AMERICAN UNIVERSITY OF BEIRUT

CHEMICAL AND MICROBIOLOGICAL SAFETY OF VEGETABLES GROWN IN THE UPPER LITANI BASIN

by
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A thesis
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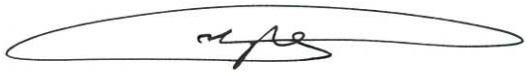
Approved by:



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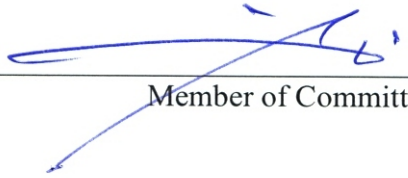

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AN ABSTRACT OF THE THESIS OF

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Title: Chemical and Microbiological Safety of Vegetables Grown in the Upper Litani Basin

The use of heavily polluted water for irrigation poses an important health hazard relating to the safety (chemical and microbiological) of grown crops. In Lebanon, the degradation in the Litani river water quality is of major concern to the agricultural production in the Bekaa region. Hence, the study objectives were to (a) identify the microbiological and chemical hazards in soils and main vegetables grown and irrigated with the Litani river water, (b) compare the levels of chemical and microbiological contaminants in irrigation water, soils and vegetables to determine the levels of contaminants and identify factors impacting the translocation and accumulation of these contaminants in grown products, (c) evaluate the magnitude of the health hazards by comparing levels of chemical and microbiological contaminants to national and international standards, and (d) determine the antibiotic resistance patterns of detected pathogenic bacteria for proper foodborne disease management.

The study methodology consisted of collecting 48 composite samples of soils and vegetables (lettuce, parsley and potato) from three different experimental sites (Bar Elias, Dalhamieh and Zahle) and a control site. The microbiological and chemical quality of the vegetables and soil was determined and evaluated. Further, the antibiotic resistant pattern was determined for the four commonly prescribed antibiotics (Ciprofloxacin, Cefotaxime, Gentamicin, and Erythromycin).

The results indicate that the irrigation with Litani River water is leading to the accumulation of microbiological (*E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, K. *oxytoca*, *S. marcescens*, *C. freundii*, *Sh. sonnei*, *C. diversus*, *Listeria spp* and *P. aeruginosa*) and chemical contaminants (barium, arsenic, lead, cadmium, chromium, zinc, iron, nickel, copper, manganese and Molybdenum) in vegetables (lettuce, parsley and potato) and soils. And, the levels in leafy vegetable are higher than in tubular crops (potato). Moreover, the results of the study showed that the exposure to the polluted irrigation water through sprinkling irrigation is the main important factor impacting the

safety of the grown crops. Furthermore, all isolated pathogens showed 100% resistance to Erythromycin, 98% resistance to Gentamicin and 93% resistant to both Ciprofloxacin and Cefotaxime.

As such, the consumption of vegetables irrigated with the Litani river water poses a major public health concern, and accordingly, it recommended to operate the existing wastewater treatment plants and follow up on the construction of planned ones, substitute sprinkler irrigation by drip irrigation to reduce exposure to contaminants, disseminate awareness on appropriate household practices to reduce the levels of contaminants in the consumed crops, and implement integrated river basin management for proper risk assessment and risk management. Additional studies are also recommended to evaluate human exposure to the identified chemical and microbiological hazards.

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LIST OF ABBREVIATION

WHO: World Health Organization

EPA: Environmental Protection Agency

FDA: Food and Drug Administration

CDC: Center for Disease Control

Ba: Barium

Pb: Lead

As: Arsenic

Mo: Molybdenum

Mn: Manganese

Cr: Chromium

Cu: Copper

Fe: Iron

Zn: Zinc

Ni: Nickel

Ca: Cadmium

CHAPTER 1
INTRODUCTION

1.1. Overview of the Chapter

This chapter presents the significance, objectives and research question of the presented study.

1.2. Significance and Objectives of the Study

The use of heavily polluted water and untreated wastewater effluents in agriculture is rising in industrial and developing countries due to water scarcity, increased population growth and the richness of such water in nutrients needed for plant growth (WHO, 2006). However, such types of irrigation water contain vast amounts of pathogens that are capable of surviving in crops and soils (WHO, 2006). The diseases mainly associated with such microbiological exposure are typhoid, shigellosis, salmonellosis, and gastroenteritis etc. (WHO, 2006). Additionally, chemical contaminants like heavy metals can be transferred from irrigation water to soils and crops. And, the accumulation of heavy metals in crops and soils may lead to immunodeficiency, impaired psycho-social behavior, intrauterine growth retardation, malnutrition and gastrointestinal cancer (Arora, 2008). As such, the use of polluted water and untreated wastewater effluents for irrigation poses an important health concern.

In Lebanon, the Bekaa region hosts 10% of the Lebanese population and is mostly characterized by agricultural (44% of the Lebanese agricultural lands), industrial (e.g. food

processing plants) and touristic activities (Assaf et al., 2008; Karam, 2002). In 2005, the vegetable production in the Bekaa valley accounted to 57 % of the overall crop production; moreover, the Bekaa region produced 80% of the local potato (Abou Zeid, 2005). The Litani River is the main source of irrigation in the region (Assaf et al 2008). Still, recent studies have shown the progressive exposure of this river to the following sources of pollution (Jurdi et al. 2010, 2011):

- Domestic wastewater effluents from sanitary sewer system outlets and cesspool leachates
- Leachates of solid waste dump sites.
- Recreational areas direct sewage discharge and solid wastes dumping
- Farm wastes (cows, sheep, poultry and swine).
- Industrial wastewater effluents (e.g. chemicals, sponge, manufacturing of batteries, paper and stone cutting, dyeing and tanning and electroplating).
- Food processing plants wastewater effluents (e.g. sugar beet, fruit jam, dairy products, juices, vegetable canning)
- Agriculture runoff (excessive use of fertilizers and pesticides)

The impact of these sources of pollution on the quality of Litani river water is more sever in the dry season due to minimal recharge from rain and melting snow (Jurdi et al., 2010; Jurdi et al., 2011). Thus the degradation of the water quality poses a major concern to agriculture production in the Bekaa region as reflected by the following major limitations (Jurdi et al., 2010; Jurdi et al., 2011):

- Increase of soil salinity due to increased levels of total dissolved solids.
- Increase of sodium and manganese levels that would lead to reduction in the water infiltrations rates.
- Increase of heavy metals levels that would lead to plant toxicity and subsequent health risks to consumers.
- Increase in microbiological contamination that would also pose a health risk to farmers and consumers.

As such, it is important to initiate risk analysis studies starting with hazards identification (chemical and microbiological) and hazard characterization to determine the safety of the grown crops. It is well known that hazard identification and hazard characterization can provide food safety regulators with the information and evidence needed for effective risk assessment and management. Hence the objectives of this study are as follows:

- Identify the microbiological and chemical hazards in the main vegetables grown in the Bekaa Region and irrigated with Litani river water.
- Determine the antibiotic resistance pattern of the isolated pathogenic bacteria for proper case management.
- Determine the microbiological and chemical quality of the soils irrigated by the Litani river water.
- Compare the levels of chemical and microbiological contaminants in Litani river water, soils and vegetables.

- Determine the levels and factors impacting the translocation and accumulation of contaminants in grown products.
- Evaluate the magnitude of the health hazard by comparing to national and international standards.
- Recommend possible mitigation measures to ensure the safety of the grown crops, reduce the exposure to health hazards, promote the need to protect the ecologic wellbeing of this major national water resource, and try to communicate the identified risk with the local community.

1.3. Hypothesis:

The research question of the presented study is:

Leafy and tubular roots irrigated by the Litani River water are microbiologically and chemically safe for human consumption.

CHAPTER 2

BACKGROUND INFORMATION

2.1. Overview of the Chapter:

This chapter presents background information on water security, water reuse in the Arabic world, water quality requirements for irrigation, the transfer and accumulation of contaminants from soil to crops and the public health significance of the use of contaminated water for irrigation.

2.2. Water Security

2.2.1. *Water Scarcity Issues*

The earth's freshwater resources are governed by increased stress from the extensive water use and pollution (Hoekestra et al., 2011). The available percentage of the annual renewable freshwater resources that is accessible to human use, accounts for 31 percent only (Asano et al., 2007).

According to UNICEF and WHO (2012), 187 million people merely depend on surface water for meeting their domestic water. In 2010, around 2.5 billion lacked access to improve sanitation and over 780 million people are still with no access to improved sources of drinking water as presented in figure 1 (WHO & UNICEF, 2012).

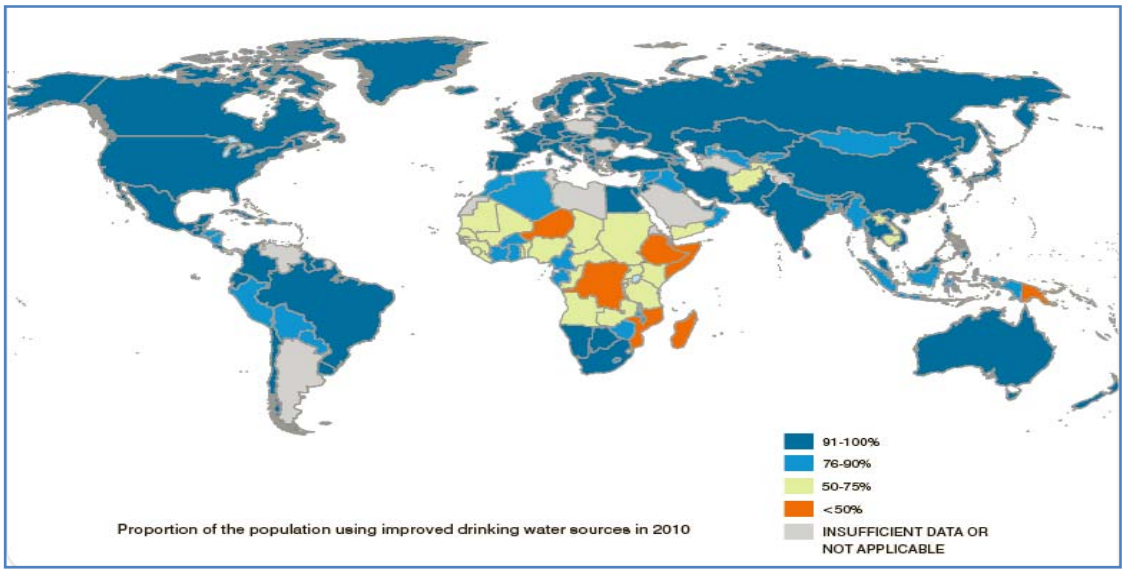

Figure 1 : Proportion of the Population with no Access to Improved Drinking Water Sources.
Source: WHO & UNICEF. (2012). Progress on Drinking Water and Sanitation: 2012 update. Retrieved from: www.unicef.org/media/files/JMPreport2012.pdf

In addition, more than one third of the world's population live under water stress conditions as presented in figure 2 (FAO, 2011). Water stress represents the ratio of the sum of the grey and blue water footprint production to the renewable water resource (WWF et al., 2010). Also, the water footprint is a measure of assessment and allocation of freshwater resources in term of water volumes polluted and consumed (incorporated in the product or evaporated) (Hoekstra et al., 2011).

The three components of the water footprint are the blue, green and grey; where the blue refers to the blue water resources consumption (ground and surface water). The green water footprint refers to the volume of the rain water (green water) that is consumed mainly in crop production (Hoekstra et al., 2011). The grey water footprint is defined as the freshwater volume needed to assimilate the pollutant loads based on the existing national

water quality standards; as such the grey water footprint is used as a freshwater pollution indicator (Hoekstra et al., 2011).

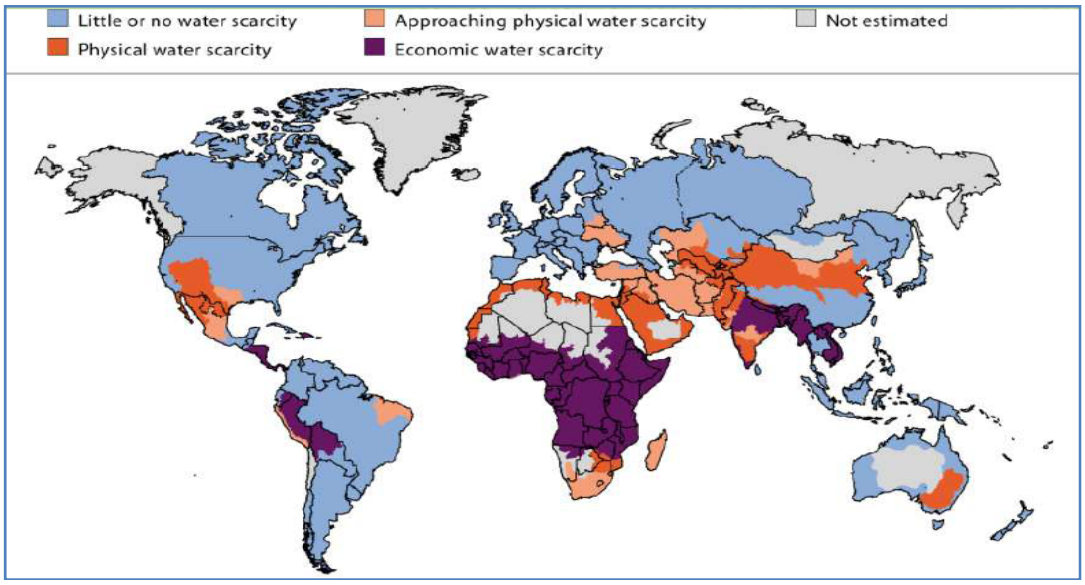

Figure 2: Global distribution of Water Scarcity around the World
Source: FAO. (2011). The state of the world's land and water resources for food and agriculture (SOLAW) - Managing systems at risk. Food and Agriculture Organization of the United Nations, Rome and Earthscan, London.

In addition, the ecological water footprint is the measure of polluted and consumed water volumes per unit time; where it constitutes of three components blue water (surface and ground water resources), green water (rain water) and grey water (degree of fresh water pollution). The ecological water footprint is high and rising in most countries of the world refer to figure 3 (WWF et al., 2010; Hoekstra et al., 2011). The global water footprint in the period 1996-2005 was 9087 Gm^3/yr where 74% accounts for green water, 11% for blue water and 15% grey water (Hoekstra et al., 2011).

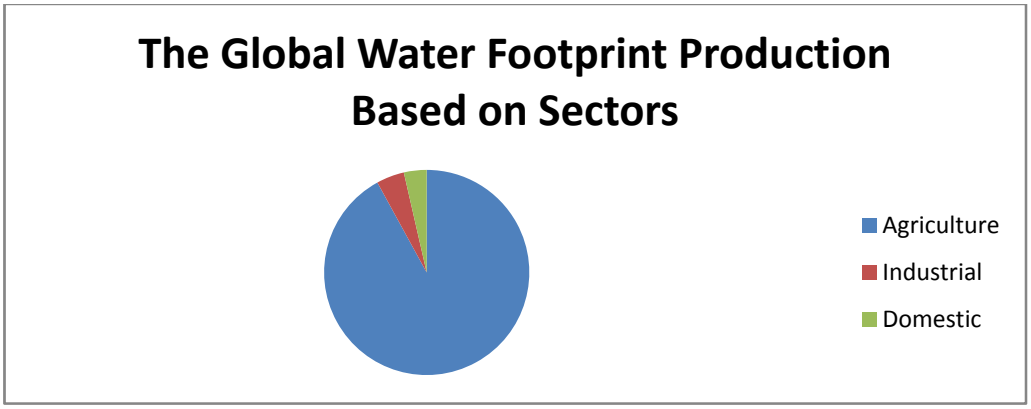

Figure 3: The Global Footprint Production Based on Sectors
Source: World Wildlife Fund, Zoological Society of London and Global Footprint Network. (2010). Living planet report 2010. Retrieved from http://awsassets.panda.org/downloads/wwf_lpr2010_lr_en.pdf

The agricultural production contributes to 92% of this total footprint; whereas, the industrial production and domestic water supply contributes only to 4.4% and 3.6 % respectively as presented in figures 3 and 4 (Hoekstra et al., 2011).

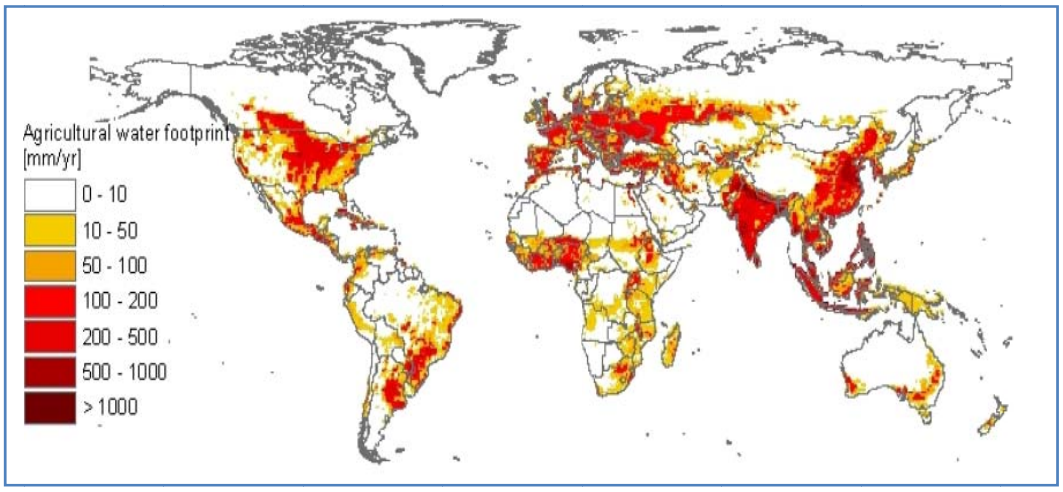

Figure 4: National Agricultural Water Footprint Between the Year 1996-2005
Source: Hoekstra, A.K., Mekonnen,M.M. (2011). National Water Footprint Accounts: The green, blue and grey water footprint of production and consumption, Value of Water Research Report Series No. 50, UNESCO-IHE, Delft, the Netherlands.

Other countries with large external water footprint, highly depend on fresh water resources, such as Malta, Kuwait, Jordan, Occupied Palestine, United Arab Emirates, Yemen, Mauritius, Lebanon and Cyprus, have large external footprint refer to figure 5 (Hoekstra et al., 2011).

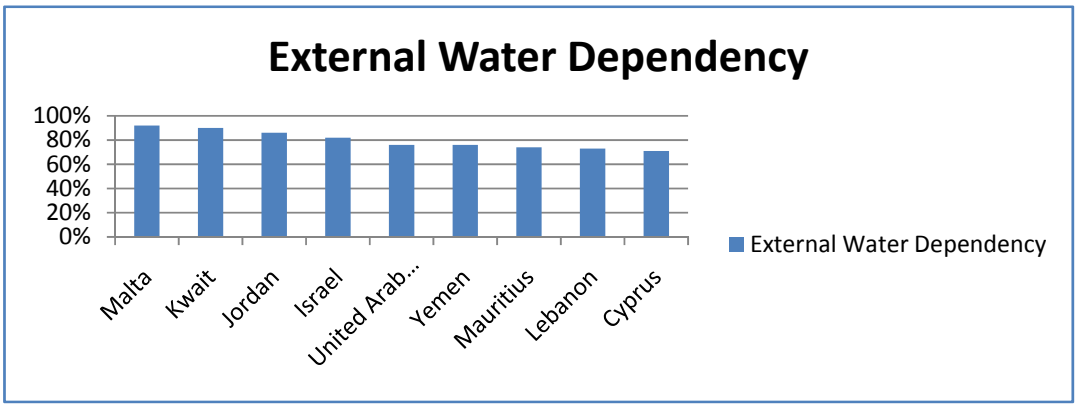

Figure 5: Highly Water-Scarce Countries with Large External Water Dependency
Source: Hoekstra, A.K., Mekonnen,M.M. (2011). National Water Footprint Accounts: The green, blue and grey water footprint of production and consumption, Value of Water Research Report Series No. 50, UNESCO-IHE, Delft, the Netherlands

Water scarcity arises when the amount of freshwater withdrawal is high and the freshwater resources are stressed and no longer able to satisfy the needs of the ecosystem and human's requirement. This would lead to increased intersectoral competition. Mostly, water scarcity reflects on an annual drop of water supply to less than 1000m^3 per person; when it drops below 500m^3 the country is considered to be facing absolute water scarcity. Regarding the water stress indicato,r if the annual water supply drops below 1700 m^3 then the area is considered facing water stress (WAAP, 2012). Figure 6 shows the areas that are experiencing water scarcity, absolute scarcity, and water stress.

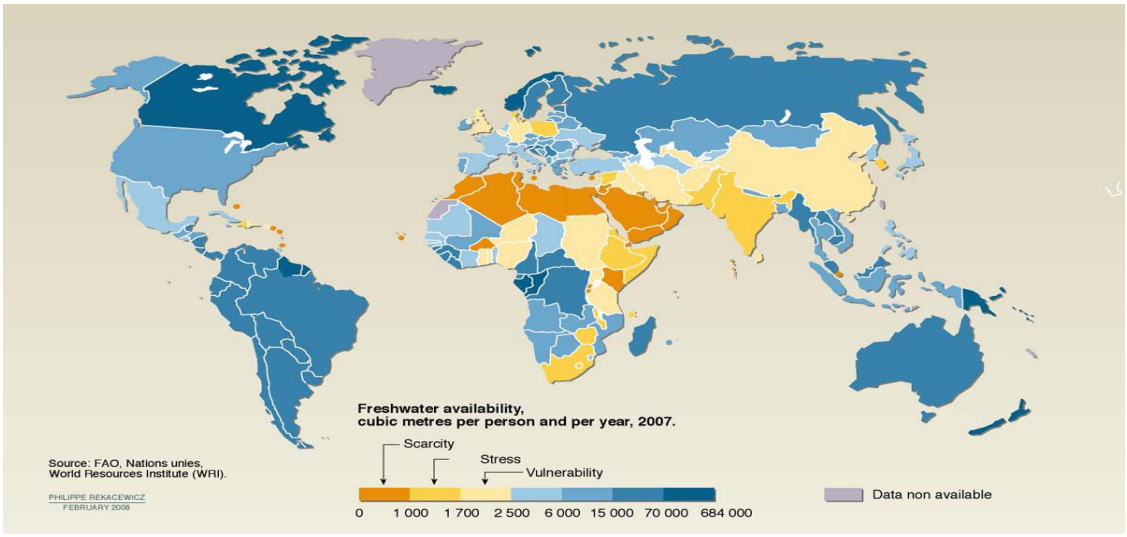

Figure 6: Global Water Stress and Water Scarcity
Source: UNEP (2008), Vital Water Graphics - An Overview of the State of the World's Fresh and Marine Waters 2nd Edition. UNEP, Nairobi, Kenya. ISBN: 92-807-2236-0

One of the most consuming sectors of renewable freshwater resources is the agricultural sector which accounts for 70 % of the world total water withdrawal as presented in figure 7 (WWAP, 2012; FAO, 2012). The total annual fresh water withdrawals for the industrial and domestic sector are 20 % and 10 %, respectively (FAO, 2012). In many countries the agricultural sector contributes up to 90% of the water demands (FAO, 2012). Developing and developed countries with severe to moderate water stress on blue include China, India, Thailand, Pakistan, Germany, Italy, Spain, Poland, South Africa, Algeria, Saudi Arabia, Morocco, Jordan, Oman, Lebanon, Kuwait, Qatar, Iraq, Armenia etc. as presented in figures 1,2,4 5, 6,7, & 8 (WWF et al., 2010; Hoekstra et al., 2011).

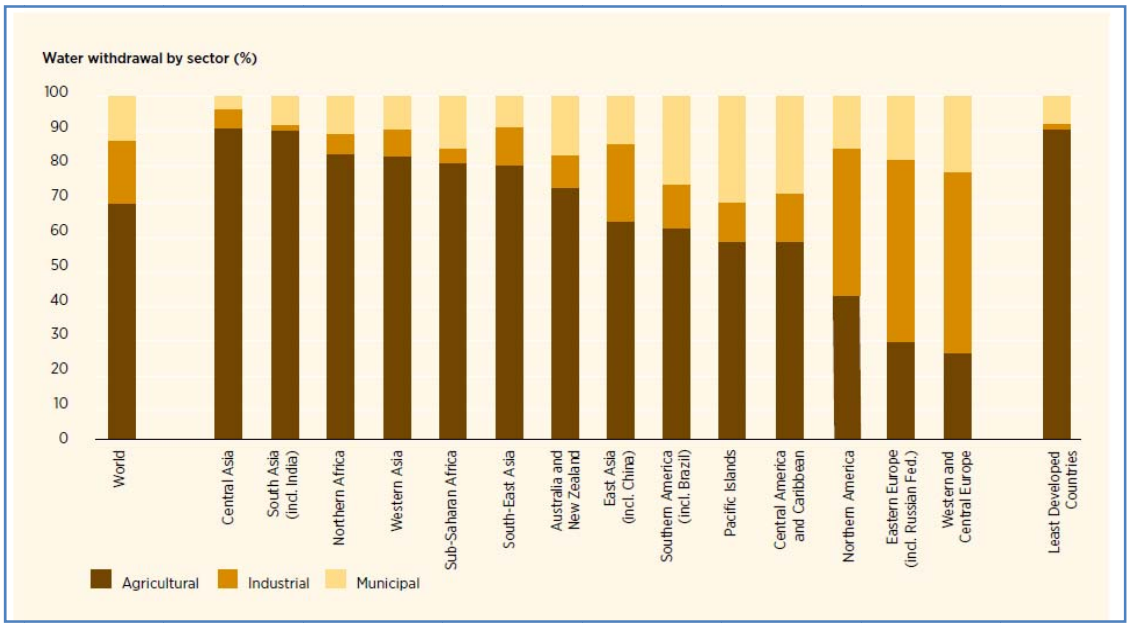

Figure 7: Water Withdrawal by Sector in the Year 2005 (Agricultural, Industrial and Municipal)
Source: WWAP (World Water Assessment Programme). 2012. *The United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk*. Paris, UNESCO.

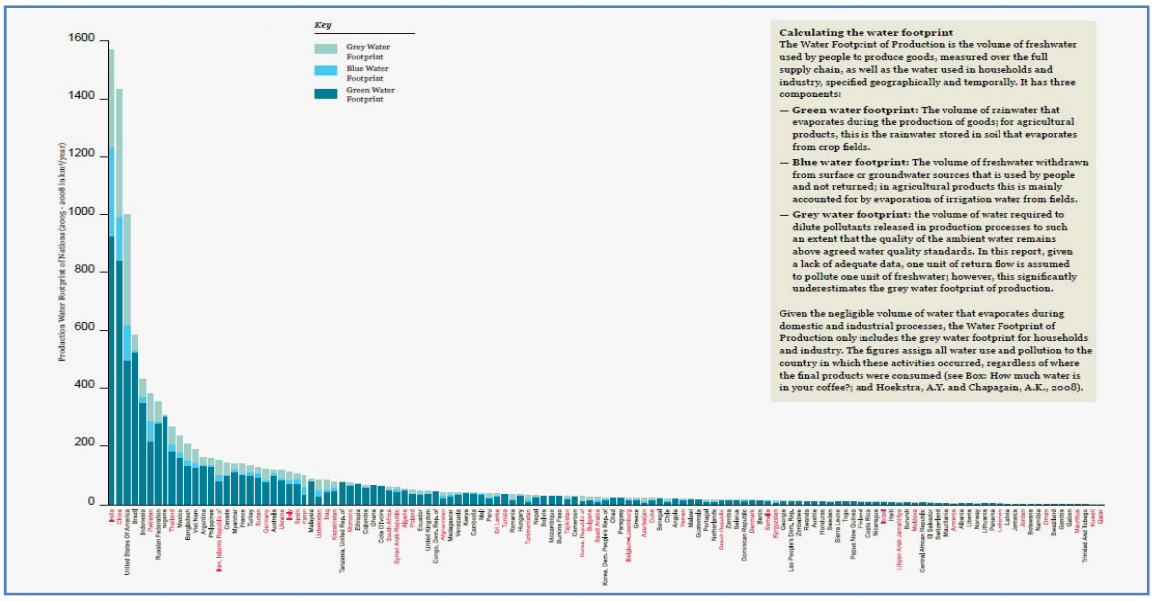

Figure 8: National Water Footprint for More 130 Countries
Source: World Wildlife Fund, Zoological Society of London and Global Footprint Network. (2010). Living planet report 2010. Retrieved from http://awsassets.panda.org/downloads/wwf_lpr2010_lr_en.pdf

2.2.2. *Population Growth*

The increase stress and demand on water resources is mainly due to the population growth; it boosts the production of waste and their discharge in the environment thus polluting freshwater resources (WHO, 2006). Based on UN population projection the world population increase from 7 billion in 2010 to 9.3 billion in 2050 (UNDESA, 2011). The increase is due to high fertility in countries of Africa (39 countries), Asia (9 countries), Oceana (6 countries) and Latin America (4 countries) (UNDESA, 2011).

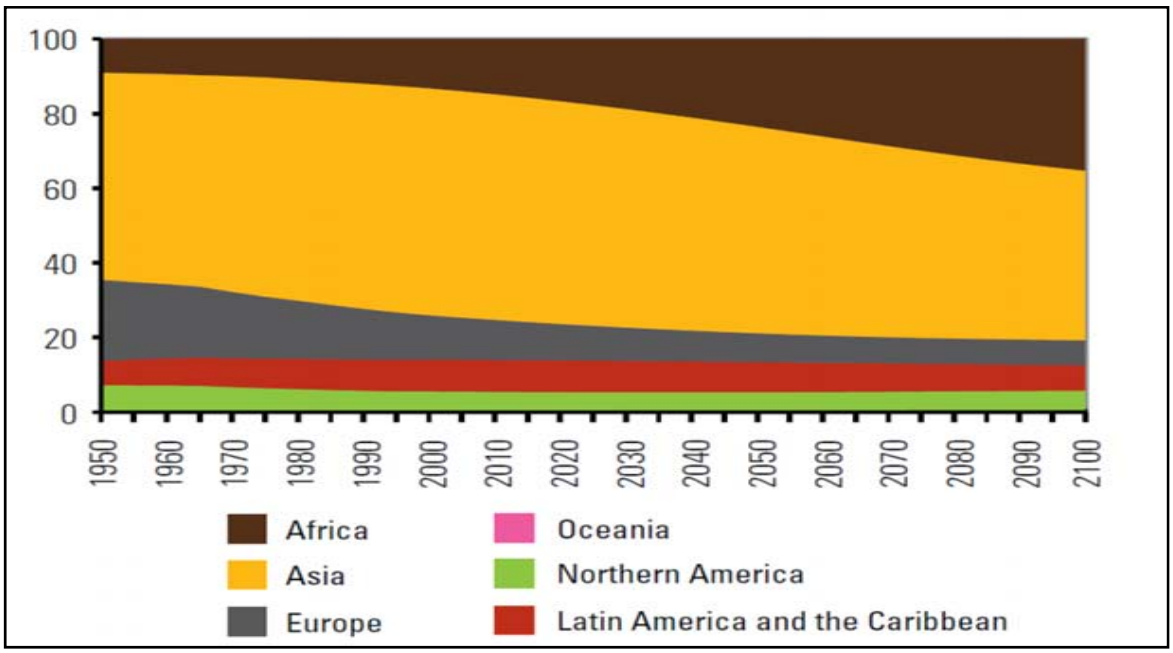

Figure 9: Percentage Distribution of Global Population
Source: United Nations, Department of Economic and Social Affairs, Population Division (2011). World Population Prospects: The 2010 Revision, Graphs and Maps from the World Population 2010 Wall Chart. Retrieved from http://esa.un.org/unpd/wpp/Documentation/pdf/WPP2010_Wallchart_Plots.pdf

The MENA region will be facing an increase of 50 to 100 % in the population such as Lebanon, Egypt, Syria, and kingdom of Saudi Arabia as figure 9 and 10 (UNDESA,

2011). Also, the total population of the Arab States was about 360 million with a population growth rate of 2 per cent between 2010 and 2015 (UNFPA, 2011). Additionally the population of urban areas of main Arabic States (Algeria, Bahrain, Djibouti, Egypt, Iraq, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Occupied Palestinian Territory, Oman, Qatar, Saudi Arabia, Somalia, Sudan, Syrian Arab Republic, Tunisia, United Arab Emirates and Yemen) is 56 % (UNFPA, 2011). In Lebanon, The total population was 4.3 million in 2011 with a projected population growth rate of 0.7% between 2010 and 2015 and the urban population is relatively high (87 %) (UNFPA, 2011).

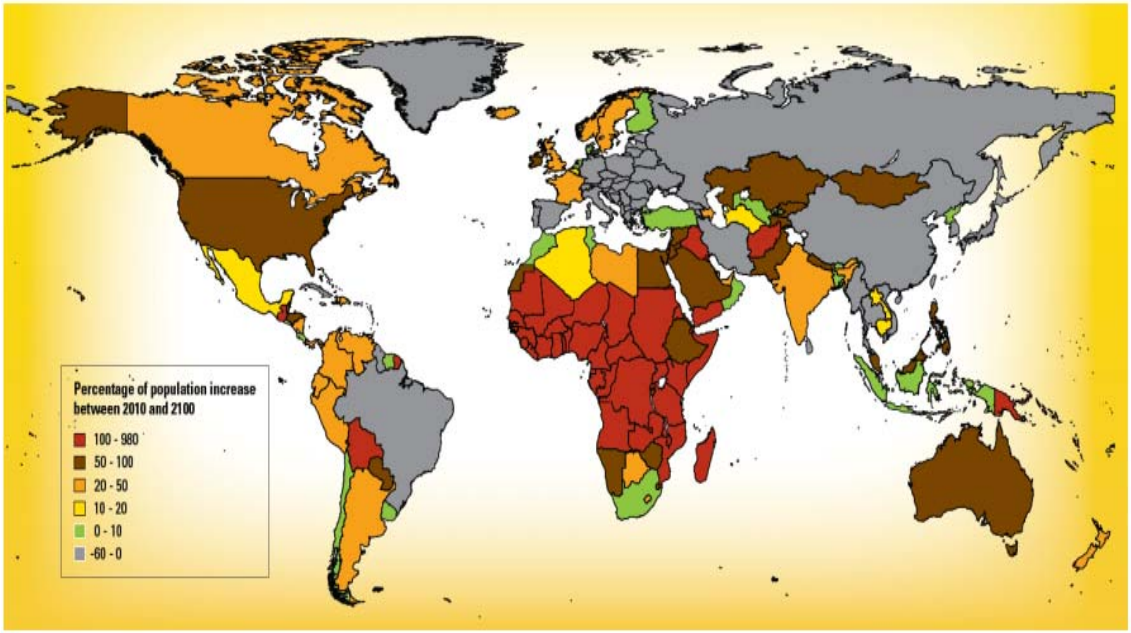

Figure 10: Percentage of Population Increase Between 2010 and 2100
Source: United Nations, Department of Economic and Social Affairs, Population Division (2011). World Population Prospects: The 2010 Revision, Graphs and Maps from the World Population 2010 Wall Chart. Retrieved from http://esa.un.org/unpd/wpp/Documentation/pdf/WPP2010_Wallchart_Plots.pdf

Coping with progressive urbanization is a major challenge to developing countries, where it is expected that by 2020 more than half of the total population of for Latin America, Africa and Asia will be residing in cities and increasing global demands of fresh water supplies (figure 11 and 12) (WWAP, 2012). And by 2020, more than 60 % of the world's population will be concentrated in urban areas. The population growth in urban areas particularly in developing countries would result in major challenges such as the increased production of solid and liquid waste and difficulty of onsite disposal (WHO, 2006). This may result in minimal wastewater treatment and direct use in agriculture, thus further contaminating ecosystems. (Qadir et al., 2010; WHO, 2006).

