

University of Zimbabwe Faculty of Engineering Department of Civil Engineering

RISK ANALYSIS OF WASTEWATER USE IN CROP PRODUCTION: A CASE OF GLEN VALLEY IRRIGATION SCHEME, BOTSWANA

By

YAONE MONYAMANE

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In collaboration with

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YAONE MONYAMANE

Supervisors

ENG. ZVIKOMBORERO HOKO DR. ROUMIANA HRANOVA

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DECLARATION

I, **Yaone Monyamane**, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Integrated Water Resources Management (IWRM) in the University of Zimbabwe. It has not been submitted before for any degree of examination in any other University.

Date: _____

Signature: _____

The findings, interpretations and conclusions expressed in this study do neither reflect the views of the University of Zimbabwe, Department of Civil Engineering nor of the individual members of the MSc Examination Committee, nor of their respective employers.

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DEDICATION

To Eurico Macuacua, Patrick Mulengera, Thulebona Precious Masuku and Rose Elisamon Mero for always being there for me.

> Mabogo dinku a a thebana. Le ka moso betsho!!!!!!!!

ABSTRACT

The Glen Valley Irrigation Scheme uses treated sewage effluent for crop production, however the potential public health risks associated with wastewater use is of major concern to workers and consumers. This research was conducted in Glen Valley in Gaborone, Botswana from February to May 2011. The study aimed at analyzing the health risks of wastewater use for crop production in the Glen Valley Irrigation Scheme.

Samples of effluent, soil and vegetables (spinach, tomatoes, and green pepper) were collected from critical points and analyzed for selected quality parameters of health significance following recommendations by the American Public Health Association (effluent), Food and Agriculture Organisation (soil) and ATSDR (vegetables). Effluent samples were collected 7 times from the filter and 3 times from the drip pipe. There were 3 soil sampling campaigns from the spinach, green pepper and tomato plots. Vegetables were collected from each of the abovementioned plots on 3 sampling campaigns.

The mean values of pH were 9.09 ± 0.15 for effluent and were within the FAO range of 6.5–8.5 for irrigation. The electrical conductivity ranged from 710 µS/cm to 760 µS/cm which was less than the guideline value of 2000 µS/cm. For effluent, cadmium and lead concentrations were lower than the 0.01 mg/l and 0.05 mg/l respectively which was within the long-term threshold limit for irrigation. Total coliforms in the effluent were within the WHO limit of 1000 CFU/100 ml ranging between 0 and 470 CFU/100ml. The pH values from all soil samples were above the FAO recommended limit of 6.5. No pathogens were detected in vegetables, but coliforms were detected. Heavy metals were detected in all the vegetables but cadmium in green pepper exceeded minimal risk levels by ATSDR.

It can be concluded that at present the health risks for the consumption of the vegetables is low except for cadmium in green pepper which is above the recommended Minimum Risk Levels. Therefore it is recommended that regular monitoring of the effluent, soil and vegetables be done to protect the health of workers and consumers.

Keywords: Cadmium; Effluent re-use; Health risks; Irrigation; Soil; Vegetables

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

APHA	American Public Health Association
ATSDR	Agency for Toxic Substances and Disease Registry
CEC	Cation Exchange Capacity
DDI	Daily Dietary Intake
DWA	Department of Water Affairs (Botswana)
DWAF	Department of Water Affairs and Forestry (South Africa)
FAO	Food Agricultural Organisation
GoB	Government of Botswana
GVIS	Glen Valley Irrigation Scheme
MRLs	Minimal Risk Levels
MoA	Ministry of Agriculture
MFDP	Ministry of Finance and Development Planning
NAFTEC	National Food Technology Research Center
NAMPAADD	National Master Plan for Arable Agriculture and Dairy Development
SAR	Sodium Adsorption Ratio
SMEC	Snowy Mountains Engineering Corporation
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WUC	Water Utilities Corporation
WWTP	Wastewater Treatment Plant
ZINWA	Zimbabwe National Water Authority

Symbols

Cd	Cadmium
CFU/100ml	Colony Forming Units per 100 ml
Р	Pula (Botswana currency)
Pb	Lead

DEFINITION OF TERMS FOR WASTEWATER USE

Beneficial uses: many ways in which water can be used either directly by people or for their overall benefit *e.g.* municipal water supply, navigation, agricultural and industrial application

Direct potable reuse: involves the incorporation of reclaimed wastewater directly into a potable water supply system, often implying blending of reclaimed water

Direct reuse: the use of reclaimed wastewater that has been transported from a wastewater reclamation plant to the water reuse site without intervening discharge to a natural body of water *e.g.* agricultural and landscape irrigation

Potable water reuse: is a direct or indirect augmentation of drinking water with reclaimed wastewater that is normally highly treated to protect public health

Reclaimed wastewater: is wastewater that as a result of wastewater reclamation, is suitable for a direct beneficial use or a controlled use that would not otherwise occur.

Wastewater reclamation: is the treatment or processing of wastewater to make it reusable. The term is often used to include delivery of reclaimed wastewater to its place of use and its actual use.

Wastewater recycling: is the use of wastewater that is captured and redirected back into the same water-use scheme. Recycling is practiced predominantly in industries such as manufacturing, and it normally involves one industry or one user.

Wastewater reuse: is the use of treated wastewater, for a beneficial use such as agricultural irrigation or industrial cooling.

Source: (Tchobanoglous and Burton, 1991)

CHAPTER 1 INTRODUCTION

1.1 Introduction

Globally, freshwater resources available for use are limited which has increased competition between uses and users (Gleick, 1998). To deal with this increased competition some people have turned to re-using wastewater which is a much cheaper alternative to freshwater. Other benefits of re-using wastewater are the conservation of freshwater resources, recovery of nutrients (nitrogen, potassium, and phosphorus). Re-use of wastewater reduces pollution load in rivers and also offers a reliable water supply throughout the year (FAO, 2010). Some of the re-uses of wastewater are for toilet flushing, cooling in industries, artificial recharge of aquifers, landscaping, irrigation and fire protection (Rowe and Abdel-Magid, 1995). The re-use of wastewater for non-potable use requires that a separate supply line be established (dual supply system) to prevent cross-contamination of potable water. Re-use in agriculture is receiving renewed attention due to increasing scarcity of freshwater resources for food production (Scott *et al.*, 2005).

In many arid and semi-arid countries, water is becoming an increasingly scarce resource and people are forced to consider other sources of water which might be used economically and effectively to promote further development (Feizi, 2001). Globally, agriculture is the largest single user of water at 70% according to (Gleick, 1998) with irrigated agriculture expanding from 50 million hectares at the turn of the century (1900) to 280 million hectares at the close of the century (Gleick, 1998). At the same time, with population expanding at a high rate, (Al-Lahham *et al.*, 2003) established that the need for increased food production is apparent. The potential for irrigation to raise both agricultural productivity and the living standards of the rural poor has long been recognized in arid regions of the Middle East, where countries like Jordan, Iran and Israel have been using treated wastewater for agricultural purposes (Ammary, 2007). Isfahan city in Iran was the first city in Iran to establish plants for the treatment of wastewater, and to introduce the use of treated water for irrigated agriculture (Feizi, 2001). In Spain where water resources are unevenly distributed, (Manas *et al.*, 2009) observed that wastewater has been used to grow lettuce, unlike in countries like Portugal and France where wastewater is not a first choice for agriculture.

In Africa, (Khouri *et al.*, 1990) stated that South Africa had used 70 Mm³/year of reclaimed wastewater for landscaping; representing 16% of the volume of sewage generated in 1998 and Tunisia had 75% of sewage re-used for irrigation in 1987. Mutengu *et al.* (2007) found that Bulawayo, being a semi-arid area, experiences frequent droughts and so farmers use treated wastewater to grow covo (*Brassica oleracea* variety, *acephala*) sugar beans (*Glycene max*) and maize (*Zea mays*). In Botswana, treated wastewater is used to irrigate lucerne (scientific name) in Lobatse, golf courses in Gaborone, Jwaneng and Orapa (SMEC *et al.*, 2003). The Glen Valley irrigation project was established by the Government of Botswana to irrigate 203 ha of farmland with treated effluent (SMEC *et al.*, 2003). The crops grown under the scheme are green pepper, okra, cucumber, sweet pepper, butternuts and olives using drip irrigation system, some of which are very sensitive to high salinity levels.

According to Gleick (1998), irrigated agriculture occupies approximately 17 percent of the world's total arable land but the production from this land comprises about 34 percent of the world total. The use of treated municipal wastewater alleviates surface water pollution problems but this wastewater also contains a variety of potentially toxic elements such as Arsenic (As), Lead (Pb), Cadmium (Cd), Chromium (Cr) and Copper (Cu) which are harmful to human health (Wise *et al.*, 2000). Localized irrigation practices (*e.g.* drip irrigation) use effluent efficiently and protect workers health as effluent is not carried as aerosols which workers might inhale. USEPA (2002) established that the drip irrigation system reduces chances of crop contamination but it requires effluent of high quality to prevent clogging of emitters and may require regular flushing. Most treated wastewaters are not very saline but a salinity level below 700 μ S/cm can achieve full yield potential with most crops (Pescod, 1992). Highly saline water with an electrical conductivity of more than 3,000 μ S/cm may not be used for sensitive crops like okra, maize, sorghum, spinach and pepper unless regular leaching is done (Pescod, 1992). This would be more expensive than choosing a more tolerant crop where there will be little or no leaching requirements.

1.2 Background

In the year 2010, the Glen Valley Wastewater Treatment Plant (WWTP) was treating a volume of 55, 000 m³ per day of sewage from the capital city, Gaborone and its satellite towns using the activated sludge process. However, the WWTP was designed to treat a volume of 40 000 m³ of sewage per day according to phase 1 of its design (Mguni, 2010). Since November 2010, the treatment plant has been undergoing an upgrade so that it can treat 65 000 m³ per day but in a few years this capacity will not be adequate. So expansion of the treatment plant will be required within a few years due to rapid expansion of the city and the satellite towns. Satellite towns around the capital are expanding due to shortage of land and accommodation in Gaborone, one such place is the Phakalane suburbs whose development has been phased thus making the planning and implementation of sewerage services more complex. The urban planners and the treatment plant operators poorly coordinate their activities such that the pace of property development is faster than the pace of developing the appropriate sewerage infrastructure. In phase 2 of the expansion of the treatment plant, the capacity will be increased to 90, 000 m³ by 2016 (MFDP, 2009).

The Glen Valley Irrigation Scheme was started in 2003 to use treated wastewater from the Glen Valley treatment plant to irrigate crops in the Glen Valley farms. The government initiated this project as a way of diversifying the economy by expanding the agricultural base which is dominated by pastoral farming (Agriculture, 2008). Botswana being a semi-arid country, the provision of drinking water and water for agricultural production is becoming more difficult and expensive so this project also addresses issues of water demand management (Emongor and Ramolemana, 2004). The project is guided by the National Master Plan for Arable Agriculture and Dairy Development (NAMPAADD) which began in 2002 (MoA, 2002). The farmers produce olives (*Olera europaea*), maize (*Zea mays*), spinach (*Spinacea oleracea*), tomatoes (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*) which are then sold to the supermarkets in Gaborone and the adjacent areas. Public health concerns are centered on pathogenic organisms (viruses, bacteria and protozoa) which can cause danger to people who irrigate (farm workers) through the inhalation of effluent aerosols and to the consumers of the produce thereof (Kirkham, 1999). Madyiwa *et al.* (2003) suggest that Lead (Pb) and Cadmium (Cd) in

wastewater irrigated soils are taken up by plants from the soil and accumulate in the plants, thereby making the plants potential sources of contamination for human and animals.

