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Al- Quds University

Microbiological Assessment of Marketed Drinking Water in Gaza City

Submitted by

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Microbiological Assessment of Marketed Drinking Water in Gaza City

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Microbiological Assessment of Marketed Drinking Water in Gaza City

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2. Dr. Yehia Abed	Internal Examiner	Signature .....
3. Dr. Nahed Al Laham	External Examiner	Signature .....

بسم الله الرحمن الرحيم

" قال الله تعالى: {أَوَلَمْ يَرَ الَّذِينَ كَفَرُوا أَنَّ السَّمَاوَاتِ وَالْأَرْضَ كَانَتَا رَتْقاً فَفَتَقْنَاهُمَا وَجَعَلْنَا مِنَ الْمَاء كُلَّ شَيْءٍ حَيٍّ أَفَلَا يُؤْمِنُونَ}. ". سورة الأنبياء أيه رقم 30

In the name of Allah, Most Gracious, Most Merciful

"Do not the Unbelievers see that the heavens and the earth were joined together (as one unit of creation), before we clove them asunder? We made from water every living thing. Will they not then believe?" The prophets, 30.

Dedication

I would like to dedicate this work to the first teacher my father, to the merciful mother, to my lovely wife, sons, brother, sisters, for their patience and lastly to my college.

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Shady Al Tartory

Date: 20/06/2009

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Shady Al Tartory

Abstract:

Water shortage is becoming one of the most important problem in the world today, specially in area as Gaza strip where it suffered from severe shortage. The microbiological quality of water in Gaza strip is so poor. So, to this reason we performed this study that aimed to investigate the microbiological quality of the different types of marketed drinking water in the Gaza city. The study population consisted of 20 samples collected from desalination plants, 12 samples from water distribution cars, 25 samples from vending machines, and 12 samples from bottled water that manufactured in the Gaza city. An experimental design was used to verify water microbiological quality using Membrane Filtration Technique (sterile nitrocellulose acetate membrane filters) placed on MacConkey, cetrimide, and Doxi's media for isolation and detection of both total and fecal coliforms, *Pseudomonas aeruginosa*, and fungi (mold and yeast) respectively.

The results of water samples that collected from desalination plants showed that 12 samples (60%) out of 20 samples were contaminated with total coliforms (1-50 cfu/100ml). 50% of samples that collected from distribution cars (6 out of 12) were found to be contaminated with total coliforms in a level of (1-20 cfu/100ml.) The water samples that collected from vending machines also showed a high percentage contamination as in the desalination plants where 15 samples out of 25 (60%) were found to be contaminated with total coliforms in a range of (1-50 cfu/100ml).
Regarding the presence of fecal coliform in the different water samples, we found 6 samples out of 20 from the desalination plants (30%) were contaminated with fecal coliforms in a range of (1-30 cfu/100ml); 5 samples out of 12 (41.7%) in the water collected from distribution cars in a range of (1-10 cfu/100ml); and 4 samples out of 25 (16%) in the water collected from vending machines.

Regarding the presence of *Pseudomonas aeruginosa* in the tested samples, 45% (9 out of 20) of samples that collected from desalination plants were found to be contaminated in the level of (1- 200 cfu/250ml). However, a 50% (6 out of 12) of samples in water collected from distribution cars were found to be contaminated in the level of (1-20 cfu/250ml). The vending machines showed 48% (12 out of 25) contamination with *P. aeruginosa* in a range of (1-65 cfu/250ml).

The presence of fungi including both yeast and molds were estimated. In the desalination plants, 25% (5 out 20) and 20% (4 out of 20) samples were contaminated with molds (1-10 cfu/100 ml) and yeast (1-50 cfu/100 ml) respectively; 10 samples (83.3%) and 6 samples (50%) out of 12 were contaminated with molds (1-15cfu/100ml) and yeast (1-20 cfu/100ml) respectively in water samples collected from distribution cars. Finally in the vending machines, we found that 14 samples (56%) and 4 samples (16%) out of 25 were contaminated with molds (1-20 cfu/100ml) and yeast (1-20 cfu/100ml) respectively.

Finally the results of water samples collected from bottled water showed that all samples were free from Total and Fecal coliform bacteria . For total bacterial count the results showed that 4 samples (33%) out of 12 samples were contaminated with bacteria (> 100 cfu /1 ml) , Meanwhile *P. aeruginosa* were found at 2 samples (16.6%) out of 12 samples and finally no Yeast or Molds were found in bottled water samples.

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Abbreviations:

ADWG	Australian Drinking Water Guidelines.
AWWA	American Water Works Association.
CBWA	Canadian Bottled Water
CFU	Colony Forming Unite.
CMWU	Coastal Municipalities Water Utility.
E.coli	*Escherichia coli.*
E D	Electrodialysis.
EPA	Environmental Protection Agency.
FC	Fecal Coliform.
HAV	Hepatitis A. Virus.
HU	Hazen Units.
MAC	Maximal Allowed Concentration.
MED	Multi-effect distillation
MOA	Ministry Of Agriculture.
MPN	Most Probable Number.
MSF	Multi-Stage Flash.
NTU	Nephelometric Turbidity Units.
P.aeruginosa	*Pseudomonas aeruginosa.*
P.glabrum	*Penicillium glabrum.*
PA	Palestinian Authority.
PCBS	Palestinian Central Bureau Of Statistics.
PFGE	Pulsed Field Gel Electrophoresis.
PHG	Palestine Hydrology Group.
PNA	Palestinian National Authority**.**
PPM	Parts Per Million**.**
PWA	Palestinian Water Authority.
RO	Reverse Osmosis.
SPSS	Statistical Package For The Social Sciences.
TC	Total Coliform.
TCU	True Color Units.
VC	Vapour Compression.
WHO	World Health Organization.

Chapter One

Introduction

Introduction

1.1 Background

Water is the source of life in the universe where it accounts for almost 75 % of the earth, and this means that the water is the basis of the universe and without water we cannot live.

Water scarcity and insufficiency is becoming the main problem in the World. The ongoing drought and the increasing demands of the growing populations are the major factors that reduce the water natural reservoirs (Sazakli et al., 2007). Moreover, the problem of water scarcity is strongly connected to the problem of water quality. Urban development, human activities and industrialization deteriorate the quality of water and, in some cases, make it unsafe for utilization.

1.2 Geography and Demography of the Gaza Strip

The Gaza Strip (Geographic coordinates 31 25 N, 34 20 E) of Palestine, is a narrow piece (378 Km^2) of land along the Mediterranean coast, just about 40km long and 10km wide (UNEP,2003). The Gaza strip is about 1.33% of the total area of mandate Palestine. Altitude and elevation extremes measurements of the Gaza strip showed the lowest point at the Mediterranean Sea level (0 meter) while the highest point is only 105 meters above sea level. The Gaza strip is divided into five governorates:, Gaza, Khan Younis, Rafah, Deirelbalah, and North governorate. Gaza strip is considered as one of the most populated areas in the world (4054/km2) with an estimated population of about 1.48 million according to July 2007 estimates (Palestine Central Bureau of Statistic, 2007).