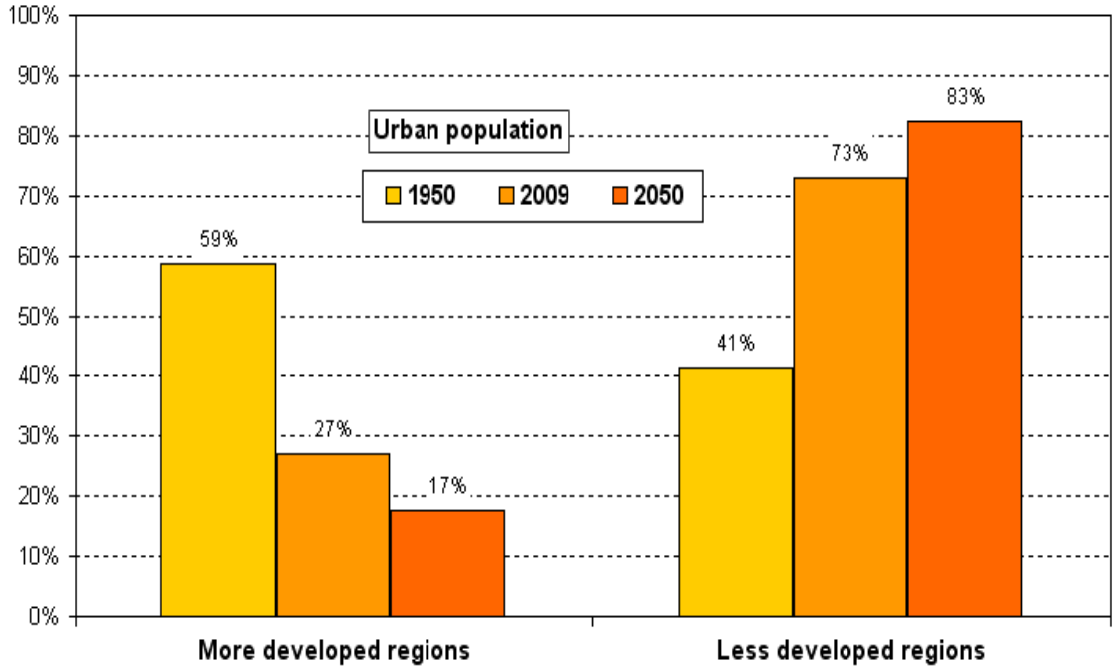

Figure 11: Distribution of the Urban Population by Development Region Based on the United Nations Department of Economic and Social Affairs
Source: United Nations, Department of Economic and Social Affairs, Population Division (2011). World Population Prospects: The 2010 Revision, Graphs and Maps from the World Population 2010 Wall Chart. Retrieved from http://esa.un.org/unpd/wpp/Documentation/pdf/WPP2010_Wallchart_Plots.pdf

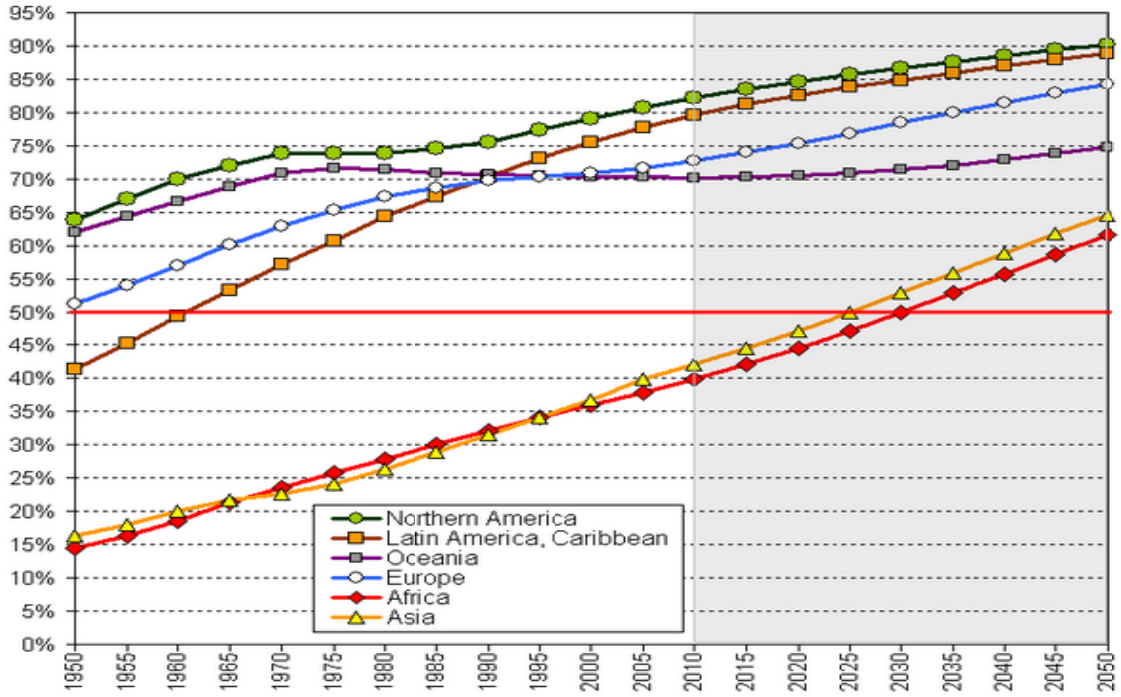

Figure 12: Urban Population by the Percentage of Total Population on Each Geographical Area.
Source: United Nations, Department of Economic and Social Affairs, Population Division (2011). World Population Prospects: The 2010 Revision,Graphs and Maps from the World Population 2010 Wall Chart. Retrieved from http://esa.un.org/unpd/wpp/Documentation/pdf/WPP2010_Wallchart_Plots.pdf

Also, the increase in urbanization would increase the stress on water resources to meet the demands of the domestic, industrial and commercial sectors (Qadir et al., 2010). Where the water withdrawal for urban use is divided like all other uses, 70 % for agriculture, 20 % industrial and 10 % for domestic use (WWAP, 2012). And, increased demand will lead to over abstraction from rivers and ground water sources (WWAP, 2012). Moreover, the excessive use of groundwater would lead to a decrease in water tables, sea water intrusion, degradation in water quality, and land subsidence, as in the case for many

cities mostly in Asia and Europe (WWAP, 2012). For example, in Beirut the capital of Lebanon, the seawater intrusion along the coastal line is leading to the degradation in water quality (Jurdi et al., 2007). Additionally, urban settlements are a main source point pollution of domestic and industrial solid and liquid waste that is dumped untreated in water bodies.

Worldwide 80 % of the produced wastewater, specifically in developing countries, is neither collected nor treated and is dumped in water bodies threatening human health, food security and safe access to domestic water supplies (figure 13) (WWAP, 2012).

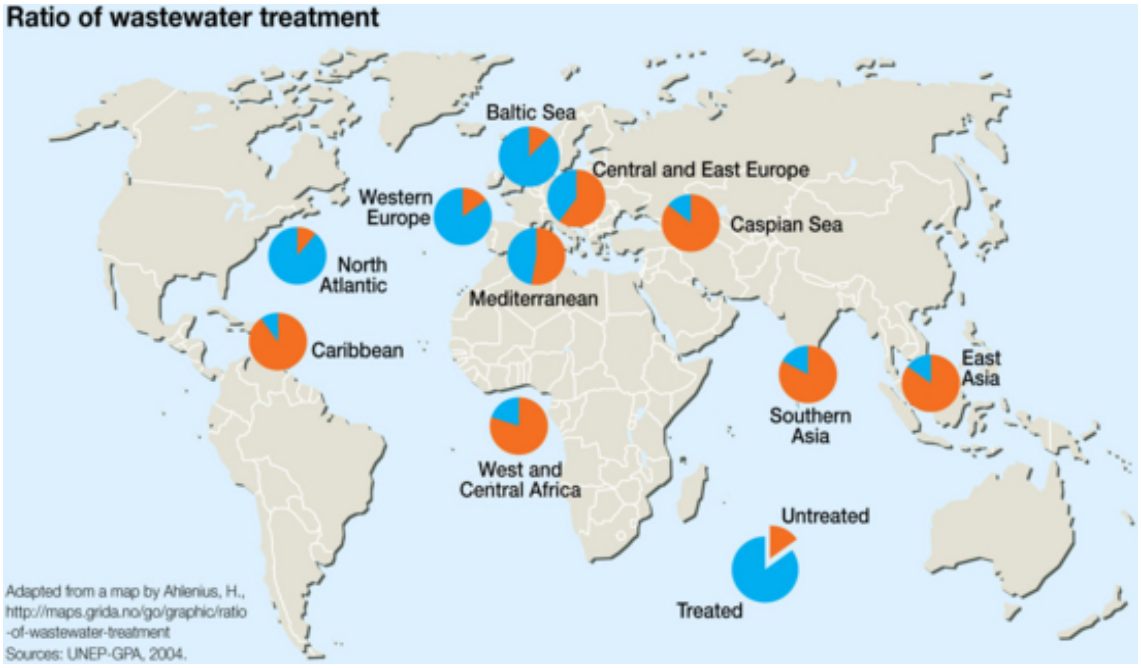

Figure 13: Ratio of Treated to Untreated Wastewater Discharged into Water bodies
Source: United Nations, Department of Economic and Social Affairs, Population Division (2011). World Population Prospects: The 2010 Revision, Graphs and Maps from the World Population 2010 Wall Chart. Retrieved from http://esa.un.org/unpd/wpp/Documentation/pdf/WPP2010_Wallchart_Plots.pdf

2.3. Water Reuse in the Arabic World

More than 12 Arabic countries are actually facing absolute water scarcity with renewable water availability of less than 500 m^3 per capita per year (WAAP, 2012). The increased demand on water resources in the Arabic countries is due to population growth, food security, regional conflicts, conflicts on shared water resources and climate change. The population growth is expected to increase from 352 million in 2009 to reach 461 million by 2025 (WAAP, 2012). It is accompanied with increase urbanization where over 55 % of the region population is urbanized, in countries such as Lebanon, Egypt, Morocco, Tunisia and Saudi Arabia. This would increase the demand on water resources accompanied with stress on water supplies mostly in urban areas (WAAP, 2012). Also, 70 % of water withdrawal in the Arabic region is for agriculture use; to meet food demands and ensure food security. In Syria, Iraq, Yemen, and Oman the agricultural water withdrawal accounts for 90 % of water use (WAAP, 2012).

Even though large amounts of water are withdrawn to meet the needs of agricultural sector, the Arab region is still unable to meet food security. As such, over 40 % or 50 % of the cereal production is imported from outside the region (WAAP, 2012). Further, climate change is expected to decrease by 20 % of the agricultural production in most of the Arabic countries by the year 2080 (WAAP, 2012).

Water reuse has been practiced for decades in Arab countries to combat desertification and land degradation; some Arab countries such as Jordan and UAE have even developed water reuse quality standards (WAAP 2012). The increased pressures on water resources and increased water scarcity have encouraged the use of treated wastewater

in agriculture, industrial, urban and environmental sectors (WAAP, 2012). UAE, Jordan, Saudi Arabia and Kuwait are among the Arab countries that use treated wastewater; Jordan and UAE use wastewater in most of the sectors including agriculture and Bahrain use grey water for cooling and landscaping (WAAP, 2012). Abu Dhabi also use treated wastewater for reforestation to increase the green cover in the desert and establish biodiversity reserves; moreover, treated wastewater is used to recharge ground water aquifers in some gulf countries (WAAP, 2012).

2.4. Water Quality Requirements

2.4.1. *Guidelines for Agricultural Water Use*

The EPA guidelines for agricultural water reuse suggest three different categories of guidelines for reuse in agriculture. These categories are for food crops that do not undergo processing before being consumed or eaten raw, processed food crops, and non-food crops. For all those categories of use secondary treatment and disinfection should be applied; while for food crops eaten raw wastewater should undergo further treatment such as filtration.

The pH for all the three categories should be between 6 and 9 and the chlorine residual should be a minimal of 1 mg/l of Cl_2. The total suspended solids (TSS) should be less than 30 mg/l. As for the biochemical oxygen demand (BOD) it should be less than 10 mg/l in water used for the irrigation of crops to be eaten raw or unprocessed and fecal coliforms should be totally absent. However, the BOD can be less than 30 mg/l in water

used for the irrigation of vegetables that will be processed and the fecal coliforms should be less than 200/100ml of water (USEPA, 2004).

2.5. Characteristics of Irrigation Water

Heavily polluted water and raw wastewater contains pathogens, salts, metals, toxic organic compounds, nutrients (nitrogen, phosphorous and potassium), organic matter, suspended solids and acids and basis.

2.5. 1. ***Microbiologic Pathogens***

The microbiological profile of such water sources includes a wide spectrum of pathogens such as bacteria, viruses, helminthes and protozoa. Pathogens are considered a primary hazard for wastewater use, particularly for untreated or inadequately treated wastewater. Pathogens can survive in the environment long enough to contaminate crops, soils, surface and ground water and to be transmitted to affect human health (WHO, 2006). Further, if contaminated wastewater is applied to areas where groundwater is close to the soil, pathogens such as viruses, bacteria, Giardia and Cryptosporidium can be transported in the soil (horizontally and vertically) to reach aquifers (WHO, 2006).

The main pathogens associated with the use of heavily polluted water and raw wastewater for irrigation water include Enterobacteriaceae (*Escherichia coli, Salmonella* spp, *Shigella* spp, *E. cloacae, C. freundii, K. oxytoca, Serratia marcescens, K. pneumoniae, E. aerogenes*), Vibrio *cholera*, *Listeria monocytogenes*, *Bacillus cereus, Staphylococcus aureus*, protozoa Cryptosporidium spp., *Cyclospora cayetanensis*, *Giardia* spp., helminthes (*Ascaris* spp.) and viruses such as Norwalk, enteroviruses, rotavirus and hepatitis A virus

(HAV). These pathogens can survive in the environment long enough to pose health risks and contaminate crops leading to disease outbreaks (mainly gastrointestinal illness).

Such types of irrigation water also contain high concentration of pathogens and especially indicator organisms; for instance it is reported that total coliform counts in untreated wastewater is 7.6 x 10^{10} per 100 ml. Indicators of fecal contamination such as E. coli are used to reflect on fecal contamination and the presence of other possible pathogens. *E. coli* is associated with gastroenteritis and *E. coli* O157:H7 leads to bloody diarrhea and hemolytic uremic syndrome. Norovirus and rotavirus are also associated with gastroenteritis. In addition, Hepatitis A is a major public health concern, mainly when untreated wastewater is applied to agriculture (WHO, 2006).

Main microbiological hazards such as *E. coli* O157:H7, *salmonella* spp., *Shigella* spp, *E. cloacae, C. freundii, K. oxytoca, S. marcescens, K. pneumoniae, E.* aerogenes and *Listeria* spp., are found in irrigation water and lead to several foodborne outbreaks resulting from the consumption of contaminated raw vegetables (Pachepsky, 2011; WHO, 2006). Such microorganisms were isolated from vegetables and fruits such as lettuce, spinaches, tomato, cabbage, celery, bean sprouts, and cucumber (Gleeson, 2004). The bacteria that are present in leafy vegetables are mainly present in grooves along the veins, epidermal cell wall junctions, stomata, and bases of trichomes; in lettuce for instance, they were present in both the lower and upper surface of lettuce (Gleeson, 2004).

2.5.1.1. *E. Coli* O157:H7

The intestinal tract of humans and warm blooded animals is the primary habitat of the *E. coli* O157:H7. The mode of transmission of *E. coli* to humans is either from animals

to humans, person to person contact and through contaminated food (Viazis et al., 2011). As such, the contamination of agricultural products with *E. Coli* O157:H7 is mainly due to the application of wastewater in irrigation, or the application of untreated animal manure as a source of natural fertilizer (Viazis et al., 2011; Cooley et al., 2007). Furthermore, as mentioned earlier contamination with *E. coli* O157:H7 leads to hemolytic uremic syndrome, and hemorrhagic colitis; where the *E. coli* O157:H7 infection dosage is very low 10–100 cells (Chang et al., 2007).

The raw vegetables and fruits that are contaminated with wastewater and manure have become an important route of transmission of *E. coli*. Further, *E. coli* O157:H7 is able to survive in soil and crops at a temperature 4°C for 70 days (Cooley et al., 2007). At 20°C, *E. coli* is able to survive in soil and crops from 15-70 days and 15-30 days, respectively (WHO, 2006). Numerous outbreaks associated with the consumption of contaminated fresh vegetables, such as spinach and lettuce, with *E. coli* O157:H7 are occurring worldwide (Viazis et al., 2011). It is estimated that yearly about 73000 cases of disease by *E. coli* O157:H7 are reported in the United States, and are associated with the consumption of food especially fresh vegetables and fruits and contaminated water sources (Cooley et al., 2007).

2.5.1.2. *Listeria monocytogenes*

Listeria monocytogenes is another common pathogen linked with irrigation and detected in sewage sludge, wastewater, contaminated soils, and crops (Oliveira, 2011; Gleeson, 2004; Donnelly, 2001). In 2008, about 76 % of reported foodborne outbreaks in USA related to Listeria spp. infections; followed with 3 deaths due to *L. monocytogenes*

infections (CDC, 2008). The survival of Listeria monocytogenes, like all other pathogens, in soil depends on several factors related to temperature, pH of the soil, type of the soil and moisture content, as well as the type of the native microbial community (Oliveira, 2011). Moreover, *L. monocytogenes* is capable to adapt and live in stressful environments increasing the probability of transfer to crops leading to increased health risks (Warriner et al., 2009; Oliveira, 2011; Gleeson, 2004). *L. monocytogenes* illness syndrome is characterized by listeriosis which is an invasive form of disease leading to flu like symptoms, meningitis, primary bacteremia, septicaemia, and encephalitis (Gleeson, 2004; Donnelly, 2001). Dairy products, meat, poultry and fresh produce (potato, lettuce, radishes, corn, green beans, broccoli, and cabbage) are detected sources of Listeria (Donnelly, 2001).

2.5.1.3. *Salmonella* spp.

Salmonella spp., an omnipresent enteric pathogen, is also one of the leading causes of foodborne illness worldwide. *Salmonella* transmission to humans is through the fecal-oral route. *Salmonella* can contaminate the environment directly through human or animal feces or indirectly through wastewater and sewage used in agriculture. *S. typhi* and *paratyphi* infect humans causing typhoid, enteric fevers; in 2003, 17 million cases of paratyphoid and typhoid fevers were reported worldwide (Levantesi et al., 2011). *Salmonella* species have been associated with several worldwide outbreaks due to the consumption of contaminated fresh vegetables and fruits (lettuce, tomatoes, melon and seed sprout) (Jacobsen et al., 2011). In 2008, in the United States, an important outbreak of *salmonella* in peppers was associated with irrigation by untreated wastewater (Holden et al., 2009; Jacobsen et al., 2011). Another outbreak (more than 450 cases) of salmonella in

tomatoes was associated with irrigation water (United States and Canada in the year 2005/2006) (Jacobsen et al., 2011; Holden et al., 2009). In 2005, about 60 confirmed cases of *S. typhimurium* were reported in Finland associated with lettuce consumption (Takkinen et al., 2005). Moreover, in 2007 salmonella was detected in fresh produce in the Europopian Union (Berger et al., 2010). In 2008, about 62% of hospitalized cases of foodborne outbreaks in USA were also associated with salmonella infection (CDC, 2008).

As such, wastewater and animal manure used in agriculture are the main source of pollution leading to the contamination of crops by salmonella (Islam, et al., 2004).This pathogenic microorganism is able to survive in the environment for long period of time due to the salmonella circulation within the farm between irrigation water, soil, plant, animals (Jacobsen et al., 2011; Levantesi et al., 2011). Moreover, salmonella strains can adhere to plant surfaces and survive for long periods; for instance *S. typhimurium*, present in contaminated irrigation water, demonstrated the ability to transfer from water to soil and then to the plant adhering on its edible parts (such as parsley, lettuce.) (Lapidot et al., 2009; Islam et al., 2004). In addition, *S. typhimurium* identified in radishes and carrots grown in fields irrigated with contaminated water and manure were able to survive in soil for several months and to be transferred to the vegetables (Islam et al., 2004).

2.5.1.4. *Klebsiella species*

Klebsiella species are opportunistic pathogens that are mainly responsible for significant nosocomial infections, where it mainly attacks hospitalized, immunocompromised individuals. In Europe and in the United States, *Klebsiella* species accounts for 8 % of the major nosocomial bacterial infections. *Klebsiella* species are

omnipresent in the environment (sewage, surface water, plants and soil) and on mammals' mucosal surfaces (humans, swine or horses) (Falomir et al., 2010, Podschun et al., 1998; Brown et al., 1973). Several studies reported the isolation of pathogenic Klebsiella species from different types of plants such as rice, potato and lettuce (Rosenblueth et al., 2004; knittel et al., 1977). Further, *Klebsiella* species also produce histamine causing seafood poisoning (Cai et al., 2008). Therefore, the presence of *Klebsiella* species especially *K. pneumoniae e* and *K. oxytoca* are identified as risk in food safety.

2.5.1.4.1. *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram negative important nosocomial pathogen that belongs to the Enterobacteriaceae family. *K. pneumoniae* is not only present in the natural environment, but also in the intestine tract of human beings (Lawlor et al., 2005). The source of infection of *K. pneumoniae* is due to its presence in the intestinal tracts of human beings, where it is one of the causes of the nosocomial infections (Legakis et al., 1995; Podschun et al., 1998; Lawlor et al., 2005; CDC, 2010). This explains its route of transmission from vegetables irrigated with wastewater. Several studies show the existence of *K. pneumoniae* in vegetables that are irrigated with wastewater and its presence in fecal material (Falomir et al., 2010, Brown et al., 1973).

Further, as opportunistic pathogens, *K. pneumoniae* infections are more significant and devastating among elderly, hospitalized and immunocompromised patients (Lawlor et al., 2005; Falomir et al., 2010). There are several risk factors for *K. pneumoniae* infections these include chronic liver disease, diabetes mellitus, biliary disease and cancer (Meatherall et al., 2009). *K. pneumoniae* is the second common cause of hospital and community

acquired gram-negative blood stream infections after *E. coli*. The blood stream infections are associated with complications of respiratory tract, gastrointestinal and fecal urinary infections (Meatherall et al., 2009). *K. pneumoniae* cause diarrhea, bacteremia, urinary tract infections, septicemia, ventilator-associated pneumonia, bronchopneumonia, typical lobar pneumonia, chronic pulmonary disease, and lung abscesses (De Souza Lopes et al., 2005; Haryani et al., 2007; Cai et al., 2008; Podschun et al., 1998; Lawlor et al., 2005; CDC, 2010).

As such, *K. pneumoniae* identification as an invasive pathogen is significantly increasing; hypermucoid strains of *K. pneumoniae* became the leading cause of pyogenic liver abscesses. In South Korea and Taiwan, *K. pneumoniae* accounts for 80 % of pyogenic liver abscess. Further, it was also reported in Australia, Europe and North America (Mandell et al., 2010; McIver et al., 2008; Wang et al., 1998).

The virulence of the *K. pneumoniae*, a mucosal pathogen, is contributed to the production of the adherence factors that are produced by the bacteria due to its colonization to the epithelial surfaces. Further, *K. pneumoniae* is known to produce capsular materials (capsular polysaccharide) that protect the bacteria from host defense mechanisms, such as neutralizing the bactericidal effect of specific antibodies (Meatherall et al., 2009; Clegg et al., 2001). As such, *K. pneumoniae* became a worldwide concern since it is capable of producing extended-spectrum β-lactamase (ESBLs), enzymes that are resistant to beta-lactam antibiotics (Liu et al., 2012). This resistance is explained by the β-lactamase enzyme that is capable of cutting the β-lactam ring of the antibiotics, rendering the antibiotic as a harmless substance to the bacteria (Liu et al., 2012).

2.5.1.4.2. *Klebsiella oxytoca*

Klebsiella oxytoca is also a gram negative pathogen that have been identified in different raw vegetables irrigated with wastewater, since contaminated water and aerosols are a source of exposure to *Klebsiella* species including *K. oxytoca* (Falomir et al., 2010; WHO, 2006). *K. oxytoca* is another opportunistic pathogen from the Klebsiella species that is also present in the environment and cause community-based and nosocomial infections. Further, *K. oxytoca* infections frequently involve immunocompromised patients. Nosocomial infections with *K. oxytoca* are usually associated with environmental reservoirs contamination such as disinfectants, ventilators, humidifiers and parenteral fluid bags (Lowe et al., 2012; Falomir et al., 2010; WHO, 2006). *K. oxytoca* infection includes hemorrhagic colitis which is due to a cytotoxin produced by the pathogen and it lead to several outbreaks (Chen et al., 2004).

2.5.1.5. *Enterobacter cloacae* and *Enterobacter aerogenes*

Enterobacter cloacae and *Enterobacter aerogenes* belongs to the family of Enterobacteriaceae, which is different than *Klebsiella* species since it is motile, urease negative and ornithine decarboxylase positive (Sanders et al., 1997). *E. cloacae* and *E. aerogenes* are the most identified human pathogens from the *Enterobacter* genus. *Enterobacter* species are becoming recognized as nosocomial pathogens. Due to the ubiquitous type of the *Enterobacter* species, its infections are acquired from different external sources such as wastewater, water, human and animal feces, plants and dairy products (Sanders et al., 1997).

Enterobacter cloacae and *Enterobacter aerogenes* are gram-negative bacteria usually found in water, sewage and soil (Falomir et al., 2010). Further, they are opportunistic pathogens that are also isolated from human gastrointestinal tract and as such they are present in high concentrations (10^7organisms/g) in sewage (Antony et al., 2011; Acolet et al., 1994; Gaston, 1988). They are also associated with urinary tract and nosocomial infection. *E. cloacae* and *E. aerogenes* infections are responsible for bacteremia, endocarditis, intra-abdominal infections, skin and soft tissues infections, septic arthritis, osteomyelitis, respiratory tract infections and ophthalmic infections (Antony et al., 2011; Krzyminska et al., 2010; Sanders et al., 1997; Paraje et al., 2005). Additionally, a recent study showed that *E. cloacae* have the ability to induce apoptosis by adhering to and penetrating into the epithelial cells (Krzyminska et al., 2010). Several others reported studies show the presence of *E. cloacae* and *E. aerogenes* in raw vegetables (lettuce, carrots and spinach) irrigated with wastewater (Falomir et al., 2010; Afolabi et al., 2010). *E. cloacae* is also responsible for many outbreaks in neonatal health care units and is associated with contaminated water, thermometers and parenteral fluid bags (Antony et al., 2011; Dalben et al., 2008, Gaston, 1988).

2.5.1.6. Citrobacter freundii

Citrobacter freundii is a gram negative ubiquitous pathogen, present in the environment as well as in the gastrointestinal tract of humans (Flegg et al., 1989; Liu et al, 2007). *C. freundii* infections include diarrhea, bacteremia, septicemia, osteomyelitis, endocarditis, meningitis, abscess formation, respiratory and urinary tract system infections

(Chen et al., 2011; Flegg et al., 1989). These infections are mainly significant and risky among elderly and immune-compromised individuals (Flegg et al., 1989; Chen et al., 2011). Moreover, *C. freundii* is associated with nosocomial infections (Liu et al., 2007; Chen et al., 2011). Several studies show the prevalence of *C. freundii* in contaminated food especially vegetables irrigated with contaminated water. For instance, *C. freundii* was isolated from contaminated vegetables in Ghana, Valencia City, Spain, and Morocco (Falomir et al., 2010; Ibenyassine et al., 2007; Mensah et al., 2002).

2.5.1.7. *Serratia marcescens*

Serratia marcescens is a motile, rod-shaped and gram negative pathogen that is also associated with nosocomial infection and isolated from different sources such as sewage, contaminated soil, water and food (Su et al., 2010; Bosi et al., 1996). *S. marcescens* causes different infections such as urinary tract infections, wound infection, pneumonia, septicaemia, endocarditis and meningitis (David et al., 2006; Voelz et al., 2010; Wu et al., 2012). Different studies show that *S. marcescens* is a rare pathogen associated with central nervous system (Wu et al., 2012). Moreover, *S. marcescens* is becoming recognized as a pathogen in neonatal health care units (Al Jarousha et al., 2008; Wu et al., 2012). *S. marcescens* was also isolated in different vegetables (e.g. in Valencia city *S. marcescens* was isolated from carrots) (Falomir et al., 2010). Further, it was isolated in Morocco from vegetables irrigated with wastewater (Ibenyassine et al., 2007).

2.5.1.8. *Shigella sonnie*

Shigella sonnie has been associated with outbreaks in several European countries such as United Kindom, Sweden, and Norway (isolated from lettuce). In 1994, outbreak of *Sh. sonnie* associated with the consumption of iceberg lettuce occurred in several countries in North West Europe (Frost et al., 1995). Further, in 2004, the Foodborne Diseases Active Surveillance Network (FoodNet) of the U.S. Centers for Disease Control and Prevention's (CDC) classified *Sh. sonnie* to be third most prevalent foodborne pathogen (Warren et al., 2007). The presence of *Sh. sonnie* in vegetables is associated with fecal contamination from humans due to the irrigation of vegetables with wastewater or the fertilization with sewage sludge and animal manure. *Shigella* infections can be either direct through the fecal-oral route or indirect through person to person transmission (Kapperud et al., 1995; Frost el at., 1995).

Shigella sonnie cannot compete with other types of enteric viruses, however its presence in small counts would be epidemiologically significant since it can infect at a low dose. As such, *Sh. sonnie* is known to infect adults at doses of 10 to 100 cells only. Moreover, *Sh. sonnie* are difficult to detect as they might be very few in number or incapable of competing with other organisms. Still, they may be present in sufficient amount to cause infection and not be detected anaaalytically (Kapperud et al., 1995). *Shigella* spp infections cause shighellosis (fever, cramps, vomiting, and bloody diarrhea) (Abaidoo et al., 2010).

2.5.1.9. Enteric viruses

Enteric viruses have been also reported in contaminated water and transferred to food surfaces (Sánchez et al., 2012; Newell et al., 2010; Cheong et al., 2009). Food, especially raw vegetables and fruits, serve a vehicle for enteric viruses' transmission, where vegetables and fruits irrigated with wastewater transmit these viruses to humans (Cheong et al., 2009; Sánchez et al., 2012). Enteric viruses can only reproduce within a host and their mechanism of transmission to humans is by the fecal-oral route where the dose needed to infect human is very low (example less than 20 virons for Hepatitis A) (Warriner et al., 2009; Haramoto et al., 2008; Newell et al., 2010). Also, enteric viruses are able to survive in stressful environments; where they are resistant to environmental degradation, freezing and most types of chemical treatment (Le Guyader et al., 2004; Warriner et al., 2009). The majority of the outbreaks caused by enteric viruses have been associated with the consumption of contaminated raw fruits and vegetables (Brassard et al., 2011; Cheong et al., 2009; Warriner et al., 2009; Mara et al., 2009). Several enteric viruses' outbreaks have been reported due to the consumption of raw vegetables and fruits; in the year 2008, 49 % of the reported foodborne outbreaks in USA have been associated with viral contamination (CDC, 2008; Butot et al., 2007).

Additionally norovirus became one of the leading cause of foodborne illness, where several outbreaks related to norovirus were associated with raspberries in Europe (Berger et al., 2010); about 65 % of foodborne illnesses from viruses in the United States were associated with norovirus (Fumian et al., 2009). Moreover, group A rotavirus (RVs) are the main cause of diarrheal diseases in infants and are associated with a large number of deaths globally (Newell et al., 2010; van Zyl et al., 2006). The annual worldwide disease

burden among young children of rotavirus is estimated at about 2.4 million hospitalizations, 24 million outpatient and 527,000 deaths (Anderson et al., 2012). In Africa, approximately about 110000 to 150000 deaths among children have been associated with rotavirus infection (van Zyl et al., 2006).

Several outbreaks of rotaviruses have been also associated with the consumption of raw vegetables such as Lettuce, carrots, strawberry, (Cheong et al., 2009). Since rotavirus survival is optimal at low temperature, the annual peak of rotavirus outbreaks worldwide occur in cooler months (fall and winter season) and drier times of the year; 40 % of severe infant diarrhea cases worldwide are associated with rotavirus infection (WHO, 2009; Atchison et al., 2009). Further, in Costa Rica outbreaks of gastroenteritis (diarrheal diseases) associated with rotavirus occur annually among children mainly in cooler months, between December and January (Herna´ndez et al., 1997). And, outbreaks of enteric viruses are linked to consumption of raw vegetables and fruits such as lettuce, Parsley, carrot green onions, strawberries and raspberries (Berger et al., 2010; Warriner et al., 2009; van Zyl et al., 2006; Newell et al., 2010). The source of virus contamination of the produce is associated with the use of wastewater or polluted water for irrigation. Infection with norovirus leads to abdominal pain, diarrhea, vomiting and nausea (Warriner et al., 2009); Hepatitis A infection results in malaise, nausea, fever, anaroxia, and abdominal discomfort; finally rotavirus infection can cause acute gastroenteritis in children and adults (CDC, 2001; Anderson et al., 2012).

Additionally, outbreaks of enteric viruses are linked to the consumption of raw vegetables and fruits such as lettuce, Parsley, carrot green onions, strawberries and raspberries (Berger et al., 2010; Warriner et al., 2009; van Zyl et al., 2006; Newell et al.,

2010). The source of the viral contamination of the produce is also associated with the use of wastewater or contaminated for irrigation. Infection with norovirus leads to abdominal pain, diarrhea, vomiting and nausea (Warriner et al., 2009); Hepatitis A infection leads to malaise, nausea, fever, anaroxia, and abdominal discomfort. Finally rotavirus infection can cause acute gastroenteritis in children and adults (CDC, 2001; Anderson et al., 2012).

2.5. 2. *Survival of Pathogens in Soil and Crops*

Several pathogens can survive for long periods in soil and crops and then be transmitted to humans and animals. Pathogens can survive in soil for longer periods than crops; however recontamination can occur after rainfall particularly for rooted crops and those that are closer to the soil (WHO, 2006). The survival of pathogens in crops and soil depends on many factors such as temperature, humidity, soil content, pH, exposure to sunlight (ultraviolet radiation), plant type and competition with the native flora and fauna (WHO, 2006). Temperature is one of the important factors for pathogen survival\die-off, where high and freezing temperatures would cause pathogen die-off (WHO, 2006). On the other hand, low temperatures, would cause prolonged pathogen survival. This is especially in the case of post-harvest storage, where pathogens can survive long enough in plants that are harvested and transported and then stored at temperature 4°C enhancing the transmission of disease agents to consumers (WHO, 2006).

Humid environments, unlike dry environments, would favor pathogen survival. Moreover, soil with high organic content, such as clay soils, also favors pathogen survival. pH also plays a role in pathogens survival\die-off, slightly alkaline to neutral soil would favor bacterial survival; however, lower pH soils are favorable to viruses survival unlike

alkaline soil that would lead to pathogen die-off (WHO, 2006). Furthermore, direct sunlight would lead to rapid pathogen die-off due to the exposure to ultraviolet radiation (WHO, 2006). The type of plant can as well affect pathogen survival\ die-off; where plants that have sticky surfaces or surface can absorb pathogens and favor prolonged pathogen survival (WHO, 2006). Also, certain native bacteria and algae can act as antagonistic factor that enhance pathogen die-off; and at the same time protozoa can prey on native bacteria thus enhancing their survival (WHO, 2006).

Moreover, the transmission of the pathogens from the soil to crops depends on two factors, the concentration of the pathogen in the soil and the distance of the edible parts of the plants from the soil (Jacobsen et al., 2011). As the concentration of the pathogens in the soil increases, the transmission of the pathogens becomes more enhanced, and when the distance of edible part of the plant to the soil decreases the transmission of the pathogen increases (Jacobsen et al., 2011). Examples of the survival time of microorganisms in sewage, crops and soils are shown in table 1.