1.3 Problem statement

Although wastewater use in irrigation poses a risk to the consumers (WHO, 2005), it appears that previous studies have estimated health risks based on total metal concentrations in the vegetables (Banerjee *et al.*, 2011) instead of the amount that is actually consumed per person per day. Ingestion of a pathogen/contaminant does not necessarily result in infection or an illness but it depends on variation in infectivity of individual pathogens, dosage ingested, virulence of different contaminants as well as variation in susceptibility of a population (Forsythe, 2002).

1.4 Objectives

1.4.1 Main objective

To assess the risk of wastewater use for crop production at Glen Valley Irrigation Scheme, in terms of coliforms and heavy metals

1.4.2 Specific objectives

1) To characterize the treated effluent and irrigated soils in Glen Valley in terms of coliforms and heavy metals

2) To determine the presence and levels of coliforms and heavy metals in different crops from Glen Valley Irrigation Scheme

3) To establish the health risk level based on pathogens and heavy metals levels present in vegetables compared to Minimal Risk Levels (MRLs)

1.5 Justification

The study seeks to assess the risk to public health of consuming the produce due to transmission of harmful substances to consumers. The produce from the irrigation scheme is sold to supermarkets in Gaborone and surrounding areas. If there is a public health risk due to the consumption of vegetables, a population of about 200 000 people, which is 10% of national population (CSO, 2009) would be affected.

1.6 Scope of the study and limitations

The study looked at aspects of production of vegetable crops irrigated with treated wastewater and their health implications. Due to high cost of testing for heavy metals, 7 sampling campaigns for effluent, 3 for soil and 3 for crops were conducted for the study. The malfunctioning of equipment did not allow for testing of coliform levels in soil.

CHAPTER 2 LITERATURE REVIEW

2.1 Wastewater re-use

The use of urban wastewater in agriculture is an old practice which is receiving renewed attention due to the increasing scarcity of freshwater resources (Scott *et al.*, 2005). It is noted by Kirkham (1999) that the rising demands for water by agricultural, industrial and domestic sector has resulted in increased competition for limited freshwater resources available for use, which is estimated to be about 1% of the total water on our planet. Water needs and withdrawals were projected to grow in the future so new interventions would be required to bridge the shortfall. Rowe and Abdel-Magid (1995) states that the shortfall in supply of freshwater is sometimes covered by re-using wastewater for toilet flushing, cooling in industries, artificial recharge of aquifers, landscaping and fire protection.

Agriculture has become water intensive according to the types of cash crops grown for a growing global population which is estimated at over 6 billion (Battilani *et al.*, 2010). According to Gleick (1998), agriculture being the largest single user of water at 70% of total available freshwater is largely inefficient with only 40% of water supplied being ultimately used to grow crops. Mutengu *et al.* (2007)suggest that the use of urban wastewater for crop production is receiving increased attention in most parts of the world due to the increasing scarcity and high cost of fresh water resources and food especially in semi-arid and arid regions.

2.2 Country examples

Jordan, which is a semi-arid country, suffers from shortages in water supply for domestic, industrial, and agricultural purposes (Ammary, 2007). The limited water supplies available require careful management for successful agricultural production especially in semi-arid countries where competition for freshwater is high and priority is usually given to domestic use (Al-Lahham *et al.*, 2003). In a study by Mutengu *et al.* (2007), it is noted that in Tunisia and South Africa, reclaimed wastewater represented 75 % and 16% of sewage respectively.

In the same study by Mutengu *et al.*, (2007), the authors state that due to frequent droughts in Bulawayo, Zimbabwe some farmers have resorted to the use of treated wastewater for irrigation of crops. On plots of about 500 m² each, farmers grow covo (*Brassica oleracea* variety, *acephala*) sugar beans (*Glycene max*) and maize (*Zea mays*) for subsistence and commercial purposes. Maize is classified by Pescod (1992) as a crop which is moderately sensitive to high salinity levels and sensitive to exchangeable sodium so it is not advisable to grow it with poor quality effluent. The best success story in the region is that of Windhoek which meets one third of daily potable water demand (21, 000m³) from treated effluent and has been reclaiming potable water since 1968 (Haarhoff and van der Merwe, 1996).

Soil Salinity Class	ConductivityofSaturationExtract (dS/m)	Effect on Crop Plants
Non saline	0-2	Salinity effects negligible
Slightly saline	2-4	Yields of sensitive crops may be restricted
Moderately saline	4-8	Yields of many crops are restricted
Strongly saline	8-16	Only tolerant crops yield satisfactorily
Very strongly saline	>16	Only a few very tolerant crops yield satisfactorily

Table 2.1Soil salinity classes and effects on crop growth

Source: Pescod (1992)

2.3 Wastewater re-use in Botswana

In Botswana, SMEC *et al.* (2003) and Emongor *et al.* (2005) state that wastewater re-use is practiced for irrigation of golf courses in Phakalane, Jwaneng and Orapa, for lucerne (*Medicago falcate*) in Lobatse, vegetables in Glen Valley and orchards and vegetable gardens at Serowe and Kanye Prisons respectively. At the Glen Valley Irrigation Scheme treated effluent is used to grow vegetables under 203 ha of farmland for supply to supermarkets in Gaborone. In response to anticipated water shortages for some countries in the region, countries are now planning to reclaim wastewater for potable use. Botswana is

planning to supply potable water from reclaimed wastewater to Gaborone using wastewater from Glen Valley Treatment Plant starting from the year 2013 drawing from experiences in Windhoek and London (WUC, 2010).

2.4 Standards for wastewater use in irrigation

According to WHO (2005), municipal wastewater contains a number of potentially toxic elements such as Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Zinc, etc. Even if toxic materials are not present in concentrations likely to affect humans from the point of view of health, a very important consideration in agricultural use of wastewater are the pathogenic micro- and macro-organisms.

Element	^a South Africa	^b Zimbabwe (ST)	^b Zimbabwe (LT)	°USEPA (ST)	°USEPA (LT)
Cd	0-1.0	0.05	0.01	0.05	0.01
Со	0-0.05	-	-	5.0	0.05
Al	0-0.15	-	-	20.0	5.0
Fe	0-0.5	-	-	20.0	5.0
Pb	0-0.2	20.0	5.0	10.0	5.0
Zn	0-0.1	-	-	10.0	2.0

Table 2.2 Irrigation water guidelines for selected heavy metals (mg/l) in different countries

^aSource: Department of Water Affairs and Forestry (1996)

^b Source: Zimbabwe National Water Authority (2000)

^c Source: United States Environmental Protection Agency (1992)

LT-Long term irrigation, ST- Short term irrigation

According to Manas *et al* (2009) pathogenic bacteria is present in wastewater at much lower levels than the coliform group of bacteria, which are much easier to identify and enumerate as total coliforms (TC/100ml). *Escherichia coli* are the most widely adopted indicator of faecal pollution and they can also be isolated with their numbers usually being

given in the form of faecal coliforms (CFU)/100 ml of wastewater (Tchobanoglous and Burton, 1991). Table 2.1 shows the different irrigation water guidelines for different countries. According to USEPA (2002), it recommends a limit of 0.01 mg/l and 0.05 mg/l of cadmium in water for short-term and long-term use respectively as it can be toxic at higher levels (see appendix B). It also recommends a limit of 5 mg/l and 10 mg/l of lead in water for short-term and long-term use respectively as it can inhibit plant cell growth at very high concentrations. The USEPA (2002) recommend a pH of 6 for reclaimed water as it affects mobility and toxicity of heavy metals.

2.5 Irrigation with sewage effluent

Battilani *et al.* (2010) note that disposal of effluent into streams is done with the assumption that the water will be sufficiently diluted by the receiving waters and therefore enhance its quality before it is used (self purification of the river). Public health concerns are centered on pathogenic organisms (viruses, bacteria and protozoa) which can cause danger to people who irrigate (farm workers) through the inhalation of effluent aerosols and to the consumers of the produce thereof (Kirkham, 1999). Agronomic aspects of wastewater irrigation, deal with effect of sewage effluent on yield of crop quantity, quality, time of harvest, accumulation of heavy metals and toxic substances in the irrigated soil (Emongor *et al.*, 2005). In a soil analysis study by Dikinya and Areola (2009) of the effluent irrigated plots in Glen Valley, Nickel and Copper were higher than the FAO guidelines. In crops, Mercury and Cadmium are highest in soils under maize and decline linearly from maize to spinach, to olive, tomato then control site. The study was inconclusive as the soils are naturally higher in some of these heavy metals so variations in results may be due to different management measures and different uptake rates by different crops.

2.6 Heavy metal uptake by crops

The level of trace elements in treated sewage effluents is determined by the chemical properties of the raw sewage from which these effluents were derived and the treatment method used (Emongor and Ramolemana, 2004). Secondary sewage treatment reduces the trace element content through the settling of suspended solids by up to 70%. According to

Pescod (1992) notes that many sewage effluents are suitable for long term irrigation with the threshold for trace elements based on the most sensitive crops. In a study by Mapanda *et al.*(2007) to determine heavy metal uptake by leafy vegetables irrigated with wastewater, Cadmium intake rates were above their recommended minimum risk levels (MRLs) while Cu, Ni, Cr and Pb had daily intakes above 40% of their MRLs so consumers' health was at risk in the long term. Trace elements are taken up by plants and tend to accumulate in plant tissues at different rates as shown in Table 2.2, but plant properties differ greatly and the effect of soil conditions is often decisive (Scott *et al.*, 2005). According to USEPA (2002), Cadmium is considered a potentially serious health hazard because of its mobility in the food chain and its toxicity to plants and humans.

High uptake	Moderate uptake	Low uptake	Very low uptake
Lettuce	Kale	Cabbage	Snap bean family
Spinach	Collards	Sweet corn	Pea
Chard	Beet	Broccoli	Melon family
Escarole	Turnip	Cauliflower	Tomato
Endive	Radish globes	Brussels sprouts	Pepper
Cress	Mustard	Celery	Eggplant
Turnip greens	Potato	Berry fruits	Fruit trees
Beet greens	Onion		
Carrot			

Table 2.3 Relative accumulation of cadmium into edible parts of crops

Source: Pescod and Arar, (1985)

The incidence of the Itai-Itai disease is associated with the presence of cadmium in the food chain. Some reports have shown that presence of high levels of trace elements in soil may result in a similar situation in plant tissues. In other cases the levels of trace element in crop constituents remained low even though large quantities of wastewater rich with trace elements were added to the soil (Scott *et al.*, 2005). Leafy vegetables have greater potential of accumulating heavy metals in their edible parts than grain or fruit crops (see Table 2.2) because heavy metals are transported passively from roots to shoots through the xylem vessels while fruit crops are dominated by phloem vessels in which heavy metals are poorly mobile (Krijger *et al.*, 1999). According to Zheljazkov and Nielsen (1996), the

concentrations of heavy metals in vegetables per unit dry matter is highest in leaves, then fresh fruits and seeds. So, contamination of the human food chains by heavy metals is not directly affected by the plants' total uptake, but rather by the concentration in those parts that are directly consumed.