Gaza Strip is a semi-arid area with subtropical climate. It is flat and sandy with little fertile soil (MOA, Report.2002). The average daily temperature ranges from 25 °C in the summer to 13 °C in the winter. The average annual rainfall varies from 400 mm/year in the north to 200 mm/year in the south, and the annual rate in the whole Gaza Strip is up to about 317 mm/year. (PWA, 2000).The winter season in Gaza starts from December and ends in March causing the precipitation that constitutes the major source of fresh water and aquifer recharge.(Isam, R, 1994). The people of the Gaza strip depend on groundwater for their agriculture, industries, and for household purposes like drinking, cooking, washing, cleaning and showering.

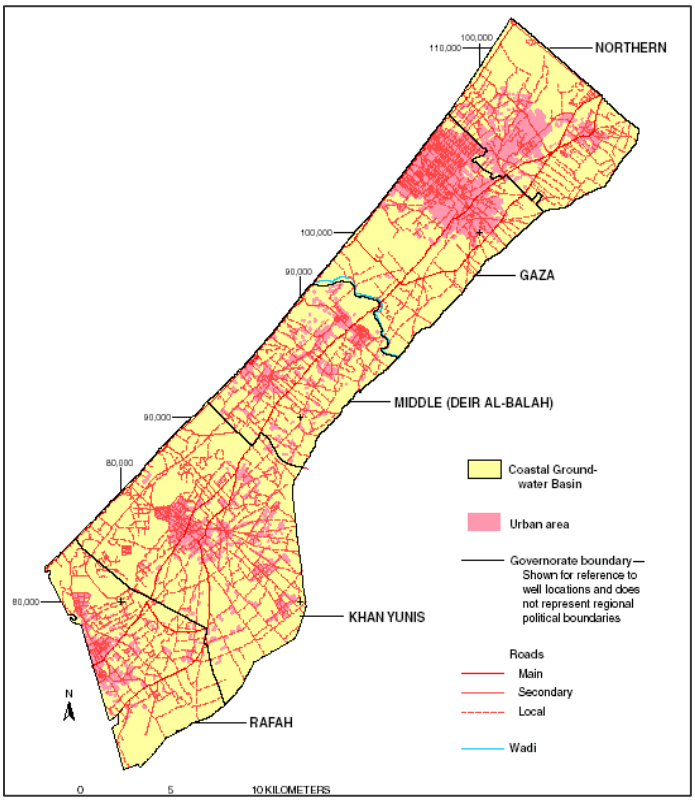


Figure 1. Location of the Gaza governorate

1.3 The Water Crisis in the Gaza Strip

Most of the Palestinian community at Gaza strip, either independently or through municipalities, depends on ground water wells as the main source of water. Controlling and monitoring the quality (biological, physical and chemical) of the water wells and water distribution networks at Gaza strip are not always possible due to the constrains applied by the Israeli occupation either on the entry of the disinfectants and instruments required for water quality assurance or some times on reaching the wells that are located near the hot and clash points. Therefore, and due to the reduced quantities and qualities of drinking water, the population try to seek other sources for drinking water which include the marketed desalinated water (produced by reverse osmosis) and the bottled water (usually misnomer as natural or mineral water) of the different drinking water trading companies in Gaza city.

Palestine and “Israeli occupation” share the Southern Mediterranean coastal aquifer. Long-term overexploitation in the Gaza Strip and the constraints by the Israeli occupation authority of using more of the ground water have resulted in the scarcity and hence decreased supply of tap water, accompanied with low water quality (Weinthal *et al*., 2007). Furthermore, Weinthal added that the rapid growth rate of population in the Gaza Strip and the dependence upon ground water as a single water source present a serious challenge for future political stability and economic development.

Many environmental problems are accelerated and exacerbated by occupation practices, which prevent effective environmental management. This problem is particularly acute in Gaza in relation to the water resources and the ongoing military conflict. The roots of Gaza’s water problem lie in the over-population of the area, due to a high influx of refugees in 1948, when approximately 200,000 people fled to Gaza from the occupied Palestine. The original population of the Gaza Strip at that time was 80,000 people; the present situation represents an increase of some 250%. Today, over three quarters of the estimated Gazian population of about 1.4 million are registered refugees. In 1994, the Gaza-Jericho agreement placed water resources in the Gaza Strip under the control of the Palestinian National Authority (PNA) and in 1995, the Palestinian Water Authority (PWA) was established and become responsible for all water issues in Palestine. It was given the mandate for managing water in the Palestinian territories. At that time, the PWA recognized serious environmental problems with the Gaza aquifer, with experts predicting that if nothing is done, the entire aquifer would become unusable by the year 2000. In addition, the water infrastructure was in a very poor state, with 50% of water being lost through leaking pipes.

A large number of agricultural wells have been drilled without any authorization or licensing , many of them unregistered, the number reaches 4000, The water supply from Israel has almost halved from 1998 to 2004 in violation of the Oslo Accords. In addition, missile strikes and Israeli military attacks repeatedly damaged and destroyed pipelines. For example, the Israeli military attacks of 2006 caused untold damage to water infrastructure, with the destruction of the Gaza power station affecting the operation of most wells, pumping stations and sewage treatment facilities. In short, Gaza teeters on the brink of a humanitarian and environmental catastrophe and urgent action is required to prevent widespread suffering. Failure to control over-pumping has led to sea-water intrusion into the aquifer to the extent that, in 2003, only 10 % of the wells produced water comparable

to the World Health Organization (WHO) drinking water standards. To compound matters, USAID have recently pulled out of the Palestinian water sector, abandoning ongoing projects and closing their contactors' offices, in an international aid embargo aimed at undermining the Hamas government (Gray, A. 2007).

More recently, the Israeli destructive war on Gaza 2008/2009 caused a heavy damage in the water sector, where the war has led to the destruction of land and soil to great depths, which led to the ease of contaminants to the aquifer. The situation has been worsened by which is increased the use of toxic and radioactive materials by the Israeli army. They hit those materials into the underground reservoir of water (which is one of the most important water sources in the Gaza Strip) which is a shallow descent and a high rate of sandy soil that makes it an easy access to the materials of an underground reservoir pollution.In addition, this war led to the destruction of a large number of water wells and its associated systems, water reservoir and water distribution networks. Furthermore, the war destroyed a large number of wastewater pumping stations, wastewater treatment plants and wastewater collection networks and its accessories. This has led to more pollution in the underground reservoir.

1.4 Water Resources and Situation in Gaza Strip

The water resources in the Gaza Strip depend mainly on groundwater, which is partially renewed from rainfall. (Sami & Ibrahim 2001). Gaza Strip is characterized by its natural water resources scarcity. The water resource in Gaza strip depends totally on groundwater to meet their domestic, agriculture and industrial needs. Wadi Gaza, the main source of surface water has a wide catchments area outside Gaza strip territories (about 3500 km2). At the present, there is no much water flowing in the Wadi because of Israelis activities who constructed catchment reservoirs in order to intercept the flow stream of water into the Wadi. (PWA, 2000).

Groundwater is the main source of water in the Gaza Strip, where the rainfalls are limited and inadequate, in addition to a number of valleys which are not so important to the water sources in the Gaza Strip, including the following:-

1.4.1 Groundwater

Groundwater is the water present in the pores of sedimentary rocks formed across different times be recent or very old for millions of years. Often the source of this water is rains or permanent or seasonal rivers or melting ice and water seeping from the Earth's surface to within what is known as the nutrition (Recharge). In the Gaza Strip, the main source of groundwater comes from the coastal aquifer (shallow aquifer), which consists mainly of sandstone, sand and gravel. The aquifer is an extension of the coastal plain aquifer in Israel. The aquifer is highly permeable with transmissivity of about 1000 m2/day and an average porosity of 25%. The depth to water ranges between 70 meters in the highly elevated area in the east and 5 meters in the low land area. The total annual recharge of the aquifer is estimated at 55 MCM. A deficit with an average of 70 MCM/year is observed in the water balance due to over pumping. Therefore, the aquifer is replenished from brackish or seawater, which results in a deterioration of quality. An average of 160 MCM/year is pumped from the groundwater aquifer and is distributed over domestic and agricultural water wells. (Mohammed, 2007).

