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Title	**PLANT GROWTH AND MICROBIOLOGICAL SAFETY OF PLANTS IRRIGATED WITH GREYWATER**
Keywords	Greywater, plant growth, microbiological quality, fertiliser
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Short CV for Introduction Purposes (100 words max)	This project is being conducted jointly by Lumka Salukazana and Siobhan Jackson, both MSc students with the School of Biological and Conservation Sciences, UKZN. Ms Salukazana holds a BSc(Hons) from the University of Fort Hare, while Mrs Jackson holds a BSc(Hons) from the University of the Witwatersrand. Mrs Jackson is head of the microbiology laboratory of eThekwini Municipality.
Photograph attached (jpg)	

INTRODUCTION

The South African government has committed itself to the provision of basic, safe, sustainable water supply and sanitation to all its people. However, it has also recognised that it is neither technologically nor financially feasible, nor necessarily environmentally wise, to provide in-house full-pressure water supply and flushing toilets linked to waterborne sewerage and wastewater treatment plants to all. Where water is not reticulated to individual households or where the water pressure is not sufficient, sanitation by flushing toilets is not feasible. Such situations require on-site sanitation systems. The Ventilated Improved Pit Latrine (VIP) has been suggested as suitable for this purpose (DWAF, 2003). A system being implemented by eThekwini Municipality (Durban), amongst others, are urine diversion toilets. Such sanitation solutions deal more or less efficiently with toilet waste (blackwater), but do not address wastewater generated from activities such as bathing, washing laundry and washing dishes, termed greywater. Greywater is most usually disposed of to the ground in the vicinity of the dwelling, leading to pooling of wastewater, which in turn leads to unpleasant odours, pollution of groundwater and surface runoff, soil erosion, health hazards and mosquito breeding. Greywater has been identified as a widespread problem in all categories of dense informal settlements in South Africa (Wood *et al.,* 2001).

On-site sanitation is also most usually associated with poor communities with poor food security. A potential solution to these co-occurring problems is reuse of greywater for irrigation of crops in small-scale urban agriculture (subsistence agriculture). Although greywater may contain grease, food particles, hair and other impurities, it does not normally contain human waste unless laundry tubs or basins are used to rinse soiled clothing or baby's nappies/diapers (Ludwing, 2000; Eriksson *et al*., 2003; Jefferson *et al*., 2004). Greywater use in urban agriculture is potentially beneficial for a number of reasons, including: (a) Water shortages can be resolved thereby lowering potable water use for irrigation; (b) Environmental degradation, eutrophication and health hazards through pooling and runoff of wastewater can be resolved; (c) Potentially wasted nutrients can be reclaimed; (d) It contributes to poverty alleviation, food security and economic benefits; (e) It encourages people to use environmentally friendly chemicals in their households. Thus greywater represents a potential resource, provided that it is managed in an environmentally responsible manner. Moreover, greywater use presents minimal risks to public health, if all safety measures are taken into consideration (Ludwing, 2000).

Health hazards associated with grey water may arise from three sources: (a) Contamination by pathogenic microorganisms, including bacteria, protozoa, viruses and parasites in concentrations high enough to present health risks; (b) Chemical pollution by dissolved salts such as sodium, nitrogen, phosphates, chloride and boron, or by organic constituents such as oils, fats, milk, soap, detergents and xenobiotic compounds which may provide food for microorganisms and either promote or restrict plant growth; (c) Physical pollution by particles of dirt, food, lint etc. If these contaminants are not managed correctly they can, in addition to posing health hazards, degrade soil structure, clog groundwater flow paths or cause non-wetting characteristics (Blumenthal *et al*., 2000). However, risk to consumers can be greatly reduced by crop restriction, modifying irrigation techniques, human exposure control and chemotherapeutic intervention in the event of disease (Carr *et al*., 2004).

