AN INVESTIGATION OF CHROMIUM AND NICKEL UPTAKE IN TOMATO PLANTS IRRIGATED WITH TREATED WASTE WATER AT THE GLEN VALLEY FARM, GABORONE, BOTSWANA

By

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DECLARATION

I, the undersigned, hereby declared that the work contained in this dissertation is my own original work except where due reference is made and has not been and will not be submitted for the award of any degree or diploma to any other institution of higher learning.

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An Investigation of chromium and nickel uptake in Tomato plants when using treated Waste water for Irrigation at the Glen Valley farm of Gaborone, Botswana

By

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DEDICATION

This work is dedicated to the glory of God who is the “Author and the finisher of my faith”. Without God the journey would have remained but a fleeting illusion. I give God all the Glory, thank you Jehovah.

SUMMARY

The use of treated waste water for irrigation of vegetable crops is on the increase in Botswana especially in the Glen Valley farms, a peri-urban settlement of Gaborone city. However, the effects of this practice on heavy metals uptake by vegetable crops are uninvestigated. Chromium and nickel have been reported to be accumulating in Gaborone crop soils and cultivating vegetables in these soils with treated waste water could potentially lead to an increased bio-availability of the heavy metals in the vegetable crops.

The main aim of this study was therefore to compare the uptake of chromium and nickel in tomato plants, a vegetable grown in sludge amended Glen Valley soils, to those grown in sludge absent Glen Valley soils using treated waste water at different pH values and tap water for irrigation. The high water uptake and high water consumption rate of tomato plants made it suitable for this study. Twenty five pots each containing 2.5 kg sludge amended Glen Valley soils and 5 pots each containing 2.5 kg sludge absent soils were utilized. Fresh treated waste water in a 50 L plastic container on a need by need basis was used. For the control experiments 5 pots each containing 2.5 kg standard commercial soils and fresh tap water were used. The potted tomato plants were cultivated from early May to middle of October 2009. One leaf and one fruit from each tomato plant was harvested and tested in this study.

The highest uptakes of chromium (0.819 mg/L) and nickel (0.327 mg/L) were experienced in the leaves where the tomato plant were cultivated in standard commercial soil and irrigated with tap water at pH 7.0. The least uptake of chromium (0.052 mg/L) and that of nickel (-0.030 mg/L) was found in the fruits, where the tomatoes were grown in sludge amended Glen Valley soil and irrigated with normal Glen Valley treated waste water at pH 8.5. Increasing the pH of the treated waste water from 5.0 to 6.0 caused increased bio-accumulation of chromium and nickel in the leaves and the fruits of the tomato plants. Normal treated waste water (pH 8.5) and treated waste water at pH 9.0, however, reduced the chromium and the nickel uptake by the tomato plants. Treated waste water at pH 10.0 bio-accumulate more chromium and more nickel in the leaves and fruits of tomato plants. The pH variation experiments suggested that the fruit tissues accumulated more chromium and the leaf tissues accumulated more nickel. The mean chromium uptake in the tomato plants exceeded the Food and Agriculture Organization permissible limits but the Botswana Bureau of Standards effluent limit was not exceeded. The mean nickel concentrations were below the threshold limits for both local and international standards. Statistical analysis showed no significant difference between the mean chromium and the mean nickel concentration in the leaves and the fruits of the tomatoes at the 5% significant level. It can be concluded from this study that cultivating tomatoes with sludge amended Glen Valley soil combined with normal treated waste water at pH 8.5 could reduce the uptake of chromium and nickel uptake in tomato plants. However, an increase in the uptake of chromium and nickel in the leaves and fruits of the tomato plants could be triggered at slightly low pH (pH 5.0 and pH 6.0) and high pH (pH 10.0) of the treated waste water.

It is recommended that the current practices of using treated waste water combined with sludge amended Glen Valley soil to cultivate tomatoes at the Glen Valley farm is good practice and should be continued. Nonetheless, further studies need to be carried out at the farm to establish possible phytotoxicity effects of these heavy metals on tomatoes when using treated waste water combined with sludge amended and sludge absent soils.

Keywords: Treated waste water, Tap water, Tomato plants, Irrigation, Sludge absent soil, Sludge amended soil, Chromium uptake, Nickel uptake, pH effect.

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Lastly, I like to thank my sister, Folasade, my brother Adeboye and mother Mary Adunni for their love, support and encouragement in supporting this project.

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LIST OF ABBREVIATIONS

AF	Areni-Haplic- Lixisol/Ferralic-Arenosol Soils
BOS	Botswana Bureau of Standards
BMC	Botswana Meat Commission
DWA	Department of Water Affairs
DWMPC	Department of Waste Management and Pollution Control
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
GVTWW	Glen Valley treated waste water
n	No of samples
OTW	Ordinary tap water
SEM	Standard Error of the Mean
UNDP	United Nations Development Program
USEPA	United States Environmental Protection Agency
VL	Vertic-Cambisol/Vertic luvisol Soils
WHO	World Health Organization

CHAPTER 1

RESEARCH PROBLEM, AIM AND OBJECTIVES

1.1 INTRODUCTION

In Botswana, rainfall is generally low and highly variable and evaporation exceeds the rainfall. Botswana thus experiences a hydro-climatological water scarcity, severely restricting its agricultural potential (Arntzen, 2006). Application of sewage waste water on agricultural land, otherwise known as irrigation, became an alternative water supply to crops as well as an alternative waste disposal method. This practice brought other positive benefits such as enhancing soil nutrients and the organic carbon content of the soils (Gupta, Narwal and Antil., 1998), promoting good crop yields and replacing chemical fertilizers. However, with increasing industrial effluent discharge, the heavy metal content and other pathogens in waste water are posing a threat to human health (Cooper, 1991; Mendoza et al., 1996). Sewage waste water should be treated for safe agricultural production (Ghulam and Al-Saati, 1999). For this reason the Gaborone sewage treatment plant was commissioned in 1997 to process about 40 000 m^3/day municipal sewage effluents in and around the city of Gaborone in Botswana. Despite the waste water treatment there is a need to assess its quality for crop production application. Some of the essential variables in the waste water include pH, salinity, major metals, anions, and heavy metals (trace metals). The pH though, has no direct effect on plant growth; however, it affects the form and availability of metal nutrients to plants (Quaghebeur et al., 2005; Kukier et al., 2004).

Heavy metals are normally present at trace amounts in water samples and they occur naturally in the earth crust. The determination of heavy metal concentrations is important because they are essential nutrients to plants, but can be toxic if they accumulate to high concentration levels (Conolly and Guerinot, 2002).

Agricultural practice in the Glen Valley farms of Gaborone, Botswana make use of treated waste water from the nearby Glen Valley waste water treatment plant. The uses of treated waste water are restricted to specific crops not used for human consumption, such as seedlings, and grass. However, current farm practices showed that these restrictions are not being strictly adhered to in the Glen Valley agricultural farms of Gaborone city.

As a consequence of the foregoing, this research would shed light on the behavior and nature of chromium and nickel uptake by the tomato plants and act as an early warning signs for the increasing levels of chromium and nickel in the food chain as a result of waste water use and sludge application to soil. Also the knowledge gained from this study can be useful by farmers who want to commence the practices of waste water use and sludge application to soils.

1.2 STATEMENT OF THE PROBLEM

Waste water quality is a concern as it contains a wide spectrum of contaminants that may be assimilated in plants depending on the level of treatment. Therefore, the use of treated waste water requires that its quality be assured in terms of its possible effects on soils, plants, animals and humans (Scheltinga, 1987). The principle for evaluating the quality of treated waste water deals with the total concentration and composition of soluble salts and trace metals in water. Soluble salts and trace metals commonly found in waste water may have undesirable effects on plants and may also be toxic to the plants. According to pilot tests conducted with the Glen Valley treated waste water (Akande, 2007) it was found that the pH of the treated waste water used for irrigation in Glen Valley was in the range of 9.52 – 10.25. This high pH of the Glen Valley treated waste water could make a significant contribution to the uptake of heavy metal contaminants in plants which could lead to the increase in concentration levels of trace elements in vegetable crops and the food chain as a whole.

Sludge (from treated waste water) application to soils is also a viable practice in Glen Valley, Gaborone, Botswana. Typical sludge consists of organic and inorganic materials including plant nutrients, organic compounds, pathogens and heavy metals. Sludge composition varies from one waste water treatment plant to the other depending on the treatment process employed and the nature of waste water received at the plant.

Carlton-Smith (1987) investigated the use of treated waste water combined with the application of sludge to soils and found that the combination poses a great concern when used to cultivate agricultural crops for human consumption. Increases in metal concentrations in the soil due to sludge application and waste water use produced significant increases in heavy metal concentrations such as cadmium (Cd), nickel (Ni), chromium (Cr), copper (Cu) and zinc (Zn) in the edible portion of most of the crops grown (wheat, potato, lettuce, red beet, cabbage and ryegrass).

Zhai et al. (2003) reported the concentration levels of chromium and nickel heavy metals to be at peak concentrations in agricultural crop soils of Gaborone, Botswana. A consequence of this could lead to an increased bio-availability of chromium and nickel trace metals and hence an increased assimilation of these contaminants into the food chain. The magnitude of the bio-availability of the heavy metals and phytotoxicity in plants depends on the interrelationship of a number of factors such as the rate and frequency of application of treated waste water and sludge, soil characteristics and the plant species. However, additional soil and plant factors further modify the uptake and the concentration of heavy metals in crops (Food and Agriculture Organization, 1992).

Presently, the agricultural crops produced in Glen Valley irrigational farms include lettuce (Appendix A), spinach, cabbage, green pepper, and tomatoes to mention a few. There is a serious lack of in-depth of information regarding the uptake of heavy metals by vegetables cultivated in the crop soils and irrigated with treated waste water in the study area. Knowledge is also lacking concerning the problems that can be caused in the food chain by heavy metals. This research will focus on tomato plants as the test plant due to its high water uptake; its fruits alone contain 92.5 – 95.0 % water (Davies and Hobson, 1981) and because it is a regular feature in most household diets in the country.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to compare the uptake of chromium and nickel between tomato plants grown in sludge-amended Glen Valley soil and those grown in sludge-absent Glen Valley soil using treated waste water and tap water. The specific objectives were to:

- Determine chromium uptake by the leaves and fruits of the tomato plants
- Determine nickel uptake by the leaves and fruits of the tomato plants
- Determine the effect of the pH of the treated waste water on chromium uptake in the leaves and fruits of tomato plants
- Determine the effect of the pH of the treated waste water on nickel uptake in the leaves and fruits of tomato plants
- Compare the chromium uptake by the leaves and fruits of the tomato plants grown in sludge absent Glen Valley soil to those grown in standard commercial soil using tap water in both cases.

- Compare the chromium uptake by the leaves and fruits of the tomato plants grown in sludge absent Glen Valley soil (using tap water) to those grown in sludge amended Glen Valley soil (using normal treated waste water at pH 8.5 and treated waste water at pH 5.0 ; pH 6.0 ; pH 9.0 and pH 10.0)
- Compare nickel uptake by the tomato leaves and fruits grown in sludge absent Glen Valley soil to those grown in standard commercial soil using tap water in both cases.
- Compare nickel uptake by leaves and fruits of tomato plants grown in sludge absent Glen Valley soil (using tap water) to those grown in sludge amended Glen Valley soil (using normal treated waste water at pH 8.5 and treated waste water at pH 5.0 ; pH 6.0 ; pH 9.0 and pH 10.0)

1.4 RESEARCH QUESTIONS

1. Will there be a significant uptake of chromium and nickel contaminants in response to treatments?
2. Will there be any observable differences in chromium and nickel concentrations in the leaf and fruit tissues of tomato plants as a consequence of variations in the pH values of the Glen Valley treated waste water?
3. At which pH value will tomato plants assimilate the highest concentrations of chromium and nickel heavy metals?
4. Will there be a statistically significant difference in concentration of chromium and nickel uptake in tomato leaves and fruits cultivated using sludge amended Glen Valley soils with treated waste water as compared with using sludge absent Glen Valley soils with tap water?

1.5 SIGNIFICANCE OF THE STUDY

- The results of this study could provide valuable insight into the behavior and nature of chromium and nickel uptake by tomato plants to the food chain and hence help farmers in developing techniques or methods that they could use for reducing the assimilation of these heavy metals in tomato plants

- The results of this study could act as an early warning signs for the increasing levels of chromium and nickel in the food chain as a result of treated waste water use and sludge application to soil.
- The knowledge acquired from this study should provide valuable information for farmers who want to start the practices of treated waste water use and sludge application to soils.

1.6 OUTLINE OF STUDY

- The introduction to the study is given in chapter 1 followed by a background of the study area in chapter 2. The relevant literature to the study is reviewed in chapter 3. Thereafter, the experimental methods and analytical techniques are detailed in chapter 4. Results and discussions are presented in chapter 5. The summary of major findings which include the conclusions and recommendations are presented in chapter 6 followed by the references and the appendices are inserted at the end.

CHAPTER 2

STUDY AREA

2.1 LOCATION

Botswana is located in Southern Africa and land locked by South Africa, Namibia, Zimbabwe and Zambia. Gaborone the capital city of Botswana is situated in the southern part of the country between latitudes 24^0 45 South and longitudes 25^0 55 East at about 1 000 meters above sea level.

Glen Valley is a peri-urban area situated in North-Eastern Gaborone. The surrounding areas are primarily residential, recreational or open space. The area is relatively flat and prone to flooding due to the proximity of river channels. The Glen Valley farms which is the study area (consist of 234 hectares) had been identified as ideal for agriculture. The soils are suitable and treated waste water from a nearby treatment plant can be utilized (Government of Botswana, 1998).

Gaborone has a semi-arid climate with a mean annual rainfall of between 250 mm and 450 mm (khupe, 1996). Almost all rain occurs during the months of October to April and its incidence is highly variable in both time and space. The use of treated waste water is becoming a viable option in the Glen Valley farms. As Botswana's population grows, water usage also grows thereby generating high volumes of effluent water discarded as waste water throughout the country. Such high volumes of water in a country with persistent drought and unreliable rainfall can be of great agronomic and economic importance. Irrigation with treated waste water can increase the available water supply or release water for alternate uses (Food and Agriculture Organization, 1992).

2.2 PHYSICAL ENVIRONMENT

The Glen Valley farms soils are predominantly sandy loam to sand occurring in an alluvial-cum-colluvial landscape, with patches of vertisolic clayey materials alternating with areas of more sandy and, even, gravelly deposits (Dikinya and Areola, 2010). The soils are all texturally very similar irrespective of taxonomic classification and when mapped on a scale 1:20 000, are classified as luvisols, lixisols, cambisols, calcisols, regosols and arenosols (Food and Agriculture Organization, 1988).

The Glen Valley farms are situated within the eastern hardveld vegetation province. The existing soil systems support crops that are cultivated under waste water irrigation and these crops include tomatoes, spinach, okra, maize, cabbage, olive, Lucerne, butternuts, and green pepper (Dikinya and Areola, 2010). Vegetable farming in the study area is exclusively dependent on treated sewage water from the Glen Valley treated waste water plant. On the ground survey showed that drip irrigation is the most common method used by farmers, furrow irrigation and drag lines were observed in a handful of the farms. The study site combines the use of drip irrigation and drag lines.

In terms of weather patterns, Botswana's annual climate ranges from months of dry temperate weather during winter to days or weeks of sub-tropical humidity interspersed with drier hot weather during summer. In summer (October to March) temperatures rise to above 34^0C (93.2^0F) in the extreme north and south-west. In winter (which lasts from April to September) there is frequent frost at night and temperatures may fall below 2^0C (35.6^0F) during the day, but skies are usually cloudless and sunny. Due to the clear skies and low relative humidity, there is maximum insulation during the day and rapid energy loss at night. This has resulted in a wide diurnal change in temperature with hotter days and relatively colder nights. Evaporation rates are consequently very high ranging from 1.8 m to 2.0 m annually for surface water (Khupe, 1996). Summer is heralded by a windy season, carrying dust from the Kalahari, from about late August to early October (Parsons, 1999). Annual rainfall, brought by winds from the Indian Ocean, averages 460 mm (18 in.), including a range from 640 mm (25 in.) in the extreme north-east to less than 130 mm (5 in.) in the extreme south-west. The rains are almost entirely limited to summer downpours between December and April, which also mark the season for ploughing and planting. Cyclical droughts, lasting up to five or six years in every two decades, can limit or eliminate harvests and reduce livestock to starvation (Parsons, 1999).

2.3 HISTORY OF GLEN VALLEY

The intent of the Glen Valley horticultural plan was to create a well-designed irrigation project which would cater to small scale commercial agricultural plots for horticultural purposes with some other activities like flower gardening and perhaps poultry and small livestock breeding. The idea was to allocate portions of land to agricultural investors who were conversant with the irrigation systems and who would utilize the land to its fullest potential, in order to produce and provide fresh agricultural produce for the city of Gaborone and its surrounding areas (Mbiba, 1995). The plan was approved in September 1998 on condition that an environmental impact assessment was clearly stipulated in the lease contract and the planning authority stated that close environmental monitoring of the project at the implementation stage was a prerequisite (Government of Botswana, 1998).

2.3.1 Overview of the sampling area

The farm area at the Glen Valley is about 10 km northeast of Gaborone city which is very close in proximity to the Notwane River where about 234 hectares of cropland are being cultivated with secondary treated waste water. The farms are located 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. There are 47 different farms, varying in size from 1 to 10 hectares being managed by private farmers raising a wide variety of arable crops (Dikinya and Areola, 2010). The size of the Glen Valley farm plots varies from 1.5 to 4 hectares. All farm plots are laid sequentially and so are easily serviced. The requirement from the Ministry of Agriculture is to reduce the buffer zone from the Notwane River and its tributaries, in order to utilize the most fertile soils along the riverbanks. As the farm area is a floodplain, investors were warned of the dangers of possible loss of investments and properties. Those areas unsuitable for horticultural purposes (e.g. with soil types susceptible to salinization) have been planned for other agricultural activities, such as the raising of small livestock, poultry, etc. As a result, 63 plots have been designated as good for horticultural purposes and 27 plots for other agricultural uses. No permanent residences are allowed in the project area apart from farm sheds and small quarters to house farm workers (Government of Botswana, 1998). A dozen or so farms have commenced operations. This study was proposed to be conducted on the oldest active farm plot in the Glen Valley area and soil samples were collected from this farm plot. Standard commercial soil samples were also obtained and used for control experiments. Figure 2.1 shows a location map of the Glen Valley project area from which soil samples were collected for this study. This area is situated about 5 km north-east of the Glen Valley waste water treatment plant.

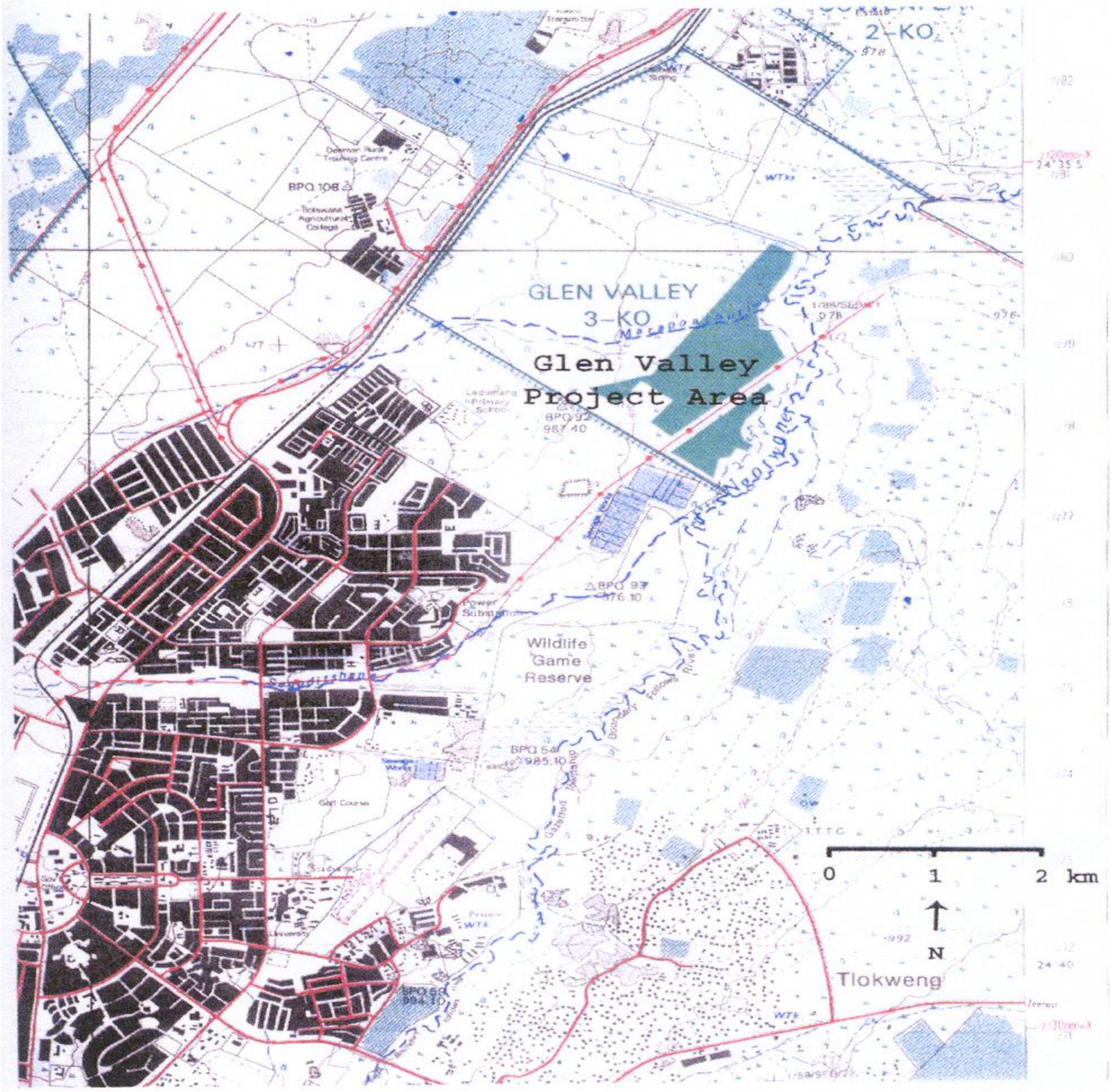


Figure 2.1 Location map of study area: Glen Valley farms

Source: Adapted from Botswana Department of Surveys and Lands (1987)

CHAPTER 3

LITERATURE REVIEW

3.1 BACKGROUND ON MUNICIPAL WASTE WATER

Municipal waste water consists of about 99.9% water, suspended organics and dissolved inorganic solids. Some of the organic substances present in sewage are carbohydrates, fats, soaps, proteins, and other natural synthetic organic chemicals from the process industries (Food and Agriculture Organization, 1992). Table 3.1 shows the compositions of the major constituents of strong, medium and weak municipal waste waters (Abdel-Ghaffar et. al, 1988). Municipal waste water includes domestic (or sanitary) waste water, industrial waste water, infiltration and inflow into sewer lines and storm water runoff (Liu and Liptak, 1997).

