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Water quality in the City of Tshwane, South Africa and its role in food safety for vegetable production

by

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DECLARATION

I declare that the thesis hereby submitted to the University of Pretoria for the degree of Magister Institutions Agrariae (Plant Protection), has not been previously submitted by me for a degree at any other University.

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CONTENTS

CHAPTER 3: Farming And Household Hygiene Practices At Tswane, ammanskraal Settlement In Gauteng Province

CHAPTER 4: Microbial Quality Of Fresh Vegetables And Apies River Water Used For Irrigation

CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

Globally there is an increase in the consumption of fresh produce (De Roever, 1999). This is the result of a change in dietary patterns motivated by governments to ensure better health in the population. However the increasing consumption of fresh fruit and vegetables in the United States of America resulted in a doubling of produce-associated diseases outbreaks between 1973 - 1992 (De Roever, 1999). According to the World Health Organisation (1993), about one third of the 51 million deaths world-wide resulted from infectious and parasitic diseases (Young, 1996). Schalekamp (1990) estimated that globally, 50 000 people die daily as a result of water related diseases (Grabow, 1996; Obi *et al*., 2002;).

Food-borne diseases occur through consumption of food that has been contaminated with microbial human pathogens. Contamination of fresh produce may take place by different means at several points from pre- to post-harvest production (Burnett and Beuchat, 2001). Production practices such as irrigation of crops with contaminated water and fertilisation with raw crown manure can result in contamination of produce (Saddik *et al.,* 1985). Irrigation water contaminated with human or animal faeces can act as a vehicle for various human pathogens (Conlo, 1999). In South Africa (SA), there is an increase in the development of informal settlements around main water resources resulting in high levels of water pollution. Contamination of water happens mainly through faecal matter from ground surfaces or pit latrines seeping below the ground into the streams (Water Research Commission, 2002). In addition, lack of proper water supplies leads to people washing their clothes in streams. Point source pollution from sewage treatment plants or other industries further contribute to water pollution (Venter *et al*., 1996).

Most commercial and small-scale farmers irrigate their crops from nearby rivers or streams, ponds, wells and dams with water that does not meet the required standard for irrigation (Westcot, 1997). Produce such as lettuce and cabbage consumed raw irrigated

with contaminated water can be a possible vehicle for spread of water-borne pathogens (Venter, 2001). In 1970, consumption of fresh vegetables such as lettuce and cucumbers irrigated with raw wastewater, resulted in cholera outbreaks in Jerusalem (Fattal *et al*. 2002). Such outbreaks could be explained by pathogenic microbiological human pathogens that can survive on crop surfaces for considerable periods of time (Rose, 1986). The quality of water that comes into contact with fresh produce dictates the potential for pathogen contamination (Food and drug Administration, 1998). Therefore, it is important to use potable water in irrigation schemes to ensure the safety of produce (Rangarajan *et al*, 1999).

Most of the people living in informal settlements are exposed to unhygienic conditions with poor sanitation facilities and inadequate water supplies. Lack of quality water for basic use can resulted in food-borne infections at home (Bloomfield, 2001). According to Geldreich and Bordner (1971), there is a concern that fruit and vegetables eaten raw, grown or processed under these conditions will transmit human pathogens. Furthermore, in SA, it has been estimated that more than 12million people do not have access to adequate supplies of potable water and about 21million lack basic sanitation facilities (Genthe and Seager, 1996). This can result in major outbreaks of water-borne diseases such as cholera that has been reported in Kwazulu-Natal and other parts of the country (www.who.int/disease-outbreak-news/n2001/april/17april2001.html).

Most of the emphasis of irrigation water is placed on chemical and physical characteristics and rarely on microbial quality (Ayers and Westcot, 1985). Conducting surveys related to food safety can provide information in terms of food safety awareness in the community. Therefore, more information on the microbial quality of water used in food production and processing and their impact on human health is required. Good Agricultural Practices, personal hygiene and improved household hygiene can have a significant impact on reducing food-borne diseases.

Investigation of produce-associated disease is difficult since food contamination could take place at any point in the food chain, from farm to fork. Furthermore, the complexity

of investigating food-borne diseases is due to the rapid increase in agricultural use and consumption of fresh water (MacCaffrey, 1997). As a result, food safety is becoming a major concern particularly for the fresh produce industry focusing mainly on export. Controlling contamination of agricultural produce that is ready for consumption, especially fruit and vegetables, present a major challenge to the industry, regulators and public health officials (Hedberg *et al.*, 1999). However, the same level of concern is not associated with produce destined for local consumption

This study aims to determine household hygienic practices in relation to fresh fruit and vegetable consumption, handling and storage patterns and determining quality of water used in agricultural production practices. It will furthermore focus on the occurrence of bacterial and viral human pathogens in the water systems and on vegetable surfaces irrigated with contaminated water.

The main objectives of this study are therefore:

- To determine the level of food safety awareness in the informal settlement around the Gauteng Province.
- To assess hygiene practices in relation to handling of water and fresh vegetables at a household level in these settlements.
- To monitor the microbial quality of water utilised by small scale and commercial vegetable growers in Gauteng Province, South Africa.
- To determine the presence of microbial pathogens on vegetable surfaces.
- To determine if untreated river or stream water used in agricultural production can contaminate vegetables and thereby increase food safety risks.

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CHAPTER 2: LITERATURE REVIEW

2.1. Global water quality

Only 2.72% of water in the world is fresh being contained in rivers, lakes, ground and permafrost of the polar caps and in glaciers high in mountains (Ganoulis, 1994). This leads to problems such as variability of water quality and quantity from one location to another (Clark *et al.*, 1977). Global water resources are currently under stress due to pollution and environmental deterioration (Morrison *et al.*, 2001). The quality of fresh water is continuously affected by natural and man-made pollutants such as dissolved solid or gaseous impurities, suspended solids and micro-organism (WRC, 1993). The source of pollution can be urban non-point source or through run-off and discharge of effluents from wastewater treatment plants next to rivers or from informal settlements (Brezonik and Stadelman, 2001)

Widespread use of agricultural chemicals is contributing to ground water contamination through leaching, run-off, deliberate spills and point source pollution (Younes and Galal-Gorchev, 2000; Nitschke and Schussler, 1997). Molefe (2002), reported that after 10 years of chemicals use by the former South African Defence Force, the Limpopo river was still found contaminated with bromacil, tebuthiuron and thidimuron. These chemicals were used between 1982-1993 to control weeds and vegetation invading the sisal hedge along the border. Environmental contamination with chemicals especially where it interferes with food production, processing and consumption presents a major health risk (Kaferstein, 2003). Through sanitary malpractices and insufficient water purification, potential pathogenic micro-organism transmitted by water is also increasing (Ford and Colwell, 1996). As a result, worldwide access to safe water remains a problem that leads to poor health and economic unproductivity, especially in developing countries (Venter, 2001).

Population growth is an important factor in terms of equitable water usage in the Southern Africa Developing Community (SADC). These countries are all situated in a water scarce region. This is mainly due to variable climatic conditions, short rainy seasons, and variable rainfall. In addition the water regional demand for domestic, industrial and production purposes are expected to increase over the next coming years (Mohamed, 2001).

In developing countries, most of the rural communities are living below the poverty line, lacking access to potable water supplies. They rely mainly on surface water (river, stream, wells and ponds) for their daily water needs (Nevondo and Cloete, 1999). As a result, more than 800 million estimated cases of diarrhoeal diseases and 4.5 million associated deaths occur yearly in these countries (Jarmey-Swan *et al*., 2001).

South African water resources are under threat particularly due to informal human settlements along the rivers and streams which lack appropriate sanitary infrastructure (Fatoki *et al*., 2003). In addition, there is a high water demand for local development within catchments areas with its increasing population densities and a growing need for water via pumping schemes (Venter *et al*., 1997; Van Riet and Slabbert, 1996). As a result, about 12million people in SA does not have adequate potable water supplies and nearly 21million lacks proper sanitation (Genthe and Seager, 1996).

Rural areas have partially treated water supplies while some communities in remote areas use untreated water directly from the source of origin (DWAF, 1993). These communities have been more exposed and prone to water-borne diseases than any other group in the country (Pegram *et al*., 1998; Obi *et al*., 2002). In addition, the commercial farmers in these regions also irrigate their crops from these sources without purification and treatment. This can result in produce being contaminated and potentially cause outbreaks of food-borne disease. Due to the current lack of information about water-borne and food-borne diseases in South Africa, the impact of poor water quality on human health and safety of crops is lacking. Therefore there is a need for the effective management of

these national water resources and ultimate more effective planning to use these resources for domestic, agricultural and industrial use.

2.2. Water quality

The term water quality is broadly applied to describe the suitability of water regarding its physical, chemical and biological characteristics (Borchardt and Walton, 1971). Ayers and Westcot (1985), also include personal preference such as taste and odour. The type of water required by different users, i.e. agriculture, industry, municipal, recreational and domestic use differs as does the quality of water (Ayers and Westcot, 1985; Borchardt and Walton, 1971). For instance, untreated water from a river may be of good quality for industrial but unacceptable for municipal usage. From this it can be concluded that water quality are determined by the needs of the user (Clark *et al*., 1977).

The quality of water resources varies widely in regional and local terms, with the supply depending on topography and meteorological conditions (Clark *et al*., 1977). River water quality differs due to the influence of geology, climate and nature of terrestrial vegetation (Day *et al*., 1998). Moreover, water quality can be affected through different ways such as intensified agriculture, loss of indigenous vegetation resulting in erosion, lack of proper water supply from the source and improper sanitation and pollution from sewage treatment plants or other industries (Venter *et al*., 1996).