Table 1: Survival in Days of Pathogens in Sewage, Crops and Soil

Organisms	Survival of organisms (days) at temperatures between 20 and 30°C		
	Sewage	Crops	Soil
Enteroviruses	Less than 120 Usually 50	Less than 60 Usually 15	Less than 100 Usually 20
Thermotolerant coliforms	Less than 60 Usually 30	Less than 30 Usually 15	Less than 70 Usually 20
Salmonella spp.	Less than 60 Usually 30	Less than 30 Usually 15	Less than 70 Usually 20

Shigella spp.	Less than 30 Usually 10	Less than 10 Usually 5	No data
V. cholerae	No data	Less than 5 Usually 2	Less than 20 Usually 10
Cryptosporidium oocysts	Less than 180 Usually 70	Less than 3 Usually 2	Less than 150 Usually 75
Tapeworm eggs	Several months	Less than 60 Usually 30	Several months

Source: World Health Organization (WHO) (2006). Guidelines for the Safe Use of Wastewater, Excreta and Grey Water. Volume 2: Excreta and Grey water Use in Agriculture. World Health Organization, Geneva.

2.5.3. *Chemical Characteristics*

2.5.3.1. Salinity

Salinity is an important parameter used to determine the suitability of water use for irrigation, where 23 % of irrigated farmlands worldwide have been damaged by increased salinity. Salinity is determined by measuring the total dissolved solids and/or electrical conductivity, sodium adsorption ratio, sodium and chloride concentration (USEPA, 2004). The soil salinity depends on water quality, organic matter content, irrigation rate, soil transmissivity, land drainage and depth to the ground water (WHO, 2006). The plants' salinity tolerance varies, where the choice of the crops should be based on the level of the salinity. The soil should be frequently prepared to tolerate the salinity in the irrigated water by draining and leaching it by applying excess irrigation to the soil to force the downward movement of the water and salt from the root zoon as such preventing soil build-up (USEPA, 2004).

Salinity can cause damage in the osmotic potential of the soil by lowering it and reducing the water uptake by the plants, which in return forces the plants to adjust the salt concentration by using big amounts of its available energy (USEPA, 2004). This would result in less energy available for the plant growth. Besides the damage in the osmotic potential of the soil, salinity can lead to the degradation of the soil physical conditions, specific ion (chloride, boron, and sodium) toxicity resulting in plant growth reduction, reduction in the yields and total crop failure in severe cases (USEPA, 2004). Further, salinity interferes with the plant uptake of nutrients (nitrogen and potassium) due to the antagonistic effect of the sulfate, chloride and sodium ions (WHO, 2006). Additionally, the ions of concern in polluted water and wastewater irrigation are mostly boron (from household detergents), chloride and sodium (from water softeners) and the concentration of these ions can cause trace metals to accumulate in the soil and in the plant resulting in phytotoxicity in plants leading to human and animal health hazards (USEPA, 2004).

2.5.3.2. Sodium

Excessive sodium in the irrigated water would result in soil structural breakdown and dispersion, causing the soil fine particles fills the empty pore spaces thus reducing water infiltration and sealing the surface (USEPA, 2004). As such wastewater that is high in sodium would lead to soil permeability problems if it wasn't properly managed.

2.5.3.3. Trace Metals

The discharge of industrial wastewater in sanitary sewers is the main source of toxic chemicals. Many types of chemicals are used in industrial sectors, households and

agricultural production. As such, the use of polluted water and untreated wastewater would introduce toxic chemicals into the soil; in return the plants absorb the chemicals from the soil leading to health impacts to consumers (WHO, 2006). Long term irrigation would increase the concentration and accumulation of heavy metals in soils (Liu et al., 2005). Furthermore, toxic chemicals accumulated in the soil would contaminate freshwater bodies. Therefore, toxic chemicals can have direct and indirect health impacts.

Direct health impacts are associated with the use of highly contaminated wastewater with industrial discharges. For instance, in Japan rice paddies irrigated from Jinzu River that is exposed to industrial wastewater discharge with elevated cadmium levels lead to chronic cadmium poisoning (Itai-Itai disease) (WHO. 2006). Indirect health impacts are related to poor irrigation practices with untreated or partially treated wastewater leading to the contamination of surface and ground water supplies. For instance, excess nitrogen and phosphorous in wastewater would contaminate surface water leading to eutrophication (WHO, 2006). Eutrophication, in return, would favor the growth of toxin producing algae and cyanobacteria causing skin irritation, gastroenteritis, liver damage and nervous system impairment; several cases associated due to direct contact recreational water and drinking water contaminated with cyanotoxins have been reported in countries such as United State of America, China, Canada, United Kingdom, Brazil and Australia (WHO, 2006).

Generally metals are affected by pH value of the soil, where metals are more bound to soils with pH values above 6.5 with high organic matter content. However, when pH levels are below 6.5 the organic matter would be consumed, thus metals become mobile and then absorbed by crops (WHO, 2006). Metals that are commonly present in wastewater

are copper, zinc, nickel, molybdenum and cadmium (WHO, 2006). Although at certain concentration copper, zinc, nickel and molybdenum are considered as essential elements for plant growth; however elevated levels in soil can induce plant toxicity. Further, the ingestion of these elements can induce adverse health effects in exposed human and animals (USEPA, 2004).

2.5.3.4. Toxic Organic Compounds

Toxic organic compounds vary in concentration between types of wastewater: domestic wastewater has low concentrations of toxic organic compounds whereas, industrial and agricultural runoff have higher concentration of toxic compounds. They include industrial compounds (such as phthalates and PCBs), pesticides (such as lindane, dieldrin, and DDT), petroleum components and disinfection products; where these compounds can be transmitted due to the use of polluted water and untreated wastewater for irrigation of crops (WHO, 2006). According to WHO (2006), exposure to organic compounds leads to different mutagenic, teratogenic and carcinogenic effects

2.5.3.5. Nutrients

Nutrients that are vital for crop production are nitrogen, phosphorus, sulfur, and boron; all these elements are present in wastewater in enough quantities. Nitrogen is one of the important nutrients for plants found in polluted water and wastewater as ammonia, nitrites, nitrate, and organic nitrogen; however excess amounts would delay crop maturity, overstimulation of growth and reduce its quality and quantity (WHO, 2006; USEPA, 2004). Nitrogen is highly soluble in water, thus when irrigating crops it can be washed and

transferred to contaminate surface and ground water supplies causing methaemoglobinaemia (WHO, 2006). Other important nutrients for plants are phosphorus and potassium; however they are present in low quantities in wastewater where it is needed to supplement the phosphorous levels in used fertilizers. Phosphorous is stable in soil and can accumulate in surface water bodies through surface runoff and soil erosion (WHO, 2006).

2.5.3.6. Organic Matter

Wastewater adds to the soil organic matter that retains metals, increases the soil moisture, and enhances microbial activities. Most organic compounds are decomposed rapidly in soils; however when the BOD concentration is high (exceeding 500 mg/l) it can lead to soil clogging (WHO, 2006).

2.5.3.7. Suspended Solids

The presence of suspended solids would lead to clogging in the irrigation infrastructure and if suspended solids are not biodegradable it will reduce water soil percolation (WHO, 2006).

2.5.3.8. Acids and Bases

Polluted water and untreated wastewater have pH values that are slightly alkaline. The acid/base equilibrium in the soil is affected when such waters are used on soil that has adequate alkalinity (WHO, 2006; USEPA, 2004).

2.6. Transfer and Accumulation of Heavy Metals from Water to Soil to Crops

Human exposure to heavy metals introduced to soils and crops by polluted irrigation water takes place through different eight pathways as shown below (WHO, 2006):

Polluted Water ➔ Soils ➔ 1. Plants →Humans

2. Plants → Animals → Humans

3. Animal → Humans

4. Humans

5. Surface runoff → Surface water →Humans

6. Airborne particles →Humans

7. Vadose zone → Groundwater → Humans

8. Atmosphere → Humans

However the primary route of heavy metals transfer to humans is through the food chain. Further the daily intake of vegetables, crops, and fruits account for 75 % of daily adult food consumption (WHO, 2006).

Polluted water ➔ Soils ➔ 1. Plants →Humans

2. Plants → Animals → Humans

3. Animal → Humans

Soil acts as a filter for toxic metals where it adsorbs heavy metals and thus retains concentrations of these metals. However, when the soil pH changes or when it is exposed to continuous loads of pollutants, the soil would reach its saturation thus reducing its capacity

to retain heavy metals (Mapanda et al., 2005). Under these conditions, the soil would release the heavy metals to the groundwater or make them available for plant uptake. Moreover, the mobilization of heavy metals in soil is a function of pH, organic matter content, clay content and cation exchange capacity. Usually increase in soil pH would decrease metal's mobility, except for metals such as molybdenum, arsenic and selenium (Mapanda et al., 2005).

Accumulation of heavy metals in plants also depends on the type of the plant species, and on its efficiency in absorption of metals (Rattan et al., 2005). The absorption of metals by the plant is assessed by two ways, either the transfer factor or plant metal uptake (Rattan et al., 2005). The transfer of heavy metals from the soil to the plant is assessed by the transfer factor (TF) (Rattan et al., 2005). The transfer factor is calculated by dividing the metal concentration in the crop (dry weight) by the metal concentration of the soil (dry weight) (Liu et al., 2005; Cui et al., 2004; Rattan et al., 2005). On the other hand, the uptake of metals by plants is a good indicator of plant absorption under controlled conditions when the metal concentration in the soil is uniform (Rattan et al., 2005). As such, the transfer factor is a more reliable indicator since it can assess the efficiency of metal absorption by plants grown in soil with variable metal contents (Rattan et al., 2005).

2.7. Public Health Impacts

Even though polluted water and raw wastewater is a resource and would have positive impacts in terms of ensuring food security and improving nutrition; however it presents a hazard due to the presence of microbiological and chemical contaminants.

Exposure or transmission of pathogens resulting from the use of such water sources in agriculture includes (WHO, 2006):

- Risk to farmers and their families, vendors and local communities due to the direct contact with such waters before, during or after irrigation.
- Risk to workers and local communities due to inhalation of water aerosols.
- Risk associated with the consumption of contaminated products Risk associated with the consumption of contaminated drinking water due to wastewater reuse activities and infiltration of pathogens in the groundwater resources.
- Risk due to the consumption of animal products of animals contaminated with polluted water

Health risks associated with microbiological contamination of crops irrigated with polluted water includes different diseases related to exposure to different kind of pathogens leading mainly to diarrhea, typhoid, Schistosomiasis, Ascariasis, Hookworm disease, Lymphatic filariasis, Hepatitis A (WHO, 2006). The highest health risk is associated with crops that are eaten raw, particularly root crops (such as onion) and those that grow close to the soil (such as lettuce, parsley and carrot) (WHO, 2006). Globally about 1798000 deaths/year and 61966000 cases of disease/year are due to diarrhea cases associated with the use of untreated wastewater for irrigation (WHO, 2006). Further, 600000 deaths/year of typhoid cases result from the use of untreated wastewater for irrigation (WHO, 2006).

Moreover, irrigation with polluted water introduces heavy metals to the plants and soils. As such, heavy metals can accumulate in the edible parts of vegetables (particularly leafy vegetables) in concentration high enough to induce adverse health effects to humans as well as animals ingesting heavy metal rich vegetables. Therefore, health risks associated with ingestion of food contaminated with heavy metals can deplete some essential nutrients in the body leading to decrease immunological defenses, disabilities associated with malnutrition, impaired psycho-social behavior, intrauterine growth retardation, and gastrointestinal cancer after chronic ingestion to certain heavy metals (Arora et al., 2008). The detailed health risks associated with the exposure to heavy metals are summarized in the table 2.

Table 2: Sources and Health Impacts of Trace Metals

Trace metals	Source	Health effects
Lead	Industrial sources: Smelting Operation, Automobile Emission, Urban Runoffs, Pesticides, Plastics, Paints, Ceramic Glaze	Vision and hearing impairment, increase blood pressure, reproductive problems (low sperm count), anemia, peripheral neuropathy, nephrotoxicity, and cerebrovascular diseases (ATSDR, 2007)
Cadmium	Batteries, Plastics, Fertilizers, Pesticides, Paints, Electroplating, leachate from landfills.	Acute exposure: vomiting, diarrhea, lung irritation and damage Chronic exposure: kidney, bone and liver damage and cancer (ATSDR, 2008)
Chromium	Industrial activities: stainless steel, alloys, ceramics, plastic, rubber, tannery	Diarrhea, vomiting, dizziness, ulcer and irritation in the stomach, hemorrhagic diathesis, and convulsions, liver and kidney damage and lung cancer (ATSDR, 2008)

Nickel	Industrial activities: stainless steel, alloys, ceramics, plastic, rubber, tannery, domestic waste, fertilizers and pesticides	Vomiting, diarrhea, cramps, neurological symptoms, allergic dermatitis, liver dysfunction and kidney damage (NJDOH, 2007; Nashalian, 2010)
Copper	Smelting and Metal plating operations, Fertilizers and Animal Feeds, Electrical Works, Pesticides and Fungicides	Anemia, liver and kidney damage, developmental toxicity and immunotoxicity (ATSDR, 2004)
Zinc	Galvanization Works, Motor Oil, Tire Wear, Pigments, Pesticides, iron and steel, zinc smelting, plastics, electroplating, and domestic wastewater	vomiting, abdominal pain, nausea and anemia (ATSDR, 2005)
Barium	cement, ceramics, glazes, glass, paper making, pharmaceutical and cosmetic products	Cardiovascular diseases (EPA, 2005)
Cobalt	Alloy, Ceramics and Paints	Respiratory Irritation, Heart Damage and Failure, Thyroid Problems
Manganese	Steel and Alloys; Fertilizer, Ceramics, and Fungicide, Dry-cell Batteries, Fireworks, Disinfectants	neurobehavioral effects , permanent neurological disorder (manganism: walking difficulties, spasms in facial muscles and tremors) (ATSDR, 2008)
Molybdenum	Steel and Alloys, Fertilizers, Ceramics and Plastics	Loss of appetite, headache, fatigue, anemia, muscle and joint pain, gout, liver and kidney damage (CDC, 1978; NJDOH, 2011)
Arsenic	Pesticides, Wood Preservatives, Glass Products	Acute exposure: vomiting, nausea, diarrhea, dermal, respiratory and cardiovascular effects Chronic exposure :preterm births, stillbirth, miscarriages, and skin and internal tumors (skin, lung, bladder, liver and kidney cancer) (ATSDR, 2007)

Source: (WHO, 2008; Perfect Life Institute 2002).

CHAPTER 3

STUDY METHODOLOGY

3.1. **Overview of the Chapter**

This chapter presents the study area, data sources, sampling framework, sample preparation, and sample analytical techniques.

3.2. **Study Area**

The Middle East is one of the water challenged region in the world (El-Fadel et al, 2003; Allan, 2003). Lebanon is considered advantaged with its water resources (rainfall and water resources) with respect to the neighboring countries. Lebanon has an area of 10,452 Km^2 with a population growth of 4,259 thousand with 87 % of its population is urbanized (UNDESA, 2011).

Furthermore, Lebanon is characterized by a Mediterranean climate characterized by heavy rain in the winter season (November to May) and throughout the rest of the year it is characterized by dry and arid conditions (FAO, 2008). The annual precipitation in Lebanon is estimated to 661 mm/year (FAO, 2012a). And, the fate of the precipitations is as follows (El-Fadel et al, 2003):

- 50 % of annual precipitation is lost through evapotranspiration
- 8% flows to the neighboring countries.
- 12 % accounts for ground water seepages.
- Only 30 % is left available for exploitation.

The Lebanese's agricultural area is about 27.5 % (288,000 hectares) of Lebanon total area. In 2009, the total irrigated agricultural area was 118,000 hectares accounting for 45 % of the total cultivated area (FAO, 2012b). Moreover, the agricultural sector in 2005 consumed 60 % (0.78 km^3/ year) of the total water (1.31 km^3/ year) in Lebanon as presented in figure 14 (FAO, 2012b).

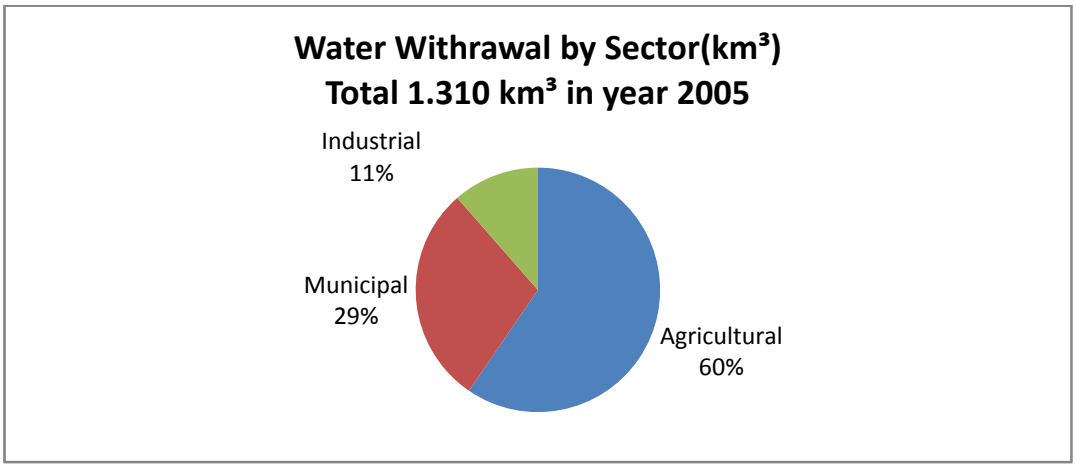

Figure 144: Lebanese Water Withdrawal by Sector (Agriculture, Domestic and Industry)
Source: FAO. (2012). Country Fact Sheet Lebanon. Retrieved from http://www.fao.org/nr/water/aquastat/data/factsheets/aquastat_fact_sheet_lbn_en.pdf

The main agricultural products in Lebanon are vegetables, potatoes, tomatoes, apples, citrus fruits, olives, poultry, sheep, and goat (FAO, 2012b). In addition, Lebanon is an exporter of vegetables and fruits to the region; in 2005 the agricultural exports accounted for US196$ million (FAO, 2012b). The vegetable consumption among Lebanese citizens was reported as 281 g/person/day; particularly the average consumption of fresh fruits and vegetables for urban Lebanese citizen was about 367 g/day (Nasreddine et al., 2010; FAO,

2010). Moreover, potato consumption in Lebanon was reported as 256 g per person per day between the years 2003 and 2005 (FAO, 2010).

3.2.1. ***Litani River***

Lebanon has about 40 major streams; where the three river basins, Litani River, Hasbani, Asi-Orontes cover 45 % of the country. The Hasbani River and the Asi-Orontes are trans-boundary Rivers, whereas the Litani River flows inside Lebanon (FAO, 2012b). The Litani River is the longest river in Lebanon with a total length of 170 km (FAO, 2012b). It rises from Nabeh Al Oleik near Baalbek city and the flows to 140 km south and west Lebanon to meet the Mediterranean Sea (Figure 15 & 16) (Assaf et al., 2008; Jurdi et al., 2010). The catchment area of the Litani is about 2180 km^2 which is equal to 20 percent of Lebanon total area (FAO, 2012b). Further, the estimated average water flowing annually in the Litani River is 475 million m^3 (FAO, 2012b).

Moreover, in the southern part of Bekaa valley and on the Upper Litani River, the largest artificial reservoir in the country is located (Qaraoun Reservoir). It has an effective storage of 160 million m^3 and total capacity of 220 million m^3 (FAO, 2012b). It does not only supply three hydroelectric power plants that contribute to 7-10 percent of Lebanon's total annual power, but also it provides a total of 140 million m^3 for irrigation purposes (FAO, 2012b).

The main sources of irrigation water in Lebanon are the Litani River and the Litani-Awali; in 2000, 44 % of the Lebanese agricultural area was irrigated from surface water (FAO, 2008). The type of irrigation schemes in Lebanon, (year 2000), varied

between sprinkler irrigation (25100 ha), surface irrigation (57200 ha) and localized irrigation (7700 ha) (FAO, 2008).

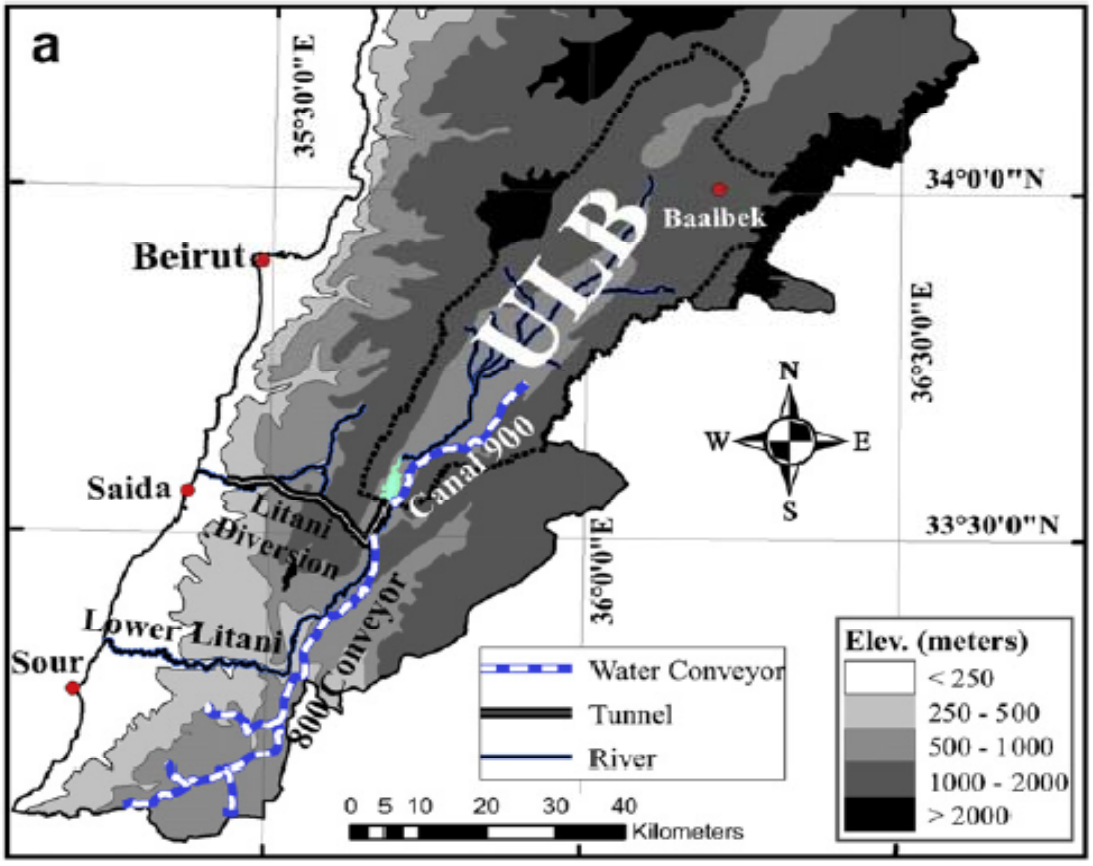

Figure 15: a: The Litani River Basin.
source: Assaf, H. & M. Saadeh (2008). Assessing Water Quality Management Options in the Upper Litani Basin, Lebanon, Using an Integrated GIS-based Decision Support System. *Environmental Modelling and Software,* 23(2008) 1327-1337. doi:10.1016/j.envsoft.2008.03.006

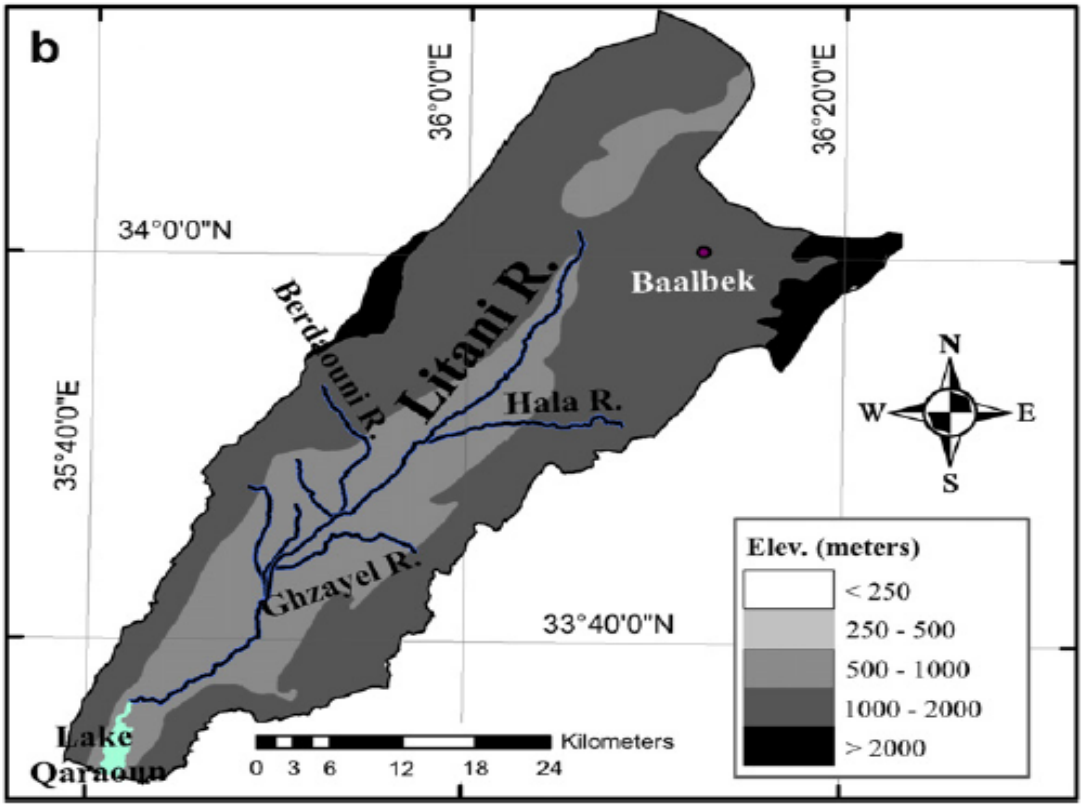

Figure 16: b: The Upper Litani River Basin.
source: Assaf, H. & M. Saadeh (2008). Assessing Water Quality Management Options in the Upper Litani Basin, Lebanon, Using an Integrated GIS-based Decision Support System. Environmental Modelling and Software, 23(2008) 1327-1337. doi:10.1016/j.envsoft.2008.03.006

In Lebanon, the Bekaa region represents 42% of the Lebanese agricultural lands and contributed to 57 % of the total production in 2005 (FAO, 2008). Additionally, the Bekaa region contributed to about 80% of the total potato production in Lebanon (Abou Zeid, 2005). Over 10 percent of Lebanese population inhabits this region which is characterized by agricultural activities, food processing and tourism as presented in figures 17 and 18 (Assaf et al., 2008). Moreover, the Upper Litani Basin is the main source of irrigation water in the Bekaa area (Assaf et al 2008).

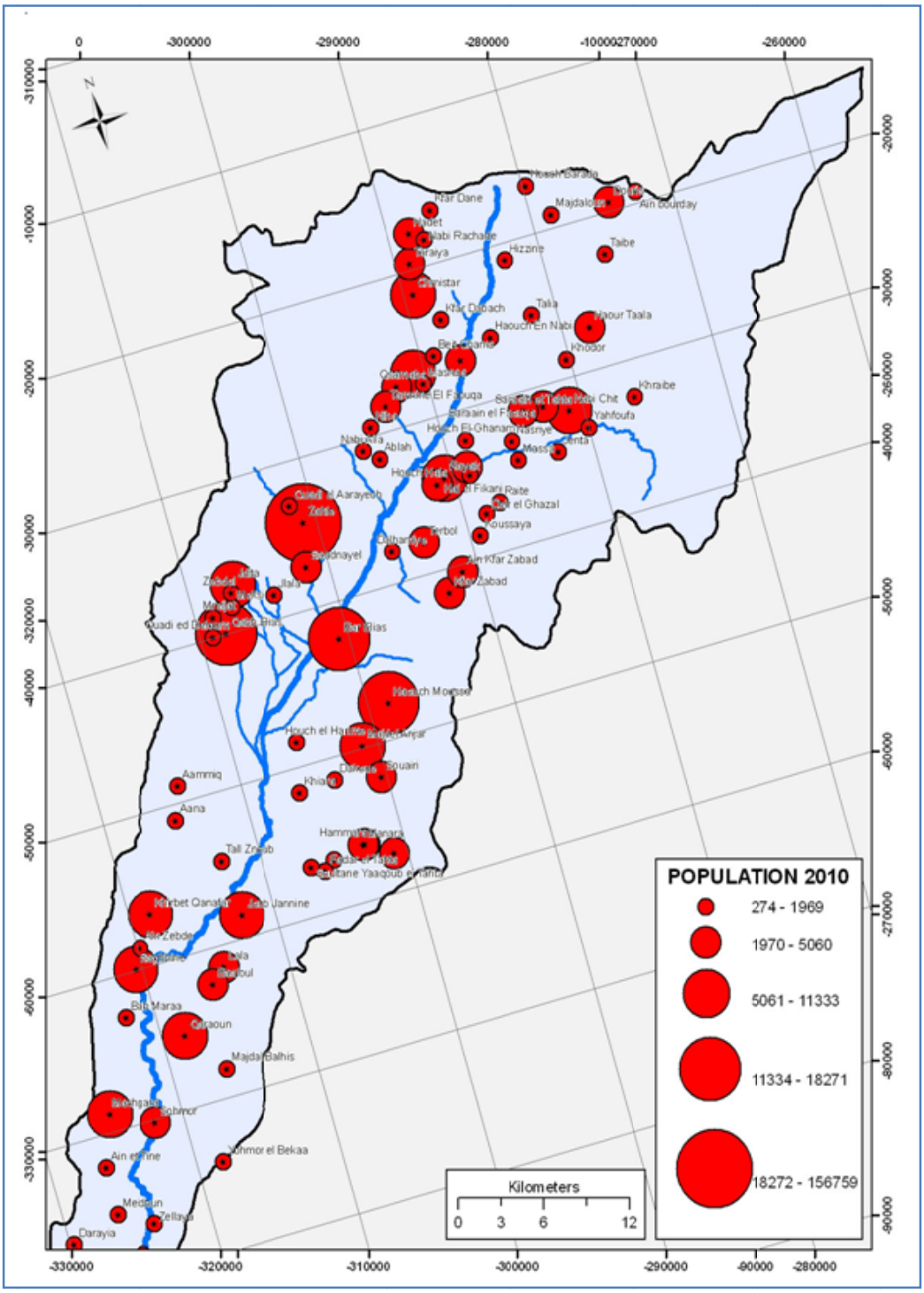

Figure 157: The Urbanization Profile of the Upper Litani Basin
Source: Jurdi et.al (2010).Dry Season Water Quality Survey of the Litani River Basin Project, Litani River Basin Management Support Program.

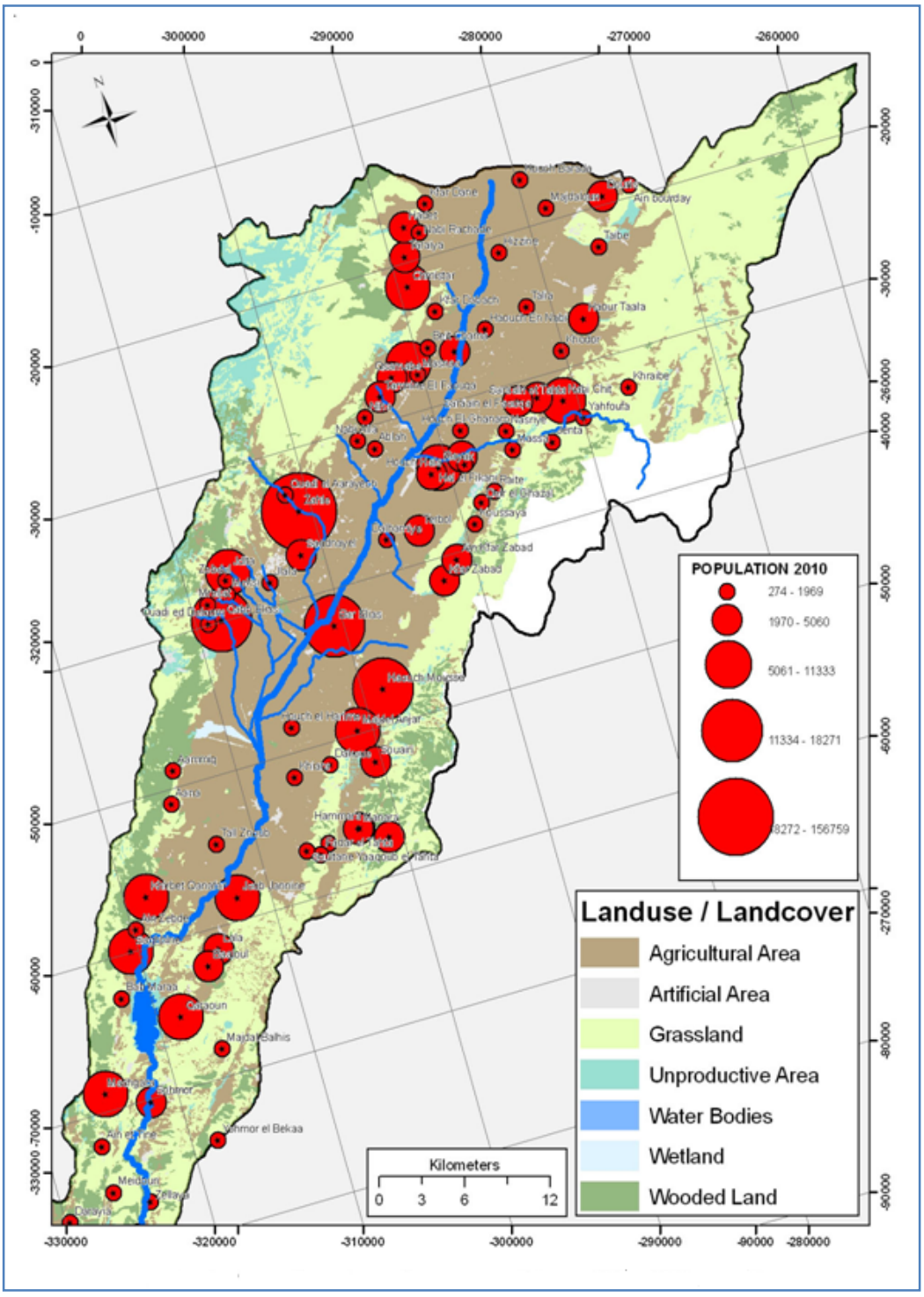

Figure 1816: The Landuse and Landcover Profile of the Upper Litani River
Source: Jurdi et.al (2010).Dry Season Water Quality Survey of the Litani River Basin Project, Litani River Basin Management Support Program.

3.2.1.2. Litani River Quality

Recent studies conducted on the Upper Litani Basin reflect on its continuous exposure to sources of pollution (point and non-point) that are distributed though out the Upper Litani Basin as presented in figure 19 (Jurdi et al., 2010; Jurdi et al, 2011) :

- Domestic wastewater effluents from the sanitary sewer system outlets and cesspool leachate
- Solid waste dump sites leachates.
- Recreational areas contributing to sewage discharge and solid wastes dumps
- Farm wastes ranging from cows, sheep, poultry and swine.
- Industrial wastewater effluents ranging from chemicals, sponge, manufacturing of batteries, paper and stone cutting, dyeing and tanning and electroplating.
- Food processing plants wastewater effluents (sugar beet, dairy products, fruit jam, juices, vegetable canning)
- Agriculture runoff resulting in fertilizers and pesticides contaminating the Upper Basin Litani River.

