HEALTH RISKS ASSOCIATED WITH CONSUMPTION OF UNTREATED WATER FROM HOUSEHOLD ROOF CATCHMENT SYSTEMS

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This thesis was prepared under the supervision of Dr. Khaled Swaileh and has been approved by all members of the examination committee.

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The findings, interpretations and conclusions expressed in this study do not necessarily express the views of Birzeit University, the views of the individual members of the MSc committee or the views of their respective employers.

بسم الله الرحمن الرحيم

وَالْعَصْرِ (1) إِنَّ الْإِنسَانَ لَفِي خُسْرٍ (2) إِلَّا الَّذِينَ آمَنُوا

وَعَمِلُوا الصَّالِحَاتِ وَتَوَاصَوْا بِالْحَقِّ وَتَوَاصَوْا بِالصَّبْرِ (3)

**By (the Token of) Time (through the ages),
Verily Human is in loss, Except such as have
Faith, and do righteous deeds, and (join
together) in the mutual teaching of Truth, and
of Patience and Constancy.**

Dedication

To my mother Fadwa Dawod for her encouragement

To my father Khalid Dawod for his continuous support and encouragement.

To my wife Afnan Sabry for her patience and support.

To my grandfather Mustafa Al Haj Hasan for his continuous support

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ABSTRACT

In Palestine, rain harvesting is a common practice especially in regions suffering from drinking water shortage or lacking drinking water distribution systems. Rain harvesting systems constitute a source of free water in a country where water prices are considered high and water supply is insufficient especially during the summer period. Rainwater is mostly harvested through roof-catchment systems and stored in underground concrete or rocky wells. Harvested rainwater is usually consumed without any treatment, although it could constitute a health hazard. The present study aims at evaluating health risks associated with drinking untreated rainwater harvested through roof catchment systems from the West Bank, Palestine.

Therefore, water samples from 21 wells were obtained during summer and winter of the years 2006-2007. Microbial and chemical quality of the harvested rainwater was investigated to determine potential health risks. Chemical and physical tests that affect water quality included: turbidity, salinity, total dissolved solids (TDS), electrical conductivity (EC), pH, temperature, nitrate and heavy metals (copper and lead). Microbial quality tests included HPC, total and fecal coliforms. In addition, 5 opportunistic and 6 pathogenic bacteria were tested using PCR technology.

Physical and chemical rainwater parameters were mostly within the WHO guidelines set for drinking water. Generally, summer samples included higher levels of TDS, salinity and EC compared to winter samples.

Classical microbial tests (HPC, total and fecal coliforms) revealed heavy contamination of rainwater with microbes. This indicates poor microbial quality especially if water is to be used for drinking purposes.

PCR results showed that opportunistic pathogens are very common in rainwater samples but pathogenic species were very rare. Only one pathogenic microbe was detected in the 2 samples out of 42 samples collected and analyzed during this study.

Harvested rainwater tested from Qalqilia and Ramallah regions needs to be disinfected before being used for drinking purposes. However, its chemical and physical quality is acceptable for drinking purposes.

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APHA: American Public Health Association,
BCYE: buffered charcoal yeast extract
bp : Base pair
ºC: Degree Centigrade
CFU: colony forming unit
Con : constant
DNA: Deoxyribonucleic Acid
EC: Electrical conductivity
EPA: Environmental Protection Agency
F: Forward primer
GV: Guideline values
H :hour
Hb: hemoglobin.
HIV: Human immunodeficiency virus
HPC : Heterotrophic plate counts
HUS: hemolytic-uremic syndrome
kb: killobase
kg: kilogram.
metHb: methaemoglobin
mg: Milligram
mM: Millimolar
Mol :mole
µM: Micromolar
NTU: Nephelometric Turbidity Units
PCR: Polymerase Chain Reaction
ppb: part per billion
ppm: part per million
PVC: **poly** vinyl chloride
R: Reverse Primer
RNA: Ribonucleic acid
Spp : species
SS: summer sample.
STEC: Shiga toxigenic *Escherichia coli*
TAE: Tris, Acetic acid, EDTA
TDS: Total Dissolved Solids
TMC: Too many to count.
TE: Tris – EDTA buffer
UV:Ultra Violet
Var: variable
WHO: World Health Organization
WS: winter sample.
µl: microliter
µs/cm: microsimmens per centimeter
‰: part per thousands.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW .1

Water shortage is becoming the number one problem in the World today. The on going drought and the increasing demand of growing populations reduce water reservoirs (Sazakli *et al.,* 2007).

Rainwater harvesting is the capture, diversion, and storage of rainwater for a number of different purposes including landscape irrigation, drinking and domestic use, aquifer recharge, and storm water abatement. (Texas Manual, 2005).

The depletion of groundwater sources, poor quality of some groundwater and surface waters, high tap fees for isolated properties, flexibility of rainwater harvesting systems, and modern methods of treatment provide excellent reasons to harvest rainwater for domestic use (Texas Manual, 2005).

According to (Krishna, 2003), there are many advantages and benefits of rainwater harvesting. The water is free; the only cost is for collection and use. There is no need for complex and costly distribution systems, because the end use of harvested water is located close to the source. Besides, rainwater harvesting provides a water source when groundwater is unacceptable or unavailable, or it can augment limited groundwater supplies. Finally, rainwater is superior for landscape irrigation. Additional advantages of rainwater harvesting include:

reduction of the volume of storm water, thereby lessening the impact of erosion and decreasing the load on storm sewers. Decreasing storm water volume also helps keep potential storm water pollutants, such as pesticides, fertilizers, and petroleum products, out of rivers and groundwater (Texas Manual, 2005).

1.1 Rainwater Harvesting System Components:

The scope of rain harvesting, method, technologies, system complexity, purpose, and end uses vary from rain barrels for garden irrigation in urban areas, to large-scale collection of rainwater for all domestic uses (Texas Manual, 2005).

At small-scale application, simple rainwater harvesting can be used by channeling rain running off an unguttered roof to a planted landscape area via contoured landscape. To prevent erosion on sloped surfaces, a bermed concave holding area down slope can store water for direct use by turf grass or plants (Waterfall, 1998).

Regardless of the complexity of the system, the domestic rainwater harvesting system (**Figure1**) comprises six basic components: 1) the catchments surface which is the collection surface from which rainfall runs off. 2) Gutters and downspouts: channel water from the roof to the tank. 3) Leaf screens, first-flush diverters, and roof washers: components which remove debris and dust from the captured rainwater before it goes to the tank. 4) One or more storage tanks, also called cisterns. 5) Delivery system: gravity-fed or pumped to the end use. 6) Treatment/purification: for potable systems, filters and other methods to make the water safe to drink (Texas Manual, 2005).

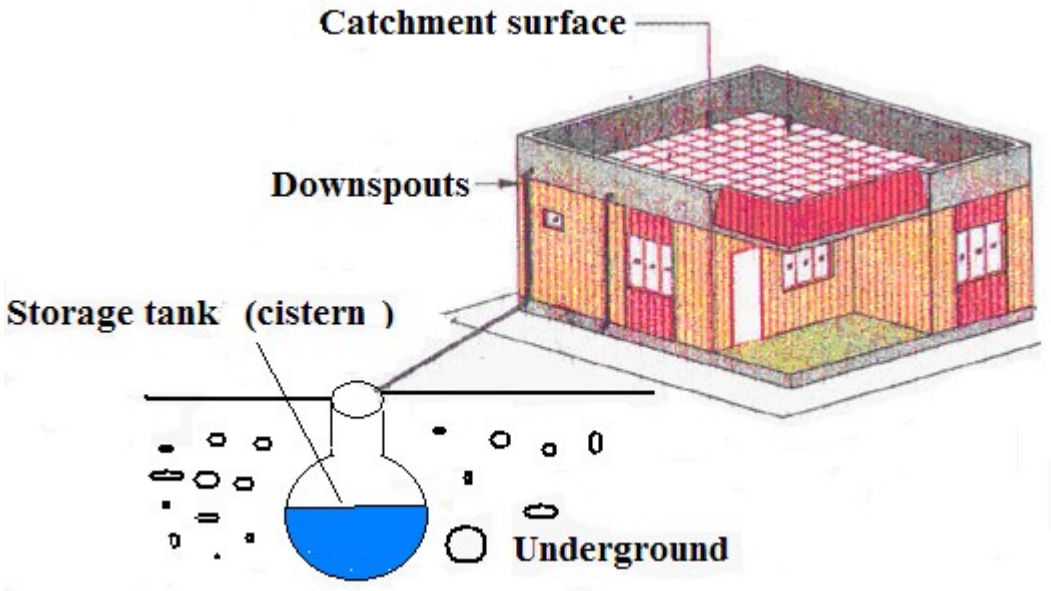


Figure 1: Typical rain water harvesting installation.

The roof of a building or house is the obvious first choice for catchment. For additional capacity, an open-sided barn – called a rain barn or pole barn – can be built. Principal component analysis revealed that microbiological parameters were affected mainly by the cleanness level of catchment areas, while chemical parameters were influenced by the sea proximity and human activities (Sazakli *et al*., 2007).

According to Vasudevan (2002) water quality from different roof catchments is a function of the type of roof material, climatic conditions, and the surrounding environment.

Gutters are installed to capture rainwater running off the eaves of a building. The most common materials for gutters and downspouts are half-round PVC, vinyl, pipe, seamless aluminum, and galvanized steel (Texas Manual, 2005).

Leaf screens remove debris that are gathered on the catchment surface, and ensure high quality water for either potable use or to prevent clogging in irrigation emitters. Essentially, mesh screens remove debris both before and after the storage tank (Texas Manual, 2005).

A roof can be a natural collection surface for dust, leaves, blooms, twigs, insect bodies, animal feces, pesticides, and other airborne residues. The first-flush diverter routes the first flow of water from the catchment surface away from the storage tank. The flushed water can be routed to a planted area.

The roof washer, placed just ahead of the storage tank, filters small debris for potable systems and also for systems using drip irrigation. Roof washers consist of a tank, usually between 30- and 50-gallon capacity, with leaf strainers and a filter (Figure 1) (Texas Manual, 2005).

The storage tank is the most expensive component of the rainwater harvesting system. The size of storage tank or cistern is dictated by several variables: the rainwater supply (local precipitation), the demand, the projected length of dry spells without rain, the catchment surface area, aesthetics, personal preference,

and budget (Texas Manual, 2005). Storage tanks should meet the following basic requirements: ability to inhibit algae growth (opaque or painted dark), must never have been used to store toxic materials especially for potable systems, must be covered and vents screened to discourage mosquito breeding, accessible for cleaning (Texas Manual, 2005).

There are many types of storage tanks that could be used in rainwater harvesting system: Fiberglass, polypropylene, wood, metal and concrete. Only concrete tanks will be discussed in this study because it is the dominant type in Palestine.

Concrete tanks are either poured in place or prefabricated (Figure 1). They can be constructed above ground or below ground. Poured-in-place tanks can be integrated into new construction under a patio, or a basement, and their placement is considered permanent.

Concrete may be prone to cracking and leaking, especially in underground tanks in clay soil. Leaks can be easily repaired although the tank may need to be drained to make the repair. Involving the expertise of a structural engineer to determine the size and spacing of reinforcing steel to match the structural loads of a poured-in-place concrete cistern is highly recommended (Texas Manual, 2005).

One possible advantage of concrete tanks is a desirable taste imparted to the water by calcium in the concrete being dissolved by the slightly acidic rainwater. For

potable systems, it is essential that the interior of the tank be plastered with a high-quality material approved for potable use (Texas Manual, 2005).

1.2 Rainwater Quality:

Because water is a universal solvent, it absorbs all kinds of contaminants on its travels to the reservoir, in addition to pathogenic organisms. While in residence in the reservoir, the water can come in contact with all kinds of foreign materials: oil, animal wastes, chemical and pharmaceutical wastes, organic compounds, industrial outflows, and trash (Texas Manual, 2005). Contamination of rainwater depends on many factors that are related to many factors like: the type of atmospheric contaminants, the harvesting process, and the storage of harvested rainwater.

Hoque *et al.* (2006) found that the contamination rate for water samples from covered house hold storage containers was significantly lower than that of uncovered containers. In addition, the rate of water contamination in storage containers was highest during the February-May period. It is shown that safe drinking water was achieved by a combination of a protected and high quality source at the initial point and maintaining quality from the initial supply (source) point through to final consumption.

The quality of harvested rainwater which is used for domestic and drinking purposes in the northern area of Kefalonia Island in Greece and the factors affecting it were assessed by Sazakli *et al*. (2007). It was found that all of the rainwater samples were within the guidelines for chemical parameters. As far as

microbiological quality is concerned, total coliforms, *Escherichia coli* and enterococci were detected in the rainwater samples, although they were found in low concentrations.

In Bangladesh high rates of diarrheal disease morbidity indicate that pathogen transmission continues through water supply chain. In a recent study, it was found that, 61% of tube-well water samples met the Bangladesh and WHO standards of faecal coliform. However, only 37% of the stored water samples met the standards during the survey in Bangladesh (Hoque *et al*., 2006).

Efe (2006) assessed rainwater samples harvested from catchments roofs in 6 rural communities of Delta State in Nigeria and found that most of physiochemical and biological characteristics of rain water samples were generally below the WHO threshold. Therefore, this rainwater was recommended for drinking and other household purposes.

Evans *et al*. (2005) studied roof run-off water at an urban housing development in Newcastle, on the east coast of Australia. They found that air borne microorganisms represented a significant contribution to the bacterial load of roof water. In addition, the overall contaminant load was influenced by wind velocities, while the profile (composition) of the load varied with wind direction.

1.2.1 Microbial quality of rainwater:

Contamination of rainwater with microbes and the possible health risks caused by these microbes necessitate developing accurate and reliable tests on harvested

rainwater to evaluate its suitability for human consumption. This lead to the development of the concept of indicator organisms as signals of fecal pollution. This became a well-developed and established practice in the assessment of drinking-water quality. The criteria determined for such indicators were that they should not be pathogens themselves and should be universally present in feces of humans and animals in large numbers; not multiply in natural waters; persist in water in a similar manner to fecal pathogens; be present in higher numbers than fecal pathogens; respond to treatment processes in a similar fashion to fecal pathogens; and be readily detected by simple, inexpensive methods (WHO, 2006). Common indicator bacteria include: Total coliform bacteria, fecal coliform bacteria, Heterotrophic plate counts and some opportunistic and pathogenic microorganisms.

Fecal coliforms are bacteria that live in the digestive tract of warm-blooded animals (humans, pets, farm animals, and wildlife) and are excreted in the feces. In themselves, fecal coliforms generally do not pose a danger to people or animals but they indicate the presence of other disease-causing bacteria, such as those that cause typhoid, dysentery, hepatitis A, and cholera **(EPA, 2005).**

Fecal contamination can arise from sources such as combined sewer overflows, leaking septic tanks, sewer malfunction, contaminated storm drains, animal feedlots, and other sources. Rainfall is frequently associated with increased abundance of fecal coliforms in water due to storm water runoff or roof catchment

systems (EPA, 2005**)**. Fecal coliforms are routinely tested in surface and ground water (drinking water, lakes, rivers, and ponds).

Total coliform bacteria include a wide range of aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 h at 35–37 °C (WHO, 2006).

Escherichia coli and thermotolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures. As part of lactose fermentation, total coliforms produce the enzyme b-galactosidase (WHO, 2006). Coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*, but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The total coliform group includes both faecal and environmental species (WHO, 2006).

Total coliforms include organisms that can survive and grow in water. Hence, they are not useful as an index of fecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (WHO, 2006).

Total coliforms should be absent immediately after disinfection, and the presence of these organisms indicates inadequate treatment. The presence of total coliforms

in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants (WHO, 2006).