Table 3.1. Composition of municipal waste water

Contaminant	Weak/(mg/L)	Medium/(mg/L)	Strong/(mg/L)
Solids, total (TS)	350	700	1200
Dissolved, total (TDS)	250	500	850
Suspended Solids (SS)	100	200	350
Nitrogen, total as (N)	20	40	85
Phosphorous, total (P)	6	10	20
Chlorides[1]	30	50	100
Alkalinity as ($CaCO_3$)	50	100	200
Grease	50	100	150
BOD_5	100	220	400

[1] The amount of TDS and chloride should be increased by the concentrations of these constituents in the carriage water.

Source: (Abdel-Ghaffar et. al, 1988)

A number of inorganic substances ranging from domestic to industrial up to and including potentially toxic elements such as copper, zinc, arsenic, lead, chromium, mercury, cadmium, etc., are also present in municipal waste water. Even if these potentially toxic elements are not at concentrations that could endanger humans, they might as well be at phytotoxic levels thus restricting their agricultural usage (Abdel-Ghaffar et al., 1988).

3.2 CONVENTIONAL WASTE WATER TREATMENT

Conventional waste water treatment follows a combination of physical, chemical, and biological processes and operations to remove solids, organic matter and, sometimes, nutrients from waste water. Different degrees of treatment, in order of increasing treatment level, are preliminary, primary, secondary, and tertiary and/or advanced waste water treatment. In some countries, disinfection to remove pathogens sometimes follows the last treatment step (Food and Agriculture Organization, 1992). A generalized waste water treatment flow diagram is shown in Figure 3.1

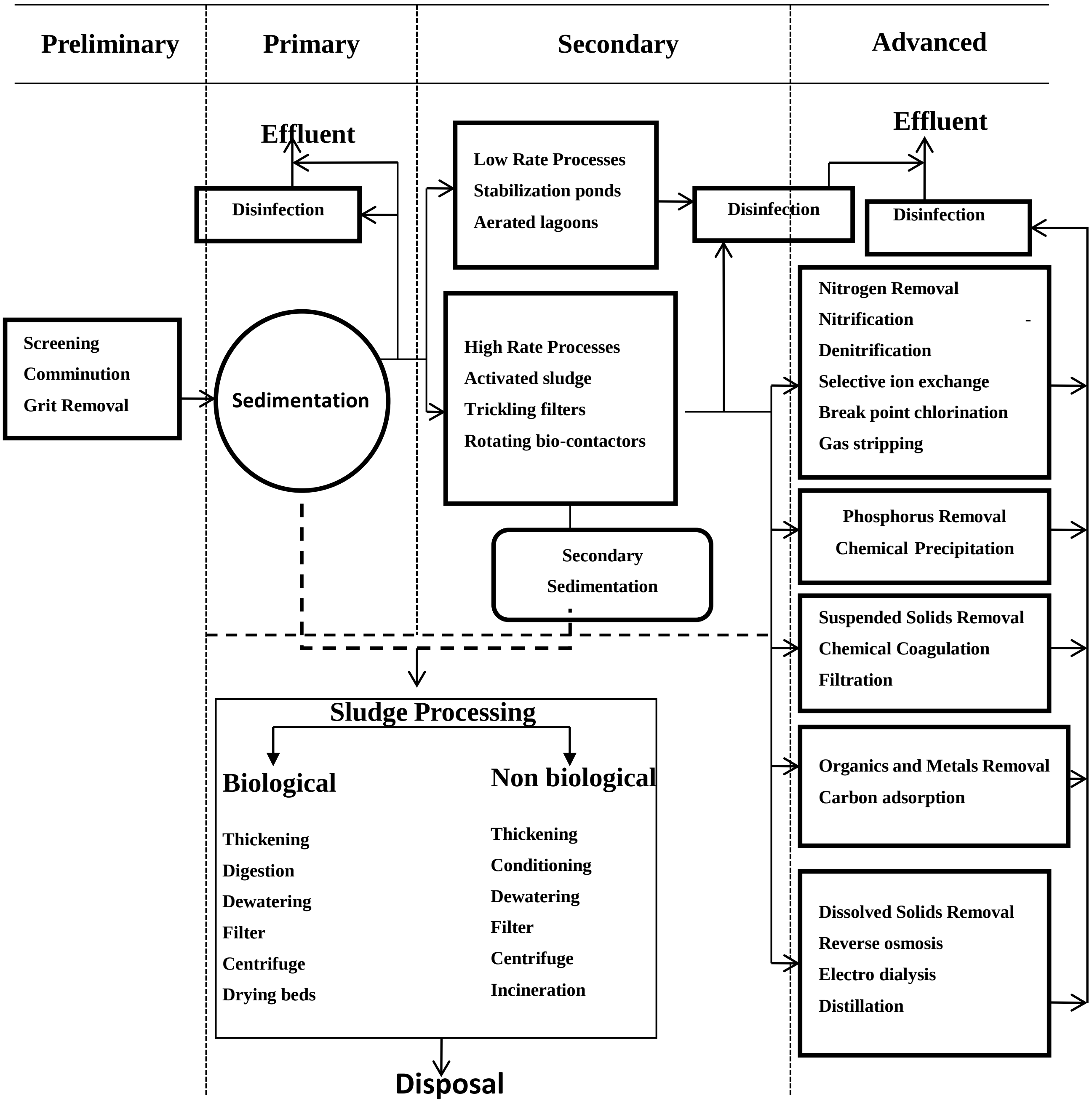


Figure 3.1 Generalized flow diagram for municipal waste water treatment

3.2.1 Preliminary treatment

The preliminary treatment aims to remove coarse solids and other large materials often found in raw waste water. Removal of these materials is necessary to enhance the operation and maintenance of subsequent treatment units. Preliminary treatment operations typically include coarse screening, grit removal and, in some cases, combination of large objects (Aganga et al., 2005).

In grit chambers, the velocity of the water through the chamber is maintained sufficiently high, or air is used, so as to prevent the settling of most organic solids. Grit removal is not included as a preliminary treatment step in most small waste water treatment plants. Comminutors are sometimes adopted to supplement coarse screening and serve to reduce the size of large particles so that they will be removed in the form of sludge in subsequent treatment processes. Flow measurement devices, often standing-wave plumes, are always included at the preliminary treatment stage (Food and Agriculture Organization, 1992).

3.2.2 Primary treatment

The objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation, and the removal of materials that will float (scum) by skimming. Approximately 25% to 50% of the incoming biochemical oxygen demand (BOD_5), 50% to 70% of the total suspended solids (SS), and 65% of the oil and grease are removed during primary treatment. Some organic nitrogen, organic phosphorus, and heavy metals associated with solids are also removed during primary sedimentation but colloidal and dissolved constituents are not affected. The effluent from primary sedimentation units is referred to as primary effluent (Aganga et. al., 2005).

The minimum level of pre-application treatment required for waste water irrigation in most industrialized countries is the primary treatment (Food and Agriculture Organization, 1992). This may be sufficient enough if the waste water is used for irrigating crops that are not consumed by humans or to irrigate orchards, vineyards and some processed food crops. However, as a precautionary measure against nuisance conditions in storage or flow-equalizing reservoirs, some form of secondary treatment is a normal requirement in these countries, even in the case of non-food crop irrigation. It may be possible to use at least a portion of primary effluent for irrigation if off-line storage is provided (Aganga et al., 2005).

3.2.3 Secondary treatment

In the secondary treatment, there is further treatment of the effluent from primary treatment to remove the residual organics and suspended solids. In most cases, secondary treatment follows primary treatment and involves the removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes. Aerobic biological treatment is performed in the presence of oxygen by aerobic micro-organisms (principally bacteria) that metabolize the organic matter in the waste water, thereby producing more micro-organisms and inorganic end-products (principally CO_2, NH_3, and H_2O). Several aerobic biological processes are used for secondary treatment differing primarily in the manner in which oxygen is supplied to the micro-organisms and in the rate at which organisms metabolize the organic matter (Asano et al., 1985). High-rate biological processes are characterized by relatively small reactor volumes and high concentrations of micro-organisms compared with low rate processes. Consequently, the growth rate of new organisms is much greater in high-rate systems because of the well-controlled environment. The micro-organisms must be separated from the treated waste water by sedimentation to produce clarified secondary effluent.

The biological solids removed during secondary sedimentation, called secondary or biological sludge, are normally combined with primary sludge for sludge processing. Common high-rate processes include the activated sludge processes, trickling filters or bio-filters, oxidation ditches, and rotating biological contactors (RBC). A combination of two of these processes in series (e.g., bio-filter followed by activated sludge) is sometimes used to treat municipal waste water containing a high concentration of organic material from industrial sources (Bhatia, 2005).The Gaborone city council sewage department waste water treatment plant is a conventional treatment plant employing processes or procedures that go only as far as secondary treatment for its waste water intake.

Among the natural biological treatment systems available, stabilization ponds and land treatment have been used widely around the world and a considerable record of experience and design practice has been documented (Food and Agriculture Organization, 1992).

3.3 WASTE WATER STANDARDS AND AGRICULTURAL WATER QUALITY

3.3.1 Quality of irrigation water used for agricultural purposes

The quality of irrigation water is of key significance in arid zones where extremes of temperature and lower relative humidity result in high rates of evaporation, with consequent deposition of salt which tends to accumulate in the soil profile. The physical and mechanical properties of the soil, such as dispersion of particles, stability of aggregates, soil structure and permeability, are very sensitive to the type of exchangeable ions present in irrigation water (Food and Agriculture Organization, 1992).

One other area of agricultural irrigation of concern is the effect of total dissolved solids (TDS) in the irrigation water on the development and growth of plants. Dissolved salts tend to increase the osmotic potential of soil water and an increase in osmotic pressure of the soil solution increases the amount of energy, which plants must expend to take up water from the soil. As a consequence, respiration is increased and the growth and yield of most plants decline progressively as the osmotic pressure increases. Although most plants respond to salinity as a function of the total osmotic potential of soil water, some plants are susceptible to specific ion toxicity (Akande, 2007).

Many of the ions which are harmless or even beneficial at relatively low concentrations may become toxic to plants at higher concentrations, either through direct interference with metabolic processes or through indirect effects on nutrients, which might be rendered inaccessible (Morishita, 1988). As a result of the foregoing, waste water quality standards (Table 3.2) are put in place to guide the discharge and possible reuse in agricultural areas where they pose no threats to plants and the food chain. It is also necessary to check if industrial effluent discharges are the sources of some selected heavy metals in the sludge (Table 3.3) and the final effluent water discharged at the Gaborone city council waste water treatment plant in Glen Valley.

Table 3.2. Botswana Bureau of Standards for waste water effluent quality

Determinant	Unit	Upper limit and range	Class 3 potable water	Comment
Colour	TCU	50	50	Acceptable
Temperature	O^0C	35		Not comparable
pH value at 25^oC		6.0-9.0	5-10	Acceptable
Chemical requirements-micro determinants	Unit			
Chromium VI as Cr	mg/L	0.25		Not comparable
Chromium as Cr (total)	mg/L	0.5	50	Acceptable
Cobalt as Co	mg/L	1.00	1000	Acceptable
Copper as Cu	mg/L	1.00	1000	Acceptable
Nickel as Ni	mg/L	0.30	20	Acceptable

Note: Acceptable means waste water standards are acceptable as class 3 drinking water

Not comparable means standards are not comparable

Source: (Botswana Bureau of Standards, 2004)

Table 3.3. Heavy metal compositions in Gaborone industrial effluent

Type of Industry	Heavy Metal Compositions in Gaborone industrial effluent				
	Ni (μgL^{-1})	Fe(μgL^{-1})	Zn(μgL^{-1})	Cd(μgL^{-1})	Pb(mgL^{-1})
Brewery	72.7	0.0	0.0	0.0	0.0
Paints	92.5	0.0	0.0	0.0	0.0
Pharmaceutical	85.9	0.0	0.0	0.0	0.0
Soaps	66.7	0.0	0.0	0.0	0.0
Phytography	87.5	669.5	0.0	0.0	0.0
Typical Gaborone Sludge	>233.8	>1443.6	>427.9	0.0	>125.5
Typical Gaborone	5.6	5.2	0.0	0.0	0.0
Gaborone city council sewer	20.0 mgL^{-1}	20.0 mgL^{-1}	20.0 mgL^{-1}	5.0 mgL^{-1}	5.0 mgL^{-1}

Sludge value: μgkg^{-1}. 0.0: means no detectable analyte

Source: (Nkegbe and Koorapetse, 2005)

3.3.2 Parameters used in the evaluation of agricultural water quality

Priority agricultural water quality parameters include a number of specific properties of water that are relevant in relation to the yield and quality of crops, maintenance of soil productivity and protection of the environment. These parameters mostly consist of certain physical and chemical characteristics of the water. Table 3.4 presents a list of some of the important physical and chemical characteristics that are used in the evaluation of agricultural water quality (Kandiah, 1990).

Table 3.4. Some parameters used in the evaluation of agricultural water quality

Parameters	Symbol	Unit
Physical		
Electrical conductivity	Ec_w	dS/m[1]
Temperature	T	°C
Colour/Turbidity		NTU/JTU[2]
Chemical		
Acidity/Basicity	pH	
Trace metals		mg/L[3]
Heavy metals		mg/L
Nitrate-Nitrogen	NO_3-N	mg/L
Phosphate Phosphorus	PO_4-P	mg/L
Potassium	K	mg/L

[1] dS/m = deciSiemen/metre in SI Units (equivalent to 1 mmho/cm)

[2] NTU/JTU = Nephelometric Turbidity Units/Jackson Turbidity Units

[3] mg/L = milligrams per litre = parts per million (ppm); also, mg/L ~ 640 x EC in dS/m

Source: (Kandiah, 1990)

3.3.3 Parameters of health significance

The regulation of water quality for irrigation is of international importance because trade in agricultural products across regions is growing and products grown with contaminated water may cause health effects at both the local and transboundary levels (Beuchat, 1998). Issues of integration of the various measures available to attain effective health protection were discussed and reported in a technical report by the World Health Organization, (1989). The effluent quality guidelines for health protection (Food and Agriculture Organization, 1992) based its standard on the view that the actual risk associated with irrigation using treated waste water is far much lower than previously reported.

3.3.4 Guidelines for interpretation of water quality for irrigation

Water quality criteria for irrigation are by nature imprecise. The end result of quality evaluation depends on plant, soil and climatic variables all of which can be interdependent. However, a guideline which serves to identify potential crop production problems inherent in the use of conventional water sources was proposed by Ayers and Westcot (Food and Agriculture Organization, 1985). Table 3.5 shows classification of irrigation water into three groups based on salinity, toxicity and miscellaneous effect.

Table 3.5. Guidelines for interpretation of water quality for irrigation

Potential irrigation problems	Units	Degree of restriction on use		
		None	Slight to moderate	Severe
Salinity (affects crop water availability)				
Ec$_w$[1]	dS/m	< 0.7	0.7 - 3.0	> 3.0
or				
TDS	mg/L	< 450	450 – 2000	> 2000
Infiltration (affects infiltration rate of water into the soil. Evaluate using EC$_w$ and SAR together				
SAR[2] = 0 - 3 and EC$_w$	=	> 0.7	0.7 - 0.2	< 0.2
= 3 - 6	=	> 1.2	1.2 - 0.3	< 0.3
= 6 - 12	=	> 1.9	1.9 - 0.5	< 0.5
= 12 - 20	=	> 2.9	2.9 - 1.3	< 1.3
= 20 - 40	=	> 5.0	5.0 - 2.9	< 2.9
Specific ion toxicity (affects sensitive crops)				
Surface irrigation	SAR	< 3	3 – 9	> 9
Sprinkler	me/L	< 3	> 3	
Chloride (Cl)				
Surface irrigation	me/L	< 4	4 – 10	> 10
Sprinkler	m^3/L	< 3	> 3	
Miscellaneous effects				
pH	Normal range 6.5-8.5			

[1] EC$_w$ = electrical conductivity, a measure of the water salinity, reported in deciSiemens per meter at 25°C (dS/m). TDS = total dissolved solids, reported milligrams per litre (mg/L)

[2] SAR = sodium adsorption ratio. At a given SAR, infiltration rate increases as water salinity increases.

Source: (Food and Agriculture Organization, 1985)

3.3.5 Trace elements and heavy metals

Several elements exist in relatively low concentrations, usually less than a few mg/L, in conventional irrigation waters and they are called trace elements. These elements are not normally included in routine analysis of regular irrigation water, but attention should be paid to them when using sewage effluents, particularly if contamination with industrial waste water discharges is suspected. These include Aluminium (Al), Beryllium (Be), Cobalt (Co), Fluoride (F), Iron (Fe), Lithium (Li), Manganese (Mn), Molybdenum (Mo), Selenium (Se), Tin (Sn), Titanium (Ti), Tungsten (W) and Vanadium (V) (Food and Agriculture Organization, 1992). Heavy metals belong to a special group of trace elements which have been shown to create definite health hazards when taken up by plants. Under this group are included, Nickel (Ni), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn). These are called heavy metals because in their metallic form, their densities are greater than 4 g/cm^3. Table 3.6 refers to chromium and nickel concentration levels in secondary treated municipal waste water effluents and irrigation water.

Table 3.6. Compositions of secondary treated municipal waste water effluents and irrigation water

Parameter	Secondary Effluent[a]		
	Range	Typical	Irrigation Water Quality Criteria [b]
pH	6.8–7.7	7.0	6.5–8.4
Nickel (*u*g/L)	5–500	10.0	200
Chromium (*u*g/L)	<1–100	1.0	100

[a] Adapted from Asano et al., (1985) and Treweek (1985)

[b] From Westcot and Ayers (1985) and National Academy of Sciences (1972)

3.4 HEAVY METAL REMOVAL METHODOLOGIES

In the past few decades several methods have been devised for the treatment and removal of heavy metals from waste water and the degree of success varies. Most commonly used procedures for removing metal ions from aqueous streams include reverse osmosis, solvent extraction, lime coagulation, chemical precipitation and ion exchange (Rich and Cherry, 1987). A particular study (Patoczka et. al., 1998) of trace heavy metal removal with ferric chloride from waste water showed that chromium and nickel concentrations of 0.10 mg/L and 0.08 mg/L, respectively, being targeted, could not be achieved by lime precipitation or ion exchange. Upon polishing of the lime precipitation supernatant with ferric chloride at 30 mg/L dose, it removed both chromium and nickel to the 0.01 mg/L range in unfiltered samples.

3.5 EFFECTS OF IRRIGATED WASTE WATER USE ON PLANTS

3.5.1 The pH factor

pH is a measure of how acidic or basic water is. It can be defined as the negative logarithm of the activity of H^+ ions: $pH = -\log [H^+]$

where $[H^+]$ is the concentration of H^+ ions in moles per liter (a mole is a unit of measurement, equal to 6.022×10^{23} atoms). The range goes from 0-14. The pH measures accurately the relative amount of free hydrogen and hydroxyl ions in the water. Pure water for example is said to be pH neutral, with a pH value close to 7.0 at 25 ^{0}C. The pH of waste water needs to remain between 6.0 and 9.0 to protect organisms. Solutions with a pH less than 7 (at 25^{0}C) are said to be acidic and solutions with a pH greater than 7.0 (at 25^{0}C) are said to be basic or alkaline. When an acid is dissolved in water, the pH will be less than 7.0 (if at 25^0C) and when a base , or alkali is dissolved in water the pH will be greater than 7.0 (if at 25^0C). A solution of a strong acid, such as hydrochloric acid, at concentration 1 mol dm^{-3}, has a pH 0.0. A solution of a strong alkali, such as sodium hydroxide, at concentration 1 mol dm^{-3} has a pH 14.0. The pH can be affected by chemicals in water and waste water; hence pH is an important indicator of water quality that is changing with chemical addition. It is reported in "logarithmic units," like the Richter scale, which measures earthquakes. Each number represents a 10-fold change in the acidity or basicity of the water. Water with a pH 5.0 is ten times more acidic than water having a pH 6.0. The pH of water and waste water can be measured with a pH meter.

3.5.1.1 Factors affecting pH

1. The concentration of carbon dioxide in the water: Carbon dioxide (CO_2) enters a water body from a variety of sources, including the atmosphere, runoff from land, release from bacteria in the water, and respiration by aquatic organisms. This dissolved CO_2 forms a weak acid. Natural, unpolluted rainwater can be as acidic as pH 5.6, because it absorbs CO_2 as it falls through the air. Because plants take in CO_2 during the day and release it during the night, pH levels in water can change during the day and at night.

2. Geology and Soils of the watershed: Acidic and alkaline compounds can be released into water from different types of rock and soil. When calcite ($CaCO_3$) is present, carbonates (HCO_3, CO_3^{-2}) can be released, increasing the alkalinity of the water, which raises the pH. When sulfide minerals, such as pyrite, or "fool’s gold," (FeS_2) are present, water and oxygen interact with the minerals to form sulfuric acid (H_2SO_4). This can significantly drop the pH of

the water. Drainage water from forests and marshes is often slightly acidic, due to the presence of organic acids produced by decaying vegetation.

3. Drainage from Mine Sites: Mining for gold, silver, and other metals often involves the removal of sulphide minerals buried in the ground. When water flows over or through sulphide waste rock or tailings exposed at a mine site, this water can become acidic from the formation of sulphuric acid. In the absence of buffering material, such as calcareous rocks, streams that receive drainage from mine sites can have low pH levels.

4. Air Pollution**:** Air pollution from car exhaust and power plant emissions increases the concentrations of nitrogen oxides (NO_2, NO_3) and sulfur dioxide (SO_2) in the air. These pollutants can travel far from their place of origin, and react in the atmosphere to form nitric acid (HNO_3) and sulfuric acid (H_2SO_4). These acids can affect the pH of streams by combining with moisture in the air and falling to the earth as acid rain or snow.

3.5.1.2 Importance of pH

The pH of water determines the solubility (amount that can be dissolved in the water) and biological availability (amount that can be utilized by aquatic life) of chemical constituents such as nutrients (phosphorus, nitrogen, and carbon) and heavy metals (nickel, copper, cadmium, chromium etc.). In the case of heavy metals, the degree to which they are soluble determines their toxicity and possible uptake by plants. Metals tend to be more toxic at lower pH because they are more soluble and more bio-available.

3.5.2 Effects on plants due to increased acidity or basicity

The pH hardly poses any problems on its own. The normal pH range for irrigated waste water is from 6.5 to 8.4; pH values that fall outside this range are indicators that the water is abnormal in quality. A low pH can result in a possible toxicity of iron, manganese, zinc and copper in certain plants. It could also cause the deficiency of calcium and / or magnesium and leads to ammonium sensitivity in plants. High basicity on the other hand could most probably cause deficiency of iron, manganese, zinc, copper and boron. Result of tests carried out during this study on the pH level of irrigated treated waste water used for tomato production in the farm site of Glen Valley farms of Gaborone, Botswana gave an average pH 8.5 and the average pH of ordinary tap water used was 7.0.

3.5.3 Effects on plants due to increased concentrations of trace elements

In conventional irrigation waters, some elements are normally present although in relatively low concentrations, say a few mg/L and these are termed trace elements. They are often excluded in routine analysis of regular irrigation water; nonetheless, care must be taken when applying sewage effluents and sewage sludge. Heavy metals, however, are species of trace elements, and are known to create definite health hazards when taken up by plants. Under this species are included, arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn). The densities of these heavy metals are greater than 4 g/ cm^3 in their metallic form (Kandiah, 1990). Table 3.7 presents phytotoxic threshold levels of some selected trace elements.

