10^{-10}	5.743×10^{-10}	1.149×10^{-7}	1.149×10^{-7}	1.149×10^{-7}	1.149×10^{-7}
	H	None	7.437×10^{-6}	7.437×10^{-6}	7.437×10^{-6}	7.437×10^{-6}	0.9980694	0.9980694	0.9980694	0.9980694
		-1	7.437×10^{-7}	7.437×10^{-7}	7.437×10^{-7}	7.437×10^{-7}	0.4875433	0.4875433	0.4875433	0.4875433
		-2	7.437×10^{-8}	7.437×10^{-8}	7.437×10^{-8}	7.437×10^{-8}	0.0651179	0.0651179	0.0651179	0.0651179
		-3	7.437×10^{-9}	7.437×10^{-9}	7.437×10^{-9}	7.437×10^{-9}	0.0067157	0.0067157	0.0067157	0.0067157
		-5	7.437×10^{-11}	7.437×10^{-11}	7.437×10^{-11}	7.437×10^{-11}	6.739×10^{-5}	6.739×10^{-5}	6.739×10^{-5}	6.739×10^{-5}
	T	None	2.252×10^{-5}	2.252×10^{-5}	$2.25\times10^{-5}2$	2.252×10^{-5}	0.294012	0.294012	0.294012	0.294012
		-1	2.252×10^{-6}	2.252×10^{-6}	2.252×10^{-6}	2.252×10^{-6}	0.0343423	0.0343423	0.0343423	0.0343423
		-2	2.252×10^{-7}	2.252×10^{-7}	2.252×10^{-7}	2.252×10^{-7}	0.0034898	0.0034898	0.0034898	0.0034898
		-3	2.252×10^{-8}	2.252×10^{-8}	2.252×10^{-8}	2.252×10^{-8}	0.0003495	0.0003495	0.0003495	0.0003495
		-5	2.252×10^{-10}	2.252×10^{-10}	2.252×10^{-10}	2.252×10^{-10}	3.496×10^{-6}	3.496×10^{-6}	3.496×10^{-6}	3.496×10^{-6}

Table 8.3: Probability of infection as a result of individual or annual consumption of Swiss chard irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
E. coli	G	None	0.1774664	7.834×10^{-7}	5.368×10^{-5}	0.062208	1	1	0.0194043	1
		-1	0.0295484	7.834×10^{-8}	5.369×10^{-6}	0.0073547	0.9999824	2.86×10^{-5}	0.0019578	0.9324161
		-2	0.0031945	7.834×10^{-9}	5.369×10^{-7}	0.0007497	0.688971	2.86×10^{-6}	0.000196	0.2394709
		-3	0.0003221	7.834×10^{-10}	5.369×10^{-8}	7.512×10^{-5}	0.1109403	2.86×10^{-7}	1.96×10^{-5}	0.0270457
		-6	3.224×10^{-7}	7.833×10^{-13}	5.369×10^{-11}	7.513×10^{-8}	0.0001177	2.859×10^{-10}	1.96×10^{-8}	2.742×10^{-5}
	H	None	0.0058293	No distribution. Maximum microbial concentration utilised		0.0058293	0.8816289	No distribution. Maximum microbial concentration utilised		0.8816289
		-1	0.0005918			0.0005918	0.1943343			0.1943343
		-2	5.928×10^{-5}			5.928×10^{-5}	0.0214037			0.0214037
		-3	5.928×10^{-6}			5.928×10^{-6}	0.0021615			0.0021615
		-6	5.929×10^{-9}			5.929×10^{-9}	2.164×10^{-6}			2.164×10^{-6}
	T	None	0.0004933			0.0004933	0.1648252			0.1648252
		-1	4.94×10^{-5}			4.94×10^{-5}	0.0178689			0.0178689
		-2	4.94×10^{-6}			4.94×10^{-6}	0.0018016			0.0018016
		-3	4.94×10^{-7}			4.94×10^{-7}	0.0001803			0.0001803
		-6	4.94×10^{-10}			4.94×10^{-10}	1.8030×10^{-7}			1.803×10^{-7}
	C	None	0.0490408			0.0490408	1			1
		-1	0.0055903			0.0055903	0.870774			0.870774
		-2	0.0005672			0.0005672	0.1870575			0.1870575
		-3	5.681×10^{-5}			5.681×10^{-5}	0.0205212			0.0205212
		-6	5.682×10^{-8}			5.682×10^{-8}	2.074×10^{-5}			2.074×10^{-5}
Enterococcus	G	None	0.6478133	1.904×10^{-5}	0.0026918	0.5301724	1	1	0.626124	1
		-1	0.4360479	1.904×10^{-6}	0.0002711	0.2724989	1	1	0.094215	1
		-2	0.1700729	1.904×10^{-7}	2.713×10^{-5}	0.0617942	1	1	0.0098522	1

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-3	0.0276694	1.904×10^{-8}	2.713×10^{-6}	0.0072973	0.9999644	6.95×10^{-6}	0.0009897	0.9309757
		-6	3.002×10^{-5}	1.904×10^{-11}	2.713×10^{-9}	7.453×10^{-6}	0.0108968	6.95×10^{-9}	9.902×10^{-7}	0.0027167
	H	None	0.5978514	0.3862585	0.0342056	0.495663	1	1	0.999997	1
		-1	0.3628418	0.0001989	0.0037447	0.231497	1	1	0.7457376	1
		-2	0.111449	1.991×10^{-5}	0.0003781	0.0458408	1	1	0.1289405	0.9999999
		-3	0.015176	1.991×10^{-6}	3.785×10^{-5}	0.0051799	0.9962338	0.0007263	0.0137207	0.8497666
		-6	1.586×10^{-5}	1.991×10^{-9}	3.785×10^{-8}	5.258×10^{-6}	0.005773	7.267×10^{-7}	1.382×10^{-5}	0.0019173
	T	None	0.2558391	0.1225969	0.0166398	0.2274112	1	1	0.9978117	1
		-1	0.0548889	0.0025337	0.0017381	0.0444349	1	1	0.4700426	0.9999999
		-2	0.0063587	0.000255	0.0001746	0.0050017	0.9025425	0.4829016	0.0617446	0.8396198
		-3	0.0006465	2.552×10^{-5}	1.747×10^{-5}	0.0005067	0.2102529	0.009272	0.0063554	0.1688956
		-6	6.477×10^{-7}	2.552×10^{-8}	1.747×10^{-8}	5.075×10^{-7}	0.0002364	9.316×10^{-6}	6.376×10^{-6}	0.0001852
	C	None	0.1506645	0.1506645	0.1506645	0.1506645	1	1	1	1
		-1	0.0230835	0.0230835	0.0230835	0.0230835	0.9998014	0.9998014	0.9998014	0.9998014
		-2	0.0024528	0.0024528	0.0024528	0.0024528	0.5919511	0.5919511	0.5919511	0.5919511
		-3	0.0002468	0.0002468	0.0002468	0.0002468	0.0861694	0.0861694	0.0861694	0.0861694
		-6	2.47×10^{-7}	2.47×10^{-7}	2.47×10^{-7}	2.47×10^{-7}	9.016×10^{-5}	9.016×10^{-5}	9.016×10^{-5}	9.016×10^{-5}
Staphylococcus	G	None	0.1488553	2.033×10^{-8}	1.077×10^{-5}	0.0503695	1	1	0.003923	1
		-1	3.677×10^{-7}	5.012×10^{-14}	2.66×10^{-11}	1.244×10^{-7}	0.0001342	1.829×10^{-11}	9.71×10^{-9}	4.541×10^{-5}
		-2	9.082×10^{-14}	0	0	3.064×10^{-14}	3.315×10^{-11}	0	0	1.118×10^{-11}
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	H	None	0.1488553	2.033×10^{-8}	1.077×10^{-5}	0.0503695	1	1	0.003923	1
		-1	3.677×10^{-7}	5.012×10^{-14}	2.66×10^{-11}	1.244×10^{-7}	0.0001342	1.829×10^{-11}	9.71×10^{-9}	4.541×10^{-5}