The Coastal Aquifer System (which includes the Gaza Aquifer) extends along 120 km of the Mediterranean coastline from Gaza in the South to Mediterranean Carmel in the North. Its width varies from 3-10 km in the North to approximately 20 km in the South. The flow is generally from east to west. It is replenished by rainfall (approximately 372 MCM yr-1 (EXACT, 1998), artificial recharge and percolation of wastewater from anthropogenic sources. The natural annual sustainable yield of the aquifer is estimated to be approximately 65 MCM yr-1 in Gaza (WRAP, 1994) and 320 MCM yr-1 in Israel (Israeli Ministry of National Infrastructures, 2001), although this figure fluctuates from year to year depending on precipitation (Sherman, 2001).

Due to the fact that the Gaza aquifer is downstream of the Israeli coastal aquifer, limiting water development in Gaza has been of no strategic interest to Israel. However, Gaza faces a different set of problems due to overpopulation of the area. By 1967, the number of wells in Gaza was approximately 1200 (Nasser, 2003), pumping 65 MCM water yr-1, which is approximately the natural replenishment rate of the aquifer (WRAP, 1994). By 1993, as the population had expanded, so too had water use. There were 2100 registered wells and an estimated 900 unregistered wells extracting between 100 and 110 MCM of water yr-1, a figure well above the aquifer’s sustainable yield (WRAP, 1994). At present,

there are thought to be 4000 agricultural wells withdrawing 82 MCM yr-1, and 125 domestic wells which extract 74.9 MCM yr1 (PCBS, 2005), creating an annual deficit of 44.8 MCM by which the aquifer is overdrawn. Coastal aquifers add a significant complication to aquifer management as salt water intrusion may render portions of the aquifer unusable (Maimone., 2002). This problem is particularly troublesome in Gaza where the aquifer provides the sole source of water for the population; however increases in chloride concentrations have also been recorded in wells in the central and northern parts of the aquifer in Israel (EXACT, 2000). Influx of salt and contaminants from anthropogenic sources are also contributing to the deterioration of water quality in the coastal aquifer in both Gaza and Israel (Bachmat and Khalid, 2000). Currently only 10 % of the water used in Gaza meets World Health Organization (WHO) drinking water standards.

1.4.2 Surface Water

Surface water includes "lakes, ponds, valleys, floods, and watercourses, whether permanent or seasonal." The runoff depends on a number of factors: the intensity and continuity of precipitation and soil type, vegetation and topography. The latter is the most important factor followed by heavy precipitation and its sustainability. The provisional run of surface water caused by rainfall in winter, the main source of surface water in the Gaza Strip, which did not last long. But there are some potential sources of surface water such as running water in the valleys, which collect in the valleys of a small valley, Beit Hanoun and Wadi al Salqa in addition to Gaza valley, which is located in the central region of the sector, which was considered the most important source of surface water in the Gaza Strip, taking to the northwest - South East up to a length of about 8.5 km. The flow of water during the rainy season at 10-15 days a year. The mountains north of Hebron and the Negev are the main sources of water to reach the valley area received about 3500 km² into the water while in the Mediterranean Sea in the area between the towns of Khan Younis, Gaza and the West. The rate of runoff in the valley annually by about 2-3 million cubic meters. However, as a result of Israeli practices towards him, which was represented in the construction of dams along its course and the conversion of some routes to within the Green Line, which led to a severe shortage of discharge. It is also the result of deliberate neglect by the Israeli occupying forces throughout the occupation years, turning the valley for many years was forced to health, as these forces have often pump waste water to the

valley from within the Green Line. For example, during the year 2005, pumping about 15,000 cubic meters per day of waste water to Wadi Gaza. Moreover, the dumping of solid waste and liquid from the central region in the valley. Also contributed to arrest the Israeli occupation of rainwater run-in Wadi Gaza is part of the Gaza Strip to prevent the course of cleansing Valley of any kind of health risks, in addition to the pollution of riverine communities and polluting the sea, as well as to worsen the problem of mosquitoes in areas along the valley, which has adversely affected the health of the Palestinian citizen. Therefore, the Wadi Gaza, in addition to other valleys in the Gaza Strip almost are useless sources of water and transforming Gaza Valley to a source of pollution (Ghanam, 2008).

1.4.3 Rainwater

The Palestinian territories in the northern temperate zone on the east coast of the Mediterranean spread between the Syrian desert and the Sinai desert. Accordingly, these influences have affected marine and desert, the climate of the Palestinian territories. This had affected the climate of the Palestinian territories, where temperatures and rainfall several factors, including, continues a series of mountains stretching from north to south of the coastal plain of the Mediterranean and desert that extends from Egypt through the Sinai to the north of Africa, the Syrian desert, which borders part of the Palestinian territories from the East .

Feeds are part of the quantities of rainfall in the Gaza Strip and ground water through seepage and leakage. This differs from region to region depending on several factors, most important, permeability and thickness of the surface layers above the reservoir and underground seepage rate hovers between 25% in the permeability of the few areas stationed in the eastern areas of Gaza and about 75% in areas of dune Sand stationed in the northern and southern parts of the sector, in addition to several factors, the rate of evaporation, wind and intensity of the falling water with the duration of rainfall. On this basis the rate was taken in for nomination as 40% of the total amount of rainfall. In other words, the total amount of water feeding the underground reservoir of rain per year to reach about 33 million cubic meters for the year 2007/2008 (Ghanam, 2008).

1.5 Water Quality in Gaza Strip

The quality of Drinking water is one of the most important environmental issues that affect the health of people. Ensuring the safety of the drinking water is one of the most important and critical issues for the public health protection. Safe drinking water has been a key in some of the greatest public health achievement of the last half century in many countries, along with other basic public health measures (Lynda et al., 2000)

Drinking water protection has played a crucial role in building a safer and healthier society. In fact when we provide people with safe water, we are sharing in the protection of the public health mainly from water born diseases.
The microbiological monitoring for the drinking water in Gaza strip is below the normal and unsatisfactory. Different microbiological contaminations and infections especially in children at school age are attributed to drinking water. The microbial monitoring and assessment is very important to manage and control the quality and safety of drinking water. Therefore, it is necessary to microbiologically examine the marketed drinking water because microbiological safety of drinking water is of paramount importance to public health.

The most commonly identified pathogens in waterborne outbreaks is *Giardia lamblia*, a flagellated protozoan that can damage the microvillous lining of the upper small intestine (McSwane et al., 1994; Craun, 1988). Another current microbiological concern in drinking water is *Cryptosporidium* spp., a newly recognized human parasite that causes cryptosporidiosis (McSwane et al., 1994). Also *Pseudomonas aeruginosa*, an opportunistic pathogen often associated with waterborne-disease transmission has a relatively high resistance to disinfection (McFeters. 1990). Total and faecal coliforms in roof tanks are higher than their relative sources. Various levels of total and faecal coliforms have also been found in water samples from 20 ground water wells located in the surrounding of the random waste water treatment pond of Beith Lahia, Gaza Strip (Melad. 2002).