The water quality of the Litani in the dry season and wet season was assessed by Jurdi, et al. (2010-2011). The analysis showed that during the dry season 48 % of the studied sites were found dry and there was minimal flow of water along the river and its tributaries which is mostly due to the use of Litani river water for irrigation purposes. In addition, the industrial and sewage wastes are discharged in the river and solid waste dumps are scattered along the river flow.

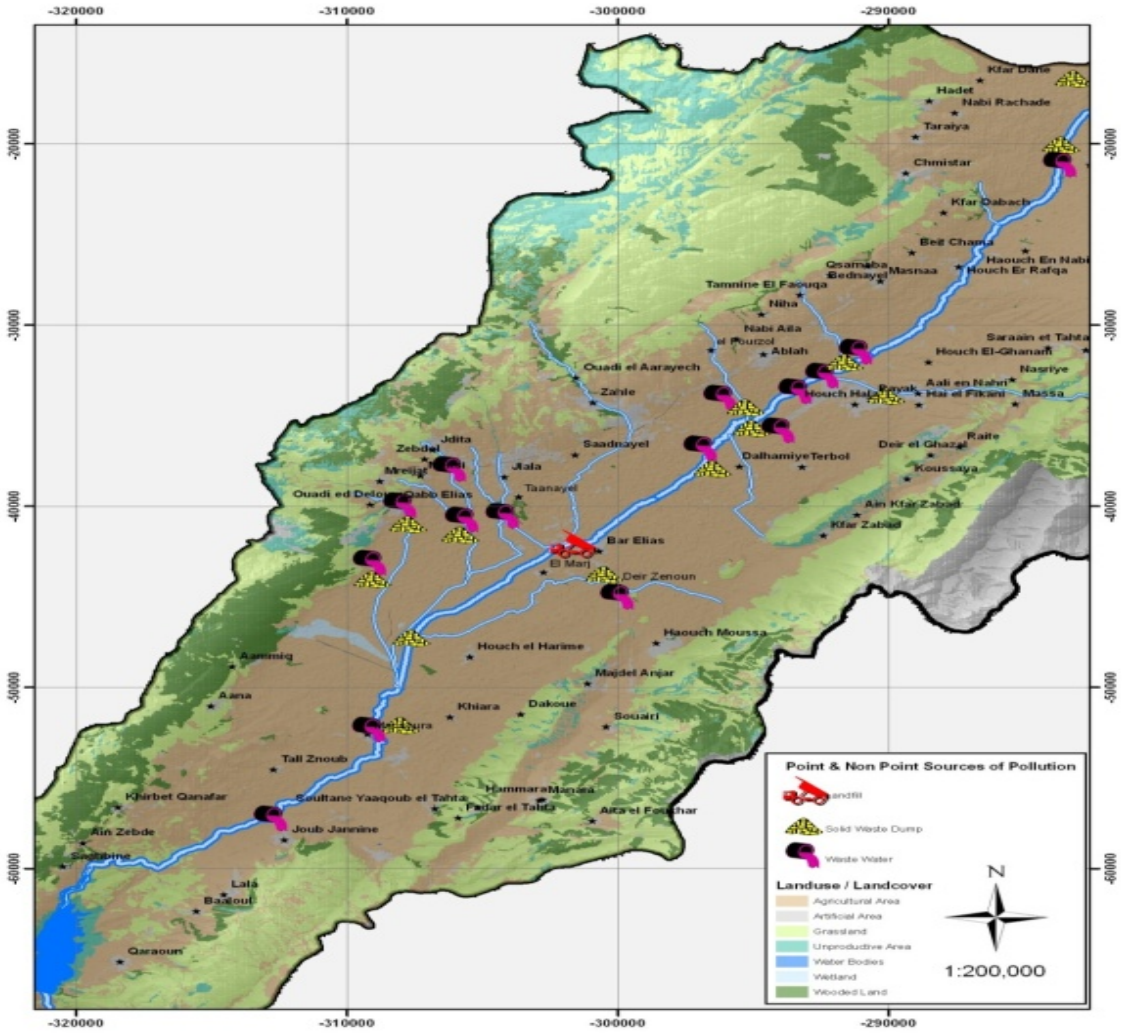

Figure 179: Sources of Pollution (Point and Non-Point) along the Upper Litani Basin. Source: Jurdi et.al (2010).Dry Season Water Quality Survey of the Litani River Basin Project, Litani River Basin Management Support Program.

The self-purification capacity and ecological viability of the river is determined by the dissolved oxygen levels, which reflect on the contamination level of the water body. The mean levels of dissolved oxygen for the dry season were reported at 4.56 mg/l compared to 4.83 mg/l in the wet season (Table 3) (Jurdi et al., 2010; Jurdi et al., 2011).

In spite of the extensive algae growth, oxygen levels dropped to less than 5 mg/l in dry and wet season in 46 % and 44 % of the samples, respectively reflecting on the continuous exposure of the river basin to sources of pollution throughout the year (Jurdi et al., 2010; Jurdi et al., 2011). Moreover, the drop of oxygen level is accompanied by an increase in the biological oxygen demand (BOD) mean levels to 548 mg/l in the dry season compared to lower levels in the wet season (19.20 mg/l) (Table 3) (Jurdi et al., 2010; Jurdi et al., 2011). The lower levels in the wet season are due to the dilution factor and the decrease in temperature which would in return decrease the duration of the decomposition of organic matter. The increase of BOD levels in both dry and wet season is also an indicator of the continuous discharge of pollutants along the Litani river.

The total dissolved solids (TDS) mean level for the dry season was reported at 503 mg/l in comparison to 255 mg/l during the wet season as presented in Table 3 (Jurdi et al., 2010; Jurdi et al., 2011). The increase in TDS levels is an indicator on the presence of inorganic salts and organic matter.

The pH mean levels were within the acceptable range for both dry and wet season 7.93 and 7.66, respectively (Jurdi et al., 2010; Jurdi et al., 2011). However, the progressive increase of pH towards alkalinity is an indicator of exposure to sources of pollution (Table 3).

The reported ammonia levels of the Litani river were higher than those of nitrates for both the dry and wet seasons, which is an indicator of decreased oxygen levels available to oxidize the ammonia into nitrate (Table 3) (Jurdi et al., 2010; Jurdi et al., 2011). The mean levels for ammonia, nitrate, sulfates, phosphates and chlorides also

reflect on the continuous exposure of Litani river to sources of pollution during both dry and wet season (Table 3) (Jurdi et al., 2010; Jurdi et al., 2011).

Trace metals were also detected during the dry season as reflected by cadmium levels exceeding the standards and high reported barium levels (Jurdi et al., 2010; Jurdi et al., 2011). However, during the wet season, the levels of cadmium, nickel, zinc, chromium and iron decreased and did not exceed the recommended limits with the exception of manganese levels that exceeded the EPA and national standards for both the dry and wet season (Jurdi et al., 2010; Jurdi et al., 2011).

Additionally, fecal contamination was observed in both dry and wet season in 50 % and 65 % of the samples, respectively (Table 3) (Jurdi et al., 2010; Jurdi et al., 2011).

Hence, the degradation in the water quality poses a major concern to agriculture production due to (a) increased soil salinity resulting from increased TDS levels, (b) increased sodium and manganese levels that would lead to reduction in the water infiltrations rates, (c) increased levels of heavy metals that would lead to plant toxicity and subsequent health risks to consumers, (d) increased microbiological contamination that would also pose a microbiological health risk to farmers and consumers. (Jurdi et al., 2010; Jurdi et al., 2011)

Table 3: Changes in the Litani River Water Quality Profile between 2005 and 2011.

Indicator	*Survey season*	*BAMAS 2005 Calculated from surface water results*			*Jurdi et al. 2010-11 study*			*Drinking Water Standards*		*Reclaimed WW for irrigation*
								LIBNOR	US EPA	
		Min.	*Mean*	*Max.*	*Min.*	*Mean*	*Max.*	*GV*[1] *(25 °C)*	*GV/MAL*[2]	*MoE guideline*
Temperature (°C)	*W*	4.1	12.39	17.7	10	17.36	25	NA[6]	NA	
	D	12	20.07	25	15.5	23.73	32.1			
TDS (mg/l)	*W*	114	202.2	415	118	254.96	533	5007	5007	
	D	88	291	706	187	502.08	1979			
pH (pH units)	*W*	6.8	7.09	8.18	4.53	7.66	8.54	6.5-8.5	6.5-8.5	
	D	6.57	7.59	7.68	7.27	7.93	8.66			
DO(mg/l O_2)	*W*	3.95	7.94	9.73	0.9	4.83	9.1	NA	NA	
	D	0	5.93	8	0.38	4.65	9.4			
BOD (mg/l)	*W*	0	6.57	45	2	19.28	70	NA	NA	Oct-45
	D	2	48.46	624	2.5	547.65	2530			
NH_4[8] (mg/l)	*W*	<0.01	1.12	11.01	0.09	3.46	31.23	NA	NA	
	D	0	12.31	120	0.1	15.26	88.22			
NO_3^- (mg/l)	*W*	<1	13.57	49.7	0.2	1.41	9.6	10 (as N)	10 (as N)	
	D	3	13.46	62	0.1	1.23	4.9			
SO_4^{2-} (mg/l)	*W*	<7	19.65	115	0	22.26	42	250	250	
	D	4	21.26	225	0	22.58	90			
P_2O_5[9] (mg/l)	*W*	0.01	0.31	2.01	0.05	2.92	44.95	NA	NA	
	D	0	10.75	197	0	8.58	72.44			
FC (CFU[10]/100,ml)	*W*	0	20122	$12x10^4$	0	190.04	400	0	0	5-2,000
	D	0	2,234, 877	$15x10^5$	1	71.61	400			

1 GV: Guideline value

2 MAL: Maximum admissible level ; USEPA: US

3 All values reported < a certain value are set equal to that

4 W: Winter sampling round, based on 94 river samples

5 S: Summer sampling round, based on 76 river samples

6NA: Not Available

7reference temperature at 25oC

87Initial value reported is NH3 , for comparison a conversion factor

8 Initial value reported is o-PO43-, for comparison a conversion factor

10 CFU: colony forming unit

Source: Jurdi et.al (2011). Wet Season Water Quality Survey of the Litani River Basin Project, Litani River Basin Management Support Program.

3.3. **Data Sources**

This study depended on primary data of soil and crops' samples (lettuce, parsley and potato) collected from the Upper Litany River Basin and on secondary data through the evaluation of the water quality of the same region (Jurdi et al., 2010, 2011).

3.4. **Sampling Frame of the Study**

The main types of crops grown in the upper Litani River Basin and irrigated with the river water were identified. And, the choice of crops mainly related to the following factors:

- Main vegetables grown in the Upper Litani River Basin and specifically in Zahle, Delhameiyeh and Bar Elias areas. Leafy vegetables (lettuce and parsley) were chosen because they are mainly consumed raw by the Lebanese consumers.
- Tubular crops and specifically potato is chosen as the Bekaa region produces 80% of the national production of this crop (Abou Zeid, 2005). In addition, the main potato chips industry in Lebanon "Master Chips" is encouraging the farmers to plant this type of crop.

In each area, one plot for each selected crop was randomly chosen. Composite samples of soils and crops samples from each plot and in each area were adopted. Composite samples were obtained by collecting equal amounts of the soil samples taken from a wide area and then placing them in sterile plastic bags (Pepper et al., 2009). A

representative volume of the composite sample was taken and prepared for chemical and microbiological analysis. All the samples were frozen for further analyses.

Each composite sample was constituted from 6 different random samples, and from different agricultural lands from the same site. As such, each composite sample is considered as a representative sample of the indicated vegetable and soil of interest from each site. Simple random sampling was adopted according to Pepper et al. (2009). Random sampling of soil involves choosing the plots of the experimented vegetables at random points within a specified plot of interest (Zahle, Delhameiyeh and Bar Elias). The depths of the leafy vegetables and potato soil samples were 15 and 30 cm, respectively (Pepper et al., 2009). Soil samples were taken by the shovel that was rinsed by a solution of ethanol (75 %) and then rinsed by sterile water between sampling to reduce the contamination between the soil samples (Pepper et al., 2009). Additionally, control samples of soils and vegetables, irrigated by ground water were taken from the same region.

As for vegetables, the same sampling procedure was adopted. Composite samples of each vegetable were obtained from each site and for each crop; samples were then placed in sterile plastic bags (Pepper et al., 2009). All the samples were frozen or stored in ice until they were prepared and analyzed.

The total numbers of samples are 6 composite samples of each soil and vegetable type from each area (Zahle, Delhameiyeh and Bar Elias) with a total of 12 samples from each site. Additionally the control samples were 12. As such, a total of 48 samples of soils and vegetables were analyzed.

3.5. **Sample Preparation**

3.5.1. ***Soil Samples***

Soil samples were prepared according to Pepper et al. (2005):

- All soil samples were air dried for a short period of time and then sieved through a 2 mm mesh to remove the debris and stones.
- The sieved soil was stored at 4°C prior to use
- The sieved soil was used for chemical and microbiological analysis.
- The rest of the samples were frozen at – 20 ºC for further analyses

3.5.2. ***Vegetables Samples***

Vegetables samples were also prepared according to Pepper et al. (2005):

- Vegetables samples from each type of crops are prepared aseptically
- Vegetables samples were stored at 4°C prior to use.
- The rest of the samples were frozen at – 20 ºC for further analyses.

3.6. **Sample Analysis**

3.6.1. ***Microbiological Analyses of Soils and Vegetables Samples***

The following main microbiological hazards were identified in soils and vegetables samples: *Escherichia Coli*: O157:H7, *Salmonella typhi, Salmonella typhimurium, Listeria monocytogenes*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, K. *oxytoca*, *Serratia marcescens*, *C. freundii*, *Shigella sonnei*, *C. diversus,* and *Pseudomonas* , Hepatitis A, Rotavirus, Norwalk virus and Enterovirus

- For vegetable samples, 20 grams of each vegetable (parsley, lettuce, potato) from each site were aseptically cut and blended with 180 ml of sterile distilled water to obtain a homogenous mixture.
- Regarding the soil samples 10 grams of each of the prepared samples was aseptically added to a sterile 95 ml of distilled water (Pepper et al., 2005). Further dilutions were done, when necessary. All microbiological analyses were performed in duplicate.

3.6.1.1. Identification of Enterobacteriaceae

Enterobacteriaceae were identified using SS agar (Difo and BBl Manual, USA, 2009) and Rapid E. coli agar (Bio-Rad, USA, 2007). The SS agar plates were incubated at 35 ± 2°C for 18-24 hours (Difo and BBl Manual, USA, 2009); as for the Rapid E. coli agar plates they were incubated at 44 ± 2°C for 21 hours (Bio-Rad, USA, 2007). After incubation colonies were isolated and Biochemical identification tests of Enterobacteriaceae were conducted.

As such, 102 bacterial suspension prepared from the SS and Rapid E. coli agar were tested for the biochemical identification, where for each sample, 9 biochemical identification test were employed; resulting in 918 tubes to be tested. These tests include Indole test, Phenol Red Lactose Broth, Phenol Red Dextrose Broth, Phenol Red Sucrose Broth, H_2S production test, Citrate agar, Urea agar, decarboxylate lysine agar, deaminate lysine agar, and Ornithine decarboxylase agar. As such colonies were identified based on biochemical tests (Isenberg et al., 2005).

3.6.1.2. Identification of *Listeria* spp. and *Listeria monocytogenes* by Classic Method

1 ml from each diluted sample was vortexed into 9 ml autoclaved Listeria Enrichment broth and incubated for 48 hours at 30°C. After incubation, 1ml of the broth was plated on Palcam agar and incubated at 37°C for 48 ± 2 hours (Difo and BBl Manual, USA, 2009). The colonies (from 31 bacterial suspensions) were identified by gram staining. The isolates were confirmed by catalase (3% H_2O_2) and Bile Esculin (Isenberg et al., 2005).

3.6.1.3. Identification of different pathogens by PCR

3.6.1.3.1. DNA extraction of all the Enterobacteriaceae and *Listeria* spp. Isolates

Isolates of bacterial cultures from the SS, Rapid *E. coli* and Palcam agar were used to prepare a bacterial suspension with a turbidity of 1 optical density (OD) at a wavelength of 600 nanometer, in order to obtain a maximum number of 2 x 10^9 cells.

The DNA was extracted from the bacterial suspensions using DNeasy Blood & Tissue kit following the DNeasy Blood & Tissue procedures (QIAGEN, 2006). The harvested cells are placed in a micro-centrifuge tube and then centrifuged for 10 minutes at 5000xg. The pellet was re-suspended in 180 µl Buffer ATL and 20 µl proteinase K and mixed thoroughly by vortexing. The suspension is then incubated at 56°C for 30 minutes in a water bath with shaking until the cells are completely lysed. After incubation, the suspension is vortexed for 15 seconds. Then 200 µl Buffer Al is added to the sample and mixed thoroughly by vortexing, followed by 200 µl ethanol (96-100 %) and mixed again by vortexing. The mixture is then pipetted with any precipitate present into a DNeasy Mini spin column and placed in a 2 ml collection tube. Then the sample is centrifuged at ≥

6000xg. 500 µl Buffer AW1 is added and the sample is centrifuged for 1 min at ≥ 6000xg. 500 µl Buffer AW2 is added and the sample is centrifuged for 3 min at 20000xg in order to dry the DNeasy membrane. Then 200 µl Buffer AE is added directly to DNeasy membrane and incubated at room temperature for 1 min. After incubation the sample is centrifuged for 1 min at ≥ 6000xg to elute. After centrifugation, the microcentrifuge is kept and stored at -80°C.

3.6.1.3.2. PCR *Listeria monocytogenes* Procedure

Listeria primers (ThermoFisher SCIENTIFIC, 2012, United States) were used to identify *Listeria monocytogenes*. The specific sequence of the primers is shown in Table 4. The amplification PCR program for *Listeria monocytogenes* was performed using C100 Thermal cycler BioRad PCR in 50 µl reaction mixture containing 25 µl Fermantas PCR Master Mix, 3.5 µl of each of the forward and reverse primers of *Listeria monocytogenes* respectively, and 10 µl water PCR-grade. The thermo cycling conditions consist of initial denaturation at 94°C at 120 sec, followed by 40 cycles of denaturation step 95°C for 10 sec, annealing at 60°C for 30 sec and extension at 72°C (Zhou et al., 2005). A final extension at 72°C for 600 sec followed by a hold at 4°C was applied. The amplified sample was stained with ethidium bromide and separated by electrophoresis on 2% agarose gel. Thermo Scientific GenerRule 100 bp-DNA ladder was inserted in each part of the gel. Finally, the gel was visualized using U.V light BioRad.

*3.6.1.3.3. Multiplex PCR for S*almonella *(Salmonella* spp*, Salmonella Typhi* and *Salmonella Typhimurium)*

Three primers for multiplex PCR for *salmonella* spp. were used (ThermoFisher SCIENTIFIC, 2012, United States), the sequence of the specific primers is shown in Table 4. The multiplex amplification procedure for the *Salmonella* spp*., Salmonella Typhi* and *Salmonella Typhimurium* was carried based on the PCR program suggested by Pui et al. (2011). The multiplex amplification PCR program was performed in 50 µl reaction mixtures containing 25µl Fermantas PCR Master Mix, 3.5µl of each of the forward and reverse primers of *Salmonella spp, Salmonella Typhi* and *Salmonella Typhimurium* respectively, and 10 µl water PCR-grade. The program consisted of initial denaturation at 94°C for 120 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, primer annealing at 53°C for 60 sec, and extension at 72°C for 60 sec. Finally, extension at 72°C for 420 sec and infinite period at 4°C holding was utilized (Pui et al., 2011). All amplifications were performed in C100 Thermal cycler BioRad PCR. And, for the visualization of the amplified samples, the samples were stained using ethidium bromide and separated by electrophoresis using 2% agarose gel (4 grams agarose and 200 mL TBE buffer). In addition, Thermo Scientific GenerRule 100 bp-DNA ladder was inserted in each part of the gel. Finally the gel was visualized under ultraviolet light (U.V. BioRad).

3.6.1.3.4. Identification of *Escherichia coli* O157:H7 by PCR

Escherichia coli 0157:H7 (ThermoFisher SCIENTIFIC, 2012, United States) primers were used to identify *Escherichia coli* 0157O157:H7. The sequence of the primers is shown in Table 4. The amplification PCR program for *Escherichia coli* 0157:H7 was

performed using C 100 Thermal cycler BioRad PCR in 50 µl reaction mixture containing 25 µl Fermantas PCR Master Mix, 3.5 µl of each of the STX1, STX2 and *eae*A Primers of *E. coli 0157:H7* respectively, and 10 µl water PCR-grade. The thermo cycling conditions consist of initial denaturation at 95°C for 600 sec followed by a denaturation at 94°C for 20 sec, annealing at 60°C for 30 sec, and 30 sec polymerization at 72°C for a total of 40 cycles. A final extension at 72°C for 300 sec followed by cooling of the samples at 4°C (Sharma et al., 1999). The amplified sample was stained with ethidium bromide and separated by electrophoresis on 2% agarose gel. Thermo Scientific GenerRule 100 bp-DNA ladder was inserted in each part of the gel. Finally, the gel was visualized using U.V light BioRad.

Table 4: Primers for PCR Analysis

Bacteria	Sequence	Reference
Salmonella spp	5'-GCCAACCATTGCTAAATTGGCGCA-3'	
	5'-GGTAGAAATTCCCAGCGGGTACTGG-3'	(Pui et al., 2011)
Salmonella typhi	5'-TGCCGGAAACGAATCT-3'	
	5'-GGTTGTCATGCCAATGCACT-3'	
Salmonella typhiurium	5'-CGGTGTTGCCCAGGTTGGTAAT-3'	
	5'-ACTGGTAAAGATGGCT-3'	
Listeria monocytogenes	5'-GCTGATTTAAGAGATAGAGGAACA-3'	(Zhou et al., 2005)
	5'-TTTATGTGGTTATTTGCTGTC-3'	
STX1	5'-CATAGTGGAACCTCACGACGCAGT-3'	
	5'-TTTGCCGAAAACGTAAAGCTTCA-3'	(Sharma et al.,

STX2	5'-GGGCAGTTATTTGCTGTGGA-3'	1999)
	5'-TGTTGCCGTATTAACGAACCC-3'	
eae A	5'-GGCGGATTAGACTTCGGCTA-3'	
	5'-CGTTTTGGCACTATTTGCCC-3'	

3.6.1.4. Antibiotic Susceptibility Analyses

Antibiotic Disk Susceptibility: (Kirby-Bauer Disk-Diffusion Method) (BD, 2011): From the suspension of the bacterial cultures that were prepared, only those that gave one type of bacterial isolates were tested for antimicrobial susceptibility following the Antibiotic Disk Susceptibility according to Kirby-Bauer Disk-Diffusion Method-BD (2011). As such, 56 isolates (50 from the experimental vegetables and soils and 6 from the control samples) were tested for antimicrobial susceptibility. These isolates include *E. cloacae* (13 isolates), *E. coli* (6 isolates), *C. freundii* (2 isolates), *K. oxytoca* (6 isolates), *Serratia marcescens* (9 isolates), *K. pneumoniae* (8 isolates), *E. aerogenes* (15 isolates) and *Shigella sonnei* (1 isolate).

The choice of the antibiotic was based on the most common types (Cefotaxime, Ciprofloxacin, Gentamicin, and Erythromycin) prescribed by physicians in the studied area. Therefore, the most common antibiotics used are. A suspension of the bacterial cultures was prepared with a turbidity of 1 optical density (OD) at a wavelength of 600 nanometers. The bacterial suspension was plated on Muller Hinton agar and after addition of the antibiotic disks (Ciprofloxacin 5µg, Cefotaxime 30µg, Gentamicin 10µg, and Erythromycin

15µg) the plates were incubated at 37ºC overnight. Based on the Clinical and Laboratory Standards Institute (CLI) standard table of antibiotic susceptibilities, each bacterial strain was classified as resistant, susceptible or intermediate to the antibiotics used, as presented in table 5 (CLSI, 2007).

Table 5: Zone Diameter Interpretive Standards (CLSI, 2007).

Antimicrobial agent	Disc Content	Zone Diameter Nearest Whole (mm)		
		Resistant	Intermediate	Susceptible
Cefotaxime	30µg	≤ 14	15-22	≥ 23
Ciprofloxacin	5µg	≤ 15	16-20	≥ 21
Gentamicin	10µg	≤ 12	13-14	≥ 15
Erythromycin	15µg	≤ 13	14-22	≥ 23

3.6.1.5. Virus Analysis for Vegetables

3.6.1.5.1. Elution Concentration Method

Viruses were recovered from the vegetable samples using elution-concentration method based on Dubois et al., (2007). The vegetable samples were placed in a plastic bag and soaked in 250 ml of elution buffer (100 mM Tris–HCl 50 mM glycine, 1% beef extract, pH 9.5), for 20 min at room temperature with constant shaking. Then the rinse fluid was removed by using filter paper and was centrifuged at 10,000 ×g for 30 min at 4 °C to pellet vegetable particles. The pH of the decanted supernatant was adjusted to 7.2 ± 0.2 by adding 5 N HCl while constantly swirling the fluid. Then 8% polyethylene glycol 6000 and 0.3 M NaCl was used to supplement the neutralized supernatant. The supernatant was incubated

for at least 2 h at 4 °C. Viruses were concentrated by centrifugation of the solution at 10,000 ×g for 30 min at 4 °C. Then the pellet was re-suspended in 400 µl of PBS and the suspension was stored at −20 °C.

3.6.1.5.2. Viral RNA Purification

The RNAs were purified from virus concentrates using High Pure Viral RNA Kit (Roche Diagnostics GmbH, Germany) according to manufacturer's instruction. Briefly, each 200 µl of sample was mixed initially with binding buffer and centrifuged for 15 sec at 8000 xg. Then inhibiter removal buffer was added and sample was centrifuged again at 8000 xg for 1min. buffer wash is added and sample is also centrifuged. Finally, after the two washing steps, Elution buffer was added to the sample and centrifuged to obtain the purified viral RNA.

3.6.1.5.3. cDNA Synthesis

The extracted RNA was reversed to cDNA using the Transciptor First Strand cDNA synthesis Kit (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. 12 µl of sample (RNA) was mixed with 1 µl of Anchored-oligo primer, 4µl of Transcriptor Reverse transcriptase, 0.5 µl of Protector RNase inhibitor, 2µl of Deoxynucleotide Mix and 0.5 µl Transciptor Reverse Transcriptase. The reagents were mixed with a final volume of 20 µl. The sample was placed in the RT-PCR for cDNA synthesis. The PCR program was 55 °C for 1800 sec, followed by 85 °C for 300 sec and followed by 4°C for cooling.

3.6.1.5.4. Primers for Virus Detection

The primers (ThermoFisher Scientific, 2012) used in the reverse-transcriptase PCR are described in Table 3. The RT-PCR amplification of viruses was performed in 25 µl–volumes using the Transcriptor Reverse Transcriptase (Roche Applied Science), to which 5 µl of the sample, 25 µl of FastStart PCR Master Mix, 5 µl of each forward and reverse primers and 10 µl of water PCR-grade. The PCR program was 900 sec denaturation at 95°C. 36 cycles of PCR with each cycle consisting of 94°C for 30 sec, 55°C for 20 sec, and 72°C for 60 sec. Followed by final extension at 72°C for 300 sec in C100 Thermal cycler BioRad PCR. The amplified samples were then separated by electrophoresis on 2% agarose gels using ethidium bromide stain and then it was visualized by U.V light BioRad. Thermo Scientific GenerRule 100 bp-DNA ladder was inserted in each part of the gel. Finally, the gel was visualized using U.V light BioRad.

Table 6: Viruses primers for PCR analysis

Virus	Sequence	Reference
Enterovirus	5´-ACCGGATGGCCAATCCAA-3'	(Fout et al., 2003)
	5'-CCTCCGGCCCCTGAATG-3'	
Rotavirus	5'-CAAAACGGGAGTGGGGAGC-3'	(Fout et al., 2003)
	5'-GCTGGCGTGTCTATGGATTCA-3'	
Norwalk virus	5'GGCGCATGGTTTGTTGATTTC-3'	(Katayama et al., 2001)
	5'-CAAGCCCCCCAAGGTGAAT-3'	
HAV	5'-CCATTTTCCCTCTGTTAGCTTTTCC-3'	(Fout et al., 2003)

	5'-CTTCTAACGTTGCTTCCCATGTCAG-3'	

3.6.2. ***Chemical Analysis***

Levels of main heavy metals (Lead, Cadmium, Chromium, Nickel, Iron, Copper, Zinc, Barium, Manganese, Molybdenum and Arsenic) were determined in soils and vegetables samples. In addition, soil pH and conductivity were also determined. All chemical analyses were performed in duplicate.

3.6.2.1. Heavy Metals Residues in Vegetables

Chemical analysis for vegetables (lettuce, parsley and potato) was performed based on procedure employed by Arora et al. (2008). Briefly, 25 grams of the previously prepared vegetables were washed using distilled water to remove airborne pollutant. Then the vegetables were air-dried for 24 hours to lessen the water content. All the samples were then oven-dried at a temp 20-80°C for 24 hours in a hot air oven to remove all the moisture content.

Pestle and mortar were used to powder the samples and then the samples were sieved through a muslin cloth. Then perchloric acid and nitric acid (1:4) solution were used to digest the ash. The samples were then left to cool and filtered through Whatman filter paper No. 42. Distilled water was added to the sample solution so that the final volume of the sample reaches 25 ml. Finally atomic absorption spectrophotometry (Thermo Electron Corporation) was used to analyze the samples. Standard solutions of Heavy metals (1000 mg/l), namely Barium (Ba), Arsenic (As), Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc

(Zn), Iron (Fe), Nickel (Ni), Copper (Cu), Manganese (Mn) and Molybdeum (Mo) were used.

3.6.2.2. Heavy Metals Residues in Soil Samples

Soil samples were dried and sieved at 0.75µm particle size. XRF analysis was based on field portable x-ray fluorescence method (US EPA Method 6200) using XRF-Niton XL3 GOLDD hand held NITON XL3t Thermo Fisher Scientific machine following manufacturer instructions (EPA, 2007; Thermo Scientific, 2010). The Handheld XRF technique (Niton XL3 GOLDD hand held, Thermo Fisher Scientific) is energy dispersive x-ray fluorescence (EDXRF) with up to 50kV x-ray tube source and optimized silicon drift detector (SDD).

3.6.3. ***Conductivity and pH of the Soil***

The electrical conductivity (ECw) was measured based on the Electrical conductivity method using SensIon 7 HACH, Conductivity Meter (Karen, 2011). The pH was measured using Electrometric method ISO 10390 (2005) using SensIon 7 HACH, pH meter. A representative sample of soil (20 grams) was added to 50 ml distilled water. The sample was shaken for 30 min, incubated for several hours at room temperature, filtered and measured for the pH and conductivity.

Table 7: Analytical Methods for the Determination of the Physical, Chemical and Microbiological Quality Parameters for Vegetables and Soil Samples

Type of sample	Analytical parameter	Standard Analytical Method	Type of Analytical Equipment
Soil	Conductivity	Electrical conductivity method	SensIon 7 HACH, Conductivity Meter
	pH	Electrometric method	SensIon 7 HACH, pH Meter
	Trace metals: Pb, Cd, Cr, Zn, Fe, As, Ni, Ba, Cu, Mn &Mo	X-Ray Fluorescence	XRF-NITON XL3T thermo scientific
Vegetable	Trace metals: Pb, Cd, Cr, Zn, Fe, As, Ni, Ba, Cu, Mn &Mo	Atomic absorption spectrophotometry	Atomic absorption spectrophotometry Thermo electron corporation
Vegetables and soils	Microbiological profile	Pour plate method and Biochemical identification	
Vegetables and soils	Antibiotic resistance	Disk Diffusion Method	
Vegetables and soils	Strains of bacteria	Identification using PCR	C100 Thermal cycler BioRad PCR
Vegetables	Viruses	Elution-concentration method	C100 Thermal cycler BioRad PCR

CHAPTER 4

RESULTS AND DISCUSSION

4.1. **Overview of the Chapter**

The microbiological and chemical hazards in soils and in the main vegetables (e.g. lettuce, parsley and potato) grown in the Bekaa Region, and irrigated by the Litani river water were identified. And, the levels of chemical and microbiological contaminants in irrigated water, soil and vegetables were compared to international standards to evaluate the magnitude of the health risk. In addition, the antibiotic resistance patterns of the isolated pathogenic microorganisms were also determined for proper case management.

4.2. **Microbiological Analysis**

The consumption of raw vegetables irrigated with wastewater by sprinklers method is the main cause of foodborne illnesses associated with the *Enterobacteriaceae* family (Nyenje et al., 2012). Hence, samples of the lettuce, parsley and potato, mainly produced in the Upper Upper Litani Basin, and irrigated by contaminated water from the river, show the presence of different types of *Enterobacteriaceae* pathogens (*E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, *K. oxytoca*, *S.marcescens*, *C. freundii*, *Shigella sonnei*, *C. diversus*), *Listeria* spp. and *Pseudomonas* (Table 8&9). In Morocco, vegetables (e.g. lettuce, potato, tomato, raddish, cucumber...) irrigated with wastewater, demonstrated similar results and were contaminated by *Enterobacteriaceae* (Nyenje et al., 2012; Karamoko et al., 2007). Further studies on *E. coli O157:H7 and Listeria* spp. showed that

these pathogens were detected on the leaves and roots of lettuce and parsley irrigated by contaminated water (Oliveira et al., 2012; Wachtel et al., 2002; Solomon et al., 2002).

Different analytical methods, as presented in the materials and methods section, were conducted to identify the strains of the mentioned microorganisms; briefly these methods include sugar tests (Indole, Lactose, Sucrose, Dextrose, Kligrer, Citrate, Urease, Lysine, and Ornithine tests) and molecular based method polymerase chain reaction (PCR) analyses for *Salmonella* species, *Listeria monocytogenes, E. coli* O157:H7 and viruses (enteric virus, rotavirus, norwalk virus and Heptatis A).

Table 8: Types of Bacterial Species Isolated from Vegetable (Lettuce, Parsley and Potato) Samples

Bacteria	Type of Vegetable Samples
K. oxytoca	Parsley (1/6)
	Lettuce (2/6)
S. marcescens	potato (1/6)
E. coli	Parsley (1/6)
	Potato (2/6)
	Lettuce (3/6)
C. Freundii	Parsley (1/6)
	Lettuce (2/6)
C. diversus	Lettuce (1/6)
E. aerogenes	Parsley (2/6)

	Lettuce (4/6)
K. pneumoniae	Parsley (2/6)
	Lettuce (2/6)
E. cloacae	Lettuce (4/6)
	Parsley (3/6)
	Potato (2/6)
Listeria spp.	Potato (1/6)

Table 9: Bacterial Species Isolated from the Soil Samples of the Grown Vegetables

Bacteria	Soil samples
S. marcescens	Parsley (4/6)
	Lettuce (2/6)
	potato (2/6)
K. oxytoca	Parsley (2/6)
	Lettuce (4/6)
E. coli	Parsley (1/6)
	Lettuce (1/6)
C. Freundii	Parsley (2/6)
	Lettuce (1/6)
Pseudomonas	Lettuce (1/6)
Sh. sonnei	Lettuce (1/6)

E. aerogenes	Lettuce (3/6)
	Parsley (3/6)
	potato (2/6)
K. pneumoniae	Lettuce (3/6)
	Parsley (2/6)
	potato (2/6)
E. cloacae	Lettuce (2/6)
	potato (4/6)
Listeria spp.	Lettuce (1/6)
	Parsley (1/6)

As such, the type, prevalence and significance of detected Microorganisms are as follows:

4.2.1. *Escherichia coli*

E. coli O157:H7 is a virulent strain responsible for enteric diseases and is of major concern as it can cause illness after ingestion of few cells (Islam et al., 2004). Outbreaks of *E. coli* O157:H7 have been reported in different studies due to the consumption of vegetables (particularly lettuce and spinach) contaminated by wastewater (Oliveira et al., 2012; Islam et al., 2004). Results of the study (the polymerase chain reaction results) show the presence of *E. coli* O157:H7 in parsley's soil at one agricultural site in Bar Elias (site 1). In addition, *E. coli* was detected in 50 % of lettuce samples, 33% of potato samples and

17% of the parsley samples; whereas, *E. coli* isolated from soil was detected in 17% of the samples for both the Lettuce's soil and parsley's soil and was not detected on potato's soil (figure 20).