Heterotrophic plate counts (HPC) measurement detects a wide spectrum of heterotrophic microorganisms, including bacteria and fungi, based on the ability of the organisms to grow on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature (WHO, 2006). The spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly proliferate in treated water in the absence of residual disinfectants (WHO, 2006). HPC can include potentially "opportunistic" pathogens such as *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Klebsiella*, *Moraxella*, *Serratia*, *Pseudomonas* and *Xanthomonas.*

The tests detect only a small proportion of the microorganisms that are present in water. The population recovered will differ according to the method and conditions applied. Although standard methods have been developed, there is no single universal HPC measurement. A range of media is available; incubation temperatures used vary from 20°C to 37 °C and incubation periods range from a few hours to 7 days or more (WHO, 2006).

The test has little value as an index of pathogen presence but can be useful in operational monitoring as a treatment and disinfectant indicator, where the objective is to keep numbers as low as possible. In addition, HPC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms (WHO, 2006).

Opportunistic pathogens are microorganisms that do not cause disease in healthy immune system, but compromised immune system presents an opportunity for the pathogens to infect. The opportunistic pathogens include: *Acinetobacter*, *Aeromonas, Pseudomonas aeruginosa,* and *Mycobacterium.*

Citrobacter *spp* is Gram negative rod, facultatively anaerobic, motile microbe that uses citrate as sole carbon source. The genus *Citrobacter* consists of three species: *Citrobacter amalonaticus, Citrobacter diversus* (also known as *Citrobacter koseri*), and *Citrobacter freundii* (Chastain and Cycle, 2000). *Citrobacter* is an opportunistic pathogen, associated with nosocomial infections; causes diarrhea and secondary infections in immunocompromised patients and occasionally severe primary septicemia. *C. koseri* is associated with meningitis in infants <2 months old.

The mode of transmission of *Citrobacter* are: Fecal-oral transmission; contaminated food; person to person spread; mother to child. The most important

reservoir of *Citrobacter* is water, sewage, foodstuffs, intestinal tract of humans and animals (Public Health Agency of Canada, 2001).

Citrobacter species are low-virulence organisms that have rarely been reported to cause serious infections in humans involving the central nervous system, or the gastrointestinal, urinary, or respiratory tracts (Canario *et al*., 2004).

Citrobacter species have rarely been involved in foreign body infections.4-6 foreign body and prosthetic infections are usually caused by *Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella, Enterobacter, Serratia, Enterococcus, or Pseudomonas* (Canario *et al*., 2004).

Acinetobacter spp. are Gram-negative, oxidase-negative, non-motile coccobacilli (Short plump rods). *Acinetobacter spp.* is ubiquitous inhabitants of soil, water and sewage environments. It has been isolated from 97% of natural surface water samples in numbers of up to 100/ml. *Acinetobacter spp*. are part of the natural microbial flora of the skin and occasionally the respiratory tract of healthy individuals (WHO, 2006). Some species and strains can be found on the skin of healthy people (Krawczyk *et al.,* 2001). *Acinetobacter spp*. Can occasionally cause infections, predominantly in susceptible patients in hospitals. They are opportunistic pathogens that may cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections (WHO, 2006).

While *Acinetobacter spp*. are often detected in treated drinking-water supplies, an association between the presence of *Acinetobacter* spp. in drinking-water and clinical disease has not been confirmed. There is no evidence of gastrointestinal infection through ingestion of *Acinetobacter* spp. in drinking-water among the general population. However, transmission of non-gastrointestinal infections by drinking-water may be possible in susceptible individuals, particularly in settings such as health care facilities and hospitals (WHO, 2006).

Acinetobacter spp. are detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. However, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be used as an index for the presence/absence of *Acinetobacter spp.* (WHO, 2006).

Aeromonas spp. are Gram-negative, non-spore-forming, facultative anaerobic bacilli belonging to the family Vibrionaceae. It includes the mesophilic motile (single polar flagellum) aeromonads that is considered of potential human health threat and consists of the species *A. hydrophila*, *A. caviae*, *A. veronii* subsp. *sobria*, *A. jandaei*, *A. veronii* subsp. *veronii* and *A. schubertii*. The bacteria are normal inhabitants of fresh water and occur in water, soil and many foods, particularly meat and milk (WHO, 2006).

Recent studies have demonstrated that the presence of *Aeromonas spp*. in drinking water is a potential risk, since these microorganisms can produce a wide range of

virulence factors (Schubert, 1991; Warburton *et al*., 1994; Kersters *et al.,* 1996; Ku¨hn *et al.,* 1997; Ivanova *et al.,* 2001; Ormen and Ostensvik, 2001; Villari *et al.*, 2002).

Entry of aeromonads into distribution systems can be minimized by adequate disinfection. Control measures that can limit growth of the bacteria in distribution systems include treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals (WHO, 2006).

There have been attempts in some countries, such as The Netherlands, Canada, and Italy, to introduce guideline standards for the presence of *Aeromonas spp*. in drinking water (Warburton *et al.,* 1994, Handfield, *et al*., 1996; Italian Ministry of Health, 1997; Italian Ministry of Health, 1998;). In Italy, provisional and cautionary limits were established in 1997 for natural mineral waters at their origin (10 CFU/100 ml) and after being bottled (100 CFU/100 ml) and maintained until the end of 1998(Villari *et al*., 2002).

Pseudomonas aeruginosa is a member of the family Pseudomonadaceae and is a polarly flagellated, aerobic, Gram-negative rod. When grown in suitable media, it produces the non-fluorescent bluish pigment pyocyanin. Many strains also produce the fluorescent green pigment pyoverdin. *P. aeruginosa*, like other

fluorescent pseudomonads, produces catalase, oxidase and ammonia from arginine and can grow on citrate as the sole source of carbon (WHO, 2006).

Pseudomonas aeruginosa is an opportunistic pathogen capable of infecting both humans and animals (Khan and Cerniglia, 1994). *P. aeruginosa* is an important cause of bacteremia in patients receiving organ transplants and is responsible for about 28% of most bacteremia episodes (Brooks and Remington, 1986; Khan and. Cerniglia, 1994).

Although *P. aeruginosa* can be significant in certain settings such as health care facilities, there is no evidence that normal uses of drinking-water supplies are a source of infection in the general population. However, the presence of high numbers of *P. aeruginosa* in potable water, notably in packaged water, can be associated with complaints about taste, odor and turbidity (WHO, 2006).

Pseudomonas aeruginosa is sensitive to disinfection, and entry into distribution systems can be minimized by adequate disinfection. Control measures that are designed to minimize biofilm growth, including treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals, should reduce the growth of these organisms. (WHO, 2006).

Mycobacterium the tuberculous or "typical" species of *Mycobacterium*, such as *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. leprae*, have only human or

animal reservoirs and are not transmitted by water. In contrast, the non-tuberculous or "atypical" species of *Mycobacterium* are natural inhabitants of a variety of water environments. These aerobic, rod-shaped and acid-fast bacteria grow slowly in suitable water environments and on culture media. Typical examples include the species *M. gordonae*, *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. xenopi*, *M. intracellulare* and *M. avium* and the more rapid growers *M. chelonae* and *M. fortuitum* (WHO, 2006).

Atypical *Mycobacterium spp.* can cause a range of diseases involving the skeleton, lymph nodes, skin and soft tissues, as well as the respiratory, gastrointestinal and genitourinary tracts. Manifestations include pulmonary disease, Buruli ulcer, and osteomyelitis. These bacteria are a major cause of disseminated infections in immunocompromised patients and are a common cause of death in HIV-positive persons (WHO, 2006).

Atypical *Mycobacterium spp.* multiplies in a variety of suitable water environments, notably biofilms. One of the most commonly occurring species is *M. gordonae*. Other species have also been isolated from water, including *M. avium*, *M. intracellulare*, *M. kansasii*, *M. fortuitum* and *M. chelonae*. High numbers of atypical *Mycobacterium spp.* may occur in distribution systems after events that dislodge biofilms, such as flushing or flow reversals. They are relatively resistant to treatment and disinfection and have been detected in well operated and maintained drinking-water supplies with HPC less than 500/ml and

total chlorine residuals of up to 2.8 mg/liter. The growth of these organisms in biofilms reduces the effectiveness of disinfection. In one survey, the organisms were detected in 54% of ice and 35% of public drinking-water samples (WHO, 2006).

In Rotterdam, Netherlands, an investigation into the frequent isolation of *M. kansasii* from clinical specimens revealed the presence of the same strains, confirmed by phage type and weak nitrase activity, in tap water. An increase in numbers of infections by the *M. avium* complex in Massachusetts, USA, has also been attributed to their incidence in drinking-water. In all these cases, there is only circumstantial evidence of a causal relationship between the occurrence of the bacteria in drinking-water and human disease. Infections have been linked to contaminated water in spas (WHO, 2006).

Pathogenic microbes: A number of studies reviewed by Gould (1999) and Lye (2002) have identified various pathogens including *Salmonella, Shigella, Vibrio, Clostridium, Legionella, Campylobacter, Cryptosporidium and Giardia spp.* in samples taken from rainwater tanks (Evans *et al.,* 2005).

Campylobacterr spp. are microaerophilic (require decreased oxygen) and capnophilic (require increased carbon dioxide), Gram-negative, curved spiral rods with a single unsheathed polar flagellum (WHO, 2006).

Campylobacter spp. are one of the most important causes of acute gastroenteritis worldwide. *Campylobacter jejuni* is the most frequently isolated species from patients with acute diarrhoeal disease, whereas *C. coli*, *C. laridis* and *C. fetus* have also been isolated in a small proportion of cases (WHO, 2006).

Clinical symptoms of *C. jejuni* infection are characterized by abdominal pain, diarrhoea (with or without blood or fecal leukocytes), vomiting, chills and fever. The infection is self-limited and resolves in 3–7 days. Relapses may occur in 5–10% of untreated patients. Other clinical manifestations of *C. jejuni* infections in humans include reactive arthritis and meningitis. Several reports have associated *C. jejuni* infection with Guillain-Barré syndrome, an acute demyelinating disease of the peripheral nerves (WHO, 2006).

Thermophilic *Campylobacter* species, particularly *jejuni* and *coli*, are recognized as the most frequently isolated bacteria that cause diarrhoeal disease in humans (Butzler *et al*. 1992; Denis *et al*., 1999).

The occurrence of *Campylobacter* in surface waters has proved to be strongly dependent on rainfall, water temperature and the presence of waterfow (WHO, 2006).

Contaminated drinking-water supplies have been identified as a source of outbreaks. The number of cases in these outbreaks ranged from a few to several thousand, with sources including unchlorinated or inadequately chlorinated

surface water supplies and fecal contamination of water storage reservoirs by wild birds (WHO, 2006).

Contaminated drinking-water supplies have been identified as a significant source of outbreaks of campylobacteriosis. The detection of waterborne outbreaks and cases appears to be increasing. Waterborne transmission has been confirmed by the isolation of the same strains from patients and drinking-water they had consumed (WHO, 2006).

Storages of treated and disinfected water should be protected from bird feces. *Campylobacter spp*. are fecally borne pathogens and are not particularly resistant to disinfection. Hence, *E. coli* (or thermotolerant coliforms) is an appropriate indicator for the presence/absence of *Campylobacter spp.* in drinking-water supplies (WHO, 2006).

Escherichia coli: pathogenic strains. *E. coli is* found in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. A limited number of enteropathogenic strains can cause acute diarrhoea (WHO, 2006).

Shiga Toxigenic *Escherichia coli* (STEC) is an important cause of gastrointestinal disease in humans, particularly since such infections may result in life-threatening

sequelae such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Paton and Paton,1997).

Within the human disease-associated strains, those producing Shiga toxin type 2 (Stx2, encoded by *stx*2) appear to be more commonly responsible for serious complications such as HUS than those producing only Shiga toxin type 1 (Stx1, encoded by *stx*1) (Kleanthous et al., 1990; Ostroff et al., 1998). Furthermore, STEC belonging to serogroup O157 and, to a lesser extent, serogroup O111 are responsible for the vast majority of HUS outbreaks (Caprioli et al., 1994; Griffin, 1995; Minami, 1997; Paton et al., 1996; Reilly, 1997; Paton and Paton., 1997).

Waterborne transmission of pathogenic *E. coli* has been well documented for recreational waters and contaminated drinking-water. A well publicized waterborne outbreak of illness caused by *E. coli* O157:H7 (and *Campylobacter jejuni*) occurred in the farming community of Walkerton in Ontario, Canada. The outbreak took place in May, 2000 and led to 7 deaths and more than 2300 illnesses. The drinking-water supply was contaminated by rainwater runoff containing cattle excreta (WHO, 2006).

Helicobacter pylori originally classified as *Campylobacter pylori*, is a Gram-negative, microaerophilic, spiral-shaped, motile bacterium. There are at least 14 species of *Helicobacter*, but only *H. pylorus has* been identified as a human pathogen (WHO, 2006).

Helicobacter pylori live in the stomach; although most infections are asymptomatic, the organism is associated with chronic gastritis, which may lead to complications such as peptic and duodenal ulcer disease and gastric cancer. Whether the organism is truly the cause of these conditions remains unclear (WHO, 2006).

The majority of *H. pylori* infections are initiated in childhood and without treatment are chronic. The infections are more prevalent in developing countries and are associated with overcrowded living conditions. Interfamilial clustering is common (WHO, 2006).

Helicobacter pylori have been detected in water. Although *H. pylori* is unlikely to grow in the environment, it has been found to survive for 3 weeks in biofilms and up to 20–30 days in surface waters. In a study conducted in the USA, *H. pylori* was found in the majority of surface water and shallow groundwater samples. The presence of *H. pylori* was not correlated with the presence of *E. coli*. Possible contamination of the environment can be through children with diarrhoea or through vomiting by children as well as adults (WHO, 2006).

Fecal-oral and person-to-person contact have been suggested as possible routes of exposure to *H. pylori*; however, drinking water may be an additional source (Hulte´n *et al*., 1996; Horiuchi *et al.,* 2001; Mazari-Hiriart *et al.,* 2001; McDaniels *et al*., 2005)

Survival of *H. pylori* in different types of waters has been reported to extend from days to weeks at temperatures between 4-15 ºC over a wide pH range (Mai *et al*., 1991; West *et al*., 1992; Beneduce *et al.,* 2003). Biofilms in drinking water systems have been reported as possible reservoirs of *H. pylori* (Mackay *et al*., 1998; Bunn *et al*., 2002; McDaniels *et al.,* 2005).

Several articles have been published using conventional PCR to detect *H. pylori* in a number of types of waters including ground water, surface water, treated and untreated wastewater, marine waters and also biofilms (Hulte´n *et al.*, 1996, 1998; Mackay *et al.,* 1998; Sasaki *et al.,* 1999; Mazari-Hiriart *et al.,* 2001; Lu *et al.,* 2002; Watson *et al.,* 2004; Carbone *et al.,* 2005, McDaniels *et al*., 2005).

Legionella is a member of the family Legionellaceae, has at least 42 species. *Legionellae* are Gram-negative, rod-shaped, non-spore-forming bacteria that require L-cysteine for growth and primary isolation. *Legionella spp.* are heterotrophic bacteria found in a wide range of water environments and can proliferate at temperatures above 25 °C (WHO, 2006).

Although all *Legionella spp*. are considered potentially pathogenic for humans, *L. pneumophila* is the major waterborne pathogen responsible for legionellosis (WHO, 2006).

Legionella spp. are members of the natural flora of many freshwater environments, such as rivers, streams and impoundments, where they occur in relatively low numbers. However, they thrive in certain human-made water environments, such as water cooling devices (cooling towers and evaporative condensers) associated with air conditioning systems, hot water distribution systems and spas, which provide suitable temperatures (25–50 °C) and conditions for their multiplication. Devices that support multiplication of *Legionella* have been associated with outbreaks of Legionnaires' disease (WHO, 2006).