Exposure to molds may cause human allergies and infections and the main route of exposure is inhalation of mold conidia from contaminated indoor air. Some studies have suggested that exposure through inhalation of aerosolised molds from water can also occur.

Marketed water may spread potentially allergenic, toxigenic and opportunistic fungal species to hospitals and private homes. Species of mold have been shown to produce unwanted flavors and odors in water. Mold spores and hyphal fragments may become aerosolised in indoor air when contaminated water passes through showerheads, taps or toilet cisterns which may result in respiratory exposure to potentially harmful species. Some mold species may survive disinfection and water treatment and could therefore contaminate the water reaching the consumer (Hageskal, et al., 2006).

In many parts of the world different studies had addressed the microbiological assessment of the drinking water. Most frequent microbial species found in drinking water are *Giardia labmila*, *Cryptosporidium* spp, *Pseudomonas aeruginosa*, coliform, fecal coliforms and others. The justification for monitoring drinking water quality is to determine whether the water supply system is being operated correctly, and implying that the water is safe for drinking or not.

1.6 Problem statement

Water scarcity and insufficiency are becoming the main problems in the World, which are strongly connected to the reduced water quality. Urban development, human activities and industrialization deteriorate the quality of water and, in some cases, make it unsafe for utilization. The people of the Gaza strip (one of the most populated areas in the world) depend on groundwater for their agriculture, industries, and for household purposes like drinking, cooking, washing, cleaning and showering. Long-term overexploitation in the Gaza Strip and the constraints by the Israeli occupation authority of using more of the ground water have resulted in a decreased supply of tap water, accompanied with low water quality. Furthermore, controlling and monitoring the quality (biological, physical and chemical) of the water wells and water distribution networks at Gaza strip are not always possible due to the constraints applied by the Israeli occupation either on the entry of the disinfectants and instruments required for water quality assurance or some times on reaching the wells that are located near the hot and clash points. Therefore, and due to the reduced quantities and qualities of drinking water, the population at Gaza strip try to seek other sources for drinking water which include the marketed desalinated water (by reverse osmosis) and the bottled water (usually known as natural water) of the different trading companies in Gaza city. The microbiological monitoring for the drinking water in Gaza

strip is below the normal and unsatisfactory. Different microbiological contaminations and infections specially in children at school age are attributed to drinking water. The microbial monitoring and assessment is very important to manage and control the quality and safety of drinking water. Therefore, it is necessary to microbiologically examine the marketed drinking water (desalinized and bottled) in the Gaza strip. The microbiological safety of drinking water is of paramount importance to public health, especially in overpopulated areas like the Gaza strip where poverty, destitute health situation, deteriorated living issues and limited sources are common.

1.7 Study justification

Most people in Gaza strip depend on marketed drinking water as an alternative source for tapped or groundwater sources. Furthermore, they do believe it is the safest water available in this local area, so nearly most of them deal with this water as pare, safe and healthy water without any consideration neither to its sources, nor to the bottling, tanking, and marketing processes. The huge number of population depending on marketed water as a major drinking and cooking source justifies the necessity for a scientific microbiological evaluation and assessment that could lead to the suitability and validity of these marketed water sources for household utilizations, mainly for drinking and cooking purposes.

1.8 Purpose of the study

The purpose of this study is to assess the microbiological quality (identify and determine the diversity, occurrence and distribution) of the marketed drinking water in Gaza City. The study covers both the safety of the marketed drinking water and also the nature and identification of these microorganisms that include molds, coliforms, fecal coliforms, and *Pseudomonas aeruginosa*.

1.9 Objectives of the study

1. To assess the Microbiological quality of the marketed drinking water at Gaza city.
2. To examine and determine the contributing factors that result in the contamination of marketed water.
3. To investigate and demonstrate the source of contamination in marketed water.
4. To recommend measures and procedures for monitoring and ameliorating the quality of marketed drinking water.
5. To assess and recommend safety measures for the production, marketing, and warehousing of marketed drinking water.

1. 10 Research questions

1. Is the marketed drinking water at Gaza city microbiologically safe?
2. What are the types of microorganisms that are found in marketed drinking water?
3. What is the number of total coliforms and fecal coliforms that are found in the marketed drinking water?
4. Is there any relationship between the warehousing (storage) conditions and the presence of microorganisms in marketed drinking water?
5. What preventive measures should be adopted to secure the safety of marketed drinking water?