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-2	9.082×10^{-14}	0	0	3.064×10^{-14}	3.315×10^{-11}	0	0	1.11×10^{-11}
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	T	None	0.1488553	2.033×10^{-8}	1.077×10^{-5}	0.0503695	1	1	0.003923	1
		-1	3.677×10^{-7}	5.012×10^{-14}	2.66×10^{-11}	1.244×10^{-7}	0.0001342	1.829×10^{-11}	9.71×10^{-9}	4.541×10^{-5}
		-2	9.082×10^{-14}	0	0	3.064×10^{-14}	3.315×10^{-11}	0	0	1.118×10^{-11}
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	C	None	0.1488553	2.033×10^{-8}	1.077×10^{-5}	0.0503695	1	1	0.003923	1
		-1	3.677×10^{-7}	5.012×10^{-14}	2.66×10^{-11}	1.244×10^{-7}	0.0001342	1.829×10^{-11}	9.71×10^{-9}	4.541×10^{-5}
		-2	9.082×10^{-14}	0	0	3.064×10^{-14}	3.315×10^{-11}	0	0	1.118×10^{-11}
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
Total coliforms	G	None	0.1016039	1.705×10^{-10}	2.96×10^{-7}	0.0237178	1	1	0.000108	0.9998433
		-1	0.0134418	1.705×10^{-11}	2.96×10^{-8}	0.0025245	0.9928419	6.224×10^{-9}	1.08×10^{-5}	0.6025128
		-2	0.0013922	1.705×10^{-12}	2.96×10^{-9}	0.0002541	0.3986173	6.224×10^{-10}	1.08×10^{-6}	0.0885883
		-3	0.0001397	1.705×10^{-13}	2.96×10^{-10}	2.543×10^{-5}	0.0497256	6.222×10^{-11}	1.08×10^{-7}	0.0092382
		-6	1.398×10^{-7}	0	2.96×10^{-13}	2.543×10^{-8}	5.102×10^{-5}	0	1.08×10^{-10}	9.282×10^{-6}
	H	None	0.0918011	5.17×10^{-10}	4.522×10^{-7}	0.0236571	1	1	0.000165	0.9998397
		-1	0.0118044	5.17×10^{-11}	4.522×10^{-8}	0.0025176	0.9868882	1.887×10^{-8}	1.65×10^{-5}	0.6015126
		-2	0.0012174	5.17×10^{-12}	4.522×10^{-9}	0.0002534	0.3589326	1.887×10^{-9}	1.65×10^{-6}	0.0883567
		-3	0.0001221	5.17×10^{-13}	4.522×10^{-10}	2.536×10^{-5}	0.0435993	1.887×10^{-10}	1.65×10^{-7}	0.009213
		-6	1.222×10^{-7}	0	4.521×10^{-13}	2.536×10^{-8}	4.459×10^{-5}	0	1.65×10^{-10}	9.256×10^{-6}
	T	None	0.1469777	6.98×10^{-12}	8.226×10^{-8}	0.041353	1	1	3.002×10^{-5}	0.9999998

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-1	0.022266	6.979×10^{-13}	8.226×10^{-9}	0.0046158	0.9997305	2.547×10^{-10}	3.002×10^{-6}	0.8152363
		-2	0.0023608	6.974×10^{-14}	8.226×10^{-10}	0.0004672	0.57798	2.546×10^{-11}	3.002×10^{-7}	0.1568006
		-3	0.0002375	0	8.226×10^{-11}	4.677×10^{-5}	0.0830557	0	3.002×10^{-8}	0.0169274
		-6	2.377×10^{-7}	0	8.216×10^{-14}	4.678×10^{-8}	8.675×10^{-5}	0	2.999×10^{-11}	1.707×10^{-5}
	C	None	0.0176449	6.627×10^{-5}	6.471×10^{-5}	0.0023183	0.9984934	0.0238985	0.0233416	0.5713752
		-1	0.001848	6.628×10^{-6}	6.472×10^{-6}	0.0002332	0.490917	0.0024163	0.0023594	0.0816162
		-2	0.0001857	6.628×10^{-7}	6.472×10^{-7}	2.334×10^{-5}	0.0655365	0.0002419	0.0002362	0.0084821
		-3	1.858×10^{-5}	6.628×10^{-8}	6.472×10^{-8}	2.334×10^{-6}	0.006758	2.419×10^{-5}	2.362×10^{-5}	0.0008515
		-6	1.858×10^{-8}	6.628×10^{-11}	6.472×10^{-11}	2.334×10^{-9}	6.781×10^{-6}	2.419×10^{-8}	2.362×10^{-8}	8.519×10^{-7}

Table 8.4: Probability of infection as a result of individual or annual consumption of green peppers irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
E. coli	G	Pinf	0.0476181	1.823×10^{-7}	1.598×10^{-5}	0.0146674	1	0.9999703	0.0058176	0.9954528
		-1	0.005407	1.823×10^{-8}	1.599×10^{-6}	0.0015241	0.8617787	6.655×10^{-6}	0.0005833	0.4269173
		-2	0.0005484	1.823×10^{-9}	1.599×10^{-7}	0.000153	0.181437	6.655×10^{-7}	5.835×10^{-5}	0.0543236
		-3	5.491×10^{-5}	1.823×10^{-10}	1.599×10^{-8}	1.531×10^{-5}	0.0198445	6.655×10^{-8}	5.835×10^{-6}	0.0055717
		-6	5.492×10^{-8}	1.823×10^{-13}	1.599×10^{-11}	1.531×10^{-8}	2.005×10^{-5}	6.655×10^{-11}	5.835×10^{-9}	5.588×10^{-6}
	H	Pinf	0.064083	1.085×10^{-5}	0.0001599	0.0200422	1	0.9999975	0.0567126	0.9993824
		-1	0.0076162	1.085×10^{-6}	1.6×10^{-5}	0.0021125	0.9386122	0.0003961	0.0058234	0.5378495
		-2	0.0007769	1.085×10^{-7}	1.6×10^{-6}	0.0002124	0.2469854	3.962×10^{-5}	0.0005839	0.0746074
		-3	7.784×10^{-5}	1.085×10^{-8}	1.6×10^{-7}	2.125×10^{-5}	0.0280145	3.962×10^{-6}	5.84×10^{-5}	0.0077272

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-6	7.786×10^{-8}	1.085×10^{-11}	1.6×10^{-10}	2.125×10^{-8}	2.842×10^{-5}	3.962×10^{-9}	5.841×10^{-8}	7.758×10^{-6}
	T	Pinf	0.0006272	2.444×10^{-5}	1.656×10^{-5}	0.0004892	0.2046773	0.0088828	0.0060252	0.1635578
		-1	6.282×10^{-5}	2.445×10^{-6}	1.656×10^{-6}	4.898×10^{-5}	0.0226706	0.0008919	0.0006042	0.0177197
		-2	6.283×10^{-6}	2.445×10^{-7}	1.656×10^{-7}	4.899×10^{-6}	0.0022908	8.923×10^{-5}	6.043×10^{-5}	0.0017864
		-3	6.284×10^{-7}	2.445×10^{-8}	1.656×10^{-8}	4.899×10^{-7}	0.0002293	8.923×10^{-6}	6.04×10^{-6}	0.0001788
		-6	6.284×10^{-10}	2.445×10^{-11}	1.656×10^{-11}	4.899×10^{-10}	2.293×10^{-7}	8.923×10^{-9}	6.04×10^{-9}	1.788×10^{-7}
	C	Pinf	0	0	0	0				
		-1	0	0	0	0				
		-2	0	0	0	0				
		-3	0	0	0	0				
		-6	0	0	0	0				
Staphylococcus	G	Pinf	0.0024271	1.19×10^{-11}	1.32×10^{-8}	0.000583	0.5880971	4.344×10^{-9}	4.818×10^{-6}	0.1917181
		-1	0.0002442	1.19×10^{-12}	1.32×10^{-9}	5.838×10^{-5}	0.0853008	4.344×10^{-10}	4.818×10^{-7}	0.0210856
		-2	2.444×10^{-5}	1.19×10^{-13}	1.32×10^{-10}	5.839×10^{-6}	0.008881	4.342×10^{-11}	4.818×10^{-8}	0.0021291
		-3	2.444×10^{-6}	0	1.32×10^{-11}	5.839×10^{-7}	0.0008917	0	4.818×10^{-9}	0.0002131
		-6	2.444×10^{-9}	0	1.31×10^{-14}	5.839×10^{-10}	8.921×10^{-7}	0	4.782×10^{-12}	2.131×10^{-7}
	H	Pinf	0.022021	3.56×10^{-13}	3.055×10^{-9}	0.0046804	0.9997047	1.299×10^{-10}	1.115×10^{-6}	0.8195605
		-1	0.0023333	0	3.055×10^{-10}	0.0004738	0.573712	0	1.115×10^{-7}	0.1588352
		-2	0.0002347	0	3.055×10^{-11}	4.744×10^{-5}	0.0821232	0	1.115×10^{-8}	0.0171653
		-3	2.349×10^{-5}	0	3.055×10^{-12}	4.744×10^{-6}	0.0085369	0	1.115×10^{-9}	0.0017301
		-6	2.349×10^{-8}	0	3.109×10^{-15}	4.744×10^{-9}	8.574×10^{-6}	0	1.135×10^{-12}	1.732×10^{-6}
	T	Pinf	0.0187026	0	2.473×10^{-9}	0.0038292	0.9989832	0	9.027×10^{-7}	0.7534859
		-1	0.0019643	0	2.473×10^{-10}	0.0003867	0.5121093	0	9.027×10^{-8}	0.1316761
		-2	0.0001974	0	2.473×10^{-11}	3.871×10^{-5}	0.0695338	0	9.027×10^{-9}	0.0140314
		-3	1.975×10^{-5}	0	2.473×10^{-12}	3.872×10^{-6}	0.0071841	0	9.027×10^{-10}	0.0014122