Legionella survive and grow in biofilms and sediments and are more easily detected from swab samples than from flowing water. Legionellae can be ingested by trophozoites of certain amoebae such as *Acanthamoeba*, *Hartmanella* and *Naegleria*, which may play a role in their persistence in water environments (WHO, 2006).

In February 2006, an outbreak of Legionnaires' disease was identified in Beachlands, a small, isolated east Auckland suburb (Simmons *et al.*, 2007). The cause of the outbreak was rainwater collected through roof catchment systems and found to be contaminated with *L. pneumophila*. The same pathogen was found in another suburb 4km east of Beachlands. This is the first outbreak of this disease linked to roof collected rainwater supplies and the first isolation of *Legionella* from these systems in New Zealand (Simmons *et al.*, 2007).

Salmonella *spp*. belong to the family Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. The classification of *Salmonella* includes 3 species (*S. enterica* or *S. choleraesuis*, *S. bongori* and *S. typhi*) (WHO, 2006).

Salmonella infections typically cause four clinical manifestations: gastroenteritis (Ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections (WHO, 2006).

The pathogens typically gain entry into water systems through fecal contamination from sewage discharges, livestock and wild animals (WHO, 2006). Waterborne typhoid fever outbreaks have devastating public health implications. However, despite their widespread occurrence, non-typhoidal *Salmonella spp.* rarely causes drinking-water-borne outbreaks. Transmission, most commonly involving *S. Typhimurium*, has been associated with the consumption of contaminated groundwater and surface water supplies. In an outbreak of illness associated with a communal rainwater supply, bird faeces were implicated as a source of contamination. *Salmonella spp*. are relatively sensitive to disinfection (WHO, 2006).

Shigella spp. are Gram-negative, non-spore-forming, non-motile, rod-like members of the family Enterobacteriaceae, which grow in the presence or absence of oxygen. Members of the genus have a complex antigenic pattern, and classification is based on their somatic O antigens, many of which are shared with other enteric bacilli, including *E. coli*. There are four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* (WHO, 2006).

Shigella spp. can cause serious intestinal diseases, including bacillary dysentery. Over 2 million infections occur each year, resulting in about 600 000 deaths, predominantly in developing countries (WHO, 2006).

In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrofils in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome. *Shigella spp*. seem to be better adapted to cause human disease than most other enteric bacterial pathogens (WHO, 2006).

Shigella spp. are enteric pathogens predominantly transmitted by the fecal–oral route through person-to-person contact, contaminated food and water. Flies have also been identified as a transmission vector from contaminated fecal waste (WHO, 2006).

A number of large waterborne outbreaks of shigellosis have been recorded. As the organisms are not particularly stable in water environments, their presence in drinking-water indicates recent human fecal pollution. Available data on prevalence in water supplies may be an underestimate, because detection techniques generally used can have a relatively low sensitivity and reliability. The control of *Shigella spp.* in drinking- water supplies is of special public health importance in view of the severity of the disease caused. *Shigella spp*. are relatively sensitive to disinfection (WHO, 2006).

1.2.2 Chemical physical quality of rainwater:

pH:

As a raindrop falls and comes in contact with the atmosphere, it dissolves naturally occurring carbon dioxide to form a weak acid. The resultant pH is about 5.7, whereas a pH of 7.0 is neutral. The pH values, ranging from 7.63 to 8.80, indicate that in the studied area the rain is not acid. In this pH range, undesirable chemical reactions that may occur during the storage are eliminated (Zhu *et al.,* 2004).

As a result of a study in Auckland, New Zealand (Simmons *et al.,* 2001), fifty-one (40.8%) supplies had pH levels outside the guideline values for drinking water of 6.5-8.5. Ninety-three (74.4%) supplies had water of alkaline pH more than 7, with 32 (25.6%) being of acid pH (5.7). Water sampled from supplies with ferrocement water storage tanks was significantly more likely to be alkaline with a median pH

of 7.5. The median pH of tap water for systems with a non-ferrocement tank (plastic, wood, fiberglass or galvanized iron) was 5.9 (Simmons *et al.,* 2001).

Turbidity:

Particulate matter refers to smoke, dust, and soot suspended in the air. As rainwater falls through the atmosphere, it can incorporate these contaminants (Texas Manual, 2005). These matters will increase the turbidity level in the rain water harvesting system.

In general, the rainwater supplies exhibited a high degree of clarity with a median turbidity of 0.56 NTU. As a result of a study in Auckland, New Zealand, only five (4.0%) supplies had turbidity levels above the maximum guideline value of the NZDWS of 2.5 NTU (Simmons *et al*., 2001).

Electrical conductivity (EC):

The seasonal variation of conductivity and chlorides demonstrate the influence of the sea environment. In winter, when the weather conditions are severe with many heavy rainfall events and strong winds, conductivity values are the highest. The conductivity values do not differ in spring, at the statistical significant level set, while in summer and autumn a decrease is detected, probably due to the sedimentation that occurs in the stored rainwater inside the water tanks (Sazakli *et al.*, 2007).

Total Dissolved Solids (TDS)/Salinity:

TDS used as a measure of the salinity of drinking water. Inorganic salts including sodium, potassium, calcium, magnesium, chloride, sulphate, bicarbonate, carbonate and nitrate ions and often a small amount of organic matter dissolved in water (Health Department of South Australia, 2008). TDS are measured in mg/L (or parts per million (ppm)). Salinity is measured in micro Siemens per cm :(μS/cm) (or EC units) and is related to TDS as follows

TDS (mg/L) = Salinity (EC) x Con

Con: constant value ranges from 0.5-0.7 depending on the water source

The World Health Organization (WHO) considers that the palatability of water with a TDS level of less than 600 mg/L is generally considered to be good; drinking-water becomes significantly and increasingly unpalatable at TDS levels greater than about 1000 mg/L. They consider that a TDS level of >1200 mg/L may be objectionable to consumers. No health-based limit since "TDS occurs in drinking-water at concentrations well below those at which toxic effects may .occur

It was found that the level of total dissolved solids (TDS) and salinity in rainwater samples collected from different roofing materials from the rural communities of Delta state were generally well below the maximum acceptable threshold of WHO (Efe, 2006).

Total dissolved solids (TDS) in rainwater, originating from particulate matter suspended in the atmosphere, range from 2-20 mg/l compared with municipal water TDS ranges of 100 ppm to more than 800 ppm. (Texas Manual, 2005).

Nitrate:

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. Nitrate is used mainly in inorganic fertilizers, and sodium nitrite is used as a food preservative, especially in cured meats (WHO, 2003).

The primary health concern regarding nitrate and nitrite is the formation of methaemoglobinaemia, so-called "blue-baby syndrome." Nitrate is reduced to nitrite in the stomach of infants, and nitrite is able to oxidize hemoglobin (Hb) to methaemoglobin (metHb), which is unable to transport oxygen around the body.

The Hb of young infants is more susceptible to metHb formation than that of older children and adults; this is believed to be the result of the large proportion of fetal Hb, which is more easily oxidized to metHb, still present in the blood of infants (WHO, 2003).

1.3 Heavy metals in drinking water:

1.3.1 Lead in drinking water:

Lead is the commonest of the heavy elements, accounting for 13 mg/kg of the Earth's crust. Several stable isotopes of lead exist in nature, including, in order of abundance, ^{208}Pb, ^{206}Pb, ^{207}Pb, and ^{204}Pb (WHO, 1996).

Lead is used in the production of lead acid batteries, solder, alloys, cable sheathing, pigments, rust inhibitors, ammunition, glazes, and plastic stabilizers. In addition, a major global source of lead is leaded gasoline. This source causes lead to be released into the atmosphere from car exhausts. As a result, rainwater may become contaminated with water.

Lead is a cumulative general poison, infants, children up to 6 years of age, the fetus, and pregnant women being the most susceptible to adverse health effects. Its effects on the central nervous system can be particularly serious (WHO, .(1996

1.3.2 Copper in drinking water:

Copper is a transition metal that is stable in its metallic state and forms monovalent (cuprous) and divalent (cupric) cations (WHO, 1996). Copper is found in surface water, groundwater, seawater and drinking-water, but it is primarily present in complexes or as particulate matter. Copper concentrations in

drinking-water vary widely as a result of variations in water characteristics, such as pH, hardness and copper availability in the distribution system (WHO, 2004). The acute lethal dose for adults lies between 4 and 400 mg of copper (II) ion per kg of body weight (Chuttani *et al.,* 1965; Jantsch *et al*., 1984 &1985; Agarwal *et al.*, 1993). Individuals ingesting large doses of copper present with gastrointestinal bleeding, haematuria, intravascular haemolysis, methaemoglobinaemia, hepatocellular toxicity, acute renal failure and oliguria (Agarwal *et al*., 1993). At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, and diarrhoea) (WHO, 2004).

1.4 Guideline values for drinking water standard:

Table (1): World Health Organization (WHO) guidelines for drinking –water quality (WHO, 1984).

Organism\constituent	**Unit**	**Guideline value**
Total coliforms	Number /100 ml	0
Faecal coliforms	Number /100 ml	0
Lead	mg/l	0.05
Nitrate	mg/l	10
Copper	mg/l	1.0
pH	---	6.5-8.5
Total Dissolved Solids (TDS)	mg/l	1000
Temperature	---	No guideline value set
Turbidity	Nephelometric Turbidity Units (NTU)	5

1.5 Polymerase Chain Reaction (PCR)

PCR, the quick, easy method for generating unlimited copies of any fragment of DNA, is one of those scientific developments that actually deserves time-worn superlatives like “revolutionary” and “breakthrough (Powledge, 2004). There are many advantages for PCR that makes it the dominant isolation methods:

1. PCR is a cell-free method of DNA replication, and requires no cleanup of unwanted cellular debris or vector DNA.
2. PCR has been shown to efficiently amplify targets up to 35 kb in length.
3. PCR is a specific process that targets only the desired DNA to be copied.
4. PCR can be end-labeled with a variety of modifications.
5. PCR is a high fidelity process, with error rates between 1 in 10,000 and 1 in 100,000. It is far superior to synthesis for long DNA sequences. The use of other novel thermostable enzymes can improve PCR’s fidelity.
6. March 28, 2005 marked the expiration of the foundational PCR patents in the U.S.A. and equivalent ex-U.S.A. foundational PCR patents expired on March 28, 2006. The expiration of these key patents allows PCR to be used for many applications without licensing fees.

PCR based on the important scientific fact that makes it very useful. The fact is: The genetic material of each living organism (plant, animal, bacterium) or virus, possesses sequences of its nucleotide building blocks (usually DNA, sometimes

RNA) that are uniquely and specifically present only in its own species(Powledge, 2004).

PCR exploits the remarkable natural function of the enzymes known as polymerases. These enzymes are present in all living things, and their job is to copy genetic material (and also proofread and correct the copies). Sometimes referred to as "molecular photocopying," PCR can characterize, analyze, and synthesize any specific piece of DNA or RNA (Powledge, 2004).

The most interesting advantage, that PCR can works even on extremely complicated mixtures, seeking out, identifying, and duplicating a particular bit of genetic material from blood, hair, or tissue specimens, from microbes, animals, or plants, some of them many thousands—or possibly even millions— of years old (Powledge, 2004).

PCR requires a template molecule—the DNA or RNA you want to copy—and two primer molecules to get the copying process started. The primers are short chains of the four different chemical components that make up any strand of genetic material. These four components are like bricks or building blocks that are used to construct genetic molecules; in the lab they are called nucleotides or bases (Powledge, 2004).

DNA itself is a chain of nucleotides. Under most conditions, DNA is double stranded, consisting of two such nucleotide chains that wind around each other in

the famous shape known as the double helix. Primers are single-stranded. They consist of a string of nucleotides in a specific order that will, under the right conditions, bind to a specific complementary sequence of nucleotides in another piece of single-stranded RNA or DNA (Powledge, 2004).

For PCR, primers must be duplicates of nucleotide sequences on either side of the piece of DNA of interest, which means that the exact order of the primers' nucleotides must already be known. These flanking sequences can be constructed in the lab, or purchased from commercial suppliers (Powledge, 2004).

There are three basic steps in PCR **Figure 2**. First, the target genetic material must be denatured—that is, the strands of its helix must be unwound and separated—by heating to 90–96°C. The second step is hybridization or annealing, in which the primers bind to their complementary bases on the now single stranded DNA. The third is DNA synthesis by a polymerase (Powledge, 2004).

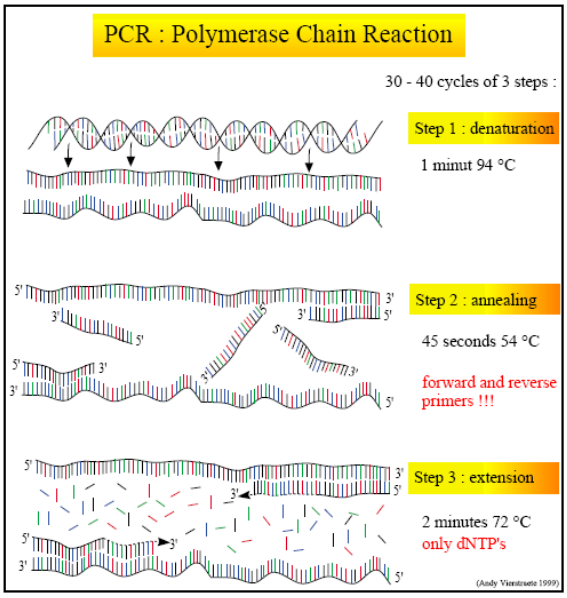


Figure 2: The different steps in PCR (Andy Vierstraete, 1999).

Starting from the primer, the polymerase can read a template strand and match it with complementary nucleotides very quickly. The result is two new helixes in place of the first, each composed of one of the original strands plus its newly assembled complementary strand (Powledge, 2004).

All PCR really requires in the way of equipment is a reaction tube, reagents, and a source of heat. But different temperatures are optimal for each of the three steps, so machines now control these temperature variations automatically (Powledge, 2004).

To get more of the DNA you want, just repeat the process, beginning by denaturing the DNA you've already made. The amount wills double every time. With the cycle of rapid heating and cooling controlled automatically, nature-aided

by scientist supplied primers, polymerase, nucleotides, and chemical reagents does the rest. Each cycle takes only 1–3 minutes, so repeating the process for just 45 minutes can generate millions of copies of a specific DNA strand. Once the primers have been characterized and obtained, PCR can do in a week's work what used to take a year (Powledge, 2004).

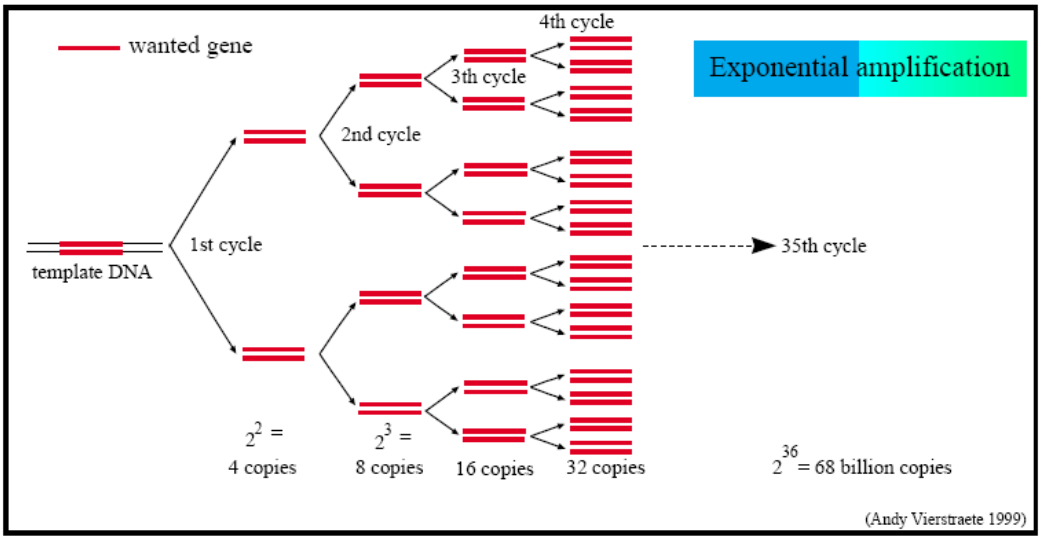


Figure 3: The exponential amplification of the gene in PCR (Andy Vierstraete, 1999).