2.7 Soil characteristics

In a study by Jung (2008) heavy metal concentrations in leaves were reported as being much higher than those in grain. The author identified factors such as soil pH, cation exchange capacity, organic matter content, texture, crop age and type of crop as those which affect availability of metals in crops and the subsequent occurrence in the crops. Uwimana *et al.* (2010) characterized sludge at Kadahowa water treatment plant where they found the CEC (Cation Exchange Capacity) to be between 28.4-33.3 cmol (+)/kg which can improve the nutrient status and water holding capacity of the soil.

	a
Description	Soil pH
•	-
Strongly acidic	<51
Strongry derdice	0.1
Moderately saidia	5260
widderatery acture	3.2-0.0
Slightly acidic	6.1-6.5
Neutral	66-73
reada	0.0 7.5
Moderately alkaline	7483
Woder atery arkainte	7.4-0.3
	0.7
Strongly alkaline	>8.5

Table 2.4	Classification	of s	oil pH
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Source: Pierzynski et al. (2005)

Soils with a high level of swelling clay and organic matter can have a CEC of more than 30 cmol (+)/kg which is 3 times the desired level of 10 cmol (+)/kg (Pierzynski *et al.*, 2005). The CEC is an indicator of soil fertility which is the ability to supply cations (Ca, Mg, K and Na). According to Wise *et al.* (2000) a high Sodium Absorption Ratio (SAR) in

water restricts permeability of water in soil but this may be offset by high levels of electrical conductivity in the soil and therefore restricts movement of cations in the soil. The solubility and toxicity of heavy metals are affected by pH, with mobility and uptake of heavy metals being reduced under neutral to alkaline conditions (Uwimana *et al.*, 2010) see Table 2.3. In the same study by Uwimana *et al.* (2010), most metals in the sludge occur as weakly mobile, non bio-degradable forms which cannot increase plant growth.

2.8 Microbial quality

Bitton (2005) found that pathogenic organisms (bacteria, viruses, protozoa) are found in raw sewage in various quantities depending on local conditions e.g. population density, lifestyle and season or time of the year. The different treatment processes remove pathogens to varying extents with stabilization ponds removing more pathogens than conventional biological treatment (Pierzynski *et al.*, 2005). Microbiological quality of the effluent determines what crops can be grown in restricted irrigation or if it can be utilised under unrestricted irrigation (see Table 2.4). This can safeguard consumers against likely diseases such as cholera, typhoid or gastroenteritis (Manas *et al.*, 2009).

Irrigation with contaminated water can therefore start a water-soil-plant contamination pathway (Battilani *et al.*, 2010). Insufficient treatment can result in food-borne diseases from protozoans like *Giardia* and *Cryptosporidium*; enteric pathogens could also reside within interiors of vegetables after root uptake (Emongor and Ramolemana, 2004). Enteric bacteria like *Salmonella spp. and Vibrio cholerae* which are pathogenic are classified as medium risk because of the low incidence of excess infection (Pescod, 1992). Other microorganisms found in wastewater are coliforms, Faecal Coliforms, Faecal Streptococci and Clostridium perfringens which are used as indicator organisms for faecal contamination but are not pathogenic (Pescod, 1992). In the USA, the state of Nevada requires a maximum faecal coliform (FC) count of less than 400 FC/100 ml (USEPA, 2002) with only surface irrigation of fruit and nut bearing trees but does not require disinfection. However, Florida requires monitoring of *Giardia* and *Cryptosporidium* (USEPA, 2002). The survival of micro-organisms in the soil is dependent on temperature, moisture levels and the presence of predator organisms (Pierzynski *et al.*, 2005). Bacteria can be retained in soil pores due to

their small size and the attraction to cations due to their negative charge on the surface. The higher the infiltration rate and permeability of water in soils also enhance movement of micro-organisms in the soil (Tarradellas *et al.*, 1997).

Category	Reuse condition	Exposed group	Intestinal nematodes ^b (arithmetic mean no. of eggs per litre ^c	Faecal coliforms (geometric mean no. per 100 ml ^c)	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks ^d	Workers, consumers, public	1	1000 ^d	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
В	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees ^e	Workers	1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal
С	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

Table 2.5 Microbiological quality for wastewater use in agriculture

Source: WHO (2005)

^{*a*} In specific cases, local epidemiological, socio-cultural and environmental factors should be taken into account, and the guidelines modified accordingly.

^b Ascaris and Trichuris species and hookworms.

^c During the irrigation period.

^{*d*} A more stringent guideline (<200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

^e In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

2.9 Effluent irrigation and human health

USEPA (2002) established that the concern about the risks to public health from the greater use of recycled wastewater is a serious obstacle to the greater spread of this practice.

According to FAO (2010) the California guidelines which were the first publications on the re-use of treated effluent have a goal of zero-risk for unrestricted reuse of wastewater for irrigation purposes. During wastewater treatment, coagulation/filtration step followed by chlorination/de-chlorination is used to achieve a zero Fecal Coliform/100 ml limit making the effluent virtually pathogen-free. This technology, referred to as the Title 22 benchmark (FAO, 2010), is considered the yardstick for unrestricted irrigation, against which all other systems are evaluated. Since wastewater quality varies with local conditions, treated effluent may be of a quality which is suitable for irrigation of some crops without requiring any further treatment but as a benchmark it has been useful in creating water quality guidelines which are applicable in all parts of the world.

The highest quality recycled water is achieved by dual membrane (micro-filtration and reverse osmosis) tertiary treatment processes (Aquarec, 2006). However, a more practical approach is to make wastewater treatment "fit-for-purpose", depending on its intended use and the degree of human contact involved. Other factors to be considered include whether the produce is eaten raw or cooked, used for fodder or as a raw material for the manufacturing industry. The FAO and WHO have developed a "Code of Hygienic Practice for Fresh Fruits and Vegetables." whose food-chain approach assesses risks of all aspects of crops from primary production to consumption (FAO, 2010). The use of micro filtration technique would be relatively expensive depending on the returns from produce sales (Battilani *et al.*, 2010) and therefore best suited for high value cash crops which can offset the cost of the technology.

As stated by WHO (2005), organic chemicals present in wastewater at low concentrations and ingestion over prolonged periods would be necessary to produce detrimental effects on human health. This is not likely to occur with irrigation of treated effluent; unless there is cross contamination with potable supplies occurs. Few epidemiological studies have established definitive adverse health impacts attributable to the practice. It appears that in areas of the world where helminthic diseases caused by *Ascaris* and *Trichuris* spp. are endemic in the population (WHO, 2005) and where raw untreated sewage is used to irrigate salad crops and/or vegetables eaten uncooked, transmission of these infections is

likely to occur through the consumption of such crops (Scott *et al.*, 2005). Therefore control measures include the provision and use of protective clothing, the maintenance of high levels of hygiene and immunization against selected infections.

2.10 Approaches to risk analysis

According to Forsythe (2002), risk is a function of probability of an adverse health effect and risk analysis focuses on the severity of that effect. Risk analysis would thus focus on consequences of ingesting a harmful substance be it chemical or microbial and its presence in the whole food chain (from farm to fork). Ingestion of a pathogen does not necessarily result in infection or an illness but it depends on factors of variation in infectivity of individual pathogens as well as variation in susceptibility of a population. For an illness to occur, the infectious agent (virus or bacteria) must be present in the raw or untreated wastewater and survive all the treatment processes applied to it. In addition to this, a person must come into direct or indirect contact with the infectious agent in the wastewater and the agent must be present in sufficient numbers to cause an illness (See appendix D). Therefore, risk of a food-borne disease is the combination of the likelihood of exposure to the pathogen through ingestion and the likelihood that the exposure will result in infection/intoxication and subsequent illness to varying degrees (Forsythe, 2002).

The dose-response relationship attempts to estimate the probability of illness upon exposure to a hazard. The dose-response relationship is a function that provides a link between the dose that is ingested and the response that occurs. The most commonly used models are the beta-Poisson model and the Exponential model (Pescod, 1992). According to Forsythe (2002), bacterial infection data are generally well described using the beta-Poisson model.

Beta-Poisson model

The Beta-Poisson model of risk analysis according to Forsythe (2002) is introduced as follows:

 $P_i = [1 - (1 + N/\beta)]^{\alpha}$ Equation 2.1

Where P_i = probability of infection

N = ingested dosage of pathogen

 α and β are parameters which are specific to the pathogen

The beta-Poisson model is used to describe the dose-response relationship (see Equation 2.1) when assessing low levels of bacterial pathogens by generating a sigmoidal dose-response relationship that assumes no threshold value for infection. Instead, it assumes that there is a small but finite risk that an individual can become infected after exposure to a single cell of bacterial pathogen (single-hit concept).

For chemical risk assessment, Minimal Risk Levels (MRLs) can be used. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used by Agency for Toxic Substances and Disease Registry (ATSDR) health assessors to identify contaminants and their potential health effects (ATSDR, 2003).

CHAPTER 3 STUDY AREA

This chapter gives the detailed description of the study area which includes the geographic location, economic activities, treatment processes and discharge of effluent into Notwane River.

3.1 Background of Glen Valley Irrigation Scheme

Under this scheme, there are 47 different farms, varying in size from 1 to 10 ha being managed by private farmers growing a wide variety of arable crops. A government agency, the National Master Plan for Arable Agriculture and Dairy Development (NAMPAAD) is running a 13 ha farm for demonstration purposes to develop and introduce new technologies (mainly olive and lucerne) to the local farmers. The scheme uses approximately 1500 m³ of wastewater per day to irrigate the crops grown there. In this scheme, about 90 % of the farmers use drip irrigation system while the remaining 10 % using sprinkler irrigation on their farms. Drip irrigation is the recommended method of irrigation for wastewater because unlike the sprinkler system it does not introduce aerosols into the air. Olives, maize, spinach, butternuts, green pepper, tomatoes and okra are some of the crops grown under this scheme. The farming and irrigation is done all year, with planting done under shaded tunnels during the winter season to protect them against frost.

The scheme has created employment for about 100 permanent employees with an additional 100 temporary staff being employed during planting and harvesting. Glen Valley irrigation scheme is an on-going pilot project (2002-2012), so the vegetable production is practiced on an informal basis with no legal backing. The scheme uses treated wastewater to irrigate 203 ha of farmland (Agriculture, 2008) for crop production. In the year 2010/2011, about P4 million (approximately ¹USD 600 000) revenue from vegetable sales of 700 tonnes was obtained from the scheme, therefore supporting local livelihoods for the farmers. The produce from the scheme is sold to supermarkets in Gaborone which cater for a population of about 200 000 people.