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-6	1.975×10^{-8}	0	2.442×10^{-15}	3.872×10^{-9}	7.21×10^{-6}	0	8.915×10^{-13}	1.413×10^{-6}
	C	Pinf	0.0024903	6.632×10^{-6}	1.312×10^{-5}	0.0007758	0.5975101	0.0024178	0.0047765	0.2466788
		-1	0.0002506	6.632×10^{-7}	1.312×10^{-6}	7.773×10^{-5}	0.0874353	0.0002421	0.0004787	0.0279748
		-2	2.508×10^{-5}	6.632×10^{-8}	1.312×10^{-7}	7.775×10^{-6}	0.0091128	2.421×10^{-5}	4.788×10^{-5}	0.0028338
		-3	2.508×10^{-6}	6.632×10^{-9}	1.312×10^{-8}	7.775×10^{-7}	0.0009151	2.421×10^{-6}	4.788×10^{-6}	0.0002837
		-6	2.508×10^{-9}	6.632×10^{-12}	1.312×10^{-11}	7.775×10^{-10}	9.155×10^{-7}	2.421×10^{-9}	4.788×10^{-9}	2.838×10^{-7}
Total coliforms	G	Pinf	0.0008265	1.239×10^{-7}	1.952×10^{-6}	0.0001932	0.260507	4.524×10^{-5}	0.0007124	0.0680975
		-1	8.283×10^{-5}	1.239×10^{-8}	1.953×10^{-7}	1.933×10^{-5}	0.0297801	4.524×10^{-6}	7.126×10^{-5}	0.0070308
		-2	8.284×10^{-6}	1.239×10^{-9}	1.953×10^{-8}	1.933×10^{-6}	0.0030192	4.524×10^{-7}	7.127×10^{-6}	0.0007053
		-3	8.285×10^{-7}	1.239×10^{-10}	1.953×10^{-9}	1.933×10^{-7}	0.0003023	4.524×10^{-8}	7.127×10^{-7}	7.056×10^{-5}
		-6	8.285×10^{-10}	4.864×10^{-11}	1.952×10^{-12}	1.933×10^{-10}	3.024×10^{-7}	1.776×10^{-8}	7.126×10^{-10}	7.056×10^{-8}
	H	Pinf	0.0004826	6.819×10^{-7}	2.333×10^{-6}	0.0001424	0.1615337	0.0002488	0.0008512	0.0506622
		-1	4.832×10^{-5}	6.819×10^{-8}	2.333×10^{-7}	1.425×10^{-5}	0.0174817	2.489×10^{-5}	8.515×10^{-5}	0.0051871
		-2	4.832×10^{-6}	6.81×10^{-9}	2.333×10^{-8}	1.425×10^{-6}	0.0017623	2.489×10^{-6}	8.515×10^{-6}	0.0005199
		-3	4.832×10^{-7}	6.819×10^{-10}	2.333×10^{-9}	1.425×10^{-7}	0.0001764	2.489×10^{-7}	8.515×10^{-7}	5.201×10^{-5}
		-6	4.832×10^{-10}	1.985×10^{-13}	2.333×10^{-12}	1.425×10^{-10}	1.764×10^{-7}	7.246×10^{-11}	8.516×10^{-10}	5.201×10^{-8}
	T	Pinf	0.0004259	2.124×10^{-7}	2.306×10^{-6}	0.0001393	0.1439891	7.751×10^{-5}	0.0008415	0.0495804
		-1	4.263×10^{-5}	2.124×10^{-8}	2.306×10^{-7}	1.394×10^{-5}	0.015441	7.751×10^{-6}	8.418×10^{-5}	0.0050738
		-2	4.264×10^{-6}	2.124×10^{-9}	2.307×10^{-8}	1.394×10^{-6}	0.0015551	7.751×10^{-7}	8.419×10^{-6}	0.0005086
		-3	4.264×10^{-7}	2.124×10^{-10}	2.307×10^{-9}	1.394×10^{-7}	0.0001556	7.751×10^{-8}	8.419×10^{-7}	5.087×10^{-5}
		-6	4.264×10^{-10}	4.957×10^{-12}	2.307×10^{-12}	1.394×10^{-10}	1.556×10^{-7}	1.809×10^{-9}	8.419×10^{-10}	5.087×10^{-8}
	C	Pinf	5.332×10^{-5}	9.181×10^{-7}	2.872×10^{-7}	1.648×10^{-5}	0.0192747	0.0003351	0.0001048	0.0059984
		-1	5.333×10^{-6}	9.181×10^{-8}	2.872×10^{-8}	1.648×10^{-6}	0.0019446	3.351×10^{-5}	1.048×10^{-5}	0.0006015
		-2	5.333×10^{-7}	9.181×10^{-9}	2.872×10^{-9}	1.648×10^{-7}	0.0001946	3.351×10^{-6}	1.048×10^{-6}	6.016×10^{-5}
		-3	5.333×10^{-8}	9.181×10^{-10}	2.872×10^{-10}	1.648×10^{-8}	1.947×10^{-5}	3.351×10^{-7}	1.048×10^{-7}	6.017×10^{-6}

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-6	5.33×10^{-11}	0	2.87×10^{-13}	1.65×10^{-11}	1.95×10^{-8}	0	1.05×10^{-10}	6.02×10^{-9}

Table 8.5: Probability of infection as a result of individual or annual consumption of chillies irrigated subsurface with greywater (G) or hydroponic solution (H) calculated according to the load of total coliforms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
Total coliform	G	None	8.6×10^{-7}	4.738×10^{-9}	5.211×10^{-9}	2.95×10^{-7}	0.0003139	1.729×10^{-6}	1.902×10^{-6}	0.0001077
		-1	8.6×10^{-8}	4.738×10^{-10}	5.211×10^{-10}	2.95×10^{-8}	3.139×10^{-5}	1.729×10^{-7}	1.902×10^{-7}	1.077×10^{-5}
		-2	8.6×10^{-9}	4.738×10^{-11}	5.211×10^{-11}	2.95×10^{-9}	3.139×10^{-6}	1.729×10^{-8}	1.902×10^{-8}	1.077×10^{-6}
		-3	8.6×10^{-10}	2.316×10^{-11}	5.211×10^{-12}	2.95×10^{-10}	3.139×10^{-7}	8.453×10^{-9}	1.902×10^{-9}	1.077×10^{-7}
		-6	8.6×10^{-13}	0	5.329×10^{-15}	2.949×10^{-13}	3.139×10^{-10}	0	1.945×10^{-12}	1.076×10^{-10}
	H	None	2.328×10^{-6}	1.493×10^{-8}	1.376×10^{-8}	7.992×10^{-7}	0.0008492	5.45×10^{-6}	5.022×10^{-6}	0.0002917
		-1	2.328×10^{-7}	1.493×10^{-9}	1.376×10^{-9}	7.992×10^{-8}	8.495×10^{-5}	5.45×10^{-7}	5.022×10^{-7}	2.917×10^{-5}
		-2	2.328×10^{-8}	1.493×10^{-10}	1.376×10^{-10}	7.992×10^{-9}	8.496×10^{-6}	5.45×10^{-8}	5.022×10^{-8}	2.917×10^{-6}
		-3	2.328×10^{-9}	1.493×10^{-11}	1.376×10^{-11}	7.992×10^{-10}	8.496×10^{-7}	5.45×10^{-9}	5.022×10^{-9}	2.917×10^{-7}
		-6	2.327×10^{-12}	0	1.377×10^{-14}	7.991×10^{-13}	8.495×10^{-10}	0	5.025×10^{-12}	2.917×10^{-10}