1.6 Objectives:

The present study aims at:

- Evaluating health risks associated with consuming rainwater without treatment by analyzing the chemical, physical and microbial quality of stored rainwater from Qalqilia and Ramallah regions.
- Investigate possible seasonal variability in the harvested rainwater.

- Applying modern molecular biology tools to investigate microbial water quality.

CHPTER TWO

2. MATERIALS AND METHODS

2.1. Water sampling and study area:

Sampling of harvested rainwater was performed twice a year, in August 2006 (summer samples) and March 2007 (winter samples). Each season, a total of 21 rainwater samples were collected from storage tanks using sterile glass bottles. Samples were taken from the middle of the storage tank. Thereafter, samples were transported in an ice box directly to the laboratory for chemical and microbiological analysis.

The study areas covered in this project were Qalqilia and Ramallah districts. In Qalqilia district, samples were obtained from Ezbit Salman, Ezbet al Ashqar and Al Nabi Elyas. In Ramallah district, samples were obtained from Atarah, Birzeit, Umsafah, Kobar and Dair Sudan. The number of samples obtained from each location within the study area is shown in **Table 2.**

Table 2: Ramallah and Qalqilia description sites

District	site	Well ID	Number of samples
Ramallah	Atarah village	12,13,14	3
	Birzeit village	15,16,17 and 18	4
	Umsafah village	19	1
	Daier al Sudan village	20	1
	Kuoper village	21	1
	Total =10		
Qalqilia	Ezbit Salman	1,2,3	3
	Ezbet al Ashqar	4,5,6	3
	Al Nabi Elyas village	7,8,9,10and 11	5
	Total =11		

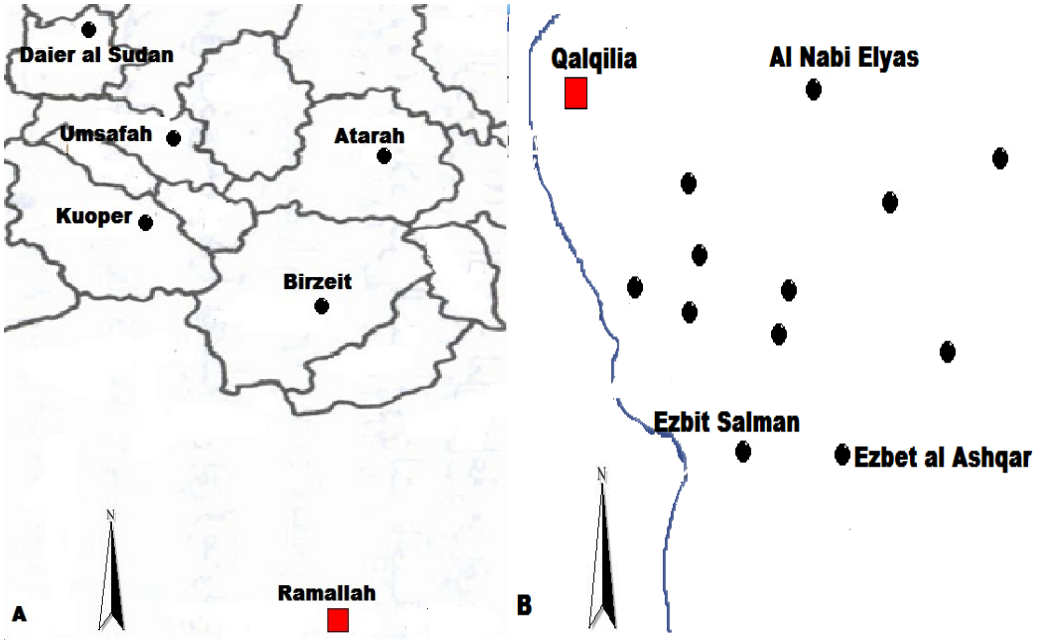


Figure 4: Sampling sites in Ramallah (A) and Qalqilia district (B)

Figure 5: Storage tanks of harvesting rainwater systems in Qalqilia District.

2.2 Microbiological Analysis:

Total, fecal coliforms and Heterotrophic Plate Count were determined in all rainwater samples by membrane filtration technique according to Standard Methods for the Examination of Water and Wastewater (APHA, 1995) using 45µm pore size filter (Sartorius, Germany). Media used for these three tests were

Endo broth, mFC broth base and m-HPC agar (Difco Laboratories, USA) for total, fecal and HPC, respectively.

Pathogens and opportunistic bacteria were tested using Polymerase Chain Reaction (PCR) as described below:

2.2.1 DNA Extraction:

A volume 100ml from each rainwater sample was filtered using 0.45 µm pores size membrane filter (Sartorius, Germany) and enriched overnight in peptone water (Merck, Germany). Thereafter, bacteria were collected by centrifugation and the pellet obtained was used for DNA extraction.

DNA extraction method was according to Elferink *et al*. (1997) with minor change as follows: the bacterial pellet was dissolved in 400 µl sterile TE (10 mmol/l TRIS/HCl + 1 mmol/l EDTA, pH= 8) in a 2.0 ml tube. Then, 200 µl TRIS/HCl buffered phenol pH= 8 were added into the tube and vortexed for 1min. The aqueous phase was separated by centrifugation for 10 minutes at maximum speed (with pre-cooled centrifuge) and transferred to a new sterile tube. Thereafter, extracted with 500µl phenol/chloroform/isoamylalcohol 25:24:1 (v:v:v) and the DNA was separated from proteins by another centrifugation as above.

The upper layer was extracted again with phenol/chloroform/isoamylalcohol 25:24:1 (v:v:v), centrifuged and extracted with 500 µl chloroform/isoamylalcohol 24:1 (v:v). After that, another centrifugation for 5 minutes at maximum speed was done. The water phase was transferred to a new tube and the volume adjusted to 0.5 ml. Consequently, DNA was precipitated with 1 ml of 96% ice-cold ethanol and 40µl sodium acetate (3 mol/l, pH= 5.2) and stored overnight at -20°C.

After storing, the samples were subjected to centrifugation for 15 minutes at maximum speed (with pre-cooled centrifuge), after which, the pellet was washed with 70% ice-cold ethanol, dried and dissolve in 100µl TE buffer.

For *Legionella* detection, a volume of 500ml was filtered and enriched in buffered charcoal yeast extract (BBL BCYE Agar Base) (Becton, Dickinson, USA**)** (Presti *et al* ., 1998). For *H. pylori* detection, samples were filtered as above and enriched in Blood Agar Base (Oxoid, England). Thereafter, DNA was extracted as described earlier (Flanigana and Rodgersa, 2003).

All *Helicobacter* strains were grown on Trypticase Soy agar plates with 5% sheep blood under microaerophilic growth conditions using a BBL CampyPak Plus microaerophilic system.(McDaniels *et al.,* 2005).

2.3 PCR reaction:

The PCR reaction was conducted in a 50µl reaction volume containing (Buffer). 0.2mM dNTPs, 1.5mM $MgCl_2$, 1.25 U Taq DNA polymerase (Promega, USA) and 0.4µM of each primer listed in **Table 3**. The temperature profile of each PCR

is listed in **Table 4**. The thermocycler used was Hybaid Omni-Gene thermocycler. The PCR product was separated in 1.5% agarose, stained with ethidium bromide, visualized with UV transluminator and documented with Kodak Polaroid Gelcam, UK.

Table 3: The primers for all microorganisms tested in this study.

Microbe	Primer Sequence (5'→ 3')	Target gene	Product band	Company	Reference
Shigella	H8:GTT CCT TGA CCG CCT TTC CGA TAC H15:GCCGGTCAGCCACC C TC	*ipaH*	620 bp	Invitrogen	Theron *et al*., (2000)
Legionella	LEG1:GTCATGAGGAATC TCGCTG LEG2:CTGGCTTCTTCCAG CTTCA	-----	900 bp	Invitrogen	Persing et al., 1993
$STEX_1$	F:ATA AAT CGC CAT TCG TTG ACT AC R:AGA ACG CCC ACT GAG ATC ATC	stx_1	180 bp	Synteeza	Paton *et al*., (1997)
$STEX_2$	F:GGC ACT GTC TGA AAC TGC TCC R:TCG CCA GTT ATC TGA CAT TCT G	stx_2	255 bp	Synteeza	Paton *et al.,* (1997)
Campylobacter	F:ATC TAA TGG CTT AAC CAT TAA AC R: GGA CGG TAA CTA GTT TAG TAT T	*16S rRNA*	857 bp	Invitrogen	Denis *et al*., (1999)
Salmonella	139:GTG AAA TTA TCG CCA CGT TCG GGC AA 141:TCA TCG CAC CGT CAA AGG AAC C	*invA*	284 bp	Invitrogen	Rahn *et al.,* (1992)
Citrobacter	Crt4F:TTG GCG TCC AGC GCA TTC A Crt4R:AAT TCC AGC CTT CGG CAA ACG	*cfa*	100 bp	Synteeza	Kacl_kov et al.,2005)
Acinetobacter	rA1(CCT GAA TCT TCT GGT AAA AC) rA2(GTT TCT GGG CTG CCA AAC ATT AC)	*recA*	425 bp	Invitrogen	Krawczyk *et al* ., (2001)

Pseudomonas aeruginosa	F:TTC CCT CGC AGA GAA AAC ATC R:CCT GGT TGA TCA GGT CGA TCT	*ETA*	339 bp	Invitrogen	Khan *et al.,* (1994)
Mycobacterium	65kDaf2:TAG GTC GGG ACG GTG AG 65kDar3:TTG CGA AGT GAT TCC TCC	(65kDa hsp)	Var	Invitrogen	Tobler *et al*., (2005)
H.pylori	F: GCT AAG AGA TCA GCC TAT GTC C R: GCG CAA TCA GCG TCA GTA ATG	16S gene	520 bp	Invitrogen	Hoshina, *et al.,* (1990)
Aromonas hydrophilia	F:GCA GTG GTT TAT GAC AAA GAC G R:TTA GAA GTT GTA TTG CAG GGC	OmpTS	1008 bp	Invitrogen	Khushiram ani *et al.,* (2006)

.**Table 4**: PCR program for pathogenic and opportunistic bacteria

Microorganisms	**PCR stages and time**				
	Pre denaturation	**Denatuoration**	**Annealing**	**extention**	**Finishing step**
Acinetobacter	C for 95 4min	C for 15 sec 95	C for 50 30sec	C for 72 40sec	C for 72 7min
Aeromonas	C for 95 3min	C for 15 sec 95	C for 59 30sec	C for 72 40sec	C for 72 7min
Pseudomonas	C for 95 4min	C for 15 sec 95	C for 58 30sec	C for 72 40sec	C for 72 7min
Mycobacterium	C for 95 4min	C for 15 sec 95	C for 53 30sec	C for 72 40sec	C for 72 7min
Campylobacter	C for 95 3min	C for 30 sec 95	C for 57 40sec	C for 72 40sec	C for 72 7min
STEC	C for 95 3min	C for 15 sec 95	C for 60 30sec	C for 72 40sec	C for 72 7min
Helicobacter pylori	C for 95 3min	C for 15 sec 95	C for 62 30sec	C for 72 40sec	C for 72 7min
legionella sps	C for 95 3min	C for 15 sec 95	C for 60 30sec	C for 72 40sec	C for 72 7min
Salmonella spp	C for 95 4min	C for 15 sec 95	C for 63 30sec	C for 72 40sec	C for 72 7min
Shigella spp	C for 95	C for 15 sec 95	C for 60	C for 72	C for 72

	4min		30sec	40sec	7min
citrobacter	C for 95 4min	C for 15 sec 95	C for 60 30sec	C for 72 40sec	C for 72 40sec

2.4 Chemical tests:

2.4.1 pH test:

The pH of each sample collected was measured using sensION1 pH/mv meter (HACH Company, USA).

2.4.2 Turbidity test:

Turbidity of the harvested rainwater samples was tested using portable turbidity meter (HACH Company, USA).

2.4.3 TDS, EC and Salinity tests:

These tests were done using sensION4-band conductivity cell (HACH Company, USA).

2.4.4 Nitrate test:

Nitrate levels in harvested rainwater were measured using capillary ion analyzer, (Millipore Water Instruments, USA).

2.4.5 Heavy metals tests

Levels of copper and lead were measured in harvested rainwater using atomic absorption spectrophotometer AAnalyst 600 (Perkin Eimer, USA).

CHAPTER THREE

3. RESULTS

3.1 Chemical Quality of Rainwater:

3.1.1 Temperature and pH:

Temperature and pH measurements were taken *in situ*. Results of these measurements in both summer and winter samples are summarized in **Table 5.** The temperature of summer samples collected from both districts ranged between 20-28.8 ^{o}C and those of winter samples from 12-19 ^{o}C. The average temperature for all summer and winter samples was 24 ^{o}C and 16.5 ^{o}C, respectively.

The pH values for the harvested rainwater samples ranged between 7.4-9.9 for summer samples and 4.8-8.6 for winter samples **Table 5.** The average pH for all summer and winter samples was 8.5 and 7.2, respectively. Water samples from 7

wells collected in summer were having pH values greater than the maximum WHO guideline value (WHO, 1984) for the pH in drinking water that equals 8.5 (**Figure 6**). Five of these wells were located in Qalqilia district, while the remaining two were from Ramallah district. On the other hand, 4 wells were having rainwater with a pH value less than the minimum guideline value for pH in drinking water that equals 6.5 (**Figure 6**). All these wells were located in Qalqilia district.

Table 5: Temperature and pH measurements for harvested rainwater samples collected from Qalqilia and Ramallah Districts during summer 2006/winter 2007.