Chapter two

Conceptual framework and Literature review

Conceptual framework and Literature review

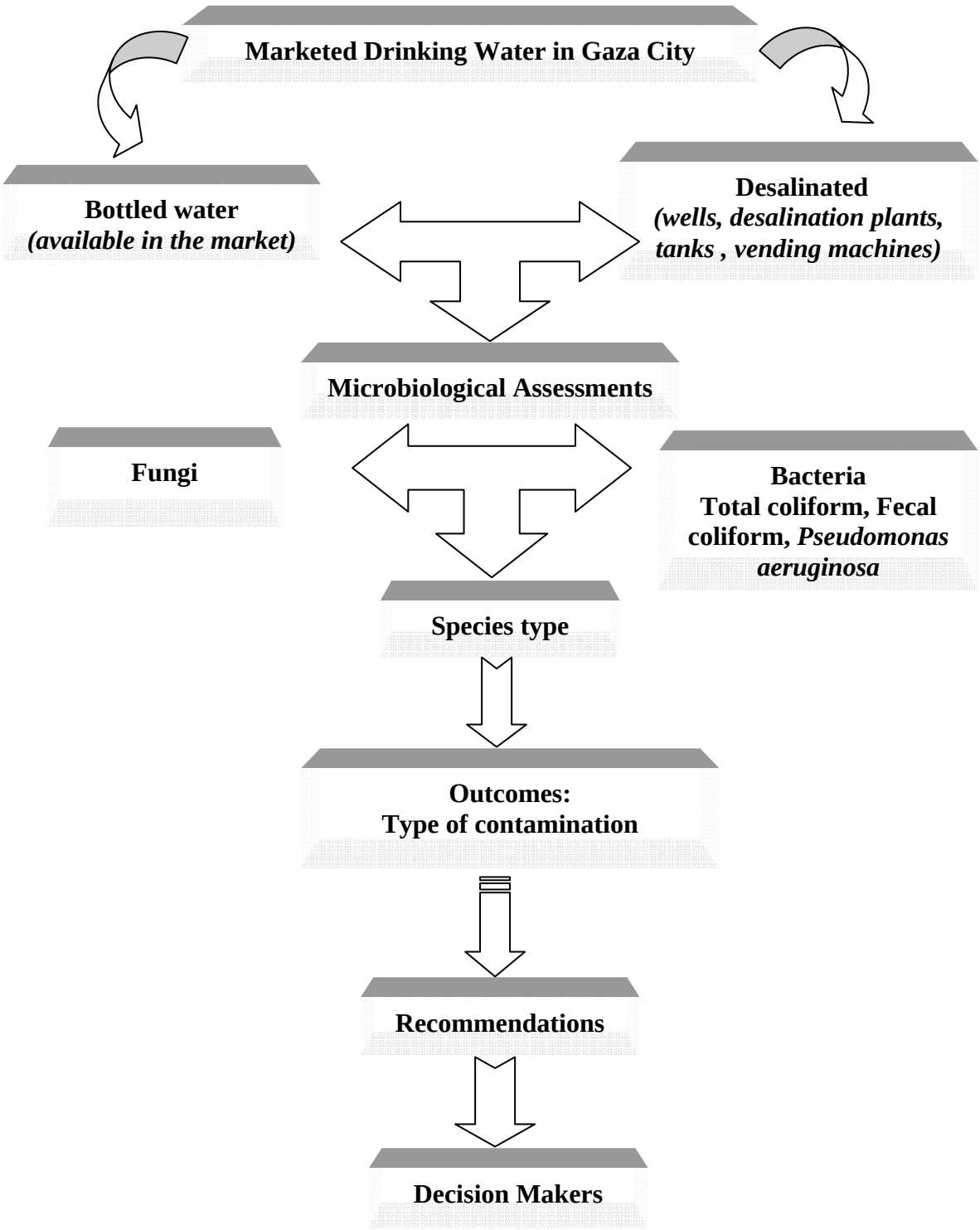
2.1 Introduction

This chapter discusses the microbial characteristics of water quality. It describes the microorganisms found in drinking water that can be harmful to health. It also discusses the 'nuisance organisms' that may affect the taste, odor or appearance of water but do not cause disease.

The most common and widespread health risk associated with drinking water is contamination, either directly or indirectly, by human or animal excreta and the microorganisms contained in faeces (ADWG, 2004). Furthermore, and addition to those contributing to the contamination that includes carriers of communicable enteric diseases (diseases of the gut), some of the microorganisms that cause these diseases may be present in the water. Drinking such contaminated water or using it in food preparation may cause new cases of infection. Those at greatest risk of infection are infants and young children, people whose immune system is suppressed, the sick, and the elderly.

The great majority of evident water-related health problems are the result of microbial (bacteriological, viral, protozoan or other biological) contamination (WHO, 2006). In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal (including bird) excreta. Faeces can be a source of pathogenic bacteria, viruses, protozoa and helminths.

The main topics which will be discussed in this chapter will include biologic components, indicators, and the guidelines that mentioned for safety drinking water.


Marketed Drinking Water in Gaza City
Bottled water
(available in the market)
Desalinated
(wells, desalination plants, tanks , vending machines)
Microbiological Assessments
Fungi
Bacteria
Total coliform, Fecal coliform, Pseudomonas aeruginosa
Species type
Outcomes:
Type of contamination
Recommendations
Decision Makers

Safe drinking water is essential to sustain life. Therefore, every effort needs to be taken to ensure that drinking water suppliers provide consumers with water that is safe to use. Access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection.

Drinking water is defined as water intended primarily for human consumption, either directly, as supplied from the tap, or indirectly, in beverages, ice or foods prepared with water. Drinking water is also used for other domestic purposes such as bathing and showering. (ADWG, 2004).

Drinking bottled water

Bottled water has been produced by distillation, deionisation or reverse osmosis. The water can come from a spring, or a public community water supply. Other suitable terms for bottled water produced by one of the above processes include “distilled water,” “deionised water,” and “reverse osmosis water.” These waters have no added minerals. (CBWA, 2002).

Bottled water

Bottled water is water sold to consumers in sealed containers. It must, therefore, be sealed in a sanitary container and must meet all applicable federal and provincial regulations for drinking water.

Bottled water cannot contain sweeteners or chemical additives and must be calorie and sugar free. (CBWA, 2002).

Biofilms are defined as a complex mixture of microbes, organic, and inorganic material accumulated amidst a microbially produced organic polymer matrix attached to the inner surface of the distribution system (USEPA, 2002).

A distribution system is defined as a system of conveyances that distributes potable water. All pipes, storage tanks, pipe laterals, and appurtenances that comprise the delivery system are included in this definition (Odom and Rotert, 2006).

An “indicator” is a parameter that can be measured and used as a surrogate for another parameter or condition which either cannot be directly measured or is difficult to directly measure (Odom and Rotert, 2006).

Desalination systems

Desalination is defined as the treatment of water (fresh water, brackish water, seawater estuarine water) to remove dissolved mineral salts and other dissolved solids by means of various water purification processes. The principal purpose of desalination is to enable sources of brackish or salty water, otherwise unacceptable for human consumption, to be used for this purpose. (WHO, 2006)

Desalination processes are used commercially to provide fresh water to many communities and industrial sectors around the world. The Middle East region has the majority (around 60%) of the currently constructed desalination capacity (WHO,2006).

Desalination technical processes

Desalination processes can be classified in two categories:

- Distillation processes; multi-stage flash (MSF), multiple-effect (MED) and vapour compression (VC) which are generally driven by thermal energy through solar thermal collectors and/or geothermal source.
- Membrane processes, reverse osmosis (RO) and electrodialysis (ED) that requires electrical energy which is generally supplied by solar photovoltaic and wind energy.(PHG-Palestine,CDER- Algeria,2004).

One of the major options for the remedy of water shortages in the Gaza Strip of Palestine, and the protection of its coastal aquifer from either depletion and/or becoming saline, is the utilization of desalination technology for brackish and seawater in that region. Other effective options for water supply may become feasible after a comprehensive peace prevails in the region. As a means of an immediate remedy to fresh water shortages in cities in central and southern Gaza and water conflict resolution, the donor countries have funded 3 relatively small brackish water reverse osmosis (RO) desalination plants, each of which produces between 45 and 75 m^3/h. Two private Palestinian water investing companies also established two similar RO plants. Several other much larger seawater RO desalination plants are planned to cover the overall shortages in the whole Gaza Strip and

allow time to restore and rejuvenate the polluted and saline Gaza aquifer. The Gaza Strip with its low rainfall and sandy soils depends greatly on its intensive agriculture production on irrigation resulting in an over utilization of the underground water resources. The high water withdrawal through about 4000, mostly unregulated, private farm wells with a capacity of 20–80 m^3/h and 95 municipality wells of 60–100 m^3/h, and lack of sufficient natural recharge is causing a deterioration of the valuable coastal aquifer system in the Gaza Strip. (Said A.A., 2001).

Due to the sharp shortage of water and the bad quality of groundwater, desalination plants were set up in the Gaza Strip area in Palestine. Currently, there are six reverse osmosis desalination plants in the Gaza Strip owned and operated by the Palestinian Water Authority (PWA) and different municipalities. In addition, there are many small desalination units owned and operated by private investors for commercial purposes. Currently there is a plan for a regional seawater desalination plant with a capacity up to 150,000 m^3/y. According to the PWA plan, desalination seems to be the only viable alternative for water resources. However, large-scale desalination plants seem to be a necessary option several years in the future. (Baalousha, 2006).

Water vendors

Vendors selling water to households or at collection points are common in many parts of the world where scarcity of water or faults in or lack of infrastructure limits access to suitable quantities of drinking-water. Water vendors use a range of modes of transport to carry drinking-water for sale directly to the consumer, including tanker trucks and wheelbarrows/trolleys. Water vending does not include bottled or packaged water or water sold through vending machines. (WHO, 2006).

Water tanks are liquid storage containers; these tanks are usually storing water for human consumption. A water tank provides for the storage of drinking water potable, irrigation agriculture, fire suppression, agricultural farming and livestock, chemical manufacturing, food preparation as well as many other possible solutions.