Table 8.6: Probability of infection as a result of individual or annual consumption of beetroot irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C), calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitgiation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
E. coli	G	Pinf	0.0008064	8.70×10^{-6}	4.68×10^{-6}	0.0002729	0.2550628	0.0031697	0.0017061	0.094819
		-1	8.081×10^{-5}	8.698×10^{-7}	4.678×10^{-7}	2.731×10^{-5}	0.0290657	0.0003174	0.0001707	0.0099183
		-2	8.08×10^{-6}	8.70×10^{-8}	4.70×10^{-8}	2.73×10^{-6}	0.0029458	3.175×10^{-5}	1.708×10^{-5}	0.0009963
		-3	8.083×10^{-7}	8.70×10^{-9}	4.68×10^{-9}	2.731×10^{-7}	0.000295	3.17×10^{-6}	1.71×10^{-6}	9.968×10^{-5}
		-6	8.08×10^{-10}	3.71×10^{-14}	4.68×10^{-12}	2.73×10^{-10}	2.95×10^{-7}	1.35×10^{-11}	1.71×10^{-9}	9.97×10^{-8}
	H	Pinf	2.155×10^{-5}	1.468×10^{-7}	1.337×10^{-7}	7.60×10^{-6}	0.0078335	5.356×10^{-5}	4.879×10^{-5}	0.0027714
		-1	2.15×10^{-6}	1.47×10^{-8}	1.34×10^{-8}	7.603×10^{-7}	0.0007862	5.36×10^{-6}	4.88×10^{-6}	0.0002775
		-2	2.155×10^{-7}	1.47×10^{-9}	1.34×10^{-9}	7.60×10^{-8}	7.864×10^{-5}	5.36×10^{-7}	4.88×10^{-7}	2.775×10^{-5}
		-3	2.15×10^{-8}	1.47×10^{-10}	1.34×10^{-10}	7.60×10^{-9}	7.86×10^{-6}	5.36×10^{-8}	4.88×10^{-8}	2.77×10^{-6}
		-6	2.15×10^{-11}	8.61×10^{-14}	1.34×10^{-13}	7.60×10^{-12}	7.86×10^{-9}	3.14×10^{-11}	4.88×10^{-11}	2.77×10^{-9}
	T	Pinf	0.000101	1.201×10^{-5}	2.63×10^{-6}	7.827×10^{-5}	0.0361902	0.0043726	0.0009583	0.028166
		-1	1.01×10^{-5}	1.20×10^{-6}	2.63×10^{-7}	7.83×10^{-6}	0.0036801	0.0004381	9.587×10^{-5}	0.0028534
		-2	1.01×10^{-6}	1.20×10^{-7}	2.63×10^{-8}	7.82×10^{-7}	0.0003686	4.382×10^{-5}	9.59×10^{-6}	0.0002857
		-3	1.01×10^{-7}	1.20×10^{-8}	2.63×10^{-9}	7.83×10^{-8}	3.687×10^{-5}	4.38×10^{-6}	9.59×10^{-7}	2.858×10^{-5}
		-6	1.01×10^{-10}	1.50×10^{-12}	2.63×10^{-12}	7.83×10^{-11}	3.69×10^{-8}	5.47×10^{-10}	9.59×10^{-10}	2.86×10^{-8}
	C	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
Enterococcus	G	Pinf	0.5424531	1.27×10^{-6}	0.0002945	0.3451193	1	1	0.1019339	1
		-1	0.2879765	1.27×10^{-7}	2.947×10^{-5}	0.0998927	1	1	0.0107003	1
		-2	0.0687751	1.27×10^{-8}	2.95×10^{-6}	0.0131497	1	4.65×10^{-6}	0.0010753	0.9920251

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitgiation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-3	0.0082821	1.27×10^{-9}	2.95×10^{-7}	0.0013609	0.9519518	4.65×10^{-7}	0.0001076	0.3916971
		-6	8.48×10^{-6}	1.27×10^{-12}	2.95×10^{-10}	1.37×10^{-6}	0.0030916	4.65×10^{-10}	1.08×10^{-7}	0.0004986
	H	Pinf	0.380769	2.141×10^{-5}	0.001108	0.2310689	1	1	0.3327951	1
		-1	0.1241452	2.14×10^{-6}	0.0001111	0.045692	1	1	0.0397505	0.9999999
		-2	0.0175537	2.14×10^{-7}	1.112×10^{-5}	0.005161	0.9984414	7.816×10^{-5}	0.004049	0.8487203
		-3	0.001838	2.14×10^{-8}	1.11×10^{-6}	0.0005231	0.4890516	7.82×10^{-6}	0.0004056	0.1738429
		-6	1.85×10^{-6}	2.14×10^{-11}	1.11×10^{-9}	5.24×10^{-7}	0.0006742	7.82×10^{-9}	4.06×10^{-7}	0.0001912
	T	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	C	Pinf	0.0504471	0.0504471	0.0504471	0.0504471	1	1	1	1
		-1	0.0057729	0.0057729	0.0057729	0.0057729	0.8791522	0.8791522	0.8791522	0.8791522
		-2	0.000586	0.000586	0.000586	0.000586	0.1926221	0.1926221	0.1926221	0.1926221
		-3	5.869×10^{-5}	5.869×10^{-5}	5.869×10^{-5}	5.869×10^{-5}	0.0211954	0.0211954	0.0211954	0.0211954
		-6	5.87×10^{-8}	5.87×10^{-8}	5.87×10^{-8}	5.87×10^{-8}	2.143×10^{-5}	2.143×10^{-5}	2.143×10^{-5}	2.143×10^{-5}
Staphylococcus	G	Pinf	0.0009088	3.67×10^{-9}	2.37×10^{-7}	0.0002246	0.2824185	1.34×10^{-6}	8.652×10^{-5}	0.0787155
		-1	9.11×10^{-5}	3.67×10^{-10}	2.37×10^{-8}	2.247×10^{-5}	0.0327049	1.34×10^{-7}	8.65×10^{-6}	0.008169
		-2	9.11×10^{-6}	3.67×10^{-11}	2.37×10^{-9}	2.25×10^{-6}	0.0033203	1.34×10^{-8}	8.65×10^{-7}	0.00082
		-3	9.11×10^{-7}	3.67×10^{-12}	2.37×10^{-10}	2.25×10^{-7}	0.0003325	1.34×10^{-9}	8.65×10^{-8}	8.203×10^{-5}
		-6	9.11×10^{-10}	0	2.37×10^{-13}	2.25×10^{-10}	3.33×10^{-7}	0	8.66×10^{-11}	8.20×10^{-8}
	H	Pinf	0.0083212	8.09×10^{-9}	1.00×10^{-6}	0.0023514	0.9526389	2.95×10^{-6}	0.0003666	0.5765249
		-1	0.0008504	8.09×10^{-10}	1.00×10^{-7}	0.0002366	0.2669305	2.95×10^{-7}	3.67×10^{-5}	0.0827368
		-2	8.522×10^{-5}	8.09×10^{-11}	1.00×10^{-8}	2.367×10^{-5}	0.0306293	2.95×10^{-8}	3.67×10^{-6}	0.0086032