Well		Temperature		pH	
ID	**Location**	**Summer samples**	**Winter samples**	**Summer samples**	**Winter samples**
1	Ezbit Salman	24.7	19.0	8.2	6.6
2	Ezbit Salman	27.0	18.0	8.0	6.0
3	Ezbit Salman	28.8	19.0	7.9	4.8
4	Ezbit al Ashqar	24.2	17.0	8.4	5.0
5	Ezbit al Ashqar	25.6	18.0	8.4	6.9
6	Ezbit al Ashqar	25.6	19.0	8.5	6.9
7	Al Nabi Elyas	26.9	14.0	9.1	5.5
8	Al Nabi Elyas	25.0	19.0	9.3	7.2
9	Al Nabi Elyas	25.2	14.0	9.7	7.0
10	Al Nabi Elyas	25.3	15.0	9.9	7.4
11	Al Nabi Elyas	21.2	18.0	9.4	6.9
12	Atarah	20.0	14.0	7.4	7.9
13	Atarah	27.0	12.0	8.3	8.4
14	Atarah	27.0	13.0	7.5	7.8
15	Birzeit	20.0	13.0	9.0	8.6
16	Birzeit	20.0	17.0	8.4	8.2
17	Birzeit	23.0	17.0	7.7	7.6
18	Birzeit	24.0	17.0	9.0	7.9
19	Umsafah	20.0	17.0	7.8	7.9
20	Daier al Sudan	21.0	19.0	7.8	8.4
21	Kuoper	22.0	17.0	8.2	8.6

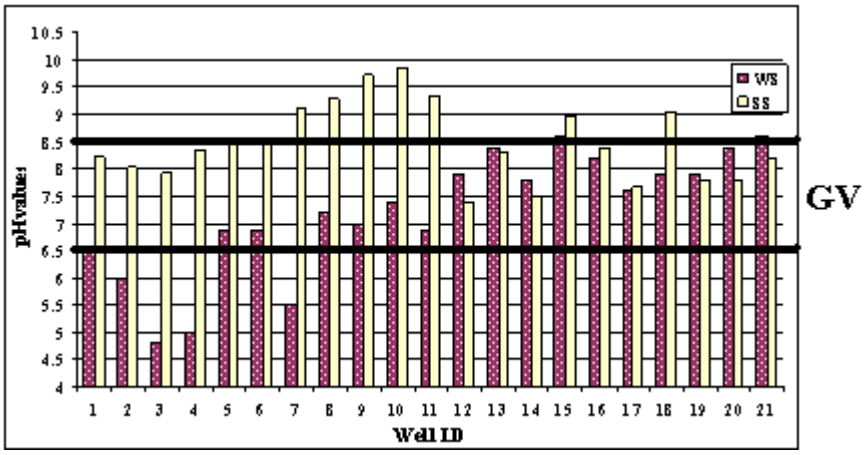


Figure 6: pH values for harvested rainwater collected from Qalqilia and Ramallah Districts in summer 2006/winter2007. SS: summer samples; WS: winter samples. GV: Guideline values.

3.1.2 EC, Salinity, TDS and Turbidity:

EC measurements for harvested rainwater samples collected in summer ranged between 180.5-834 µs/cm with an average value of 378.5 µs/cm (**Table 6**). In winter samples the EC values ranged between 121.5- 628 with an average of 286.4 µs/cm. As shown in **Figure 7**, most EC values were below 400; however, the EC value for rainwater sample collected from well 17 in summer was exceptionally high.

Table 6: Electrical Conductivity (EC), Salinity, Total Dissolved Solids (TDS) and turbidity for harvested rainwater samples collected from Qalqilia and Ramallah Districts during summer 2006 and winter 2007.

Well I.D	EC (µs/cm)		Salinity (‰)		TDS (mg/l)		Turbidity (NTU)	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
1	566	509	0.3	0.2	270	246	0.40	0.42
2	421	334	0.2	0.2	197.3	160.6	1.28	5.31
3	411	364	0.2	0.2	193.3	174.7	1.84	4.11
4	250	184.1	0.1	0.1	116.7	87.8	0.26	0.92
5	512	342	0.2	0.2	242	163.3	0.79	0.49
6	469	431	0.2	0.2	226	208	0.36	0.34
7	254	195.4	0.1	0.1	118.0	93.4	0.42	1.25
8	358	313	0.2	0.1	166.5	150.2	0.27	0.29
9	227	222	0.1	0.1	105.7	106.2	0.13	0.91
10	288	264	0.1	0.1	135.0	126.4	0.14	0.39
11	183.3	141.1	0.1	0.1	87.0	67.2	0.32	0.31
12	259	218	0.1	0.1	121.2	104.5	0.17	0.73
13	217	121.5	0.1	0.1	101.2	57.6	0.44	2.70
14	180.5	147.8	0.1	0.1	83.8	70.2	0.87	0.48
15	185.7	137.7	0.1	0.1	86.3	65.7	0.26	0.72
16	599	628	0.3	0.3	281	303	0.30	0.26
17	834	353	0.4	0.2	394	169.8	0.17	0.60
18	319	177.5	0.1	0.1	148.1	84.7	0.94	0.59
19	224	133.0	0.1	0.1	103.6	63.2	0.51	0.33
20	648	432	0.3	0.2	302	208	1.31	0.23
21	544	367	0.2	0.2	251	176.5	1.31	1.90

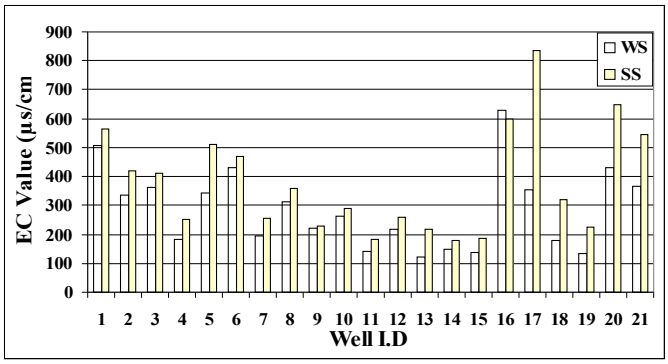


Figure 7: EC values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples.

Salinity measurements for harvested rainwater samples collected from storage wells in summer ranged from 0.1-0.4 ‰ with an average of 0.17‰ and during winter, values ranged from 0.1-0.3‰ with an average of 0.15‰. Exceptionally high value (0.4‰) was recorded in well number 17 (summer sample) (**Figure8**).

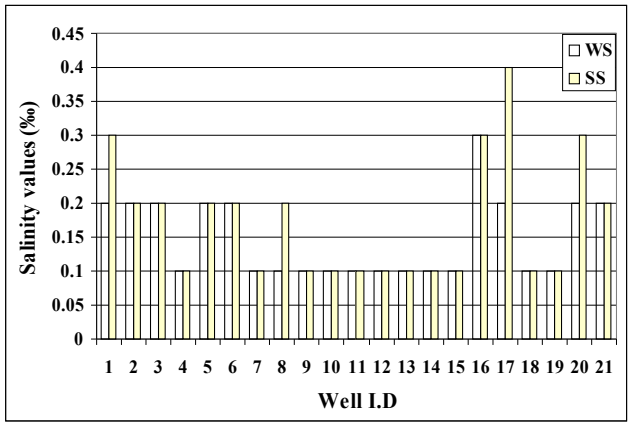


Figure 8: Salinity values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples. GV: Guideline value.

TDS measurements for harvested rainwater samples collected from storage wells in summer ranged from 83.8-394 mg/l with an average of 177.6 mg/l. In winter, values ranged from 57.6-303 mg/l with an average of 137.5 mg/l. As shown in **Figure 9**, all TDS values were well below the maximum guideline value of 1000mg/l (WHO, 1986).

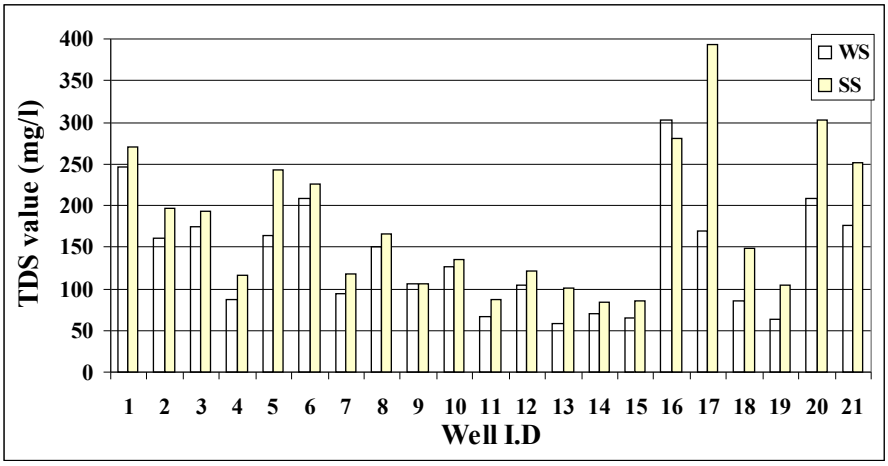


Figure 9: TDS values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples. GV: Guideline value.

Turbidity measurements for harvested rainwater samples collected from storage wells in summer ranged from 0.13-1.84 NTU with an average of 0.59 NTU. In winter, values ranged from 0.23-5.31 NTU with an average of 1.11 NTU. **Figure 10**, shows that all turbidity values were below the maximum guideline value of 5

NTU (WHO, 1986), except sample from well 2 where the turbidity value was 5.3 NTU.

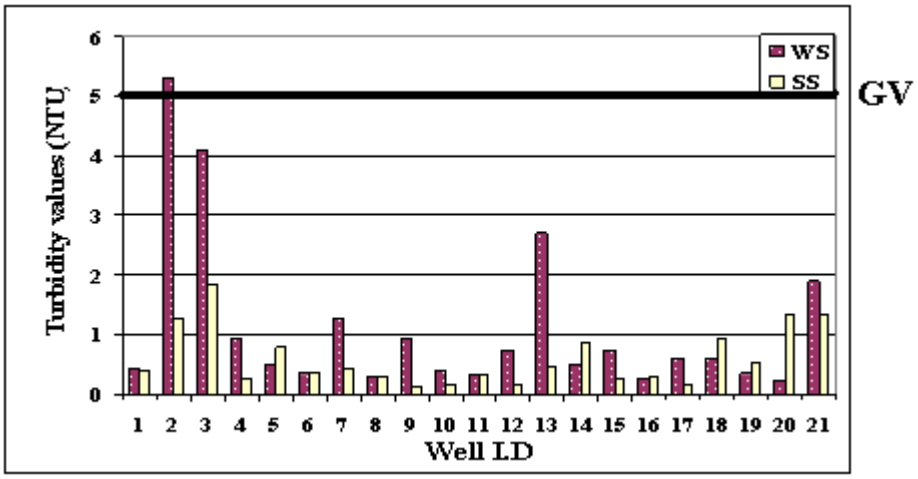


Figure 10: Turbidity values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples. GV: Guideline value.

3.1.3 Nitrate:

Nitrate measurements for harvested rainwater samples collected from storage wells in winter ranged from <0.01-5.78 ppm with an average of 3.06 ppm (**Table 7)**. As shown in **Figure 11**, all nitrate values were below 10 ppm (WHO, 1986).

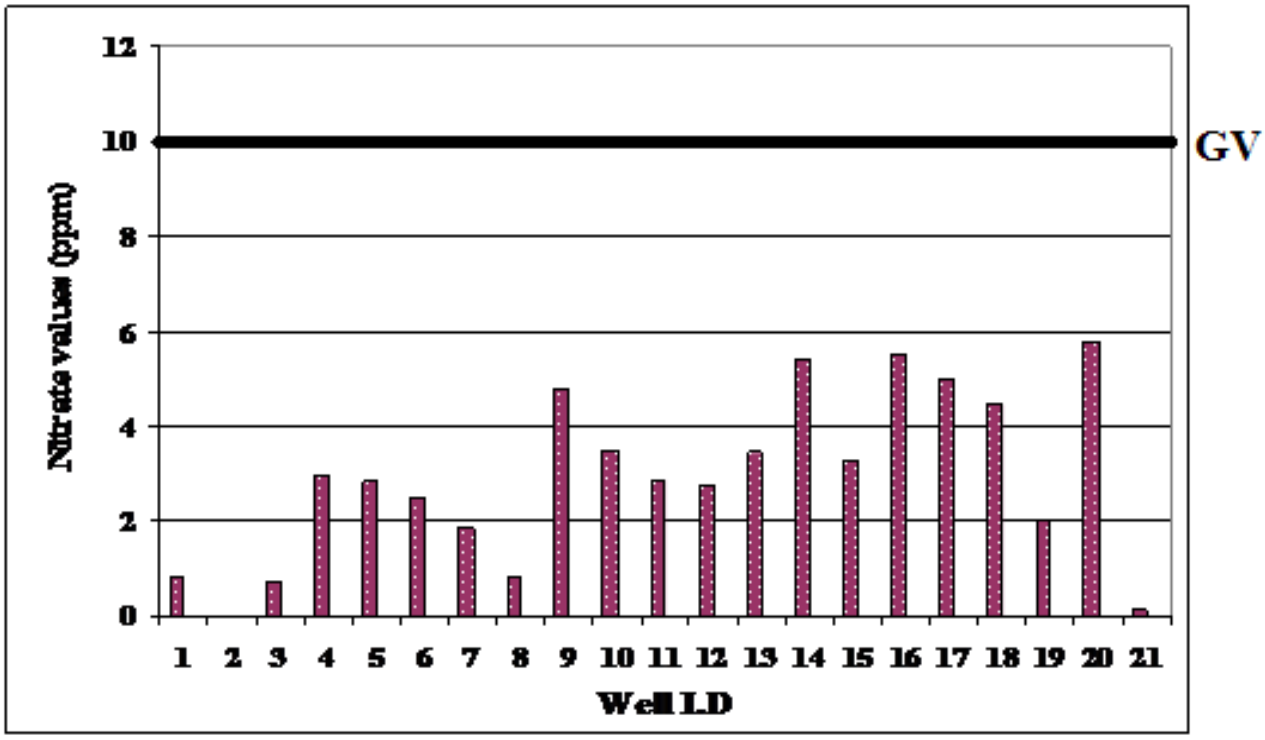


Figure 11: Levels of nitrate in harvested rainwater samples collected in winter from Qalqilia and Ramallah. GV: Guideline value.

Table 7: Nitrate results for harvested rainwater samples collected from Qalqilia and Ramallah Districts during winter 2007.

Well I.D	[NO_3]	Well I.D	[NO_3]
1	0.79	12	2.72
2	<0.01	13	3.45
3	0.73	14	5.39
4	2.92	15	3.26
5	2.84	16	5.52
6	2.49	17	4.99
7	1.86	18	4.45
8	0.795	19	2.03
9	4.78	20	5.78
10	3.49	21	0.11
11	2.85		

3.1.4 Heavy Metals:

Copper measurements for harvested rainwater samples collected from storage wells in summer ranged from 0.616-54.35 ppb with an average of 7.78 ppb and during winter, values ranged from 2.617- 30.84 ppb with an average of 8.76 ppb **Table 8**. As shown in **Figure 12**, all copper values were clearly below the maximum guideline value of 1000 ppb (WHO, 1986).

Table 8: Copper and lead results for harvested rainwater samples collected from Qalqilia and Ramallah Districts during summer 2006 and winter 2007.