¹ Exchange rate US 1 = P6.50

3.2 Location

Botswana is a landlocked country bordered by Namibia to the west, Zambia to the north, Zimbabwe to the north east and South Africa to the south (SMEC *et al.*, 1991), as shown in Fig. 3.1. This study was carried out in the Glen Valley, about 10 km northeast of Gaborone City beside the Notwane River where about 234 ha of cropland are being cultivated with secondary treated wastewater. The farms lie between the Botswana Defence Force camp and the Gaborone sewage ponds between Latitudes 24°35'23.56''S and 24°37'01.14''S and between Longitudes 25°58'43.29''E and 25°5816.74''E (Dikinya and Areola, 2009).

3.3 Climate and geology

Gaborone is very sunny, the hottest temperatures of the year are usually in January of February, maximum temperatures can be as high as 40°C in summer and as low as 0°C in winter from May to July. Gaborone receives rainfall of about 350 mm per year with almost all rain falling from October to April and its incidence is highly variable in both space and time (Khupe, 1996). In Glen Valley, the surface soil texture for most crop farms is characterized as loamy sand to sandy loam but there are also sandy clay loams where the clay fraction is on average over 30 % (Sakuringwa, 2007). Soil pH values indicate the soils to be generally slightly acidic to neutral in reaction (Dikinya and Areola, 2009).

3.4 Demography and socio-economic activities

The population of Gaborone was estimated at 192, 000 based on a 2006 survey and is estimated to be 224, 000 in 2011 at a growth rate of 3.1 % per annum (CSO, 2009). Gaborone is the administrative capital and the most developed city in Botswana. Government ministries and headquarters of financial institutions are located in the city center. Orapa house which is owned by Debswana is in the city centre where diamonds from Orapa and Jwaneng mines are sorted before they are exported to the European market (SMEC *et al.*, 2003). There are some manufacturing industries (chemical and food), brewery and slaughterhouses but most commodities are imported from South Africa.

3.5 Glen Valley wastewater treatment process

The Glen Valley WWTP is owned and operated by the Gaborone City Council to treat wastewater from Gaborone and the adjacent areas using the activated sludge process (Emongor *et al.*, 2005). According to Podile (2005), the wastewater is mostly domestic but it also comes from chemical and institutions with the industrial component being about 25%. The treatment plant was commissioned in 1997, to treat 40 000 m³ per day of sewage from Gaborone and adjacent areas (Nkgebe *et al.*, 2005).

According to Nkgebe *et al.* (2005), the treatment process involves screening, grit removal and settling out of inorganic and organic solids (See appendix A). The wastewater then undergoes secondary treatment using activated sludge process at Glen Valley WWTP. The maturation ponds reduce the concentration of coliforms by starving the micro-organisms to death and by UV (Ultra Violet) radiation so, the retention time (33 days) is sufficient enough to cause pathogen die-off (Emongor and Ramolemana, 2004). Huwa San disinfectant is then added to the effluent at the pumping station for removal of algae and also inactivation of pathogens in wastewater. It does not have any carcinogenic disinfection by-products like Chlorine.

3.5 Treated effluent discharge

Notwane River is the only perennial river in south-eastern Botswana (SMEC *et al.*, 2003) due to effluent discharge from Glen Valley WWTP (Figure 3.1) and is a major tributary to the Limpopo River. The river was transformed from a natural ephemeral stream to permanently flowing effluent-dominated river due to continuous discharge of effluent into the river (Mladenov *et al.*, 2005). In a study by Emongor *et al.* (2005) the authors note that regulation is lacking for indiscriminate use of treated effluent for agriculture. The transformation of Notwane River into a perennial river due to effluent discharge provides downstream private farmers with nutrient enriched water supply, which is reliable and inexpensive.



Figure 3.1 Location of the Glen Valley Irrigation Scheme (Source: Department of Surveys and Mapping)

CHAPTER 4 MATERIALS AND METHODS

This chapter presents the methods used for conducting the research. It also includes criteria for the selection of sampling sites, parameters analysed, time and frequency of sampling and quality assurance steps taken during study.

4.1 Study design

According to Schitz (1995), there are four components of sampling operations *i.e.* parameter selection, location of sampling sites, sampling frequency, sample collection and analysis methods. The component varies according to the objectives of the research project.

4.1.1 Sampling sites

Effluent samples were collected at 2 points in selected farm, at the filter and from drip pipe. The soil and crop samples were taken from the same farm growing tomatoes, spinach and green pepper (see Figure 4.1). The effluent was collected at the filter because it is the first point of entry into the farm into the farm from the maturation ponds. The tomato and green pepper were chosen because they are vegetables which can be eaten either raw or cooked (wastewater-irrigated crops should not be eaten raw). The spinach is a leafy vegetable which is usually not recommended for wastewater irrigation. The green pepper is a vegetable whose fruit is consumed and is usually eaten cooked. The soil samples were collected from the plots where tomato, green pepper and spinach are grown to compare variables in crops to their respective plots.

4.1.2 Selection of parameters

The water quality parameter selection was based on the nature of the wastewater treated by the Glen Valley WWTP. Cadmium and Lead were chosen for this study because they are two of the most prevalent as well as two of the most nephrotoxic metals known to man (Gonick, 2008). Heavy metals (cadmium and lead) are of health concern as they can accumulate in the food chain (USEPA, 2002). Microbiological parameters chosen for this study were Enteropathogenic *E. coli* and *Salmonella spp*. because they are the most common bacterial pathogens in wastewater (USEPA, 2002; Pescod, 1992) which have

caused several outbreaks in the USA and are the most persistent pathogens in the environment (see appendix C). According to the Ministry of Health, these pathogens have been previously detected in Botswana. Other selected parameters were EC, pH, which are important factors in effluent and soils for crop production. SAR was also chosen because it is an important criterion in wastewater-irrigated soils.

4.2 Frequency of sampling

For the physico-chemical parameters (pH, EC, temperature, SAR, salinity) the effluent, soil and crop samples were collected for a period of 12 weeks. The effluent samples were collected almost every week from the 25th February 2011 to 18th March 2011, and then from the 6th April to 20th April 2011. The soil samples were collected once a month because cost of analysis could not allow for more sampling campaigns. The effluent samples were collected between 0900hrs and 1200hrs every fortnight.



Figure 4.1 Sampling points for effluent, soil and vegetables

4.3 Data collection and analysis

Effluent samples taken from 2 sampling points of a selected farm. Samples were collected 7 times from the pipe after filter and 3 times from the drip pipe and tested for pH, temperature, EC, *E. coli*, Cadmium and Lead. Soil samples were collected from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from each plot and tested for pH, SAR, EC, Cd and Pb. vegetables samples taken from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from each plot and analysed for Cd, Pb, Total coliforms, *Salmonella spp.* and Enteropathogenic *E. coli*.

Grab samples of effluent were collected at the 2 sampling points using sterilized glass bottles covered with foil to prevent interference by light for microbiological analysis. The pH, temperature and salinity were measured on-site using a 340i conductivity meter; temperature and pH using an electrical pH meter (see Table 4.1). Samples for metal analysis were collected in clean plastic bottles, acidified in 1.0 ml of concentrated nitric acid (to pH less than 2), digested with nitric acid and analysed using the Inductively Coupled Plasma spectrometry (ICP-MS) at the Glen Valley WWTP Laboratory. *E. coli* concentration was analysed at the Wellfield Laboratory following recommendations by using (APHA, 2001). Samples were filtered on a membrane filter and incubated on mTEC agar for 24 hours.

Composite soil samples were collected at a depth of 0.2 m from the surface using a soil auger and packed into polythene bags. Soil samples were diluted in water at a ratio 1:2.5, then pH was measured using an electrical pH meter. Analysis of heavy metals was done following recommendations by van Reeuwijk (1993) at the Geological Surveys Laboratory. Samples were dissolved in Nitric acid, centrifuged and the supernatant was analyzed for lead with an AAS. Sodium, Magnesium and calcium ions were determined in the laboratory using Flame photometry (Na⁺ and K⁺) and titration (Mg²⁺). Metals were extracted from soil samples using appropriate extraction solutions and put in a centrifuge. The suspension was filtered and metal concentrations determined using Flame AAS.

The concentrations for these ions were used to calculate the SAR using equation 1 (Pierzynski *et al.*, 2005).

SAR = $\frac{Na^{+}}{\sqrt{[Ca^{2+} + Mg^{2+}]/2}}$Equation (1)

Where

SAR is the Sodium Adsorption Ratio

Na⁺ is the concentration of Sodium ions (meq/l),

 \mbox{Ca}^{2+} is the concentration of Calcium ions (meq/l)

 Mg^{2+} is the concentration of Magnesium ions (meq/l).

Table 4.1	Analytical	methods for	different	parameters in	effluent,	soil and	vegetables
	•			1			

	Parameter	Method	Equipment
Soil	рН	Electrometric	pH meter
	SAR	Flame photometry	Flame photometer (Na ⁺ , K ⁺)
			Titration (Mg ²⁺)
	Salinity	Electrical Conductivity	EC meter
	Pb and Cd	Atomic absorption spectrometry	Flame AAS
Сгор	Salmonella spp.	Membrane filtration	Membrane filter
	Enteropathogenic E. coli	Membrane filtration	Membrane filter
	Pb and Cd	Dry Ashing	Flame AAS
Effluent	рН	Electrometric	Electronic pH meter
	E. coli	Plate count method	Agar media and 0.24 mm filter membrane
	Salinity and temperature	Electrical Conductivity	EC meter
	Pb and Cd	Mass spectrometry	Inductively Coupled Plasma-MS

Composite samples of vegetables were collected from the field, then packed into polythene bags, placed in a cooler box and taken to the laboratory for analysis. This sampling method is the most representative method as it accounts for horizontal and vertical variations. Determination of Enteropathogenic *E. coli* and *Salmonella spp.* was done at the National

Food Laboratory according to FAO (1994). A 50g sample of vegetable was blended with 450ml Butterfield's phosphate buffer, diluted and filtered on membrane filter and incubated. The analysis of heavy metal metals in vegetables was done following recommendations by FAO/WHO (1999) method AOAC 999.11 at NAFTEC (National Food Technology Research Center) Laboratory. The vegetable samples were dried, ashed in an oven. Hydrochloric acid was added to residue and evaporated to dryness, nitric acid was added and the analysed using Flame AAS.

4.4 Data interpretation

For the physico-chemical parameters (pH, EC and SAR) data interpretation was done by comparing with the WHO guidelines and FAO guidelines for wastewater re-use in agriculture (Pescod, 1992). The Percent Rank (% Rank) function in Excel was used to determine what percentage of the results was meeting the standards or guidelines. For Microbiological analysis of vegetables the Food Standards Australia and New Zealand (December 2010): Guidelines for the Microbiological Examination of Ready-to-Eat foods were used. The Codex (Joint FAO/WHO Food Standards) was used for chemical analysis of vegetables to compare the results with the recommended levels safe for human consumption. Risk analysis index of transfer factor (TF) were used. TF compares heavy metal concentration in the crops to the heavy metal content in the irrigated soil. Minimal Risk Levels (MRLs) by ATSDR were compared to Daily Dietary Intake (DDI) levels to determine risk as a result of consumption of the vegetables using equation 2 (Mapanda *et al.*, 2007).