The low levels of *E. coli* detected in soil samples could be highly linked to the sprinkling irrigation method used that would allow minimal amount of the pathogen reaching the soil, and retained on soil. Oliveira et al. (2012) observed that the surface soil is exposed to several environmental conditions such as UV light and wind-mediated drying that would contribute to the pathogen die off when sprinkling irrigation method is used.

E. coli was not isolated from vegetables of Dalhamieh's site (site 2) and from parsley of Zahle's site (site 3). The presence of *E. coli* in different vegetable samples is an indicator of fecal contamination, mainly from Litani river water used for irrigation. These results are concurrent with Halablab et al. (2011) study on the microbiological safety of raw vegetables irrigated with Litani river water; where lettuce and parsley were contaminated with *E. coli*. *E. coli* in the contaminated irrigation water or soil can be easily transferred from the root of the plant to the edible parts of leafy vegetables such as lettuce and Parsley (Oliveira et al., 2012; Islam et al., 2004; Solomon et al., 2002).

Also, *E. coli* can survive and persist on the plant's leaf surface for 20-30 days after the irrigation with contaminated water by sprinkler method (Solomon et al., 2003). It is enough to irrigate with contaminated water once for vegetables to become contaminated by *E. coli*. *E. coli* survive better in the fall season than in summer and spring. Furthermore, *E. coli* survive easily at average temperature (10°C) and humidity (82 %) than at hot temperatures of summer and spring, since higher temperatures lead pathogen survival reduction. Moreover, *E. coli* have been reported to survive less than 4 weeks in lettuce

leaves in the spring season when spray irrigated, whereas it survives for more than 5 weeks in the fall season (Oliveira et al., 2012).

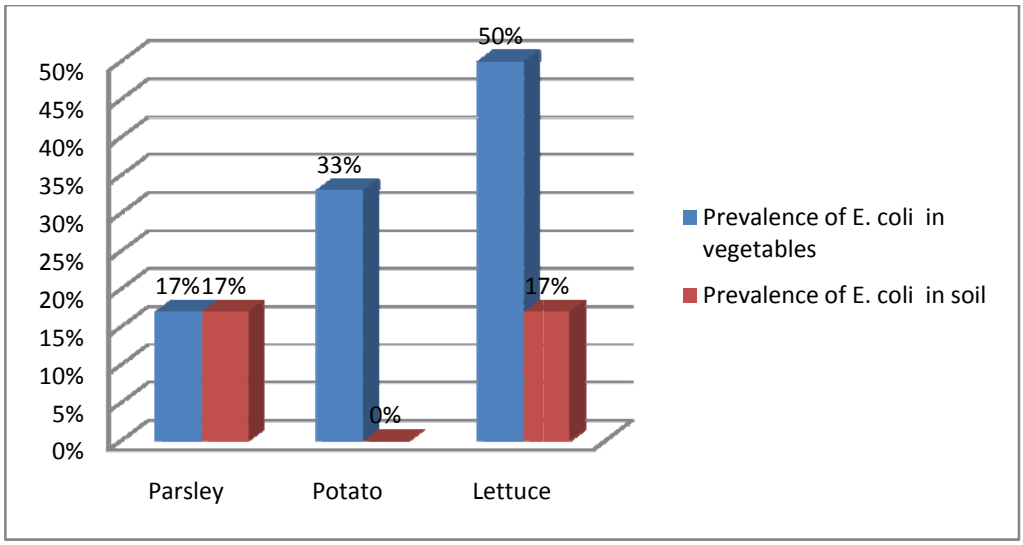

Figure 180: The Prevalence of E. coli in Vegetables and Soil Samples

4.2.2. ***Escherichia cloacae and Escherichia aerogenes***

Various studies reported the isolation of *E. cloacae* and *E. aerogenes* from different food samples such as vegetables (lettuce, tomato and carrot), milk, cereals and grains (Shaker et al., 2007; Falomir et al., 2010). In this study, *E. cloacae* was detected in 67 % of lettuce samples, 50 % of parsley's samples, and 33 % of potato samples; where *E. cloacae*, isolated from the soil samples, was detected in 67 % of potato's soil samples, 33 % of lettuce's soil samples but was not detected in any of parsley's soil samples (figure 21). In addition, *E. cloacae* weren't identified on the parsley of Zahle's site and potato of Dalhamieh's site (site 2). The detection of *E. cloacae* in lettuce samples in frequencies higher than that in parsley could be attributed to the large surface area of lettuce leaves in

which pathogens are entrapped compared to that of parsley. Moreover, lettuce provides a good medium for bacterial inhabitants since it has a foliar surface with many fissures and folds (junction zones); additionally the bacteria can penetrate the inner tissues of lettuce due to its fragile leaves (Tagoe et al., 2011; Halablab et al., 2011; Solomon et al., 2001).

As for *E. aerogenes* it was detected in 67 % and 33 % on lettuce and parsley, respectively. Whereas, *E. aerogenes* was present in the soil samples with 50 % prevalence on both lettuce and parsley's soil and 33 % in potato's soil samples as presented in figure 22. The absence of *E. cloacae* and *E. aerogenes* in parsley and potato's soil samples might be attributed to sprinkler irrigation method since lettuce soil is more exposed to irrigation than parsley and potato soil. Furthermore, the difference in contamination between leafy vegetables (lettuce and parsley) and potato could be attributed to the type of irrigation methods used and plant type. Sprinkler irrigation leads to the direct contact between contaminated irrigation water and leafy vegetables' leaves in comparison to potato plants in soil. Further, the lettuce and parsley leaves are characterized by the phyllosphere that supports the growth of a wide variety of microbial community (Whipps et al., 2007; Lindow et al., 2003).

As such, the results of pathogens that are isolated in this study are in line with other studies where *E. cloacea* was one of the most isolated pathogen from vegetables irrigated with wastewater (Nyenje et al., 2012; Afolabi et al., 2010; Falomir et al., 2010).

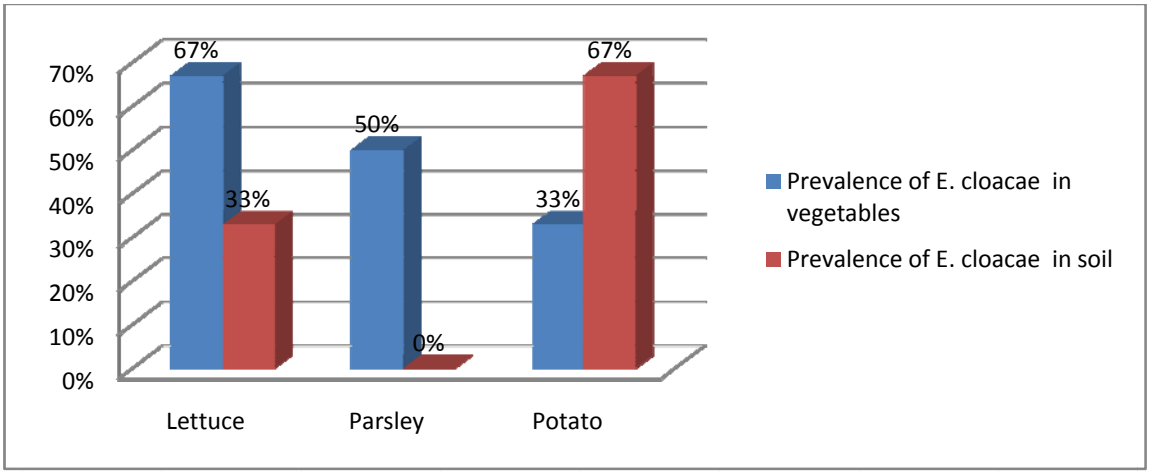

Figure 191: Prevalence of E. cloacae in Vegetables and Soil samples

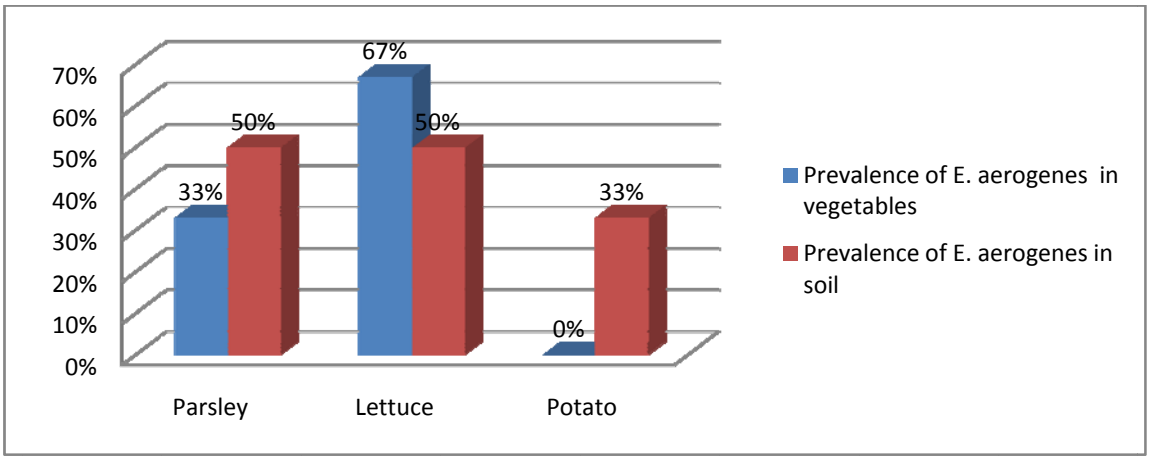

Figure 202: Prevalence of E. aerogenes in Vegetables and Soil Samples

4.2.3. ***Klebsiella pneumonia and Klebsiella oxytoca***

K. pneumoniae and *K. oxytoca* were also isolated from lettuce and parsley vegetable samples but were not detected in potato vegetable samples. *K. pneumonia* was present in 33 % of parsley samples and similarly in 33% of lettuce samples. As for the soil

samples, it was identified in all the three types with 50 % occurrence in lettuce's soil samples, and 33 % occurrence in potato and parsley's soil samples as presented in figure 23. Furthermore, *K. oxytoca* was isolated in 33 and 17% of lettuce and parsley samples, respectively; as for the soil samples, *K. oxytoca* was present in 67% of lettuce's soil and 33 % of parsley's soil; however it was not detected in potato's soil as presented in figure 24. The absence of *K. oxytoca* in potato's soil could be due to the inability of this bacterial organism to compete with other soil microorganisms. Moreover, the difference in contamination between leafy vegetables (lettuce and parsley) and potato, could be also attributed to the type of irrigation method used (sprinkler irrigation) and plant type (leafy compared to tubular) (Forslund et al., 2010; Whipps et al., 2007; Lindow et al., 2003).

These results are in accordance with reported studies that show the survival capability of *Klebsiella* species mainly *K. pneumoniae* and *K. oxytoca* in soil and vegetable (lettuce, carrot and tomato) samples irrigated with wastewater (Falomir et al., 2010, Brown et al., 1973). The presence of these opportunistic microorganisms in the studied crops and soils, mainly originates from human gastro-intestinal tract (Lawlor et al., 2005; WHO, 2006) and is associated with the fecal contamination of Litani River (Jurdi et al., 2010). The results of pathogens that are isolated in this study are also in line with other studies where *K. oxytoca* was among the most isolated pathogen from vegetables irrigated with wastewater (Nyenje et al., 2012; Falomir et al., 2010). Further Afolabi et al., (2010), reported the presence of *K. pneumonia* in lettuce irrigated with polluted stream.

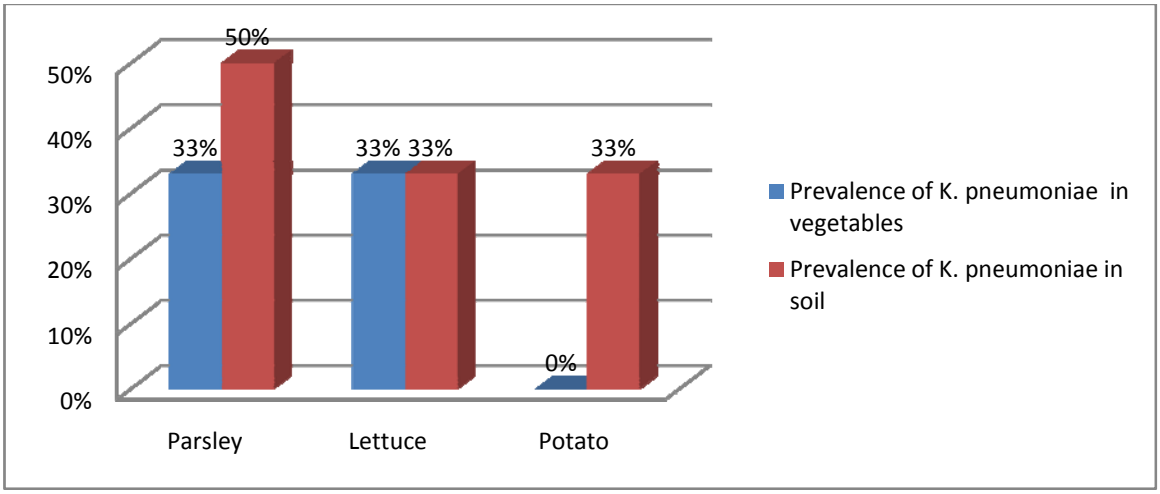

Figure 213: Prevalence of *K. pneumoniae* in Vegetables and Soil Samples

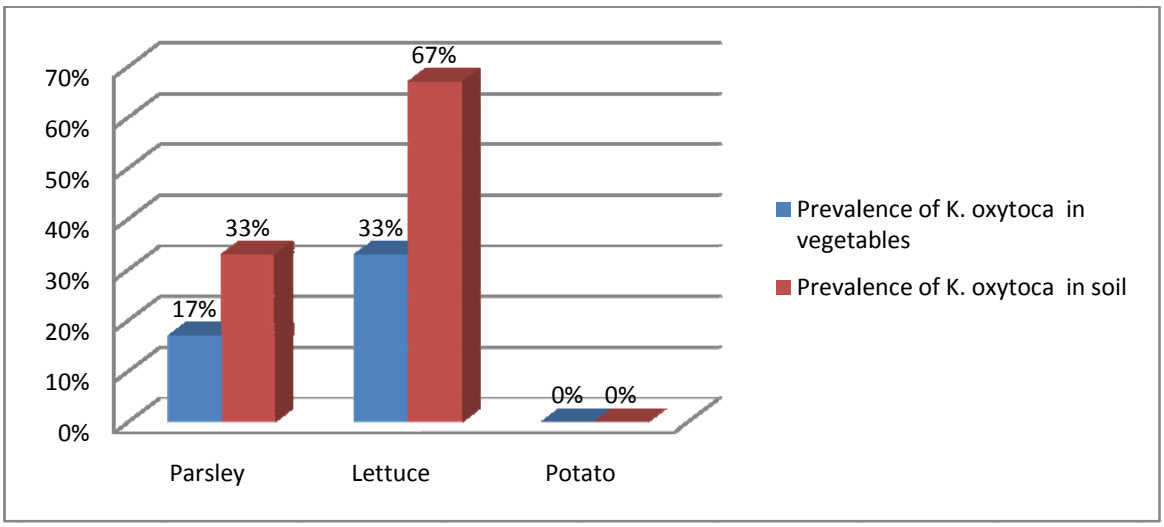

Figure 224: Prevalence of *K. oxytoca* in Vegetables and Soil Samples

4.2.4. ***Citrobacter spp.***

C. freundii was detected in 33 % of lettuce samples and 17 % of parsley's sample with the same prevalence of *K. oxytoca*. These findings concur with that of Falmoir et al.

(2010) that showed a prevalence of *C. freundii* in lettuce and salad. Other studies of salad and salad ingredients also showed contamination of lettuce and carrot by *C. freundii* (Uzeh et al., 2009; Soriano et al., 2001). Moreover, studies in Morocco showed that *C. Freundii* and *E. Cloacea* were the most frequently detected pathogens on vegetables (including lettuce and potato) irrigated with wastewater (Karamoko et al., 2007). *C. freundii* wasn't detected in potato's soil; however, it was found in 33 % of parsley's soil and 17 % of lettuce soil (figure 25). The absence of *C. freundii* in potato could be explained by its inability to compete with other soil microorganisms.

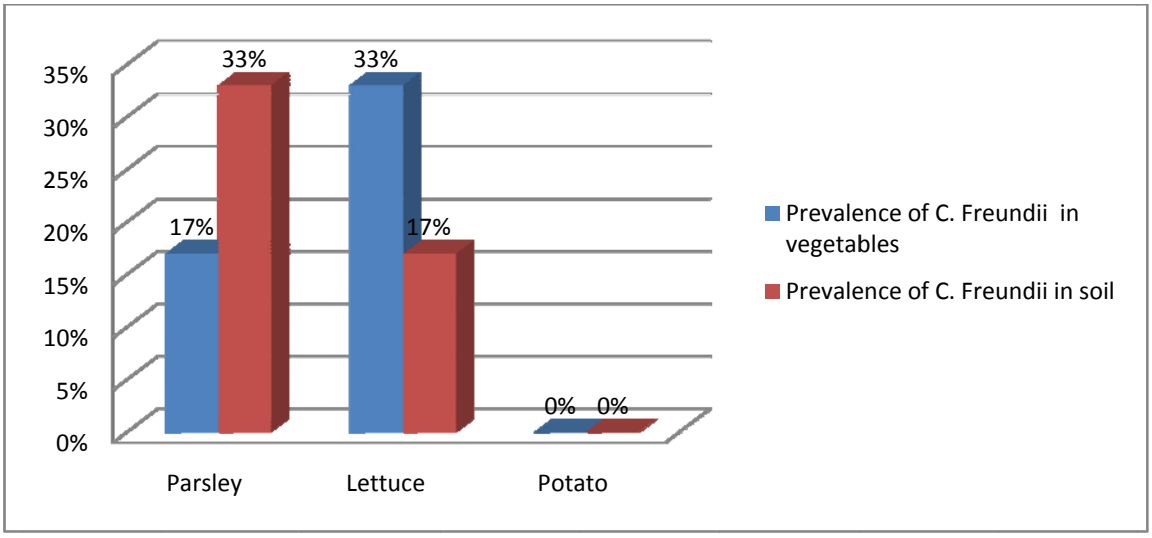

Figure 235: Prevalence of C. Freundii in Vegetables and Soil Samples

In addition, *Citrobacter diversus* was isolated from lettuce samples of the Dalhamieh's area. The results of this study are in line with the study done by wright et al. (1976) which demonstrated the presence of isolates of *C. diversus* on lettuce. Another study about the occurrence of human enteric pathogens in raw vegetables and fruits, showed the

presence of isolates of *C. diversus* present in tomato, coriander leaves, and blackberry (Shaid et al., 2009). *Citrobacter diversus* is also an opportunistic gram negative bacillus belonging to the Enterobacter family, and is associated with opportunistic infections such as brain abscess and neonatal meningitis (Soriano et al., 1991; Badger et al., 1999).

4.2.5. ***Lactose Non Fermenter Enterobacteriaceae***

As for the lactose non-fermenter Enterobacteriaceae, only *Shigella sonnei* and *Serratia marcescens* were detected in the collected samples. *S. marcescens* isolates were only identified in 17 % of potato vegetable; however, *S.marcescens* was identified in 67 % of parsley's soil samples and 33 % in potato and lettuce's soil samples as presented in figure 26.

These results are in accordance with those of a study conducted in the suburban area of Abidjan, where manure application and soil contamination were identified as the major factors leading to vegetables contamination with S. *marcescens* and *E. coli* (Koffi-Nevry et al., 2011). Likewise, several studies showed the prevalence of S. *marcescens* in various vegetables such as lettuce, potato, carrots, tomato and cabbage irrigated with wastewater (Karamoko et al., 2007, Falomir et al., 2010).

Still, the absence of S. *marcescens* in lettuce and parsley vegetables could be attributed to the inability of the pathogen to colonize and adhere to the vegetable leaves in the presence of other pathogens.

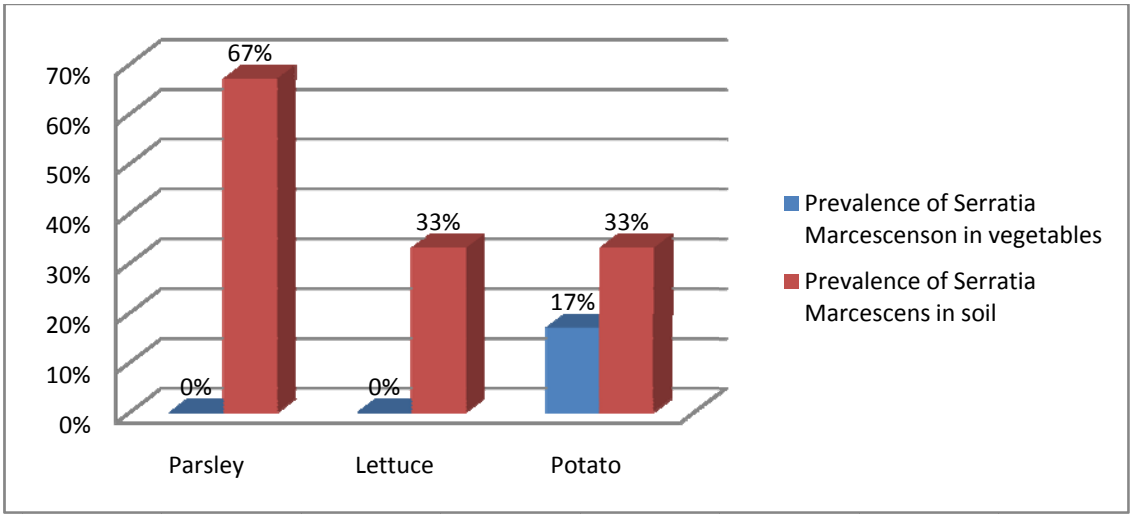

Figure 246: Prevalence of Serratia Marcescens in Vegetables and Soil Samples

Furthermore, *Shigella sonnei* was just identified in lettuce's soil; it was absent in all the other samples. This is because *Sh. sonnei* cannot compete with other organisms. However, the presence of *Sh. sonnei* in few cells is sufficient to cause infection (Kapperud et al., 1995; Abaidoo et al., 2010). Several outbreaks are associated with *Sh. sonnei* presence on vegetables that are irrigated with wastewater or contaminated water (Kapperud et al., 1995; Frost et al., 1995). Another study showed prevalence of *Shigella* spp. on different vegetables such as parsley, lettuce and celery (Buck et al., 2003).

4.2.6. ***Listeria spp.***

Listeria species are capable of surviving in water soil and manure for long periods and as such can contaminate vegetables (Oliveira et al., 2011). Several outbreaks of listeriosis have been associated with the consumption of vegetables like lettuce and

cabbage, irrigated with wastewater or contaminated by animal manure (Nyenje et al., 2012; Oliveira et al., 2011; Buck et al., 2003).

In this study, *Listeria spp.* was only present in 17 % of potato samples, and 17 % of both parsley and lettuce's soil. The survival of *Listeria spp.* in soil is impacted by several biotic and abiotic factors such as soil type, soil moisture, pH, temperature and type of microbial community (Oliveira et al., 2011). As such, irrigation with the Litani River under favorable conditions would lead to the transfer of Listeria species from contaminated soil to vegetables during their growth or directly via sprinkler irrigation of the leafy vegetables posing health threats and possible listeriosis outbreaks.

However, and based on polymerase chain reaction (PCR) analyses, none of the vegetables or soil samples are contaminated by *Listeria monocytogenes.* The absence of *Listeria* spp. and *L. monocytogenes* on leafy vegetables such as lettuce and parsley is justified by the inability to compete for water and nutrients with other pathogens, or they cannot adapt to the stressful environmental conditions given that sampling was done during the hot season of the year (August). Studies by Jablasone et al. (2005) showed that the presence of *E. cloacae* detected in different vegetables and soils samples, as presented before, leads to the significant reduction in the numbers of *L. monocytogenes* and *E. coli* colonizing the vegetables such as lettuce, spinach and carrots.

Further, it is noted that *Listeria* species and specially *L. monocytogenes* favor survival at cold temperature (≤ 5°C) which is more in winter in comparison to other seasons. Higher temperatures reduce the survival rate of this pathogenic microorganism by increasing energy expenditure and stress in comparison to lower temperatures (Oliveira et al., 2011). Moreover, the type of irrigation method used has also a great influence on

pathogen survival in soil. Sprinkling irrigation, the method mostly used along the Upper Litani River Basin, leads to the decline in the pathogen population in soil compared to surface irrigation that provides the soil with higher water saturation (Oliveira et al., 2011).

4.2.7. ***Pseudomonas spp***.

Pseudomonas aeruginosa was detected only in parsley's soil of site 2. However, several studies did show the prevalence of *P. aeruginosa* in lettuce, tomato, carrot and cucumber (Littlewood, 2007; Minion, 2010; Nyenje et al., 2012). *P. aeruginosa* is a gram negative rods opportunistic pathogen that mainly infect immune-compromised patients specially patients with cystic fibrosis. *P. aeruginosa* can also survive in harsh environments and can adapt to high temperatures 42°C (Minion, 2010). *P. aeruginosa* is usually found in environments contaminated by animal or human waste and in environments containing high organic loads where it can be isolated from contaminated plants, soil and surface water. Additionally, sprinkling irrigation would favor the colonization of *P. aeruginosa* in soil and plants. In addition, high humidity (80-95%) and a temperature of 27°C are optimal conditions for their growth. As such, and although *P. aeruginosa* isolates were not detected in vegetable samples, the risk is still apparent. The irrigation by the contaminated Litani River using sprinkling irrigation may allow the transfer of this pathogenic microorganism to the crops and may cause severe foodborne illnesses.

4.2.8. ***Salmonella spp.***

Vegetables play an important role as vehicles for the transfer of S*almonella* to humans (Lapidot et al., 2008; Levantesi et al., 2011). However, *Salmonella spp*.,

S*almonella typhimurium* and *Salmonella typhi* were not detected in vegetables samples and in soils samples (biomedical identification analyses as well as in PCR analyses). As such, the findings of this study, regarding the identification of salmonella, are not in agreement with reported studies that show the contamination with *Salmonella spp.* of raw vegetables (lettuce, tomato, potato, parsley) irrigated with wastewater. The transfer of *Salmonella spp*. from the irrigation water directly to the plant is stronger than that of transfer from the soil; the pathogens that are tightly attached to the soil particles become absorbed by the soil and can only move short distances (Lapidot et al., 2008).

Different factors affect the survival of *Salmonella* spp. in the environment. Temperature is a critical factor affecting *Salmonella* spp. survival; where high temperatures (25°C compared to lower temp 15 and 5°C) could result in the pathogen die-off (Jacobsen et al., 2011). Further, the survival of salmonella in soil is influenced by the presence of other types of bacterial organisms and protozoa such as protozoa that prey on *Salmonella* spp. (Jacobsen et al., 2011). As such, the absence of detected *Salmonella* spp. can be possibly attributed to the higher summer temperatures (32-40^{0}C) and the presence of other competing microorganisms.

4.2.9. ***Viruses***

Viruses (Enteric, Rota, Norwalk and Heptatis A virus) were not detected in all types of samples tested. This finding is not in line with results of other reported studies that detected viruses in vegetables irrigated with contaminated water. Several studies show that irrigation with sewage or wastewater effluents would lead to the transfer of viruses (Norwalk, Rotavirus, Enterovirus and Hepatits A) from the irrigation water to the soil and

the vegetables (Beuchat et al., 1997; Badawy et al., 1985; Ward et al., 1987; Dubois et al., 2007; Fumian et al., 2009). Vegetables that are eaten raw and that have short growth period (such as radish, lettuce and parsley) are of particular concern when irrigated with wastewater (Badawy et al., 1985). Several other studies also showed the presence of hepatitis A and rotavirus on lettuce irrigated with sewage (Herna´ndez et al., 1997). Another study showed that rotavirus is capable of surviving in lettuce, carrots and radish up to 30 days at a refrigeration temperature of 4°C (Badawy et al., 1985). And, experimental studies on the recovery of viruses from vegetables (lettuce and radish) that were spray irrigated with wastewater showed positive results for poliovirus (Rzeżutka et al., 2004).

As such, the reasons why viruses were not detected in the vegetables irrigated by the Litani river water could be due to the level of contamination of viruses in vegetables; where low levels would make it harder for the viruses to be detected in samples. Further, the presence of humic substances would also interfere with the detection of viruses since they inhibit the taq polymerase and reverse transcriptase (Dubois et al., 2007).

Moreover, it is to be noted that high temperatures and direct exposure to sunlight are detrimental to virus survival and would lead to virus drying of from the vegetables (Bitton et al., 1979; Bosch et al., 2006). A study done by Kott and Fishelson (1974) on lettuce, celery and tomato spray irrigated with wastewater, showed a poliovirus decrease by more than 90 % in the vegetables due to exposure to solar radiation (Rzezutka et al., 2004; Bosch et al., 2006). Another study on the presence of rotavirus and poliovirus on grass irrigated with sewage showed that temperatures in summer decrease the virus survival (Badawy et al., 1985). As such, the survival of viruses, in this study, may be challenged by high temperatures and direct exposure to sunlight.

4.2.10. ***Microbiological Profile of Control Vegetable and Soil Samples***

Lettuce, parsley and potato control samples grown in the Upper Litani Basin area and irrigated with ground water sources were also studied. Enterobacter species were detected in several control samples as presented in table 10. Control parsley soil showed the prevalence of *S. marcescens*, *C. diversus*, and *C. freundii;* whereas the control parsley vegetable didn't show any prevalence of pathogenic bacteria.

E. cloacae was isolated from both potato vegetable and soil control samples; where control potato soil also showed occurrence of *K. pneumonia*. As for *E. aerogenes* it was detected in control lettuce vegetable samples. Lettuce soil showed presence of *Serratia marcescens*. The presence of these Enterobacter in control samples is mainly attributed to the use of contaminated ground water for irrigation (Jurdi et al., 2010). Other possible sources could be associated with the natural fertilizers (improperly composted manure), insects as vehicle of transferring pathogens, presence of animals or due to the contamination of irrigation water (Berger et al., 2010; Beuchat et al., 1997).

Table 10: Types of Bacterial Isolates from Control Samples

Type of Vegetable Sample	Soil/Vegetable Sample	Bacteria
Potato	Soil	*K. pneumonia*
	Soil	*E. cloacae*
	vegetable	*E. cloacae*
Lettuce	Vegetable	*E. aerogenes*
	Soil	*S. marcescens*
Parsley	soil	*C. Freundii*
	soil	*C. diversus*
	soil	*S. marcescens*

To conclude vegetables contamination with pathogens is mostly due to pre-harvest conditions reflecting on the quality of irrigation water, application of animal manure, contaminated soil, wastes of domestic animals, and improper handling of vegetables in the field (Oliveira et al., 2012; Buck et al., 2003; Beuchat et al., 1997). Several experimental studies provide supported evidence showing that irrigation water and manure lead to the contamination of grown vegetables. The vegetables are either contaminated directly from irrigation with wastewater or from contaminated soils which becomes a reservoir of pathogens once irrigated with sewage (Oliveira et al., 2012).

The Upper Litani Basin quality is deteriorated, as explained earlier, due to the uncontrolled discharge of sewage and industrial wastewater, municipal and industrial solid waste and animal waste throughout the river basin. In the study by Jurdi et al. (2009, 2010) fecal coliforms were detected throughout the river flow, verifying the source of pathogenic microorganisms in the grown vegetables (lettuce, parsley and potato) and the soil tested. And, of the studied vegetables, lettuce is the most contaminated with 19 different types of pathogenic isolates, followed by parsley with 11 isolates and potato with 7 isolates. As for soil samples, also lettuce soil is the most contaminated followed by parsley soil and potato soil as presented in table 11.

Table 11: Pathogens Isolated from Different Types of Samples.

Lettuce Vegetable	*E. aerogenes (4/6)*
	K. oxytoca (2/6)
	C. Freundii (2/6)
	E. cloacae (4/6)
	E. coli (3/6)
	K. pneumoniae (2/6)
	C. diversus (1/6)
Parsley Vegetable	*K. pneumoniae(2/6)*
	E. aerogenes(2/6)
	K. oxytoca(1/6)
	E. cloacae(3/6)
	E. coli (1/6)
	C. Freundii (1/6)
Potato vegetable	*S. marcescens(1/6)*
	E. coli (2/6)

	Listeria spps (1/6)
	E. cloacae (2/6)
Lettuce soil	*E. aerogenes* (3/6)
	K. oxytoca (4/6)
	C. Freundii (1/6)
	E. cloacae (2/6)
	S. marcescens (2/6)
	Pseudomonas (1/6)
	E. coli (1/6)
	Listeria spps *(1/6)*
	Sh. sonnei (1/6)
	K. pneumoniae(3/6)
Parsley soil	*K. pneumoniae*(2/6)
	E. aerogenes(3/6)
	K. oxytoca(2/6)
	S. marcescens(4/6)
	E. coli (1/6)
	C. Freundii (2/6)
	Listeria spps *(1/6)*
Potato soil	*E. aerogenes*(2/6)
	K. pneumoniae(2/6)
	S. marcescens(2/6)
	E. cloacae(4/6)

The results of this study are consistent with other reported studies that show the presence of pathogenic bacteria and specially *Enterobacteriaceae* family (*E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, K. *oxytoca*, *S. marcescens*, *C. freundii*, *Shigella*

sonnei, *C. diversus,* and *P. aeruginosa*) in vegetables irrigated by wastewater. Moreover, the attachment of the pathogens (ex. *E. coli*) to the roots of the plant would lead to contamination to the edible roots of crops such as potato (Wachtel et al., 2002). *Enterobacteriaceae* family and *Listeria* spp. have the ability to adhere to the edible parts of leafy vegetables, leafy vegetable roots, interior of the stomatal pores and to the deep grooves of seed coats (Wachtel et al., 2002; Oliveira et al., 2011). It should be noted that these adherence mechanisms induce bacterial resistance to chemical interventions (like chlorination) and physical methods of seed surface disinfection.

As such, the edible parts of leafy vegetables are contaminated with pathogens due to irrigation with contaminated Litani river water. Contamination of vegetables (lettuce, parsley and potato) in this study is mainly due to the initial and direct contact of polluted irrigation water with the vegetables specially when utilizing the sprinkling irrigation method. Other routes of contamination that are suggested by different studies indicate also that the edible parts of vegetables could become contaminated with pathogens by internal transport of the pathogen via the root system of the vegetable where the interior tissues of the vegetable would become colonized by the pathogen. Another route of contamination is by the external transport via rain or wind that would favor the transfer of pathogen from the contaminated soil to the vegetable (Islam et al., 2004; Oliveira et al., 2012; Deering et al., 2012).