Characterisation of microbiologically safe irrigation practices combined with knowledge transfer, behaviour change and personal responsibility when using greywater has the potential to directly benefit large number of poor communities in the eThekwini municipal area. In 2003-2004, preliminary trials were conducted by eThekwini Municipality in two communities, one rural and one informal peri-urban. Householders were provided with water-collecting containers, seeds and implements, and received training. All participants were satisfied with the crop quantity and quality achieved. However, before the use of greywater for urban agriculture can be widely promoted by the municipal authority, the effect on plant growth, sustainability of greywater irrigation (in terms of medium to long term effects on soil quality), community acceptance of the practice, and associated health risks both during irrigation and as a result of crop consumption need to be investigated. This paper presents preliminary results of a semi-field trial conducted to determine the effect of greywater on plant growth and yield, and to investigate the microbiological safety of vegetables irrigated with greywater, in the eThekwini context.

MATERIALS AND METHODS

Experimental design

Cato Crest is a peri-urban settlement located in Durban, in close proximity to the University of KwaZulu-Natal. Greywater from eight households in this community was collected by eThekwini Municipality, and delivered to the University for use in semi-field trials. Cato Crest was selected for greywater collection on the basis of convenience of access. The selection of households was conducted in a preliminary study and was based on the number of people per household, ages, gender and washing applications (bath, hand basin, laundry and dish washing). Interviews and questionnaires were used to measure the greywater-producing activities. The total number of residents was 53, comprising 37 adults (18-100 yr), 12 children (2-18 yr) and 4 infants (0-2yr). These households were supplied with 20 litre plastic buckets for greywater collection, which was collected by eThekwini Municipality, weekly from Monday to Friday. Greywater from various households were mixed in one 200 litre container prior to use for irrigation.

Semi-field growth trials were conducted at an experimental site situated on the University premises. Representative above-ground (spinach, green pepper) and rooted (potatoes, madumbes) crops were selected, in consultation with eThekwini Municipality. These were grown in plastic bags at the site, which was plumbed for both potable water and greywater.

Water was delivered to directly to the root zone of each plant by a low-technology form of drip irrigation, comprising 500 ml plastic bottle which had been punctured at the base and buried to half its length beside each plant.

Three experimental treatments were employed. Tap water containing no nutrients served as a negative control, water amended with a commercially available plant nutrient medium served as a positive control, and greywater served as 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 nutrient medium was applied once weekly and tap water was used on the remaining days for the positive control plants. A total of 25 plants of each crop type were used for each treatment. Of the 25 plants of the same type, 10 were sacrificed for microbiological analysis at the end of the crop cycle. To date, one crop cycle has been completed.

Greywater characterisation
Greywater was characterised chemically and microbiologically for the parameters indicated in Table 1 at the beginning of the study.

Plant growth monitoring
Plant growth was measured weekly. Growth parameters included stem height, stem diameter, leaf area, number of leaves, number of fruits, fresh weights and dry weight. Harvested crops were assessed for fresh and dry weights. In this paper, only stem height and yield will be reported.

Microbiological analysis
Harvested crops were analysed for microbiological contaminants. These included *E. coli*, total coliforms, *Staphylococcus,* somatic coliphages and *Ascaris* ova. For the detection of *E. coli* and total coliforms, Chromocult agar (Merck) was used. Baird Parker agar (Bio lab) was used for the detection of *Staphylococcus*. Detection of coliphages was by standard double layer plaque assay. Analysis for *Ascaris* was according to Gaspard and Schwartzbrod (1995). Bacterial analyses were conducted on both surface and interior of the plant. Plant samples to be analysed were submerged in plastic jars with 100 ml sterile deionised water and placed on a platform shaker at 200 rpm for 1 hour in order to suspend surface microbial contaminants. The plastic jars were previously sterilised with 20% chlorine bleach for ten minutes and rinsed three times with sterile tap water. Aliquots of the suspended liquid were plated onto selective media for the required microoganisms. The same washed plants were then placed in a 20% solution of chlorine, and were shaken as above for 10 minutes. The plants were then placed in 100 ml sterile deionised water and macerated using a Heidolph blender. Aliquots of the resulting liquid were used for analysis as above. All plates were incubated according to the standard procedure.