Table 3.7. Threshold levels of some trace elements for crop production

Symbol /(Element)	Recommended maximum concentration (mg/L)	Remarks
Cd/(cadmium)	0.01	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg/L in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants
Co/(cobalt)	0.05	Toxic to tomato plants at 0.1 mg/L in nutrient solution. Tends to be inactivated by neutral and alkaline soils.
Cr/(chromium)	0.10	Not generally recognized as an essential growth element. Conservative limits recommended due to lack of knowledge on its toxicity to plants.
Ni/(nickel)	0.20	Toxic to a number of plants at 0.5 mg/L to 1.0 mg/L; reduced toxicity at neutral or alkaline pH.
Zn/(zinc)	2.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured or organic soils.

Source: (Food and Agriculture Organization, 1985)

3.6 BACKGROUND INFORMATION ON TEST CROP (TOMATO)

The Tomato (*Lycopersicon esculentum*) is an herbaceous, usually sprawling plant in the nightshade family that is typically cultivated for the purpose of harvesting its fruit for human consumption. The fruit of most varieties ripens to a distinctive red color.

Tomato plants typically reach 1–3 metres (3–10 feet) in height, and have a weak, woody stem that often vines over other plants. The leaves are 10–25 centimeters (4–10 inch) long, odd pinnate, with 5–9 leaflets on petioles (Acquaah, 2002) and each leaflet measures up to 8 centimeters (3 inch) long, with a serrated margin; both the stem and leaves are densely glandular-hairy. The flowers are 1–2 centimeters (0.4–0.8 inch) in width, yellow, with five pointed lobes on the corolla; they are borne in a cyme of 3–12 together. Tomatoes are perennial and are often grown outdoors in temperate climates as an annual crop.

There are two types of tomatoes commonly grown; determinate and indeterminate tomatoes. Most commercial varieties are determinate. These “bushy” types have a defined period of flowering and fruit development. Most heirloom garden varieties and greenhouse tomatoes like the ones used in this research are indeterminate which means they produce flowers and fruit throughout the life of the plant (Kelley and Boyhan, 2006). Specifically, tomato variety used in the project was the “25 tomato super beef steak seeds”. This variety grows firm and strong, offer better resistance to blight and fungus though it takes longer time to produce fruits; nonetheless of good quality (see appendix B)

Most cultivated tomatoes require around 75 days from transplanting to first harvest and can be harvested for several weeks before production declines. Ideal temperatures for tomato growth are 70^0F-85^0F or 21^0C-29^0C during the day and 65^0F-70^0F or 18^0C-21^0C at night.

Significantly higher or lower temperatures can have negative effects on fruit set and quality. The optimum pH range for tomato production is 6.2 to 6.8 (Kelley and Boyhan, 2006). Jones (2008) provided instructions on how a grower can produce transplants for greenhouse tomato production. He stated that it is best to have a seedling greenhouse, or growth chamber, where the growing conditions (temperature, humidity, light etc.) can be precisely controlled. The common procedure is to transplant seedlings from a seedling bed or cube into a larger cube or pot before transplanting. The transplants should be set into the soil 1 inch (2.54 centimeters) deeper than previously grown, or up to the cotyledon leaves.

3.7 IRRIGATION FOR TOMATOES

For a tomato plant, irrigation is used to replace the amount of water lost by transpiration and evaporation. This amount is also called crop evapotranspiration (ETc) (Simonne, 2003). Tomato water requirement (ETc) depends on the stage of growth and evaporative demand. ETc can be estimated by adjusting the reference evapotranspiration (ETo) with a correction factor called the crop factor (Kc; equation (1)):

Crop water requirement = crop coefficient **X** reference evapotranspiration

ETc = Kc **X** ETo…………………….. Eq. (1)

3.8 TOMATO IRRIGATION REQUIREMENTS (IR)

Irrigation systems are generally rated with respect to application efficiency (Ea) which is the fraction of the water that has been applied by the irrigation system and that is available to the plant for use (Simonne, 2003). In general, Ea is 60-80% for overhead irrigation, 20-70% for seepage irrigation, and 90-95% for drip irrigation.

A tomato irrigation requirement (IR) is determined by dividing the desired amount of water to provide to the plant (ETc) by Ea as a decimal fraction (Eq. 2).

Irrigation requirement (IR) = Crop water requirement divided by application efficiency

IR = ETc/Ea…………………………… Eq. (2)

3.9 NATURE OF HEAVY METALS OF CONCERN (CHROMIUM AND NICKEL)

3.9.1 Chromium

Chromium is a non-essential element to plants and its compounds are highly toxic to plants.

There are many valence states of chromium which are unstable and short lived in biological systems however, the most stable forms of Chromium are the trivalent Chromium (III) and the hexavalent Chromium (VI) species. Its complex electronic chemistry has been a serious challenge in unraveling its uptake and bio-accumulation pattern in plants. It is probably due to this reason that chromium has received little attention from plant scientists as compared to other toxic trace metals like mercury, cadmium, aluminum and lead. In soil, chromium concentrations range between 1 and 3 000 mg/kg, in sea water 5 to 800 µg/L, and in rivers and lakes 26 µg/L to 5.2 mg/L (Kotaś and Stasicka, 2000). The relation between chromium (III) and chromium (VI) strongly depends on the pH and oxidative properties of the location; however, the chromium (III) is the dominating species (Kotaś and Stasicka, 2000). In some areas the ground water can contain up to 39 µg/L of total chromium of which 30 µg/L is present as chromium (VI) (Gonzalez, Ndung'u and Flegal, 2005).

3.9.2 Chromium in water and waste water

Many chromium compounds are relatively water insoluble. Chromium (III) oxides are only slightly water soluble; therefore, concentrations in natural waters are limited. Cr^{3+} ions are rarely present at pH values over 5.0, because hydrated chromium oxide is hardly water soluble. Hexavalent chromium in industrial waste waters mainly originates from tanning and painting. Waste water usually contains about 5 ppm of chromium. Kotlhao, Ngila and Emongor (2006) gave an assessment of 111.00 ± 6.56 µg/L in the month of August 2004 and 98.35 ± 4.81 µg/L in the month of March 2005, respectively, for the level of Cr^{3+} detected in secondary treated sewage water for crop irrigation in Gaborone, Botswana. The maximum allowable value of hexavalent chromium for irrigation water is 0.1 mg/L (Food and Agriculture Organization, 1985). Botswana Bureau of Standards (2004) also set a maximum limit of 0.50 mg/L for chromium effluent quality.

3. 9.3 Chromium in soil

Chromium solubility in soil water is lower than that of other potentially toxic metals. This explains the relatively low plant uptake. The solubility of chromium (III) in soil is dependent on pH (Palmer and Wittbrodt, 1991) and decreases dramatically at $pH > 4.5$. Chromium (VI) compounds are toxic at low concentrations for both plants and animals. The mechanism of toxicity is also pH dependent. Chromium (VI) is more mobile in soils than chromium (III) compounds, but is usually reduced to chromium (III) compounds within a short period of time. Soluble chromates are converted to insoluble chromium (III) salts and consequently, availability for plants decreases. This mechanism protects the food chain from high amounts of chromium. Chromate mobility in soils depends on both soil pH and soil sorption capacity, and on temperature. Adsorption of chromium (VI) is considerably less at neutral to alkaline pH than at more acidic pH values (Bartlett and Kimble, 1976; Bartlett and James, 1983). The intensity of adsorption will depend on the type and quantity of soil components, as well as the pH and the presence of competing ligands such as phosphate. The availability of soil chromium to the plant depends on the oxidation state of chromium, pH, and the presence of colloidal binding sites and chromium-organic complexes that would influence its total solubility (Hossner et al., 1998).

3.9.4 Chromium concentration in plants (case studies)

The first interaction that chromium has with a plant is during the uptake process (Shanker et al., 2005). In a study carried out by Mangabeira et al. (2005) of the uptake, transport and localization of chromium in tomato plants using Secondary Ion Mass Spectrometry (SIMS) and Electron Probe Microanalysis (EPMA) they detected chromium in decreasing order of concentration in the roots, stems and leaves. They reported no detection of chromium in the fruits of tomato plants. Golovatyj et al., (1999) showed that chromium distribution in crops had a stable character which did not depend on the soil properties and concentration of this element in the water; the maximum quantity of element contaminant was always contained in the roots and a minimum in the vegetative and reproductive organs. The use of metabolic inhibitors diminished chromium (VI) uptake whereas it did not affect chromium (III) uptake, indicating that chromium (VI) uptake depends on metabolic energy and chromium (III) does not (Skeffington et al., 1976). In contrast, an active uptake of both chromium species, slightly higher for chromium (III) than for chromium (VI), was found in the same crop (Ramachandran et al., 1980). In 7 out of 10 crops analyzed, more chromium accumulated when plants were grown with chromium (VI) than with chromium (III) (Zayed et al., 1998). Table 3.8 gives an account of chromium uptake and accumulation by some crops.

Table 3.8. Chromium uptake in plants (Case studies)

Chromium concentration in medium	Uptake and accumulation pattern	Crop/plant	Reference
0, 5, 30, 45, 60, 75, 90, 105, 120 and 135 mg/Kg Cr(III) and Cr(VI)	2.8 Cr(III) and 3.14 Cr(VI) µg/g	Spinach	Singh (2001)
6, 12, 24 mg/L Cr	Cr more in roots than shoots in A and more in shoots than roots in B	A:*Dactylis glomerate* B:*Medicago sativa*	Shanker (2003)
0.5, 1.5, 25 µg/mL ^{51}Cr radio-labeled (^{51}Cr is a radioactive isotope of chromium)	Progressive increase with more Cr in roots than shoots	Rice	Mishra et al., (1997)
0, 50, 100 mg/L Cr(III)	Roots took up more than shoots and not detected in fruits	Tomato	Moral et al. (1996)
0-200 mg/Kg	Progressive increase with more Cr in roots than shoots	Sunflower, maize and Viciafaba	Kocik and Ilavsky (1994)
0.25 and 1.0 mg/L	75-100% steady state removal; 1-2 mg/Kg dry weight at the rate of 250-667 mg/day m^2	*Lemna minor*	Wahaab et al. (1995)

Source: (Shanker et al., 2005)

3.9.5 Nickel

Nickel is the twenty-fourth most abundant chemical element in the earth's crust, occurring at an average concentration about 75 µg/g. Nickel has an atomic number of 28 and an atomic weight of 58.71. Although it has oxidation states of -1, 0, +1, +2, +3, and +4, the most common valence state in the environment is Ni^{2+} (Cotton and Wilkinson, 1988; Nieboer et al., 1988). Nickel occurs in nature as a trace constituent in a wide variety of minerals, particularly those containing large amounts of iron and magnesium, such as olivine and pyroxenes (Avias, 1972). In minerals in which it is an essential component, it occurs most frequently in combination with sulphur, arsenic, or antimony. Examples include millerite (NiS), red nickel ore (mainly niccolite (NiAs), pentlandite $(Ni, Fe)_9S_8$, and deposits consisting primarily of NiSb, $NiAs_2$, NiAsS, or NiSbS. In Botswana, the most important commercial deposits of nickel contain up to 8% Ni as 70% pyrrhotite, 20% pentlandite and 7% pyrite. This exists in the Phoenix mine (Palmer and Johnson, 2005) and is operated by the Tati nickel mining company near Francistown, Botswana.

3.9.6 Nickel in water and waste water

Nickel is a naturally occurring element that is present in the environment principally in the divalent state (Ni^{2+}). Nickel in dissolved and the particulate form enters the aquatic environment in effluents and leachates, as well as through atmospheric deposition after release from anthropogenic sources (Canadian Environmental Protection Act, 1994). The most water-soluble nickel compounds are nickel chloride hexahydrate (2 500 g/L), nickel sulphate hexahydrate (660 g/L), nickel sulphate heptahydrate (760 g/L), and nickel nitrate hexahydrate (2 400 g/L) (Lide, 1993). Less soluble nickel compounds include hexa-ammine nickel nitrate (45 g/L), nickel (II) hydroxide (0.13 g/L), and nickel carbonate (0.09 g/L) (Lide, 1993). Nickel subsulphide and nickel oxide are considered to be "insoluble" in water, but both are soluble in acids (Cotton and Wilkinson, 1980; International Program on Chemical Safety, 1991). Nickel is a relatively mobile heavy metal. In natural waters, nickel is transported in both particulate and dissolved forms. The pH, oxidation-reduction potential, ionic strength, type, and concentration of organic and inorganic ligands (in particular, humic and fulvic acids), and the presence of solid surfaces for adsorption (in particular, hydrous iron and manganese oxides) can all affect the transport, fate, and biological availability of nickel in fresh water and seawater (Semkin, 1975; Callahan et al., 1979). Nkegbe and Koorapetse (2005) reported the level of nickel concentrated in Gaborone industrial effluent at 5.6 µg/L and the amount concentrated in the Glen Valley sludge varied from 27.5 – 33.1 mg/Kg (Nkegbe, 2005). The maximum allowable value of nickel for irrigation water is 0.2 mg/L (Food and Agriculture Organization, 1985). Botswana Bureau of Standards (2004) has a permissible limit of 0.30 mg/L for nickel effluent quality.

3.9.7 Nickel in soil

The bio-availability of nickel in soils varies, depending in particular upon the forms of nickel present and the soil pH. Nickel that is bound in the lattice of naturally occurring silicate minerals (e.g., olivine or pyroxenes) is relatively unavailable for uptake by plants compared to water soluble forms, such as nickel sulphate, which may be deposited on surface soils from the atmosphere (Canadian Environmental Protection Act, 1994). Generally, bio-availability increases with decreasing soil pH. In acidic soils, nickel-bearing sulphide and, to a lesser extent, silicate minerals (and possibly nickel oxide) can dissolve over time, and relatively little nickel is removed from soil pore waters by adsorption processes. Nickel complexed by organic ligands dissolved in soil pore waters is expected to be less bio-available than free nickel ions (Canadian Environmental Protection Act, 1994).

3.9.8 Nickel concentration in plants (case studies)

Nickel is very easily extracted from soils by plants and the nickel contents of plants are simple functions of the nickel content of soils (Kabata-Pendias and Mukherjee, 2007). The concentration of nickel in plant tissues provides an indication of the concentrations of bio-available forms of nickel in the soils in which they are growing. Although the transport and storage of nickel by plants seems to be metabolically controlled, it is mobile in plants and is likely to accumulate in both the leaves and seeds (Kabata-Pendias and Pendias, 2001). Both plant and soil factors affect nickel uptake by plants, although the most important factor is the influence of soil pH; uptake is reduced significantly by increasing soil alkalinity (Kabata-Pendias and Pendias, 2001). Table 3.9 summarizes the uptake of nickel by some plants.

Table 3.9. Nickel Uptake in plants (Case studies)

A study of uptake of trace metals by tomato plants from a nutrient solution.	They observed that nickel accumulated in the fruit more than observed with other metals including chromium and cadmium.	(Moral et al., 1994)
The uptake of nickel and cobalt by tomato plants in a series of pot experiments	Observation was that nickel was concentrated in the roots but was transferred to all parts of the plant	(Woodward *et al*., 2003)
The study of uptake of nickel by tomato and squash plants from soil amended with coal fly ash.	Nickel concentration generally declined as the plant matured, suggesting that early uptake was diluted by growth. Daily watering might have leached available nickel from the rhizosphere soil.	(Brake *et al*., 2004)
Study of uptake of nickel by tomatoes in a series of pot experiments with soil amended with nickel chloride solution	Of the nickel taken up by the tomato plants about 75% was translocated to the shoots and only 25% to the fruits	(Poulik, 1999)

Source: (Environment Agency Science Report, 2009)

3.10 WASTE WATER USE CASE STUDIES

3.10.1 Waste water re-use for agricultural irrigation: Case study in León-Guanajuato, central Mexico.

The city of León-Guanajuato with a population 1.2 million is one of the fastest growing cities in Mexico, North America, and is highly dependent on groundwater for public supply (United Nations Environment Program, 2003). Groundwater is abstracted mainly from aquifers downstream of the city, including areas where waste water is used for agricultural irrigation. Studies (Foster, 1992; Chilton et al, 1998) showed that high rates of recharge from excess waste water irrigation on alfalfa and maize south-west of the city (coupled with no agricultural abstraction) have helped maintained groundwater levels within 10 metres depth, despite intensive abstraction from deeper horizons for municipal water supply. In adjacent areas water levels are falling at 2 to 5 m/a.

Further observation (United Nations Environment Program, 2003) showed that though the waste water contained large concentrations of chromium salts, the chromium content of the groundwater remained low. Test carried out on the soil samples showed that both chromium and other heavy metals were accumulating in the soil, with very little passing below a depth of 0.3 metre. These accumulations of heavy metals on the surface soil could potentially impact on their uptake by plants. Experimental results from solution culture and greenhouse potted plants have shown that plant uptake usually increases with increased trace-element concentration in the growing medium (Chang and Page, 1977).

3.10.2 Waste water use case studies in California

Beneficial use of waste water has been in practice since the 1890s in California, USA. By the turn of the century, say around 1987, more than 0.899 Mm^3/d of municipal waste water (7-8% of the production) was used for various farm applications. Historically, agricultural use has dominated, and continues to play significant roles; however, the past decade has seen reclaimed waste water utilized for landscape irrigation in urban areas and for ground water recharge. Most of the reclaimed water (78%) is used in the central valley and south coastal regions of California. In agricultural use of treated effluent, at least twenty different food crops are being irrigated as well as at least eleven other crops and nursery products as indicated in Table 3.10.

Table 3.10. Types of crops irrigated with reclaimed water in California

Food Crops	Non-food Crops
Apples, corn, asparagus, grapes, peaches, Avocados, lettuce, barley, beans, plums, Peppers, broccoli, pistachios, cabbage, Cauliflower, squash, celery, sugar beets Citrus and wheat	Alfalfa ,Christmas trees, Clover, Corn, Cotton, eucalyptus trees, flower seeds hay, sod trees, vegetable seeds

Source: (California State Water Resources Control Board, 1990)

In several surveys reported in the review of the California municipal waste water reclamation in 1987, all the waste water treatment plants producing effluents for beneficial uses were found to provide at least secondary treatment.

3.10.3 Current and future use of waste water in Tunisia

Waste water use for agricultural purposes has been practiced in Tunisia for several decades and currently it is an integral part of the national water resources strategy. In the year 1988, the volume of treated waste water available was 78 million m^3 and in the year 2000 it would probably exceed 125 million m^3 (Bahri, 1988). Use of treated effluents is seasonal in Tunisia (spring and summer time) and the effluent is often mixed with groundwater before being applied to irrigate citrus and olive trees, forage crops, cotton, golf courses and hotel lawns.

In the period 1981 to 1987, the Ministries of Agriculture and Public Health, with assistance from the United Nations Development Program carried out studies designed to assess the effects of using treated waste water and dried, digested sewage sludge on crop productivity and on the hygienic quality of crops and soil. Treated waste waters and dried, digested sludge from the La Cherguia (Tunis) and Nabeul activated sludge plants were used in the studies and irrigation with groundwater was used as a control. At La Soukra, tests were conducted on sorghum (*Sorghum vulgare*) and pepper (*Capsicum annuum*) using flood irrigation and furrow irrigation, respectively. Clementine and orange trees were irrigated at Oued Souhil Nabeul (Bahri, 1988).

The average quality characteristics of the treated waste water from La Soukra are shown in Table 3.11. The effluent contains moderate to high salinity but presents no alkalization risk and trace element concentrations are below toxicity thresholds.

Evaluation of the fertilizing value of the effluent in relation to crop uptake suggests that the mean summer irrigation volume of 6 000 m^3/ha would provide an excess of nitrogen (N) and potassium (K_2O) but a deficit of phosphorus (P_2O_5). Application of treated effluent would balance the fertilizing elements but could provide an excess for crop requirements. Excess nitrogen would be of concern from the point of view of crop growth and in relation to groundwater pollution (Bahri, 1988). The application of treated waste waters and sewage sludge at the La Soukra and Oued Souhil experimental stations, where the soils are alluvial and sandy-clayey to sandy, has not adversely affected the physical or bacterial quality of the soils. However, the chemical quality of the soils varied considerably, with an increase in electrical conductivity and a transformation of the geochemical characteristics of the soil solution from bicarbonate-calcium to chloride-sulphate- sodium. Trace elements concentrated in the surface layer of soil, particularly zinc, lead (Pb) and copper (Cu), but did not increase to phytotoxic levels in the short term of the study period (Bahri, 1988).

Table 3.11. Average characteristics of treated waste water (TWW) and well waters (WW) used for irrigation (in mg/L) in La Soukra compared to FAO recommended maximum concentrations.

Parameter	TWW	WW	FAO
pH	7.6	7.6	6.5-8.5
EC	2.97	2.61	3.0
TDS (g/L)	1.82	1.71	2.0
Cr	0.02	NA	0.1
Ni	0.06	0.05	5
Fe	0.33	0.11	5
Pb	0.19	0.16	2
Co	0.05	0.04	0.05
Mn	0.05	0.01	0.2
Cd	NA	NA	0.01

NA: Not Available, EC: Electrical Conductivity (in dS/m at 25°C)

TDS: Total Dissolved Solids

Source: (Bahri, 1988)

3.10.4 Waste water use case study in Botswana

In Botswana Aganga et al. (2005) conducted a study to determine the effect of sewage water on soils and forages irrigated with treated sewage water at the Botswana College of

Agriculture’s farm. The study was conducted for a period of 120 days using established forage pastures of ryegrass (*Lolium multiforum*) and Lucerne (*Medicago sativa*). Heavy metals determined were Fe, Mn, Cu, Zn, Ni, Pb and Cd. Generally the treated sewage water contained relatively low levels of heavy metals. Zn, Ni and Mn concentrations were below the detectable levels in the sewage water while soils and plants had low levels of heavy metals. Comparatively the soil and plants heavy metal levels were much higher than those in the water and the difference was significant ($p<0.05$). There was a low correlation between trace element contents in the water and soil. In addition there was some significant difference ($p<0.05$) in the heavy metal concentration in the sewage water between the months during which the analyses were carried out. However, the sewage water, soils and forage mineral concentrations were within the internationally allowable heavy metal concentration with respect to irrigation, soil loadings and animal feeds. The results showed that the water contained Fe, Cu, Zn, Pb and Cd. The concentration of Mn and Ni were below levels detectable by the Atomic Absorption Spectrophotometer (AAS) procedure. There was no variation in heavy metal concentration with months except for Fe which increased with respect to seasons. The decline was significantly different at $p<0.05$ and similarly the increase in Fe was significantly different at $p<0.05$. The mineral concentrations were within the typical and allowable concentrations required for irrigation water compared to the levels in Table 3.12. Both forages contained some heavy metals including non-essential trace elements such as Pb and Cd. There were some significant differences ($p<0.05$) in concentrations of most constituents determined for Lucerne and rye grass except for Cu (Table 3.12.)