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitgiation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-3	8.52×10^{-6}	8.09×10^{-12}	1.00×10^{-9}	2.37×10^{-6}	0.0031065	2.95×10^{-9}	3.67×10^{-7}	0.0008637
		-6	8.52×10^{-9}	0	1.00×10^{-12}	2.37×10^{-9}	3.11×10^{-6}	0	3.67×10^{-10}	8.64×10^{-7}
	T	Pinf	0.001366	6.56×10^{-6}	7.16×10^{-6}	0.000407	0.3928247	0.0023904	0.0026109	0.1380616
		-1	0.0001371	6.56×10^{-7}	7.16×10^{-7}	4.074×10^{-5}	0.0488088	0.0002393	0.0002614	0.0147601
		-2	1.371×10^{-5}	6.56×10^{-8}	7.17×10^{-8}	4.07×10^{-6}	0.004993	2.393×10^{-5}	2.614×10^{-5}	0.001486
		-3	1.37×10^{-6}	6.56×10^{-9}	7.16×10^{-9}	4.07×10^{-7}	0.0005004	2.39×10^{-6}	2.61×10^{-6}	0.0001487
		-6	1.37×10^{-9}	1.16×10^{-12}	7.16×10^{-12}	4.07×10^{-10}	5.01×10^{-7}	4.22×10^{-10}	2.61×10^{-9}	1.49×10^{-7}
	C	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
Total coliforms	G	Pinf	0.0013619	2.38×10^{-6}	8.07×10^{-6}	0.0004784	0.3919054	0.0008691	0.002941	0.1602551
		-1	0.0001367	2.38×10^{-7}	8.07×10^{-7}	4.79×10^{-5}	0.048664	8.694×10^{-5}	0.0002945	0.0173317
		-2	1.367×10^{-5}	2.38×10^{-8}	8.07×10^{-8}	4.79×10^{-6}	0.0049778	8.69×10^{-6}	2.945×10^{-5}	0.001747
		-3	1.37×10^{-6}	2.38×10^{-9}	8.07×10^{-9}	4.79×10^{-7}	0.0004989	8.69×10^{-7}	2.94×10^{-6}	0.0001748
		-6	1.37×10^{-9}	3.79×10^{-11}	8.07×10^{-12}	4.79×10^{-10}	4.99×10^{-7}	1.38×10^{-8}	2.94×10^{-9}	1.75×10^{-7}
	H	Pinf	0.0551375	1.772×10^{-5}	7.87×10^{-6}	0.0009804	1	0.0064485	0.00287	0.3009514
		-1	0.0063919	1.77×10^{-6}	7.87×10^{-7}	9.829×10^{-5}	0.9037238	0.0006468	0.0002874	0.0352428
		-2	0.0006499	1.77×10^{-7}	7.87×10^{-8}	9.83×10^{-6}	0.2112421	6.469×10^{-5}	2.874×10^{-5}	0.0035822
		-3	6.51×10^{-5}	1.77×10^{-8}	7.87×10^{-9}	9.83×10^{-7}	0.0234826	6.47×10^{-6}	2.87×10^{-6}	0.0003588
		-6	6.51×10^{-8}	5.33×10^{-12}	7.87×10^{-12}	9.83×10^{-10}	2.377×10^{-5}	1.94×10^{-9}	2.87×10^{-9}	3.59×10^{-7}
	T	Pinf	0.0003473	1.22×10^{-8}	4.36×10^{-7}	0.0001193	0.1190645	4.46×10^{-6}	0.0001591	0.0425956
		-1	3.476×10^{-5}	1.22×10^{-9}	4.36×10^{-8}	1.193×10^{-5}	0.0126064	4.46×10^{-7}	1.591×10^{-5}	0.0043446
		-2	3.48×10^{-6}	1.22×10^{-10}	4.36×10^{-9}	1.19×10^{-6}	0.0012679	4.46×10^{-8}	1.59×10^{-6}	0.0004353

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitgiation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-3	3.48×10^{-7}	1.22×10^{-11}	4.36×10^{-10}	1.19×10^{-7}	0.0001269	4.46×10^{-9}	1.59×10^{-7}	4.354×10^{-5}
		-6	3.48×10^{-10}	0	4.36×10^{-13}	1.19×10^{-10}	1.27×10^{-7}	0	1.59×10^{-10}	4.35×10^{-8}
	C	Pinf	0.0008579	0.0008579	0.0008579	0.0008579	0.2689353	0.2689353	0.2689353	0.2689353
		-1	8.598×10^{-5}	8.598×10^{-5}	8.598×10^{-5}	8.598×10^{-5}	0.0308957	0.0308957	0.0308957	0.0308957
		-2	8.6×10^{-6}	8.6×10^{-6}	8.6×10^{-6}	8.6×10^{-6}	0.003134	0.003134	0.003134	0.003134
		-3	8.6×10^{-7}	8.6×10^{-7}	8.6×10^{-7}	8.6×10^{-7}	0.0003138	0.0003138	0.0003138	0.0003138
		-6	8.6×10^{-10}	8.6×10^{-10}	8.6×10^{-10}	8.6×10^{-10}	3.14×10^{-7}	3.14×10^{-7}	3.14×10^{-7}	3.14×10^{-7}

Table 8.7: Probability of infection as a result of individual or annual consumption of potato irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C), calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
Staphylococcus	G	Pinf	0.2096806	0.0025206	0.0027622	0.1176383	1	1	0.6356385	1
		-1	0.0386815	0.0002537	0.0002782	0.0163146	0.9999995	0.9999535	0.0965743	0.997531
		-2	0.0042864	2.539×10^{-5}	2.784×10^{-5}	0.0017027	0.7915149	0.009224	0.0101107	0.4631319
		-3	0.0004334	2.54×10^{-6}	2.78×10^{-6}	0.000171	0.146355	0.0009263	0.0010158	0.0605192
		-6	4.34×10^{-7}	2.54×10^{-9}	2.78×10^{-9}	1.71×10^{-7}	0.0001584	9.27×10^{-7}	1.02×10^{-6}	6.24×10^{-5}
	H	Pinf	0.1480372	0.0003711	0.0012395	0.0631155	1	1	0.3640983	1
		-1	0.0224993	3.71×10^{-5}	0.0001244	0.0074809	0.999753	0.3033567	0.0443769	0.9354815
		-2	0.002387	3.71×10^{-6}	1.24×10^{-5}	0.0007628	0.5820082	0.0013551	0.0045301	0.2431066
		-3	0.0002402	3.71×10^{-7}	1.24×10^{-6}	7.64×10^{-5}	0.0839437	0.0001356	0.0004539	0.0275133
		-6	2.40×10^{-7}	3.71×10^{-10}	1.24×10^{-9}	7.64×10^{-8}	8.77×10^{-5}	1.36×10^{-7}	4.54×10^{-7}	2.79×10^{-5}
	T	Pinf	0.0037232	4.49×10^{-5}	1.97×10^{-5}	0.00109	0.7437245	0.0162601	0.0071653	0.3283836