2.2 Chemical and physical assessment

Water of good chemical and physical quality is necessary from the point of view of its acceptability to the consumer, protection of consumer health, and conservation of water

system. Situations are encountered in which offending chemical substances have made a water source unacceptable to the public event through its bacteriological quality was excellent.

2.2.1 Physical assessment

2.2.1.1 Odor and taste

Odor and taste are the primary criteria consumers use to judge the quality and acceptability of drinking water. People's senses of taste and smell tend to vary, and so the acceptability of the same water can vary from person to person, and from day to day for the same person. Similarly, one individual within a group may be more or less sensitive to a particular substance than the group as a whole (look table 2:1).

Taste and odor in drinking water can be naturally occurring, or the result of chemical contamination and may also develop during storage and distribution due to microbial activity.(WHO,2006).

Odor in potable water may indicate pollution of the water or malfunction during water treatment or distribution. Odors of a biological origin can indicate increased biological activity.

2.2.1.2 Color

Two terms are used to describe color. 'True color' is the color after particulate matter has been removed (usually by filtration through a 0.45 micrometer pore size filter). 'Apparent color' is what one actually sees; it is the color resulting from the combined effect of true color and any particulate matter, or turbidity. In turbid waters, the true color is substantially less than the apparent color. (Look table 2:1).

In natural waters, color is due mainly to the presence of dissolved organic matter; surface water can also be colored by waste discharges, for example from dyeing operations in the textile industry.

Most people can detect colors above 15 true color units (TCU) in a glass of water. Levels of color below 15 TCU are usually acceptable to consumers, but acceptability may vary. High color could also indicate a high propensity to produce by-products from disinfection processes. No health-based guideline value is proposed for color in drinking-water.

True color is preferred analytically, as the measurement is more precise than for apparent color, and not as dependent on site or time. If both true color and turbidity are at the guideline values (i.e. true color of 15 Hazen units (HU) and turbidity of 5 nephelometric

turbidity Units (NTU)), the apparent color could be 20 HU. This is considered to be acceptable. (ADWG, 2004).

2.2.1.3 PH

PH is a measure of the hydrogen ion concentration of water. It is measured on a logarithmic scale from 0 to 14. A pH of 7 is neutral, greater than 7 are alkaline, and less than 7 is acidic (look table 2:1). Monitoring for pH is one of the most common tests conducted for water (Addy et al. 2004). Environmental protection agency (EPA) recommends pH monitoring to establish baseline water quality in the distribution system. In well-buffered waters, pH should remain fairly constant throughout the distribution system, as long as the water has come into equilibrium with the pipes and there are no significant corrosion problems (AWWA, 1999a).

The pH of water in the distribution system is an important factor in nitrification activity (Harrington et al., 2003).

A reduction in pH can be an indication of problematic biofilm growth. For example, a decrease in pH can result from growth of sulfur-reducing bacteria such as *Thiobacillus*. These bacteria generate hydrogen ions which lowers the pH (AWWA, 1995). A growth of nitrifying bacteria may also decrease the pH by oxidizing ammonium to nitrate and other nitrogen compounds (Schock, 1999).

2.2.1.4 Temperature

Temperature is primarily an aesthetic criterion for drinking water. Generally, cool water is more palatable than warm or cold water. In general, consumers will react to a change in water temperature. Complaints are most frequent when the temperature suddenly increases. High water temperature enhances the growth of microorganisms and may increase taste, odor, color and corrosion problems (look table 2:1).

Temperature is a very important parameter for many physical and chemical water treatment applications (AwwaRF, 2002). Changes in temperature are also important to predicting distribution system integrity breaches including mains breaks, corrosion, nitrification and changes in hydraulic conditions (NRC, 2006). Temperature difference between storage tanks and entry to the distribution system can suggest stratification in storage tanks and hence degradation of water quality that could lead to microbial regrowth in the distributions system (Mahmood *et al*., 2005). Many systems conduct online

temperature monitoring both at entry points and within the distribution system.Warmer temperatures are associated with increased growth rates of bacteria (Besner et al., 2002). Increases in summer occurrences of total coliform-positive samples have been reported (Colbourne et al., 1991; Olstadt et al., 1998). Coliform positive samples occur more frequently when the distribution system water temperature is above 15 ºC (Volk and Joret, 1994; Volk and LeChevallier, 2000; Besner et al., 2001).

2.2.1.5 Turbidity

Turbidity is caused by the presence of fine suspended matter such as clay, silt, colloidal particles and other microscopic organisms**.** Turbidity can have a significant effect on the microbiological quality of drinking water. High turbidity can both interfere with the detection of bacteria and viruses (look table 2:1).

Turbidity is also an important operational parameter in process control and can indicate problems with treatment processes, particularly coagulation/sedimentation and filtration. Turbidity can be used as an indicator for identifying contamination entry, hydraulic problems or finished water reservoir rehabilitation frequencies in the distribution system. Sudden increases in turbidity can indicate main breaks, backflow, fire fighting or hydrant opening, flushing, scheduled maintenance or repairs, valve failures, and treatment failures in the distribution system (Kirmeyer et al., 2002b).

Microorganisms can adhere to particles that protect them from disinfection, provide a source of nutrients, and facilitate their movement within the distribution system (Gauthier et al. 1999a; Morin et al. 1999). Furthermore, an increase in turbidity in the distribution system will exert a greater chlorine demand which could lead to inadequate disinfection of the distributed water (Kirmeyer et al., 2002b). Increased turbidity may be due to contamination in the storage tank, water age or mixing issues or tank material degradation (Kirmeyer et al., 2002b).

Table (2-1): Water Physical Standards

Physical assessment			
	WHO standard	**Australian standard**	**Palestinian standard**
Odor and Taste	acceptable to avoid consumer complaints.	should be acceptable to most people.	Acceptable to all customers (taste on 20C° acceptable)
Color	recommend a value of 15 HU.	true color in drinking water should not exceed 15 HU	Maximum acceptable 15HU
PH	a pH range of 6.5 to 8.5.	a pH range of 6.5 to 8.5.	6.5 -8.5 and maximum acceptable range of PH 9.5
Temperature	Temperature should be acceptable to avoid consumer complaints.	above 20°C may result in an increase in the number of complaints.	acceptable temperature 8-25 C°
Turbidity	turbidity above 5 NTU may give rise to consumer complaints	turbidity should not exceed 5 NTU.	Turbidity should not exceed 5 NTU

2.2.2 Chemical assessment

2.2.2.1 Chloride

Chlorides are compounds of chlorine with other elements or radical present in nearly all natural waters. There is a wide range of concentration but the most abundant concentration is with sodium (NaCl, common salt) (look table 2:2).

Chloride in drinking-water originates from natural sources, sewage and industrial effluents. The main source of human exposure to chloride is the addition of salt to food, and the intake from this source is usually greatly in excess of that from drinking water. (WHO, 2006).

Chloride is essential for humans and animals. A normal 70 kg human body contains approximately 80 g of chloride. (ADWG, 2004)

Chloride is present in agricultural, industrial, and domestic wastewaters that are discharged to surface waters. Home water softeners contribute a significant amount of chlorides as a result of the regeneration process. Human excreta are another significant source of chlorides with an average of about 6 grams of chloride per person per day (Metcalf and Eddy, 1991).