2.2.1. Effects of water quality

Water users can experience a range of impacts as a result of poor water quality. The effects can be broadly categorised as health, aesthetic and economic impacts. It is therefore, important that water intended for human consumption is safe, palatable and aesthetically pleasing. This means that the water should be free of pathogenic micro-organism and other substances that may present a health risk (Genthe and Kfir, 1995). In addition, the water should be free from unpleasant or objectionable tastes, odours, colours and turbidity (Gower, 1980). Since all water sources contain some impurities (WRC,

1993), no water intended for human consumption can be assumed free from pollution. This includes water from the following sources: rainwater (as rain or snow), surface water from streams, rivers, lakes, dams or ponds and groundwater including well, boreholes or springs (The Microbiology of Water, 1994).

Water promotes the economic and general well-being of a society in various ways, such as the quality of its water supply, recreational potential and aquatic life (Hammer, 1975). In contrast, it has been found that water can also be a source of disease (Casemore, 1994). Pathogenic micro-organism recognised to be excreted in faeces of infected people and transmitted by water are bacteria, viruses and protozoa (Venter *et al.*, 1996; Hammer, 1975). To prevent this situation it is always important to monitor and determine the microbiological quality of water resources.

2.2.1.1. Water-borne diseases

Water-borne diseases are those that are acquired through ingestion of contaminated water. Examples of these diseases are cholera and typhoid fever. Oral transmission of infectious agents of bacteria, viral and protozoa may be from human to human or zoonotic transmission (Cairncross and Feachem, 1997).

2.2.1.2. Water-washed diseases

Water-washed diseases are diseases that are closely related to poor hygiene and lack of sanitation. The availability of water plays a more important role than its quality, especially in rural communities. Examples of water-washed diseases are trachoma, scabies and leprosy. These diseases are transmitted from one person to another, either directly or indirectly through contaminated food, utensils or via faecal contaminated hands (Craun, 1986; Venter *et al*., 1996).

2.2.1.3. Water-based diseases

Water-based diseases are caused by pathogens which are water dependent or aquatic organisms through completion of part of their life cycles. Examples of such diseases include schistosomiasis (Bilharzia) and guinea worm disease (Craun, 1986; Cairn cross and Feachem, 1997).

2.2.1.4. Water-vectored diseases

Water-vectored diseases are transmitted by insects which live and breed in or near water. Such diseases include malaria, yellow fever, dengue and anchocerciasis (Cairncross and Feachem, 1997). Diseases in this group are not associated with drinking water (Croun, 1986; Venter *et al.*, 1996).

2.2.2. Water-borne pathogen groups

2.2.2.1. Bacteria

Bacteria are the most common and cause many different water-borne illnesses (Jones, 1992). Bacteria mostly responsible for human diseases include *Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Shigella* spp, enterovirulent *Escherichia coli, Brucella* spp, *Yersinia enterocolitica* and *Campylobacter* spp, *Salmonella* spp, *Clostridium botulinum*, *Listeria monocytogenes*, *Clostridium perfringens and some Bacillus spp* (European commission, 2002). Potential Pathogens and diseases they cause have been listed in table 2.1.

2.2.2.2. Protozoan parasites

Human parasitic protozoa are a major cause of world-wide morbidity and mortality, affecting primarily the gastrointestinal tract (Girdwood, 1994). Most of these organisms produce cysts that survive outside their hosts under adverse conditions (Bitton, 1994). Parasites are mainly transmitted by faecal-oral routes and inadequate sanitation. In

addition, potable water supply systems also play a major role in epidemiology and transmission of these organisms (Girdwood. 1994). The most important species in outbreaks of water-associated diseases are *Giaria* and *Cryptosporidium* (Casemore, 1994).

2.2.2.3. Viral pathogens

Viruses are the cause of a broad-spectrum of human diseases ranging from skin rash, fever, respiratory infections and conjunctivitis to gastroenteritis and paralysis (Bitton, 1994). Acording to European commission Hepatitis A and Norwalk-like viruses are most commonly associated with food contamination (European commission, 200).

Table 2.1. Potential water – borne and water - related pathogenic microbial organisms of concern (Droste, 1997)

Name of organisms	Pathogen group	Disease
	Bacterial pathogens	
Escherichia coli		Enterohymogic fever
Legionella spp		Pneumonia
Leptospira spp		Leptospirosis
Mycobacterium spp		Tuberculosis
Pasturella tularensis		Tularemia
Shigella spp		Food poisoning, salmonellosis.
Salmonella typhi		Typhoid fever
Salmonella paratyphi		Paratyphoid fever
Salmonella spp		salmonellosis

Vibrio cholera	Cholera
Vibrio spp	Diarrhoea
Yersinia spp	Yersiniosis

Viral pathogens

Adeno Viruses	Upper respiratory and Gastrointestinal illness.
Coxsackievirus	Numerous conditions.
Hepatitis A virus	Infectious hepatitis.
Norwalk virus (Norovirus)	Gastroenteritis.
Reoviruses	Mild upper respiratory and Gastrointestinal illness.
Rotavirus	Gastroenteritis.
Polioviruses	Poliomyelitis.

Protozoa parasites

Balantidium coli	Mild diarrhoea, colonic ulceration.
Cryptosporidium	Cryptosporidiosis.
Entamoeba histolytica	Colonic ulceration
Giadia lamlia	Giadiasis

2.3. Determination of microbial quality of water

There are different methods described to determine whether water is safe and clean. This can be typically determined by microbial presence; especially faecal coliform bacteria and physico-chemical properties (Bezuidenhout *et al*., 2002). Most methods used for the detection and test of water-borne pathogens presence are expensive, complex and time-consuming. These methods are often also not very accurate in providing mostly quantitative results. As a result, it is common practice to manage microbial water quality on the basis of levels of indicator organisms (Venter *et al*., 1996). Water quality indicators are microbial, chemical or physical parameters, which indicate the potential risk of infectious disease causing organisms, present in water.

An indicator organism should ideally fulfil the following criteria (Genthe and Kfir, 1995):

- It should be present when other pathogens are present and absent in unpolluted water.
- It should be present in greater numbers than the pathogen.
- Its survival in the environment and resistance to treatment process should be comparable to that of pathogens.
- It should not be harmful to human health
- It should be easy to identify and isolate

Although there is no perfect indicator that complies with all the above requirements. The wide variety of indicators used has their own advantages and disadvantages. There is no universal guideline for combination of indicators and frequency of testing. Each situation has to be considered in its own right (DWAF, 1996).

2.3.1. Bacterial indicators

Bacteriological examination of water is particularly important because it remains the most sensitive method of detecting faecal and potentially dangerous pollution (The microbiology of Water, 1994). It is, however, becoming increasingly clear that bacterial indicators are not adequate for the assessment of microbial water quality under all circumstances. This is because of failure to indicate the presence of viruses and parasites. Therefore, it is important that methods for testing, modelling and management of microbial water quality should address a large range of organisms and should not be limited to common indicator bacteria (Venter *et al*., 1996).

2.3.2. Viral indicators

Viruses can be detected in water using different methods. Immunological techniques can be used for detection of viruses that do not cause cytopathogenic effects (DWAF, 1993). According to Grabow (1991), coliphages are also used to indicate the presence and behaviour of viruses, particularly enteric viruses present in water intended for domestic use. Unfortunately, coliphages are not used as indicators of viruses in water because of a lack of supporting information and difficulty with monitoring. The method for isolating coliphages is simple, inexpensive and fast. Coliphages are usually detected by their ability to form visible plaques on a bacterial plaque assay using *E. coli* as a host (DWAF, 1993).

2.3.3. Protozoa parasites

It is difficult to monitor protozoa parasites in environmental samples. Their detection relies on direct examination of water samples. In addition, large volumes of water need to be concentrated for examination and the procedure require an experienced person using microscopy to identify protozoan parasites after staining with fluorescent antibodies (Fricker, 1995).

2.3.4. Algae

Photosynthetic plants may indicate the quality of river water in a wide variety of ways (Whitton, 1979). Chlorophylous algae are preferable indicators of algal biomass estimates, but ways to monitor algae are not included in most guidelines (DWAF, 1993).

2.4. Treatment to improve the microbiological quality of water

Prevention of water-borne disease and its transmission can be achieved through water treatment processes such as coagulation-flocculation, sedimentation, filtration and disinfections. Such processes can inactivate or remove indicator bacteria and pathogenic organisms from raw water (DWAF, 1993). Water softening by activated carbon adsorption is also effective in this regard (Bitton, 1994).

2.4.1. Coagulation-flocculation

Coagulation involves the destabilization of colloidal particles by coagulants and sometimes by coagulant aids, with the most common coagulants being alum, ferric chloride and ferric sulphate (Nevondo and Cloete, 1997). Flocculation is the process of gentle, continuous stirring for the purpose of forming larger flocks of particles present in the water. It is thus the conditioning of water to form flocks that can readily be removed by settling or filtration (WRC, 1993). In general, coagulation transfers pathogens from water to the flocculated material.

2.4.2. Sedimentation

Because of high level of silt in most tropical rivers, sedimentation is usually the first necessary stage of water treatment (Cairncross and Faechem, 1997). Sedimentation is the settling and removal of suspended particles, which takes place in a slow flowing basin (Nevondo and Cloete, 1997). Turbulence is negligible and particles having a mass density greater than that of water will settle to the bottom of the settling basin. The efficiency of

sedimentation process is affected by settling velocity and concentration of suspended solids in the fluid (Water Research Commission, 1993).