- DDI = Vegetable consumption rate (kg/day)*Metal conc. in vegetable (mg/kg)Equation (2)
- WhereDDI- Daily dietary intake (mg/day)Vegetable consumption rate (kg/day)Metal concentration in vegetable (mg/kg)

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Effluent quality

This section on effluent quality is based on results of effluent samples taken from 2 sampling points of a selected farm (Figure 4.1). Samples were collected 7 times from the pipe after filter and 3 times from the drip pipe and tested for pH, temperature, salinity, *E.coli*, Cadmium and Lead (see appendix E).

5.1.1 Effluent pH and temperature

Figure 5.1 shows the pH recorded from the effluent samples.



Figure 5.1 pH levels in the effluent

The effluent pH range was between 8.91 and 9.29, at an average of 9.09 ± 0.15 at the filter with average temperature of 22.8° C. A pH range of 8.64-9.2 was measured with an average of 8.93 ± 0.28 at the drip and an average temperature of 22.3° C. All pH values are above the 6.5-8.4 range (Figure 5.1) recommended by Pescod (1992).The high pH values are due to presence of algal blooms in the maturation ponds (Emongor *et al.*, 2005). The results from this study show similar mean pH values to those found by Emongor *et al.* (2005) of 9.08 in Glen Valley, but higher than 6.33 found by Usman and Ghallab (2006) in Aswan,

Egypt. The high pH of effluent makes it unsuitable for irrigation as it may cause nutritional imbalance in soil. The high pH lowers the bioavailability of some nutrients in the soil so plant uptake of these nutrients will be low while other nutrients will be taken up by plants.

5.1.2 Salinity of effluent

Figure 5.2 shows electrical conductivity (EC) levels in the effluent samples collected for the study.



Figure 5.2 Electrical conductivity levels in effluent

Electrical conductivity values had a range between 710 μ S/cm and 760 μ S/cm at an average of 735 μ S/cm at the filter (Figure 5.2). At the drip, values between 700-730 μ S/cm were measured, with a mean value of 713 μ S/cm. These values were within the recommended limit of 2,000 μ S/cm (Pescod and Arar, 1985) but cannot achieve full yield potential and regular leaching may have to be done. Most treated wastewaters are not very saline but a salinity level less than 700 μ S/cm can achieve full yield potential with most crops (Pescod, 1992). The results from this study are higher than the 510 μ S/cm value found by Emongor *et al.* (2005) at the Glen Valley WWTP but lower than the 1100 μ S/cm found in Aswan, Egypt. The values obtained in the study within the normal range for

wastewaters of 700-3000 μ S/cm. The salinity levels in effluent indicate that the effluent is suitable for irrigation and it poses a low salinity hazard in the soil.

5.1.3 Bacteriological quality of effluent

Figure 5.3 shows the *E. coli* levels found in the in effluent.



Figure 5.3 Bacteriological quality of effluent

The *E. coli* concentrations were ranging between zero to 470 CFU/100ml as shown in Figure 5.3. The mean concentrations were ranging from 0-470 CFU/100 ml at the filter and between 2 and 26 CFU/100 ml at the filter. The WHO (2005) guideline for irrigation allows for a geometric mean of ≤ 1 , 000 no. per 100 ml of faecal coliforms during the irrigation period and 100% of the samples were within this guideline value. The presence of coliforms in effluent implies that the treatment process is not completely effective in removing all the coliform bacteria. The long retention time in the Broadhurst and Phakalane ponds destroys micro-organisms using UV radiation after the primary treatment using activated sludge. The application of disinfectant further reduces the concentration of coliforms to make it suitable for irrigation (WHO, 2005). During this study, it was observed that the application of disinfectant was irregular; when the disinfectant was finished the effluent was pumped directly from the ponds to the farmers without any form of treatment. The results where disinfection was applied are similar to those reported by

Emongor *et al.* (2005) of 194 CFU/100ml at the Phakalane ponds but much lower than those recorded by Mutengu *et al.* (2007) of 5,836 CFU/100ml. The *E. coli* are much lower than the 78, 000 CFU/100ml found in Italy (Palese *et al.*, 2009). The effluent is suitable for irrigation in terms of microbiological quality.

Although the risk of consumption of vegetables is low, preliminary discussions with the workers suggest that the workers know that a certain risk is involved in using effluent. Despite this, workers were not wearing protective clothing at most times during this study (see appendix H). At times the workers were seen having direct contact with the effluent especially when they were changing the disk filter and therefore may be exposing themselves to pathogens. The reason offered for this was that there was not enough protective clothing for everyone and the owners do not seem keen to invest in it.

5.1.4 Heavy metals (Cd and Pb) in effluent

Cadmium and lead concentrations found in effluent are shown in Table 5.1. The range was 0.002-0.003 mg/l with a mean Cd level of 0.002 mg/l at the filter and at the drip. The Cd concentrations are within the USEPA guideline value of 0.01 mg/l. Cadmium is capable of accumulating in food chain and can be toxic to beans at low concentrations of 0.1 mg/l (USEPA, 2002). The Cd in the effluent could be due to batteries and car fluids containing Cd which are discharged into sewer system (Mbongwe et al., 2010). Pb concentration at the filter was ranging between 0.32-0.35 mg/l with a mean concentration of 0.33 mg/l. At the drip, values were in the range 0.33-0.35 mg/l with a mean concentration of 0.33 mg/l. The Pb content is within the recommended limit of 5 mg/l (USEPA, 2002). Mbongwe et al. (2010) suggests that car lubricants e.g. gear oil containing Lead naphthenate are the source of Pb in effluent. The results from this study are lower than 0.037 mg/l and 0.79 mg/l for Cd and Pb respectively found by Mutengu et al. (2007). In a study by (Sahu et al., 2007) in India, higher values were measured, ranging from 0.36 to 0.59 mg/l for Pb and 0.02-0.09 mg/l for Cd. The low results in this study are due to little manufacturing activity around Gaborone. The cadmium and lead content in the effluent is low, therefore the effluent is suitable for irrigation purposes.

Date	Sampling point	Cadmium (mg/l)	Lead (mg/l)
06/04/2011	Filter	0.002	0.35
	Drip	0.003	0.35
13/04/2011	Filter	0.002	0.33
	Drip	0.002	0.34
15/04/2011	Filter	0.003	0.32
	Drip	0.002	0.33
Mean		0.002	0.34
Std dev		0.01	0.0005
Guideline ²		0.01	5

Table 5.1 Cadmium and lead concentrations in effluent

5.2 Soil quality

This section on soil quality is based on results of soil samples taken from the spinach, tomato and green pepper plots of a selected farm. Samples were collected 3 times from each plot and tested for pH, SAR, salinity, Cd and Pb (see appendix F).

5.2.1 Soil pH

Soil pH in soil samples from different vegetable plots are shown in Figure 5.4. In the tomato plot, the pH values were ranging between 7.82-7.94 at an average of 7.89. For the soil sample from green pepper, the range was 8.1-8.4 with an average of 8.27. The values were in the range 8.1-8.4 at an average of 8.23 in the spinach plot. The pH values from all soil samples were above the FAO recommended limit of 6.5. Acidic conditions in the soil with a pH less than 5 enhance the solubility and mobility of heavy metals (Uwimana *et al.*, 2010).

² USEPA, 2002



Figure 5.4 pH levels in different vegetable plots

The results obtained from this study are higher than the 6.7-7.8 by Mutengu *et al.* (2007) in Bulawayo, Zimbabwe, and Dikinya and Areola (2009) of 6.60-7.14 in Glen Valley Farms, Botswana. The results from this study could be attributed to lime application on the field under study which raised the soil pH. The high soil pH is unsuitable for crop production because high pH levels reduce nutrient uptake thus affecting plant growth.

5.2.2 Soil conductivity

Soil conductivity in soil samples from the different vegetable plots is shown in Figure 5.5. In the tomato plot, the EC values were ranging between 300-320 μ S/cm at an average of 310± 10 μ S/cm. For the soil sample from green pepper, the range was 300-310 μ S/cm with an average of 305±5 μ S/cm. The values were in the range 300-320 μ S/cm at an average of 310± 10 μ S/cm in the spinach plot. The values measured on all the plots were lower than the 4, 000 μ S/cm recommended by Pescod and Arar (1985). Drip irrigation system is used for the farm which was studied, drip irrigation system maintains high soil-water potential and minimizes the effect of salinity (Pescod, 1992). The results from this study are similar to average EC of 316 μ S/cm found by Dikinya and Areola (2009) in Glen Valley, Botswana because the scheme is still relatively new so the values of EC tend to be low. However the results from this study are lower than the value found by Usman and Ghallab (2006) of 1500 μ S/cm in Aswan, Egypt. Therefore based on these results, the conductivity of the soil is suitable for crop production



Figure 5.5 Soil conductivity in different vegetable plots

5.2.3 Sodium Absorption Ratio (SAR)

SAR is a ratio of exchangeable sodium ions to Calcium and Magnesium ions which tends to influence soil properties. A high SAR (>15) causes dispersal of soil particles (Pescod, 1992). SAR values determined from different vegetable plots are shown in Figure 5.6. SAR was calculated using equation 1. SAR values ranged from 1.74 to 1.85, with an average of 1.79 ± 0.055 in the soil sample from tomato plot. The soil sample from the tomato plot ranged from 2.65 to 2.7, with an average of 2.68 ± 0.03 . A range from 2.65 to 2.85, with an average of 2.72 ± 0.11 was calculated in soil sample from the spinach plot (Figure 5.5). All the values were below the recommended limit of 15 (Pescod, 1992). A high concentration of Sodium ions (above 15) can cause substitution of Sodium ions for other cations in the soil thus dispersing clay particles in soil. The dispersal of clay particles reduces permeability of soil and infiltration of water in soil (Pescod and Arar, 1985). The values recorded in this study are lower than the 3.21 obtained by Mutengu et al. (2007) although both studies indicate a low sodicity hazard which is suitable for plant growth. However a much wider range of 0.3-4.47 was found for soils in Pakistan (Faryal et al., 2007). Lower values for study are due to the application of lime which replaces sodium ion with calcium ions which lowers SAR (Pescod and Arar, 1985). Based on these results, the SAR values show that the soil is suitable for plant growth.



Figure 5.6 SAR from different vegetable plots

5.2.4 Lead (Pb) concentration in soil

Lead concentration in soil samples from different vegetable plots are shown in Figure 5.7. The range in the soil from spinach plot was 19.2-20.4 mg/kg, with average Pb concentration of 19.7 mg/kg. The soil from the green pepper plot had a range between 15.9 and 16.1 mg/kg, with a mean concentration of 16.2 mg/kg. The values from the tomato plot were ranging between 14.7-15.1 mg/kg at an average value of 14.9 mg/kg. The difference between the soil samples could be due to the different uptake rates of metals by different crops which were planted in previous cropping seasons. All the soil samples have lower values than limit of 190 mg/kg recommended by USEPA (1992) for long term irrigation. The presence of lead in the soil is due to the application of sewage sludge from Glen Valley WWTP as a soil conditioner in the farm. The results of this study are lower than 900 mg/kg found by Muchuweti *et al.* (2007) in Zimbabwe. Higher values with the range between 64.2 and 77.4 mg/kg were obtained in China (Li *et al.*, 2009). Lower values were obtained in the study due to low application rate of effluent through the use of drip irrigation. The lead content in soils from this study shows that, the soils are suitable for crop production because they are below the threshold limits for crop production.