Moreover, the survival of pathogens in the soil and crop, as explained earlier, is affected by different factors such as the pH, humidity, temperature, plant type and competition with native flora and fauna (WHO, 2006; Oliveira et al., 2011). It is noted that the sampling time of the vegetables was done in summer (month of August), during hot

temperatures, which has high influence on the detection of the different types of microorganisms such as (*Salmonella* spps., *Listeria* spp. *E. coli* and viruses); higher temperatures would contribute to high energy expenditure and stress on the bacteria than lower temperatures (Oliveira et al., 2011). As such, high temperature would play important role in decreasing pathogens survival in soil and vegetables.

Furthermore, the method of irrigation plays an important role in the transfer of pathogens from the irrigation water to vegetables; where sprinkler irrigation favors pathogen transfer more than surface irrigation. In addition, the spray irrigation would favor the persistence of the pathogens in growing leafy vegetables for long periods (Solomon et al., 2002; Solomon et al., 2003). As such, in the study area, the irrigation method used is mostly sprinkler irrigation which would further explain the transfer and survival of pathogens in vegetables. However, sprinkling irrigation is detrimental to pathogen survival in soil. Use of this irrigation method would lead to the decline of pathogens population, where minimal amounts of water reach the soil surface, and with the exposure to solar UV and wind this would result in pathogen die off in soil (Oliveira et al., 2011).

Hence, leafy (such as Lettuce and parsley) and tubular vegetables are prone to become contaminated with pathogens and as supported by the increase in the number of reported outbreaks related to the consumption of vegetables irrigated with wastewater (CDC, 2006; Lynch et al., 2009, Elizaquível et al., 2011; Frost et al., 1995). In the Upper Litani river basin, the detection and survival of these pathogens is mostly associated with the quality of irrigation water posing a potential health risk; where pathogens from the Litani river water are transported from the irrigation water to the soil and vegetables and are able to survive in amounts sufficient to be detected, and to cause disease.

4.3. **Antibiotic Resistance**

A total number of 56 vegetables and soil isolates: *E. cloacae* (13 isolates), *E. coli* (6 isolates), *C. Freundii (2* isolates*), K. oxytoca (6* isolates*), S. marcescens* (9 isolates), *K. pneumoniae* (8 isolates), *E. aerogenes* (15 isolates) and *Sh. sonnei* (1 isolate), were tested for antimicrobial susceptibility to Cefotaxime, Ciprofloxacin, Gentamicin, and Erythromycin. The choice of the antibiotic was based on a field survey that identified the most common types of antibiotics prescribed by physicians in the Bekaa region.

All vegetables and soil isolates showed 100 % resistance to Erythromycin and Gentamicin with the exception of *E. aerogenes* that had 93 % of resistance to Gentamicin. *E. coli, K. oxytoca*, *K. pneumoniae, E. aerogenes* and *Sh. sonnei* isolates showed 100 % resistance to Cefotaxime; whereas *E. cloacae, C. Freundii,* and *S. marcescens* showed 91%, 50% and 71% resistance, respectively. Also, *E. cloacae*, *C. Freundii, K. oxytoca* and *K. pneumonia* showed 100% resistance to Ciprofloxacin; whereas *E. coli*, *S. marcescens, E. aerogenes* and *Sh. sonnei* showed 83 %, 86%, 93% and 0% resistance, respectively (figure 27). Moreover, none of the isolates was totally susceptible to any of the four antibiotics used. The resistance pattern for the antibiotics used on all the isolates showed 100% resistance to Erythromycin, 98% resistance to Gentamicin and 93% resistant to both Ciprofloxacin and Cefotaxime as presented in figure 28.

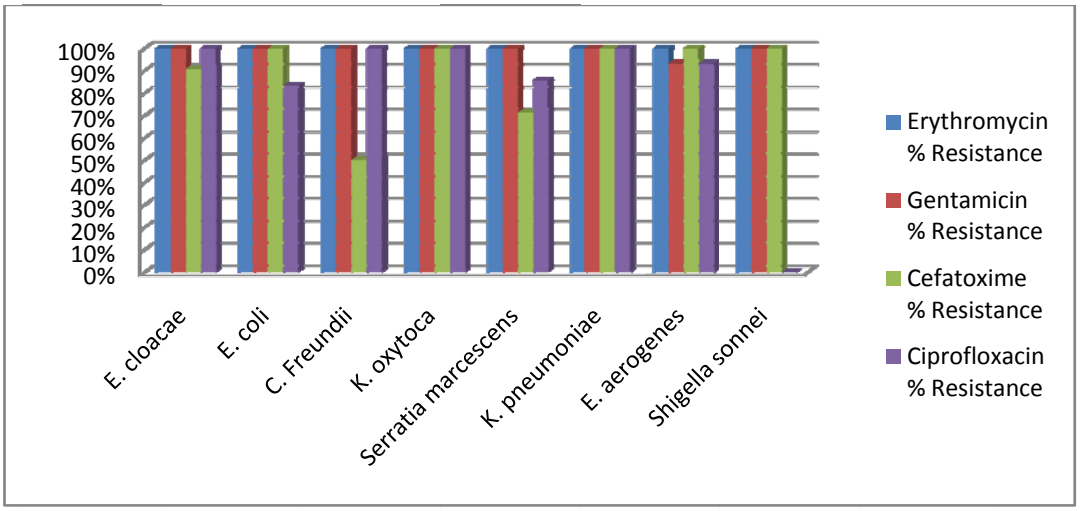

Figure 27: Percentage of Bacterial Resistance to Antibiotics

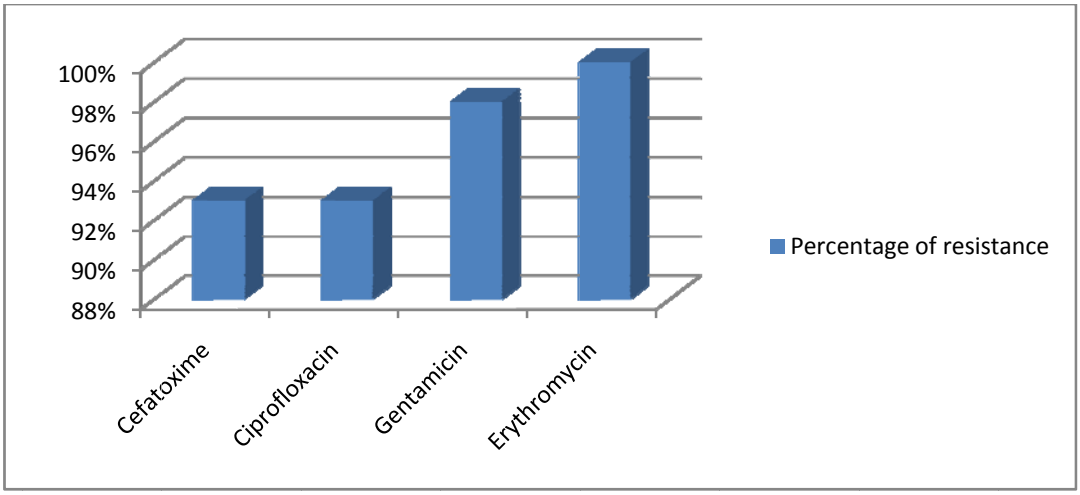

Figure 2825: Percentage of Resistance of Isolates to all the Antibiotics

4.3.1. ***E. coli***

Hundred percent of *E. coli* isolates of soil and vegetable samples were resistant to Erythromycin, Gentamicin and Cefotaxime. As for Ciprofloxacin, *E. coli* isolates from soil showed 100% resistance; whereas, isolates from vegetables showed 75% resistance with

only one isolate of E. coli from potato of site 3 showed intermediate resistances to ciprofloxacin (figure 29). Clinical isolates of *E. coli* in Lebanon were reported to be 77% and 50% resistant to Ciprofloxacin and Gentamicin, respectively (Araj et al., 2008). Further, *E. coli* isolates from dairy products in Lebanon showed just 31% resistance to Cefatoxime (Saleh et al., 2009; Kamleh et al., 2012). Therefore, an increase of resistance pattern of *E. coli* isolates was mainly observed for Ciprofloxacin and Gentamicin. In contrast to previously reported results, *E. coli* isolates from meat-based fast food (Shawarma sandwiches and meat pie) in Lebanon showed 100% and 77.8% susceptibility to Gentamicin and Cefotaxime, respectively and 88.9 % were resistant to Erythromycin (Harakeh et al., 2005). Hence this study shows also an increase in the resistance of *E. coli* from the year 2005 to the year 2012. Moreover, studies by Falomir et al. (2010) and Viswanathan et al. (2001) showed different resistance patterns of *E. coli* isolates from vegetables in Spain and India that were susceptible to Gentamicin, Cefotaxime and Erythromycin.

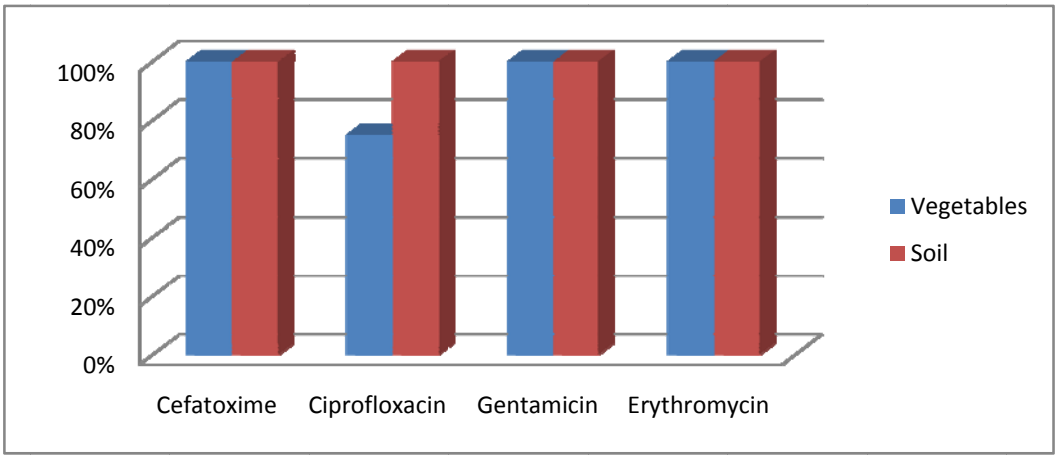

Figure 2926: Antibiotic Resistance Pattern of E. coli Isolates from Vegetable and Soil Samples

4.3.2. ***E. cloacae***

The antibiotic resistance pattern of the *E. cloacae* isolates from both soil and vegetable samples revealed 100% resistance for Erythromycin, Gentamicin and Ciprofloxacin. Regarding Cefotaxime, isolates from soil samples showed 100% resistance whereas, isolates from vegetable samples showed 86% as presented in figure 30. One isolate in lettuce from site 2 only showed intermediate resistance to Cefotaxime as presented in table 12. In addition, two isolates from control potato and potato soils were resistant to all the tested antibiotics.

This resistance pattern could be attributed to the repeated and overuse of antibiotics in Lebanon (Kamleh et al., 2012). Falmoir et al. (2010) also found that *E. cloacae* isolates from vegetables in Valencia City-Spain were resistant to Cefotaxime, and susceptible to Gentamicin and Ciprofloxacin. These results are in line with Falomir et al. (2010) where one strain of *E. cloacae* was also resistant to cefotaxime.

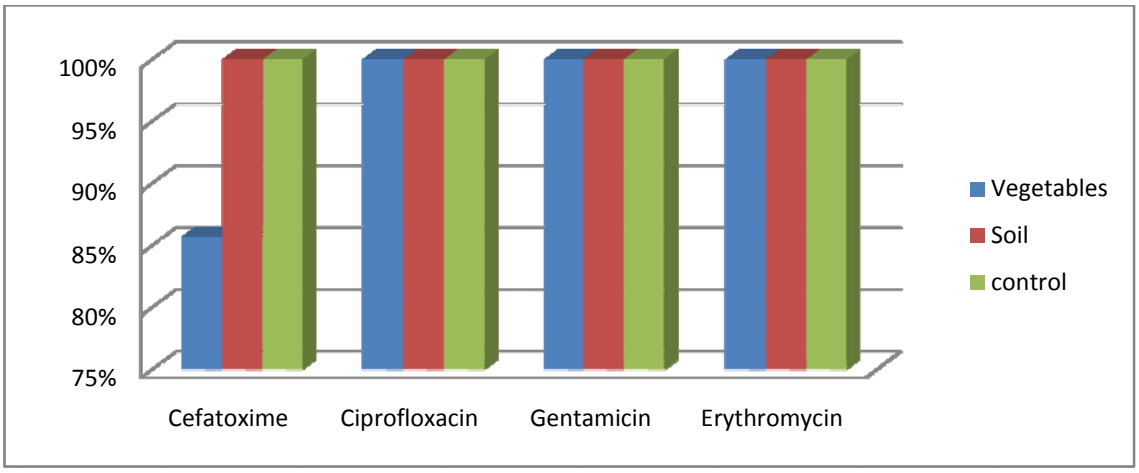

Figure 270: Antibiotic Resistance Pattern of E. cloacae Isolates from Vegetable and Soil Samples (experimental and control)

4.3.3. ***E. aerogenes***

E. aerogenes isolates from vegetables were 100% resistant to the four tested antibiotics (figure 31). Whereas, isolates from soil samples showed 100% resistance to Cefotaxime and Erythromycin, and 86% resistant to Ciprofloxacin and Gentamicin (figure 31). One isolate from lettuce soils of site 3 showed intermediate resistance to both Ciprofloxacin and Gentamicin (table 12). Moreover, *E. aerogenes* isolates from lettuce control vegetables samples were resistant to the four tested antibiotics. Results of this study are not in line with those of Falomir et al. (2010) reporting susceptibility of *E. aerogenes* isolates from raw vegetables (in Valencia City)- to Cefotaxime, Ciprofloxacin and Gentamicin. Further, Araj et al. (1994) study on clinical Enetrobacter species in Lebanon revealed 88% and 98% susceptibility to Gentamicin and Cefotaxime and resistance only to Erythromycin; this shows an increase in acquired resistance in *E. aerogenes* from year 1994 to 2012.

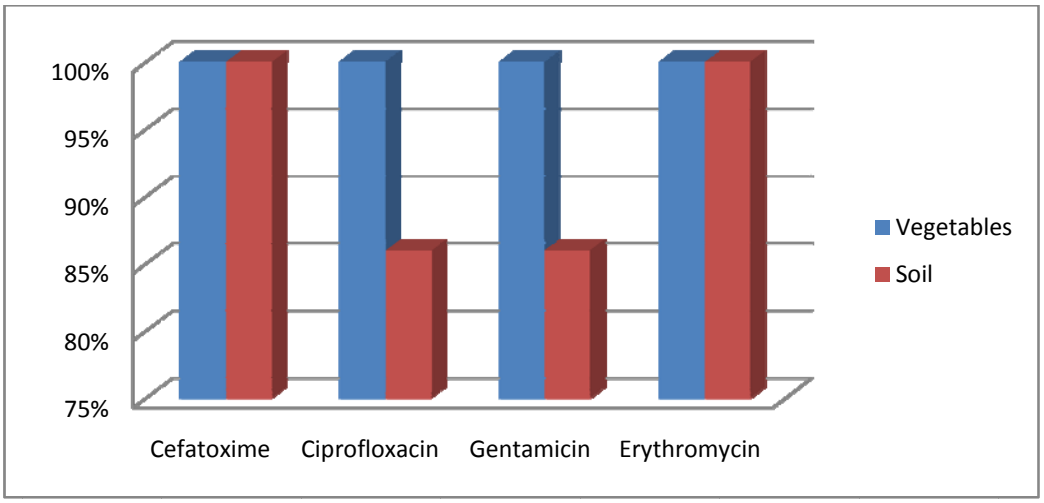

Figure 281: Antibiotic Resistance Pattern of E. aerogenes Isolates from Vegetables and Soil Samples (experimental and control samples)

4.3.4. ***C. freundii***

C. freundii isolates showed resistance to Erythromycin, Gentamicin and Ciprofloxacin and intermediate resistance to Cefotaxime (table 12). Whereas, *C. freundi* isolates form control parsley soil showed resistance to all the antibiotics except to Ciprofloxacin, showing intermediate resistance. These results are in line with those of Chen et al. (2011) reporting the resistance of clinical *C. freundi* isolates, in China, to Ciprofloxacin and Cefotaxime. Liu et al., (2007) also reported the resistant of 58% of clinical *C. freundi* isolates, in Taiwan, to Cefotaxime. On the contrary, Falomir et al. (2010) showed the susceptible of *C. freundii* isolates, from vegetables in Valencia City- Spain, to Ciprofloxacin, Gentamicin and Cefotaxime. On the contrary, Araj et al. (1994) study on clinical isolates of *Citrobacter* species in Lebanon showed that 83 % and 58% of the isolates were susceptible to Gentamicin and Cefotaxime respectively and all isolates were resistant to erythromycin; this also shows an increase in acquired resistance in *C. freundii* from year 1994 to 2012.

4.3.5. ***K. oxytoca and K. pneumoniae***

K. oxytoca and *K. pneumoniae* isolates from vegetables and soil samples showed 100% resistance to the four tested antibiotics as presented in figures 32 and 33. These results show an increase in acquired resistance among *K. pneumonia* since clinical isolates of *K. pneumoniae* in Lebanon were just 51% resistant to Gentamicin, and 46 % resistant to Ciprofloxacin (Araj et al., 2008). Moreover, the antibiotic resistance pattern of *K. pneumonia* isolates, from street foods in Malysia, showed resistance to Erythromycin (100%), Gentamicin (32%) and Ciprofloxacin (36%) (Haryani et al., 2007). Contrary, these

results are not in accordance with those reported by Falomir et al. (2010) that showed significant susceptibility of *K. oxytoca* and *K. pneumonia* isolates to Ciprofloxacin, Gentamicin and Cefotaxime.

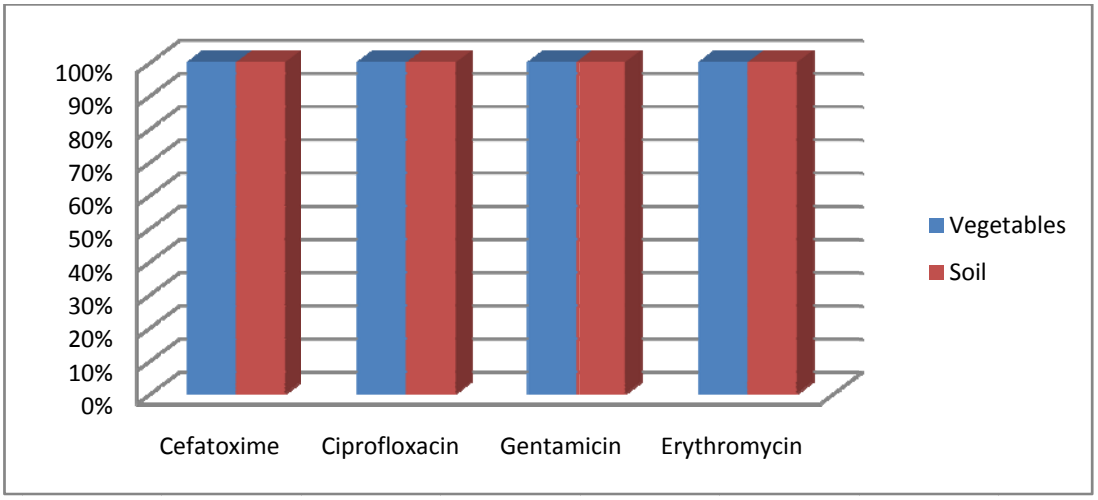

Figure 292: Antibiotic Resistant Pattern of *K. oxytoca* Isolates from Vegetables and Soils

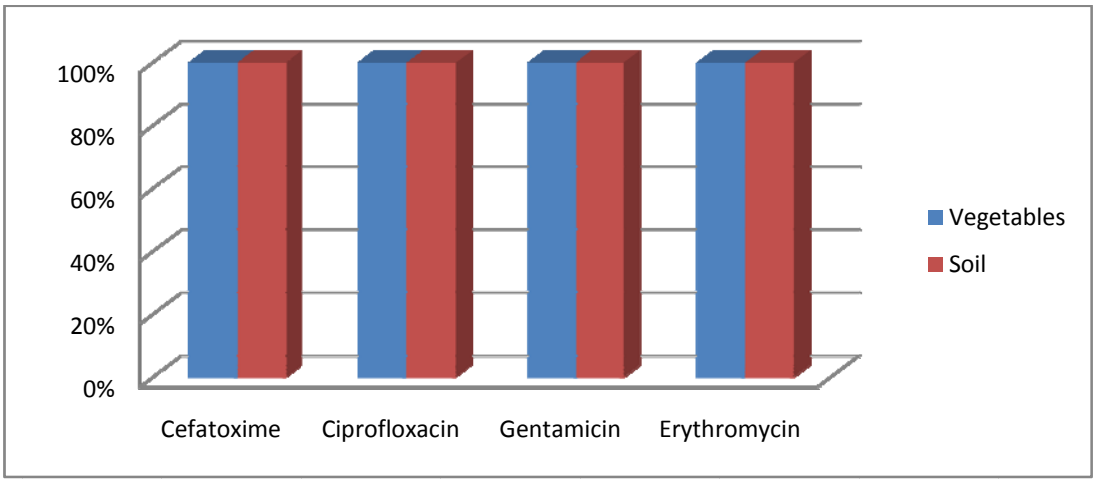

Figure 303: Antibiotic Resistance Pattern of *K. pneumoniae* Isolates from Vegetables and Soils

4.3.6. ***Serratia marcescens***

Serratia marcescens isolates from vegetable were resistant to Gentamicin, Erythromycin and Ciprofloxacin; whereas theyshowed intermediate resistance to Cefotaxime (Table 12). Regarding isolates from soil, 100 % resistance was observed to Gentamicin, Erythromycin and 83 % resistance to both Cefotaxime and Ciprofloxacin as presented in figure 34; just two isolates from parsley soils of site 1 and 2 had intermediate resistance to Cefotaxime and Ciprofloxacin (table 12). Alternatively, clinical isolates of *Serratia* spp. in Lebanon are reported to be 100% susceptible to Gentamicin and Cefotaxime and 100% resistant to Erythromycin (Araj et al., 1994). In addition, isolates of *S. marcescens* from different vegetables in Spain were susceptible to Ciprofloxacin, Gentamicin and Cefotaxime (Falomir et al., 2010). Viswanathan et al. (2001) showed that *S. marcescens* isolates from raw vegetables in India were susceptible to Ciprofloxacin and Cefotaxime. Clinical isolates of *S. marcescens* from Ghaza- Palestine showed 24% resistance to Ciprofloxacin, 72% resistant to Cefotaxime and 93% resistant to Gentamicin (Al Jarousha et al., 2008). As for isolates from control parsley and lettuce soils results showed 100% resistance to all the tested antibiotics.

The acquired resistance among isolates of control samples could be related to overuse of antibiotics in Lebanon (Kamleh et al., 2012; Harakeh et al., 2005). Further, the use of antibiotics in the animal husbandry would introduce resistant bacteria through the application of animal manure used as an organic fertilizer (Heuer et al., 2011).

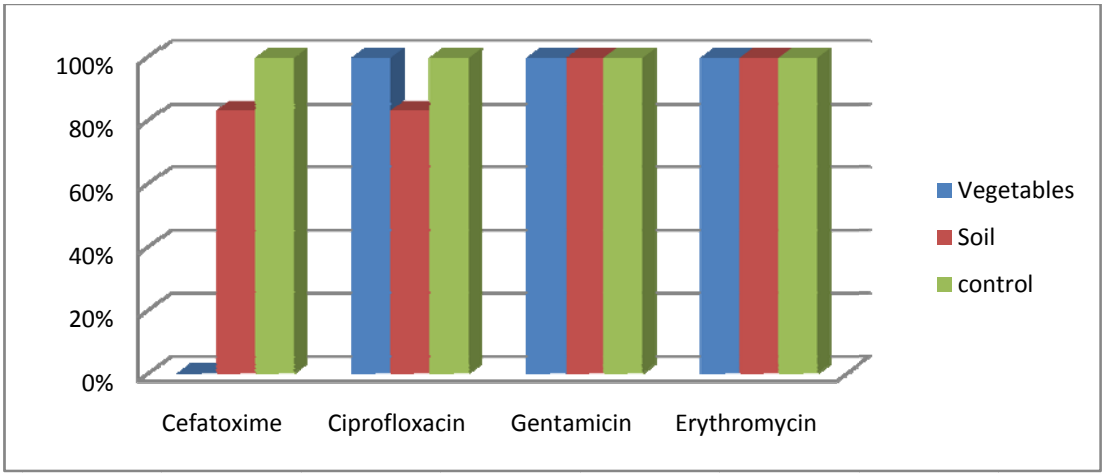

Figure 314: Antibiotic Resistance Pattern of *Serratia marcescens* Isolates from Vegetable and Soil Samples (experimental and control samples)

4.3.7. ***Shigella sonnei***

Shigella sonnei isolate was resistant to all the antibiotics (Erythromycin, Gentamicin and Cefotaxime) and showed intermediate resistance to Ciprofloxacin as presented in table 12; *shighella* clinical isolates in Lebanon have been reported as 100 % susceptible to Gentamicin and Cefotaxime and resistant to Erythromycin (Araj et al., 1994).

To conclude the antibiotic resistance pattern of all isolates (*E. cloacae, E. coli, C. freundii, K. oxytoca, S. marcescens, K. pneumoniae, E. aerogenes,* and *Sh. sonnei*) from vegetable and soil samples is associated with the irrigation with contaminated Litani river water. Most of the organisms showed comparable resistance to all antibiotics tested and isolates were not susceptible to any of them. The high percentage of antibiotic resistance, presented in this study, is mostly related to the over prescription of antibiotics in the treatment of infectious diseases in Lebanon. Moreover, the excessive and repeated use of

antibiotics in agriculture in animal husbandry and in crop production (to inhibit plant pathogens) plays a significant role in the emergence of resistant strains (Kamleh et al., 2012; Falomir et al. 2010; Levantesi et al., 2012; Harakeh et al., 2005; Warriner et al., 2009).

The presence of resistant microbial strains is alarming and poses a serious public health threat. With the emergence of antibiotic resistance pathogens, the treatment of infections becomes ineffective, since antibiotic options become limited, more expensive, less effective and more toxic with serious side effects. Further, when infections are not effectively treated, the pathogens would persist and spread to infect others (CDC, 2012; FDA, 2012, Frieden, 2010). Hence, infections caused by resistant strains are critical, especially for children, elderly and immune-compromised patients, and would lead to increase fatality (Kamleh et al., 2012; Harakeh et al., 2005). Moreover, the emergence of antibiotic resistance is a major public health concern that reflects directly on the management of the foodborne disease burden (Kamleh et al., 2012).

Table 12: Antibiotic Resistance Pattern of Different Isolates of Vegetables and Soil Samples from ULB

Sample	Veg/Soil	Sample type	Bacteria	Cefatoxime	Ciprofloxacin	Gentamicin	Erythromycin
S1	Veg	Potato 2	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Potato	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Parsley	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S2	veg	Parsley	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S2	Veg	Lettuce 2	*E. cloacae*	Intermediate	Resistant	Resistant	Resistant
S3	Veg	Potato 2	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S3	Veg	Lettuce 2	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S1	soil	Potato 2	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Potato	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S2	Soil	Potato 2	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S3	soil	Lettuce	*E. cloacae*	Resistant	Resistant	Resistant	Resistant
S1	veg	Lettuce	*E. coli*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Potato	*E. coli*	Resistant	Resistant	Resistant	Resistant
S3	veg	Potato	*E. coli*	Resistant	Intermdiate	Resistant	Resistant
S3	veg	Lettuce	*E. coli*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Parlsey	*E. coli*	Resistant	Resistant	Resistant	Resistant
S3	soil	Parsley	*E. coli*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Parsley 2	*C. Freundii*	Intermediate	Resistant	Resistant	Resistant
S1	Veg	Parsley 2	*C. Freundii*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Lettuce	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S2	veg	Parsley 2	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S3	Veg	Lettuce	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S2	Soil	Lettuce	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S3	Soil	Parsley 2	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S3	Soil	Lettuce	*K. oxytoca*	Resistant	Resistant	Resistant	Resistant
S3	Veg	Potato	*S. marcescens*	Intermediate	Resistant	Resistant	Resistant
S1	soil	Lettuce 2	*S. marcescens*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Parsley	*S. marcescens*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Potato	*S. marcescens*	Resistant	Resistant	Resistant	Resistant
S1	soil	Parsley 2	*S. marcescens*	Intermediate	Resistant	Resistant	Resistant
S2	Soil	Parsley 2	*S. marcescens*	Resistant	Intermdiate	Resistant	Resistant
S2	Soil	Lettuce	*S. marcescens*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Lettuce	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S2	veg	Parsley	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S1	soil	Potato	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S1	soil	Potato 2	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Parsley	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S1	soil	Lettuce 2	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S2	soil	Lettuce 2	*K. pneumoniae*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Potato 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Lettuce 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S1	Veg	Parsley	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S1	veg	Parsley 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S1	veg	Lettuce	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S2	Veg	Lettuce	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S3	Veg	Lettuce 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S1	Soil	Lettuce	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S2	Soil	Lettuce 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S2	soil	Parsley 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S3	soil	Lettuce	*E. aerogenes*	Resistant	Intermediate	Intermediate	Resistant
S3	Soil	Potato	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S3	Soil	Potato 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S3	Soil	Lettuce 2	*E. aerogenes*	Resistant	Resistant	Resistant	Resistant
S3	Soil	Lettuce	*Sh. sonnei*	Resistant	Intermediate	Resistant	Resistant

4.4. **Chemical Analysis**

4.4.1. ***Soils characteristics***

The use of polluted water and wastewater effluents for irrigation contributes to the introduction of toxic chemicals in soil and plants; long term irrigation with polluted waters would lead to the accumulation of heavy metals in soils (Liu et al., 2005; Mapanda et al., 2005 and Khan et al., 2008). The soil acts as a filter medium that can adsorb and retain heavy metals. Still, when the soil becomes saturated, or when its capacity is reduced due to the continuous exposure, as indicated before, or to changes in pH, it would no longer retain the heavy metals load leading to its uptake by the plants.

Moreover, the mobilization of heavy metals such as Mo, As, Cd, Cu, Ni, Pb and Zn in soils is impacted by the soil organic content, clay content, pH, total metal content and cation exchange properties (Mapanda et al., 2005). When the pH level of the soil decreases, the heavy metals become more mobilized and readily available for plant uptake. The only exceptions to this pattern are the elements of molybdenum and arsenic that become more mobile with the increase in soil pH (WHO, 2006; Mapanda et al., 2005).

In this study, the pH of the soils of the three experimental sites that are irrigated by polluted Litani river water ranged between 7.6 and 8.52 with a mean level of 8.14 ± 0.25. The pH values for sites 1, 2 and 3 were 8.07 ± 0.24, 8.34 ± 0.13 and 8.01± 0.24, respectively as presented in table 13. These reported values are in accordance with the results obtained by Jurdi et al. in 2010 for the ULB soil samples. The ULB study also reported a mean dry season pH levels of 7.93 for Litani river water and 7.77 for ground water wells; which reflected on high exposure to sewage that shifts the pH towards

alkalinity (Jurdi et.al 2010). Given that the soil pH values are highly influenced by the pH of the water used for irrigation this would lead to a shift in soil pH towards alkalinity (Khan et al., 2008; Al-Lahham et al., 2007).

As for the pH of the control soil samples, they ranged from 7.69 to 8.19 with a mean level of 7.98 which is less by 0.16 units from the mean of the soil samples irrigated by Litani River. The slight difference between the control sample and soil samples irrigated by Litani River could be related to the use of ground water (mean pH of 7.77) for irrigation in comparison to the highly polluted Litani river water (mean pH of 7.93).

The results of this study are coherent with the results of Khan et al. (2008) study, showing that the pH of the soil irrigated with wastewater ranged between 7.8 and 8.2. However, Mapanda et al. (2007) reported soil pH values varying between 5.6 and 8.2 for agricultural areas irrigated by the contaminated Mukuvisi River Zimbabwe (industrial and sewage discharge). The same study also showed that irrigation with Mukuvisi River water increased the pH of the soil by 0.5-3 units when compared to control samples that were not irrigated by river water. On the other hand, the use of treated wastewater effluents slightly decreased pH values by 0.39; however, the pH of the soil remained alkaline (8.05) (Jun-feng et al., 2007).

Table 13: Reported Soil pH for Experimental and Control Sites

Experimental Site*	N	Mean	Std. Deviation	Minimum	Maximum
S1	6	8.07	0.24	7.72	8.37
S2	6	8.3383	0.13	8.15	8.52
S3	6	8.0083	0.24	7.6	8.32
Average for Experimental Sites	18	8.1389	0.25	7.6	8.52
Control Site	6	7.98	0.17	7.69	8.19

*Site 1: Bar Elias; Site 2: Dalhamieh; Site 3: Zahle

The soil total dissolved solids ranged between 157.44 mg/l and 245.12 mg/l with a mean level of 194.06 ± 23.37 mg/l which is lower than that of the control samples ranging between 112.6 and 311.4 mg/l with a mean level of 208.96 ± 79.94 mg/l. The total dissolved solid content of site 1 was slightly lower than that of site 2 and site 3 as presented in table 14. These results show that the soil is not saline based on FAO classifications (FAO, 1985). Further, the mean TDS levels of the surface water of the ULB is 502 mg/l in comparison to the high TDS levels of the ULB industrial and domestic wastewater effluents (1248.43 and 762.67 mg/l, respectively) which is considerably higher than the levels in analyzed soil samples. Moreover, the use of Litani river water is not restrictive to irrigation based on the salinity of this source.

The TDS levels of the soil samples of the ULB are much lower than those reported by Singh et al. (2012) with reported TDS levels of 352-307 mg/l and 1094.4 to 1072.64 mg/l. The difference in soil TDS values in these reported studies and the current study is

attributed to the direct use of wastewater effluents with elevated TDS values as a source of irrigation water in comparison to the use of Litani river water with the relatively lower TDS levels (mean of 502 mg/l).

Table 14: Reported Soil TDS (mg/l) for Experimental Sites

Site	N	Mean	Std. Deviation	Minimum	Maximum
site 1	6	186.453	12.03245	167.68	198.4
site 2	6	197.227	25.57252	167.68	235.52
site 3	6	198.507	30.88069	157.44	245.12
Total	18	194.062	23.37385	157.44	245.12

4.4.2. ***Heavy Metals Profile in Irrigation Water, Soil and Crops***

The mean levels of heavy metals in the ULB surface water were reported at 0.27313 mg/L for barium, 0.00994 mg/l for cadmium, 0.00237 mg/l for molybdenum, 0.00118 mg/l for chromium, 0.00307 mg/l for nickel, 0.00083 mg/l for copper, 0.00007 mg/l for manganese, 0.00016 mg/l for iron, and 0.02222 mg/l for zinc. Lead and arsenic were not detected samples as presented in table 15 (Jurdi et al., 2010). These levels of trace metals reflected on various point sources of pollution ranging from domestic wastewater to industrial wastewater, leachate of solid waste dumps and non-point sources such as agricultural runoff. Based on FAO standards for irrigation water, the only element approaching the permissible limit of 0.1 mg/l is cadmium. Still, although the concentrations of the other indicated metals are still within the permissible limits they can still pose a major risk by accumulating in soils and grown crops.

The accumulation of metals in soils would lead to elevated heavy metals' uptake by plants thus affecting the quality and safety of the grown crops (Khan et al., 2008; Rattan et al., 2005). Further, since sprinkler irrigation is the method used heavy metals are also introduced directly from water to plants (FAO 1992; FAO, 1994).