2.4.3. Filtration

Filtration is one of the oldest processes used for water treatment (Modise and Krieg, 2004). Filtration is defined as the passage of fluids through porous media to remove suspended solids such as clays or microbial cells (Bitton, 1994). The effectiveness of this process depends on the filter medium, concentration and type of solids to be filtered, and the operation of the filter. An examination of water-borne disease outbreaks around the world shows that filtration has been instrumental as a barrier against pathogenic micro-organism and has largely contributed to the reduction of water-borne diseases (Bitton, 1994). The further removal of microbial contaminants, although variable, may exceed a 99% success rate. Combining the process of filtration, coagulation and flocculation is of particular importance in water treatment.

There are different types of filtration systems and they are listed as follows (Bitton, 1994; WRC, 1993):

- Slow sand filtration
- Rapid sand filtration
- Roughing filtration
- Diatomaceous earth filtration
- Surface filtration with ceramic filters.

2.4.4. Disinfection

The most important requirement for drinking water is that it should be free from micro-organism that could cause disease to the consumer (WRC, 1993). Although the earlier stages of water treatment will remove large percentage of bacteria, present in raw water, separate disinfection will still be required (Stevenson, 1997). Disinfection is the most useful method in killing human pathogenic micro-organisms in the water. Disinfection

alone is only sufficient if raw water originates from a protected source. In all other instances, additional treatment processes may be required prior to disinfections in order to improve its effectiveness.

Disinfection can simply be defined as the destruction or the complete inactivation of micro-organism capable of causing diseases (WRC, 1993; Bitton, 1994). This can be carried out using physical, chemical and photochemical means (Acher *et al*., 1997).

The factors influencing the efficiency of the water disinfection process are the nature and number of organisms to be destroyed, the type and concentration of the disinfectant used the temperature of water to be disinfected, the time of contact, the nature of water to be disinfected and pH of the water (Bitton, 1994).

2.4.4.1. Chemical disinfection methods

Chemical disinfection methods are based on the oxidation potential of chemicals that can oxidize and damage the cells of micro-organism causing their death (Acher *et al*., 1997). The most commonly used chemicals for disinfecting water are: chlorine (Cl_2), hypochlorite (ClO^-), chloramines (RNHCl), chlorine dioxide (ClO_2), bromine (Br_2) and ozone (O_3).

2.4.4.2. Other disinfection methods

There are other methods that may be used to disinfect water other than the use of chemicals. These disinfection methods may include photochemical, UV, solar pasteurization and physical methods such as boiling and storage (Nevondo and Cloete, 1999).

2.5. Water quality guidelines and standards

Water quality guidelines and standards provide concentration levels and management practices that have been set to protect public health (Berry and Hortson, 1974). The guideline specifies concentration of water quality variables, in quantitative terms, to ensure that all water users are protected (Moore *et al*., 1991). On the other hand the standards are legal requirements, which may be generally applicable or which may apply to specific discharge. The standards are generally used as the basis of pollution control (Moore, 1991).Water quality standards define the application of water for use in the public water supply, wild life, recreation, agriculture and industry. In addition, the standards include criteria based on the uses and plans to implement and enforce (Berry and Hortson, 1974). The standards are expressed in terms of microbial, chemical and physical characteristics of water (Cairncross and Feachem, 1997).

The World Health Organization guidelines for irrigation with treated wastewater specify that the geometric mean limit of faecal coliforms for unrestricted irrigation must not exceed 1000 in100 ml and one viable egg of intestinal nematodes per litre (WHO, 1989). The South African Department of National Health and Population set guidelines for standards of irrigation water when used as treated wastewater and specify that the level of *E. coli* or faecal coliforms not exceeding 1000/100 ml. The guidelines also specify that for irrigation of crops eaten raw and for contact landscape irrigation there may be no *E. coli* or faecal coliforms per 100ml (Venter *et al*., 1996).

2.6. Vegetables as a vector of pathogens

Consumption of fresh fruit and vegetables promote health and reduce the risk of some diseases thus making it an important part of the human diet. Consequently, there is an increase in consumption of fresh produce such as vegetables and fruit. Food safety is a global issue especially in relation to fresh vegetables, due to the outbreak of food-borne disease outbreaks. This is mainly due to infectious pathogens being transmitted after the consumption of contaminated produce (Meng and Doyle, 2002; Molins *et al*., 2001).

Micro-organism can contaminate food, resulting in various human illnesses which can occur in two forms, namely food poisoning or food intoxication and food infection after consumption of produce containing these pathogens or their toxins (Marth, 1981; Jones, 1992; Tauxe, 2002).

It has been documented that fresh produce consumed raw also act as a vehicle for human pathogens which, is of great concern regarding for safe food products (Venter, 2001). Okafo *et al*. (2003) found that wastewater used for irrigation contributes to produce contamination. A clasical example is the *Cholera* outbreak in Jerusalem in 1970 that involved 200 cases of food-borne diseases through consumption of vegetables such as lettuce and cucumber that were illegally irrigated with raw wastewater (Fatal *et al*., 2002). Another outbreak occurred in 1990 in Illinois, Michigan, Minosota, and Wisconsin. The causal organism was *Salmonella javiana* and was found on fresh tomatoes. The initial source of contamination was thought to be the water bath used in the pack house (Wood *et al*., 1991). Potential food-borne pathogens and the produce they have been isolated from are listed on table 2.2.

Table 2.2 examples of fresh produce from which bacterial pathogens have been isolated, adopted from (Buck *et al*., 2003)

Pathogens	**Products**
Aeromonas	alfalfa sprouts, asparagus, broccoli, cauliflower, celery, lettuce, pepper, spinach.
Bacillus cereus	alfalfa sprout, cress sprouts, cucumber, mustard sprouts, soybean sprout.
Campylobacter jejuni	green onions, lettuce, mushroom, potato, parsley, pepper, spinach.
Clostridium botulinum	cabbage, mushrooms, pepper.
E. coli 0157: H7	alfalfa sprout, apple juice, cabbage, celery, cilantro, coriander, cress sprouts, lettuce.
Listeria monocytogenes	bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushrooms, potatoes, radish, salad vegetables, tomato.
Salmonella spp	alfalfa sprouts, artichokes, beet leaves, celery, cabbage, cantaloupe, cauliflower, chilli, cilantro, eggplant, endive, fennel, green onions, lettuce, mungbean sprouts, mustard cress, orange juice, parsley, pepper, salad greens, spinach, strawberries, tomato, water melon.
Staphylococcus spp	alfalfa sprouts, carrots, lettuce, onions sprouts, parsley, radish.
Vibrio cholera	cabbage, coconut milk, lettuce.

2.6.1. Source of vegetables contamination

Fresh produce can harbor diverse numbers of micro-organisms including pathogens, which can contaminate fruit and vegetables through various ways and at different points, along the fruit chain. Potential source of both pre and post-harvest contamination have been listed by Burnett and Beuchat (2001) and include:

- Reclaimed wastewater irrigation
- Soil fertilised with un composed crown manure
- Crop fertilised with sewage sludge
- Faecal pollution of the areas in which food products are obtained
- Non-point pollution such as land run-off into watersheds and discharge of human waste from boats
- Water used to apply pesticides
- Dust
- Insects
- Inadequately composted manure
- Wild and domestic animals
- Improper unhygienic handling
- Recontamination after cooking or processing
- Inadequate facility sanitation
- Cross contamination of processed products from raw food
- Contaminated harvest equipment
- Unhygienic transport containers
- Polluted rinse water and ice.

2.6.2. Economic importance of food-borne and water-borne diseases

It has been estimated that water borne diseases are the cause of 2-5 million deaths and 900 million illnesses each year (World bank, 1992). In addition, millions of people suffer from diseases caused by contaminated food world-wide (Meng and Doyle, 2002). It has

been reported in US, that 76 million illnesses, 325 000 hospitalisations and 5000 deaths occurs annually as a results of consumption of contaminated food. *Salmonella* is estimated to cause 300-400 000 cases of illnesses (DeRezende *et al.*, 2001). In developing countries, it is estimated that 80% of all diseases are transmitted through water (Safe Water Systems, 1996). The cost per country is 6.9 billion for medical services, lost productivity and premature death because of water-borne diseases (Buzby, 2002). In addition, it has been suggested that the annual number of food-borne and water-borne disease outbreaks will double (De Roever, 1999).

The availability of safe, clean water is also a serious problem for a large number of the South African population particularly in rural areas. It is estimated that more than 12 million South Africans do not have access to an adequate supply of potable water (Genthe and Seager, 1996).

2.6.3. Factors contributing to outbreaks of food-borne diseases

Industries responsible for ensuring safe drinking water supplies and food are experiencing problems with several emerging and re-emerging pathogens. The various factors contributing to the emergence and re-emergence of microbial pathogens are: an increasing number of people that are susceptible to potential infectious pathogens, people with compromised immune systems, due to Human Immunodeficiency Virus (HIV) infection, immunosuppressive therapy, and elderly or children whose immune systems are not as active as healthy young adults (Du Preez, 2001; Prier and Solnic, 2000). Since these persons have weak immune responses they are subject to infection that would not likely occur in healthy adults or if they do occur, may be less severe.