Figure 5.7 Lead concentrations in different vegetable plots

5.2.5 Cadmium (Cd) concentration in soil

Cadmium concentration in soil samples from different vegetable plots are shown in Figure 5.8. The range measured in the soil from spinach plot was 1.1-1.4 mg/kg, with average of 1.28 mg/kg. The soil from the green pepper plot had a range of 0.9-10.95 mg/kg, with a mean concentration of 0.93 mg/kg. The values from the tomato plot were ranging between 0.7-0.8 mg/kg at an average value of 0.75 mg/kg (Figure 5.8). All the soil samples have lower than USEPA (1992) recommended limit of 20 mg/kg.

The differences between the soil samples could be attributed to the previous cropping practices on the same soil. The presence of cadmium is due to the application of sewage sludge from Glen Valley WWTP as a soil conditioner in the farm and the use of pesticides which contain cadmium. Rattan *et al.* (2005) reported that Cd is a very mobile metal unlike Pb which is immobilized in the soil and therefore uptake rate of Cd is more than of Pb. Cd levels can increase in soil due to the excessive use of phosphate fertilizers, pesticides and also from atmospheric deposition (Mejáre and Bülow, 2001).



Figure 5.8 Cadmium concentrations in different vegetable plots

The results from the study are much lower than those obtained by Muchuweti *et al.* (2006) of 6-15 mg/kg and 0.03-0.08 mg/kg reported by (Bahmanyar, 2008). Lower results for the study may be due to the short period for which this scheme has been in use (8 years), and the low application rate of effluent. The lead concentrations in soils from this study indicate that the soils are suitable for crop production as they are below the threshold limits for crop production.

5.3 Crop quality

This section on crop quality is based on results of vegetables samples taken from the spinach, tomato and green pepper plots of a selected farm (See appendix G). Samples were collected 3 times from each plot and analysed for Cd, Pb, total coliforms, *Salmonella spp*. and Enteropathogenic *E. coli*.

5.3.1 Bacteriological quality of vegetables

No pathogens were detected in the selected crops for this study, but some faecal coliforms were detected in all the crops (see Table 5.2). In spinach, coliform levels were ranging between 240 and 1100 CFU/100ml while in green pepper the range was between 4 and 150 CFU/100ml. In the tomato samples the values were below 5 CFU/100ml. The presence of

coliforms in the vegetables may be due to direct contact of the vegetables with the soil which had been irrigated with the effluent. In a study by Palese *et al.* (2009), no pathogens were detected in wastewater-irrigated vegetables except for one which had 10 CFU/100 ml. No pathogens were detected in this study because the effluent has been disinfected and drip irrigation is used for crop production.

Vegetable	Sampling date	Total coliforms (CFU/100ml)	Salmonella spp. (CFU/100 ml)	<i>E. Coli</i> (CFU/100ml)	Cd (mg/kg)	Pb (mg/kg)
Tomato	3/18/2011	<3	Not detected	0	0.017	0.2
	4/13/2011	4	Not detected	0	0.032	0.21
	5/9/2011	<3	Not detected	0	0.04	0.27
Green						
pepper	3/18/2011	4	Not detected	0	0.0054	0.28
	4/13/2011	150	Not detected	0	0.12	0.72
	³ 5/9/2011	-	-	-	-	-
Spinach	3/18/2011	240	Not detected	0	0.028	0.36
	4/13/2011	1100	Not detected	0	0.019	0.12
	5/9/2011	900	Not detected	0	0.03	0.2

Table 5.2 Microbiological quality of vegetables (Salmonella spp., total coliforms and E. coli)

5.3.2 Heavy metals in vegetables

Tables 5.3 and 5.4 present heavy metals concentration in different vegetables and their transfer factors from soil to vegetables. The transfer factor (TF) was used to compare concentrations of metals in vegetables compared to metal concentrations to the corresponding soils. The TF for lead is below 1 for all the vegetables (Table 5.3) which shows that the vegetables contain less heavy metals than the soil.

Table 5.3 Concentration of lead in different vegetables (mg/kg) and the transfer factors

	18-March	13-April	9-May	average	⁴ TF
Tomato	0.2	0.21	0.27	0.23	0.02
Green					
pepper	0.28	0.72	-	0.5	0.03
Spinach	0.36	0.12	0.2	0.23	0.01

⁴ TF- transfer factor

The ratio of metals between soil and plants is an important criterion for the contamination assessment and selection of crop plants for cultivation on contaminated soil, and the ratio >1 means higher accumulation of metals in plant parts than soil (Nayek *et al.*, 2010). The ratio may have been influenced by the mobility of different heavy metals which influence the uptake rate of the metals. Lead is a relatively immobile metal as compared to cadmium (Table 5.4) and bio-availability of different heavy metals based on soil properties (Chary *et al.*, 2008). The TF values from this study are lower than those found by Bahmanyar (2008) of 0.07 for spinach grown in Iran and by Chary *et al.* (2008) of 0.99 found in India. The differences in results could due to differences in crops/varieties grown and soil properties which affected plant uptake. The results for TF show that lead has very low uptake rates due to poor mobility is soil.

	18-March	13-April	9-May	average	TF
Tomato	0.017	0.032	0.04	0.030	0.88
Green					
pepper ⁵	0.0054	0.12	-	0.063	0.69
Spinach	0.028	0.019	0.03	0.026	1.03

Table 5.4 Concentration of cadmium in different vegetables (mg/kg) and the transfer factors

The TF for cadmium was found to be above 1 (Table 5.4) indicating that the vegetables contain more heavy metals than the soil (Nayek *et al.*, 2010). The results of the study are lower than those reported by Chary *et al.* (2008) and Mapanda *et al.* (2007) of 0.99 and 3.0 respectively due to differing soil conditions under which the spinach and *B. napus* were grown.

In alkaline pH, the metals are more firmly bounded with organic matter and their phytoavailability is reduced. Significant correlations between total metal content with their bioavailable fractions for Fe, Cd, Mn and Cu revealed their higher availability and translocation in soil–plant system than Pb. The TF values from this study are higher than those found in Iran of 0.19 (Bahmanyar, 2008).

⁵ Dash (-) indicates that no sampling was done because all the green pepper had been harvested and the plot was cleared

This may be due to differences in crops grown and the high soil pH found in this study which affected plant uptake of the Cd. The TF values from this study show that cadmium is a mobile element which is readily taken up by plants.

5.4 Risk analysis

This section on risk analysis is based on results of Cd and Pb levels in vegetables samples taken from the spinach, tomato and green pepper plots of a selected farm.

5.4.1 Bacteriological risk

There is risk of infection from consuming the spinach, green pepper and tomato from this scheme due to the detection of coliforms on all the vegetables considered for this study. Although coliforms are not pathogenic (Mara *et al.*, 2007), they are an indicator of the presence of pathogens. The bacteriological quality of vegetables was the main concern raised by people when asked about their opinion on the scheme. However they assumed that the quality is good for consumption since the health department has not issued a warning to the contrary. During some discussions with the farmers, they revealed that the decision to grow certain types of crops over others was based on recommendations by agricultural officers and also profitability/marketability of the produce. The main buyers of the produce are the local supermarkets and the government. Therefore from these results, the risk of transmission of pathogens through the faecal-oral route is low.

5.4.2 Minimum Risk Levels (MRLs)

MRLs were calculated from Dietary Daily Intake (DDI) rate which is the amount of heavy metals that a person ingests daily through contaminated food. The DDI values of heavy metals were estimated from average concentrations of heavy metals in vegetables and vegetable consumption rate of 183-219 mg/day for a 60 kg adult (Mapanda *et al.*, 2007). Table 5.5 shows minimal risk levels and the DDI which was calculated using equation 2.

	Vegetable	Ave. Metal Conc. (mg/kg)	⁶ DDI (mg/day)	⁷ MRL (mg/day)
Cadmium (Cd)	Spinach	0.026	0.005-0.006	0.012
	Green pepper	0.063	0.01-0.014	0.012
	Tomato	0.03	0.005-0.007	0.012
Lead (Pb)	Spinach	0.23	0.04-0.05	0.18
	Green pepper	0.5	0.09-0.11	0.18
	Tomato	0.23	0.04-0.05	0.18

Table 5.5 Heavy metal intake rate relative to Minimal Risk Levels (MRLs)

The daily intake rates for Cd and Pb are generally higher in green pepper than in tomatoes and spinach. The upper limit of the DDI for Cadmium in green pepper (0.014 mg/day) is more than the MRL of 0.012 mg/day while the intake rates for other vegetables were below the MRLs for Cd and Pb. Therefore 66% of samples were below the MRLs for the Cadmium in all vegetables while all vegetables were below the MRLs for lead. This could be because the green pepper was planted earlier than tomato and spinach and therefore had more time to accumulate Cd in the plant. Typical daily intakes of Pb by adults range from 0.015 to 0.1 mg/day, depending on the composition of the diet and where the consumer lives. The daily intake rate for Pb in spinach from this study is lower than the 0.05-0.09 found by Mapanda *et al.* (2007). However the daily intake rate for Cd in spinach for this study is higher than the 0.02-0.04 mg/day reported in the same study.

Cadmium and lead are two of the most prevalent as well as two of the most nephrotoxic metals known to man with the former causing itai-itai-byo or "ouch-ouch" disease, after prolonged exposure, the name derived from the crippling and painful osteomalacic component of the disease (Gonick, 2008).

⁶ DDI calculated using equation 2

⁷ MRLs from ATSDR (2003)

The results of this study are lower than those reported by Muchuweti *et al.* (2006) for tomato fruits with a much higher concentration of 5mg/kg. The Cd and Pb concentrations reported by Banejee *et al.* (2011), for different vegetables were found to be above permissible levels recommended by WHO/FAO of 0.3 mg/kg for Pb and 0.2 mg/kg for Cd. Heavy metal concentrations were highest in unwashed samples followed by washed and boiled samples.

Even after washing and boiling, Pb content of all the vegetables remained higher than the recommended value. The variation in results could be due to the irrigation with a treated wastewater in this study unlike the mixtures of wastewater and sewage used in the study by Muchuweti *et al.* (2006). The results of this study are also lower than those reported by Mapanda *et al.* (2007) where partially treated sewage is diverted into gardens for horticultural production. Therefore it can be concluded that consumption of the vegetable by consumers has low risk of disease and may not have adverse health effects on the consumer as the MRLs are used as screening levels to identify a health hazard.

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study the following conclusions were made:

- The treatment process is effective in removing pathogens and the reducing heavy metal concentration thus making the effluent safe for irrigation. The soil has been contaminated with Cd as a result of irrigation with treated effluent but the Pb levels decreased due to plant uptake.
- 2) The vegetables translocate metals from the soil through the roots to the shoot then to the fruit. Cd is more mobile than Pb and therefore rate of plant uptake of Cd was higher than that of Pb. Coliform levels in the crops are from the direct contact of crops with effluent-irrigated soil.
- 3) There is a low health risk posed by consumption of Cadmium as levels found in green pepper are above the levels considered safe for human consumption on a daily basis. Pb and Cd levels in all other vegetables do not seem to pose any risk as a result of long term consumption as they are below the chronic levels to cause diseases.