Table 3.12. Mean ±SEM mineral concentration (mg/L) in the soils of the ryegrass and Lucerne fields

Months	Forage	Mn	Fe	Cu	Zn	Ni	Pb	Cd
September	Rye Field	11.344	4.471	0.120	0.367	0.293	0.180	0.030
	Lucerne field	12.019	5.127	0.216	0.233	0.280	0.260	0.035
October	Rye Field	11.344	4.471	0.120	0.367	0.293	0.180	0.030
	Lucerne field	12.019	5.127	0.216	0.233	0.280	0.260	0.035
November	Rye Field	12.442	5.896	0.109	0.188	0.203	0.244	0.040
	Lucerne field	10.849	6.149	1.163	0.206	0.206	0.244	0.040
December	Rye Field	13.037	2.929	0.112	0.291	0.325	0.330	0.035
	Lucerne field	9.7465	3.498	0.180	0.333	0.241	0.366	0.040
SEM	Rye Field	0.2053	0.0506	0.0014	0.0207	0.0164	0.0134	0.0014
	Lucerne field	1.139	0.7288	0.5024	0.0239	0.0222	0.0522	0.0020

Source: (Aganga et al., 2005)

3.11 SUMMARY OF FINDINGS AND PROBLEM TO BE ADDRESSED

There is a serious lack of in-depth of information regarding the uptake of heavy metals by vegetables cultivated in the crop soils irrigated with treated waste water in the Glen Valley farms of Gaborone, Botswana. Nevertheless the use of treated waste water combined with application of sludge to soils could pose a great concern when used to cultivate agricultural crops for human consumption. Reports had shown that agrochemical activities in Gaborone crop soils was causing chromium and nickel accumulation on the surface soils and this could potentially impact on their uptake by plants. One chief factor that could influence the transport, fate and biological availability of these heavy metals in plants is the pH of the treated waste water irrigation. At the Glen Valley farms, the average pH value of the treated waste water used for tomato production is pH 8.5. This pH is beyond the optimum pH range of 6.2 to 6.8 which is suitable for tomato production.

Henceforth, the present study would compare the uptake of chromium and the uptake of nickel between tomato plants (leaves and fruits) grown in sludge-amended Glen Valley soils to those grown in sludge-absent Glen Valley soils using treated waste water at pH 8.5 and tap water at pH 7.0. This study will also compare the chromium uptake by the leaves and fruits of the tomato plants grown in sludge absent Glen Valley soils using tap water at pH 7.0 to those grown in sludge amended Glen Valley soil using treated waste water at pH 8.5 and treated waste water at pH 5.0 , pH 6.0, pH 9.0 and pH 10.0. The results of this pH variation experiments could tell if there is any significant contribution to the uptake of chromium and nickel in the tomato leaves and fruits which invariably could lead to the increase in concentration levels of these heavy metals in tomato plants, the food chain as well as impact on human health.

CHAPTER 4

EXPERIMENTAL METHODS AND ANALYTICAL TECHNIQUES

4.1 AN OVERVIEW OF THE PROJECT DESIGN

The project design consisted of three sets of experiments conducted in the greenhouse after on the ground survey had been carried out at the actual research site in the oldest Glen Valley farm of Gaborone, Botswana. The treated waste water used for the experiments was transported from the Glen Valley site in 50 liter plastic containers. Physical and chemical checks of agricultural significance were carried out on the treated waste water. Standard commercial soil was also collected and used in the control experiment. Chemical parameters analyzed were pH and heavy metals determined by Atomic Absorption Spectrometry. The soil type in the Glen Valley project site was a mixture of Vertic-Cambisol/Vertic-Luvisol soils and Areni-Haplic-Lixisol Ferralic-Arenosol soils. The soil used was uniform for two sets of the experiments which came from the Glen Valley farm site. The third soil type came from a standard commercial soil and contained a mixture of Virgin Vertisol and Cleared Vertisol soils. Parameters such as soil pH, soil electrical conductivity, and Cation Exchange Capacity (CEC) were not the primary focus of this research and hence were not determined. However, the soil analytical data for the Glen Valley farm are presented (Appendix C) for reference purposes. Further routine laboratory analysis of the soil samples had been reported elsewhere (Dikinya and Areola, 2010). Tissue analysis was conducted to determine concentrations of chromium and nickel within the fruits and leaves of the plants; because the fruit is the edible portion of the tomato and also in order to compare between the fruits and the leaves. This was primarily achieved through nitric and sulphuric acid digestions and the quantitative determination of heavy metals concentration was done using a Varian Spectr AA-10/20 Atomic Absorption Spectrometer (AAS).

4.2 THE GREENHOUSE EXPERIMENTS

The greenhouse preparation started in late April of 2009. The greenhouse was constructed with the help of a commercial farmer and expert advice of the Botswana College of Agriculture (BAC). The greenhouse was made out of treated timbers as the base and the fascia; the gusset was made of treated plywood and the covering was ultra violet resistant net shedding as shown in Figure 4.1.

Figure 4.1 Frontal view of the greenhouse used for the tomato production

The next step was to collect sludge amended soil and sludge absent soil of the same type and put them into 30 flower pots. The soil was collected within a 2 m x 2 m grid in a central location on the farm to ensure uniformity and the soil that was dug was at the root level zone. Standard commercial soil was purchased for the control experiments and 5 pots were utilized. At the greenhouse each pot was weighed to ensure that it contained exactly 2.5 kg of soil and the soil was kept wet until seedlings were planted. Tomato cultivation was originally started on Monday 16 March 2009 but after about 7 weeks, a heavy rainstorm destroyed everything. Subsequently, fresh seeds were planted on Sunday 10 May 2009. A total of 35 pots with each pot containing about 2.5 kg of soil were again collected from the Glen Valley farm site. All pots were watered with 100 mL/day of ordinary tap water from 10 May to 24 July (11 weeks) -germination and early growth phase (Tomatoes grow best in slightly acidic soil with an optimum pH level between 6.5 and 7.0 hence the need to use tap water for the initial germination and early development stage).

Treatment commenced on 25 July 2009 with all the 35 germinating potted tomato plants receiving closer monitoring. Treatment with tap water at pH 7.0, treated waste water at pH 8.5 and adjusted treated waste water (at pH 5.0, 6.0, 9.0 and 10.0) was carried out on a need by need basis of each plant. However, to ensure uniformity and consistency in pattern each plant received an equal amount of water (250 mL) by the end of each week; the rate and time of day(s) were determined by the individual plant response to treatment. Some of the plants were watered early in the day to cut down on evaporation losses and also to give the plants plenty of time to dry out. A drip irrigation technique which delivered water right at the soil surface and not on the leaves was used; this was to make sure that water was made available all the time. Irrigation at midday was avoided because that was when evaporation losses were the highest. The average characteristics of representative samples of the Glen Valley treated waste water and ordinary tap water used for experimental irrigation treatments (in mg/L) compared to the Food and Agricultural Organization (FAO) recommended maximum concentrations are shown in Table 4.1.

Table 4.1. Some characteristics of Glen Valley treated waste water (GVTWW) and ordinary tap water (OTW) used for experimental irrigation compared to FAO recommended maximum concentrations

PARAMETER	GVTWW[a]	OTW[b]	FAO[c](mg/L)
pH	9.700	7.200	6.5 to 8. 5
pH with LAN fertilizer	10.400	7.900	NA
Nitrate nitrogen	0.400	0.020	0.0 - 10
Phosphate	0.014	0.013	0.0 - 2.0

Source: a, b (Akande, 2007) c (Food and Agriculture Organization, 1992)

The project contained three experimental designs which were the control, bio-accumulation and pH variation experiments.

4.3 THE CONTROL EXPERIMENTS

The control experiments had 5 tomato pots each filled with 2.5 kg standard commercial soil and treated with ordinary tap water (the pots were labeled CON1, CON2, CON3, CON_{123}a and CON_{123}b for the purpose of identification and analysis). A summary of the control experiments is shown in Table 4.2.

Table 4.2. The Control experiments

TREATMENT TYPE	CODE
T1: Standard commercial soil irrigated with tap water (pH 7.0)	CON1 (Replicates:CON2, CON3, $CON_{123}a$ and $CON_{123}b$)

4.4 BIO -ACCUMULATION EXPERIMENTS

The bio-accumulation experiments had 10 tomato pots each filled with 2.5 kg soil. The pots were labeled B1, B1a, B1b, B1c, B1d, B2, B3, B4, B5 and B6. B1 contained Sludge Absent Glen Valley soil irrigated with ordinary tap water at pH 7.0. B1a, B1b, B1c, and B1d were replicates of B1. B2 contained Sludge Amended Glen Valley soil irrigated with treated waste water collected from the farm site. B3, B4 and B5 and B6 were replicates of the B2 set up. A summary of the bio-accumulation treatments is shown in Table 4.3.

Table 4.3. Bio-accumulation treatments

TREATMENT TYPE	CODE
T2: Sludge absent (ordinary) Glen Valley soil irrigated with tap water (pH 7.0)	B1 (Replicates: B1a, B1b, B1c, B1d)
T3: Sludge amended Glen Valley soil irrigated with treated waste water (pH 8.5)	B2 (Replicates: B3, B4, B5 and B6)

4.5 pH VARIATION EXPERIMENTS

The experimental set-up consisted of 20 tomato pots which were sub divided into 4 set-ups. Set up 1 contained 5 tomato pots each filled with 2.5 kg sludge amended Glen Valley soil and irrigated with treated waste water at pH 5.0 (the pots were labeled pHa, pHb, pHc, $pHabc_1$, and $pHabc_2$ respectively). Set-up 2 contained 5 tomato pots each filled with 2.5 kg Glen Valley sludge amended soil and irrigated with treated waste water at pH 6.0 (The pots were labeled pHd, pHe, pHf, $pHdef_1$, and $pHdef_2$). Set-up 3 contained 5 tomato pots each filled with 2.5 kg sludge amended Glen Valley soil and irrigated with treated waste water at pH 9.0 (The pots were labeled pHg, pHh, pHi, $pHghi_1$, and $pHghi_2$). Set-up 4 also contained 5 tomato pots each filled with 2.5 kg sludge amended Glen Valley soil and irrigated with treated waste water at pH 10.0 (the pots were labeled pHj, pHk, pHl, $pHjkl_1$, and $pHjkl_2$). Dilute 0.1 M hydrochloric acid (HCL) was used as acid pH adjuster and dilute 0.1 M sodium hydroxide (NaOH) was used to adjust the basicity level. The acid and base were used to increase or reduce the level of acidity of the treated waste water to pH 5.0 or pH 6.0 or increase the level of basicity to pH 9.0 or pH 10.0. The potted tomato plants were irrigated regularly with the treated waste water at different pH values (Appendix B). A summary of the pH variation experiments is shown in Table 4.4.

Table 4.4. pH variation experiments

TREATMENT TYPE	CODE
T4: Sludge amended Glen Valley soil irrigated with treated waste water at pH 5.0	pHa (Replicates: pHb, pHc, pHabc$_1$, and pHabc$_2$)
T5: Sludge amended Glen Valley soil irrigated with treated waste water at pH 6.0	pHd (Replicates: pHe, pHf, pHdef$_1$, and pHdef$_2$)
T6: Sludge amended Glen Valley soil irrigated with treated waste water at pH 9.0	pHg (Replicates: pHh, pHi, pHghi$_1$, and pHghi$_2$)
T7: Sludge amended Glen Valley soil irrigated with treated waste water at pH 10.0	pHj (Replicates: pHjkl$_{2,}$ pHl, pHjkl$_1$, and pHjkl$_2$)

4.6 SAMPLE COLLECTION AND PREPARATION

Tomato leaf and fruit samples were hand harvested at the full red stage (vine-ripe) by mid October 2009. The largest leaf and the biggest fruit from each tomato plant were selected for testing (financial considerations also played a role in making this decision). Harvested leaves and fruits were transported immediately to the Department of Waste Management and Pollution Control, Gaborone for laboratory preparation and subsequent analysis by the laboratory of the Department of Water Affairs also in Gaborone, Botswana.

4.7 OPEN DIGESTION TECHNIQUE FOR THE TOMATO LEAVES AND FRUITS

The tomato plants (leaves and fruits) were harvested on 14 October 2009. The leaves and fruits were washed thoroughly with ordinary tap water, rinsed with deionized water and oven dried at 60^0 C. Each sample was weighed (about 2 g each of leaves and of fruits) and then 10 mL of concentrated HNO_3 was added to the samples and the samples were covered with a ribbed watch glass. The samples were then brought to the boil on a hot plate and evaporated to 15 – 10 mL. Thereafter 5 mL of concentrated HNO_3 and 10 mL of concentrated H_2SO_4 were added and the flask was cooled between additions. The flask and its content were then transferred to a hot plate to allow its contents to evaporate until dense white fumes of SO_3 just appear. Heat was then applied to remove all the HNO_3 before treatment. The next step was to cool and to dilute the flask contents to about 50 mL with water and subsequently heated to almost boiling to dissolve the soluble salts. Finally, the samples were filtered and ready for analysis (Jackson, 1967). A blank was also run under similar conditions.

4.8 ATOMIC ABSORPTION SPECTROMETRY

The concentrations of chromium were determined with the Varian SpectrAA 10/20 system (SpectrAA-10/20, 1985) and that of nickel with the Shimadzu AA6300 Atomic Absorption Spectrometer (Shimadzu AA6300, 2003). In atomic absorption spectrometry, a light beam is directed through a flame, into a monochromator and then onto a detector that measures the amount of light absorbed by the atomized element in the flame. For some metals, atomic absorption exhibits superior sensitivity over other techniques such as the flame emission technique. Since each metal has its own characteristic absorption wavelength, a source lamp composed of that element was used; this makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range (Wilis, 1962).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 OVERVIEW

The chromium and nickel concentrations in the leaves and fruits of the tomato plants after treatment with Glen Valley soils using tap water and treated waste water are presented and discussed in this chapter.

5.2 CHROMIUM BIO-ACCUMULATION (CONTROL)

The chromium concentration in the leaves and the fruits of the tomato plants after treatment with standard commercial soil and sludge absent Glen Valley soil and irrigated with tap water are shown in Table 5.1 and Figure 5.1

Table 5.1. Chromium uptake in the leaves and the fruits of the tomato plants: Tap water (at pH 7.0) irrigation with standard commercial soil and sludge absent Glen Valley soil.

Treatment Type	Chromium concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error
T1:Standard commercial soil with tap water at pH 7.0	0.819	0.242	0.599	0.153
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.740	0.028	0.511	0.009

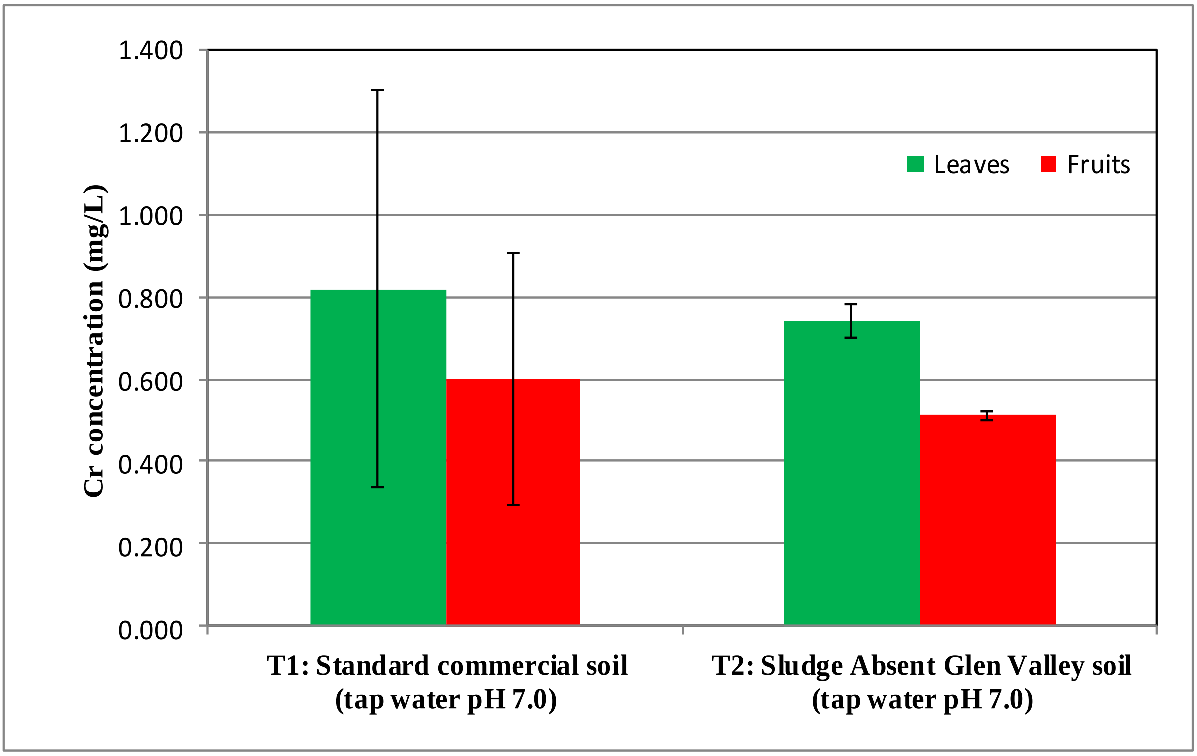


Figure 5.1 Average concentration of chromium in the tomato plants (control). Bars represent SEM (n=5)

Table 5.1 and Figure 5.1 show that more chromium bio-accumulate in the leaves than in the fruits with both treatments. The highest concentration of chromium was found in the leaves (0.819 mg/L) where the tomatoes were grown in standard commercial soil irrigated with tap water and the lowest concentration (0.511 mg/L) in the fruits where the tomatoes were grown in sludge absent Glen Valley soil irrigated with tap water. These results agree with findings of Mangabeira et al. (2005). The mean concentrations of chromium bio-accumulation in both treatments exceeded the 0.10 mg/L permitted limit suggested by the Food and Agriculture Organization (1985) for crop production. The 0.50 mg/L effluent quality limits set by the Botswana Bureau of Standards (2004) was also exceeded in both cases. Other studies

(Kirkham, 1986 and Omran et al., 1988) have reported high concentrations of heavy metals bio-accumulation in tomato plants when using tap water as a source of irrigation water. This could be ascribed to high concentrations of heavy metals in the soil irrigated with the tap water source. The sludge absent Glen Valley soil bio-accumulates somewhat less chromium in the leaves and the fruits as compared with the standard commercial soil when using tap water for irrigation. In conclusion, the standard commercial soil or possibly the tap water contains chromium levels above the Food and Agriculture Organization safe limits.

To determine if there were any significant differences in the concentration levels of chromium uptake in the tomato leaves and fruits cultivated using sludge amended Glen Valley soils with treated waste water compared with using sludge absent Glen Valley soils treated with tap water, a statistical tool known as the Graph Pad software which uses the student t-test was employed (this was necessary to analyze, graph and organize the data sets). The student t-test determines if the mean values of two data columns are equal. The hypotheses used are the null and alternate hypothesis and they are stated as follows:

The null hypothesis (H_O): There are no statistically significant differences in concentration levels of chromium uptake in the tomato leaves and fruits cultivated using sludge amended Glen Valley soils with treated waste water compared with using sludge absent Glen Valley soils treated with tap water.

The alternate hypothesis (H_A): There are significant differences in concentration levels of chromium uptake in the tomato leaves and fruits cultivated using sludge amended Glen Valley soils with treated waste water compared with using sludge absent Glen Valley soils treated with tap water.

To determine whether the null hypothesis is accepted or rejected statistically, the probability value (P-value) is used. The P-value ranges from 0 to 1, and it has been statistically accepted that if the P-value is > 0.5 then the null hypothesis is accepted, but if the P-value is < 0.5 then the null hypothesis is rejected and the alternate hypothesis is accepted (Appendix D). By conventional criteria (Appendix D), the difference observed in the concentrations of chromium in the leaves and fruits of the tomato plants in this study are considered to be not statistically significant as shown in Table 5.2, therefore the null hypothesis is accepted.

Table 5.2. t-test for chromium to determine any significant differences in chromium concentrations in the tomato leaves and fruits.

Heavy Metal	Treated waste water irrigation with sludge amended Glen Valley soil (Mean values)	Tap water irrigation with sludge absent Glen Valley soil (Mean values)	t-test values	P-values	Remarks
Chromium	0.2970	0.2930	0.0253	0.9803	No significant difference

5.2 CHROMIUM UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER PH 5.0

Chromium concentrations in the tomato leaves and fruits using sludge amended Glen Valley soils treated with waste water (normal waste water at pH 8.5 and waste water adjusted to pH 5.0) compared with chromium concentrations in tomato leaves and fruits treated with sludge absent Glen Valley soils irrigated with tap water are presented in Table 5.3 and Figure 5.2

Table 5.3. Chromium uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 5.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils).