Emerging and re-emerging water-borne and food-borne diseases are also caused by changes in human demographics, food preferences, food production and distribution systems, lack of support through public health resources and infrastructure (Grabow, 1996). According to Meng and Doyle (2002), the epidemiology of microbial food-borne

illnesses has changed because of the increase in disease susceptible populations, changes in lifestyle that include adventurous eating, more convenience foods, less time devoted to food preparation, emerging newly recognised microbial pathogens and ever-evolving technologies for food production, processing and distribution. Some of the food-borne diseases have been found to occur after eating restaurant or canteen food as well as prepared commercially available food (Schlundt, 2002).

2.7. Detection of pathogens

Pathogenic micro-organisms which occurred in food, on fresh fruit and vegetables and in water need to be isolated, identified and tested for pathogenicity. Various methods can be used which include selective media, selective culturing methods and molecular based techniques such as polymerase chain reaction (PCR) and DNA hybridization, biochemical reactions, API and serological tests such as immunofluorescence (Theron, 2001).

2.8. Conclusion

Water is being used for various purposes such as aquaculture, recreational, industry, agriculture and domestic purpose. It is becoming a scarce resource and is often highly contaminated with both chemicals and microbes. Water used for purposes of direct or indirect consumption must be good quality since contaminated water can cause diseases.

Currently there is a growing awareness of the indirect impact of contaminated produce on human health. The presence and number of micro-organism differ between produce, production practice and geographical areas. Poor water quality used for irrigation or in processing plants during washing can contaminate food. Water used in the production or food processing plant must therefore be monitored regularly. Environmental conditions and handling of produce after harvesting can also greatly influence the microbial ecosystem on the surface of fresh fruit and vegetables (Burnett and Beuchat, 2000). Incorporation of good agricultural practices (GAP), including the use of potable water for

irrigation, composting of manure and in sanitation facilities such as toilets and running water in production areas can therefore help in reducing produce contamination levels. Hazard analysis and critical control point (HACCP) may further help reducing product contamination.

High levels of food-borne infections due to consumption of fresh produce at home might be increased by the lack of knowledge in terms of produce handling and hygienic practices. Therefore, there is a general need for consumer awareness and education in terms of general hygiene practices as well as handling of food products at home. General food safety programmes can successfully be achieved through the full integration of food safety principles from farm to fork with interdisciplinary collaboration linking production sites, retailers and consumers and promoting good general hygienic practices (Schludt, 2002).

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CHAPTER 3: FARMING AND HOUSEHOLD HYGIENE PRACTICES IN HAMMANSKRAAL INFORMAL SETTLEMENT, GAUTENG PROVINCE

Abstract

A survey was conducted by means of a questionnaire to assess practices of small-scale vegetable growers' and to determine the impact of contaminating produce. The consumption level of fresh produce and knowledge of hygiene practices in informal settlements was also determined in a similar way. The results indicated that some farming practices, may contribute to food safety risks. This includes the use of raw crown manure on crops and untreated surfaces water. Furthermore, it was found that consumption of various fresh fruit and vegetables is common practice among the informal settlements. General hygiene knowledge and practices were found to be good despite limitations in restricted water supplies. People were using municipal tap water, but were complaining about the quality. Furthermore, insignificant low numbers of residents reported regularly suffering from gastroenteritis diseases such as diarrhoea and stomach pains.

3.1. Introduction

Research showed that diets, which are low in fat and high in fiber, are protective against many cancers and lessen the risk of coronary heart disease (De Roever, 1999). As a result there is a considerable recent increase in the consumption of fresh fruit and vegetables through out the world (Wessinger *et al*., 2000). On the other hand, the increase in consumption of fresh produce is followed by an increase in associated diseases. As a result, fresh produce has been implicated as a vehicle for food-borne pathoge (Saddik *et al*., 1985). Major outbreaks of human diseases have been associated with common food-borne pathogens. It has been found that fresh produce can become contaminated with food-borne pathogens while being cultivated in the field, during harvest, post-harvest handling, processing or distribution (Burnett and Beuchat 2001; Beuchat and Ryu, 1997; Beuchat, 1996).

According to Jones, (1992), most consumers are not concerned about microbial contamination of food. The way in which consumers handle fresh produce has an effect on its shelf life and also contributes to food-borne illnesses (De Roever, 1999). Viswanathan and Kaur (2001), found that vendors contaminate salads during slicing, chopping and hand mixing. As a result, it is documented that pathogenic intestinal diseases are often transferred through food handlers in the kitchen (Curtis *et al*., 2003). This could mean that gastro-intestinal infection may arise from unhygienic practices at home.

Applications of irrigation water to crops can expose the produce to direct contact with potentially contaminated water increasing the risk of disease. Spray irrigation is more likely to increase contamination than drip or flood irrigation (De Roever, 1999). Therefore, if such irrigation systems are used, care should be taken that water is not contaminated, resulting in negative health effects in humans (Morrison *et al*., 2001). Research shows that vegetables harvested from soils exposed to animal waste or irrigated with sewage-contaminated water will result in enteric pathogen transmission (Saddik *et al*., 1985; Beuchat, 2002). Furthermore, the use of manure has been considered as one of the major factors that can result in pre-harvest contamination of produce with human pathogens (De Roever, 1999).

The aim of this study was to survey farming practices along the Apies river and determine the level of food safety knowledge and hygienic practices in Hammanskraal households. The surveys were also used to assess possible sources of produce contamination both on farms and in the homes of informal settlements.

3.2. Materials and Methods

3.2.1. Household handling of fresh vegetables, fruit and hygiene practices

Study area and design: The study was conducted at Hammanskraal (Themba). Eighty households were randomly selected for this study and one member of each household was interviewed.

Data collection: A second questionnaire (Appendix 2) was compiled which covered hygiene practices, socio-economic factors, and consumption patterns of fresh raw vegetables and fruit. As stated before the questionnaire was interpreted to the people using their main local language (mostly Tswana).

Ethical consideration: Prior to use, the questionnaire was submitted to the ethical committee of the University of Pretoria for clearance and the statistician at the Agricultural Research Council, Ms Marie Smith Department of Biometry. The purpose and confidentiality of information of the questionnaire was first explained to interviewees. Furthermore, the importance of this study and benefits for the community as a whole were also explained.

Data analysis: All data were analysed by use of frequency tables.

3.2.2. Agricultural farming practices

Study area and design: The study was conducted along the Apies river from Wonderboom to Hammanskraal about 55 km north of Pretoria. Forty-nine individuals farming alongside the Apies river were interviewed. From each farm one person (owner or worker) was interviewed.

Data collection: A questionnaire (Appendix 1) was designed to obtain information concerning production practices, hygiene standards, knowledge of sanitation and environmental factors. All participants were interviewed in their local language (Tswana, Pedi, Ndebele etc).

Ethical consideration: A Similar approach of first submitting the questionnaire to the ethical committee of the University of pretoria and explaining the purpose and confidentiality to interviewees were followed.

Data analysis: All data were analysed as described before

3.3. Results

3.3.1. Household questionnaires

3.3.1.1. Age

A summary of the age distribution of the people that were interviewed during the study is reflected in table 3.1. The largest group of respondents was aged between 20 and 30.

Table 3.1 age distribution of people who were interviewed

Age (Years)	Respondents (%)
11-20	8.75
21-30	28.75
31-40	25.0
41-50	12.5
51-60	10.0
>60	15.0

3.3.1.2. Socio-economic factors

The majority of people interviewed were Tswana (55%). Other ethnic groups include Ndebele (18%) and Pedi (15%). Smaller groups (12%) were a mix of Tsonga, Venda, Zulu, Swazi and Xhoza. Out of the interviewed group, 43% were homeowners and 59% of these were Tswana. In addition, most interviewed people were unemployed (77.5%) and of the employed group, the majority of them earned less than R1000 per month (Table 3.2).

Table 3.2 summary of income distribution of people interviewed in the Hamanskraal area

Income in Rand	Respondents (%)
Unemployed	77.5
100-1000	18.5
1000.-4000	3.0
>4000	1.0

3.3.1.3. Consumption patterns of fresh vegetables and fruits

All people interviewed consumed fresh raw vegetables and fruit, either on a daily or weekly basis (46%) while 49% consumed them on monthly, and 5% on yearly basis.

3.3.1.4. Hygiene practices

Most of the interviewed people (76%) said they always washed their vegetables and fruit, while 23% responded that they frequently washed their produce before consumption. Although respondents stated that they washed their produce before consumption, 52% of

them did not use running water. The majority of respondents (64%) always washed their hands before handling food and 23% frequently did so, while the rest (13%) hardly ever followed basic hygiene practices. Furthermore, 80% washed their hands more than four times a day, which were mostly in the morning when they woke up, after working with dirty items, after visiting toilet facilities, before working with food and before and after eating (Table 3.9). However, 59 % of those who washed their hands more than four times per day, did not use soap in the washing process.

Table 3.3 Number of times respondents washed their hands per day

Occasions	Respondents (%)
2	2.5
3	2.5
4	15.0
>4	80.0

3.3.1.5. Produce accessibility

Most of the interviewed people (50%) obtained their produce from either a local supermarket or street vendor, while 43% got it from supermarkets and only 7% were buying produce from the street market. The majority of people (96%) stored their produce at home, of which most of them (93%) stored it in an electric refrigerator.

3.3.1.6. Source of water and uses

The majority of people interviewed (99%) obtained water from the local Municipal tap in the area. However, most of them were complaining about the quality of the water in terms of colour, sediments and turbidity. From those who used tap water, about 60% stored it indoors. Although the local people complained about the quality of water, 82% of the interviewed people did not treat their water at home. Most of them stored water,

particularly in closed plastic containers, for one to two days. The reason for storage was to allow the sediments in the water to settle prior to usage. In the case of cleaning the containers, 88% did so with water and soap before refilling the containers.