Well I.D	Copper (ppb)		Lead (ppb)	
	Summer samples	Winter samples	Summer samples	Winter samples
1	6.87	13.8	1.17	1.24
2	1.20	15.4	1.10	2.74
3	3.40	5.16	1.71	2.03
4	5.46	8.24	1.14	0.84
5	2.17	6.05	0.79	1.50
6	4.39	5.65	1.39	0.93
7	9.32	9.13	1.16	1.81
8	7.97	4.75	1.68	2.01
9	3.36	4.84	1.97	1.16
10	1.62	7.44	3.54	0.853
11	6.07	15.3	1.18	0.949
12	4.62	3.79	1.19	3.29
13	0.616	11.1	1.19	30.7
14	2.61	3.20	1.03	1.44
15	54.3	6.69	0.37	2.07
16	6.86	30.8	0.500	5.62
17	7.89	13.1	0.412	3.36
18	3.12	4.94	1.03	1.78
19	3.02	2.63	0.464	18.1
20	10.21	5.44	1.25	1.01
21	18.2	6.49	0.889	2.24

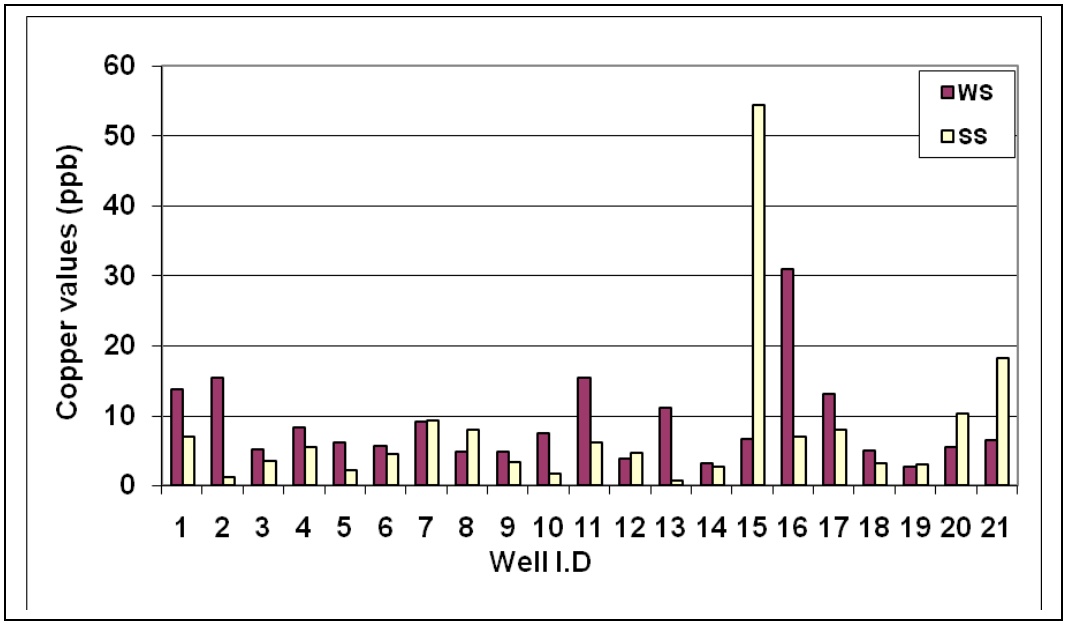


Figure 12: Copper values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples.

Lead measurements for harvested rainwater samples collected from storage wells in summer ranged from 0.37-3.54 ppb with an average of 1.28 ppb **Table 8**. In winter, values ranged from 0.84-30.67 ppb with an average of 4.08 ppb. As shown in **Figure 13**, all lead values were below 50 ppb (WHO, 1986).

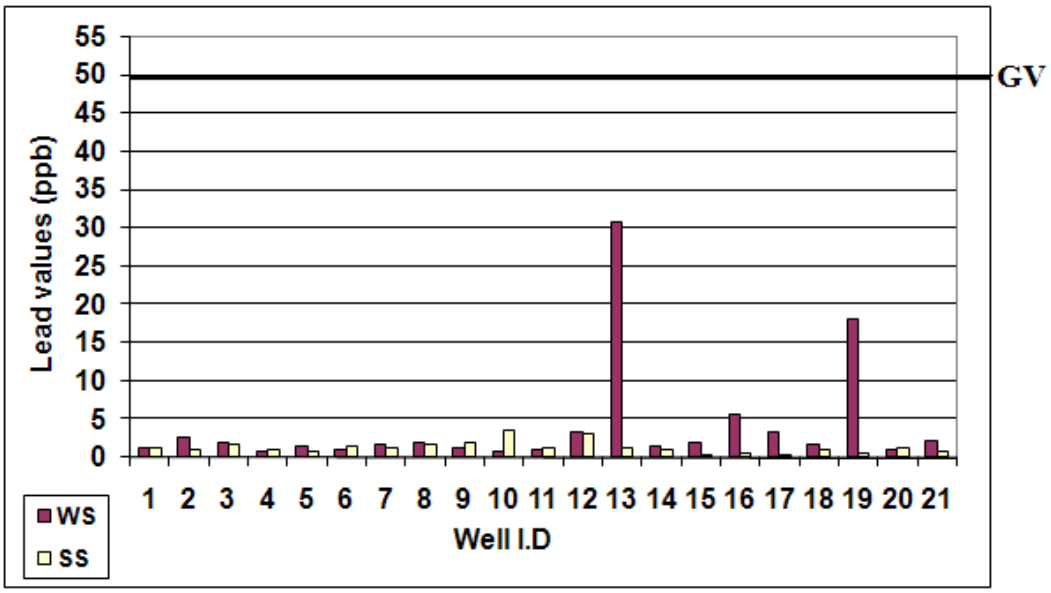


Figure 13: Lead values for rainwater samples collected from Qalqilia and Ramallah in summer 2006/winter 2007. SS: summer samples; WS: winter samples. GV: Guideline value.

3.2 Microbial Quality of Rainwater:

3.2.1 Indicator organisms:

Results for total coliforms, fecal coliforms and heterotrophic plate count (HPC) are summarized in **Table 9**. All summer and winter samples contained total coliform colonies, the number of which ranged from 2 colonies to more than 1000 colony (Too many to count"TMC"). Fourteen wells were found to contain more total coliform colonies in winter than in summer. In total, 12 wells were containing too many to count total coliforms 10 of which were recorded in winter samples.

Fecal coliform results are shown in **Table 9**. Samples from 10 wells out of 21 were found to contain more fecal coliforms in winter than in summer and only I well was found to contain too many to count colonies (Well 2, winter). A total of 14 well samples (6 summer and 8 winter) showed negative results regarding fecal coliforms.

HPC results showed that among the 21 wells tested, 10 wells were found to have higher HPC colonies in winter than in summer **Table 9**. One well was found to have TMC colonies, while the remaining wells were found to have from 2-770 colony/1ml.

Table 9: Total coliform, fecal coliform and heterotrophic plate count (HPC) results for harvested rainwater samples collected from Qalqilia and Ramallah Districts during summer 2006 and winter 2007.

Well I.D	Total coliform (colony/100ml)		Fecal coliform (colony/100ml)		HPC (colony/1ml)	
	Summer	Winter	Summer	Winter	Summer	Winter
1	700	TMC	33	100	102	TMC
2	400	TMC	37	TMC	75	140
3	100	15	3	5	210	106
4	100	TMC	40	210	300	106
5	55	20	10	0	250	150
6	70	950	8	9	375	150
7	600	TMC	40	11	180	250
8	700	22	28	80	37	63
9	35	TMC	24	10	71	TMC
10	47	110	0	0	20	7
11	125	TMC	56	0	55	160
12	9	2	1	0	46	75
13	8	50	0	1	6	48
14	90	TMC	7	71	47	360
15	21	TMC	0	2	2	49
16	50	5	1	0	11	160
17	11	TMC	0	0	30	84
18	42	16	0	0	7	9
19	TMC	TMC	400	80	150	150
20	TMC	65	10	13	770	150
21	2	24	0	0	15	180

3.3 Molecular Biology Testing:

3.3.1 DNA quality:

To check the quality of DNA isolated and used in the PCR reactions, samples of DNA were tested for integrity as shown in **Figure 14**.

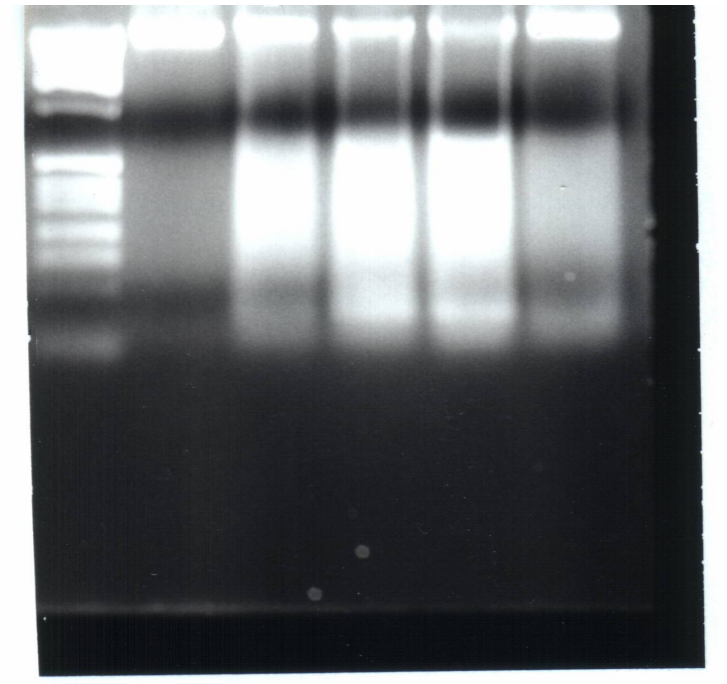


Figure 14: DNA quality. Lane (1) marker, lanes (2, 3, 4, 5 and 6) DNA from different samples.

3.4 Detecting Pathogens Using Molecular Biology:

3.4.1 Opportunistic pathogens:

Results obtained from the PCR for the presence/absence of opportunistic pathogens are summarized in **Table 10.** The PCR fingerprints for these tests are shown in **Figures 15-18**

Table 10 shows that all wells, except one, were having *Acinetobacter spp* in summer samples and 15 wells were having *Acinetobacter* in winter.

12 wells were found to be contaminated with *Aeromonas spp* in summer samples. Whereas the contamination in harvested rainwater wells in winter samples was in 10 wells from both districts **(Table 10).**

The contaminations with *Pseudomonas aeruginosa* was present only in 2 wells in summer samples one from Qalqilia and the other from Ramallah districts. Whereas no contamination was presents in winter samples **(Table 10).**

No contamination with *Mycobacterium* in winter or summer samples was observed.

All summer samples were contaminated with *Citrobacter*, whereas only 14 wells from both districts were contaminated in winter.

Table 10: PCR results for opportunistic pathogens for both summer samples (SS) and winter samples (WS) in Ramallah and Qalqilia districts.

Well I.D	*Acinetobacter spp*		*Aeromonas spp*		*Pseudomonas aeruginosa*		*Mycobacterium*		*Citrobacter*	
	SS	WS	SS	WS	SS	WS	SS	WS	SS	WS
1	+	-	-	-	-	-	-	-	+	-
2	+	-	+	-	-	-	-	-	+	-
3	+	-	+	-	-	-	-	-	+	-
4	+	+	+	+	+	-	-	-	+	+
5	+	+	+	+	-	-	-	-	+	+
6	+	+	+	+	-	-	-	-	+	+
7	+	-	+	+	-	-	-	-	+	+
8	+	-	+	-	-	-	-	-	+	-
9	+	-	+	-	-	-	-	-	+	-
10	+	+	+	-	-	-	-	-	+	-
11	+	+	+	+	-	-	-	-	+	+
12	+	+	-	-	+	-	-	-	+	-
13	+	+	-	-	-	-	-	-	+	+
14	-	+	+	+	-	-	-	-	+	+
15	+	+	-	-	-	-	-	-	+	+
16	-	+	+	+	-	-	-	-	+	+
17	+	+	-	+	-	+	-	-	+	+
18	+	+	-	+	-	-	-	-	+	+
19	+	-	-	-	-	-	-	-	+	+
20	+	+	-	+	-	-	-	-	+	+
21	+	+	-	-	-	-	-	-	+	+

Pseudomonas aeruginosa was found in summer water samples from 2 wells **(Figure 15A)**. One was from well 4 in Qalqilia region and the other was from well 12 in Ramallah region. In winter, *P. aeruginosa* was detected only in one well (Well 17) from Ramallah region. All other wells were negative with regard to *P. aeruginosa* (**Figure 15B).**

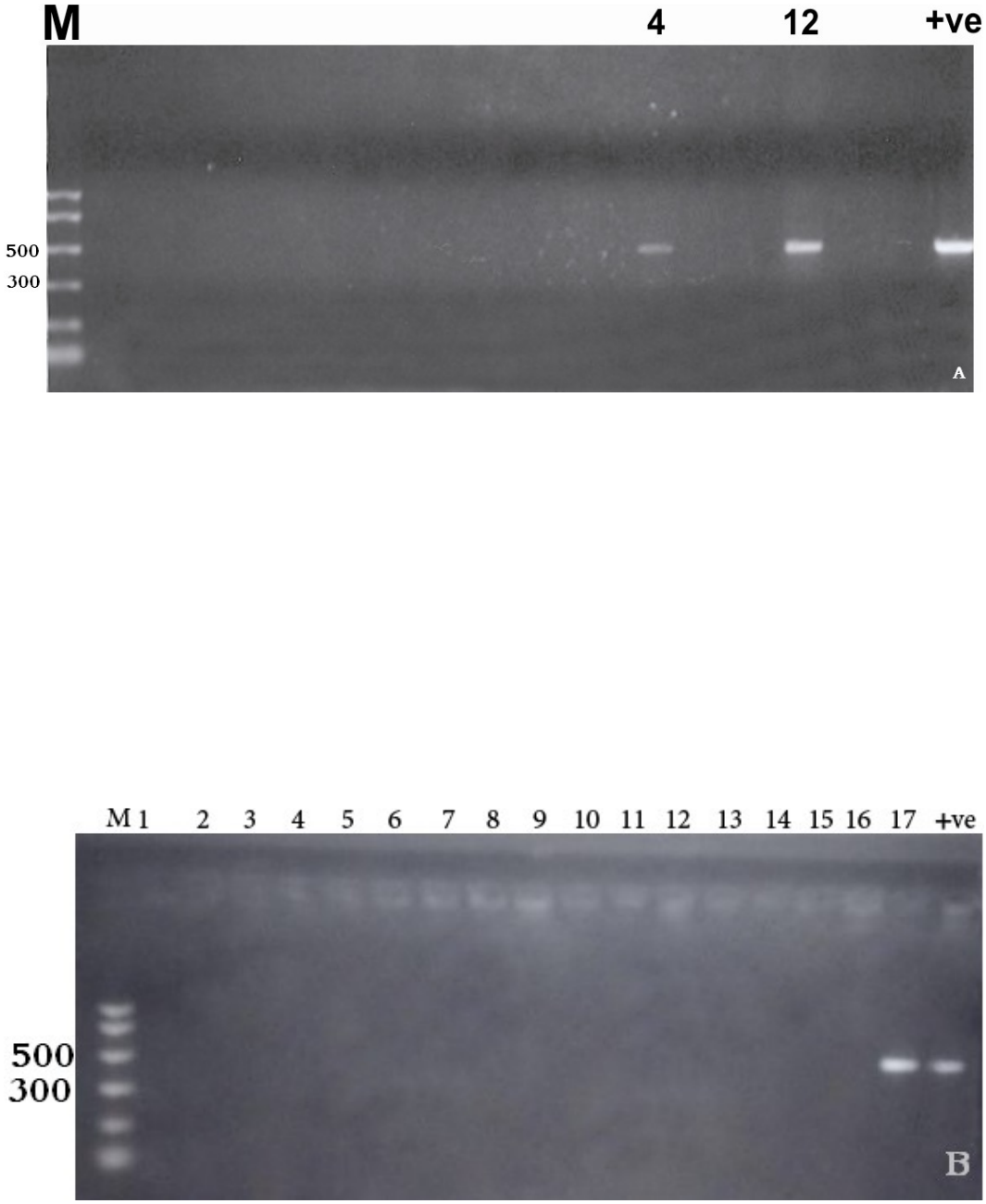


Figure 15: PCR results for *Pseudomonas aeruginosa* in summer (A) and winter (B) harvested rainwater samples collected from storage wells in Qalqilia and Ramallah regions in summer 2006/winter 2007. Lane (M) is a ladder marker, other lanes are DNA samples.

Acinetobacter was detected in all summer samples from Qalqilia region and all water samples, except wells 14 and 16, from Ramallah region (**Figure 16A**). In winter, samples from all wells were having *Acinetobacter* except wells 1, 2, 3, 7, 8, 9 (from Qalqilia region) and 19 (from Ramallah region) (**Figure 16 B**).