Treatment Type	Chromium concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.740	0.028	0.511	0.009
T3: Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	0.052	0.007	0.063	0.009
T4: Sludge amended Glen Valley soil with treated waste water at pH 5.0	0.231	0.090	0.165	0.067

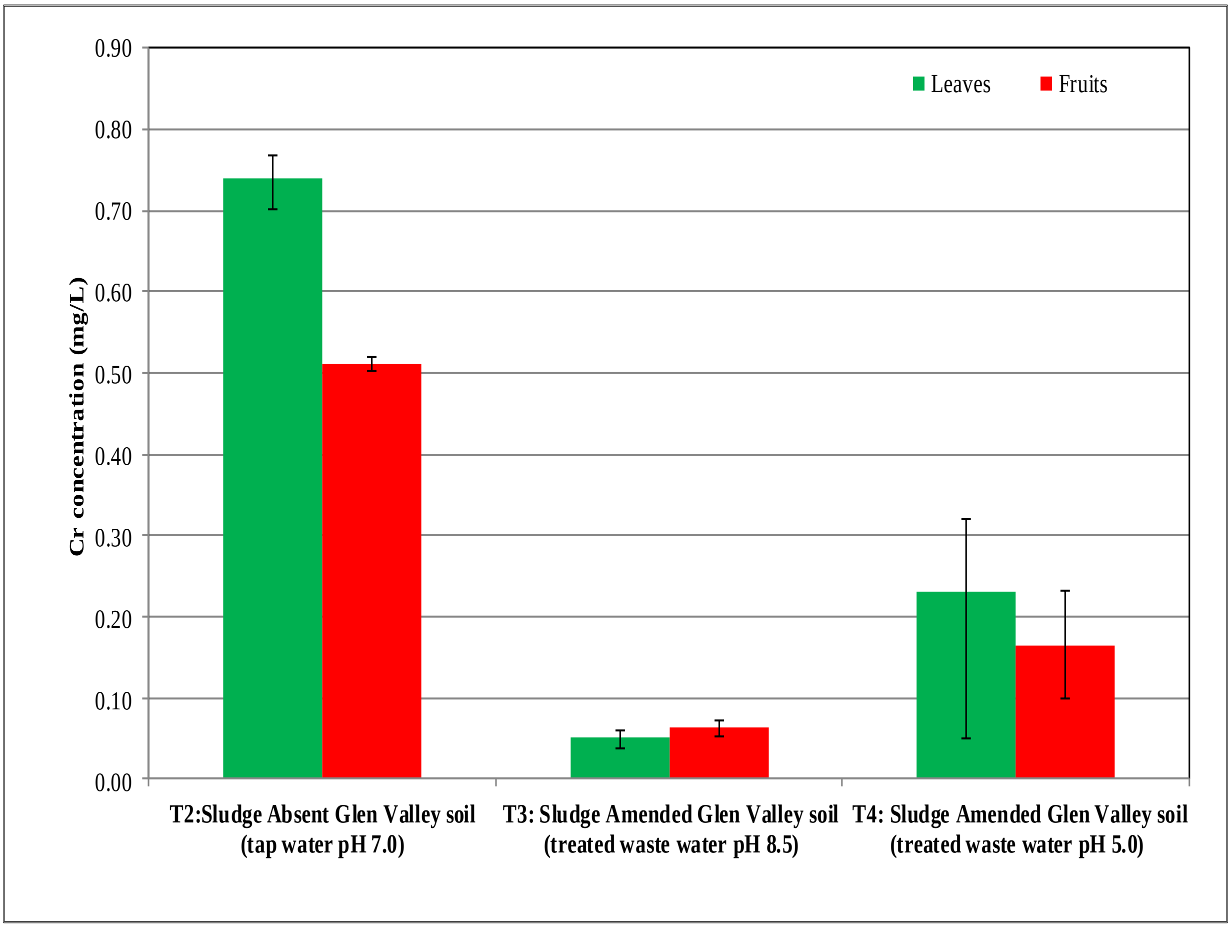


Figure 5.2 Average concentration of chromium in the tomato plants for the different treatments (pH 5.0). Bars represent SEM (n=5)

Gaborone crop soils which include the Glen Valley soils are enriched in chromium (59 mg/kg – 240 mg/kg) (Zhai et al. 2003). Growing tomato plants in sludge amended Glen Valley soils with treated waste water has been shown to reduce chromium translocation to the leaves and fruits of the tomatoes. This pattern is observed (Figure 5.2) where the uptake of chromium was significantly different for the different treatments. The highest uptake of chromium was observed in the tomato plants treated with sludge absent Glen Valley soil and tap water (0.740

mg/L for leaves and 0.511 mg/L for fruits). This could be due to chromium having the affinity to be sorbed to a slightly greater extent at pH 7.0 compared to other pH values. Chromium uptake was reduced in tomato plants cultivated in sludge amended Glen Valley soil using treated waste water at pH 5.0 (0.231 mg/L for leaves and 0.165 mg/L for fruits). This could be ascribed to chromium species (particularly trivalent chromium) which tend to form hydroxides that precipitate at low pH 5.0 (Appendix E1). This hydroxide of chromium formed is mostly retained in the roots and minimally translocated to the leaves and fruits of the tomato plants. Shewry and Peterson (1974) observed a similar trend when studying the uptake and translocation of $Cr0_4^{2-}$ from nutrient solutions by barley seedlings. They suggested that most of the chromium were retained in the roots and very little translocation of chromium took place from the roots to the tops. The lowest chromium uptake in the tomato plants was obtained when using normal treated waste water at pH 8.5 with sludge amended Glen Valley soil (0.052 mg/L for leaves and 0.063 mg/L for fruits). This is an indication of the minimum solubility of chromium at this treated waste water pH 8.5 (Appendix E1) and consequently less chromium uptake. On the average the tomato leaves tend to accumulate more chromium than the fruits because the rate of transpiration is higher in the leaves compared with the fruits. Moreover, fruits are mostly phloem loaded and heavy metals are generally poorly mobile in the phloem. The work of Zheljazkov and Neilsen (1996) on concentrations of heavy metals in vegetables buttressed this point. They found that the concentrations of heavy metals in vegetables per unit dry matter generally follow the order: leaves >fresh fruits >seeds.

5.3 CHROMIUM UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER pH 6.0

The chromium concentration in the leaves and fruits of tomatoes after treatment with normal treated waste water and treated waste water at pH 6.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Table 5.4 and Figure 5.3

Table 5.4. Chromium uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 6.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils).

Treatment Type	Chromium concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.740	0.028	0.511	0.009
T3: Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	0.052	0.007	0.063	0.009
T5: Sludge amended Glen Valley soil with treated waste water at pH 6.0	0.406	0.009	0.427	0.036

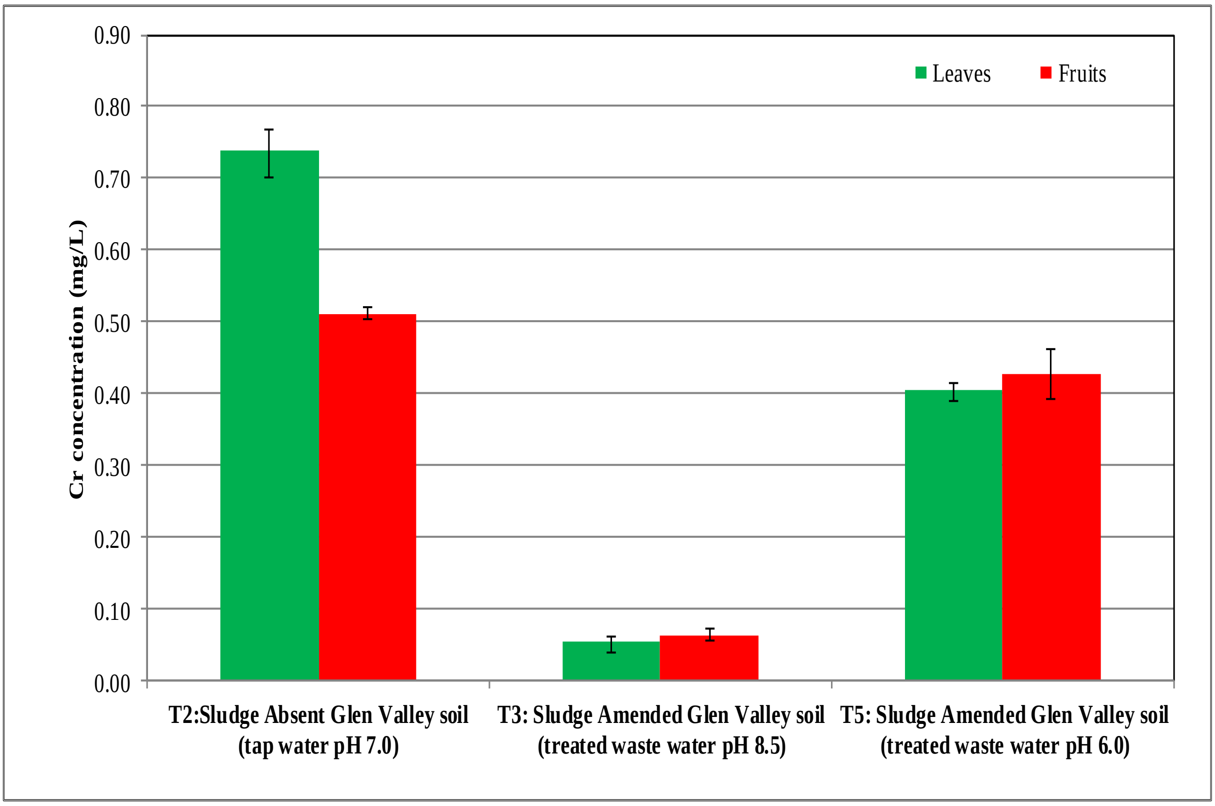


Figure 5.3 Average concentration of chromium in the tomato plants for the different treatments (pH 6.0). Bars represent SEM (n=5)

Treatment with sludge amended Glen Valley soil (Figure 5.3; T5) using treated waste water at pH 6.0 increased the chromium uptake in the leaves and fruits of the tomato plants compared with treatment using treated waste water at pH 5.0 (Figure 5.2; T4). The chromium uptake in the leaves increased from 0.231 mg/L (Figure 5.2; T4) to 0.406 mg/L (Figure 5.3; T5). Similarly, the chromium uptake in the fruits increased from 0.165 mg/L (Figure 5.2; T4) to 0.427 mg/L (Figure 5.3; T5). These observation showed that at pH 6.0 chromium accumulation in both leaf and fruit is roughly double that at pH 5.0. However, the chromium uptake in the leaves and fruits of the tomato plants with tap water (pH 7.0) treatments in sludge absent Glen Valley soils (Figure 5.3; T2) still remain higher compared with normal treated waste water at pH 8.5 in sludge amended Glen Valley soils (T3) and treated waste water at pH 6.0 in sludge amended Glen Valley soil (T5). The trend of increasing chromium uptake in the tomato leaves and fruits from pH 5.0 to 6.0 and further up to pH 7.0 show evidence for the strong dependence of chromium uptake on the pH of the water or waste water used for irrigation. Thus far, these results agree with the findings of Cary et al., (1975) where they studied the effect of pH on the uptake of chromium from solutions of pH 5.0, 6.0, 7.0 and 8.0 using wheat plants 25 to 30 cm tall. They reported that chromium (VI) which is highly soluble and more mobile than chromium (III) was sorbed to a slightly greater extent at a pH 6.0 to 7.0.

5.4 CHROMIUM UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER PH 9.0

The chromium concentrations in the leaves and fruits of tomatoes after treatment with normal treated waste water and treated waste water at pH 9.0 using sludge amended Glen Valley soils compared with treatment using tap water in sludge absent Glen Valley soils are shown in Table 5.5 and Figure 5.4.

Table 5.5. Chromium uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 9.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils)

Treatment Type	Chromium concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.740	0.028	0.511	0.009
T3: Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	0.052	0.007	0.063	0.009
T6: Sludge amended Glen Valley soil with treated waste water at pH 9.0	0.079	0.011	0.054	0.008

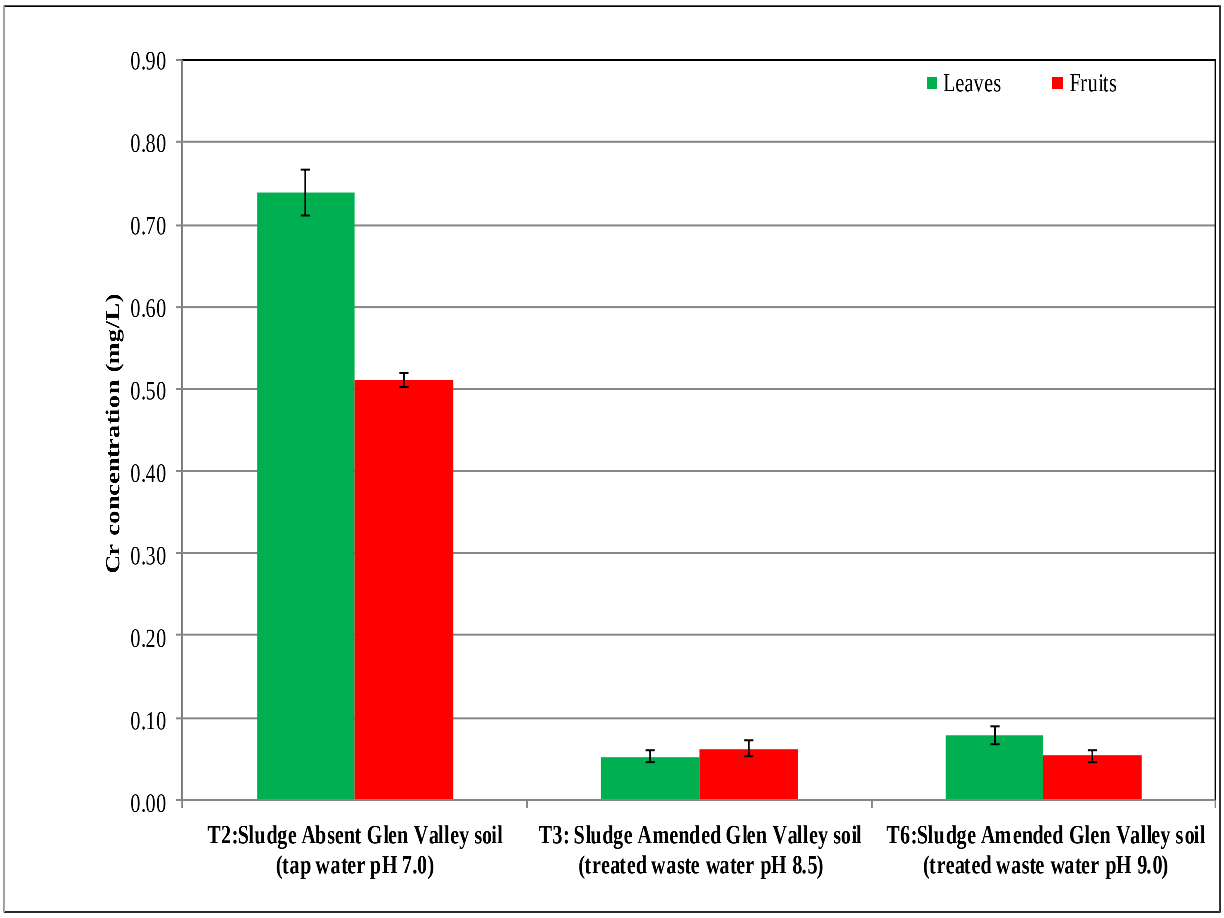


Figure 5.4 Average concentration of chromium in the tomato plants for the different treatments (pH 9.0). Bars represent SEM (n=5)

Cultivating tomatoes with sludge amended Glen Valley soil (Figure 5.4; T6) using treated waste water at pH 9.0 decreased the chromium uptake in the leaves and fruits of the tomato plants compared with treatments at pH 6.0 (Figure 5.3; T5). Chromium was reduced from 0.406 mg/L (Figure 5.3; T5) to 0.079 mg/L (Figure 5.4; T6) and from 0.427 mg/L (Figure 5.3; T5) to 0.054 mg/L (Figure 5.4; T6) in the leaves and fruits, respectively. The result of T6 treatments (sludge amended Glen Valley soil) where treated waste water at pH 9.0 was used was close to T3 treatments (sludge amended Glen Valley soil) where normal treated waste water at pH 8.5 was the source of irrigation. This close trend that was observed could be ascribed to the optimum precipitation of chromium occurring around a pH slightly greater than 8.5 (Appendix E1). It thus makes chromium less available for plant uptake. Another possible cause of the low chromium uptake when using treated waste water at pH 9.0 is the lower solubility of chromium hydroxide at this pH. Therefore, less chromium is available for uptake by the tomato plants.

5.5 CHROMIUM UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER pH 10.0

The chromium concentration in the leaves and fruits of the tomatoes after treatment with normal treated waste water and treated waste water at pH 10.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Tables 5.6 and Figure 5.5

Table 5.6. Chromium uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 10.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils).

Treatment Type	Chromium concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.740	0.028	0.511	0.009
T3:Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	0.052	0.007	0.063	0.009
T7:Sludge amended Glen Valley soil with treated waste water at pH 10.0	0.271	0.047	0.538	0.151

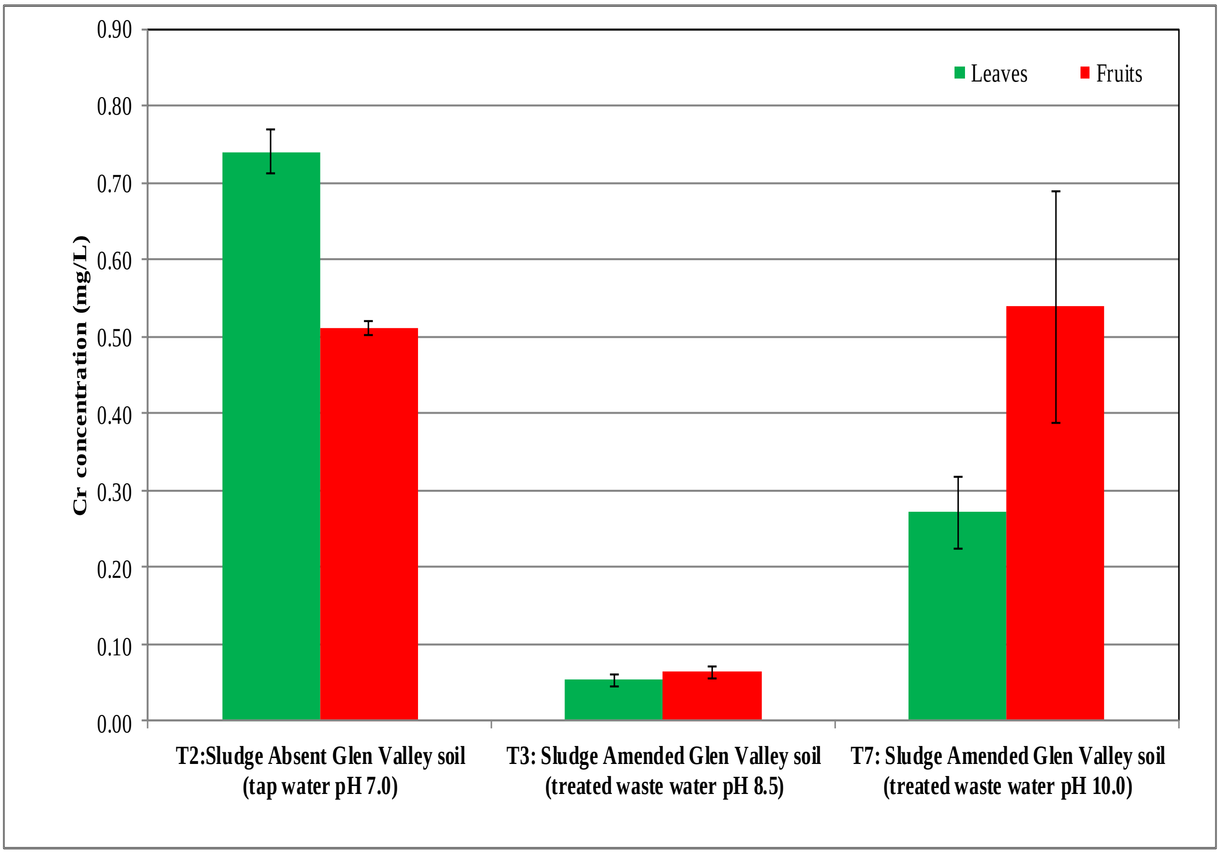


Figure 5.5 Average concentration of chromium in the tomato plants for the different treatments (pH 10.0). Bars represent SEM (n=5)

Using sludge amended Glen Valley soil (Figure 5.5; T7) and treated waste water at pH 10.0 increased the chromium uptake in the leaves and fruits of the tomato plants compared to treatment with sludge amended Glen Valley soil and treated waste water at pH 9.0 (Figure 5.4; T6). This effect could be linked to the amphoteric nature of chromium hydroxide which formed from the precipitation reaction when adding sodium hydroxide (to adjust the pH to 9.0) to the normal treated waste water at pH 8.5. This amphoteric nature of chromium hydroxide makes it increasingly soluble in the treated waste water at both low and high pH values. At pH 10.0 the chromium hydroxide formed goes back into solution and makes chromium available for tomato plant uptake. Another reverse trend that was observed when using treated waste water at pH 10.0 was that more chromium, 0.538 mg/L, bio-accumulate in the fruits of the tomatoes planted (Figure 5.5; T7) compared to a lesser amount of chromium, 0.054 mg/L, in the fruits of the tomatoes planted with treated waste water at pH 9.0 (Figure 5.4; T6). This reverse trend could be traceable to the tomato roots surface where increasingly negative charges could have built up at high pH (pH 10.0) of the treated waste water that could have attracted the positively charged chromium ions more strongly into the fruits. There is also more chromium in the fruits compared with the leaves when the pH of the treated waste water was raised to 10.0. This agrees with research findings of Khairiah et al. (2002) where they reported higher chromium concentration in the fruits and roots than in the leafy vegetables in their study of the bioavailability of chromium in vegetables of selected agricultural areas in Malaysia. However, other researchers (Grubinger et al., 1994; Soane and Saunder, 1959) pointed to the fact that chromium uptake and distribution in plants are often dictated by the type of cultivar.

The role of pH in water or treated waste water irrigation is significant albeit controversial in the uptake of chromium in tomato leaves and fruits. The contribution of the sludge amended soil to chromium uptake is also not well defined and further research needs to be carried out on the combined use of waste water and sludge amended soil. One thing that emerged from the pH variation experiments is that the cultivation of tomatoes with the Glen Valley treated waste water at pH 8.5 on sludge amended Glen Valley soil has been shown to reduce the level of chromium uptake compared to using tap water on sludge absent Glen Valley soil. This should be good news for farmers in the Glen Valley farms. However caution must be exercised to avoid prolonged use of treated waste water that may trigger the buildup of chromium and cause possible harm to the food chain and impact adversely on human health.

5.6 SUMMARY OF CHROMIUM UPTAKE IN THE TOMATO PLANTS

A summary of the chromium concentration in the leaves and fruits of the tomatoes after treatment with normal treated waste water and treated waste water at different pH levels (in sludge amended Glen Valley soils) and tap water (in sludge absent Glen Valley soil and standard commercial soil) are shown in Figure 5.6

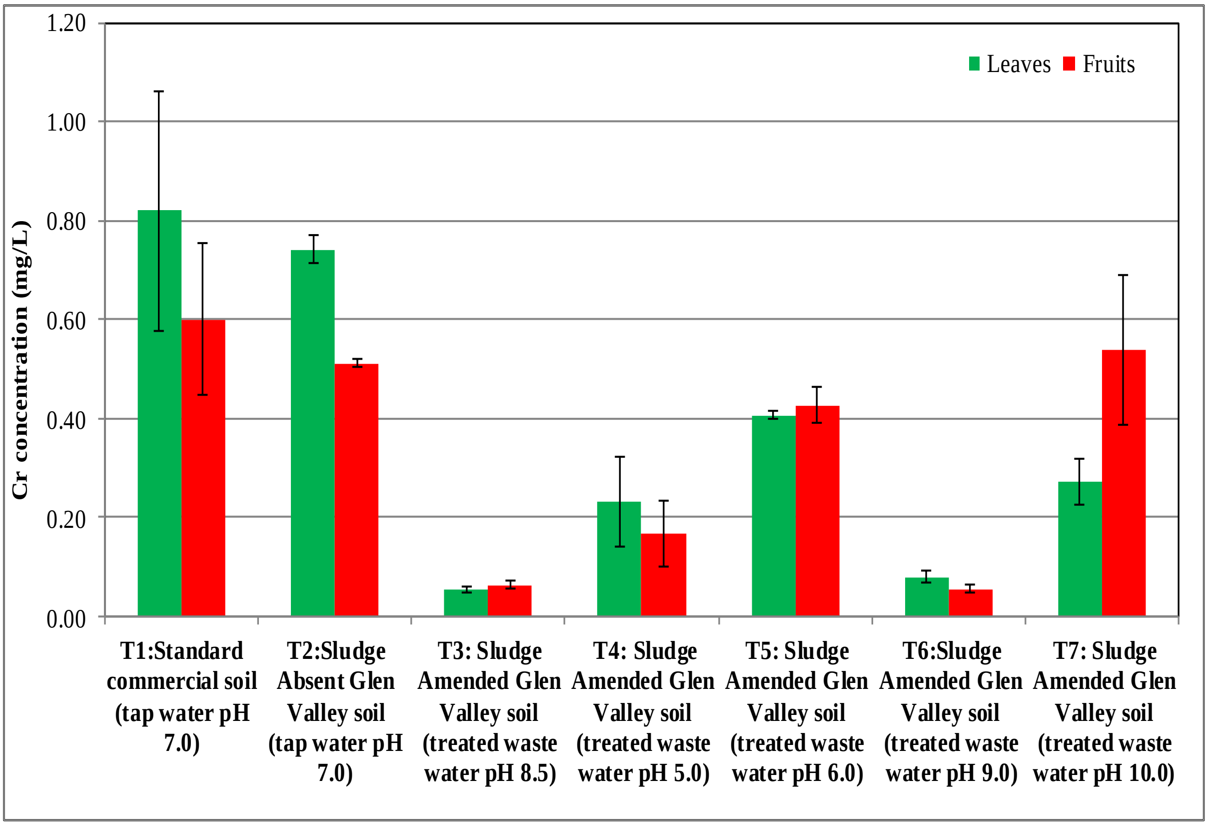


Figure 5.6 Average concentration of chromium in the tomato plants for the different treatments (summary). Bars represent SEM (n=5)

Tap water irrigation of the tomato plants in standard commercial soil induced the highest accumulation of chromium, 0.819 mg/L (Figure 5.6; T1), in the tomato leaves. Chromium accumulation, 0.740 mg/L (Figure 5.6; T2), was also high in the tomato leaves when using tap water to irrigate tomatoes planted in sludge absent Glen Valley soil. This could be ascribed to gpre-existing high chromium levels in standard commercial soil and sludge absent Glen Valley soil which is being translocated easily to the tomato leaves. Another factor could be the rate of transpiration that is higher in the leaves than the fruits and again the fruits are mostly phloem loaded where heavy metals are generally poorly mobile. The lowest accumulation of chromium, 0.052 mg/L (Figure 5.6; T3), was recorded in the tomato leaves when using treated waste water irrigation with sludge amended Glen Valley soils. This could be ascribed to a strong affinity of chromium for organic matter which may be present in the normal treated waste water and also sludge amended Glen Valley soils, making it easily complexed and reducing its availability for plant uptake.