Table 3.4 General volumes of water usages per household on daily basis in Hammanskraal

Quantity per household	Household (%)
0-50l	23.7
51-100l	70.0
100-150l	6.0

Table 3.5 Number of days that water was stored in household in Hammanskraal

Days	Respondents (%)
1-2	41.0
3-4	7.0
5-6	2.0
7-8	6.0
>8	4.0
Not applicable	40.0

3.3.1.7. Health

The majority of respondents (61%) had no complaints of stomach pains. In addition, most of the respondent's children (84%) did not experience runny stomachs or diarrhoea.

3.3.2. Farming practices questionnaires

3.3.2.1 Farm owners

The majority of respondents (61%) were farmers of which 76% owned the land they cultivated. From the interviewed people, 55% were working on farms smaller than 10ha.

3.3.2.2. Commodities

Most of the interviewees (53%) were producing less than three crops on their land per season. From the respondents, 53% were experiencing plant diseases problems with their crops. Among these farmers, 35% knew the names of most of the diseases that affected their crops. Furthermore, 82% of respondents have experienced insect problems on their crop, of which 70% were familiar with the local (common) names of the pest.

3.3.2.3. Rainfall, water sources and irrigation systems used

The region receives relatively low rainfall from September until February (typical summer rainfall). Either the Apies river or Bon Accord dam were their only source of water for their crops during this period. As a result, growers irrigated their crops at various intervals. Most of the interviewees (68%) used water from either the Apies river or Bonacord dam for irrigation, applied with sprinkler systems (Table 3.6), while the rest (32%) use tap or borehole water.

3.3.2.4. Use of manure

Only 43% of the people interviewed were using crown manure for fertilisation and 48% used manure untreated. They indicated that they got crown manure from the livestock farmers around the Hammanskraal. All respondents used commercial fertiliser with the majority (79%) applying different types mainly NPK.

Table 3.6 Different types of irrigation systems used by vegetable farmers in the Hammanskraal area

Type	Respondents (%)
Furrow	34.7
Sprinkler	51.0
Flood	10.2
Drip	4.1

3.3.2.5. Chemical storage and withholding periods

In terms of pest and disease control, 98% of respondents used chemicals to control pests and diseases. Of these, 82% always used scales to measure the chemicals prior to mixing and applying to the crop (Table 3.7). It was found that most farmers were applying different kinds of chemicals depending on the pest. When asked how do they know which chemical to apply for a specific pest they indicated that there are some extension officers from the department of Agriculture who advice them on what chemical to apply. Depending on the chemicals used, most respondents harvested their crop a week after their last spray (Table 3.8). Fifty five percent of the interviewees used tap and borehole water for their spray mixes. Furthermore, 55% of respondents were using a spray bottle or knapsack to apply their chemicals. With regard to storage, 39% of the farmers were storing their chemicals in a storeroom and another 39% were storing them in a shed on their farm, while the rest were keeping them at home.

Table 3.7 methods used to measure chemicals for pest and disease control

Instruments	Respondents (%)
Tin	2.0
Cup	16.0
Commercial scale	82.0

Table 3.8 withholding period practices by small scale vegetable farmers at Hammanskraal

Time	Respondents (%)
Within a week	18.4
After one week	24.5
After two weeks	24.5
After three weeks	14.3
After more than three weeks	18.4

3.4. Discussion

From this study it was found that consumption of fresh fruit and vegetables such as lettuce and cabbages were high. The reason could be that most of the people were unemployed and grow these produce for own consumption. Although some of them where producing for own consumption, they supplemented through buying from different places such as streets market and supermarkets. Some studies have shown a considerable recent increase in consumption of fresh produce in many parts of the world (Viswanathan and Kaur, 2001; Wessinsinger *et al*., 2000). De Roever (1999) indicated that the increased consumption patterns of fresh produce have been followed by an increase in produce-associated diseases. In contrast, in this study the number of people complaining about food-borne related diseases was relative low. This could be because most of the people were resistance to illnesses associated to fresh produce. The other reason might because of good hygiene practices such as washing produce before consumption. Furthermore, the other reason might be that some cooked most of their fresh vegetables before consumption and seldom eat fresh salads.

Although most people had access to municipal water and local municipal authorities have water purification plants and were responsible to manage water quality, local residents in Hammanskraal still complained about the quality and consistency of supplies. The reason

and cause for poor water quality was not assessed. In SA, it has been estimated that more than 12million people do not have access to adequate supplies of potable water and about 21million lack basic sanitation facilities (Genthe and Seager, 1996). As a result many people were storing their water in closed plastic containers prior to use for various reasons such as to allow the sediments to settle and for future use since the supply were not consistence. Jagals *et al*. (1999), found that during storage the water deteriorated to a quality often unsuitable for human consumption. Furthermore, he indicated that deterioration differed from poor container hygiene, individual manner of water handling and within open containers due to pollution from the environment. Although most of the respondents washed their plastic containers prior to re-filling, Momba and Kaleni (2002) found that plastic base materials supported more attachment of indicator micro-organism than metal based ones.

Most of the growers were producing their vegetables under dry land conditions. The source of irrigation water was mostly from either the Apies river or Bonacord dam. Furthermore, the majority used sprinkler irrigation systems. It is common in developing countries, to use untreated irrigation water (Okafo *et al*., 2003). Such water is often contaminated and may pose a food safety risk if crops are irrigated with. South African surface water resources are under increasing threat of pollution (Venter *et al*., 1997). In addition, Westcott (1997) found that water from ponds, wells, streams and drains did not meet the general standard for irrigation of produce consumed raw.

In this study, it was found that a low percentage of vegetable growers use crown manure to fertilise their soil. Some of the crown manure was applied untreated. According to Geldreich (1970), crown manure used on crops in many parts of the world is sources of hazardous bacterial and viral pathogens even parasites of enteric origin. As a result, in countries were animal waste is used as fertiliser, it is expected that vegetables harvested from these fields will be contaminated with enteric pathogens (Saddik *et al*., 1985). Further more, it was observed that most of the farms do not have sanitation facilities such as toilets and washing basins.

In this study it was found that there were some production practices used that are known to contribute to produce contamination. Such practices include use of untreated polluted water for irrigation and application of untreated manure. Furthermore, the water is being applied through sprinkler irrigation systems on produce that is more commonly consumed raw. It was found that the production practices used in the study area were generally not good while the people living in the region mostly consume these vegetables raw. The level of food-borne related diseases reported was found very low. This could be because the household hygiene practices were generally good in the study area. The low number of people experiencing stomach pain and children encountering running stomachs might be caused by poor water quality or it might be from low percentage number of people that doesn't wash their produce before consumption. This study found that there is an urgent need for quality and quantity water supply in this area.

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CHAPTER 4: MICROBIAL QUALITY OF FRESH VEGETABLES AND WATER FROM THE APIES RIVER USED FOR IRRIGATION

Abstract

The microbial quality of Apies river water used for irrigation of fresh produce in the Tshwane metropolitan area was monitored. In addition, the microbiological quality of vegetables was assessed to determine safeness for human consumption. The water source studied ie. Apies river were mostly sampled at nine points for bacterial and viral water-borne pathogens. Faecal coliform, *E. coli* and coliphages tests were included as indicators of pollution and to determine the microbiological quality of the water. Samples of vegetables, cultivated on three farms alongside the Apies river, were also collected on a monthly basis for microbial analysis. Surface washings were plated on various selective media to test for the presence of food-borne bacteria. In addition part of the surface wash was tested for human pathogenic viruses. The results showed that the water at all sampling points was highly contaminated with faecal coliform and *E. coli.* The high microbial counts were higher than the acceptable water quality standard. Indicator organisms (coliphages) suggesting the presence of viruses were also detected in the water. Furthermore, potentially pathogenic human viruses such as adenoviruses, astrovirus, hepatitis A virus and rotavirus were detected in the water sources and on the vegetable surfaces.

4.1. Introduction

Man has been irrigating his crops to facilitate maximum production of food supplies over centuries. Approximately 40% of the world's food supply is currently produced on irrigated land (Kirby *et al*., 2003). In South Africa, 1.2 million hectors of agricultural land has been developed for farming and is currently under irrigation (DWAF, 1993). However, there is no source of water intended for human use that can be assumed to be free from pollutants (The Microbiology of Water, 1994). This is mainly due to water sources having different qualities, influenced by natural or human induced pollution.

World-wide, water resources are continuously deteriorating as a result of increased population pressures, human settlement near these sources and industrial development.

Microbial human pathogens transmitted by water are considered as water-borne (Theron, 2001). Disease outbreaks occur due to water-borne pathogens through direct consumption of contaminated water or indirectly through consumption of contaminated fresh produce. Water has been identified as one of the main sources of faecal contamination of fresh produce (De Roever, 1999). The use of contaminated waste water can result in the introduction of food-borne pathogens in the food chain (De Roever, 1999; Croci et *al*., 2002). Cholera outbreak in Jerusalem in 1970 was attributed to consumption of fresh vegetables such as lettuce and cucumber, irrigated with raw wastewater (Fattal *et al*., 2002).

The hypothesis of this investigation was that contaminated irrigation water could also contaminate fresh vegetables produced by small scale growers. The main aim of this study was therefore to determine the microbiological quality of irrigation water and the presence of pathogens on vegetable surfaces that has been irrigated with water from the Appies river.