Table 15: Physical and Chemical Characteristics of ULB Surface Water

physical and chemical characteristics of ULB surface water	pH	TDS mg/L	Ba µg/L	Cd µg/L	Mo µg/L	As µg/L	Pb µg/L	Cr µg/L	Ni µg/L	Cu µg/L	Mn µg/L	Zn µg/L	Fe mg/L
mean	7.930	502.1	0.273	0.010	0.002	ND	ND	0.001	0.003	0.001	0.000072	0.022	0.160
SD	0.370	429.8	0.108	0.020	0.001	-	-	0.002	0.003	0.000	0.000064	0.012	0.250
Min	7.270	187.0	0.031	0.001	0.002	-	-	0.000	0.000	0.000	0.000009	0.001	0.010
Max	8.660	1979.0	0.388	0.070	0.004	-	-	0.005	0.009	0.002	0.000272	0.044	1.150
FAO irrigation water standards	6.5-8.4			0.010	0.010	0.100	5.000	0.100	0.200	0.200	0.200	2.000	5.000

4.4.2.1. Barium

The detected barium levels in lettuce, parsley and potato soils were 352.5 ± 24.37 mg/kg, 296.96 ± 87.51 mg/kg, and 286.94 ± 119.05 mg/kg, respectively (Table 16). Among the lettuce soil samples, the highest concentration was observed at site 3 (377.5 ± 11 mg/kg) followed by site 2 (342.428 ± 9.7 mg/kg) and then by site 1 (337.7 ± 29.4 mg/kg). In all lettuce soil samples, barium levels were above the maximum permissible concentration in soil (WHO, 2002). Whereas, in parsley soils, the highest levels of barium were reported at site 3 with a concentration of 393.3 ± 11.7 mg/kg; however, lower values were detected in the other sites with levels of 296.34 ± 4mg/kg and 201.2 ± 35.2 mg/kg,

respectively. Soil samples of site 3 only exceeded the WHO Guidelines. On the other hand, the highest barium concentrations were observed in potato's soil at sites 3 and 2 with concentrations of 376.3 ± 32.1 mg/kg and 339.62 ± 49.8 mg/kg, respectively; and both levels exceed the WHO Guideline level (WHO, 2002). The lowest value was at site 1 with a level of 145 ± 74.1 mg/kg and not exceeding the WHO Guideline.

As for the control soil samples barium levels were as following: 499.4895 ± 3.89 mg/kg for lettuce soils, 285.37775 ± 12.8 mg/kg for parsley soils and 481.52025 ± 71.7 mg/kg for potato soils; barium levels of lettuce and potato soils exceeded the WHO guideline as presented in table 16. The detection of high barium levels in the control samples could be related to a previous contamination of soil that led to barium accumulation. Moreover, the results of this study reflect on the detection of barium in the ULB sediments and soils (Jurdi et al., 2010).

For vegetable samples, the detected barium levels were 10.03 ± 20.38 mg/kg in lettuce, 10.61 ± 7.2 mg/kg in parsley, and 1.45 ± 0.6 in potato samples as presented in table 17. Among the lettuce samples, the highest barium concentration was detected in one lettuce sample at site 1 at site 1 with a concentration of 51.65 mg/kg contributing to a mean level of 26.7 ± 35.2 mg/kg, followed by site 2 with a mean concentration of 2.07 ± 0.18 mg/kg and a mean concentration of 1.29 ± 0.04 at site 3; barium concentration in control lettuce samples was just 1.40 ± 0.17 mg/kg. As for the parsley vegetable samples the highest barium concentration was at site 2 with a mean level of 18.91 ± 0.07 mg/kg, followed by site 1 and 3 with concentrations of 8.8 ± 5.8 mg/kg and 4.127 ± 0.25 mg/kg, respectively. And, barium concentrations in potato's samples were lower than those of

lettuce and parsley samples. Levels of barium in potato samples were 1.873 ± 1.22 mg/kg, 1.3 ± 0.2 mg/kg and 1.159 ± 0.15 mg/kg, in site 3, 1 and 2 respectively.

Barium concentrations in parsley and lettuce samples were comparable with a mean value of 10.6 ± 7.2 mg/kg and 10.03 ± 20.38 mg/kg respectively; however, the mean concentration in potato samples was 1.45 ± 0.64 mg/kg. In the control samples, the mean levels of barium were 2.275 ± 0.21 mg/kg for parsley, 1.057 ± 0.02 mg/kg for potato and 1.403 ± 0.17 mg/kg for lettuce (table 17). Moreover, the concentrations of barium in vegetables irrigated with the Litani river water were higher than those of the control samples, indicating that the use of the Litani River for irrigation is a main source of pollution.

The main sources of Barium are mainly industrial sources relating to cement, cosmetic and pharmaceutical products, glass, glazes and paper making. Jurdi et al. (2010) reported the detection of barium in the Litani river water, as indicated before, at an average concentration of 0.273 mg/L; whereas the mean levels in industrial wastewater effluents was much higher (0.916 mg/l) and the levels in domestic wastewater effluents was much lower (0.00317mg/l). Barium was also detected in the irrigation canal soils and ULB soils (Jurdi et al., 2010) (table 18). So mostly barium is attributed by industrial sources of pollution feeding into the water flow, especially as a significant number of cement, and paper industries were identified in the river basin (Jurdi et al., 2010). There is no reported standard for barium in vegetables/food. However the ATSDR and EPA derived an oral minimal risk level for barium of 0.2 mg/kg/day as it is associated with cardiovascular diseases (EPA, 2005).

4.4.2.2. Cadmium

The levels of cadmium detected in all soils are higher than those recommended by EC and WHO guidelines. Among the lettuce soil samples, site 3 samples showed the highest concentration of cadmium (14.3 ± 3.4 mg/kg) followed by site 2 samples (13.4 mg/kg) and site 1 samples (13.1 ± 5 mg/kg). And the cadmium mean level in the control lettuce soil sample is 12.317mg/kg. The same trend was observed for parsley soil samples, where site 3 showed the highest concentration of cadmium (13.4 ± 2.2 mg/kg), followed by site 2 (11.409 mg/kg) and site 1(10.5 ± 2.7 mg/kg). Potato's soil samples showed high concentration of cadmium at site 2 (14.5 ± 2.7 mg/kg), followed by site 3 (11.2 ± 1.7 mg/kg) and lowest concentration was observed at site 1 (10.5 mg/kg); while the mean cadmium level in potato soil was 12.38 ± 2.52 mg/kg. The cadmium concentration in control parsley, potato and lettuce soil was 10.61 mg/kg, 12.99mg/kg and 12.32 mg/kg respectively. It is noticed that only one sample of each control lettuce, parsley and soil showed also detectable cadmium levels.

These reported results are in accordance with the detection of cadmium in soils of the ULB (Jurdi et al., 2010). However, cadmium levels reported for ULB soil samples are much higher than levels reported by other studies conducted in Beijing, and the Beijing-Tianjin city cluster in China, where average concentrations of 0.18 mg/kg and 0.46 mg/kg were reported (Wang et al., 2012; Liu et al., 2005). Further the results of this study were also higher than those found by in soils irrigated with the contaminated Mukuvisi River in Zimbabwe (Mapanda et al., 2005).

The mean levels of cadmium detected in potato, lettuce and parsley samples were 0.083 mg/kg, 0.043 ± 0.048 mg/kg and 0.022 ± 0.027 mg/kg, respectively and cadmium was not detected in the control samples as presented in table 17. Furthermore, among the potato samples, cadmium was only detected in one sample at site 2 with a concentration of 0.083 mg/kg. As for the lettuce samples, the cadmium level was highest at site 2 with a concentration of 0.068 ± 0.068 mg/kg (one lettuce sample had 0.11665 mg/kg of cadmium), and 0.019 ± 0.003 mg/kg at site 3; but cadmium was not detected at site 1. Parsley's samples showed concentrations of 0.0416 mg/kg and 0.0028 mg/kg at site 1 and 3, respectively; whereas cadmium was not detected in any of the samples of site 2. Therefore, the mean levels of cadmium in vegetables samples are as follows: 0.08335mg/kg in potato, 0.043± 0.048 mg/kg in lettuce and 0.022± 0.027mg/kg in parsley. These results are in accordance with the results reported by Wang et al. (2012) on cadmium levels detected in leafy vegetables irrigated with wastewater. However, cadmium results of this study are lower than those reported by (Liu et al., (2005) in Beijing, China that ranged from 0.03-0.7 mg/kg. Moreover, the results of our study are lower than those reported in vegetables (lettuce, spinach, and radish) irrigated by wastewater of 0.39 mg/kg to 0.93 mg/kg (Khan et al., 2008).

The lower levels reported for cadmium in the studied vegetables compared to the results reported by other studies could be justified by the initial higher cadmium concentrations in wastewater or due to different soil properties (Liu et al., 2005; Khan et al., 2008). All the cadmium levels in parsley and lettuce were below the recommended international standards (EC and joint FAO/WHO) for leafy vegetables. In addition, cadmium in potato samples was only detected in one sample at site 2 with a concentration

of 0.083 mg/kg which is also lower than the EC and FAO/WHO recommended standard of 0.1 mg/kg potato.

The presence of cadmium in soil and water of the ULB was reported by Jurdi et al. in 2010 (table 18). And, cadmium levels were associated with point sources like plastic industries, leachates of solid waste dumps and agricultural runoff (excessive use of fertilizers and pesticides). Cadmium detection in leafy vegetables is of major concern as it can accumulate in high levels in the leaves posing a major health risk. Food containing high levels of cadmium cause vomiting, diarrhea, lung irritation and damage; and chronic exposure leads to kidney, bone and liver damage and cancer (ATSDR, 2008). Further, cadmium has several toxicity effects on plants ranging from injuries in the plant, growth inhibition, root tips browning and death. This is mostly due to the interference of cadmium with the uptake of other essential plant elements such as potassium, calcium, magnesium, and phosphorous. Cadmium also interferes in the absorption and transport of nitrate by plants (Nagajyoti et al., 2010).

4.4.2.3. Arsenic

The concentration of Arsenic in potato soil samples of site 3 was 14.7 ± 0.8 mg/kg. Site 2 and site 1 showed lower concentrations of 12.2 ± 0.3 mg/kg and 7.7 ± 0.4 mg/kg, respectively (Table 16). Arsenic concentrations in lettuce soil samples were highest at site 1 and 2 with concentration of 13.9 ± 0.9 mg/kg and 14 ± 0.9 mg/kg and lowest at site 3 (11.6 ± 0.5 mg/kg). Parsley soil samples showed highest arsenic concentration at site 3 (14 ± 0.3 mg/kg) and lowest concentrations at sites 1 and 2 (11.0 ± 1.6 mg/kg). Moreover, arsenic levels in control samples were higher than that of the soil samples irrigated with the Litani

river water where arsenic levels were 20.3115 ± 1.5 mg/kg in lettuce soils, 24.206 ± 1.4 mg/kg in parsley soils and 22.7975 ± 0.6 mg/kg in potato soils.

The high levels of arsenic identified in soil control samples could be attributed to the accumulation of arsenic due to contamination whether from contaminated irrigation water or from excessive use of pesticides. Moreover, the levels of arsenic in all soil samples were above the WHO standards (WHO, 2002). In addition, they were much higher than those reported in the Beijing- Tianjin city cluster of China (4.7-9.8 mg/kg) (Wang et al., 2012).

Moreover, the arsenic concentrations of all vegetable samples were above the FAO/WHO levels and below the EC standards. Arsenic levels in lettuce samples were 0.482 ± 0.05mg/kg in site 2, 0.332 ± 0.04 mg/kg in site 3 and 0.1553 mg/kg in site 1; while arsenic was only detected in one control lettuce sample (0.0033 mg/kg) (table 17). As for the parsley's sample, arsenic concentrations were 0.5939 ± 0.05 mg/kg in site 1 and 0.522 ± 0.01 mg/kg in site 2. The lowest concentration was observed at site 3 (0.370 ± 0.05 mg/kg); whereas arsenic was only detected in one parsley with a concentration of 0.0027mg/kg (table 17). In potato samples arsenic levels at sites 1, 2 and 3 were 0.450 ± 0.05 mg/kg, 0.367 ± 0.005 mg/kg, 0.323 ± 0.01 mg/kg, respectively; however, arsenic was not detected in any of the potato control samples (table 17). Therefore, mean arsenic concentrations in vegetables irrigated by Litani River were 0.495± 0.1mg/kg in parsley, 0.4 ± 0.07 mg/kg in potato and 0.35 ± 0.14 mg/kg in lettuce**.** The results of arsenic in vegetables are consistent with the results reported by Wang et al. (2012). Also, Jurdi et al. (2010) reported that the Litani river sediments, agriculture soils and irrigation Canal 900 soils were contaminated

with arsenic (Table 18); the source of arsenic was mostly attributed to agricultural runoff due to the excessive application of pesticides (Jurdi et al., 2010).

At high concentration, arsenic leads to phytotoxicity by interfering in the plant metabolism, inhibiting its growth and development (Rahman et al., 2007). Arsenic levels in lettuce, parsley and potato samples irrigated by the Litani River poses a major public health concern because levels exceed international standards. It is to be noted that exposure to arsenic is highly toxic causing vomiting, nausea, diarrhea, and dermal hyperkeratinization, hyperpigmentation of the skin, respiratory malfunction, cardiovascular problems, cyanosis, and circulatory and high blood pressure problems. And, chronic exposure in the diet would lead to preterm births, stillbirth, miscarriages, and skin and internal tumors (skin, lung, bladder, liver and kidney cancer) (ATSDR, 2007).

4.4.2.4. Lead

Lead was not detected in soil samples of site 3 as well as in lettuce soil samples. It was only detected in potato soil of site 1 and site 2 with a concentration of 8.8 ± 0.4 mg/kg and 9 ± 0.8 mg/kg respectively. Also, it was only detected in parsley soil of site 2 with a concentration of 4.4 ± 6.2 mg/kg; lower than that of potato soil samples (Table 15). Lead concentrations in all soil samples were lower than those recommended by international standards (EC and Canadian standards and WHO guidelines). However, lead was detected in high levels in potato and lettuce control samples with an average concentration of 42.54 ± 21.98 mg/kg and 10.87mg/kg respectively but was not detected in parsley control samples. The presence of lead at high levels in the control soil could be attributed to the

accumulation of lead due to soil contamination from irrigation water (ground water) or excessive use of pesticides.

The results of lead in this study are in accordance with Jurdi et al. (2010) study that reported accumulated levels in agricultural soil irrigated with the Litani river water. Also irrigation with wastewater is considered as an important source of soil contamination as reported by Mapanda et al. (2007), Wang et al. (2005). Khan et al. (2008) and Wang et al. (2012).

Fortunately, lead values in vegetable samples were below the EC and FAO/WHO standards. The highest lead concentration was observed at site 1 in one lettuce sample (0.07 mg/kg) followed by potato samples (0.05445 mg/kg) as presented in table 17. Lead was not detected in any of the lettuce and parsley's samples of site 3; whereas, it was only detected in two parsley samples of site 1 and site 2 with a concentration of 0.0069 mg/kg and 0.0077 mg/kg, respectively. Lead levels were also found in two lettuce samples only of site 1 (0.07 mg/kg) and site 2 (0.03225mg/kg). Also, lead was detected in three potato samples among all the sites (1, 2, and 3) with concentrations of 0.05445 mg/kg, 0.01 mg/kg and 0.0098mg/kg, respectively.

As such, lead concentration in vegetables could be arranged in the following order Lettuce (0.051125 ± 0.026 mg/kg) > potato (0.02475 ± 0.025 mg/kg) > parsley (0.0073 ± 0.0005 mg/kg)**.** As for the control samples, lead was detected in all vegetable samples with average concentrations of 0.055 ± 0.03 mg/kg in parsley, 0.0277mg/kg in one lettuce sample and 0.021 ± 0.01 mg/kg in potato. The presence of lead in control vegetable samples could be related to the accumulation of lead in the soil of potato and lettuce control samples; whereas lead detection in parsley's vegetable only and not in the soil might be

attributed to fresh contamination due to the contaminated irrigation ground water and/or the use of pesticides.

Still, lead concentrations reported in this study were lower than those detected in vegetables irrigated with wastewater (Mapanda et al., 2007; Wang et al, 2012; Khan et al., 2008; Liu et al. ,2005). The detection of elevated lead levels in these studies compared to the current study Litani River could be attributed to the high deposition of lead from vehicles and industrial fumes in China and India compared to Bekaa area of Lebanon and to the capability of leafy vegetables to accumulate air-borne lead in the foliar surface of the leaves (Mapanda et al., 2007; Wang et al., 2012).

It is to be noted that Jurdi et al. (2010) also reported the presence of lead in irrigation canal soils and agricultural soils however, it was not present in significant concentrations (Table 18). The possible sources of lead along the Litani river basin are mostly industrial sources such as plastic industries, and agricultural runoff (excessive, haphazard use of pesticides). The oral exposure to lead would cause vision and hearing impairment, increase blood pressure, reproductive problems (low sperm count), anemia, peripheral neuropathy, nephrotoxicity, and cerebrovascular diseases (ATSDR, 2007). Moreover, lead has several effects on plants; it inhibits the enzymes' activities, seed germination, leaf expansion, and root elongation. At high levels, lead causes plant abnormal morphology and induces reduction in plant's growth, mainly in lettuce (Nagajyoti et al., 2010).

4.4.2.5. Chromium

The average concentration of chromium was 239.591 ± 15 mg/kg in lettuce soil samples, 233.35 ± 26.21 mg/kg in parsley's soil samples, and 207.80 ± 66.2 mg/kg in potato soil samples (Table 16). In lettuce soils samples chromium levels ranged between 222.4 ± 9.2 mg/kg at site 3, 243.3 ± 7 mg/kg at site 1 and 253.09 ± 2.8 mg/kg at site 2. The same profile of chromium levels was observed in parsley's soil samples ranging from 225.9 ± 35.6 mg/kg at site 3 to 232.683 ± 6.4 mg/kg at site 2 and 241.5 ± 43.4 mg/kg at site 1. Potato's soil showed the lowest chromium concentrations compared to lettuce and parsley; chromium concentrations in potato's soils were 127 ± 39 mg/kg, 234.2 ± 6 mg/kg, and 262.2 ± 4.7 mg/kg in site 1, 2 and 3, respectively. Chromium was also detected in high levels in control samples with a concentration of 490.52 ± 25.2 mg/kg in lettuce, 285.37 ± 12.8 mg/kg in parsley and 424.28 ± 11.9 mg/kg in potato; this could be attributed to accumulation of chromium in the soil due to contamination from irrigation water (ground water) and/or contaminated manure or compost. All the chromium levels were above the EC standards except for potato samples of site 1.

The results of this study are concurrent with the detection of chromium in agricultural soils irrigated by the Litani River (with an average concentration of 142.91 ± 57mg/kg) (Jurdi et.al 2010). However, reported levels are higher than the levels reported for soils irrigated with wastewater in China, (Wang et al., 2012; Liu et al., 2005; and Khan et al. 2008) and in Zimbabwe (Mapanda et al., 2007).This may be due to progressive irrigation with polluted water leading to the accumulation of chromium in the agricultural soil.

Chromium concentrations in vegetable samples were respectively high in potato samples of site 1 (1.9 ± 0.2 mg/kg) and low at site 3 mainly in parsley's samples (0.885 ± 0.03 mg/kg) (table 16). Chromium concentration did not considerably vary in lettuce samples among the sites; where it was 1.125 ± 0.32 mg/kg, 1.440 ± 0.01 mg/kg and 1.574 mg/kg at site 1, 2 and 3, respectively. However, among parsley's samples, site 1 and 2 showed higher concentrations of 1.6 ± 0.1 mg/kg and 1.670 ± 0.19 mg/kg, respectively, than in site 3 which had a concentration of 0.885 ± 0.03 mg/kg. Concerning the potato samples, chromium concentrations were 1.9 ± 0.2 mg/kg, 1.5431 ± 0.262 mg/kg and 1.081 ± 0.31 mg/kg for sites 1, 2 and 3 respectively. Therefore, chromium concentrations in vegetables was highest in potato (1.55 ± 0.4 mg/kg) followed by parsley (1.39 ± 0.4) and then lettuce (1.37 ± 0.253); with lowest chromium concentrations was observed at site 3 among all the vegetable samples. Moreover, chromium was detected in all control vegetable samples with a mean average of 1.717 ± 0.3 mg/kg in parsley, 2.223 ± 0.38 mg/kg in lettuce and 1.282 ± 0.64 mg/kg in potato (table 17); these results could be associated with plant uptake from the soil, since chromium was detected in high levels in soil samples. Moreover, the presence of chromium in control samples could be due to atmospheric deposition of chromium from several industries (such as cement, plastic, stainless steel and alloy) along the ULB and to a lesser extent from road dust (catalytic converter emissions) (ATSDR, 2008; Wang et al., 2012).

The EC standards stated that chromium is not recommended to be present in vegetables; whereas a maximum permitted level (2.3 mg/kg) was set by the joint committee of the FAO/WHO. As such, the levels are not acceptable according to EC standards; however, they are lower than the FAO/WHO standards. These results are concurrent to

results reported by Wang et al. (2012) of chromium in vegetables irrigated with wastewater. However, Khan et al. (2008) and Liu et al. (2005) reported higher levels of chromium in vegetables irrigated with wastewater; this could be explained by the initial high concentration of chromium in the wastewater or different soil properties (Khan et al., 2005). Other justification could be due to the air-borne deposition of chromium on leafy vegetables from industrial sources or road dust (ATSDR, 2008; Wang et al., 2012) .

The possible sources of chromium along the ULB could be associated with cement, plastic, rubber, stainless steel, and alloy industries (Jurdi et al., 2010). Due to the contamination of ULB by these industrial activities, chromium was detected in surface water and sediments of the ULB and in the irrigation canal soil (Table 18) (Jurdi et al., 2010). Therefore, chromium is transported to agricultural areas contaminating plants and agricultural soils. The presence of chromium in plants and soil has several toxic effects on plants as it can inhibit the seed germination and decrease the root growth (Nagajyoti et al., 2010).

Moreover, several health effects are associated with exposure of chromium by the oral route like diarrhea, vomiting, dizziness, ulcer and irritation in the stomach, hemorrhagic diathesis, and convulsions. Furthermore, the ingestion of significant levels of chromium causes liver and kidney damage; however, chronic exposure to chromium would lead to lung cancer (ATSDR, 2008).

4.4.2.6. Zinc

The mean values of zinc in lettuce soil samples for sites 2, 3 and site 1 were 72.2 ± 1.8 mg/kg, 69.5 ± 3.8 mg/kg and 67.1 ± 0.3 mg/kg, respectively as presented in Table 16.

And, levels are comparable to Jurdi et al. (2010) reported concentrations in irrigation canal 900 (136.41 mg/kg) and agricultural soils (94.91 mg/kg) (Table 18). Moreover, the average zinc concentrations in parsley soils were 66.6 ± 2.2 mg/kg at site 1, 68.2 ± 6.5 mg.kg at site 3 and 75.1 ± 2.7 mg/kg being the highest at site 2. In potato soils high levels were observed at site 3 (88.6 ± 7.1 mg/kg) and low levels at site 1 (50.2 ± 6.6 mg/kg); where the zinc concentration was 77.2 ± 2.3 mg/kg at site 2 (table 16). However, soil control samples showed elevated levels of zinc in lettuce, parsley and potatoes with average concentrations of 145.7835 ± 1.5 mg/kg, 84.98025 ± 1.4 mg/kg and 128.323 ± 1.3 mg/kg, respectively. High levels of zinc in the soil control samples could be attributed to a high accumulation of zinc due to the use of contaminated irrigation water (ground water) and /or excessive use of pesticides (Perfect Life Institute, 2002).

All the detected zinc levels in soil samples were below standards. These results are also in line with other reported studies by Liu et al. (2005), Mapanada et al. (2005) and Wang et al. (2012) on the levels of zinc in soils irrigated with wastewater (40-100 mg/kg, 26-190 mg/kg and 58-191 mg/kg, respectively). Conversely, Rattan et al. (2005) reported lower levels of zinc in soils irrigated with wastewater 0.67- 36.9 mg/kg. However, in Khan et al. (2008) study, zinc concentrations in wastewater irrigated soils showed higher levels ranging from 136 mg/kg to 176 mg/kg. The higher levels reported by Khan are attributed to originally higher zinc concentration in reference/background soil (72.9 mg/kg) in Beijing, China.

All the vegetable samples showed acceptable zinc concentrations below the maximum EC and FAO/WHO permitted limits. The average zinc concentrations for vegetables were in the following order: Lettuce (38.96 ± 7 mg/kg), parsley (35.09 ± 4.6

mg/kg) and potato (34.7 ± 4.05 mg/kg). Lettuce samples had the highest zinc concentration at site 3 (43.975 ± 1.2 mg/kg) followed by site 1 (38.575 ± 5.2 mg/kg) and site 2 (34.35 ± 11.17 mg/kg). Similarly, the same trend was observed in parsley samples where site 3 showed highest concentration of zinc (37.425 ± 1.8 mg/kg) followed by site 1 and 2 with comparable zinc concentrations of 33.675 ± 7.2 mg/kg and 33.225 ± 2.5 mg/kg, respectively (table 17). And, mean zinc concentrations in control samples were 36.625 ± 7.04mg/kg in lettuce, 37.475 ± 1.66 mg/kg in parsley and 29.55 ± 10.68 mg/kg in potato (table 17). These results are consistent with the results of Arora et al. (2008) where vegetables irrigated with wastewater accumulated zinc concentrations ranging between 22 and 46 mg/kg. Further, other studies by Wang et al. (2012), Khan et al. (2008) and Liu et al. (2005) in China showed similar results. However, the study results are lower than those reported by Rattan et al. (2005) for crops irrigated with wastewater in India. This was attributed to the characteristics of the wastewater effluents. In Rattan et al. (2005) study, the wastewater had elevated mean zinc concentration of 61µg/l compared to zinc levels in the Litani river water (0.022 µg/l) that lead to an increase in plant uptake from the soil with a high transfer factor 9.5-24.6.

The main anthropogenic sources of zinc in the Litani river water are industrial wastewater effluents (mainly plastic), domestic wastewater discharge and leachate of solid waste dumps (ATSDR, 2005; Jurdi et al., 2010). Even though zinc is an essential micronutrient for plant growth; however high levels of zinc would lead to plant phytotoxicity by decreasing the metabolism, growth and development of the plant, and inducing chlorosis in plant leaves (Nagajyoti et al., 2010). Further, human ingestion of

elevated levels of zinc would cause several adverse effects like vomiting, abdominal pain, nausea; whereas chronic exposure to high zinc levels leads to anemia (ATSDR, 2005).

4.4.2.7. Copper

The average copper concentrations were 37.08 ± 1 mg/kg in parsley soils, 35.59 ± 4.98 mg/kg in potato soils and 33.34 ± 4.6 mg/kg in lettuce soils. Copper concentrations in soil parsley were comparable among the three sites with values at sites 1, 2 and 3 of 37 ± 0.4 mg/kg, 37.3 ± 2.2 mg/kg and 36.9 ± 0.1 mg/kg, respectively. As for lettuce soil samples, copper concentrations at sites 1, 2, and 3 were 30.9 ± 2.2 mg/kg, 31.8 ± 5.4 mg/kg and 37.3 ± 5 mg/kg, respectively. Copper concentration in potato soil showed equal concentrations for site 2 (38.9 ± 0.7 mg/kg) and site 3 (38.2 ± 0.4 mg/kg); however, it differed in site 1 with a concentration of 29.7 ± 4.2 mg/kg (table 16). Moreover, control samples showed higher concentrations of copper than in the agricultural soils irrigated by Litani river water, with a concentration of 490.52 ± 25.2 in lettuce soil, 339.93 ± 28.5 in parsley soil and 424.28 ± 11.9 in potato soil (table 16).

The results of copper in soil are in accordance with the data reported by Wang et al. (2012), Khan et al. (2008), and Mapanda et al. (2007). On the other hand, the results of copper concentrations in soils were much higher than that in Beijing, China (15-30 mg/kg) (Liu et al., 2005). The difference in the copper concentration between Litani irrigated soil and that of Beijing, China could be attributed to the low background levels of copper in Beijing soil (10.07 mg/kg). All the copper concentration detected on the soil samples were below the standards. The presence of elevated copper concentration in control soil could be

attributed to the buildup of copper in the soil from geological formation, fertilizers and pesticides or due to contamination from irrigation water (Perfect Life Institute, 2002).

In the analyzed vegetables, the average copper concentrations were 5.59 ± 4.1 mg/kg in parsley, 4.6 ± 1.3 mg/kg in potato, and 3.57 ± 0.7 mg/kg in lettuce. Further, it is to be noted that one parsley sample at site 1 had an elevated level of copper (14.07 mg/kg) similar to the concentration of copper in vegetables (lettuce, parsley, cabbage, spinach and radish) irrigated with wastewater as reported by Khan et al. (2008) and Rattan et al. (2005). And, the average concentration of copper in parsley's samples was almost the same for site 2 and 3 with copper values of 3.71 ± 0.64 mg/kg and 4.05 ± 0.45 mg/kg respectively. Lettuce samples among the three sites showed lower concentration of copper than that in parsley, ranging at sites 2, 3 and 1 between 2.99 ± 0.3 mg/kg, 3.26 ± 0.24 mg/kg and 4.44 ± 0.2 mg/kg, respectively. Furthermore, potato samples had a range of copper concentrations with a maximum level of 5.32 ± 0.72 mg/kg at site 3, followed by 4.63 mg/kg at site 1 and a minimum at site 2 with a concentration of 2.91 ± 0.25 mg/kg. Based on the sites, copper concentration among all the vegetables was highest at site 1 and lowest at site 2.

On the other hand, control samples showed higher levels of copper in lettuce, parsley and potato soils with a mean concentration of 7.79 ± 1.86 mg/kg, 10.47± 0.91mg/kg and 9.16 ± 1.44 mg/kg, respectively. This could be related to the plant uptake of copper from the soil since it is present in elevated levels. Moreover, the levels of copper detected in vegetables are compatible with data reported by Wang et al. (2012). Copper levels in vegetables were also similar to those reported in Beijing showing a range of 3.58 to 18.59 mg/kg (Liu et al., 2005). Furthermore, copper results of parsley in site 1 and potato samples in site 3 are comparable to copper levels reported in vegetables irrigated with wastewater

(Arora et al., 2008). All the samples were below the joint FAO/WHO and EC standards, except for that of parsley soil sample at site 1 (14.0737 mg/kg) that was above the joint FAO/WHO standard.

The major sources of copper are the geological formation, and various anthropogenic sources. Mostly, anthropogenic sources along the Litani river water include domestic wastewater discharge, leachate from solid waste dumps, excessive use of copper sulfate in the treatment of algae, and agriculture runoff (excessive use pesticides and fertilizers). All these sources are associated with the increase of copper concentration in the Litani river water, sediments, and irrigation canal soil as reported by Jurdi et al., (2010) (Table 18). The presence of copper in soil has several effects on plants even though it is an essential micronutrient; excessive copper amounts in soil would lead to stress and injuries in plants leading to plant impaired growth and chlorosis (Nagajyoti et al., 2010). Not only copper is essential for plant growth but it is also important for human health. Still, excessive oral exposure to copper could lead to anemia, liver and kidney damage, developmental toxicity and anemia, and immunotoxicity (ATSDR, 2004).

4.4.2.8. Molybdenum

The average molybdenum concentrations were comparable in lettuce (0.267 ± 0.36 mg/kg) and parsley (0.209 ± 0.2 mg/kg) and potato (0.17 ± 0.1 mg/kg) samples. Site 3 showed the highest levels of molybdenum in lettuce (0.545 ± 0.66 mg/kg), parsley (0.466 ± 0.1 mg/kg) and potato (0.413 ± 0.001 mg/kg). Lowest levels were detected in parsley (0.0739 ± 0.02 mg/kg) and potato samples (0.0605 ± 0.02 mg/kg) of site 1 and in lettuce of site 2 (0.074 ± 0.01 mg/kg). Whereas, molybdenum concentration in control samples was

0.118 ± 0.06 mg/kg in lettuce, 0.319 ± 0.22 mg/kg in parsley, and 0.120 ± 0.02 mg/kg in potato; showing lower concentration than that of experimental samples, except for parsley (table 17).

Molybdenum is an essential micronutrient for plant growth required for nitrogen metabolism and protein synthesis and molybdenum induced toxicity in plants is rare (FAO, 1992; Abd El-Samad et al., 2005). Moreover, results show that molybdenum in all the soil samples and control soil samples were below the detection limits. These results are concurrent with Jurdi et al. (2010), reporting molybdenum in soil and sediments of the Litani River (table 18).

The sources of molybdenum in the ULB river water is attributed to the agricultural runoff (excessive use of fertilizers) and plastic industry. The difference in the results between the soil and vegetable samples could be explained to the type of irrigation method applied; where sprinkling irrigation would directly contaminate the plants (FAO, 1992; FAO, 1994).

There are no published standards for molybdenum in vegetables but the acceptable daily intake (ADI) set by the EPA is 0.005 mg/Kg body weight (IRIS, 1993). Molybdenum is an essential trace metals for human health where it is important for Fe- and flavin-containing enzymes (EC, 2000; CDC, 2012). However, several health effects are associated with increased exposure to molybdenum, including loss of appetite, headache, fatigue, anemia, muscle and joint pain, gout, liver and kidney damage (CDC, 1978; NJDOH, 2011).

4.4.2.9. Nickel

Soil samples had elevated concentration of nickel particularly in lettuce soils (119.72 ± 11.9 mg/kg) and parsley soil (106.15 ± 19.58 mg/kg) where potato soils had lower nickel levels (92.134 ± 26.95 mg/kg); reflecting on higher levels than those reported by Khan et al. (2008). As for nickel concentration in soil irrigated with Litani River highest levels of nickel were found in soil samples of site 2 (119.25 mg/kg) and site 3 (113.68mg/kg) followed by site 1 (85.05 mg/kg) (Table 16). Lettuce and parsley soils showed nickel concentration was relatively higher at site 2 (128.5 ± 0.5 mg/kg, 126.4 ± 0.2 mg/kg, respectively) and lower at site 1 (111.2 ± 15.6 mg/kg and 84.6 ± 2.6 mg/kg, respectively). As for the potato soils, nickel concentrations ranged between 59.4 ± 11.6 mg/kg at site 1, 100.7 ± 2.7 at site 2 and 114.3 ± 12.2 mg/kg at site 3. However, control soil samples had higher concentrations of nickel in lettuce, parsley and potato soils with an average of 131.85675 ± 3.82 mg/kg, 117.0915 ± 0.65mg/kg and 190.9445 ± 2.6 mg/kg, respectively (Table 16).

The elevated levels of nickel in the control soils are attributed to the accumulation of this metal due to the excessive use of fertilizers such as phosphates that are rich in nickel or from contaminated irrigation groundwater (Martin et al., 2012). The results of nickel detected in soil are much higher than those reported by Mapanda et al. (2007) and Rattan et al. (2005). All the samples, except for parsley in site 1, exceeded the EC standards for nickel concentration in soil. In comparison to WHO Guidelines, all the sites were above the recommended except for potato soil of site 1 and 2 and parsley soil of site 1.

As for the vegetable samples, the averages of nickel concentrations were comparable in lettuce (0.923 ± 0.4 mg/kg), parsley (0.903 ± 0.3 mg/kg) and potato (0.884 ±

0.1mg/kg); while the mean nickel concentration in control samples was higher in lettuce and parsley samples with levels of 2.028 ± 1.76 mg/kg and 1.5199 ± 0.89 mg/kg, respectively and lower in potato with a mean value of 0.742 ± 0.002 mg/kg. And, nickel concentration in lettuce ranged between 0.64 ± 0.35 mg/kg at site 3 and 1.358 ± 0.6 mg/kg at site 1. Moreover, parsley samples showed similar results for sites 1 (0.851 ± 0.1mg/kg) and 2 (0.807 ± 0.13 mg/kg); whereas the concentration of nickel at site 3 was 1.055 ± 0.69 mg/kg. On the other hand, potato samples showed similar results for site 1 (0.938 ± 0.1 mg/kg) and 3 (0.962 ± 0.12 mg/kg), but lower levels for site 2 (0.486 ± 0.13 mg/kg). The reported nickel concentrations were however lower than those reported by Khan et al. (2008) and Rattan et al. (2005). This could be associated with high plant uptake of nickel in these studies.