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-1	0.0003759	4.49×10^{-6}	1.97×10^{-6}	0.0001093	0.1282422	0.0016382	0.0007189	0.0391144
		-2	3.76×10^{-5}	4.49×10^{-7}	1.97×10^{-7}	1.09×10^{-5}	0.0136415	0.0001639	7.19×10^{-5}	0.003983
		-3	3.76×10^{-6}	4.49×10^{-8}	1.97×10^{-8}	1.09×10^{-6}	0.0013727	1.64×10^{-5}	7.19×10^{-6}	0.000399
		-6	3.76×10^{-9}	4.49×10^{-11}	1.97×10^{-11}	1.09×10^{-9}	1.37×10^{-6}	1.64×10^{-8}	7.19×10^{-9}	3.99×10^{-7}
	C	Pinf	0.3063492	0.0038005	0.0052755	0.1793676	1	1	0.8549472	1
		-1	0.0778221	0.0003838	0.0005348	0.030044	1	1	0.1773865	0.9999854
		-2	0.0096143	3.84×10^{-5}	5.36×10^{-5}	0.0032524	0.9705833	0.013926	0.0193594	0.6954966
		-3	0.0009858	3.84×10^{-6}	5.36×10^{-6}	0.000328	0.3023301	0.0014016	0.0019532	0.1128497
		-6	9.89×10^{-7}	3.84×10^{-9}	5.36×10^{-9}	3.28×10^{-7}	0.0003608	1.40×10^{-6}	1.95×10^{-6}	0.0001198
Total coliforms	G	Pinf	0.0036105	0.0018305	0.0013623	0.0024163	0.7329181	0.487645	0.3920009	0.5864621
		-1	0.0003644	0.0001839	0.0001367	0.0002431	0.1245769	0.0649329	0.0486791	0.0849346
		-2	3.65×10^{-5}	1.84×10^{-5}	1.37×10^{-5}	2.43×10^{-5}	0.0132269	0.0066938	0.0049794	0.0088413
		-3	3.65×10^{-6}	1.84×10^{-6}	1.37×10^{-6}	2.43×10^{-6}	0.0013307	0.0006714	0.0004991	0.0008877
		-6	3.65×10^{-9}	1.84×10^{-9}	1.37×10^{-9}	2.43×10^{-9}	1.33×10^{-6}	6.72×10^{-7}	4.99×10^{-7}	8.88×10^{-7}
	H	Pinf	0.0019221	0.0019221	0.0019221	0.0019221	0.504534	0.504534	0.504534	0.504534
		-1	0.0001932	0.0001932	0.0001932	0.0001932	0.0680871	0.0680871	0.0680871	0.0680871
		-2	1.93×10^{-5}	1.93×10^{-5}	1.93×10^{-5}	1.93×10^{-5}	0.0070297	0.0070297	0.0070297	0.0070297
		-3	1.93×10^{-6}	1.93×10^{-6}	1.93×10^{-6}	1.93×10^{-6}	0.0007052	0.0007052	0.0007052	0.0007052
		-6	1.93×10^{-9}	1.93×10^{-9}	1.93×10^{-9}	1.93×10^{-9}	7.05×10^{-7}	7.05×10^{-7}	7.05×10^{-7}	7.05×10^{-7}
	T	Pinf	0.0018821	0.0018821	0.0018821	0.0018821	0.4972232	0.4972232	0.4972232	0.4972232
		-1	0.0001891	0.0001891	0.0001891	0.0001891	0.0667099	0.0667099	0.0667099	0.0667099
		-2	1.89×10^{-5}	1.89×10^{-5}	1.89×10^{-5}	1.89×10^{-5}	0.0068829	0.0068829	0.0068829	0.0068829
		-3	1.89×10^{-6}	1.89×10^{-6}	1.89×10^{-6}	1.89×10^{-6}	0.0006905	0.0006905	0.0006905	0.0006905
		-6	1.89×10^{-9}	1.89×10^{-9}	1.89×10^{-9}	1.89×10^{-9}	6.91×10^{-7}	6.91×10^{-7}	6.91×10^{-7}	6.91×10^{-7}
	C	Pinf	0.0243682	0.0243682	3.44×10^{-6}	0.0243681	0.9998772	0.9998772	0.0012538	0.9998772

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-1	0.0025982	0.0025982	3.44×10^{-7}	0.0025982	0.6130941	0.6130941	0.0001255	0.6130931
		-2	0.0002616	0.0002616	3.44×10^{-8}	0.0002616	0.0910712	0.0910712	1.25×10^{-5}	0.0910709
		-3	2.62×10^{-5}	2.62×10^{-5}	3.44×10^{-9}	2.62×10^{-5}	0.0095087	0.0095087	1.25×10^{-6}	0.0095087
		-6	2.62×10^{-8}	2.62×10^{-8}	3.44×10^{-12}	2.62×10^{-8}	9.55×10^{-6}	9.55×10^{-6}	1.25×10^{-9}	9.55×10^{-6}

Table 8.8: Probability of infection as a result of individual or annual consumption of onion irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
Enterococcus	G	Pinf	0.3204052	1.29×10^{-5}	0.0007004	0.1751691	1	1	0.2256592	1
		-1	0.0853378	1.29×10^{-6}	7.02×10^{-5}	0.0289564	1	1	0.0252875	0.999978
		-2	0.0107713	1.29×10^{-7}	7.02×10^{-6}	0.0031256	0.9808	4.72×10^{-5}	0.0025584	0.6810157
		-3	0.0011078	1.29×10^{-8}	7.02×10^{-7}	0.0003151	0.3327462	4.72×10^{-6}	0.0002561	0.1086614
		-6	1.11×10^{-6}	1.29×10^{-11}	7.02×10^{-10}	3.15×10^{-7}	0.0004056	4.72×10^{-9}	2.56×10^{-7}	0.0001151
	H	Pinf	0.0695308	0.0068733	0.0021295	0.0331842	1	1	0.5407262	0.9999955
		-1	0.0083909	0.0004796	0.0002141	0.0036229	0.9538385	0.3002267	0.0751903	0.7341327
		-2	0.0008576	4.80×10^{-5}	2.14×10^{-5}	0.0003657	0.2688774	0.0252634	0.0077898	0.1249821
		-3	8.60×10^{-5}	4.80×10^{-6}	2.14×10^{-6}	3.66×10^{-5}	0.030888	0.0017513	0.0007818	0.0132727
		-6	8.60×10^{-8}	4.80×10^{-9}	2.14×10^{-9}	3.66×10^{-8}	3.14×10^{-5}	1.75×10^{-6}	7.82×10^{-7}	1.34×10^{-5}
	T	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0

		Single exposure					Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	C	Pinf	0.1350527	0.1350527	0.1350527	0.1350527	1	1	1	1
		-1	0.0197317	0.0197317	0.0197317	0.0197317	0.9993067	0.9993067	0.9993067	0.9993067
		-2	0.002078	0.002078	0.002078	0.002078	0.5319918	0.5319918	0.5319918	0.5319918
		-3	0.0002089	0.0002089	0.0002089	0.0002089	0.0734306	0.0734306	0.0734306	0.0734306
		-6	2.09×10^{-7}	2.09×10^{-7}	2.09×10^{-7}	2.09×10^{-7}	7.63×10^{-5}	7.63×10^{-5}	7.63×10^{-5}	7.63×10^{-5}
Staphylococcus	G	Pinf	0.0035128	7.12×10^{-9}	5.85×10^{-7}	0.0007711	0.7231889	2.60×10^{-6}	0.0002136	0.2453968
		-1	0.0003545	7.12×10^{-10}	5.85×10^{-8}	7.73×10^{-5}	0.1213906	2.60×10^{-7}	2.14×10^{-5}	0.0278089
		-2	3.55×10^{-5}	7.12×10^{-11}	5.85×10^{-9}	7.73×10^{-6}	0.0128678	2.60×10^{-8}	2.14×10^{-6}	0.0028168
		-3	3.55×10^{-6}	7.12×10^{-12}	5.85×10^{-10}	7.73×10^{-7}	0.0012944	2.60×10^{-9}	2.14×10^{-7}	0.000282
		-6	3.55×10^{-9}	0	5.85×10^{-13}	7.73×10^{-10}	1.29×10^{-6}	0	2.14×10^{-10}	2.82×10^{-7}
	H	Pinf	0.0034259	1.38×10^{-6}	1.62×10^{-5}	0.0011476	0.7142426	0.0005049	0.005906	0.3423612
		-1	0.0003457	1.38×10^{-7}	1.62×10^{-6}	0.0001151	0.1185495	5.05×10^{-5}	0.0005922	0.0411429
		-2	3.46×10^{-5}	1.38×10^{-8}	1.62×10^{-7}	1.15×10^{-5}	0.0125486	5.05×10^{-6}	5.92×10^{-5}	0.0041935
		-3	3.46×10^{-6}	1.38×10^{-9}	1.62×10^{-8}	1.15×10^{-6}	0.0012621	5.05×10^{-7}	5.92×10^{-6}	0.0004202
		-6	3.46×10^{-9}	1.38×10^{-12}	1.62×10^{-11}	1.15×10^{-9}	1.26×10^{-6}	5.05×10^{-10}	5.92×10^{-9}	4.20×10^{-7}
	T	Pinf	0.0039298	1.76×10^{-8}	1.35×10^{-6}	0.000911	0.7624128	6.41×10^{-6}	0.0004925	0.2829944
		-1	0.000397	1.76×10^{-9}	1.35×10^{-7}	9.13×10^{-5}	0.1349265	6.41×10^{-7}	4.93×10^{-5}	0.0327828
		-2	3.97×10^{-5}	1.76×10^{-10}	1.35×10^{-8}	9.13×10^{-6}	0.0144017	6.41×10^{-8}	4.93×10^{-6}	0.0033283
		-3	3.97×10^{-6}	1.76×10^{-11}	1.35×10^{-9}	9.13×10^{-7}	0.0014497	6.41×10^{-9}	4.93×10^{-7}	0.0003333
		-6	3.97×10^{-9}	0	1.35×10^{-12}	9.13×10^{-10}	1.45×10^{-6}	0	4.93×10^{-10}	3.33×10^{-7}