Tap water irrigation (pH 7.0) in tomato plants cultivated in standard commercial soil and in sludge absent Glen Valley soil showed a higher accumulation of chromium in their leaves and their fruits compared to treated waste water irrigation (and treated waste water at different pH values; T4, T5, T6, T7) in the tomato plants cultivated in sludge amended Glen Valley soils. It is important to note that tomato plants cultivated in sludge amended Glen Valley soil using treated waste water at pH 10.0 increased the chromium uptake in the leaves and fruits of the tomato plants compared to treatment with sludge amended Glen Valley soil and treated waste water at pH 9.0. All these show that chromium solubility and subsequent bioavailability in tomato plant are pH dependent. Also, the theoretical solubility of chromium hydroxide (Appendix E1) showed how chromium is directly controlled by pH. The affinity and binding capacity of chromium in solution for organic matter contained in soil as well as its tendency to form complexes become reduced as pH increases from pH 5.0 to pH 6.0 increasing availability for plant uptake (Guertin et al., 2004).

Mean chromium concentration in the leaves was higher than in the fruits but statistical analysis shows no significant difference between them at the 5% significant level. The mean chromium concentration in the leaves and fruits of tomato plants in this study exceed the 0.1 mg/L recommended maximum level of chromium for crop production (Food and Agriculture Organization, 1985). However, the maximum limits of 0.50 mg/L for chromium effluent quality set by the Botswana Bureau of Standards (2004) was only exceeded when tomato plants were irrigated with tap water at pH 7.0 and treated waste water at pH 10.0 (in case of the tomato fruits).

5.7 NICKEL BIO-ACCUMULATION (CONTROL)

The nickel concentration in the leaves and fruits of the tomato plants after treatment with standard commercial soil and sludge absent Glen Valley soil with tap water are shown in Table 5.7. and Figure 5.7.

Table 5.7. Nickel uptake in the leaves and the fruits of tomato plants: Tap water (at pH 7.0) irrigation with standard commercial soil and sludge absent Glen Valley soil.

	Nickel concentration (mg/L)			
	Leaves		Fruits	
Treatment Type	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T1:Standard commercial soil with tap water at pH 7.0	0.327	0.204	0.224	0.174
T2: Sludge Absent Glen Valley soil with tap water at pH 7.0	0.217	0.000	-0.003	0.000

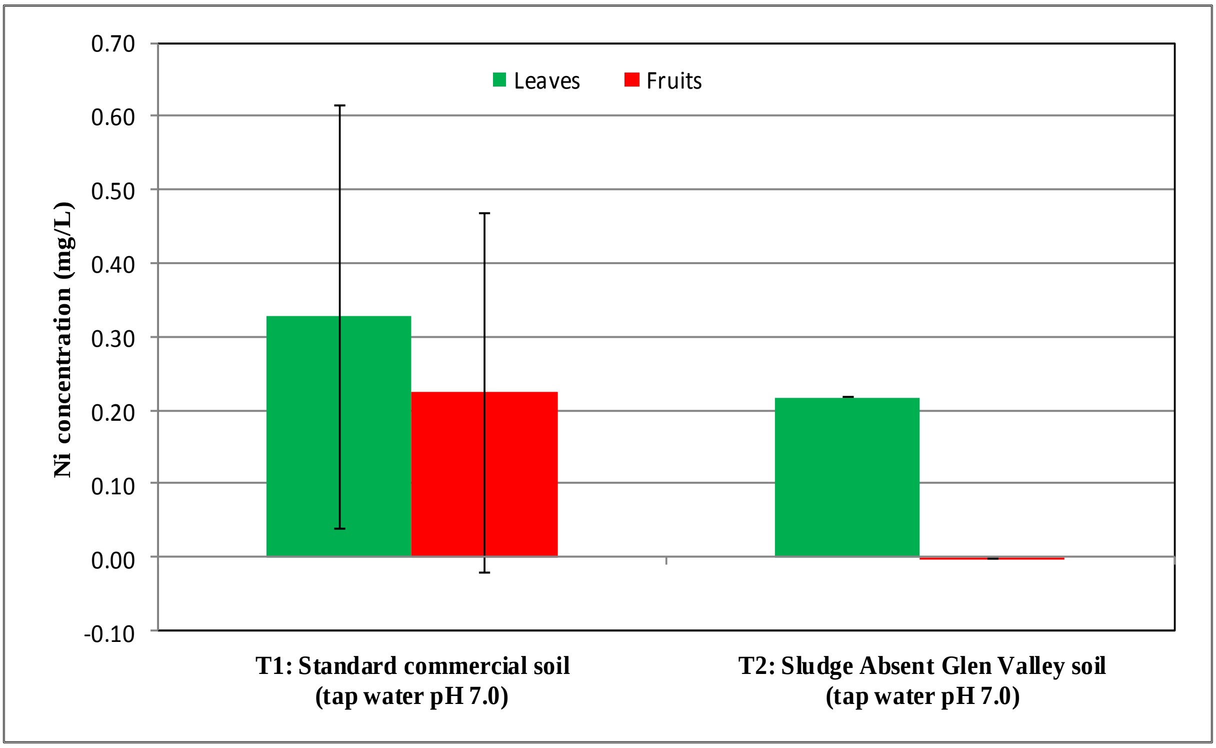


Figure 5.7 Average concentration of nickel in the tomato plants for the different treatments (control). Bars represent SEM (n=5)

Crop soils of Gaborone (including the Glen Valley farm soils) are high in nickel, ranging from 40 mg/kg to 161 mg/kg (Zhai et al. 2003). Using standard commercial soil and tap water for irrigation, T1 of Figure 5.7, and sludge absent Glen Valley soil with tap water, T2 of Figure 5.7, to cultivate tomato plants produced significant differences in the nickel uptake in the leaves and the fruits of the tomato plants. Nickel uptake was 0.327 mg/L in the leaves when standard commercial soil with tap water was used and 0.217 mg/L in the leaves when sludge absent Glen Valley was used with tap water. There was also a high nickel (0.224 mg/L) uptake in the fruits when standard commercial soil with tap water was used. However, nickel desorption was experienced in the case of the sludge absent Glen Valley soil treated with tap water (-0.003 mg/L) for the fruits. The unavailability of nickel in the tomato fruits when irrigating sludge absent Glen Valley soil could be due to the low transpiration rates of the fruits as compared with the leaves which are more tolerant to nickel at pH 7.0. Again, the fruits as storage organs are largely phloem-loaded and heavy metals are generally poorly mobile in the phloem (Krijger et al., 1999). Al-Lahham et al., (2003) observed a similar trend in their study where they recorded no accumulation of nickel in tomato fruits when irrigating with potable water.

The tomato leaves in the case of the standard commercial soil and sludge amended Glen Valley soil (treated with tap water at pH 7.0) and the fruits in the case of the standard commercial soil (treated with tap water at pH 7.0) accumulate nickel beyond the limit of 0.20 mg/L as set by the Food and Agricultural Organization (1985) for crop production. It also exceeds the 0.30 mg/L permissible limits set by the Botswana Bureau of Standards (2004). The nickel concentration in the tomato fruits (-0.003 mg/L) when using tap water at pH 7.0 to irrigate tomatoes planted in sludge absent Glen Valley soil is not significant. This indicates that tap water at pH 7.0 combined with sludge absent Glen Valley soil could reduce the uptake of nickel in the fruits but not in the leaves of the tomato plants. However, this indication is not conclusive and further studies need therefore be carried out to properly understand the uptake mechanism of nickel when using tap water to irrigate tomato plants in sludge absent soils.

To determine if there were any significant differences in the concentration levels of nickel uptake in the tomato leaves and fruits cultivated using sludge amended Glen Valley soils with treated waste water compared with using sludge absent Glen Valley soils with tap water, the same statistical tool described in section 5.1 for chromium was also used for nickel and the same convention followed (Appendix D). By conventional criteria, the differences observed in the leaves and fruits were considered to be not statistically significant (Table 5.8).

Table 5.8. t-test for nickel to determine any significant differences in tomato leaves and fruits

Heavy Metal	Treated waste water irrigation with sludge amended Glen Valley soil (Mean values)	Tap water irrigation with sludge absent Glen Valley soil (Mean values)	t-test values	P-values	Remarks
Nickel	0.0918	0.0545	0.5180	0.6157	No significant difference

5.8 NICKEL UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER PH 5.0

The nickel concentration in the leaves and the fruits of the tomatoes after treatment with normal treated waste water and treated waste water at pH 5.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Table 5.9. and Figure 5.8.

Table 5.9. Nickel uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 5.0 (sludge amended soil) compared with normal treated waste water at pH 8.5 (sludge amended soil) and with tap water at pH 7.0 (sludge absent soils).

Treatment Type	Nickel concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error Of the Mean	Mean	Standard Error Of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.217	0.000	-0.003	0.000
T3:Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	-0.025	0.078	-0.030	0.030
T4:Sludge amended Glen Valley soil with treated waste water at pH 5.0	0.085	0.022	0.020	0.047

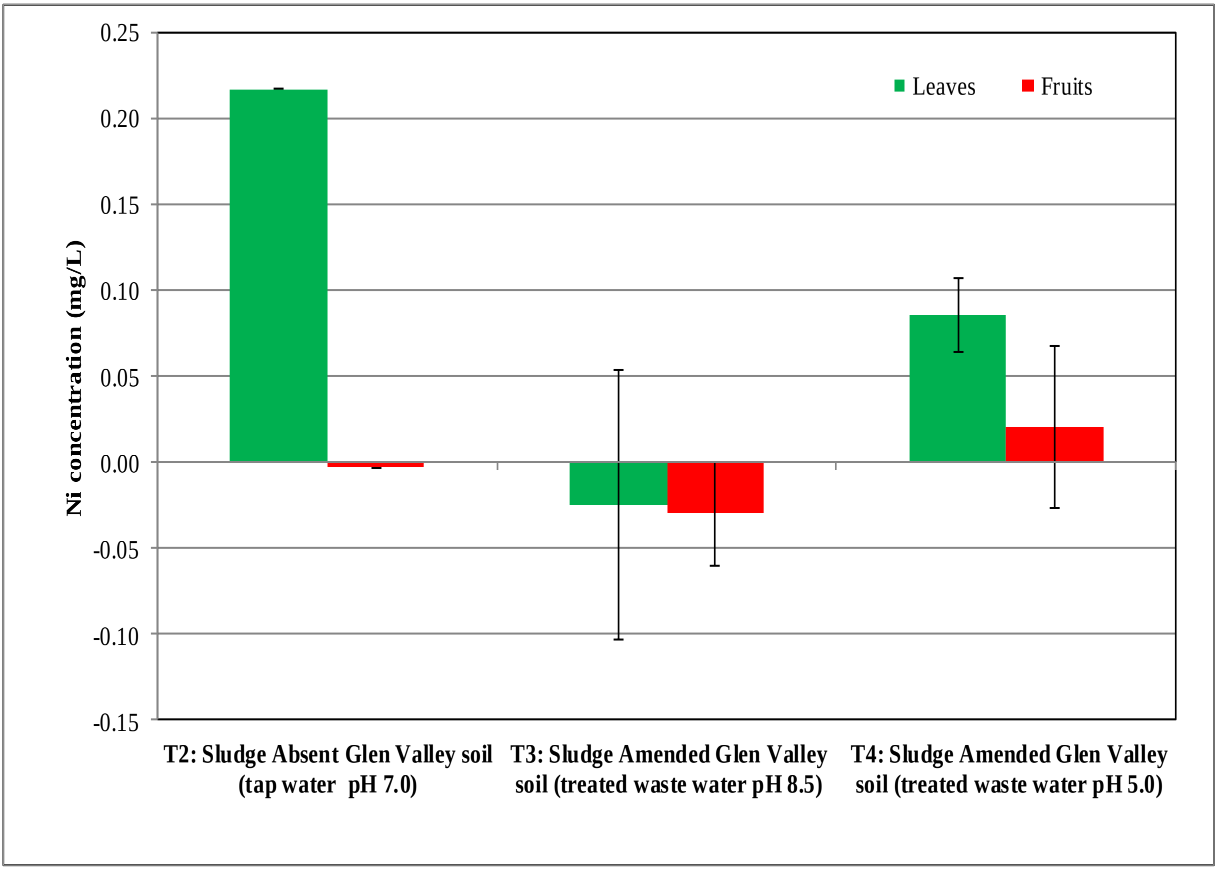


Figure 5.8 Average concentration of nickel in the tomato plants for the different treatments (pH 5.0). Bars represent SEM (n=5)

Figure 5.8 compares tap water irrigation of tomato plants (in sludge absent Glen Valley soil) with treated waste water irrigation (in sludge amended Glen Valley soil). It can be deduced that irrigating tomato plants with treated waste water at pH 5.0 in sludge amended Glen Valley soils has been shown to reduce its translocation to the leaves, 0.085 mg/L (Figure 5.8; T4). The level of nickel was quite high, however, in the tomato leaves, 0.217 mg/L, when using tap water in sludge absent Glen Valley soil (Figure 5.8; T2). There was nickel desorption in the tomato fruits, -0.003 mg/L when using tap water to irrigate tomatoes planted in sludge absent Glen Valley soil. No nickel was taken up in the tomato leaves and fruits when irrigating sludge amended Glen Valley soil with normal treated waste water and a possible reason could be the higher basicity (pH 8.5) of the normal treated waste water which caused reduced solubility and mobility of the nickel in plants at the higher pH of the irrigation water (Kabata-Pendias and Mukherjee, 2007). The concentration of nickel in the tomato plants (leaves and fruits) was below the recommended threshold of 0.30 mg/L set by the Botswana Bureau of Standards and also lower than the 0.2 mg/L limits set by the Food and Agricultural Organization for crop production when irrigating the sludge amended Glen Valley soil with treated waste water at pH 5.0. This could be correlated with the free nickel ion activity in the soil solution because the plant uptake of nickel is dependent on the soil pH as well as other factors such as the organic matter, iron and manganese oxide content of the soil (Ge et al., 2000; Kabata-Pendias and Mukherjee, 2007). Another possibility is that the tomato plants might have less tolerance for nickel at pH 8.5 and this observation agreed with work by the Environment Agency (2009) that plants generally differ in their tolerance and ability to uptake nickel. These differences could be ascribed to the plants ability to respond differently to nickel ion activity in the soil solution. Nickel uptake becomes more readily available in its simple ionic form (Ni^{2+}) than as inorganic and organic complexes (Kabata-Pendias and Mukherjee, 2007)

5.9 NICKEL UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER pH 6.0

The nickel concentration in the leaves and the fruits of tomatoes after treatment with normal treated waste water and treated waste water at pH 6.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Table 5.10. and Figure 5.9.

Table 5.10. Nickel uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 6.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils).

	Nickel concentration (mg/L)			
	Leaves		Fruits	
Treatment Type	Mean	Standard Error Of the Mean	Mean	Standard Error Of the Mean
T2: Sludge Absent Glen Valley soil with tap water at pH 7.0	0.217	0.000	-0.003	0.000
T3: Sludge Amended Glen Valley soil with normal treated waste water at pH 8.5	-0.025	0.078	-0.030	0.030
T5: Sludge amended Glen Valley soil with treated waste water at pH 6.0	0.262	0.138	0.147	0.097

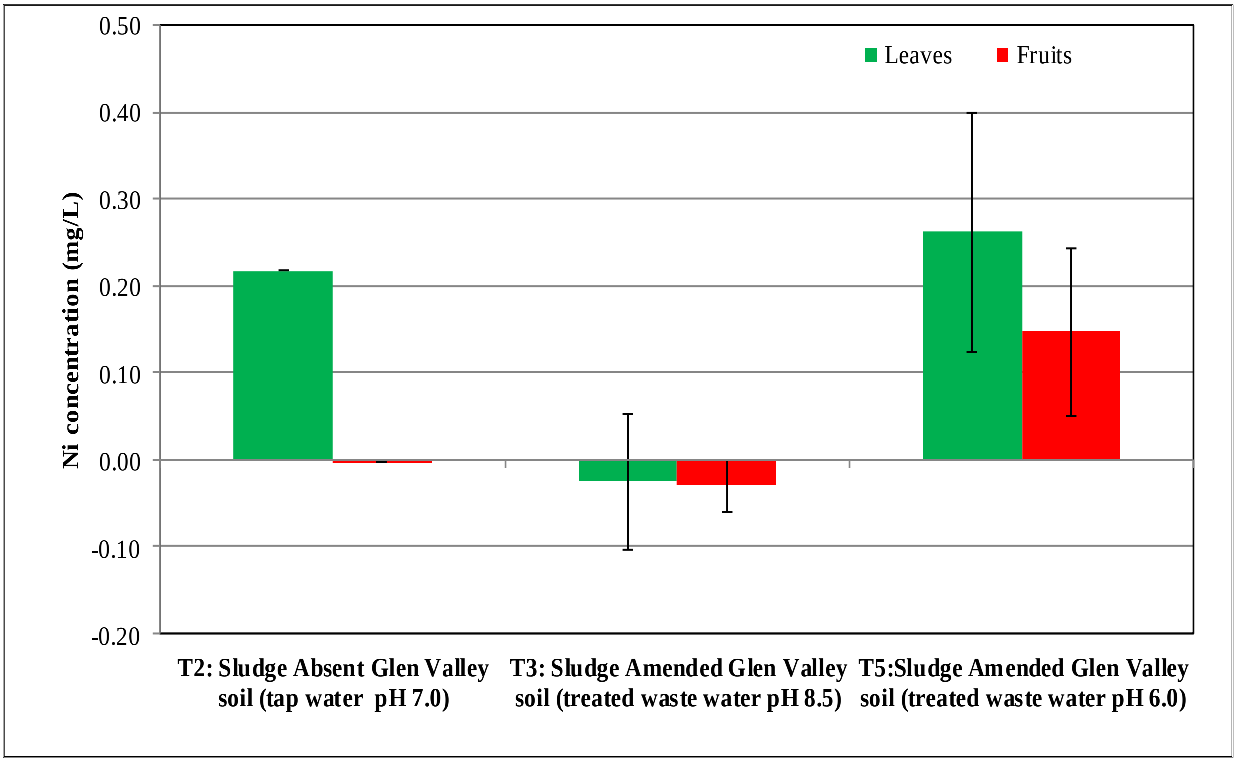


Figure 5.9 Average concentration of nickel in the tomato plants for the different treatments (pH 6.0). Bars represent SEM (n=5)

As shown in Figure 5.9., there was an increased uptake of nickel when treated waste water at pH 6.0 was used to irrigate sludge amended Glen Valley soil compared to irrigation of treated waste water at pH 5.0 (Figure 5.8; T4). This might be ascribed to nickel being rather weakly sorbed to clay and iron minerals in the soil at the higher pH than at the lower pH and thus becoming more mobile and available for plant uptake (Agency for Toxic Substance and Disease Registry, 2005; McGrath, 1995). However, one contrary report stated that it was possible that the type of plant species affects the pH behavior of nickel uptake. Cataldo et al., (1978) reported in their study of nickel in plants that nickel uptake in soybean seedlings lack a pH effect; they reported nickel uptake from 20 µM/L solutions by 15-day old plant to be independent of pH from 4.5 to 7.0

5.10 NICKEL UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER PH 9.0

The nickel concentration in the leaves and the fruits of tomatoes after treatment with normal treated waste water and treated waste water at pH 9.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Table 5.11 and Figure 5.10.