On the other hand, findings of this study are in line with the results of Jurdi et al. (2010), where nickel was detected in all the agricultural soil, irrigation canal soil and sediment samples of the Litani river basin and river (Table 18). The possible sources of nickel were attributed to the prevailing industrial activities such as plastic, ceramics, alloys and stainless steel industries (Jurdi et al., 2010). Other possible sources of nickel could be associated with domestic wastewater discharge and agriculture runoff (excessive use of fertilizers) (Martin et al., 2005; Cempel et al., 2005; ATSDR, 2005). Hence, irrigation with Litani river water results in the accumulation of nickel in soils and vegetables. Moreover, the presence of nickel in significant concentration in control samples could be attributed to the plant uptake of nickel from the control soils since it is present in elevated levels due to contaminated irrigation water or excessive use of fertilizers that are rich in nickel such as phosphates (Martin et al., 2005).

Nickel is a beneficial nutrient for plant growth at low levels; still at elevated levels could cause toxicity by inducing necrosis, chlorosis and physiological alterations in plants. Besides, nickel would interfere in the water uptake by plants (Nagajyoti et al., 2010). Additionally, nickel is an essential trace metal for human health, but its ingestion in high amounts leads to adverse health effects. The health effects of nickel resulting from oral ingestion include vomiting, diarrhea, cramps, neurological symptoms, allergic dermatitis, liver dysfunction and kidney damage (NJDOH, 2007; Nashalian, 2010). And, The EPA reference oral dose is set at 0.02 mg/Kg body weight (IRIS, 1996).

4.4.2.10. Manganese

The detected manganese levels were 538.6 ± 18.9 mg/kg in lettuce soil, 513 ± 37.13 mg/kg in parsley soil, and 409.09 ± 117.38 mg/kg in potato soil. Manganese levels in lettuce soil samples were comparable: 522.5± 11.8 mg/kg at site 3, 541.02 ± 12 mg/kg at site 2 and 552.6 ± 24.4 mg/kg at site 1. Similarly manganese levels were almost equal in parsley soil of site 1 and 2 with a concentration of 501.1 ± 3.7 mg/kg and 496.3 ± 18.4 mg/kg, respectively but differed at site 3 (556.8 ± 25.7 mg/kg). Moreover, manganese concentrations varied among the different potato sites ranging from 265.4 ± 13.4 mg/kg at site 1, 440.9 ± 2.5 mg/kg at site 2 to 521 ± 19.4 mg/kg at site 3 (table 16). The manganese levels in vegetable soils irrigated by the Litani river water are concurrent with the results of Jurdi et al. (2010) that reported comparable manganese levels in agricultural soils and irrigation canal soils. But, the levels of manganese detected in parsley, lettuce and potato soils are higher than those reported by Rattan et al. (2008).

On the other hand, the control soil samples showed elevated levels of manganese higher than those of the experimental samples irrigated with the Litani river water (concentration of 1608.2 ± 49 mg/kg in lettuce, 1134.79 ± 55.6 mg/kg in parsley and 1218.93 ± 7 mg//kg in potato) (table 16). The presence of high manganese levels in the control soil is associated with the excess use of fertilizers that lead to its accumulation in the control soil.

Moreover, the highest levels of manganese were detected in potato vegetable samples with an average concentration of 19.38 ± 14.8 mg/kg followed by lettuce and parsley with average concentrations of 15.9 ± 8.54 mg/kg and 14.2 ± 6.7 mg/kg respectively; whereas lettuce, parsley and potato control samples showed concentrations of 21.45 ± 25.67 mg/kg, 3.125 ± 0.04 mg/kg and 6.525 ± 6.61 mg/kg, respectively. And, the highest levels were detected in potato at site 3 with an average concentration of 33.8 ± 17.9 mg/kg; however, levels at site 1 (10.175 ± 9.9 mg/kg) and 2 (11.4 ± 2.26 mg/kg) were lower than that at site 3. Additionally, lettuce samples at site 1 had the highest levels of 23.875 ± 8.1 mg/kg followed by site 3 (14.925 ± 2.7 mg/kg) and site 2 (9.175 ± 8.5 mg/kg). Besides, manganese concentration in parsley samples ranged between 11.3 ± 12.09 mg/kg in site 3 and 17.45 ± 5.16 mg/kg in site 2. The high concentration of manganese in control lettuce can be related to contaminated irrigation water and soils and/or the excess use of fertilizers.

The results of the study are in accordance with those reported by Arora et al. (2008) and Rattan et al. (2008) reflecting on manganese levels of 12-69 mg/kg and 16.7-39.3 mg/kg in carrot, cabbage, spinach, cucumber and min irrigated with wastewater.

The reported data by Jurdi et al. (2010) show that manganese was detected in the Litani river water and sediments as well as irrigation canal and agricultural soils (table 18).

Manganese sources are associated with geological formations and anthropogenic activities such as steel and alloy industries and fertilizers (Jurdi et al., 2010). Therefore, the irrigation with Litani river water introduces high levels of manganese and leads to the accumulation in vegetables and soil. And, although manganese is an essential nutrient for human diet and for plant growth, however its presence in excess amount would produce adverse health effects and would induce plant phytotoxicity. Manganese toxicity in plants is associated with reduction in the photosynthesis rate, chlorosis, and necrotic brown spots on plant leaves (Nagajyoti et al., 2010).

The oral exposure to manganese levels causes neurobehavioral effects mainly in children and could result in permanent neurological disorders (e.g. manganism, walking difficulties, facial spasms and tremors) (ATSDR, 2008). There is no identified standard for levels in vegetables; however the dietary intake limit is between 2 and 20 mg/kg body weight (WHO 1996). And, the oral reference dose of 0.14 mg/kg/day is set by EPA (IRIS, 1998).

4.4.2.11. Iron

The highest iron concentrations in soil samples were detected in lettuce soil (33032.41± 2855.37 mg/kg) followed by parsley and potato soils with concentration of 31285.11 ± 826.67 mg/kg and 29978.98 ± 8678.914 mg/kg, respectively. The average concentration of iron in lettuce soils ranged between 30007.8 ± 583.4 mg/kg at site 3,

32788 ± 294 mg/kg at site 1 and 36301.4 ± 741.4 mg/kg at site 2. In parsley soil, iron concentrations ranged from 30615.6 ± 511.2 mg/kg at site 1, to 31154.4 ± 613.3 mg/kg at site 2 and 32085 ±755.2 at site 3. As for potato soils, iron concentrations were the highest at site 3 (39422.5 ± 467.8 mg/kg) and the lowest at site 1 (20041 ± 201.4 mg/kg); site 2 had an iron concentration of 30472.9 ± 9.4mg/kg as presented in Table 15.

These levels of iron are much higher than those reported by Rattan et al. (2008); this could be explained by the accumulation of iron in the agricultural soils along the Litani River. Moreover, control samples showed elevated levels of iron compared to soil samples irrigated by the Litani river water. And, the average concentrations of iron in control lettuce, parsley and potato soil samples were 62404.8 ± 567 mg/kg, 77208.5 ± 995.4 mg/kg and 66401.06 ± 1099.3 mg/kg, respectively. Moreover, the high concentrations of iron in control soils could be attributed to accumulation of iron in soils irrigated with groundwater or exposed to excessive use of phosphate fertilizers.

In vegetables, the highest mean iron levels were detected in potato with a concentration of 79.575± 44.3 mg/kg followed by parsley and lettuce with concentrations of 60.4 ± 50.1 mg/kg and 38.31 ± 22.23 mg/kg, respectively; whereas control samples showed lower iron concentration: 31.325 ± 32.28 mg/kg in lettuce, 10.8 ± 0.78 mg/kg in parsley and 23.825 ± 32.92 mg/kg in potato samples (table 17). Vegetables of site 2 showed the highest mean concentration of iron 85.741 ± 26.3 mg/kg followed by site 1 (52.7 ± 59.2 mg/kg) and by site 3 (39.8 ± 21.07 mg/kg) (table 17). Iron concentration in potato samples ranged between 52.35 ± 10.1 mg/kg at site 3 and 84.875 ± 25.42 mg/kg at site 2. A similar trend of iron level distribution was observed in parsley samples among the sites, ranging from 26.775 ± 7.3 mg/kg at site 3 to 84.375 ± 94.43 mg/kg at site 2. Lowest concentrations

of iron were observed in lettuce samples ranging between 29.125 ± 8.2 mg/kg at site 1 to 45.45 ± 26.8 mg/kg at site 2.

Still, the levels of iron in vegetables irrigated with the Litani river water are much lower than those reported by Arora et al. (2005); where iron levels in vegetables irrigated with wastewater ranged from 111 to 378 mg/kg. In addition, Rattan et al. (2005) reported higher iron levels in different vegetables irrigated with wastewater.

Although iron is an essential microelement required for plant growth, high concentrations would induce plant phytotoxicity. Excess of Fe^{2+} in plants induces the production of free radicals that damages the cell structure, protein, DNA and membranes (Nagajyoti et al., 2010). There are no specified standards for iron in vegetables and WHO set an ADI ranging between 10 and 50 mg/kg body weight (WHO, 1996). And, even though iron is an essential trace element for the production of red blood cells, at high intake it induces adverse health effects such as nephric malfunction, myocardial infarction, and hepatic megaly (Kumar et al., 2007; Jagrati et al., 2012).

Table 16: Heavy Metal Concentrations in Soil Samples

Soil samples		Lettuce	Parsley	Potato	Standards EC	WHO 2002 guidelines	Canadian guidelines
Ba (mg/kg)	Site 1	337.7 ±29.4	201.2 ± 35.2	145 ± 74.1		300	750
	Site 2	342.428 ± 9.7	296.34 ± 4	339.62 ± 49.8			
	Site 3	377.5 ± 11	393.3 ± 11.7	376.3 ± 32.1			
	Control	499.4895 ± 3.89	285.37775 ± 12.8	481.52025 ± 71.7			
Cd (mg/kg)	Site 1	13.1 ± 5	10.5 ± 2.7	10.5	3	4	1.4
	Site 2	13.400	11.409	14.5 ± 2.7			
	Site 3	14.3 ± 3.4	13.4 ± 2.2	11.2 ± 1.7			
	Control	12.317	10.607	12.998			
Mo (mg/kg)	Site 1	ND	ND	ND		0.06	5
	Site 2	ND	ND	ND			
	Site 3	ND	ND	ND			
	Control	ND	ND	ND			
As (mg/kg)	Site 1	13.9 ± 0.9	11.0 ± 1.6	7.7 ± 0.4		8	12
	Site 2	14 ± 0.9	11.8 ± 1.1	12.2 ± 0.3			
	Site 3	11.6 ± 0.5	14 ± 0.3	14.7 ± 0.8			
	Control	20.3115 ± 1.5	24.206 ± 1.4	22.7975 ± 0.6			
Pb (mg/kg)	Site 1	ND	ND	8.8 ± 0.4	300	84	70
	Site 2	ND	4.4 ± 6.2	9 ± 0.8			
	Site 3	ND	ND	ND			
	Control	10.877	ND	42.5365 ± 21.98			
Cr (mg/kg)	Site 1	243.3 ± 7	241.5 ± 43.4	127 ± 39	150		64
	Site 2	253.09 ± 2.8	232.683 ± 6.4	234.2 ± 6			
	Site 3	222.4 ± 9.2	225.9 ± 35.6	262.2 ± 4.7			
	Control	490.5215 ± 25.2	339.937 ± 28.5	424.285 ± 11.9			
Ni (mg/kg)	Site 1	111.2 ± 15.6	84.6 ± 2.6	59.4 ± 11.6	75	107	50
	Site 2	128.5 ± 0.5	126.4 ± 0.2	100.7 ± 2.7			
	Site 3	119.1 ± 12.7	107.6 ± 12.8	114.3 ± 12.2			
	Control	131.85675 ± 3.82	117.0915 ± 0.65	190.9445 ± 2.6			
Cu (mg/kg)	Site 1	30.9 ± 2.2	37 ± 0.4	29.7 ± 4.2	140		63
	Site 2	31.8 ± 5.4	37.3 ± 2.2	38.9 ± 0.7			
	Site 3	37.3 ± 5	36.9 ± 0.1	38.2 ± 0.4			
	Control	66.592 ± 2.01	47.74325 ± 2.5	62.978 ± 0.4			
Mn (mg/kg)	Site 1	552.6 ± 24.4	501.1 ± 3.7	265.4 ± 13.4			470
	Site 2	541.02 ± 12	496.3 ± 18.4	440.9 ± 2.5			
	Site 3	522.5 ± 11.8	556.8 ± 25.7	521 ± 19.4			
	Control	1608.2095 ± 49	1134.79875 ± 55.6	1218.93475 ± 7.06			
Fe (mg/kg)	Site 1	32788 ± 294	30615.6 ± 511.2	20041 ± 201.4			
	Site 2	36301.4 ± 741.4	31154.4 ± 613.3	30472.9 ± 9.4			
	Site 3	30007.8 ± 583.4	32085 ±755.2	39422.5 ± 467.8			
	Control	62404.816 ± 567	77208.4945 ±	66401.0605 ±			
Zn (mg/kg)	Site 1	67.1 ± 0.3	66.6 ± 2.2	50.2 ± 6.6	300		200
	Site 2	72.2 ± 1.8	75.1 ± 2.7	77.2 ± 2.3			
	Site 3	69.5 ± 3.8	68.2 ± 6.5	88.6 ± 7.1			
	Control	145.7835 ± 1.5	84.98025 ± 1.4	128.323 ± 1.3			

Table 17: Heavy Metal Concentration in Vegetable Samples

vegetable samples		Lettuce	Parsley	Potato	standards FAO/WHO	EC Standards
Ba (mg/kg)	Site 1	26.7 ± 35.2	8.8 ± 5.8	1.3 ± 0.2		
	Site 2	2.065 ± 0.18	18.905 ± 0.07	1.159 ± 0.15		
	Site 3	1.299 ± 0.04	4.127 ± 0.25	1.873 ± 1.22		
	Control	1.403 ± 0.17	2.275 ± 0.21	1.057 ± 0.02		
Cd (mg/kg)	Site 1	ND	0.0416	ND	0.2 leafy and 0.1 roots	0.2 leafy and 0.1 roots
	Site 2	0.068 ± 0.068	ND	0.083		
	Site 3	0.019 ± 0.003	0.0028	ND		
	Control	ND	ND	ND		
Mo (mg/kg)	Site 1	0.184 ± 0.02	0.0739 ± 0.02	0.0605 ± 0.02		
	Site 2	0.074 ± 0.01	0.088 ± 0.02	0.069 ± 0.01		
	Site 3	0.545 ± 0.66	0.466 ± 0.1	0.413 ± 0.001		
	Control	0.118 ± 0.06	0.319 ± 0.22	0.120 ± 0.02		
As (mg/kg)	Site 1	0.155	0.5939 ± 0.05	0.450 ± 0.05	0.05	NR
	Site 2	0.482 ± 0.05	0.522 ± 0.01	0.367 ± 0.005		
	Site 3	0.332 ± 0.04	0.370 ± 0.05	0.323 ± 0.01		
	Control	0.003	0.0027	ND		
Pb (mg/kg)	Site 1	0.070	0.0069	0.05445	0.3 leafy and 0.1 roots	0.3
	Site 2	0.032	0.0077	0.01		
	Site 3	ND	ND	0.010		
	Control	0.028	0.055 ± 0.03	0.021 ± 0.01		
Cr (mg/kg)	Site 1	1.574	1.6 ± 0.1	1.9 ± 0.2	2.3	NR
	Site 2	1.440 ± 0.01	1.670 ± 0.19	1.5431 ± 0.262		
	Site 3	1.125 ± 0.32	0.885 ± 0.03	1.081 ± 0.31		
	Control	2.223 ± 0.38	1.717 ± 0.3	1.282 ± 0.64		
Ni (mg/kg)	Site 1	1.358 ± 0.6	0.851 ± 0.1	0.938 ± 0.1		
	Site 2	0.773 ± 0.03	0.807 ± 0.13	0.486 ± 0.13		
	Site 3	0.64 ± 0.35	1.055 ± 0.69	0.962 ± 0.12		
	Control	2.028 ± 1.76	1.5199 ± 0.89	0.742 ±0		
Cu (mg/kg)	Site 1	4.448 ± 0.2	9.01 ± 7.2	4.62915	9.4	20
	Site 2	2.9996 ± 0.3	3.718 ± 0.64	2.914 ± 0.25		
	Site 3	3.267 ± 0.24	4.054 ± 0.45	5.321 ± 0.72		
	Control	7.789 ± 1.86	10.471± 0.91	9.164 ±1.44		
Mn (mg/kg)	Site 1	23.875 ± 8.1	13.925 ± 3.7	10.175 ± 9.9		
	Site 2	9.175 ± 8.5	17.45 ± 5.16	11.4 ± 2.26		
	Site 3	14.925 ± 2.7	11.3 ± 12.09	33.8 ± 17.9		
	Control	21.45 ± 25.67	3.125 ± 0.04	6.525 ± 6.61		
Fe (mg/kg)	Site 1	29.125 ± 8.2	70.075 ± 1.2	59.975 ± 40.1		
	Site 2	45.45 ± 26.8	84.375 ± 94.43	84.875 ± 25.42		
	Site 3	40.375 ± 37.5	26.775 ± 7.3	52.35 ± 10.1		
	Control	31.325 ± 32.28	10.8 ± 0.78	23.825 ± 32.92		
Zn (mg/kg)	Site 1	38.575 ± 5.2	33.675 ± 7.2	40.7725 ± 2.4	73.5	50
	Site 2	34.35 ± 11.17	33.225 ± 2.5	37.675 ± 7.672		
	Site 3	43.975 ± 1.2	37.425 ± 1.8	31.275 ± 0.17		
	control	29.55 ± 10.68	36.625 ± 7.04	37.475 ± 1.66		

Table 18: Heavy Metal Concentration in Different Types of Samples

Samples/ Heavy metals	Litani River Water*	Mean of Experimental Vegetable Samples (mg/kg)	Mean of Experimental Soil Samples (mg/kg)	Irrigation Canal* Soil (mg/kg)	Agricultural Soil*
Ba	0.273	1.451	286.943	208	220.091
Cd	0.010	0.083	12.385	13	2.837
Mo	0.002	0.178	ND	ND	ND
As	ND	0.400	11.548	19.25	17.59
Pb	ND	0.025	8.900	9.5	25.87
Cr	0.00118	1.552	207.803	202.920	142.920
Ni	0.00307	0.885	92.135	156.330	98.040
Cu	0.00083	4.630	35.591	56.170	46.920
Mn	0.00007	19.383	409.092	683.330	593.330
Fe	0.00016	79.575	29978.988	38088.833	32805.458
Zn	0.02222	35.092	72.035	136.417	94.917

* Jurdi et.al (2010).Dry Season Water Quality Survey of the Litani River Basin Project, Litani River Basin Management Support Program.

4.4.3. ***Transfer Factor***

The human exposure to soil contamination is through two pathways, either directly from accidental ingestion of contaminated soil or from ingestion of plants grown on contaminated soils (Cui et al., 2004). As such, the transfer factor (soil to plants) is one of

the indicators used to assess exposure to metals through the food chain (Khan et al., 2008). The absorption of metals is assessed by the transfer factor calculated by dividing the metal concentration of the crop (dry weight) by the metal concentration of the soil (dry weight) (Liu et al., 2005; Cui et al., 2004; Rattan et al., 2005).

The mean values of transfer factor of the eleven heavy metals in lettuce were 0.0284 for barium, 0.003 for cadmium, 0.027 for arsenic, 0.005 for chromium, 0.007 for nickel, 0.107 for copper, 0.029 for manganese, 0.001 for iron and 0.559 for zinc as presented in table 19. The transfer factor for molybdenum and lead could not be calculated since these metals were not detected in the experimental and control soils.

As for the transfer factor of parsley it was 0.0357 for barium, 0.0018 for cadmium, 0.04 for arsenic, 0.0008 for lead, 0.0059 for chromium, 0.0085 for nickel, 0.150 for copper, 0.027 for iron, and 0.49 for zinc as presented in table 19.

Moreover, the potato transfer factor was 0.005 for barium, 0.006 for cadmium, 0.034 for arsenic, 0.0027 for lead, 0.007 for chromium, 0.009 for nickel, 0.13 for copper, 0.047 for manganese, 0.002 for iron and 0.487 for zinc as presented in table 19. And the transfer factor for molybdenum in parsley and potato was not calculated since it was not detected in any of the soil samples.

The TF values in all the vegetables (lettuce, parsley and potato) showed the following trend:

Zn > Cu > Mn > As > Ba > Ni > Cr > Cd > Fe > Pb.

The high values of zinc and copper indicate high accumulation of metals in plants. The reported TF values still are much lower than results reported by Liu et al. (2005),

Rattan et al. (2008), and Khan et al. (2008); which might be related to the differences in soil properties, and soil nutrient management (Ciu et al., 2004). Further, the difference can be also attributed to the concentration of metals in the soil to that of vegetables; where accumulation in soils irrigated by the polluted Litani river water due to progressive irrigation is higher compared to levels reported by the indicated studies. Further, all the vegetable soil samples at the three experimental sites had a pH values above 8.14; as such the mobilization of the heavy metal in these soils would decrease and the heavy metals uptake by the plants would be minimal. Therefore, this would affect the values of transfer factor and plant to soil metal ratio; resulting in lower TF values compared to other studies.

On the other hand, the TF values of zinc were relatively comparable to the results reported by Liu et al. (2005) and Khan et al. (2008); TF values of chromium were similar to these reported by Khan et al., (2008) and Wang et al. (2012). Moreover, copper, lead and arsenic TF values were comparable with TF results stated by Wang et al. (2012).

It is to be noted that the irrigation method utilized for crop irrigation is sprinkler irrigation whereby the metals detected in crops is not only from the plant uptake of metals from the soil but also due to absorption through sprinkling (FAO, 1992; FAO, 1994). Therefore, the transfer factor reflects only on the accumulation in plants from metal uptake from soil, and it does not take into account the metals introduced by the sprinkler irrigation. Further, the long term application use of wastewater for irrigation leads to elevated metal concentration in soil but it doesn't lead to linear increase in the plant uptake of metals as reported by Rattan et al. (2008).

Table 19: Transfer Factor of Heavy Metals from Soil to Crops (dry weight)

Sample type		Lettuce	Parsley	Potato
Ba (mg/kg)	Samples	0.0285	0.0357	0.0051
	Control	0.0028	0.0080	0.0022
Cd (mg/kg)	Samples	0.0032	0.0019	0.0067
	Control	ND	ND	ND
Mo (mg/kg)	Samples	ND	ND	ND
	Control	ND	ND	ND
As (mg/kg)	Samples	0.0271	0.0403	0.0347
	Control	0.0001	0.0001	ND
Pb (mg/kg)	Samples	ND	0.0008	0.0028
	Control	0.0013	ND	0.0005
Cr (mg/kg)	Samples	0.0058	0.0060	0.0075
	Control	0.0045	0.0051	0.0030
Ni (mg/kg)	Samples	0.0077	0.0085	0.0096
	Control	0.0154	0.0130	0.0039
Cu (mg/kg)	Samples	0.1071	0.1509	0.1301
	Control	0.1170	0.2193	0.1455
Mn (mg/kg)	Samples	0.0297	0.0277	0.0474
	Control	0.0133	0.0028	0.0054
Fe (mg/kg)	Samples	0.0012	0.0019	0.0027
	Control	0.0005	0.0001	0.0004

Zn (mg/kg)	Samples	0.5600	0.4971	0.4871
	Control	0.2027	0.4310	0.2920

To conclude, the detected levels of heavy metal concentrations in lettuce, parsley and potato irrigated by Litani river water show that leafy vegetables (lettuce and parsley) accumulated barium, cadmium, arsenic, lead, molybdenum, nickel and zinc at levels higher than that for potato samples. Whereas, chromium, copper, manganese, and iron were accumulated at higher levels in potato samples compared to leafy vegetables as presented in the table 20.

Table 20: Heavy Metals Concentrations in Leafy and Root Vegetables

Vegetable types	Ba mg/kg	Cd mg/kg	Mo mg/kg	As mg/kg	Pb mg/kg	Cr mg/kg	Ni mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Zn mg/kg
Leafy vegetables	10.32 ± 14.5	0.036 ± 0.041	0.23 ± 0.28	0.43 ± 0.13	0.029 ± 0.029	1.387 ± 0.32	0.913 ± 0.38	4.583 ± 3.04	15.108 ± 7.38	49.36 ± 38.73	36.87 ± 5.88
Potato	1.451 ± 0.6	0.08	0.17 ± 0.18	0.4 ± 0.07	0.024 ± 0.025	1.55 ± 0.42	0.88 ± 0.16	4.63 ± 1.03	19.383 ± 14.81	79.57 ± 44.32	35.09 ± 4.62

And, the mobilization of heavy metals in lettuce, parsley and potato soils is highly affected by the soil pH and would increase with decreased pH due to the release of free metal ions in soil solution leading to higher metal uptake by plants. However, all the vegetable soil samples at the three experimental sites had a pH values above 7; as such the mobilization of the heavy metal in these soils would decrease and the heavy metals uptake by the plants would be minimal (Mapanda et al., 2007).

Further, the TF values of metals differed among the two types of vegetables (leafy and root). The TF values of lead, chromium, nickel, manganese, cadmium and iron in the experimental samples were highest in root vegetable (potato) compared to leafy vegetables (parsley and lettuce) as presented in table 19. Except for TF values of barium, arsenic and copper that were higher in parsley, followed by potato and then lettuce as presented in table 19. The difference of the TF values between types of vegetables was also noted by other studies. Wang et al. (2012) reported that TF values were dependent on vegetables type and species. Further, Wang et al. (2012) reported different TF values for vegetable types; leafy vegetables (lettuce) reported higher TF values compared to radish.

Hence, the irrigation by the Litani river water leads to the accumulation of heavy metals in vegetables (lettuce, parsley and potato) and soils. The mean content of heavy metals per dry weight of leafy vegetables (lettuce, parsley), potato and soil are summarized in Table 20. The levels of arsenic in all the vegetable samples exceeded the permissible limits set by the European Commission (EC) and joint FAO/WHO Food Standard Program. Chromium levels in all the vegetable samples also exceeded the EC standards. As for copper, only parsley samples of site 2 exceeded the EC standards.

As for soil samples the levels of barium, cadmium, arsenic, chromium and nickel exceeded the permissible limits set by European Commission (EC) and WHO Guidelines; and the levels of manganese exceeded the Canadian standard.

As such, the ingestion of vegetables irrigated with the Litani River is a major public health concern especially due to the exposure to arsenic, chromium and copper at levels exceeding the permissible limits. Moreover, irrigated soils can also pose a health risk

(mostly to farmers) since metals in the irrigated soil might be transferred to humans through metal inhalation or ingestion of soil dust (Liu et al., 2005).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1. **Overview of the Chapter**

This part of the study presents the main conclusions relating to the microbiological and chemical safety of vegetables and soils irrigated by the Litani river water as well as the antibiotic resistance pattern of the isolated pathogens. Furthermore, this chapter will recommend possible mitigation measures to ensure the safety of the grown crops, reduce the exposure to health risks, promote the need to protect the ecologic wellbeing of this major national water resource, and try to communicate the identified risk with local community.

5.2. **Conclusions**

5.2.1. ***Microbiological Safety***

The Litani river water quality is progressively exposed to uncontrolled discharge of sewage and industrial wastewater effluents, municipal and industrial solid waste and animal waste leading to the deterioration of water quality. This is exposing irrigated soils and crops (lettuce, parsley and potato) to the accumulation of microbiological pathogens (*E. coli*, *E. cloacae*, *E. aerogenes*, *K. pneumonia*, K. *oxytoca*, *Serratia marcescens*, *C. freundii*, *Shigella sonnei*, *C. diversus*, *Listeria spp* and *Pseudomonas*).

Furthermore, the results of the study showed that the exposure to polluted irrigated water by sprinkling irrigation is the main important factor impacting the safety of the grown experimental vegetables. Other routes of contamination could be related to the internal transport of the pathogen via the root system of the vegetables (interior tissues of the vegetable become colonized by the pathogen) and external transport via rain or wind (favoring the transfer of pathogen from the contaminated soil to the vegetable) (Islam et al., 2004; Oliveira et al., 2012; Deering et al., 2012).

Among the experimental vegetables, lettuce was found to be contaminated with 7 different types of pathogens (*E. aerogenes, K. oxytoca, C. Freundii, E. cloacae, E. coli, K. pneumonia,*and *C. diversus*), followed by parsley with 6 pathogens (*K. pneumonia, E. aerogenes, K. oxytoca, E. cloacae, E. coli* and *C. Freundii*) and potato with 4 pathogens (*S. marcescens, E. coli, Listeria* spps *and E. cloacae*) as was presented in table 11. This is mostly because *Enterobacteriaceae* family and *Listeria* spp have the ability to adhere to the edible parts of leafy vegetables, leafy vegetable roots, interior of the stomatal pores and to the deep grooves of seed coats. And, these adherence mechanisms would also induce bacterial resistance to chemical interventions (Wachtel et al., 2002; Oliveira et al., 2011).

As for soil samples, also the lettuce soils were found to be the most contaminated (*E. aerogenes*, *K. oxytoca, C. Freundii,* E. cloacae, *Serratia marcescens, P. aeruginosa, E. coli, Listeria spps, Sh. sonnei* and *K. pneumoniae*) followed by parsley soils (*K. pneumonia, E. aerogenes, K. oxytoca, Serratia marcescens, E. coli, C. Freundii,* and *Listeria* spps*)* and potato soils (*E. aerogenes*, *K. pneumonia, Serratia marcescens,* and *E. cloacae)* as was presented in table 11.

Moreover, the survival of pathogens in soils and vegetables is also affected by different factors such as pH, humidity, temperature, plant type and competition with native flora and fauna (WHO, 2006; Oliveira et al., 2011). And, as the study was conducted in summer (August), the hot temperatures influenced the detection of different types of microorganisms such as *Salmonella* spps., *Listeria* spp. *E. coli* and viruses (Oliveira et al., 2011). Also the use of sprinkler irrigation favors pathogen transfer from the polluted water to the crops; still it is detrimental on pathogen survival in soils (Solomon et al., 2002; Solomon et al., 2003).

5.2.2. ***Antibiotic Resistance***

The resistance pattern of all the isolates (*E. cloacae*, *E. coli, C. Freundii, K. oxytoca, Serratia marcescens, K. pneumoniae, E. aerogenes* and *Shigella sonnei*) to the experimental antibiotics (Erythromycin, Gentamicin, Ciprofloxacin and Cefotaxime) showed 100% resistance to Erythromycin, 98% resistance to Gentamicin and 93% resistant to both Ciprofloxacin and Cefotaxime; where none of the isolates was totally susceptible to any of the four antibiotics used.

This antibiotic resistance pattern of all isolates from vegetable and soil samples is associated with the irrigation with contaminated Litani river water. The high percentage of antibiotic resistance, presented in this study, is mostly related to the over prescription of antibiotics in the treatment of infectious diseases in Lebanon, and to the excessive and repeated use of antibiotics in agriculture (animal and crop production) (Kamleh et al., 2012; Falomir et al. 2010; Levantesi et al., 2012; Harakeh et al., 2005; Warriner et al., 2009).

The presence of resistant microbial strains is alarming and poses a serious public health threat. With the emergence of antibiotic resistance pathogens, the treatment of infections becomes ineffective, since antibiotic options become limited, more expensive, less effective and more toxic with serious side effects. Further, when infections are not effectively treated, the pathogen would persist and spread to infect others (CDC, 2012; FDA, 2012, Frieden, 2010). Hence, infections caused by resistant strains are critical, especially for children, elderly and immune-compromised patients, and would lead to increase fatality (Harakeh et al., 2005, Kamleh et al., 2012). And, the emergence of antibiotic resistance is a major public health concern that reflects directly on the management of the foodborne disease burden (Kamleh et al., 2012).

5.2.3. ***Chemical Safety (Heavy Metals)***

As for the chemical safety of crops, the study showed that the irrigation water is leading to the introduction and accumulation of heavy metals (barium, cadmium, chromium, copper, lead, zinc, manganese, molybdenum, iron, nickel, magnesium) in soils and vegetables (lettuce, parsley and potato); where the levels of arsenic in all the vegetable samples exceeded the permissible limits set by the European Commission (EC) and joint FAO/WHO Food Standard Program. Chromium levels in all the vegetable samples also exceeded the EC standards. As for copper, only parsley samples of site 2 exceeded the EC standards. In addition, leafy vegetables (lettuce and parsley) accumulated barium, cadmium, arsenic, lead, molybdenum, nickel and zinc at levels higher than those of potato samples. Whereas, chromium, copper, manganese, and iron were accumulated at higher levels in potato samples compared to leafy vegetables.

As for soil samples, the levels of barium, cadmium, arsenic, chromium and nickel exceeded the permissible limits set by European Commission (EC) and WHO Guidelines; and the levels of manganese exceeded the Canadian standard. The mobilization of heavy metals in lettuce, parsley and potato soils is highly affected by the soil pH and would increase with decreased pH (promotes the release of free metal ions in soil leading consequently to higher metal uptake by plants) (Mapanda et al., 2007). However all the vegetable soil samples at the three experimental sites had a pH values above 7; as such the mobilization of the heavy metal in these soils would decrease and the heavy metals uptake by the plants would be minimal.

Further, the Transfer values of metals differed among the two types of vegetables (leafy and root). In the experimental samples root vegetable (potato) had higher TF values for heavy metals compared to leafy vegetables (parsley and lettuce); except for TF values of barium, arsenic and copper that were higher in parsley, followed by potato and then lettuce.

As such, the ingestion of vegetables irrigated with the Litani river water is a major public health concern especially due to the exposure to arsenic, chromium and copper at levels exceeding the permissible limits. Moreover, irrigated soils could also pose a health risk, since metals in the irrigated soil might be transferred to humans (mostly to farmers), through metal inhalation or ingestion of soil dust (Liu et al., 2005).

5.3. **Recommendations**

The use of polluted Litani river water for irrigation leads to the microbiological and chemical contamination of vegetables and soils. Hence, the following is recommended:

5.3.1. ***Short term Recommendations***

- Operate the three constructed wastewater treatment plants (Ablah, Ferzol and Jeb Jannine), and complete the construction of the remaining planned plants.
- Reduce exposure to contaminants by substituting the sprinkler irrigation by drip irrigation, as the latter would reduce the levels of microbiological and chemical contaminants especially in leafy vegetables.
- Disseminate awareness on appropriate household practices to reduce the levels of contaminates in consumed crops.

5.3.2. ***Long Term Recommendations***

Implement integrated river basin management (IRBM) to:

- Identify point and non point sources of pollution
- Manage properly domestic and industrial wastewater effluents, and municipal solid waste Enforce onsite treatment of industrial wastewater effluents.
- Control the discharge of municipal and industrial solid waste discharge along the river flow.
- Implement good agricultural practices (GAPs) and integrated pest management (IPM) to reduce/prevent the hazards to control the excessive use of pesticides and fertilizers.
- Enforce the use of the services of local laboratories (Lebanese Agricultural Research Institute and Litani Water Authority) to determine the levels of nutrients in soils and the need to apply fertilizers.

- Control the use of antibiotics to reduce the emergence of antibiotic resistant microorganisms.

Conduct further studies to:

- Identify the chemical and microbiological hazards in other types of crops irrigated with the Litani river water.
- Assess the probability of human exposure to the identified chemical and microbiological contaminants and accordingly recommend appropriate interventions.

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