		Single exposure					Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
	C	Pinf	3.14×10^{-6}	3.14×10^{-6}	3.14×10^{-6}	3.14×10^{-6}	0.0011439	0.0011439	0.0011439	0.0011439
		-1	3.14×10^{-7}	3.14×10^{-7}	3.14×10^{-7}	3.14×10^{-7}	0.0001144	0.0001144	0.0001144	0.0001144
		-2	3.14×10^{-8}	3.14×10^{-8}	3.14×10^{-8}	3.14×10^{-8}	1.14×10^{-5}	1.14×10^{-5}	1.14×10^{-5}	1.14×10^{-5}
		-3	3.14×10^{-9}	3.14×10^{-9}	3.14×10^{-9}	3.14×10^{-9}	1.14×10^{-6}	1.14×10^{-6}	1.14×10^{-6}	1.14×10^{-6}
		-6	3.14×10^{-12}	3.14×10^{-12}	3.14×10^{-12}	3.14×10^{-12}	1.14×10^{-9}	1.14×10^{-9}	1.14×10^{-9}	1.14×10^{-9}
Total coliform	G	Pinf	0.7612635	8.07×10^{-6}	7.63×10^{-6}	0.0068682	1	1	0.0027812	0.9191817
		-1	0.6136082	8.07×10^{-7}	7.63×10^{-7}	0.0006992	1	0.0002946	0.0002785	0.2253182
		-2	0.3854361	8.07×10^{-8}	7.63×10^{-8}	7.00×10^{-5}	1	2.95×10^{-5}	2.78×10^{-5}	0.0252445
		-3	0.1276219	8.07×10^{-9}	7.63×10^{-9}	7.01×10^{-6}	1	2.95×10^{-6}	2.78×10^{-6}	0.002554
		-6	0.0001922	8.07×10^{-12}	7.63×10^{-12}	7.01×10^{-9}	0.0677616	2.95×10^{-9}	2.78×10^{-9}	2.56×10^{-6}
	H	Pinf	0.0454526	2.52×10^{-12}	1.11×10^{-8}	0.0081016	0.9999999	9.21×10^{-10}	4.07×10^{-6}	0.9486512
		-1	0.0051305	2.52×10^{-13}	1.11×10^{-9}	0.0008274	0.8470231	9.21×10^{-11}	4.07×10^{-7}	0.2607659
		-2	0.0005199	0	1.11×10^{-10}	8.29×10^{-5}	0.1729001	0	4.07×10^{-8}	0.0298142
		-3	5.21×10^{-5}	0	1.11×10^{-11}	8.29×10^{-6}	0.0188247	0	4.07×10^{-9}	0.0030227
		-6	5.21×10^{-8}	0	1.11×10^{-14}	8.29×10^{-9}	1.90×10^{-5}	0	4.05×10^{-12}	3.03×10^{-6}
	T	Pinf	0.001653	3.08×10^{-9}	2.78×10^{-7}	0.0004407	0.4532849	1.13×10^{-6}	0.0001015	0.1486227
		-1	0.000166	3.08×10^{-10}	2.78×10^{-8}	4.41×10^{-5}	0.0587978	1.13×10^{-7}	1.01×10^{-5}	0.0159762
		-2	1.66×10^{-5}	3.08×10^{-11}	2.78×10^{-9}	4.41×10^{-6}	0.0060435	1.13×10^{-8}	1.01×10^{-6}	0.0016094
		-3	1.66×10^{-6}	3.08×10^{-12}	2.78×10^{-10}	4.41×10^{-7}	0.000606	1.13×10^{-9}	1.01×10^{-7}	0.0001611
		-6	1.66×10^{-9}	0	2.78×10^{-13}	4.41×10^{-10}	6.06×10^{-7}	0	1.02×10^{-10}	1.61×10^{-7}
	C	Pinf	6.06×10^{-5}	6.06×10^{-5}	6.06×10^{-5}	6.06×10^{-5}	0.0218821	0.0218821	0.0218821	0.0218821
		-1	6.06×10^{-6}	6.06×10^{-6}	6.06×10^{-6}	6.06×10^{-6}	0.0022103	0.0022103	0.0022103	0.0022103
		-2	6.06×10^{-7}	6.06×10^{-7}	6.06×10^{-7}	6.06×10^{-7}	0.0002213	0.0002213	0.0002213	0.0002213
		-3	6.06×10^{-8}	6.06×10^{-8}	6.06×10^{-8}	6.06×10^{-8}	2.21×10^{-5}	2.21×10^{-5}	2.21×10^{-5}	2.21×10^{-5}

		Single exposure					Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-6	6.06×10^{-11}	6.06×10^{-11}	6.06×10^{-11}	6.06×10^{-11}	2.21×10^{-8}	2.21×10^{-8}	2.21×10^{-8}	2.21×10^{-8}

Table 8.9: Probability of infection as a result of individual or annual consumption of carrot irrigated subsurface with greywater (G), hydroponic solution (H), tap water (T) or purchased commercially (C) calculated according to the selected indicator organisms

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
E coli	G	Pinf	0.1590408	0.0032882	0.0013216	0.0626604	1	1	0.3828888	1
		-1	0.0250032	0.0003316	0.0001326	0.0074176	0.9999031	0.1140248	0.0472547	0.9339603
		-2	0.0026704	3.32×10^{-5}	1.33×10^{-5}	0.0007562	0.6231867	0.0120423	0.0048304	0.2412832
		-3	0.0002689	3.32×10^{-6}	1.33×10^{-6}	7.58×10^{-5}	0.0934977	0.0012109	0.0004841	0.0272785
		-6	2.69×10^{-7}	3.32×10^{-9}	1.33×10^{-9}	7.58×10^{-8}	9.82×10^{-5}	1.21×10^{-6}	4.84×10^{-7}	2.77×10^{-5}
	H	Pinf	0.0064175	3.87×10^{-5}	3.59×10^{-5}	0.0019463	0.9046246	0.0140228	0.0130227	0.5088989
		-1	0.0006526	3.87×10^{-6}	3.59×10^{-6}	0.0001956	0.2120039	0.0014113	0.0013101	0.0689182
		-2	6.54×10^{-5}	3.87×10^{-7}	3.59×10^{-7}	1.96×10^{-5}	0.0235772	0.0001412	0.0001311	0.0071184
		-3	6.54×10^{-6}	3.87×10^{-8}	3.59×10^{-8}	1.96×10^{-6}	0.0023834	1.41×10^{-5}	1.31×10^{-5}	0.0007142
		-6	6.54×10^{-9}	3.87×10^{-11}	3.59×10^{-11}	1.96×10^{-9}	2.39×10^{-6}	1.41×10^{-8}	1.31×10^{-8}	7.14×10^{-7}
Enterococcus	G	Pinf	0.129365	0.1273528	0.0094205	0.12527	1	1	0.9684053	1
		-1	0.0185798	0.0181808	0.0009655	0.0177723	0.9989357	0.9987653	0.2971196	0.9985631