Table 5.11. Nickel uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 9.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils)

Treatment Type	Nickel concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error of the Mean	Mean	Standard Error of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.217	0.000	-0.003	0.000
T3:Sludge amended Glen Valley soil with treated waste water at pH 8.5	-0.025	0.078	-0.030	0.030
T6:Sludge amended Glen Valley soil with treated waste water at pH 9.0	-0.050	0.020	-0.007	0.053

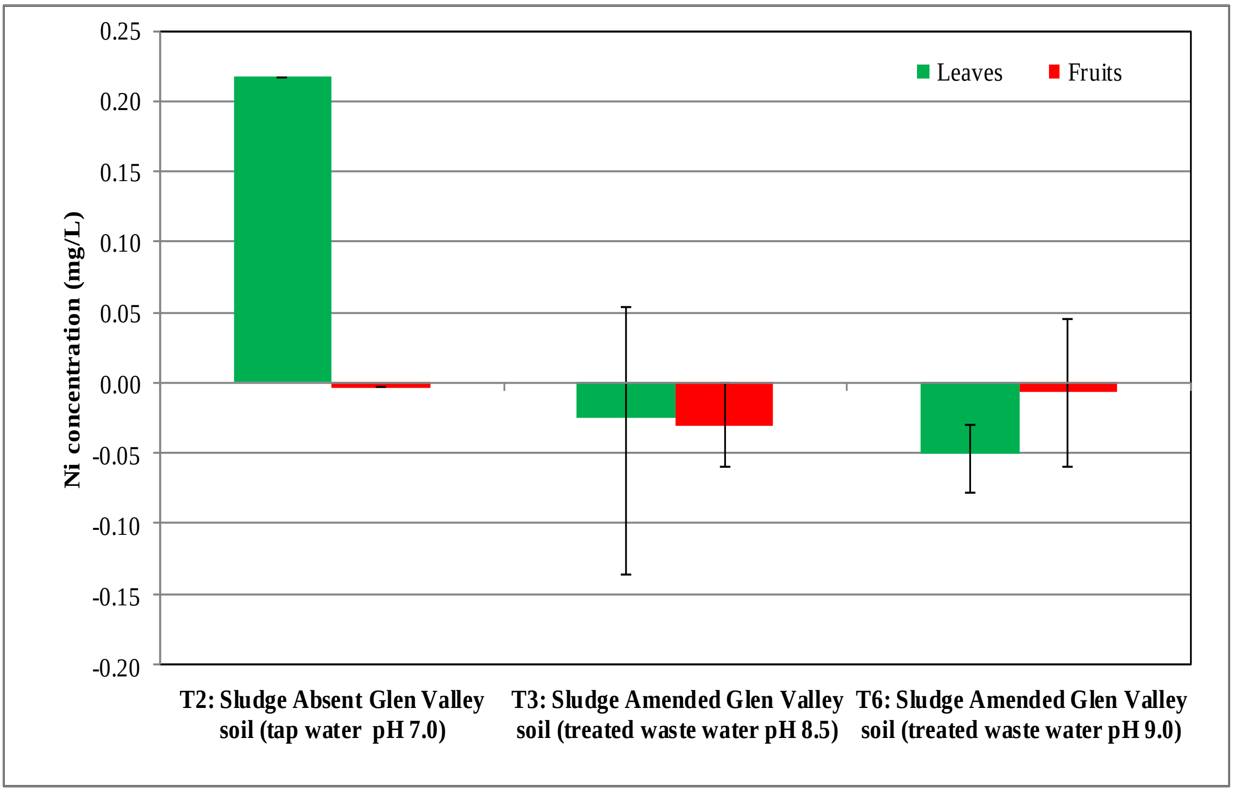


Figure 5.10 Average concentration of nickel in the tomato plants for the different treatments (pH 9.0). Bars represent SEM (n=5)

Treatment with sludge amended Glen Valley soil (Figure 5.10; T6) using treated waste water at pH 9.0 showed a negative correlation of nickel uptake in the leaves and fruits of the tomato plants compared with treatments at pH 6.0 (Figure 5.8; T5). Nickel was reduced from 0.262 mg/L (Figure 5.8; T5) to -0.050 mg/L (Figure 5.10; T6) and from 0.147 mg/L (Figure 5.8; T5) to -0.007 mg/L (Figure 5.10; T6) for the leaves and fruits, respectively. The observed trend in the T6 treatments with nickel desorption in both the leaves and the fruits of tomato plants cultivated in sludge amended Glen Valley using treated waste water at pH 9.0 is similar to the T3 treatments (sludge amended Glen Valley soil with normal treated waste water at pH 8.5) where there was also nickel desorption in both the leaves and the fruits of the tomato plants. The similar trend observed could be ascribed to the reduced availability of the free nickel ion in the soil water at high pH (pH 9.0) (Vijayakumaranj et al., 2009). From these observations it can be concluded that the measured and controlled Glen Valley treated waste water at pH 8.5 can significantly lower if not eliminate the uptake of nickel in the tomato plants, especially the edible fruit portion of it.

5.11 NICKEL UPTAKE IN THE TOMATO PLANTS AT TREATED WASTE WATER PH 10.0

The nickel concentration in the leaves and the fruits of tomato plants after treatment with normal treated waste water and treated waste water at pH 10.0 using sludge amended Glen Valley soils compared with treatment using tap water on sludge absent Glen Valley soils are shown in Table 5.12 and Figure 5.11.

Table 5.12. Nickel uptake in the leaves and the fruits of the tomato plants: Treated waste water at pH 10.0 (sludge amended soils) compared with normal treated waste water at pH 8.5 (sludge amended soils) and with tap water at pH 7.0 (sludge absent soils).

Treatment Type	Nickel concentration (mg/L)			
	Leaves		Fruits	
	Mean	Standard Error Of the Mean	Mean	Standard Error Of the Mean
T2: Sludge absent Glen Valley soil with tap water at pH 7.0	0.217	0.000	-0.003	0.000
T3: Sludge amended Glen Valley soil with normal treated waste water at pH 8.5	-0.025	0.078	-0.030	0.030
T7: Sludge amended Glen Valley soil with treated waste water at pH 10.0	0.062	0.008	0.200	0.237

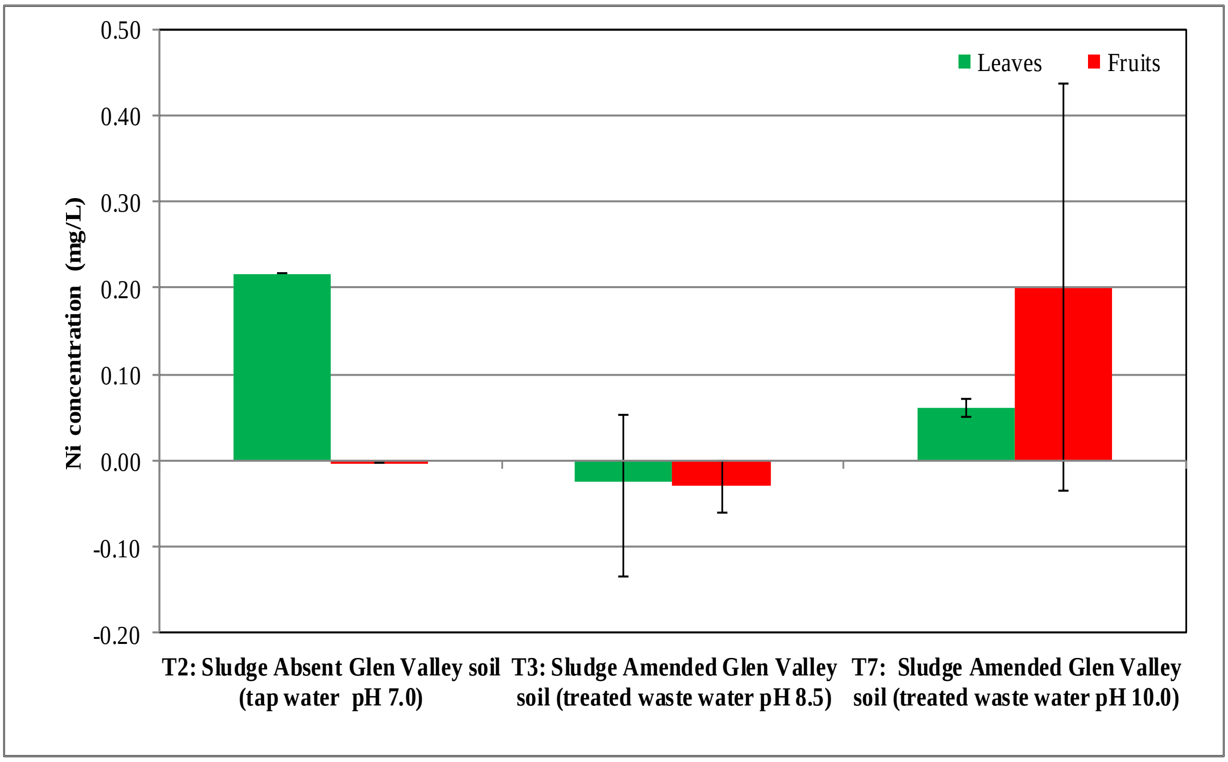


Figure 5.11 Average concentration of nickel in the tomato plants for the different treatments (pH 10.0). Bars represent SEM (n=5)

Treatment with sludge amended Glen Valley soil (Figure 5.11; T7) and treated waste water at pH 10.0 increased the nickel uptake in the leaves and fruits of the tomato plants compared to treatment with sludge amended Glen Valley soil and treated waste water at pH of 9.0 (Figure 5.10; T6) where there was nickel desorption. This effect could be linked to the amphoteric nature of the hydroxide of nickel that formed from the precipitation reaction when adding sodium hydroxide to the normal treated waste water at pH 8.5. This amphoteric nature of nickel hydroxide makes it increasingly soluble in the treated waste water for irrigation at high pH (pH 10.0) so that the nickel goes back into solution and makes it available for tomato plant uptake. One opposite pattern observed when cultivating tomato plants in sludge amended

Glen Valley soil using treated waste water at pH 10.0 compared with cultivating tomato plants in sludge amended Glen Valley soil using treated waste water at pH 9.0 was that more nickel bio-accumulate in the fruits of the tomato at pH 10.0 than at pH 9.0 (Figure 5.11; T7). This could be ascribed to the presence of root surface hydroxyl (OH^-) radicals at pH value higher than 9.0 (Argun and Dursun, 2007) and thus creating a competition between nickel ions, decreasing the aggregation of nickel and thereby causing an increase in the adsorption of nickel into tomato shoots and partioning it into the fruits and the leaves.

5.12 SUMMARY OF NICKEL UPTAKE IN THE TOMATO PLANTS

A summary of the nickel concentration in the leaves and fruits of the tomatoes after treatment with normal treated waste water and treated waste water at different pH levels (sludge amended Glen Valley soils) and tap water (in sludge absent Glen Valley soil and standard commercial soil) are shown in Figure 5.12.

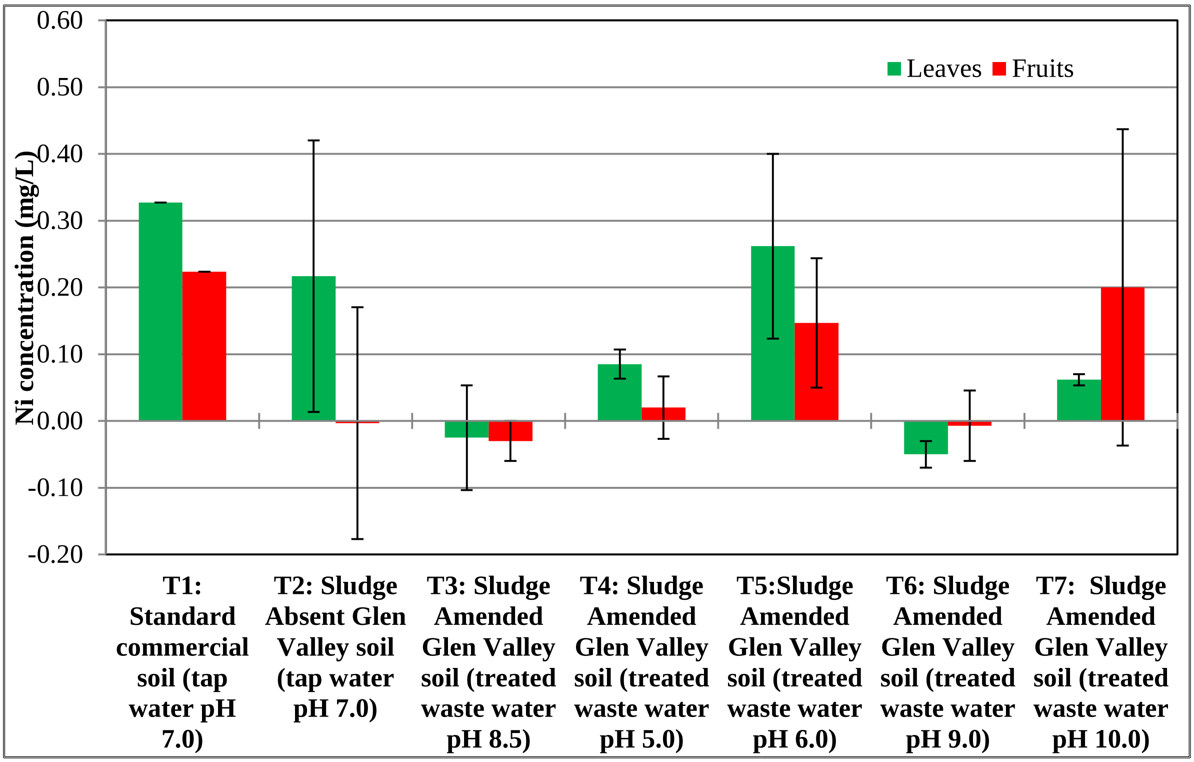


Figure 5.12 Average concentration of nickel in the tomato plants for the different treatments (summary). Bars represent SEM (n=5)

Nickel accumulation in the tomato plants tends to fluctuate. The highest accumulation occurred in the tomato leaves, 0.327 mg/L (Figure 5.12; T1), when using tap water irrigation for tomatoes planted in standard commercial soil. There was also a very high concentration of nickel in the leaves (0.262 mg/L) of the tomato plants irrigated with treated waste water at pH 6.0 (in sludge amended Glen Valley soil) and the leaves (0.217 mg/L) (Figure 5.12; T2), of the tomato plants irrigated with tap water at pH 7.0 (in sludge absent Glen Valley soil). High concentration of nickel was also experienced in the fruits (0.224 mg/L) of the tomato plants irrigated with tap water (in standard commercial soil) and in the fruits (0.200 mg/L) of the tomato plants irrigated with treated waste water at pH 10.0 (in sludge amended Glen Valley soil). Again, nickel concentration was high in the fruits (0.147 mg/L) of the tomato plants irrigated with treated waste water at pH 6.0 (in sludge amended Glen valley soil). In contrast, nickel desorption took place in the tomato plants cultivated in the sludge amended Glen Valley soils using treated waste water at pH 8.5 and pH 9.0. This could be ascribed to a combination of factors such as reduced availability of the free nickel ion in the soil water at high pH and raising of the pH of the normal treated waste water solution to 9.0 with sodium hydroxide which precipitated nickel hydroxide from the solution. The foregoing trends are comparable to the theoretical solubility of nickel hydroxide (Appendix E2) where the solubility of the metal is directly controlled by pH. Statistical analysis shows no significant difference between the mean nickel concentration in the leaves and that of fruits of the tomatoes at the 5% significant level. Most of the mean nickel concentrations in the leaves and fruits of the tomato plants in this study were lower than the 0.2 mg/L recommended maximum level for crop production (Food and Agriculture Organization, 1985). The recommended permissible limit of 0.30 mg/L set by the Botswana Bureau of Standards (2004) was also not exceeded. However there were a few exemptions where these limits were exceeded. One is the mean nickel concentration in the tomato plant leaves cultivated in standard commercial soil using tap water irrigation and another is the mean nickel concentration in the tomatoes planted in sludge amended Glen Valley soil with treated waste water (pH 6.0) for irrigation.

CHAPTER 6

SUMMARY OF MAJOR FINDINGS

6.1 SUMMARY OF FINDINGS

The objective of this study is to compare the uptake of chromium and nickel in tomato plants irrigated with treated waste water (using sludge amended Glen Valley soils) to that irrigated with tap water (using sludge absent Glen Valley soils). The summary, conclusions and recommendations that can be made are as follows;

6.1.1 CHROMIUM

- ✓ Chromium was detected in significant concentrations in the leaves (0.052 mg/L – 0.819 mg/L) and the fruits (0.054mg/L – 0.599mg/L) of the tomatoes for most treatments.

- ✓ Irrigation of the tomato plants with normal Glen Valley treated waste water at pH 8.5 in sludge absent Glen Valley soils has been shown to reduce the uptake of chromium in the leaves and the fruits of the tomato plants compared to irrigation of the tomato plants with tap water in sludge absent Glen Valley soils. A similar reduction pattern was observed in the leaves and the fruits of the tomato plants irrigated with treated waste water at pH 9.0 in sludge amended soils
- ✓ Accumulations of chromium in the tomato leaves and the fruits tend to increase as the pH of the treated waste water increased from slightly acidic (pH 5.0 to pH 6.0) and close to a neutral pH of 7.0. Chromium uptake increased more than two fold in the tomato leaves and fruits when the pH of the irrigating waste water was raised from 5.0 to 6.0.

- ✓ Tomato leaves and fruits cultivated in standard commercial soil with tap water take up slightly more chromium than tomato plants cultivated in sludge absent Glen Valley soil with tap water.
- ✓ Uptakes of chromium in the tomato leaves and fruits grown in sludge absent Glen Valley soils using tap water are greater than the uptake of chromium in the tomato leaves and fruits grown in sludge amended Glen Valley soil using treated waste water at pH 5.0; 6.0; 8.5 and 9.0. However, there was an exception when the tomato plants were irrigated with treated waste water at pH 10.0 (in sludge amended Glen Valley soil); the fruits of the tomato bio-accumulate more chromium than the other treatments in this case.
- ✓ The Food and Agriculture Organization permissible limits and the Botswana Bureau of Standards effluent quality limits for chromium were exceeded as a result of treated waste water irrigation in sludge amended Glen Valley soil for tomato production.

6.1.2 NICKEL

- ✓ Nickel was detected in the tomato leaves (-0.050 mg/L – 0.327 mg/L) and fruits

  (-0.030 – 0.224 mg/L) but the concentrations were not significant for most treatments. Desorption of the nickel occurred in the fruits of the tomato plants cultivated with sludge absent Glen Valley soil using tap water for irrigation. Nickel was also desorbed in the tomato leaves and fruits cultivated with sludge amended Glen Valley soils using normal treated waste water at pH 8.5 and treated waste water at pH 9.0 for irrigation
- ✓ Irrigation of the tomato plants with normal Glen Valley treated waste water at pH 8.5 in sludge absent Glen Valley soils has been shown to eliminate the uptake of nickel in

the leaves and the fruits of the tomato plants compared to irrigation of the tomato plants with tap water in sludge absent Glen Valley soils. A similar reduction pattern was observed in the leaves and the fruits of the tomato plants irrigated with treated waste water at pH 9.0 in sludge amended soils

- ✓ Accumulations of nickel in the tomato leaves and fruits tend to increase as the pH of the treated waste water increased from slightly acidic to neutral pH values. Nickel uptake increased more than two fold in the tomato leaves and fruits when the pH of the irrigating waste water was raised from pH 5.0 to 6.0.
- ✓ Uptake of nickel in the leaves of the tomato plants grown in sludge amended Glen Valley soil with treated waste water at a pH 6.0 was slightly higher than uptake of nickel in the leaves of the tomato plants grown in sludge absent Glen Valley soil using tap water.
- ✓ The Food and Agriculture Organization permissible limits and the Botswana Bureau of Standards effluent quality limits for nickel were not exceeded as a result of treated waste water irrigation in sludge amended Glen Valley soil for tomato production.

6.2 CONCLUSIONS

In terms of chromium uptake, use of treated waste water at pH 8.5 was the most suitable for the production of tomatoes. At this of pH 8.5 chromium concentration in the leaves and fruits of the tomato plant was found to bio-accumulate the least amount of chromium compared to other treatments. For nickel uptake, desorption occurred in the tomato leaves and fruits when using treated waste water at pH 8.5 and this may be good because nickel toxicity may be avoided at this pH of 8.5 but it may also be bad because repeated application of treated waste water at pH of 8.5 could trigger leaching of nickel in the soil and consequently to the ground water table. Irrigation of tomato plants by Glen Valley treated waste water at pH 5.0; 6.0 and 10.0 has increased mean chromium concentration in the tomato leaves and fruits to quantities above the maximum permitted concentrations, hence usage at these pH levels should not be allowed. However, the mean nickel concentration is reduced at these pH levels.

In the final analysis, irrigation of tomato plants with normal Glen Valley treated waste water at pH 8.5 appears to be safe and should be continued, but with caution. As highlighted in this study, the mean chromium uptake in the tomato plant samples exceeded the permissible safe limit for agriculture production. Consequently, the edible portions of the tomato plants grown with treated waste water and sludge amended Glen Valley soils should be subject to testing and analysis before passing them on to consumers.

6.3 RECOMMENDATIONS

- ✓ The Glen Valley farmers should consider reducing the pH of the treated waste water by acid addition to pH 8.5 or pH 9.0 because this should help to reduce or minimize the uptake of chromium by tomato plants and possibly eliminate the nickel uptake.
- ✓ Further studies into the uptake and the phytotoxicity effects of other heavy metals in tomato plants when irrigating with treated waste water should be carried out. It should pose questions on the bio-assimilation of heavy metals in tomato plants.
- ✓ Analyze the content of chromium, nickel and other heavy metals in the tomato leaves and fruits and, compare them to the levels in the soil around the root zone.
- ✓ Utilization of treated waste water in the long term irrigation plans at the Glen Valley should be based on an environmentally sound, accurate, and a well-managed

approach. Also, programs need to be monitored periodically for the quality of the properties of the water, treated waste water and plants, in order to minimize the risk of negative effects to the food chain and human health.

✓ Government should partner farmers to initiate, fund and encourage more research work on chromium, nickel and other heavy metals of concerns as a result of treated waste water use. Also, due to few empirical data available from Botswana for this present study it is imperative for the government of Botswana to actively take a leading role in the campaign for academicians, researchers, financial backers and the private sector to contribute in improving the situation.

REFERENCES

ABDEL-GHAFFAR AS, EL-ATTAR HA and ELSOKARY IH (1988) Egyptian experience in the treatment and use of sewage and sludge in agriculture. Ch. 17. Treatment and Use of Sewage Effluent for Irrigation. M.B. Pescod and A. Arar (eds). Butterworths, Sevenoaks, Kent.

ACQUAAH G (2002) Horticulture; Principles and Practices. New Jersey, Prentice Hall.

AGANGA AA, MACHACHA S, THEMA T and MAROTSI BB (2005) Minerals in Soils and Forages Irrigated with Secondary Treated Sewage Water in Sebele. Botswana Journal of Applied Sciences 5(1): 155 - 161.

AGENCY for TOXIC SUBSTANCE and DISEASE REGISTRY (2005) Toxicological Profile for nickel. Atlanta, GA. Available online at www.atsdr.cdc.gov/ToxProfiles

AKANDE CB (2007) A performance study on the use of treated wastewater and sludge for spinach production in Glen Valley. MSc Thesis, University of Botswana, Gaborone, Botswana (Unpublished).

AL-LAHHAM O, EL ASSI NM and FAYYAD P (2003) Impact of treated waste water irrigation on quality attributes and contamination of tomato fruit. Agricultural Water Management. 61, 51–62.

ARGUN EM and DURSUN S (2007) A new approach to modification of natural adsorbent for heavy metal adsorption. Bioresource Technology 99 (2008) 2516-252.

ARNTZEN J (2006) Mainstreaming waste water through water accounting: the example of Botswana; Centre for Applied Research, Gaborone, Botswana.

ASANO T, SMITH RG and TCHOBANOGLOUS (1985) Municipal waste water: Treatment and reclaimed water characteristics. Irrigation with Reclaimed Municipal Waste water. A Guidance Manual, G.S. Pettygrove and T. Asano (eds). Lewis Publishers Inc., Chelsea, Mississippi.

AVIAS J (1972) Nickel: Element Geochemistry. In: The Encyclopedia of Geochemistry and Environmental Sciences, R.W. Fairbridge (ed.), Van Nostrand Reinhold Co., New York, NY, pp. 790-793.

AYRES DM, DAVIS AP and Gietka PM (1994) Removing heavy metals from waste water. Engineering research center report, University of Maryland, USA. (Accessed online at www.ummap.umd.edu/documents/rm_wastewater.html)

BAHRI A (1988) Present and future state of treated waste waters and sewage sludge in Tunisia Presented at Regional Seminar on Wastewater Reclamation and Reuse, 11-16 December, 1988, Cairo, Egypt.

BARTLETT RJ and JAMES B (1983) Behavior of chromium in soils: Adsorption and reduction of hexavalent forms. J. Environ. Qual. 12(2):177-181.

BARTLETT RJ and KIMBLE JM (1976) Behavior of chromium in soils: I. trivalent forms. J. Environ. Qual. 5:379-382.