6.2 Recommendations

From the conclusions drawn, the following recommendations are made:

- Monitoring programmes should be maintained to ensure efficiency of treatment and compliance with effluent standards. A filtration step should be introduced before the application of disinfectant to reduce treatment costs and carryover of suspended solids.
- 2) Long-term monitoring of selected parameters especially heavy metals should be carried out to assess trends and potential long-term effect on the soil and plant uptake. Bioremediation techniques should be employed to reduce metal concentrations in the soil and therefore reduce the amount which is available for plant uptake.

3) There is need for further research into the practice of wastewater use for crop production. Crop studies should be done to investigate types of vegetables which are more tolerant to heavy metals and pathogens but can give optimum yields under local conditions. Regular monitoring of the effluent, soil and crops is required to protect the health of workers and consumers. Long-term monitoring of selected parameters especially heavy metals should be carried out to assess trends and potential long-term effects on the soil and plant uptake.

REFERENCES

Agriculture, 2008. National investment brief: Botswana. Water for Agriculture and Energy in Africa: The Challenges of Climate Change Retrieved 11 November 2010, from http://www.sirtewaterandenergy.org/docs/reports/Botswana-Draft2.pdf.

Al-Lahham, O., El Assi, N. M., Fayyad, M., 2003. Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. Agricultural Water Management 61, 51-62.

Ammary, B. Y., 2007. Wastewater reuse in Jordan: Present status and future plans. Desalination 211, 164-176.

APHA, 2001. Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington D.C.

Aquarec, 2006. Water reuse system management manual. Retrieved 15 February 2011, from <u>http://www.amk.rwth-</u>aachen.de/fileadmin/files/Forschung/Aquarec/AQUAREC_SUMMARY_REPORT.pdf.

ATSDR, 2003. Minimal Risk Levels (MRLs) for hazardous substances. Agency for Toxic Substances and Disease Registry (ATSDR). Retrieved 10 February 2011, from <u>http://www.atsdr.cdc.gov/mrls.html</u>.

Bahmanyar, M. A., 2008. Cadmium, nickel, chromium, and lead levels in soils and vegetables under long-term irrigation with industrial wastewater. Communications in Soil Science and Plant Analysis 39, 2068-2079.

Banerjee, D., Kuila, P., Ganguli, A., Das, D., Mukherjee, S., Ray, L., 2011. Heavy metal contamination in vegetables collected from market sites of Kolkata, India. Electronic Journal of Environmental, Agricultural and Food Chemistry 10, 2160-2165.

Battilani, A., Steiner, M., Andersen, M., Soren, N. B., Lorenzen, J., Sweitzer, A., Dalsgaard, A., Forslund, A., Gola, S., Klopmann, W., Plauborg, F., Andersen, M. N., 2010. Decentralised water and wastewater treatment technologies to produce functional water for irrigation. Agricultural Water Management 98, 385-402.

Bitton, G., 2005. Wastewater microbiology. Lewis publishers, United States of America.

CSO, 2009. Botswana demographic survey 2006, Ministry of Finance and Development Planning, Gaborone.

Dikinya, O., Areola, O., 2009. Comparative assessment of heavy metal concentration in treated wastewater irrigated soils cultivated to different crops in the Glen Valley, Botswana. African Crop Science Uganda, 351 – 355.

DWAF, 1996. South African water quality guidelines, agricultural use: Irrigation 4, Department of Water Affairs and Forestry (DWAF), Pretoria.

Emongor, V. E., Ramolemana, G. M., 2004. Treated sewage effluent (water) potential to be used for horticultural production in Botswana. Physics and Chemistry of the Earth Parts A/B/C 29, 1101-1108.

Emongor, V. E., Ramolemana, G. M., Machacha, S., 2005. Water quality of Notwane River. Retrieved 10th December 2010, from <u>www.bscw.ihe.nl/pubs/bscw.cgi/d2606463/Emongor.pdf</u>.

FAO, 1994. Microbiological analysis. FAO Food and Nutrition Paper. 14/4 Rev., FAO, Rome.

FAO, 2010. Wealth of waste: The economics of wastewater use in agriculture. FAO Water Report no. 35, Food and Agriculture Organisation (FAO), Rome.

FAO/WHO, 1999. Expert committee on food additives: Summary and conclusions. 53rd meeting, Food and Agriculture Organisation (FAO)/World Health Organisation (WHO), Rome.

Faryal, R., Tahir, F., Hammed, A., 2007. Effect of wastewater irrigation on soil along with its micro and macro flora. Pakistan Journal of Botany 39, 193-204.

Feizi, M., 2001. Effect of treated wastewater on accumulation of heavy metals in plants and soil. International Workshop on Wastewater Reuse Management Retrieved 19-20 September 2001, from http://www.nwl.ac.uk/research/cairoworkshop/papers/WASTEWATER/ws2_17.PDF.

Forsythe, S. J., 2002. The microbiological risk assessment of food. Blackwell Publishing, Oxford.

Gleick, P. H., 1998. The world's water 1998-1999: The biennial report on freshwater resources. Island Press, Washington, D.C.

Gonick, H. C., 2008. Nephrotoxicity of cadmium and lead. Indian Journal of Medical Research 128, 335-352.

Haarhoff, J., van der Merwe, B., 1996. Twenty-five years of wastewater reclamation in Windhoek, Namibia. Water, Science and Technology 33, 25-35.

Jung, C. M., 2008. Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. Applied Geochemistry 11, 53-59.

Khouri, N., Kalbermatten, M., Bartone, J., Carl, R., 1990. Reuse of wastewater in agriculture. A guide for planners, UNDP Washington D.C.

Khupe, J. S., 1996. Water supply, sewage and wastewater management for Gaborone Botswana. AMBIO 25, 138-143.

Kirkham, M. B., 1999. Water use in crop production. Food Products Press, New York.

Krijger, G. C., Vliet, P. M., Wolterbeek, H. T., 1999. Metal speciation in xylem exudate of *lycopersicon esculentum*. Plant Soil 212, 165–173.

Li, P., Wang, X., Allinson, G., Li, X., Xiong, X., 2009. Risk assessment of heavy metals in soil previously irrigated with industrial wastewater in Shenyang, China. Journal of Hazardous Materials 161, 516-521.

Madyiwa, S., Chambari, M. J., Schutte, C. F., Nyamangara, J., 2003. Greenhouse studies on the phyto-extraction capacity of *cynodon nlemfluensis* for lead and cadmium under irrigation with treated wastewater. Physics and Chemistry of the Earth Parts A/B/C 28, 859-867.

Manas, P., Castro, E., De las Heras, J., 2009. Irrigation with treated wastewater: Effects on soil, lettuce (*lactuca sativa* l.) crop and dynamics of micro-organisms. Journal of Environmental Science and Health Part A 44, 1261-1273.

Mapanda, F., Mangwayana, E. N., Nyamangara J., K.E., G., 2007. Uptake of heavy metals by vegetables irrigated using wastewater and subsequent risk in Harare, Zimbabwe. Physics and Chemistry of the Earth Parts A/B/C 32, 1399-1405.

Mara, D. D., Sleigh, P. A., Blumenthal, U. J., Carr, R. M., 2007. Health risks in wastewater irrigation: Comparing estimates from quantitative microbial risk analyses and epidemiological studies. Journal of Water and Health 5, 39-50.

Mbongwe, B., Barnes, B., Tshabang, J., Zhai, M., Rajoram, S., Mpuchane S., Mathee, A., 2010. Exposure to lead among children aged 1-6 years in the city of Gaborone, Botswana. Journal of Environmental Health Research 10, 17-26.

Mejáre, M., Bülow, L., 2001. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends in Biotechnology 19, 67-73.

MFDP, 2009. National development plan: 2009-2015, Ministry of Finance and Development Planning (MFDP), Gaborone.

Mguni, M., 2010, No.172. Gaborone upgrades 'bloated' sewerage plant. Mmegi, 27, from <u>http://www.mmegi.bw/index.php?sid=4&aid=6523&dir=2010/November/Tuesday16</u>.

Mladenov, N., Strzepek, K., Serumola, O. M., 2005. Water quality assessment and modelling of an effluent dominated stream: Notwane River in Botswana. Environmental Monitoring and Assessment 109, 97-121.

MoA, 2002. National master plan for arable agriculture and dairy development (nampaadd) guidelines, Ministry of Agriculture (MoA), Gaborone.

Mutengu, S., Hoko, Z., Makoni, F. S., 2007. An assessment of public health hazard potential of wastewater use for crop production. Physics and Chemistry of the Earth Parts A/B/C 32, 1195-1203.

Nayek, S., Gupta, S., Saha, R. N., 2010. Metal accumulation and its effects in relation to biochemical response of vegetables irrigated with metal contaminated water and wastewater. Journal of Hazardous Materials 178, 588-592.

Nkgebe, E., Sankwasa, S., Koorapetse, I., Keikanetswe, I., 2005. An overview of cod load removal at glen valley re-activated sludge wastewater treatment plant. Journal of Applied Sciences 5, 1178-1181.

Palese, A. M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S., Xiloyannis, C., 2009. Irrigation of olive groves in southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. Agriculture, Ecosystems and Environment 129, 43-51.

Pescod, M. B., 1992. Wastewater treatment and use in agriculture. FAO Irrigation and Drainage Paper 47: 125, FAO, Rome.

Pescod, M. B., Arar, A., 1985. Treatment and use of sewage effluent for irrigation. FAO Regional Seminar on the Treatment and Use of Sewage Effluent for Irrigation. Nicosia, Cyprus, 7-9 October 1985.

Pierzynski, G. M., Sims J.T., Vance, G. F., 2005. Soils and environmental quality. Taylor and Francis, USA.

Podile, T. E., 2005. Water demand management- wastewater reuse and recycling potential for the greater Gaborone area, Unpublished MSc thesis, University of Zimbabwe.

Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K., Singh, A. K., 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. Agriculture, Ecosystems and Environment 109, 310-322.

Rowe, D. R., Abdel-Magid, I. M., 1995. Handbook of wastewater reclamation and reuse. CRC Press Incorporated, United States of America.

Sahu, R. K., Katiyar, S., Tiwari, J., Kisku, G. C., 2007. Assessment of drain water receiving effluent from tanneries and its impact on soil and plants with particular emphasis on bioaccumulation of heavy metals. Journal of Environmental Biology 28, 685-690.

Sakuringwa, S., 2007. An assessment of the public health hazard potential of wastewater reuse for crop production: A case of Gaborone, Botswana, Unpublished MSc thesis, University of Dar es Salaam.

Scott, C. A., Faraqui, N. I., Richard-Sally, I., 2005. Wastewater use in irrigated agriculture: Coordinating the livelihoods and environmental realities. CABS International, IWMI and IDRC, United Kingdom.