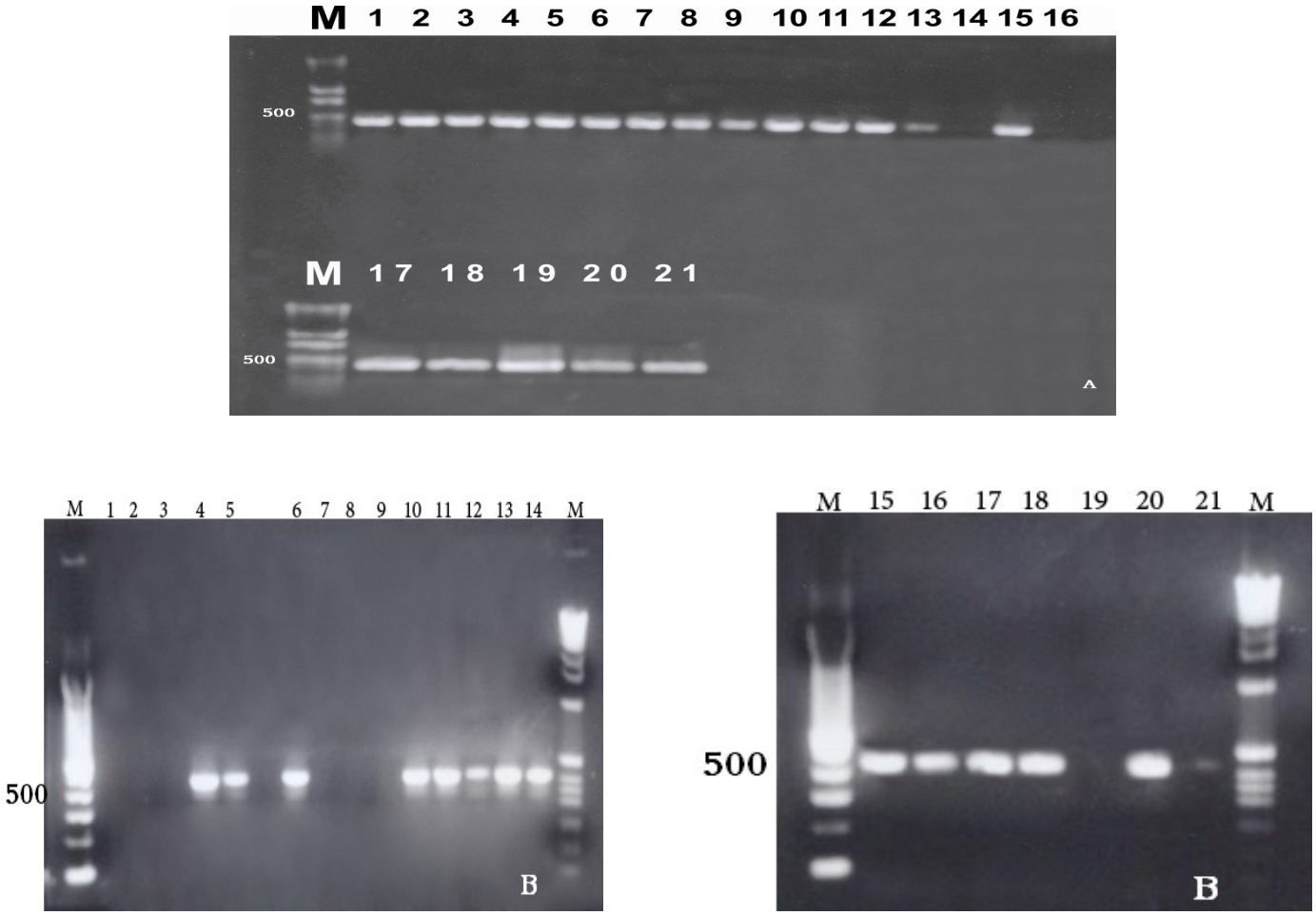
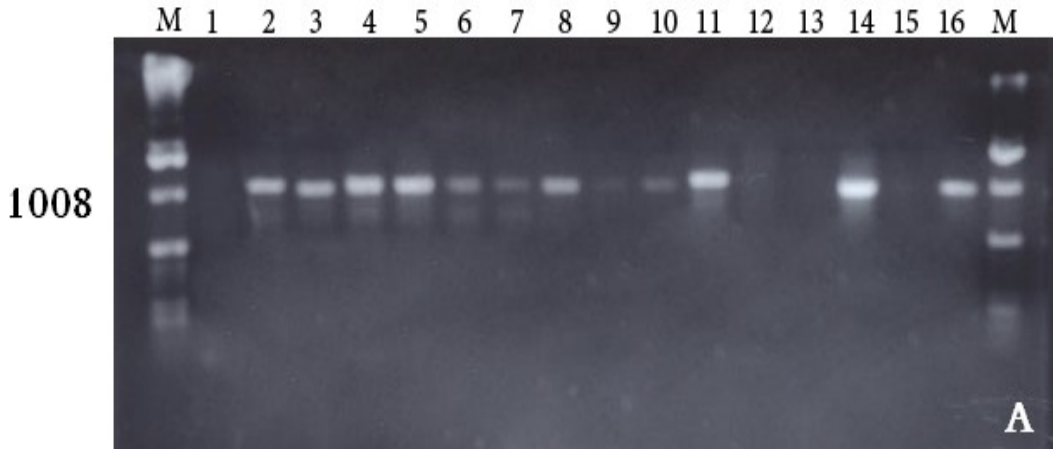
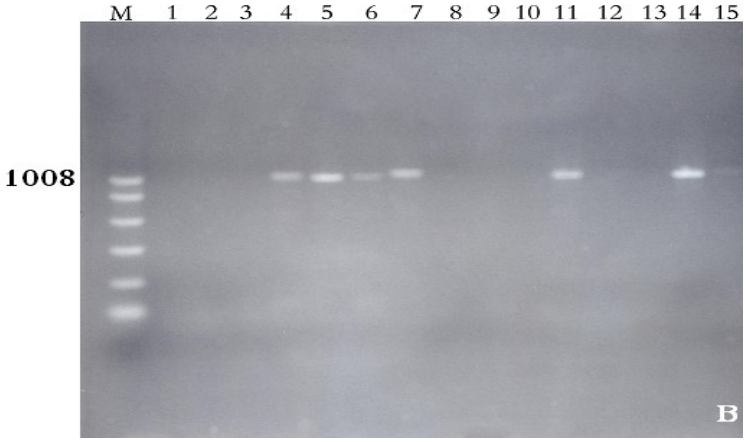


Figure 16: PCR results for *Acinetobacter* in summer (A) and winter (B) in harvested rainwater samples collected from storage wells in Qalqilia and Ramallah regions in summer 2006/winter 2007. Lane (M) is a ladder marker, other lanes are DNA samples.

A. hydrophilya was detected in all summer samples except wells 1 (Qalqilia), 12, 13, and 15 (Ramallah) (**Figure 17A)**. Winter RAPD fingerprints show that about half of the wells (4, 5, 6, 7, and 11 from Qalqilia and 14, 16, 17, 18, and 20 from Ramallah) were contaminated with *A. hydrophilia* (**Figure 17B).**





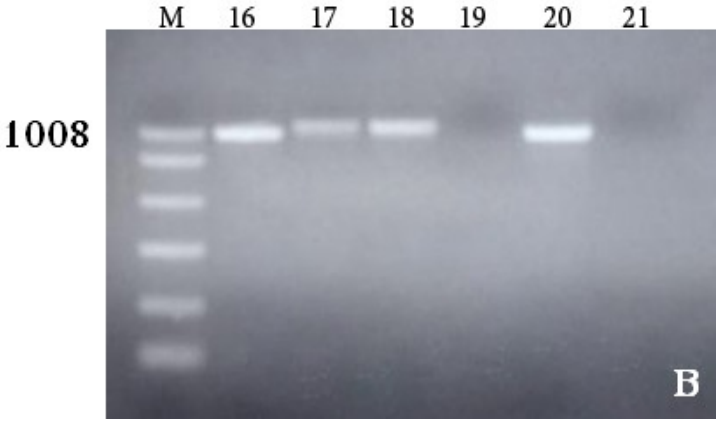


Figure 17: PCR results for *A. hydrophilya* in summer (A) and winter (B) in harvested rainwater samples collected from storage wells in Qalqilia and Ramallah regions in summer 2006/winter 2007. Lane (M) is a ladder marker, other lanes are DNA samples.

PCR for water samples show that all wells were contaminated with *Citrobacter* in summer (**Figure 18A**). In winter, water samples from 11 wells were found to be contaminated with *Citrobacter*. These wells were: 4, 5, 6, 7, 11, 13, 14, 15, 16, 17, and 18 (**Figure 18B**).

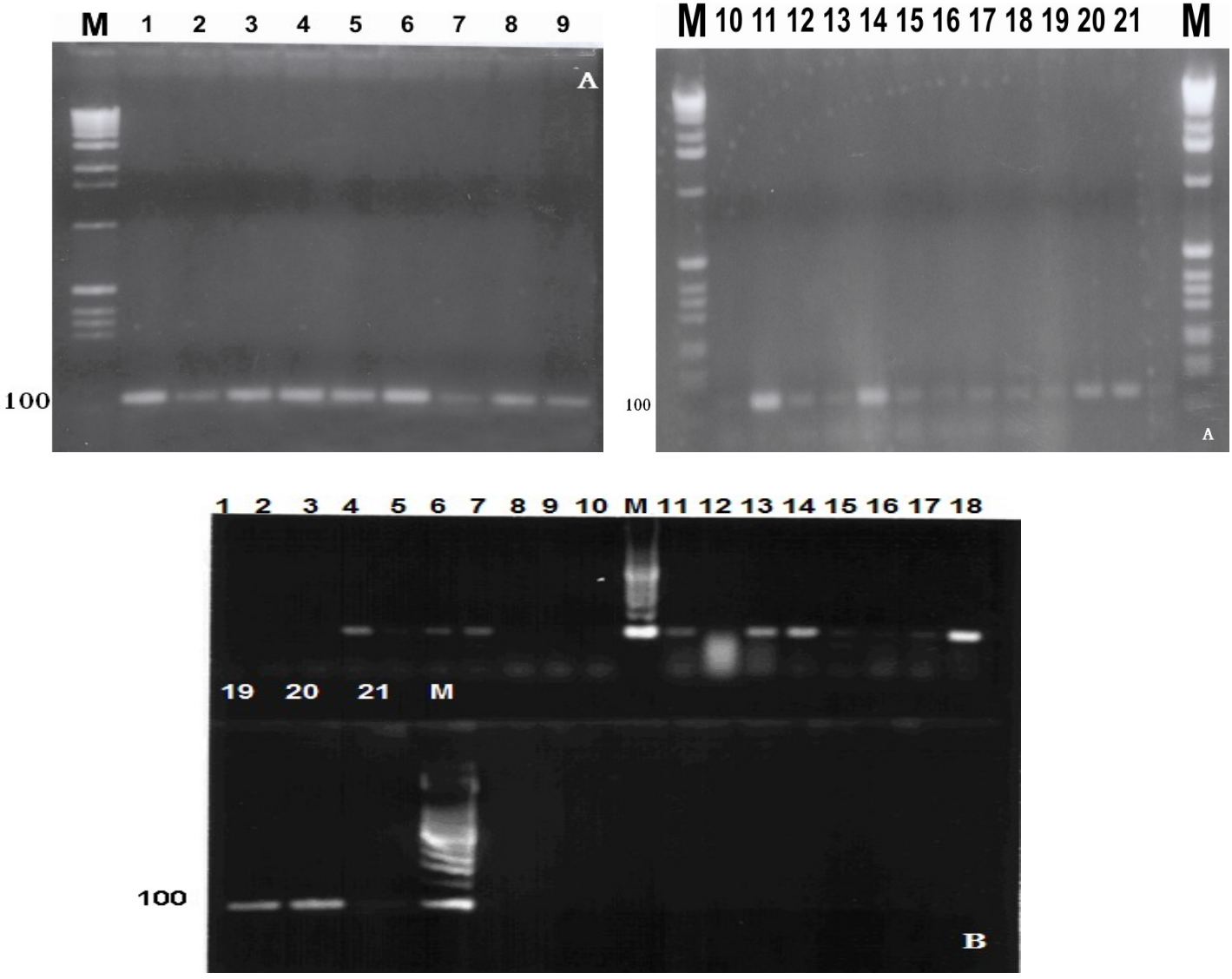


Figure 18: PCR results for *Citrobacter* in summer (A) and winter (B) rainwater samples collected from storage wells in Qalqilia and Ramallah regions in summer 2006/winter 2007. Lane (M) is a ladder marker, other lanes are DNA samples.

3.4.2 PCR results for pathogenic bacteria:

From all pathogenic bacteria only *Campylobacter* were detected. As **Figure 19** showing only well 14 and well 17 in summer sample from Ramallah district were detected whereas the other are negative .

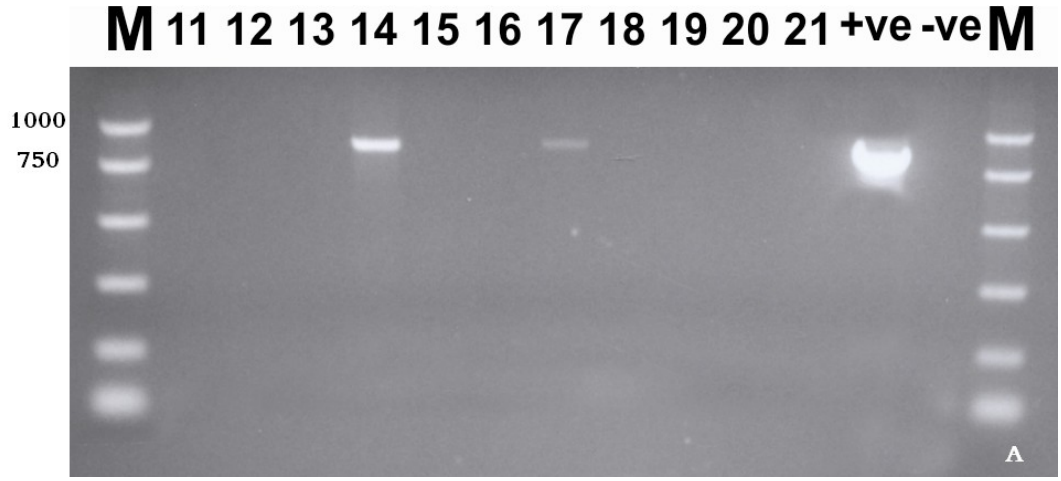


Figure 19: PCR results for *Campylobacter* in summer (A) rainwater samples collected from storage wells in Qalqilia and Ramallah regions in summer 2006/winter 2007. Lane (M) is a ladder marker.

CHAPTER FOUR

4. DISCUSSION:

Water shortage is becoming the number one problem in the world today. The on going drought and the increasing demand of growing population reduce reservoirs. Rain water harvesting may serves as an alternative solution to the above problem. It has been a common practice in many nations all over the world for thousands of years, especially in arid or remote areas, where the provision of water through piped net works is uneconomic or not technically feasible (Sazakli et al., 2007)

Solutions seems to be so attractive from an ecological point of view, potential health risks from ingesting of harvested rainwater related to microbiological and chemical contaminants should be taken into account (Sazakli et al., 2007)

Microbial pathogens originate in fecal contamination by birds, mammals and reptiles that have access to catchment areas or water storage tanks. Chemical contamination of the rain water can occur due to traffic emissions and industrial

pollution in urban areas or due to agricultural usage of fertilizers and pesticides in rural areas (Sazakli et al., 2007)

While in more recent ones, either chemical or microbiological contaminants have been in the collected rainwater, often in levels exceeding the international or national guidelines set for safe drinking water (Sazakli et al., 2007).