RESULTS AND DISCUSSION

Results of physico-chemical and microbiological characterisation of the raw greywater, as received at the experimental site, are shown in Table 1. Typically, chemical characteristics (ammonia, COD, orthophosphate, total nitrogen and total phosphorus) were greater than literature values by a factor of 2 to 10 (Elmitwalli *et al.*, 2003; Jenssen and Vråle, 2003; Zifu *et al.*, 2003). This is consistent with the respective origins of the greywater. The cited studies were all conducted in Europe, with households receiving unlimited water supply. In contrast, households contributing greywater in this study were from a low income peri-urban community, restricted to 200 litres/day free basic water supply, and would be expected to yield a more concentrated waste. Nonetheless, when the ratios of nutrients were compared to published values for greywater, based on European conditions, there was no difference for those elements for which information was available. Palmquist and Jönsson (2003) report a ratio of 2:1:2:3 for N:P:K:S in greywater, comparing this with a corresponding ratio of 4-10:1:1-8:0.3-1 for crop uptake of these elements. The ratio of N:P:S in this study was 2:1:3 (no results available for K). Palmquist and Jönsson (2003) comment that the excess of sulphur added via greywater may result in acidification of the soil. Also of concern are the high concentration of chloride and the high conductivity of the greywater, which may lead to salinisation of the soil (Table 1). These are potential problems which will require monitoring in subsequent growth cycles.

Table 1: Characteristics of pooled greywater from 8 households

Alkalinity	300-334	mg/l
Ammonia (free)	20	mg/l
BOD	280-310	mg/l
Cadmium	<0.05	mg/l
Calcium	<5.0	mg/l
Chloride	210	mg/l
Chrome	0.11	mg/l
COD	1135	mg/l
Conductivity	144-148	mSiemens/m
Copper	0.1	mg/l
Lead	0.2	mg/l
Magnesium	5.6	mg/l
Nickel	<0.1	mg/l
Nitrate + Nitrite	<0.1-1.2	mg N/l
Ortho phosphate	11	mg P/l
pH	5.8-6.3	
Selenium	<0.05	mg/l
Sulphate	113	mg/l
Total Kjeldahl Nitrogen	24-30	mg N/l
Total phosphate	13	mg/l
Zinc	0.22	mg/l
Total coliforms	cfu/100ml	$4x10^5$
E. coli	cfu/100ml	$4x10^5$
Coliphage	pfu	0
***Ascaris* spp**	ova	0

Unlike the chemical quality, microbiological quality of the greywater used in this study was in the same range as reported in the studies cited above. According to Carr *et al*. (2004), proposing a modification of the 1989 WHO Guidelines for the Safe Use of Wastewater in Agriculture, this quality would be considered suitable only for restricted use, specifically localised irrigation of cereal crops, fodder crops, pasture and trees by trickle, drip or bubbler irrigation. This corresponds to the type of use, but not to the crops, for which greywater irrigation was piloted in communities by eThekwini Municipality and for which it was tested in the present study. Minimum treatment by primary sedimentation was also prescribed (Carr *et al*. , 2004). In the present study, greywater was used without treatment.

Comparison of plant growth as indicated by stem height (Fig.1) showed that there was a consistent increase in plant height when crops were irrigated with nutrient solution and with greywater, as compared with tap water. Growth with greywater irrigation was less than with nutrient solution, but still significantly greater than with tap water. Plants irrigated with tap water did not increase significantly in height throughout the crop cycle, whereas height increased steadily over time with both nutrient solution and greywater irrigation. An exception to this was observed for potatoes (Fig. 1C), where plant growth increased over the entire crop cycle with nutrient solution, but declined significantly in the final two weeks with greywater irrigation.