4.2. Materials and methods

4.2.1. Study site and design of the experiment

The study was conducted at various points along the Apies river near Wonderboom, Lusthof and Hammanskraal where small-scale vegetable growers were utilising the water directly for irrigation. Three small scale growers producing vegetable crops were randomly selected along the Apies river. Water samples and leafy vegetables (cabbages and lettuce) were randomly collected monthly and analysed for microbiological contamination.

4.2.2. Water

4.2.2.1. Water sample collection for bacterial analysis

From March 2003 until February 2004, water samples were collected monthly from nine sampling points along the Apies river for microbiological analyses. Samples were collected where the river enters the farm boundaries, at the point where water was withdrawn for irrigation and just after the farm boundaries. Water samples were taken aseptically in 100ml disinfected plastic bottles. Each water sample was collected by immersing the plastic bottle into the river and filling it to the top. Labelled bottles were packed in a cooler box and transported on ice to the Plant Pathology Laboratories, Faculty of Natural and Agricultural Science, University of Pretoria for bacterial analysis.

4.2.2.2. Bacterial analyses of water

Microbiological analyses were conducted within 6 h after samples were collected. To determine the microbiological quality of water, faecal coliform and *E. coli* tests were used as indicator organisms. Microbiological tests were conducted using a modification of the SANAS accredited ISO 17025 technique (PPL 006) of the Plant Pathology Laboratories. Laboratory tests were done using the Colilert-18, IDEXX Laboratories, Inc. According to manufacturer's instructions, one ampule of Colilert-18 was added to 100 ml water sample and allowed to dissolve. The water was carefully poured into a 98-well quanti tray, which was then placed in a 98 well rubber mould and sealed with a quanti tray sealer. Trays were incubated at 37°C for 18-22h. A colour change to yellow after incubation was regarded as a positive faecal coliform reaction. Positive reactions were further checked under fluorescence light to confirm the presence of *E. coli*. The most probable number of coliforms and *E. coli* were determined using the standard table for the Colilert test.

4.2.2.3. Spot water sample collection for bacterial analysis

From January 2004 until March 2004, water samples were collected once per month from three sampling points along the Apies river for specific bacteria analyses. Samples were collected at irrigation water points as described before (4.2.2.2). Water samples were taken aseptically in 1ℓ sterile bottles as described in 4.2.2.1 and transported to the Plant Pathology Laboratories for bacterial analysis.

4.2.2.4. Bacterial analysis of spot water sample

Spot water samples were serially diluted before plated on different selective media (i.e. MaConkey (*E. coli*), TBS (*Vibrio cholera*), XLD (*Salmonella* spp) and Listeria agar (*Listeria* spp). The plates were then incubated at 36^0C for 24 to 48 hours. Pure cultures were prepared by streaking isolates on nutrient agar (NA), incubated as described above. Further identification were conducted by using API 20E, Omnimed (PTY) LTD. RSA.

4.2.2.5. Water sample collection for virus analysis

Four separate samples were collected with sterile plastic bottles at the point where farmers withdraw water for irrigation. At each sampling point, two 5ℓ and 1ℓ samples were collected for virus and coliphages tests, respectively. Before sampling, the bottles were rinsed at the water source. After sampling the bottles were sealed, labelled and transported to the Medical Virology Department, Faculty of Health Sciences, University of Pretoria for viral and coliphage analysis.

4.2.2.6. Virus analyses of water

Viruses were recovered from water samples using a glass wool adsorption elution technique (Grabow & Taylor, 1993). Virus analyses was performed using published methods, for Hepatitis A virus (Taylor, 1997; Taylor *et al*., 2001), astroviruses (Marx *et al*., 1998;

Taylor *et al*., 2001), adenoviruses (Van Heerden *et al*., 2003), and rotaviruses (Van Zyl *et al*., 2004) and enteroviruses (Vivier *et al*., 2004).

4.2.3. Vegetables

4.2.3.1. Vegetable sample collection

Vegetable samples (lettuce and cabbage) were collected from three vegetable producers along the Apies river near (Wonderboom, Lusthof and Hammanskraal). On each farm, three vegetable samples were collected. The samples were collected monthly between 2003and 2004. Each sample was placed in a plastic bag, labelled put in a cooler box and transported to the Plant Pathology Laboratory for immediate analyses.

4.2.3.2. Bacterial analysis of vegetables

The outer leaves of the cabbage and lettuce were aseptically removed and one gram of leaf tissues was then washed with 100ml of ¼ strength ringer's solution. Vegetable wash were serially diluted before plating on different selective media, MaCconkey, TBS, XLD and Listeria agar. The plates were then incubated at 36^0C for 24 to 48 hours. The pure cultures were prepared by streaking isolates on nutrient agar. The plates were incubated as described before in 4.2.2.2.

4.2.3.3. Viral Analyses of vegetables

A 50 g vegetable sample was placed in a sterile plastic bag with 25 ml phosphate buffer, pH 7.4. The bag was shaken manually to wash off the viruses from vegetable surfaces. Washed samples were immediately taken to the Department of Medical Virology for virus analyses. The samples were tested for bacteriophages, as they are proposed indicators of virus presence in water sources (Erb *et al*., 1995). The vegetable washed sample were further tested for enteric viruses as described in 4.2.2.6.

4.3. Data analysis

Analysis of variance (ANOVA) was used to test for differences between the three farms (A,B, and C) and the three samples taken before the farm, at the point where the farmer normally withdraws water for irrigation and after the boundaries of each farm. Treatment means were separated using Fishers protected t-test least significant difference (LSD) at the 1% level of significance (Snedecor and Cochran, 1980) for greater discriminatory power. Data were analysed using the statistical program Genstat (2000).

4.4. Results

4.4.1. Bacterial indicators in the water

The data was acceptable normal distributed, with heterogeneous treatment variances (fig.1.). There was no significant difference of faecal coliforms between farm A and B sampling points. Although there was a slight increase in faecal coliform numbers at farm A, were water is taken for irrigation and after the farm boundaries, there was no significant difference. Faecal coliforms numbers were generally higher before and after farm A and B boundaries. Sampling point before farm C boundary and where the farmer withdraw water for irrigation where significantly lower than Sampling point before the farm boundary and sampling point after the farm boundary A. At farm C, before sampling point and where the farmer withdraw water for irrigation were significant different with that of Farm A and B. The sampling point after the farm boundary shows no difference compared to farm A and B. The sampling points after the farm boundary were mostly contaminated (Fig. 1).

The most probable numbers of *E.coli* were higher at the sampling point before the farm boundaries, lower at the point water extraction but higher again after the farm boundaries. It can be seen that from farm A sampling point before the farm boundaries the pollution were higher decreasing at farm B and continues to decrease at farm C (fig. 2). The

contamination decreases with the flow of the river. From all the farms contamination were high at sampling point after farm boundaries.

The faecal coliform and *E. coli* levels were higher between November till March and decreased between April to September (Fig. 3 and 4). In figure 4 contamination levels for *E. coli* were higher from November till February and decreased between March till July while the contamination was relatively lower between August to October.

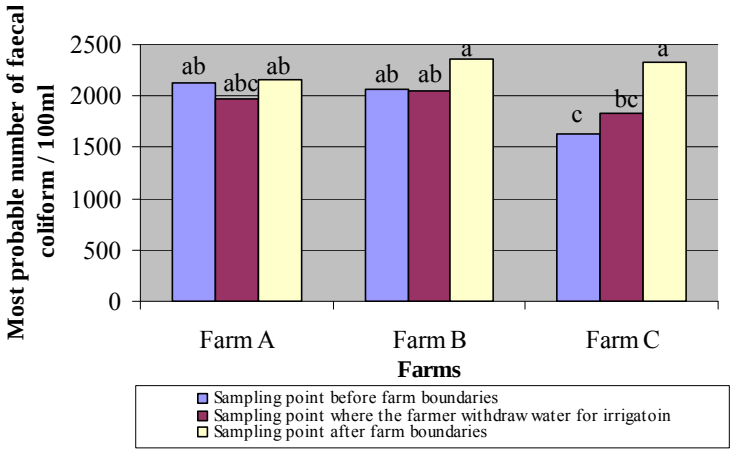

Fig.1 Most probable number of faecal coliform per 100ml in water samples collected along the Apies river. Different letters above bars indicate significant differences at a 1% level of significance.

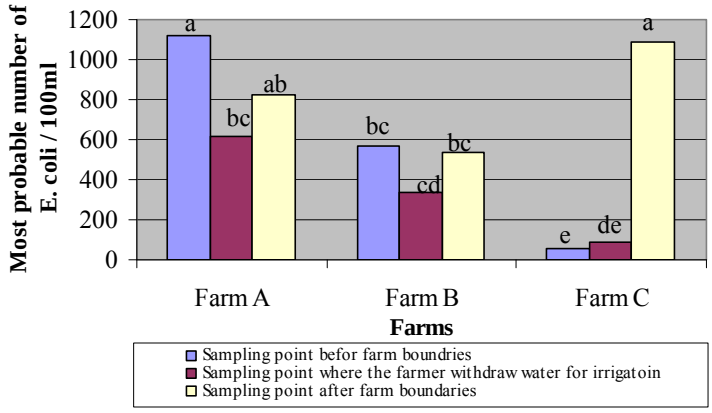

Fig.2 Most probable number of *Escherichia coli* per 100ml along the Apies. Different letters above bars indicate significant differences at 1% level of significance.