BEUCHAT LR (1998) Food safety issues: Surface decontamination of fruits and vegetables eaten raw: A Review. Food Safety Unit, World Health Organization (WHO), Geneva, Switzerland, pp 42

BHATIA SC (2005) Textbook of Biotechnology: Conventional Wastewater treatment processes. New Delhi, Atlantic.

BOTSWANA BUREAU of STANDARDS (2004) Water quality standards for waste water. BOS 93.

BOTSWANA DEPARTMENT of SURVEYS and LANDS (1987) Location map of study area: Glen Valley farms.

BRAKE SS, JENSEN RR and MATTOX JM (2004) Effect of coal fly ash amended soils on trace element uptake in plants. Environmental Geology, 45, 680-689.

CALIFORNIA STATE WATER RESOURCES CONTROL BOARD (1990) California Municipal waste water reclamation in 1987. Sacramento, California, USA.

CALLAHAN MA, SLIMAK MW, GABEL NW, MAY IP, FOWLER CF, FREED JR, JENNINGS RL, DURFEE RL, WHITMORE B, MAESTRI B, MABEY WR, HOLT BR and GOULD C (1979) Nickel In: Water-related Environmental Fate of 129 Priority Pollutants, Vol. I: Introduction and Technical Background, Metals and In organics, Pesticides and PCBs, U.S. Environmental Protection Agency Report EPA-440/4-79-029a, U.S. EPA, Office of Water and Waste Management, Washington, DC, USA.

CANADIAN ENVIRONMENTAL PROTECTION ACT (1994) Nickel and its compounds, priority substances list assessment report. National printers (Ottawa) Inc.

CARLTON-SMITH CH (1987) Effects of metals in sludge-treated soils on crops. Report TR 251, Water Research Centre, Medmenham, UK.

CARY EE, ALLAWAY WH and OLSON EO (1975) Control of chromium concentration in food plants. 1. Absorption and Translocation of chromium by plants. J. Agric. Food Chem. 23, 479

CATALDO DA, GARLAND TR and WILDUNG RE (1978) Uptake kinetics using intact soybean seedlings. Plant physiology. 62: 563-565

CHANG CA and PAGE LA (1977) Trace elements in waste water. California Agricultural Journal pp 32-33

CHILTON PJ, STUART ME, ESCOLERO O, MARKS RJ, GINZALES A and MILNE CJ (1998) Groundwater recharge and pollution transport beneath waste water irrigation: the case of Leon, Mexico. 153-168 in groundwater pollution, aquifer recharge and vulnerability. Robins, N.S. ed. Geological Society of London Special Publication, No.130.

CONOLLY EL and GUERINOT ML (2002) Iron stress in plants. Genome Biology 3(8): 1024 -1026.

COOPER RC (1991) Public health concerns in waste water use. Water Science and Technology 24: 55 - 65.

COTTON FA and WILKINSON G (1988) Advanced Inorganic Chemistry: A Comprehensive Text. Wiley-Interscience, New York, NY, (4th edition, 1980, 5th edition, 1988).

DAVIES JN and HOBSON GE (1981) The constituents of tomato fruit - the influence of environment, nutrition, and genotype. Critical Reviews in Food Science and Nutrition 15, 205-280

DIKINYA O and AREOLA O (2010) Comparative analysis of heavy metal concentration in secondary treated wastewater irrigated soils cultivated by different crops. Int. J. Environ. Sci. Tech., 7 (2), 337-346.

ENVIRONMENT AGENCY (2009) Supplementary information for the derivation of SGV for Nickel. Plant uptake review. Report SC050021. Bristol: Environment Agency.

FOOD and AGRICULTURE ORGANIZATION (1992) Wastewater treatment and use in agriculture. FAO Irrigation Paper 47: Food and Agriculture Organization of the united nations, Rome, Italy.

FOOD and AGRICULTURE ORGANIZATION (1988) Revised legend of the FAO and UNESCO soil map of the world. World soils resources report No. 60. FAO/UNESCO/ISRIC, Rome.

FOOD and AGRICULTURE ORGANIZATION (1985) Water quality for agriculture. AYERS RS and WESTCOT DW. Irrigation and Drainage paper 29 Rev.1. FAO, Rome. p174

FOSTER SD (1992) Unsustainable development and irrational exploitation of groundwater resources in developing nations – an overview. IAH Selected Papers, Vol. 3, 321-336.

GE Y, MURRAY P and HENDERSHOT WH (2000) Trace metal speciation and bioavailability in urban soils. Environmental Pollution, 107, 137 – 144.

GHULAM H and AL-SAATI A (1999) Wastewater quality and its reuse in agriculture in Saudi Arabia. Desalination 123: 241 - 251.

GOLOVATYJ SE, BOGATYREVA EN and GOLOVATYI SE (1999) Effect of levels of chromium content in a soil on its distribution in organs of corn plants. Soil Res. Fert. 197-204.

GONZALEZ R, NDUNG'U K and FLEGAL R (2005) Natural Occurrence of Hexavalent chromium in the Aromas Red Sands Aquifer, California. Environmental Science and Technology 39 (15): 5505–5511.

GOVERNMENT OF BOTSWANA (1998) Glen Valley Detailed Layout, Department of Town and Regional Planning, Ministry of Lands, Housing and Environment of the Republic of Botswana, Gaborone, Botswana.

GRUBINGER VP, GUTENMANN WH, DOSS GJ, RUTZKE MO and LISK DJ (1994) Chromium in Swiss chard grown on soil amended with tannery meal fertilizer. Chemosphere. 4:717-720.

GUERTIN J, JACOBS JA and AVAKIAN CP (2004) Chromium (VI) handbook, CRC Press, London.

GUPTA AP, NARWAL RP and ANTIL RS (1998) Biosolids management update. Biocycle 39: (1), 69 - 74.

HOSSNER LR, LOEPPERT RH and NEWTON RJ (1998) Phytoaccumulation of Chromium, Uranium and Plutonium in plant systems. Amarillo National Resource Center for Plutonium, Amarillo Texas, USA.

INTERNATIONAL PROGRAM ON CHEMICAL SAFETY (1991) Environmental Health Criteria Series #108: Nickel", World Health Organization, Geneva, Switzerland, ISBN 92-4-157108, 383+ p.

JACKSON ML (1967) Soil chemical analysis. Prentice-Hall India Part. Ltd., New Delhi, India.

JONES JB (2008) Tomato plant Culture: in the field, greenhouse and home garden: Seed and seedling production. 2nd ed. Florida, RCR Press.

KABATA-PENDIAS A and MUKHERJEE A (2007) Trace Elements from Soil to Human. Berlin: Springer-Verlag.

KABATA-PENDIAS A and PENDIAS H (2001) Trace Elements in Soils and Plants, Third Edition. Boca Raton: CRC Press LLC.

KANDIAH A (1990) Water quality management for sustainable agricultural development. Natural resources forum. 14(1): 22-32.

KELLEY WT and BOYHAN G (2006) History, Significance, Classification and Growth: Commercial Tomato Production Handbook (Available online at: http://pubs.caes.uga.edu/caespubs/pubcd/)

KHAIRIAH JI, YIN HY, IBRAHIM NK, WEE WA, AMINAH AS, MAINO AA, ZALIFAH MK and GIBER KG (2002) Bioavailability of chromium in Vegetables of selected agricultural areas of Malaysia. Pakistan Journal of Biological Sciences 5(4): 471-473.

KHUPE SN (1996) Water supply sewage and waste management for Gaborone, Botswana. AMBIO, 25. (2) 138-143.

KIRKHAM MB (1986) Problems of using waste water on vegetable crops. Hort Science 21 (1), 24–27.

KOCIK K and ILAVSKY J (1994) Effect of Strontium and chromium on the quantity and quality of the biomass of field crops. Production and utilization of agricultural and forest biomass for energy: Proceedings of a seminar held at Zvolen, Slovakia, p.168– 78.

KOTAS J and STASICKA Z (2000) Chromium occurrence in the environment and methods of its speciation. Environmental Pollution 107 (3): 263–283.

KOTLHAO K, NGILA C and EMONGOR VE (2006) Metal determination in secondary treated sewage water for crop irrigation in Gaborone, Botswana. Botswana Journal of Agriculture and Applied Sciences 2(1):11-12, ISSN 1815-5574.

KRIJGER GC, VLIET PM and WOLTERBEEK HT (1999) Metal Speciation in Xylem Exudates of *Lycopersicon esculentum*. Plant soil, 212; 165-173.

KUKIER U, PETERS CA, CHANEY LR and ANGEL AJ (2004) The effect of pH on metal accumulation in two ayssum species. Journal of Environmental Quality 33: 2090 - 2102.

LIDE DR (1993) CRC hand book of chemistry and physics. 73rd ed. Boca Raton, FL: CRC Press, Inc

LIU DF and LIPTAK BG (1997) Environmental engineering handbook: Nature of wastewater. 2nd ed. Florida, CRC Press.

MANGABEIRA P, MUSHRIFAH E, SCAIG F, ALMEIDA F, LAFFRAY D, SEVERO G, OLIVEIRA H and GALLE P (2005) Accumulation and distribution of chromium in the Tomato Plant studies using SIMS and Electro Probe X-Ray Microanalysis. Thesis Université Paris XII, p. 172.

MBIBA B (1995) Urban Agriculture in Zimbabwe, Ashgate publishing Ltd. Averbury, UK.

MCGRATH SP (1995) Nickel in Heavy Metals in Soils (2nd edn.) (Ed. B.J. Alloway). London: Blackie Academic & Professional.

MISHRA S, SHANKER K, SRIVASTAVA MM, SRIVASTAVA S, SRIVASTAVA R, DASS S (1997) A study on the uptake of trivalent and hexavalent chromium by paddy (*Oryza sativa*): possible chemical modifications in rhizosphere. Agriculture Ecosystem Environment 62:53 – 8.

MORAL R, GOMEZ I, PEDRENO J and MATAIX J (1996) Absorption of chromium and effects on micronutrient content in tomato plant (*Lycopersicum esculentum M*). Agrochemical; 40:132–8.

MORAL R, PALACIOS G, GOMEZ I, NAVARRO-PEDRENO J and MATAIX J(1994) Distribution and accumulation of heavy metals (Cd, Ni, and Cr) in tomato plant. Fresenius Environmental Bulletin, 3, 395-399.

MORISHITA T (1988) Environmental hazards of sewage and industrial effluents on irrigated farmlands in Japan. Ch 6, Treatment and Use of Sewage Effluent for irrigation. M.B. Pescod and A. Arar (eds). Butterworths, Severoaks, Kent.

MOTULSKY H (2002) The link between error bars and statistical significance (Accessed online at: http://www.graphpad.com/articles/errorbars)

NATIONAL ACADEMY OF SCIENCES (1972) pp.150. Accessed online at http://books.google.de/books

NIEBOER E, TOM RT and SANFORD WE (1988) Nickel metabolism in man and animals. in: Sigel H, editor. Metal ions in biological systems. Vol 23. New York: Marcel Dekker, Inc; p. 91-121.

NKEGBE E (2005) Assessment of heavy metal compositions in Glen Valley dry sludge. Journal of Applied Science 5(8): 1399-1401, 2005 ISSN 1812-5654

NKEGBE E and KOORAPETSE I (2005) Heavy metal compositions in Gaborone Industrial Effluent, Journal of Applied Science 5(8): 1418-1419, 2005 ISSN 1812-5654.

OMRAN MS, WALY TM, ABDELNAIM EM and EL NASHAR BMB (1988) Effect of sewage irrigation on yield tree components and heavy metals accumulation in Navel orange trees Boil. Wastes 23, 7–24.

PALMER CD and WITTBRODT PR (1991) Processes affecting the remediation of chromium contaminated sites. Environmental health perspectives, 92:25-40.

PALMER CM and JOHNSON GD (2005) The activox process: Growing significance in the nickel industry. JOM Journal of the Minerals and Materials Society Volume 57, Number 3, 40-47.

PARSONS N (1999) Botswana history pages (Accessed online at: http//www.thuto.org/ubh)

PATOCZKA JJ, JOHNSON KR and SCHERI G (1998) Trace heavy metals removal with ferric chloride. In: Hatch Mott Macdonald. Water Environment Federation Industrial Wastes Technical Conference, Nashville, TN (available at: www.patoczka.net)

POULIK Z (1999) Influence of nickel contaminated soils on lettuce and tomatoes. *Scientia Horticulurae*, 81, 243-250.

QUAGHEBEUR M, RATE A, RENGEL Z and HINZ C (2005) Desorption kinetics of arsenate from kaolinite as influenced by pH. Journal of Environmental Quality 34: 479 - 486.

RAMACHANDRAN V, D'SOUZA TJ and MISTRY KB (1980) Uptake and transport of chromium in plants. Journal of Nuclear Agric. Biology; 9: 126-9.

RICH G and CHERRY K (1987) Hazardous waste treatment technologies, Pudvan Publishers, New York.

SCHELTINGA HMH (1987) Sludge in agriculture: The European approach. Wat. Sci. tech. 19(8):9 -18.

SEMKIN RG (1975) A Limnogeochemical Study of Sudbury Area Lakes", M.Sc. Thesis, McMaster University, Geology Department, Hamilton, Ont., 248 p.

SHANKER AK, CERVANTES C, LOZA-TAVERA C and AVUDAINAYAGAM S (2005) Chromium toxicity in plants. Science Direct, Environment International 31: 739– 753.

SHANKER AK (2003) Physiological, biochemical and molecular aspects of Chromium toxicity and tolerance in selected crops and tree species. PhD Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

SHEWRY PR, PETERSON PJ (1974) Journ. Exp. Bot. 25, 785.

SHIMADZU 6300 (2003) Operation Manuals, Shimadzu Deutschland GmbH, Duisburg, Germany.

SIMONNE E (2003) Water Management for Tomato. Florida, September 3, 2003. (Available online at: gcrec.ifas.ufl.edu/TOMATO)

SINGH AK (2001) Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea L*). Environ Ecol; 19:807–10.

SKEFFINGTON RA, SHEWRY PR and PETERSON PJ (1976) Chromium uptake and transport in barley seedlings (*Hordeumvulgare L.*) Planta 132:209-214.

SOANE BD and SAUNDER DH (1959) Nickel and chromium toxicity of serpentine soils in Southern Rhodesia. Soil Sci. 84:322-329.

SPECTR AA-10/20 (1985) Operation Manuals, Varian Techtron Pty. Ltd., Springvale, Australia.

TREWEEK, GP (1985). Pretreatment processes for ground water recharge. In: T. ASANO, ed. Artificial Recharge of Ground Water. Butterworth Publishers, Boston, pp. 205-248

UNITED NATIONS ENVIRONMENT PROGRAMME (2003) Waste water reuse for agriculture: A global assessment of the problem and options for management. Early warning and assessment report series, RS.03-3 United Nations Environment Programme, Nairobi, Kenya.

VIJAYAKUMARAN V, ARIVOLI S and RAMUTHAI S (2009) Kinetic, Thermodynamic and Equilibrium Studies. In: E. Journal of Chemistry, 6(S1), PP S347-S357

WAHAAB RA, LUBBERDING HJ, ALAERTS GJ and EL GOHARY F (1995) Copper and chromium (III) uptake by duckweed. Water Sci Technol; 32:105– 10.

WESTCOT DW and AYERS RS (1985) Irrigation water quality criteria. In: Irrigation with reclaimed municipal waste water – A guidance manual", (ed. G.S. Pettygrove, and T. Asano), Lewis Publishers, Inc., Chelsea.

WHO (1989) Health guidelines for the use of waste water in agriculture and aquaculture. Technical report No 778. World Health Organization, Geneva 74 p.

WILLIS JB (1962) Determination of lead and other heavy metals by atomic absorption spectrometry, Analytical Chemistry 36: 614

WOODWARD TL, THOMAS RJ and XING B (2003) Potential for phytoextraction of cobalt by tomato. Communications in Soil Science and Plant Analysis, 34(5, 6), 645-654.

ZAYED A, LYTLE CM, JIN-HONG Q, TERRY N and QIAN JH (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta; 206: 293-9.

ZHAI M, KAMPUNZU HAB, MODISI MP and TOTOLO O (2003) Distribution of heavy metals in Gaborone urban soils (Botswana) and its relationship to soil pollution and bedrock composition, Journal of Environmental Geology (45):171–180.

ZHELJAZKOV VD, NIELSEN NE (1996) Effect of heavy metals on peppermint and cornmint. Plant and Soil. 178: 59-66.

APPENDICES

Appendix A

Production of vegetables (Lettuce) using treated waste water in Glen valley

Agricultural production of vegetable (green pepper) using treated waste water in Glen Valley

Appendix B

Potted tomato plants irrigation with treated waste water at different pH values

Ripen tomato plants just before harvesting

Appendix C

Soil analytical data

Soil pH was reported to be basically neutral in the Glen Valley sludge amended soils and sludge absent soils as shown in table 16.

Table C1: Active and inactive soil pH of Glen Valley sludge amended and sludge absent soils

Soil sample	Active pH	Inactive pH kcl
Glen Valley sludge absent soils	7.90 at temp 23.20^0C	6.05 at temp 23.20^0C
Glen Valley sludge amended soils	7.34 at temp 23.20^0C	5.19 at temp 23.20^0C

Source: (Akande, 2007)


P1


P2


P3

PLATES: P1, P2 and P3 (some potted tomato plants containing withered plants after the second day of treatment with nutrients, N: P: K)


P4


P5


P6


P7

PLATES**:** P4, P5, P6 and P7 (some potted tomato plants containing sludge absent and sludge amended Glen Valley soil and Standard commercial soil after second day of treatment)

Appendix D

t-test calculator

The *t*-test used compares one variable (chromium/nickel) between two groups; using treated waste water with sludge amended soil on one hand and tap water with sludge absent soil on the other hand. Group one represents leaves of tomatoes and group two represents fruits of tomatoes. The data sets are entered according to Table D1 and the GraphPad software calculates the t-tests. Data set for chromium is presented below (nickel values were obtained in a similar version).

Table D1: Chromium concentration in the leaves and fruits of tomato plants

Treatment	Group one (treated waste water with sludge amended soil) Mean values	Group two (tap water with sludge absent soil) Mean Values
T2	0.217	-0.003
T3	-0.025	-0.030
T4	0.085	0.020
T5	0.262	0.147
T6	-0.050	-0.007
T7	0.062	0.200

Unpaired t-test results-Chromium

P value and statistical significance:

The two-tailed P (probability of the result) value, assuming the null hypothesis (Ho) equals 0.9803. By conventional criteria; this difference is considered to be not statistically significant.

The conventional criteria assume the following:

- The null hypothesis (Ho) has priority and is not rejected unless there is strong evidence against it.
- If one of the two hypotheses is 'simpler' it is given priority so that a more 'complicated' theory is not adopted unless there is sufficient evidence against the simpler one.
- In general, it is 'simpler' to propose that there is no difference between two sets of results than to say that there is a difference.
- The outcome of a hypothesis testing is "reject H_0" or "do not reject H_0". If we conclude "do not reject H_0", this does not necessarily mean that the null hypothesis is true, only that there is insufficient evidence against H_0 in favor of H_A. Rejecting the null hypothesis suggests that the alternative hypothesis may be true.

Confidence interval:

The mean of Group One minus Group Two equals 0.00350

95% confidence interval of this difference: From -0.30433 to 0.31133

Intermediate values used in calculations:

t-test value = 0.0253

degree of freedom = 10

standard error of difference = 0.138

Unpaired t-test results for nickel: P value and statistical significance

The two-tailed P (probability of the result) value, assuming the null hypothesis (Ho) equals 0.6157. By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:

The mean of Group One minus Group Two equals 0.03283

95% confidence interval of this difference: From -0.10839 to 0.17406

Intermediate values used in calculations:

t-test value = 0.5180

degree of freedom = 10

standard error of difference = 0.063

The Relationship between error bars and statistical significance

From the data in this research a figure as the one shown in Figure 6 is drawn and one may be tempted to draw conclusions about the statistical significance of differences between group means by looking at whether the error bars overlap. Let's look at two contrasting examples of chromium uptake between the leaves and fruits when cultivating tomato using sludge absent Glen Valley soil with tap water on one hand and using sludge amended Glen Valley soil with treated waste water on the other hand. Figure 6: Chromium concentration in tomato plants in different treatment (Error bars and statistical significance compared). Bars represent SEM (n=5)

What can be concluded when standard error bars do not overlap?

When standard error (SE) bars do not overlap, one cannot be sure that the difference between two means is statistically significant. Even though the error bars do not overlap in experiment T2 (sludge absent Glen Valley soil with tap water), the difference is not statistically significant (P=0.9803 by unpaired *t* test).

What can be concluded when standard error bars do overlap?

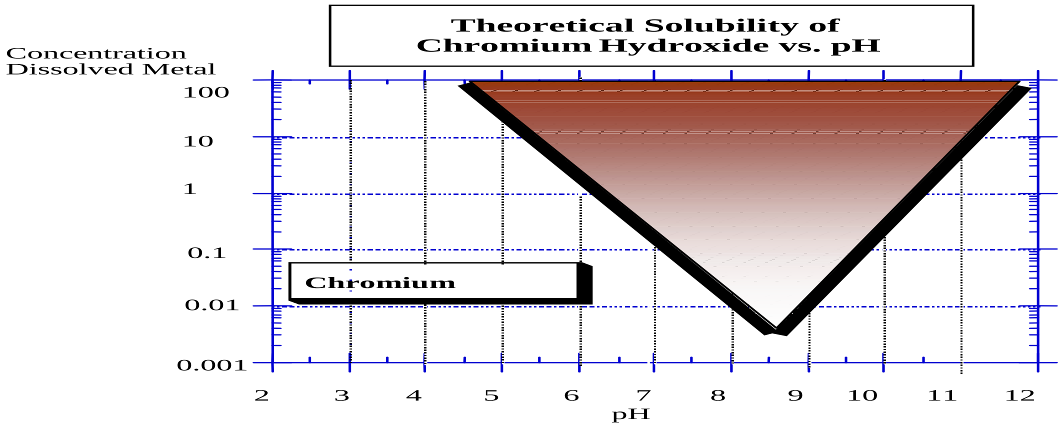
When Standard Error bars overlap, as in experiment T3 (sludge amended Glen Valley soil with treated waste water) one can be sure the difference between the two means is not statistically significant (P>0.05).

In conclusion, if two Standard Error bars overlap one can conclude that the difference is not statistically significant, but that the converse is not true (Motulsky, 2002).

Appendix E

Both of the Figures in appendix E1 and E2 illustrate how the solubility of chromium and nickel is directly controlled by pH. The y-axis displays the concentration of dissolved metal in the waste water, in milligrams/liter (mg/L). There is a wide variation in scale. The upper part of the scale shows a dissolved concentration of 100 mg/L. The lowest number on the scale is 0.001 mg/L. These solubility graphs display regions where the metals are soluble or insoluble. The region above the dark lines (the shaded areas) for each metal signifies that the metals should precipitate as metal hydroxides. This is referred to as the precipitation region.The region below or outside of the dark lines illustrates where the metals are dissolved in solution, no precipitation occurs, and no metal removal takes place.

Appendix E1: Theoretical Solubility of Chromium Hydroxide (Ayres et al., 1994)



Appendix E2: Theoretical Solubility of Nickel Hydroxide (Ayres et al., 1994)