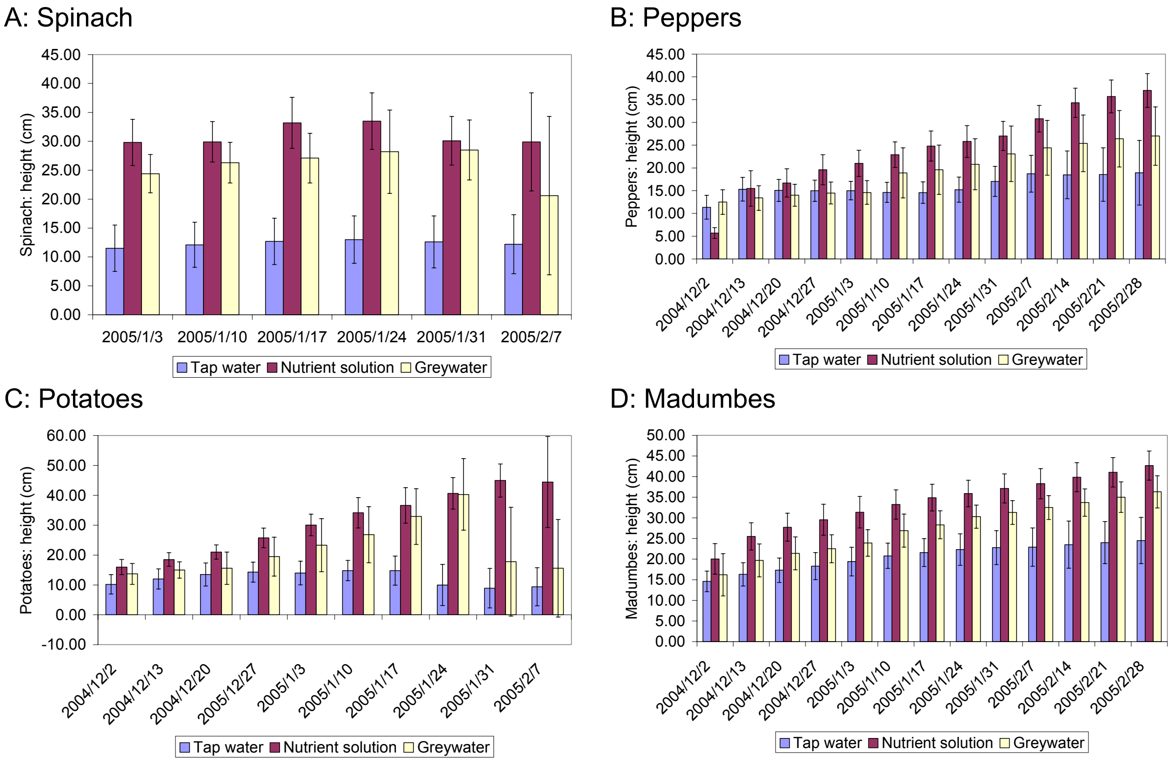

Figure 1: Stem height of spinach (A), green peppers (B), potatoes (C) and madumbes (D), monitored throughout one crop cycle. Figures show means and standard deviations for 25 plants.

Spinach (A): Crops irrigated with nutrient solution and with greywater were consistently significantly taller than crops irrigated with tap water. Crops irrigated with nutrient solution and grey water were similar in height after 24/1/2005
Peppers (B): Crops irrigated with nutrient solution and greywater were generally significantly taller than crops irrigated with tap water.
Potaoes (C): Crops irrigated with nutrient solution were significantly taller than the rest throughout. Crops irrigated with greywater were taller than cops irrigated with tap water except on 20/12/2003, 31/1/2005 and 7/2/2005.
Madumbes (D): Crops irrigated with nutrient solution were significantly taller than crops irrigated with greywater, which in turn were significantly taller than crops irrigated with tap water.
All comparisons by 1-way ANOVA, $p \leq 0.05$.

Comparison of crop yields over time was possible only for spinach, for which plants regrew after harvesting, and for green peppers, for which fruits could be harvested without sacrificing the entire plant. For spinach (Fig. 2A), irrigation with nutrient solution increased yield markedly on the first harvest. On subsequent harvests, yield was still higher than both greywater and tap water treatments, but less markedly so. Greywater-irrigated spinach also produced greater yield on the first harvest than on subsequent harvests, but this effect was less pronounced. Yield of tap water-irrigated spinach was very low throughout. Green pepper plants irrigated with nutrient solution produced the highest number of fruits per plant (Fig 2B), while plants irrigated with tap water produced the lowest number. Yield per plant from greywater-irrigated plants was intermediate between nutrient solution and tap water treatments. Interesting differences in the timing of fruit production can be noted among the three treatments. Tap water-irrigated plants showed the greatest productivity early in the crop cycle, while yield per plant with nutrient solution peaked in the middle of the cycle. Greywater-irrigated plants showed the latest peak in number of fruits per plants.