2.2.2.2 Nitrate

Nitrate (NO3) is an inorganic chemical composed of nitrogen and oxygen. Nitrate contamination of drinking water usually results from runoff of agricultural fertilizers or from human or animal wastes (EPA, 2005). (Look table 2:2).

Contamination of groundwater by nitrate is considered a global problem. Nitrates are introduced in the groundwater from a variety of sources like agricultural activities, poor sewer system, wastewaters, and industrial activities. (Abdulrahman et al , 2008).

Nitrogen is essential for all living things, but high levels of nitrate-nitrogen in drinking water can be dangerous to health. The susceptibility of infants to nitrate has been attributed to their high intake relative to body weight, to the presence of nitrate-reducing bacteria in the upper gastrointestinal tract, and to the greater ease of oxidation of foetal haemoglobin (present in this form for the first few months of life). The problem of methaemoglobinaemia “blue-baby syndrome,” which can be fatal, does not arise in adults. Increased sensitivity may also occur when infants suffer gastrointestinal disturbances, which increase the numbers of bacteria that can convert nitrate into nitrite. The reconstitution of powdered milk, as opposed to other forms of milk, has also been regarded as increasing the sensitivity to nitrate content in water. (EPA, 2005) and (Abou_Nasser, 2003).

2.2.2.3 Fluorides

Shomar *et al* (2004) who studied Fluorides in groundwater found that a high positive correlation was found between fluoride concentrations in groundwater and occurrence of dental fluorosis. Among 353 school children of the five geographic areas of the Gaza Strip the prevalence of dental fluorosis was 60%, and 40% had no signs of fluorosis in their permanent dentitions. (look table 2:2)

The highest occurrence, 94%, was in Khan Yunis, followed by 82% in Rafah, 68% in the middle area, 29% in Gaza and the lowest occurrence of 9% was in the northern area. These percentages were directly proportional to the average content of fluoride in groundwater of each area: 2.6, 0.9, 1.7, 1.2, and 0.7 ppm, respectively. The exception was Rafah where people drinking from new groundwater wells that have been dug in the last 10 years.

Table (2-2): Water Chemical Standards

Chemical Assessment			
	WHO standard	**Australian standard**	**Palestinien standard**
Chloride	should not exceed 250 mg/L.	should not exceed 250 mg/L.	should not exceed 250 mg/L.
Nitrate	the maximum acceptable limit of nitrate is 50 mg/L as NO_3.	the maximum acceptable limit of nitrate is 50 mg/L as NO_3.	the maximum acceptable limit of nitrate is 50 mg/L as NO_3.
Fluoride	1.5 mg/L	1.5 mg/L	0.6- 1mg/L and maximum to 1.5 mg/L

2.3 Biological contamination

Safe drinking-water, as defined by the guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. Those at greatest risk of waterborne disease are infants and young children, people who are debilitated or living under unsanitary conditions and the elderly. Safe drinking-water is suitable for all usual domestic purposes, including personal hygiene (WHO, 2006).

Pathogenic organisms of concern include bacteria, viruses and protozoa; the diseases they cause vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis, cholera or typhoid fever (ADWG, 2004). However, the classic waterborne diseases are caused by organisms originating in the gut of humans or other animals.

Whereas, many organisms of environmental origin that are not normally associated with the gastrointestinal system are found in water, and some of these organisms may, under certain circumstances, cause disease in humans. Such organisms include the protozoan *Naegleria fowleri*, a number of bacteria, including *Pseudomonas, Klebsiella* and *Legionella* spp, and some species of environmental Mycobacteria (ADWG, 2004). Infection is the main, but not the only, problem associated with microorganisms in drinking water. For instance, certain algae and bacteria can produce toxins that affect humans; the toxins may remain in the water even when the organisms responsible have been removed. Other ‘nuisance organisms’ can cause problems of taste, odor or colour, or promote deposition and corrosion.

Waterborne gastrointestinal infections remain one of the major causes of morbidity and mortality worldwide (World Health Organization, 2002b; World Health Organization, 2003a). The most important microbes causing infections or epidemics through drinking water include the bacteria *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and *Yersinia enterocolitica*, viruses such as: adeno-, entero-, hepatitis A- and E-, noro-, sapo- and rotaviruses and the protozoa: *Cryptosporidium parvum*, ,*Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia duodenalis* and *Toxoplasma gondii* and the helminthes nematode *Dracunculus medinensis* (World Health Organization,2004).

2.3.1 Parasites

The most common waterborne pathogens and parasites are those that have high infectivity and either can proliferate in water or possess high resistance to decay outside the body (WHO, 2006). The most commonly identified pathogen in waterborne outbreaks is *Giardia lamblia*, a flagellated protozoan that can damage the microvillous lining of the upper small intestine (Craun, 1988, McSwane *et al*, 1994).

Enteric parasites such as *Giardia* spp. and *Cryptosporidium* spp. are well recognized as emerging pathogens in drinking water and as being able to cause severe waterborne enteritis even with small doses, especially in immunocompromised persons (Franzen and Muller, 1999; Szewzyk *et al*., 2000). Giardia spp. And Cryptosporidium spp. are common causes of human diarrhoeal diseases in the developed and developing countries (Marshall *et al*., 1997; Clark, 1999).

The genus *Giardia* comprises six species that can infect a variety of hosts. *Giardia duodenalis* (also referred to as *G. intestinalis* or *G. lamblia*) is infectious for humans but can also cause infections in other hosts (Monis *et al.*, 2003). The spectrum of clinical giardiasis varies from asymptomatic carriers to severe diarrhoea and malabsorption. Acute giardiasis develops after an incubation period of 1-14 days (mean 7 days) and usually lasts 1-3 weeks.

Yassine et al., (2006) found that giardiasis was strongly correlated with fecal coliform contamination in water networks (r=0.7) compared with diarrheal diseases and hepatitis A (r=0.3 and 0.1, respectively).

Ashbollt (2004) in study of Microbial contamination of drinking water and disease outcomes in developing regions found that in addition to the traditional pathogens (helminths, *Entamoeba histolytica, Giardia lamblia* hepatitis A and E) various enteroviruses, *Campelobacter jejuni* and *Helicobacter pylori* are emerging issues in adults.

Waterborne outbreaks of giardiasis have been reported for some 30 years (Moore *et al*., 1969; Brodsky, Spencer & Schultz, 1974; Craun, 1990). In the USA, *Giardia* is the most commonly identified pathogen in outbreak investigations, with more than 100 waterborne outbreaks, based on epidemiological evidence (Craun, 1990). Waterborne outbreaks have also been reported in Australia, Canada, New Zealand, Sweden, and the United Kingdom. These outbreaks have been linked to consumption of untreated surface water contaminated by human sewage (Craun, 1990) or by wild rodents (Moore *et al*., 1969; Dykes *et al*., 1980), to groundwater that was contaminated by human sewage or contaminated surface water, to surface water systems treated only by disinfection (Craun, 1984; Kent *et al*., 1988) or by ineffective filtration (Dykes *et al*., 1980; Craun, 1990), and to cross-connections or damage in water-distribution systems (Craun, 1986).

2.3.2 Viruses

Viruses are among the smallest of all infectious agents. The viruses of most significance for drinking water are those that multiply in the human intestine and are excreted in large numbers in the faeces of infected individuals. Although they cannot multiply outside the tissues of infected hosts, some enteric viruses can survive in the environment and remain infective for long periods. Human enteric viruses occur in water largely as a result of contamination with sewage and human excreta. The numbers of viruses present and their species distribution will reflect the extent to which they are being carried by the population. Water is often only one of various routes of transmission; it is not always the major route (ADWG, 2004).