4.1Chemical and Physical Quality of Harvested Rainwater:

4.1.1 Temperature

Harvested rainwater temperature ranged from 20-28 ^{o}C in summer to 12-19 ^{o}C in winter. The average for both seasons was 24 ^{o}C and 16.5 ^{o}C for summer and winter samples, respectively. Temperature of harvested rain water affects bacterial growth rates and thus, the microbial quality of rainwater as a drinking water source.

4.1.2 pH

Results of the pH measurements indicated that in from a total of 42 samples collected in winter and summer, only 11 samples were found to have values outside the guideline values recommended by the WHO for drinking water **(Figure 6)**. The pH values of other rainwater samples were meeting the WHO guideline value of 6.5-8.5 (WHO, 1986).

In winter samples, rainwater from wells 2, 3, 4 and 7 (Qalqilia region) were having pH values below the guideline value. This indicates that water from these

four wells is more acidic than allowed for drinking water purposes. These wells were found in bad hygienic conditions with many pollutants on the surface of water inside the wells. Moreover, these wells are not subjected to regular cleaning on yearly basis. These waters cause corrosion in pipes and may contain heavy metals either from pipe corrosion or as a result of weathering the rocky walls of the storage wells. The high acidity of harvested rain water can be neutralized by a slight buffering using 1 tablespoon of baking soda to 100 gallons of water in the tank (Texas Manual, 2005).

On the other hand, 7 wells (7, 8, 9, 10, 11, 15 and 18) from summer sample were having pH values above the allowed WHO values for drinking water. The high alkalinity of these water samples could be due to the concrete walls of the some storage wells that shift the pH of water to the alkaline range (Texas Manual, 2005) or due to the neutralization of the water acidity from winter time with the calcareous rocky walls of some other wells.

High alkalinity water requires a longer contact time or a higher free residual chlorine level at the end of the contact time for adequate disinfection (0.4–0.5 mg/liter at pH 6–8, rising to 0.6 mg/liter at pH 8–9; chlorination may be ineffective above pH 9 (WHO, 2006).

4.1.3 EC, salinity and TDS:

These three properties are interrelated. Generally, all three were found to be higher in summer samples than in winter samples.

The average value of EC for summer samples was 378.5 µs/cm. This value decreased in winter to reach 286.4 µs/cm **(Figure 7)**.

Salinity measurements followed the same pattern as EC. Its values were higher in summer than in winter. The averages were 0.17‰ and 0.15‰ for summer and winter samples, respectively **(Figure 8).**

Values of TDS were found to be much less than the maximum allowed limit set by the WHO for drinking water as 1000 mg/l. All rainwater samples were having TDS values less than 400 mg/l with an average of 157.5 mg/l for all winter and summer samples collected **(Figure 9)**. Seasonal variations in TDS values were obvious especially when the average value of TDS in summer samples (177.6 mg/l) is compared to the winter value of 137.5 mg/l.

This trend in EC, TDS and salinity indicates that rainwater contains less ions at the beginning and with the storage process its ionic contents start to increase due to getting more ions from the storage process itself. Besides, the high evaporation rates in summer cause dissolved ions to increase with respect to the small amount of water left in most of the wells at this time.

4.1.4 Turbidity:

All turbidity measurements (except that from well 2) were less than the maximum WHO guideline value of 5 NTU set for drinking water **(Figure 10).** Turbidity values in winter samples were higher than summer ones. The averages were 0.59 NTU (in summer) and 1.11 NTU (in winter). In winter, rainwater seems to collect turbidity from the catchment surface or through mixing within the well. Thereafter, stored water starts loosing turbidity due to settling of turbidity-causing particles. Well number two had a metal cover that does not close the well opening properly. This causes soil and dust particle to accumulate in the well increasing its turbidity.

Turbidity determination is important for the type and level of treatment and disinfection needed (WHO, 2006). In addition, suspended particles can carry bacteria fixed on their surface, which protect them from disinfection (Ridgway and Olson, 1982; Camper *et al*., 1986; Herson *et al*., 1987; Gauthier *et al*., 1998).

4.1.5 Copper and Lead:

All rainwater samples collected during the study were having lead and cooper levels much less than the maximum guideline values of the WHO for drinking water (**Figures 12and 13)**.

The average copper levels in rainwater were 7.78 ppb in summer and 8.76 ppb in winter. These are much less than the maximum guideline value for copper in drinking water set by the WHO as 1000 ppb. This means that no health risks are associated with this metal in summer and winter samples.

Lead average levels in rainwater (1.28 ppb in summer and 4.08 ppb in winter) were also well below the maximum value of 50ppb set by the WHO for drinking water.

4.1.6 Nitrate:

Nitrate was measured in winter samples **(Figure 11)**. Nitrate levels in all samples were well below the maximum guideline value set by the WHO of 10 ppm. The average nitrate concentration in all winter samples was 3.06 ppm. This indicates good rainwater quality and no health risks regarding nitrate could result from the consumption of rainwater.

4.2 Microbial Rainwater Quality:

4.2.1 HPC, Total and Fecal Coliforms.

Results of HPC indicated that all wells were heavily contaminated with microbes. As a result, colonies were too many to count in all wells. Therefore, all samples were diluted and colonies/1m instead of 100 ml water were counted **(Table 9)**. Heterotrophic microorganisms include both members of the natural (typically nonhazardous) microbial flora of water and organisms present in a range of

pollution sources. They occur in large numbers in natural water sources. The high number of heterotrophic bacteria and coliforms in these wells, increase due to long residence times, corrosion and high temperatures (Haudidier *et al*., 1988; Pedersen, 1990).

Total coliforms were found in all rainwater samples collected in summer and winter. In 12 rainwater samples, too many colonies were observed that couldn't be counted. According to WHO standards for drinking water, no total coliforms are allowed in drinking water. Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the feces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms (WHO, 2006).

About 1/3 of rainwater samples (14/42) were not containing fecal coliforms. The remaining wells were contaminated with fecal coliforms to a variable degree. According to WHO standards for drinking water, no fecal coliforms are allowed in drinking water. Fecal contamination can arise from sources such as combined sewer overflows, leaking septic tanks, sewer malfunction, contaminated storm drains, animal feedlots, and other sources. Rainfall is frequently associated with increased abundance of fecal coliforms in water due to stormwater runoff or ctachment surfaces (EPA).

Fecal indicator bacteria have been used as surrogates for pathogens in assessing water quality in many countries. The fecal indicator bacterial groups most commonly used as indexes of water quality are total coliform (TC) and fecal coliform (FC) (Savichtcheva *et al.*, 2007).

Despite widespread implementation of TC and FC monitoring programs, these conventional fecal indicators have numerous limitations associated with their application including short survival in water body (McFeters *et al*., 1974), nonfecal source (Scott *et al*., 2002; Simpson *et al*., 2002), ability to multiply after releasing into water column (Desmarais *et al.*, 2002; Solo-Gabriele *et al*., 2000), great weakness to the disinfection process (Hurst *et al*., 2002), inability to identify the source of fecal contamination (point and nonpoint) (Field *et al*., 2003) and low levels of correlation with the presence of pathogens (Horman *et al*., 2004; Winfield and Groisman, 2003).

Contamination with fecal coliform in harvested rainwater wells occur from natural wildlife as well as from anthropogenic sources (Mudge and Duce, 2005). Excessive influx of fecal materials from anthropogenic sources and animal farms can pose major problems due to the potential adverse health impacts when the waters are to be used for potable, recreational and shellfish harvesting purposes (Reeves *et al*., 2004).

Other source of fecal contamination is the contamination from non-point agricultural pollution which can also cause deterioration of water quality by emitting significant levels of fecal bacteria and nutrients to waterways (Scholefield *et al*., 1993; Ledgard *et al*., 1996; Gillingham and Thorrold, 2000; Monaghan *et al*., 2007;).

The use of coliforms as an indicator organism has been questioned because pathogens have been isolated when there have been low concentrations of fecal coliforms, and many viruses and protozoan cysts are more resistant to disinfection than coliforms (AWWA, 1999).

4.2.2 Specific PCR:

PCR results for the 42 rainwater samples collected in summer and winter indicated the presence of all opportunistic pathogens tested in the present study in all samples. *Mycobacterium* was the only opportunistic pathogen that was not detected in any of the samples. All pathogenic bacteria tested were negative except *Campylobacter* that was detected in wells 14 and 17 of summer samples.

Pseudomonas aeruginosa was found in summer water samples from 2 wells **(Figure 15A)**. One was from well 4 in Qalqilia region and the other was from well 12 in Ramallah region. In winter, *P. aeruginosa* was detected only in one well (Well 17) from Ramallah region. All other wells were negative with regard to *P. aeruginosa* (**Figure 15B).** Rainwater may become contaminated with p.

aeruginosa from the catchments surface, since it may be polluted by dusts and bird feces.

***Acinetobacter* was** detected in all summer samples from Qalqilia region and all water samples, except wells 14 and 16, from Ramallah region (**Figure 16A**). In winter, samples from all wells were having *Acinetobacter* except wells 1, 2, 3, 7, 8, 9 (from Qalqilia region) and 19 (from Ramallah region) (**Figure 16B**).

The widespread of *Acinetobacter* in rainwater samples may be due to that fact that some species and strains of *Acinetobacter* can be found on the skin of healthy people (Krawczyk *et al*., 2001). So the water container and the rope which is used to obtain water from the well may constitute the source of contamination to well waters.

A. hydrophilya, About half of the wells in summer and as well as in winter were found to be contaminated with ***A. hydrophilya* (Figure 17A & B).** This could be due to the high residence time of water in the well which causes an increase in microbial population (WHO, 2006). Most of the wells are characterized by high residence time, because of the lack of regular annual cleaning of these wells.

Other possible source of contamination is the catchment surface itself as many catchment surfaces are not cleaned before directing water into the storage wells. Catchment surfaces are usually contaminated with dust, feces, plant leaves and other debris (WHO, 2006).

Citrobacter was found in all wells in summer and about half of the wells in winter **(Figure 18 A & B).** *Citrobacter* is a commensal in human intestine that is reported to occur in soil, water and sewage (Sedlak, 1973) as well as in the rhizosphere (Zablotowicz *et al*., 1995).

Citrobacter spp. are Gram-negative bacteria and are associated with neonatal meningitis with unacceptable rates of morbidity and mortality (Kinzler *et al.*, 1996). The unique feature of meningitis caused by *Citrobacter spp*. is their frequent association with brain abscess formation (Huang *et al*., 2000).

Campylobacter is the only pathogenic microbe that was detected in 2 wells (14 and 17) in summer samples **(Figure 19)**. Contamination with *Campylobacter* may be origination from roof catchment that may polluted with feces of birds and animal. Other sources of *Campylobacters* could include sewage discharges or agricultural run-off (Atabay and Corry, 1998; Diergaardt *et al*., 2004). *C. jejuni* and *C. coli* are among the most common causes of human diarrheal diseases.

CHAPTER FIVE

5. CONCLOSION AND RECOMENDATIONS

5.1Conclusions:

1. The physical and chemical properties of harvested rainwater are generally within the WHO guideline values indicating good water quality for drinking purposes.
2. No clear differences were observed with regard to physical and chemical properties between wells of Ramallah and Qalqilia.
3. Rainwater microbial quality in the studied regions is poor and imposes health risks if consumed without pretreatment.
4. Most wells were found to be contaminated with one or more microbe.
5. Opportunistic pathogens were found to be common in rainwater, however, only one pathogenic bacterium was observed in 2 wells in summer.
6. Generally, summer samples were more contaminated with microbes than winter samples.

7. No obvious differences between wells from Qalqilia region and Ramallah region with regard to microbial quality were observed.

8. Molecular biology techniques provide quick, precise and reliable results when compared to classical microbiology techniques.

5.2 Recommendations:

1. Roof catchment surfaces must be washed carefully before directing water into the storage wells.

2. First rainfall water in winter must be directed outside of the well as the first rain fall might contain dust and pathogens in large amounts.

3. Storage wells must be cleaned on yearly basis in order to reduce possible microbial contamination.

4. Wells must be kept properly closed in order to reduce possible contamination after storage.

5. Disinfection practices are highly encouraged especially when rainwater is intended to be used for household purposes.

6. Birds should be kept away from the roof catchment systems as they contaminate the roof with feces and fecal microbes.

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الخلاصة

في فلسطين، آبار جمع مياه الامطار , تقليد معروف خصوصاً في المناطقِ التي تَعاني مِنْ نَقْصِ الماء الصالح للشربِ أَو تَفتقرُ إلى أنظمةِ توزيعِه. تُشكّلُ آبار جمع مياه الامطار مصدرا للماء المجاني في الوقت الذي بلغت فيه أسعار المياه مستوى عاليا وفي فلسطين تجمع مياه الامطار في الغالب من خلال أنظمةِ معينة منها :مصبِّ السقفِ وخَزانَ ماء يتكون عادة من الخرسانة او الصخر . مياه المطر المجموعة تُستَهلكُ عادة بدون أيّ معالجة، بالرغم من أنّه يُمْكِنُ أَنْ تشكّلَ خطرا على الصحة.

الدراسة الحالية تَستهدفُ تَقييم أخطارِ الصحةِ المرتبطة باستهلاك مياه المطر والمجموعة من اسطح البيوت في الضفة الغربية.لذا، تم اخذ 21 عينة ماء من 21 بئرا من منطقتين مختلفتين هما قلقيلية ورام الله خلال فترتي الصيف والشتاء من عامي 2006-2007.تم اختبار عينات الماء باستخدام الفحوصات الميكروبية والكيميائية لتَقْرير المخاطرِ الصحيةِ المحتملةِ و الإختبارات الكيميائية والطبيعية التي تُؤثّرُ على نوعيةِ الماءِ تَضمّنتْ: فحص العكورة، الملوحة، مجموعَ المواد الصلبةَ الذائبة (TDS)، التوصيل الكهربائي (EC) , درجة الحموضة(pH)، درجة الحرارة، فحص النترات والمعادن الثقيلة (نحاس ورصاص). و تَضمّنتْ ايضا الإختباراتُ الميكروبيةُ: فحص (total and fecal coliform ، HPC). إضافة الى ذلك تم فحص، خمسة انواع من البكتيريا الإنتهازية و ستة انواع من البكتيريا المسبّبة للمرضِ باستخدام تقنيةِ تفاعل البناء المتسلسل للمادة الوراثية عرف اختصارا ب(PCR).

أظهرت نتائج الفحوصات الكيميائية للعينات ان معظم عينات الصيف والشتاء تتطابق في الغالب مع الارشادات الصحية لمنظمة الصحة العالمية(WHO) .لكن وجد ان نتائج فحوصات (TDS,EC, salinity) في عينات الصيف اعلى من عينات الشتاء.

اما الإختبارات الميكروبية (HPC ، total , fecal coliform) فقد أظهرت تلوثا كبير لمياه الآبار بالجراثيمِ. وهذا يُشيرُ إلى بعض المخاطر الصحية و لا سيما إذا ُستَعملُ هذا الماء لأغراضِ الشرب.

اظهرت نَتائِج (PCR) بأنّ التلوث بالبكتيريا الانتهازية في عينات الصيف والشتاء كان كبيرا جدا مقارنة مع البكتيريا المسببة للمرض والتي اظهرت تلوث بعض الابار بنوع واحد من البكتيريا المسببة للمرض.

اخيرا مَنْ الضَّرُوري أَنْ يُطهّرَ ماء المطر المجموع قبل أن يُستَعملَ للأغراضِ الشرب وضرورة المحافظة على نظافة اسطح الجمع(المنازل) وخزانات الجمع وتنظيفها بشكل دوري لضمان الحصول على ماء نظيف صالح قدر الامكان للاستهلاك المنزلي.

المخاطر الصحية المرتبطة باستهلاك مياه الأمطار غير المعالجة والمجموعة بوساطة أسطح البنايات

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