Yield measured by total fresh weight per treatment is shown in Fig 2C, while the statistical analysis of yield per plant is presented in the caption. Yield was significantly highest in nutrient solution treatments for all plants. There was no significant difference in yield between nutrient solution and greywater-irrigated spinach plants. Green pepper yield measured by fresh weight was greater with greywater irrigation than with tap water, but not significantly so. This contrasts with yield assessment on the basis on number of fruits, for which production with greywater was markedly greater than with tap water. This suggests that fruits produced with greywater irrigation were more numerous, but of lower relative yield on a fresh weight basis, when compared with tap water irrigation. Greywater did not increase the yield of potatoes (in fact, total yield was lower than with tap water), but this may be related to a restriction on tuber growth posed by the size of the bags in which the plants were grown. For madumbes, yield in greywater-irrigated plants was significantly greater than tap water-irrigated plants, but significantly lower than plants irrigated with nutrient solution.

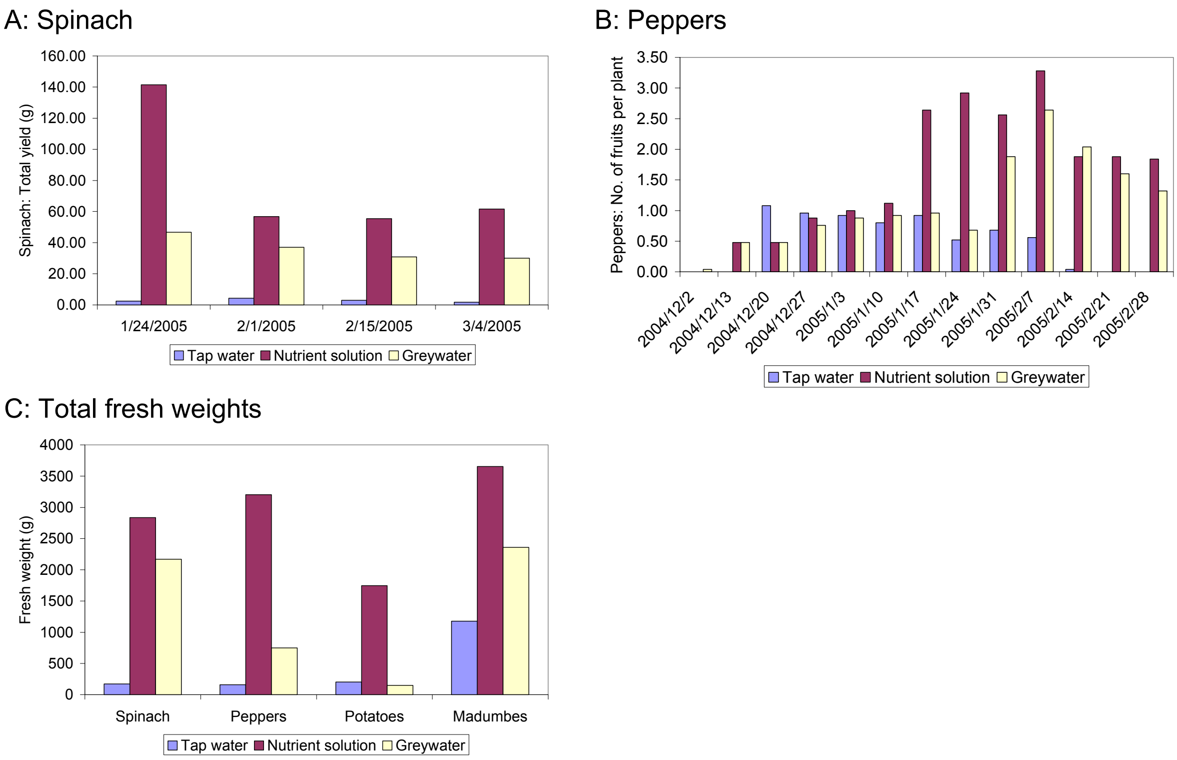

Figure 2: Yield over time for spinach (A) and green peppers (B), and total yield for all crops for one crop cycle (C). A and C show cumulative yield for all plants; B shows mean number of peppers produced per plant.