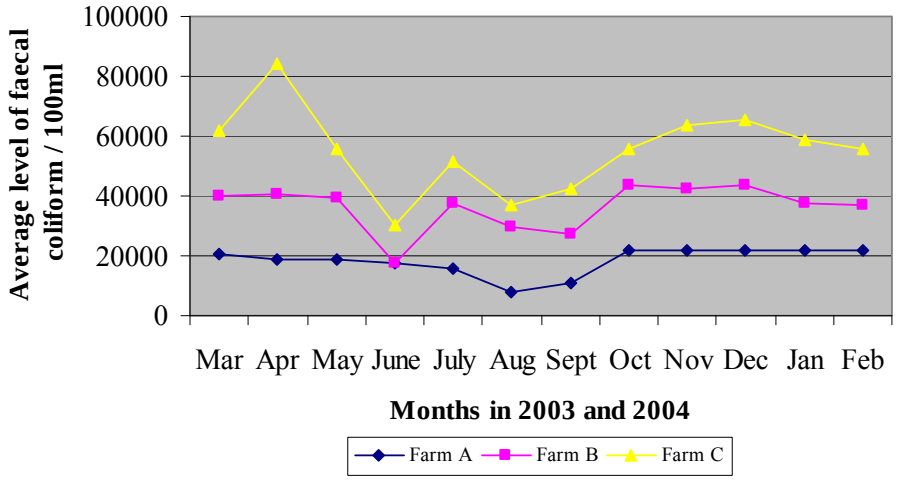

Fig. 3 Most probable numbers of faecal coliforms per 100 ml along the Apies river over a period of 12 months from March 2003-February 2004.

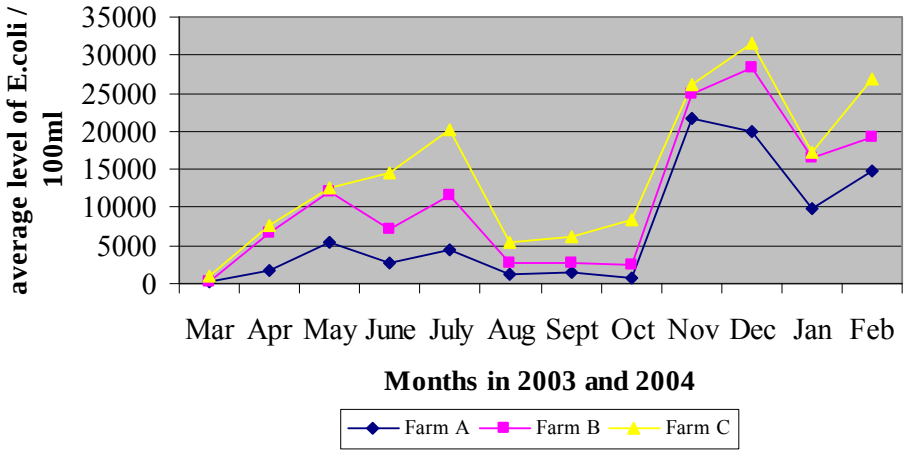

Fig. 4 Most probable number of *Escherichia coli* per 100 ml along the Apies river over a period of 12 months from March 2003 till February 2004.

4.4.2. Specific bacteria detected in spot water analyses and on vegetables surfaces

Specific human pathogens tested on the surface of vegetables and in the spot water samples could not be identified with API 20E, Omnimed (PTY) LTD. RSA.

4.4.3. Viruses detected in water and on vegetables surfaces

Viruses were detected in the water samples on numerous occasions. Viruses such as enterovirus were detected in both water and vegetable matching samples. Hepatitia A virus (HAV) and astroviruses were not detected in either water or vegetable samples. Coliphages (Somatic and F-RNA) were found in most of the water samples.

Table 4.1 Sumarry of viral pathogens and coliphages tested from the Apies river Water and on the surface of vegetables

Organisms		**Water**	**Vegetables**
Phages	Somatic	+	
	F-RNA	+	
Viruses	Adeno	+	_
	Astro	_	_
	Entero	+	+
	HAV	_	_
	Rota	+	_

4.5. Discussion

The Apies river water used for irrigation was found highly polluted with faecal coliform bacteria and *E. coli*. The water quality from all three farms investigated was below the national standard set by DWAF for irrigation water for crops to be consumed raw. The level of pollution was more than 1000 cfu/100ml, while the recommended standard by DWAF is less than 1000 coliforms per 100ml (DWAF, 1993). In addition, some specific microbial human pathogens (viruses) were also detected. Brackett (1999) and Lingeng *et al*. (2004), indicated that irrigation water can be a source of pathogens that can ultimately contaminate agricultural products. Other studies have showed that Bacteria such as *Vibrio cholera*, *Listeria*, *Salmonella* and *Shigella* spp are mostly associated and have been

isolated from fresh produce such as vegetables. This is more likely in situation like this, where the producers are using sprinkler irrigation. Irrigation water should be potable, because the presence of human pathogens in irrigation water can be a serious public health concern (Lemerchand and Lebaron, 2003).

In this study, it was found that there was a slight decrease in the level of contamination from Wonderboom, Lusthof and Hammanskraal but it was not significant. This is in agreement with Venter *et al*. (1997) who indicated that microbial die-off does not play a significant role in the improvement of microbial water quality. High level of contamination at the sampling point after farms boundaries might be due to farming practices e.g. use of crown manures as fertilisers.

Although the quality of irrigation water was highly contaminated with faecal coliform and *E.coli*, bacterial isolates tested in the water and on the vegetables surfaces could not be positively identified. The bacterial isolates were gram negative-rod but could not be positively identified using API 20E. It could be that the water was the source of contamination of vegetable, since both isolates from water and vegetables were gram-negative rods but could not be identified with API 20E.

Coliphages are bacterial viruses which infect and replicate in *E. coli* and may infect related coliform bacteria. Furthermore, they are subdivided into somatic and male specific coliphages (Department of Water Affairs and Forestry, 1993). Their presence in water intended for domestic use indicates the presence of viruses, particularly enteric viruses (Grabow, 1991). Somatic coliphages and F-RNA coliphages were detected in selected samples indicating that viruses may be present. Adenoviruses were detected in irrigation water, and their presence may be of major concern for human health. These viruses are considered a public health risk, since they are associated with clinical manifestations (Van Heerden *et al*., 2003). It has been documented that adenoviruses account for 5-20% of US hospitalisation for diarrhoea, mainly in children below the age of two years (Carter, 2005). Enteroviruses were detected in both water and on the

vegetable surfaces. They are ubiquitous agents, mostly inducing sub-clinical infections. However several of them are associated with significant diseases in man resulting in diarrhoea, vomiting, hepatitis or meningitis (Carter, 2005). Another group of viruses detected was the rotavirus. The presence of rotaviruses in the water could pose a potential risk for consumers. Rotaviruses are indicated as the cause of water-borne outbreaks particularly in developing countries (Jaykus, 1997). World-wide, rotaviruses affect all children in the first few years of life representing 80% of recognised viral aetiologies and 140 million cases of diarrhoea per year (Ashbolt, 2004). Water borne rotavirus infections in developed world are usually linked to post treatment contamination of clean water (Carter, 2005). The last group of viruses tested in this study belonged to the astroviruses. Since they are excreted in human faeces, they have the potential to become water pollutants (Taylor *et al*., 2001). It is interesting that human astrovirus (HAstVs) were not detected in the water samples and on the vegetables samples surface. This virus survives in aquatic environments (Barnes and Taylor, 2004). Although Kurtz (1994) indicated that the transmission of HAstVs via food is rare, it is still one of the major viruses associated with food-borne outbreaks. It was identified as a causative agent in one of the largest food-borne outbreaks in Osaka Japan (Oishi *et al*., 1994).

Taylor *et al*. (2001) have indicated that hepatitis A is hyper-endemic in South Africa. Although HAV were not detected, Taylor (1997) found HAV in selected rivers and dams of South Africa. In addition, HAV is globally associated with contaminated water and food (Grabow, 1976). It has been reported that its infection is fairly high in various countries (Croci *et al*., 2002).

Specific human pathogenic viruses were detected in the water used for irrigation and on the surfaces of vegetables. Because some of the viruses detected in the water were also detected on the surfaces of vegetables, it implies that the source of vegetable contamination was irrigation water. It is advisable to wash the produce with potable water before consumption, since they were contaminated with human viruses. Failure to do so

may results in food borne outbreaks. This is because most of disease outbreaks are being associated with consumption of contaminated fresh raw vegetables or fruit.

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CHAPTER 5: GENERAL DISCUSSIONS

Globally there is rapid increase in water pollution levels particularly in high density areas where the increased population put major pressures on scarce water resources. Growing numbers of informal settlements along national water ways particularly in developing countries have contributed to this problem. This resulted in an increased in number of water-borne and food-borne diseases, caused by various bacteria, viruses and protozoa parasites. Added to this, industrialisation with its growing demand for water and resultant pollution there of. It is inevitable that water quality is under severe pressure. The impact of water quality adversely affects agriculture that requires safe and sustainable water resource for food production.

There are various ways to monitor microbiological water quality and different chemical tests exist that can be used to determine levels of pollution. Microbiological contamination indicator organism such as colliphages, faecal coliform and coliphages that gives an indication of the level of microbial pollution and presence of pathogenic organisms in water are routinely used. Water treatment process prevents the build up of pathogens and subsequent transmission of waterborne diseases. Various treatment methods may be used such as coagulation-flocculation, sedimentation and chemical disinfection. Managing water quality can therefore be achieved and assured by using water quality guidelines and standards set by the Department of Water Affairs and Forestry.