SMEC, Ninham Shand Consulting Services, Department of Sanitation and Wastewater, 1991. Botswana national master plan for wastewater and sanitation 6, Government of Botswana, Gaborone.

SMEC, Ninham Shand Consulting Services, Department of Sanitation and Wastewater, 2003. Botswana national master plan for wastewater and sanitation 8, Government of Botswana, Gaborone.

Tarradellas, J., Bitton, G., Rossel, D., 1997. Soil ecotoxicology. Lewis Publishers, United kingdom.

Tchobanoglous, G., Burton, F. L., 1991. Wastewater engineering: Treatment, disposal and reuse. McGraw-Hill, Singapore.

USEPA, 1992. Preparation of soil sampling protocols: Sampling techniques and strategies, United States Environmental Protection Agency (USEPA), Washington DC.

USEPA, 2002. Guidelines for water reuse, United States Environmental Protection Agency (USEPA), United States of America.

Usman, A. R. A., Ghallab, A., 2006. Heavy-metal fractionation and distribution in soil profiles short-term-irrigated with sewage wastewater. Chemistry and Ecology 22, 267-278.

Uwimana, A., Nhapi I., Wali, U. G., Hoko Z., Kashaigili, J., 2010. Sludge characterization at Kadahokwa water treatment plant, Rwanda. Water Science and Technology: Water Supply 10, 847-858.

van Reeuwijk, L. P., 1993. Procedures for soil analysis. Technical paper. 19, ISRIC (International Soil Reference and Information Centre), Netherlands.

WHO, 2005. Guidelines for the safe use of wastewater and excreta in agriculture and aquaculture, World Health Organisation (WHO), Switzerland.

Wise, D. L., Trantolo, D. J., Cichon, E. J., Inyang, H. I., 2000. Bioremediation of contaminated soils. Marcel Dekker Incorporated, USA.

WUC, 2010. Annual report 2010/2011, Water Utilities Corporation (WUC), Gaborone.

Zheljazkov, V. D., Nielsen, N. E., 1996. Effect of heavy metals on peppermint and commint. Plant Soil 178, 59-66.

ZINWA, 2000. Water (waste and effluent disposal) regulations. Statutory Instrument 274 of 2000, Zimbabwe National Water Authority (ZINWA), Harare.

APPENDICES



Appendix A: Layout of treatment process at Glen Valley WWTP

Appendix B:	US EPA recom	nended limits for	r irrigation	with 1	reclaimed	water
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Constituent	Long-term	Short-term	Remark
	use (mg/l)	use (mg/l)	
Aluminium	5.0	20	Can cause non-productiveness in acid soils, but soils at pH 5.5 to 8.0 will precipitate the ion and eliminate toxicity.
Arsenic	0.1	2.0	Toxicity to plants varies widely, ranging from 12 mg/L for Sudan grass to less than 0.05 mg/L for rice.
Berylium	0.1	0.5	Toxicity to plants varies widely, ranging from 5 mg/L for kale to 0.5 mg/L for bush beans.
Boron	0.75	2.0	Essential to plant growth, with optimum yields for many obtained at a few-tenths mg/L in nutrient solutions. Toxic to many sensitive plants (e.g., citrus) at 1 mg/L. Usually sufficient quantities in reclaimed water to correct soil deficiencies. Most grasses are relatively tolerant at 2.0 to 10 mg/L.
Cadmium	0.01	0.05	Toxic to beans, beets, and turnips at concentrations as low as 0.1 mg/L in nutrient solution. Conservative limits recommended.
Chromium	0.1	1.0	Not generally recognized as an essential growth element. Conservative limits recommended due to lack of knowledge on toxicity to plants
Cobalt	0.05	5.0	Toxic to tomato plants at 0.1 mg/L in nutrient solution. Tends to be inactivated by neutral and alkaline soils.
Copper	0.2	5.0	Toxic to a number of plants at 0.1 to 1.0 mg/L in nutrient solution
Fluoride	1.0	15.0	Inactivated by neutral and alkaline soils.
Iron	5.0	20.0	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of essential phosphorus and molybdenum.
Lead	5.0	10.0	Can inhibit plant cell growth at very high concentrations.
Lithium	2.5	2.5	Tolerated by most crops at concentrations up to 5 mg/L; mobile in soil. Toxic to citrus at low doses - recommended limit is 0.075 mg/L.
Manganese	0.2	10.0	Toxic to a number of crops at a few-tenths to a few mg/L in acidic soils.
Molybdenum	0.01	0.05	Nontoxic to plants at normal concentrations in soil and water. Can be toxic to livestock if forage is grown in soils with high levels of available molybdenum
Nickel	0.2	2.0	Toxic to a number of plants at 0.5 to 1.0 mg/L; reduced toxicity at neutral or alkaline pH.
Selenium	0.02	0.02	Toxic to plants at low concentrations and to livestock if forage is grown in soils with low levels of selenium.
Tin, Tungsten and Titanium	-	-	Effectively excluded by plants; specific tolerance levels unknown
Vanadium	0.1	1.0	Toxic to many plants at relatively low concentrations.
Zinc	2.0	10.0	Toxic to many plants at widely varying concentrations; reduced toxicity at increased pH (6 or above) and in fine-textured or organic soils.
рН	6.0		Most effects of pH on plant growth are indirect (e.g., pH effects on heavy metals' toxicity described above).
TDS (Total Dissolved Solids)	500-2000		Below 500 mg/L, no detrimental effects are usually noticed. Between 500 and 1,000 mg/L, TDS in irrigation water can affect sensitive plants. At 1,000 to 2,000 mg/L, TDS levels can affect many crops and careful management practices should be followed. Above 2,000 mg/L, water can be used regularly only for tolerant plants on permeable soils.
Free Chlorine Residual	<1.0		Concentrations greater than 5 mg/l causes severe damage to most plants. Some sensitive plants may be damaged at levels as low as 0.05 mg/l.

Source: Rowe and Abdel-Magid, 1995

Pathogen		Survival time in days					
		Sewage	Crops	Soil			
Viruses Enteroviruses		<120 but usually <50	<60 but usually <15	<100 but usually <20			
Bacteria	Faecal coliforms	<60 but usually <30	<30 but usually <15	<70 but usually <20			
	Salmonella spp.	<60 but usually <30	<30 but usually <15	<70 but usually <20			
	Shigella spp.	<30 but usually <10	<10 but usually <5				
	Vibrio Cholerae	<30 but usually <10	<5 but usually <2	<20 but usually <10			
Protozoa	Entamoeba Hytolytica cysts	<30 but usually <15	<10 but usually <2	<20 but usually <10			
Helminths	Ascaris Lumbricoides eggs	Many months	<60 but usually <30	Many months			

Appendix C: Survival time of pathogens in sewage, soil and crops

Source: USEPA, 2002

Organism	Minimal infective dose (organisms per
	100ml)
Salmonella spp.	$10^4 - 10^7$
Shigella spp.	10^{1} - 10^{2}
Escherichia coli	$10^{6} - 10^{8}$
<i>E. coli</i> 0157:H7	<100
Vibrio cholerae	10^{3}
Ascaris	1-10 eggs

Appendix D: Minimal infective dose for some pathogens and parasites

Source: Bitton (2005)

Appendix E: Effluent quality results

			EC			
Sampling date	pН	Temp (°C)	(µS/cm)	E. Coli (CFU/100 ml)	Cd (mg/l)	Pb (mg/l)
25/2/2011	9.01	22.6	760	470	0.001	0.4
4/3/2011	8.94	22.3	760	37	0.004	0.32
11/3/2011	9.29	22	740	0	0.002	0.35
18/03/2011	8.91	23.8	710	0	0.002	0.35
6/4/2011	9.05	23.4	740	15	0.002	0.345
13/4/2011	9.23	22.3	730	14	0.002	0.33
15/4/2011	9.22	23	710	2	0.003	0.322
Min	8.91	22	710	0	0.001	0.32
Max	9.29	23.8	760	470	0.004	0.4
Ave	9.09	22.77	735.71	76.86	0.002	0.35
Std dev	0.152	0.655	20.702	173.851	0.001	0.027

Samples from the filter

Samples from drip

Sampling date	pН	Temp (°C)	EC (µS/cm)	E. Coli (CFU/100 ml)	Cd (mg/l)	Pb (mg/l)
6/4/2011	8.95	22.5	710	6	0.003	0.35
13/4/2011	8.64	22.5	730	3	0.002	0.337
15/4/2011	9.2	21.9	700	26	0.002	0.333
Min	8.64	21.9	700	3	0.002	0.333
Max	9.2	22.5	730	26	0.003	0.35
Ave	8.93	22.3	713.3	11.7	0.002	0.34
Std dev	0.28	0.35	15.3	12.5	0.001	0.0089

Appendix F: Soil quality results

Samples from Tomato plot

Sampling date	рН	EC (µS/cm)	SAR	Cd (mg/kg)	Pb (mg/kg)
23-Mar	7.94	310	1.74	0.8	15.1
15-Apr	7.82	300	1.79	0.75	15
9-May	7.9	320	1.85	0.7	14.7
Min	7.82	300	1.74	0.7	14.7
Max	7.94	320	1.85	0.8	15.1
Ave	7.89	310	1.79	0.75	14.93
Std dev	0.061	10	0.055	0.05	0.21

Samples from Green pepper plot

Sampling date	pН	EC (µS/cm)	SAR	Cd (mg/kg)	Pb (mg/kg)
23-Mar	8.4	300	2.65	0.95	16.5
15-Apr	8.1	305	2.7	0.9	15.9
9-May	8.3	310	2.7	0.94	16.1
Min	8.1	300	2.65	0.9	15.9
Max	8.4	310	2.7	0.95	16.5
Ave	8.27	305	2.68	0.93	16.17
Std dev	0.15	5	0.03	0.03	0.31

Samples from Spinach plot

Sampling date	рН	EC (µS/cm)	SAR	Cd (mg/kg)	Pb (mg/kg)
23-Mar	8.4	300	2.65	1.4	20.4
15-Apr	8.1	310	2.67	1.1	19.5
9-May	8.2	320	2.85	1.35	19.2
Min	8.1	300	2.65	1.1	19.2
Max	8.4	320	2.85	1.4	20.4
Ave	8.23	310	2.72	1.28	19.70
Std dev	0.15	10	0.11	0.16	0.62

Vegetable	Sampling date	Total coliforms	Salmonella spp.	<i>E. Coli</i> (FC/25g)	Cd (mg/kg)	Pb (mg/kg)
Tomato	3/18/2011	<3	Not detected	0	0.017	0.2
	4/13/2011	4	Not detected	0	0.032	0.21
	5/9/2011	<3	Not detected	0	0.04	0.27
Green pepper	3/18/2011	4	Not detected	0	0.0054	0.28
	4/13/2011	150	Not detected	0	0.12	0.72
	5/9/2011	-	-	-	-	-
Spinach	3/18/2011	240	Not detected	0	0.028	0.36
	4/13/2011	1100	Not detected	0	0.019	0.12
	5/9/2011	900	Not detected	0	0.03	0.2

Appendix G: Crop quality results

Appendix H: Photo gallery



a) No use of protective clothing by the workers



b) Use of boots as protective clothing



c) Broadhurst maturation ponds