Statistical comparison of yield per plant showed that spinach yields were significantly greater for nutrient solution and greywater treatments, when compared to tap water; pepper and potato yields were significantly enhanced relative to tap water by nutrient solution but not by greywater; madumbe yield with greywater treatment was significantly greater than with tap water, and significantly greater with nutrient solution treatment than with greywater treatment. When yield of pepper fruits was compared by treatment, greywater treatment yielded significantly more fruits (total) than tap water, and nutrient solution treatment yielded significantly more fruits (total) than greywater. All comparisons by 1-way ANOVA, $p \leq 0.05$

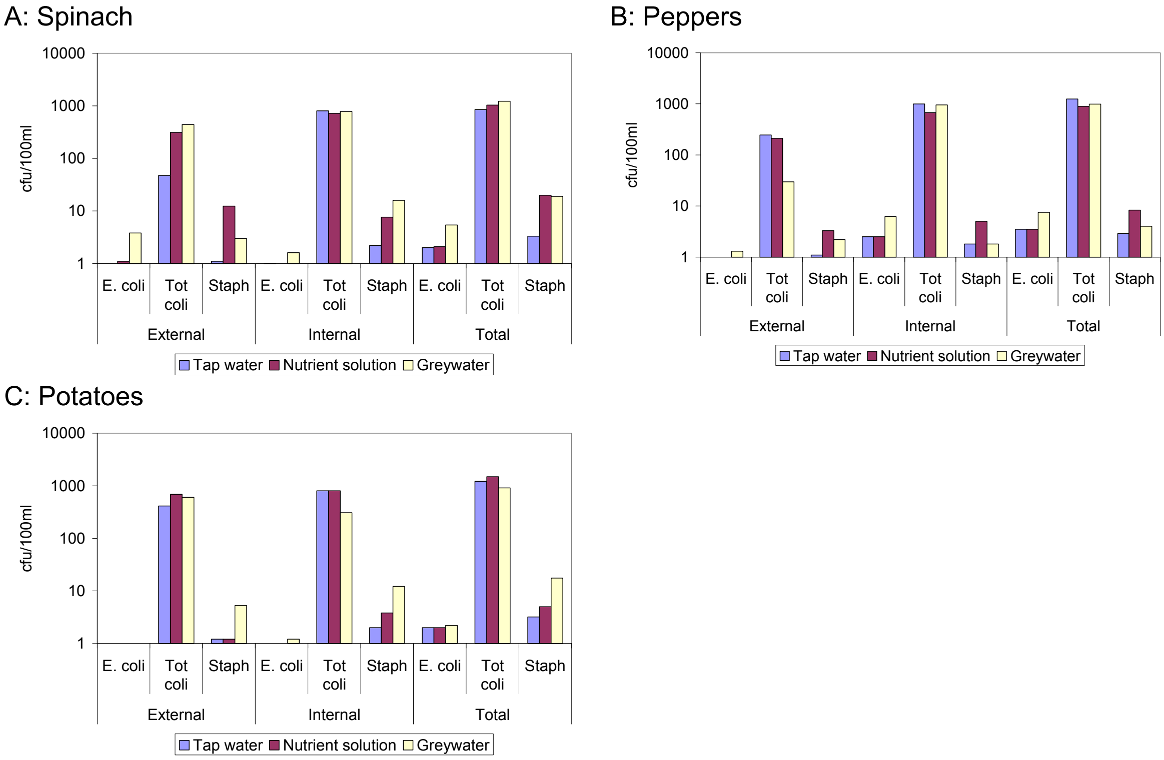

Figure 3: Microbiological analyses of crops harvested at the end of the first crop cycle. Figures show geometric means for yield from 10 plants.

Statistical comparisons were conducted on total counts only. Spinach (A): *E. coli* counts were significantly higher for irrigation with greywater; total coliforms showed significant differences between tap water and nutrient solution, and tap water and greywater; *Staphylococcus* showed significant differences among all treatments. Peppers (B): *E. coli* and total coliforms were not significantly different among the 3 treatments; *Staphylococci* were significantly higher for irrigation with nutrient solution than tap water, but not greywater. Potatoes (C): No significant differences among treatments. All comparisons by pairwise Mann-Whitney test of log-transformed data; $p \leq 0.05$. Neither log transformed nor untransformed data conformed to normal distribution, as per the Kolmogorov-Smirnov test.