Consumption of fresh fruits and vegetables improves the health status of human beings. On the other hand, increased consumption of fresh produce has been found to be related to the increased number of food-borne disease outbreaks. Food-borne disease outbreaks occurs through consumption of food contaminated with microbial human pathogens. Food contamination may take place by various means at several points in the farm to the fork chain. This includes pre harvest and post harvest practices, distribution and retail. There are known sources of produce contamination such as untreated irrigation water, raw crown manure, unhygienic human handling of food during picking, packing and distribution and unsanitary environments. Inappropriate unhygienic handling of food and incorrect storage at

cool temperatures may contribute in outbreaks of food-borne diseases. Lack of good quality water supplies may lead to produce contamination and ultimately affect the health of the end user.

In this study informal settlement were surveyed by means of a questionnaire to determine consumption levels of fresh produce and knowledge of hygiene practices in informal settlements. Furthermore, small scale vegetable producers along the Apies river were also surveyed to assess production practices and likely hood and impact of contamination of produce.

In a preliminary survey done in this study, it was found that consumption of fresh fruit and vegetables were high in informal settlements. The quality of domestic water used within these informal settlements was not consistent throughout the year and turbidity and sediments was prevalent in most situations. Water storage is a common practice in informal settlements where regular water supplies were absent. The reported diseases incidences associated with water were perceived to be relatively low in informal settlements. At a household level the produce handling and hygiene practices were reportedly good. From the interview it was found that the number of complains regarding stomach pains and children experiencing diarrhoea were very low.

Vegetables were found to be produced under dry land conditions. The growers were irrigating their crops directly with water from the Apies river. Most of the farmers interviewed in this study were using sprinkler irrigation and raw crown manure as fertilisers.

The Apies river water was found to be highly contaminated with faecal coliform and *E. coli* through out the year. The level of contamination was above the recommended level by the Department of Water Affairs and Forestry standards. Specific human pathogenic viruses were detected in the water, Adeno virus, Entero virus and Rota virus. Further more, one of these viral pathogens were also detected on the surfaces of vegetables i.e. Entero virus. Bacteria detected in water samples and on the surfaces of vegetable could not be positively identified with API 20E. Effective methods for detection of bacteria in the water and on the

vegetable surfaces should in future be evaluated. Parasitic protozoa parasites should also be tested and monitored on vegetable and in irrigation waters to get a true picture of the level of risk. Furthermore, chemical residues and heavy metal pollution should also be monitored as part of a holistic approach to water quality management.

This preliminary study found that the quality of the Apies river water was not fit for human consumption, fluctuated in levels of pollution, was not suitable for irrigation of crops particularly ones to be consumed raw. This study confirmed the general need to improve the quality and supply of water in informal settlements of Gauteng.

Appendix 1

Urban small-scale growers

Water used in Agriculture: Questionnaire 2003

Compiled by the University of Pretoria- Department of Microbiology and Plant Pathology

READ THE FOLLOWING QUESTIONS AND CIRCLE THE MOST APPROPRIATE ANSWER TO EACH QUESTION

Name:…….

Farm area:……

1. What position do you have in the farm?

Owner	Manager	Worker	Other

2. Do you own the land?

Yes	No

3. When do you have your most rainfall?

Spring	Summer	Autumn	Winter

4. Approximately how many hectares are you working on?

<1	1-5	6-10	>10

5. Which are the main crops you farming with?

Tomatoes	Cabbage	Lettuce	Carrots	Spinach	Others (state)

5.1 What are your secondary crops, if any?

Tomatoes	Cabbage	Lettuce	Carrots	Spinach	Others (state)

5.2. Do you have problems with diseases on your crops?

Yes	No

7. If yes, do you know the name of the diseases?

Yes	No

8. Do you have problems with insects?

Yes	No

9. If yes, do you know the names of insects?

Yes	No

10. Do you manage the diseases and pests on your crops?

Yes	No

11. If yes, how do you manage them?

Chemical spray	Organic spray	Nothing	Other

12. Do you use chemical sprays?

Yes	No

13. Where do you store your chemicals?

Home	Shed	Store room	Others

14. How do you mix your sprays?

By hand	With a stick	Others

15. How do you measure your chemicals for spraying?

With a tin	With a cup	With a scale	Other

16. What water do you use to mix your sprays?

Dam	River	Tap	Bore hole	Canal	0ther

17. What do you spray your crops with?

Nap sack	Spray bottle	Spray car	Other

18. After how long from your last spray do you harvest?

Within a week	One week	Two weeks	Three weeks	Four weeks	Five weeks

19. Do you use protective clothing when you spray your crops?

Yes	No

20. Do you use protective masks for your face when you spray your crops?

Yes	No

21. Do you wash your hands before harvesting?

Yes	No

22. Do you irrigate your crops?

Yes	No

23. How many days between irrigation

1-2 days	3-4 days	5-6 days	7-8 days	9-10 days

24. What is your source of irrigation water?

Dam	River	Tap	Bore hole	Canal

25. What is the name of your river or dam?

Appies river	Ritvlei river	Bonacord dam	Others

26. Do you treat your water?

Yes	No

27. What types of irrigation systems do you use?

Furrow	Sprinkler	Flood	Drip	Bucket	Other

28. List other different sources of water you use.

Dam	River	Tap	Bore hole	Other

29. Do you fertilise your crops with manure?

Yes	No

30. Do you compost the manure?

Yes	No

31. How is your manure?

Fresh	Old

32. If it is old, how old is your manure?

1-2 Months	3-4 Months	5-6 Months	7-8 Months	9-10 Months	11-12 months

33. If it fresh how fresh is it?

One week	Two weeks	Three weeks	Four weeks

34. Do you use fertilisers?

Yes	No

35. Which type of fertiliser do you use?

NPK	LAN	Lime	Gypsum	Other

Please note this information will be kept confidential and will be used only for research purposes

Thank you for your time and effort. It is appreciated.

Itani Tshivhandekano

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Appendix 2

Urban Households

Water used in Households: Questionnaire 2003

Compiled by the University of Pretoria- Department of
Microbiology and Plant Pathology

READ THE FOLLOWING QUESTIONS AND CIRCLE THE MOST APPROPRIATE ANSWER TO EACH QUESTION

Name:…….

Area/suburb:……

1. Are you a homeowner?

Yes	No

2. What is your gender?

Female	Male

3. What is your age?

1 - 10 yrs	11 - 20 yrs	21 - 30 yrs	31 - 40yrs	41 - 50 yrs	51 - 60 yrs	Above 60

4. What is your race or cultural group?

Venda	Zulu	Tzonga	Pedi	Sotho	Xhoza	Tswana	Ndebele	Others

5. What is roughly your monthly income?

Unemployed	R0 - R1000	R1000 – R4000	R>4000

6. Do you consume fresh fruits?

Always	Frequently	Hardly	Never	Not applicable

7. Do you consume fresh fruit and vegetables?

Daily	Weekly	Monthly	Yearly	Never

8. Which vegetables do you mostly consume?

Potato	Tomato	Salads	Herbs	Onions
Carrots	Beans	Cabbage	Lettuce	Other

9. Which fruits do you mostly consume?

Apples	Peas	Mango	Banana	Avocado
Peaches	Grapes	Guava	Orange	Papaya

10. Do you wash your fruits / vegetables before you eat?

Always	Frequently	Hardly	Never

11. Do you wash Fruit and vegetables with running water?

Yes	No

12. Do you wash your hands before you handle fruits / vegetables?

Always	Frequently	Hardly	Never	Not applicable

13. Do you thoroughly wash your hands with soap before working with food?

Yes	No

14. When do you wash your hands?

Before and after eating	After visiting toilet facilities	Before working with food	Early in the morning	After working with items which are dirty

15. Do you wash your hands thoroughly with soap before eating?

Yes	No

16. Do you have a nailbrush?

Yes	No

17. Do you brush under your nails while washing your hands?

Yes	No

18. Where do you get your main supply of fruits and vegetables?

From a farm	Super markets	Street market	Own cultivation	Other

19. Do you store fruits and vegetables at home?

Yes	No

20. Do you store fruits and vegetables in the refrigerator at home?

Yes	No

21. From where do you obtain the main supply of water that you use at home?

River	Wells	Dam	Tap	Bore hole

22. Do you drink your river, wells, dam, and bore holes water?

Yes	No

23. Do you store your water?

Yes	No

24. Where do you store your water?

In doors	Out doors

25. In what kind of containers do you store water?

Open plastic	Open metal	Closed plastic	Closed metal	Others

26. If yes, for how long do you store water in containers?

1-2 Days	3-4 Days	5-6 Days	7-8 Days	9-10 Days

27. Do you clean the containers?

Yes	No

28. How often do you clean your containers?

When refilling container	Within a week	After a week	After 2 weeks	After 3 weeks	After a month	After > one month

29. What do you clean your containers with?

Water	Water +soap	Other

30. Do you treat the water?

Yes	No

31. How do you treat you water?

Boil	Jik	None	Others

32. Roughly how many liters of water do you use daily?

10-50 L	50-100L	100-150L	>150L

33. Do you experience some stomach pains?

Yes	No

34. How often?

Daily	Weekly	Monthly	Yearly	Never

35. Do your children experience running stomach?

Yes	No

36. How often?

Daily	Weekly	Monthly	Yearly	Never

Please note this information will be kept confidential and will be used for research purposes only

Thank you for your time and effort. It is appreciated.

Itani Tshivhandekano

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