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-2	0.0019507	0.0019068	9.68×10^{-5}	0.001862	0.5096902	0.5017522	0.0347131	0.4935115
		-3	0.0001961	0.0001916	9.68×10^{-6}	0.0001871	0.0690696	0.067561	0.0035275	0.0660176
		-6	1.96×10^{-7}	1.92×10^{-7}	9.68×10^{-9}	1.87×10^{-7}	7.16×10^{-5}	7.00×10^{-5}	3.53×10^{-6}	6.83×10^{-5}
	H	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	T	Pinf	0	0	0	0	0	0	0	0
		-1	0	0	0	0	0	0	0	0
		-2	0	0	0	0	0	0	0	0
		-3	0	0	0	0	0	0	0	0
		-6	0	0	0	0	0	0	0	0
	C	Pinf	0.2660909	0.2660909	0.2660909	0.2660909	1	1	1	1
		-1	0.0590658	0.0590658	0.0590658	0.0590658	1	1	1	1
		-2	0.0069224	0.0069224	0.0069224	0.0069224	0.9207734	0.9207734	0.9207734	0.9207734
		-3	0.0007048	0.0007048	0.0007048	0.0007048	0.2269038	0.2269038	0.2269038	0.2269038
		-6	7.06×10^{-7}	7.06×10^{-7}	7.06×10^{-7}	7.06×10^{-7}	0.0002578	0.0002578	0.0002578	0.0002578
Staphylococcus	G	Pinf	0.1078061	2.37×10^{-7}	2.36×10^{-5}	0.0347978	1	1	0.0085729	0.9999976
		-1	0.0145235	2.37×10^{-8}	2.36×10^{-6}	0.0038157	0.9952038	8.64×10^{-6}	0.0008607	0.7522607
		-2	0.0015086	2.37×10^{-9}	2.36×10^{-7}	0.0003854	0.4236545	8.64×10^{-7}	8.61×10^{-5}	0.1312384
		-3	0.0001514	2.37×10^{-10}	2.36×10^{-8}	3.86×10^{-5}	0.0537831	8.64×10^{-8}	8.61×10^{-6}	0.0139816
		-6	1.51×10^{-7}	2.37×10^{-13}	2.36×10^{-11}	3.86×10^{-8}	5.53×10^{-5}	8.64×10^{-11}	8.61×10^{-9}	1.41×10^{-5}

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
	H	Pinf	0.0007535	2.00×10^{-6}	3.91×10^{-6}	0.0002331	0.240529	0.0007314	0.0014273	0.0815594
		-1	7.55×10^{-5}	2.00×10^{-7}	3.91×10^{-7}	2.33×10^{-5}	0.0271816	7.32×10^{-5}	0.0001428	0.0084759
		-2	7.55×10^{-6}	2.00×10^{-8}	3.91×10^{-8}	2.33×10^{-6}	0.0027524	7.32×10^{-6}	1.43×10^{-5}	0.0008509
		-3	7.55×10^{-7}	2.00×10^{-9}	3.91×10^{-9}	2.33×10^{-7}	0.0002756	7.32×10^{-7}	1.43×10^{-6}	8.51×10^{-5}
		-6	7.55×10^{-10}	5.55×10^{-14}	3.91×10^{-12}	2.33×10^{-10}	2.76×10^{-7}	2.03×10^{-12}	1.43×10^{-9}	8.51×10^{-8}
	T	Pinf	0.0002956	9.28×10^{-7}	1.35×10^{-6}	8.81×10^{-5}	0.1022791	0.0003389	0.0004933	0.0316496
		-1	2.96×10^{-5}	9.28×10^{-8}	1.35×10^{-7}	8.81×10^{-6}	0.0107384	3.39×10^{-5}	4.93×10^{-5}	0.0032116
		-2	2.96×10^{-6}	9.28×10^{-9}	1.35×10^{-8}	8.81×10^{-7}	0.0010791	3.39×10^{-6}	4.93×10^{-6}	0.0003216
		-3	2.96×10^{-7}	9.28×10^{-10}	1.35×10^{-9}	8.81×10^{-8}	0.000108	3.39×10^{-7}	4.93×10^{-7}	3.22×10^{-5}
		-6	2.96×10^{-10}	3.10×10^{-12}	1.35×10^{-12}	8.81×10^{-11}	1.08×10^{-7}	1.13×10^{-9}	4.93×10^{-10}	3.22×10^{-8}
	C	Pinf	0.0110822	0.0001394	5.56×10^{-5}	0.0033864	0.9828812	0.0496154	0.0201005	0.7100748
		-1	0.0011407	1.39×10^{-5}	5.56×10^{-6}	0.0003416	0.3407219	0.0050775	0.0020287	0.1172534
		-2	0.0001144	1.39×10^{-6}	5.56×10^{-7}	3.42×10^{-5}	0.040903	0.0005089	0.0002031	0.0124033
		-3	1.14×10^{-5}	1.39×10^{-7}	5.56×10^{-8}	3.42×10^{-6}	0.0041686	5.09×10^{-5}	2.03×10^{-5}	0.0012474
		-6	1.14×10^{-8}	1.39×10^{-10}	5.56×10^{-11}	3.42×10^{-9}	4.18×10^{-6}	5.09×10^{-8}	2.03×10^{-8}	1.25×10^{-6}
Total coliforms	G	Pinf	0.5467111	1.60×10^{-5}	2.32×10^{-5}	0.0059743	1	0.9999911	0.008429	0.887764
		-1	0.2934454	1.60×10^{-6}	2.32×10^{-6}	0.0006068	1	0.0005857	0.0008462	0.1987202
		-2	0.0713792	1.60×10^{-7}	2.32×10^{-7}	6.08×10^{-5}	1	5.86×10^{-5}	8.46×10^{-5}	0.0219391
		-3	0.008659	1.60×10^{-8}	2.32×10^{-8}	6.08×10^{-6}	0.958176	5.86×10^{-6}	8.46×10^{-6}	0.0022162
		-6	8.88×10^{-6}	1.60×10^{-11}	2.32×10^{-11}	6.08×10^{-9}	0.0032356	5.86×10^{-9}	8.46×10^{-9}	2.22×10^{-6}
	H	Pinf	0.0074878	8.87×10^{-5}	4.07×10^{-5}	0.0006686	0.9356436	0.0318609	0.0147438	0.2165974
		-1	0.0007635	8.87×10^{-6}	4.07×10^{-6}	6.70×10^{-5}	0.2433031	0.0032333	0.0014844	0.0241495
		-2	7.65×10^{-5}	8.87×10^{-7}	4.07×10^{-7}	6.70×10^{-6}	0.0275386	0.0003238	0.0001485	0.002442
		-3	7.65×10^{-6}	8.87×10^{-8}	4.07×10^{-8}	6.70×10^{-7}	0.0027891	3.24×10^{-5}	1.48×10^{-5}	0.0002445

			Single exposure				Annual exposure			
Organism	**Irrigation**	**Mitigation**	**Max**	**Mode**	**5%**	**95%**	**Max**	**Mode**	**5%**	**95%**
		-6	7.65×10^{-9}	8.87×10^{-11}	4.07×10^{-11}	6.70×10^{-10}	2.79×10^{-6}	3.24×10^{-8}	1.49×10^{-8}	2.44×10^{-7}
	T	Pinf	0.005121	1.78×10^{-7}	5.04×10^{-6}	0.0012831	0.8464839	6.51×10^{-5}	0.0018392	0.3741362
		-1	0.000519	1.78×10^{-8}	5.04×10^{-7}	0.0001287	0.1726026	6.51×10^{-6}	0.0001841	0.0459044
		-2	5.20×10^{-5}	1.78×10^{-9}	5.04×10^{-8}	1.29×10^{-5}	0.0187893	6.51×10^{-7}	1.84×10^{-5}	0.0046894
		-3	5.20×10^{-6}	1.78×10^{-10}	5.04×10^{-9}	1.29×10^{-6}	0.0018952	6.51×10^{-8}	1.84×10^{-6}	0.0004699
		-6	5.20×10^{-9}	1.78×10^{-13}	5.04×10^{-12}	1.29×10^{-9}	1.90×10^{-6}	6.51×10^{-11}	1.84×10^{-9}	4.70×10^{-7}
	C	Pinf	0.0004012	5.05×10^{-5}	1.75×10^{-5}	0.0001648	0.1362391	0.0182571	0.0063753	0.058377
		-1	4.02×10^{-5}	5.05×10^{-6}	1.75×10^{-6}	1.65×10^{-5}	0.0145516	0.0018411	0.0006394	0.0059991
		-2	4.02×10^{-6}	5.05×10^{-7}	1.75×10^{-7}	1.65×10^{-6}	0.0014649	0.0001843	6.40×10^{-5}	0.0006016
		-3	4.02×10^{-7}	5.05×10^{-8}	1.75×10^{-8}	1.65×10^{-7}	0.0001466	1.84×10^{-5}	6.40×10^{-6}	6.02×10^{-5}
		-6	4.02×10^{-10}	1.63×10^{-11}	1.75×10^{-11}	1.65×10^{-10}	1.47×10^{-7}	5.94×10^{-9}	6.40×10^{-9}	6.02×10^{-8}