Microbiological analyses included total coliforms, *E. coli*, *Staphylococcus*, coliphages and *Ascaris* ova. No coliphages or *Ascaris* ova were detected in any sample (results not presented graphically). Analysis of spinach, green pepper and potato crops did not yield consistent trends in the distribution of organisms between the interior and the exterior of the plant, nor in total counts relative to irrigation treatment (Fig. 3). It is possible that microorganisms apparently detected on the interior of crops had, in fact, not been adequately removed during previous wash procedures. The lack of a clear trend in total counts with irrigation treatment contrasted sharply with the trends observed in plant growth. This gave rise to a concern that levels of bacteria on tap water and nutrient solution irrigated crops may have been the result of contamination during irrigation. To determine whether the overall levels of bacteria detected were likely to represent a true lack of consistent significant difference with irrigation treatment, spinach and green peppers were purchased at a street market and at two chain stores. The same analyses were performed on these crops (Fig. 4) and the results compared to Fig 3. Bacterial levels on the commercial samples was generally similar to or, in the case of produce obtained from a street market, markedly higher than levels recorded in the crops from our growth trials. This indicated that the similarity observed in bacterial levels on crops from all three irrigation treatments was probably a valid result. A noteworthy observation was that total coliform levels were consistently high across all treatments, a trend which was replicated in the commercial samples. Analyses during future crop cycles will therefore focus on identifying the bacteria in the total coliform population and assessing, firstly, whether different members of the total coliform group are favoured by different irrigation treatments, and secondly, the extent to which opportunistic pathogens are represented among the total coliforms detected.

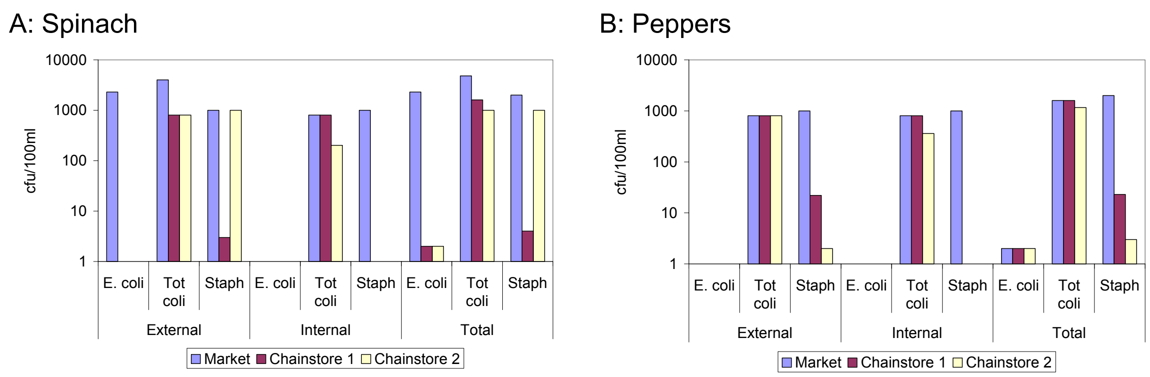

Figure 4: Microbiological analyses of commercially available crops, obtained from one street market and two chain stores.

Thus, overall, it can be concluded with some confidence that greywater holds nutrient value for above-ground leafy crops and probably also for below-ground root crops. This is suggested both by the nutrient ratios in the greywater and by the growth and yield of greywater-irrigated crops. Furthermore, levels of bacteria detected on crops from the three irrigation treatments suggest that contamination with faecally-derived bacteria (indicated by *E. coli*) was minimal to negligible, and that irrigation with greywater did not produce consistent increases in bacterial, viral or helminth ova levels on the final crops. This is despite a 'worst case' scenario being evaluated, with no waiting period between irrigation with greywater and harvesting, and no allowance made for inactivation of bacteria during food preparation such as cooking. These results confirm reports by other authors of the beneficial application of greywater in agriculture (Jeppeson, 1996; Carr *et al.*, 2000; Ludwing, 2000; Choi *et al.*, 2004; Scott *et al.*, 2004). They also suggest that restrictions regarding pretreatment of greywater and the types of crops grown, as per revised recommendations for wastewater use in agriculture (Carr *et al.*, 2004), may be more stringent than necessary, provided water is delivered below the soil surface.

While results from a single crop cycle cannot be considered conclusive, preliminary results reported here indicate that greywater represents a potentially important resource for food production in poor peri-urban communities with minimal additional risk to health associated with consumption of the irrigated produce.

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