Enteric viruses are extremely small microorganisms that multiply only in the gastrointestinal tract of humans and other animals. Enteric viruses cannot multiply in the

environment, but they can survive longer in water than most intestinal bacteria and are more infectious and resistant to disinfection than most other microorganisms. The presence of *E. coli* is an indication that enteric viruses could also be present. However, because enteric viruses are more resistant to disinfection, the absence of *E. coli* does not necessarily mean that enteric viruses are also absent. Enteric virus always found in sewage samples, even in smaller communities, indicating their widespread presence. For example, HAV has been detected in sewage, in polluted rivers and in drinking water (Gerba et al. 1985; Bloch et al. 1990; AWWA 1999a; Jothikumar et al. 2000; Scipioni et al. 2000; Pina et al. 2001). Enteric virus in drinking water can be the result of lack of treatment, insufficient disinfection or inadequate treatment of surface water containing high levels of viruses (Payment and Armon 1989; Gerba and Rose 1990).

Human enteric viruses can be found in environmental waters contaminated by wastewater and direct discharge of human waste.

These microbes are of particular significance to public health and can cause disease by ingestion of contaminated drinking water.(Lipp, 2005).

Luksamijarulkul *et al*., (1994) in a study found that HAV and coliform contamination rates of drinking water were 25.26% and 64.21%, respectively. The rain water had the highest contamination (60.00% and 80.00%). Tap water was 23.73% for HAV (14/59 samples) and 64.41% for coliforms (38/59 samples) whereas running water had the least contamination (2.94% for HAV and 5.88% for coliforms). The contamination rates of used water were 10.69% for HAV and 68.67% for coliforms.

In a study by Locas *et al*., (2008), They found that when testing samples of water from municipal wells, total coliforms and *E. coli* remains the best approach to detect contamination of source water by fecal pollutants and accompanying pathogens. The absence of total coliforms at a site appears to be a good indication of the absence of human enteric viruses mainly (HAV, and HEV).

Barbara *et al*,.(2008) in their study revealed that enteroviruses are being isolated from all types of water: ground, sea, sewage and fresh water environments but also - and what is the most important from the epidemiological point of view - drinking water. They are resilient organisms, able to withstand high concentrations of sodium chloride (NaCl) and large changes in temperature. These abilities allow the viruses to flourish in a water environment.

However, Botzenhart K. (2007) in his study found that viruses in drinking water can cause infectious diseases. In the past, hepatitis A and E were the most frequently observed drinking- water-borne viral infections, but in recent years several small- and large-scale norovirus epidemics have been described, even in Europe. All virus species spread via drinking water are of fecal origin. Virological tests are not reliable enough to ensure that drinking water is sufficiently virus-free. The examination of 100 mL of water for *E. coli* and coliform bacteria is not adequate proof either. If potentially contaminated raw water is used.

Sarguna P., *et al* (2007) found that overcrowding and poor sanitation and living conditions contributed to the rapid spread of the outbreak of acute viral hepatitis.
(HAV) and (HEV) viruses are associated with inadequate water supplies and poor sanitation and hygiene, leading to infection and inflammation of the liver. Poor sanitation in developing regions, however, results in early infection of HAV and lifelong protection from the severe ill effects seen in unexposed people (in developed regions) of 50 years or older (Kindhauser, 2003).

Bae J. *et al* (2008) found that Human noroviruses (NoVs) are a significant cause of nonbacterial gastroenteritis worldwide, with contaminated drinking water as a potential transmission route.

2.3.3 Bacteria

Currently, water quality is assessed using proxies for pathogens. The most commonly used fecal indicator bacteria include total and fecal coliform bacteria, *E. coli* , enterococci and *P. aeruginosa* and *A. hydrophila* were studied as water quality indicators (Miescier and Cabelli, 1982).

Total coliform and *E. coli* counts are used worldwide as indicators for faecal contamination of drinking and recreational bathing water (Edberg *et al*., 2000; Havelaar *et al*., 2001; Rompre *et al*., 2002; Scott *et al*., 2002).

Microbial indicators of drinking water quality and faecal contamination should 1) be absent in unpolluted water and present when a source of pathogenic microorganisms is

present, 2) not multiply in the environment, 3) be present in greater numbers than the pathogenic microorganisms, 4) respond to natural environmental conditions and water treatment processes in a manner similar to that of the pathogens and 5) have methods available for their isolation, identification and enumeration (Medema *et al*., 2003a).

Total coliform, fecal coliform, and *E. coli* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. The fecal coliform group is a sub-group of total coliform and has fewer kinds of bacteria. *E. coli* is a sub-group of fecal coliform (EHFS, 2004).

Table (2:3) water bacterial standards

Water Bacterial Standard		
	WHO standard	**Palestine standard**
Total coilfrom	Due to the lack of direct health significance, No guideline value is proposed for coliforms (excluding *E. coli).*	Must be (Zero) when using membrane filter technique should be not detected
Fecal colifrom thermotolerant coliforms	Should not be detected in a minimum 100 mL sample of drinking water	*Must be (Zero) when using MFT* should not be detected in a minimum 100 mL sample of drinking water
Pseudomonas aeruginosa	No guideline value has been established for *Pseudomonas aeruginosa* in drinking water	
Fungi (molds & yeast).	No significance in drinking water	

2.3.3.1 Total coliforms

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. Total coliforms include organisms that can survive and grow in water. Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals.

Total coliform bacteria are commonly found in the environment (e.g. soil or vegetation) and are generally harmless. If only total coliform bacteria are detected in drinking water, the source is probably environmental, and fecal contamination is not likely. However, if environmental contamination can enter the system, there may be a way for other pathogens to enter the system. Therefore, it is important to determine the source and resolve the problem (EHFS, 2004).

Total coliforms have, in the past, been used as indicators for pathogens; they are no longer recommended for this purpose. Total coliforms are now recognised as being a poor parameter for measuring the potential for faecal contamination in water because they are present as normal inhabitants of soil and water, and can grow in water distribution systems in the absence of faecal contamination.

Total coliforms include *E. coli*, *Citrobacter, Enterobacter*, *Klebsiella* and other related enterobacteria. Coliform bacteria other than E. coli are capable of multiplying in water to high numbers, given the right conditions (ADWG,2004).

2.3.3.2 Fecal coliform:

Coliforms are gram-negative, nonsporing rod-shaped bacteria capable of aerobic and facultative anaerobic growth in the presence of bile salts or other surface active agents with similar growth-inhibiting properties.

They are found in large numbers in the faeces of humans and other warm-blooded animals. Thermotolerant coliforms, including *E. coli*, can ferment lactose (or mannitol) at 44.5± 0.2°C with the production of acid within 24 hours (WHO, 2006).

Thermotolerant coliforms are now the preferred designation for the group of bacteria previously referred to as fecal coliforms (WHO, 1996). This group of organisms, which are distinguished from other members of the total coliform group by their ability to ferment

lactose at a specified elevated temperature, includes thermotolerant strains of the genera *Klebsiella*, *Escherichia*, *Enterobacter* and *Citrobacter* (WHO, 2004). In drinking water, *E. coli* typically comprises the majority of the thermotolerant coliforms isolated.

Fecal coliform bacteria are a sub-group of the total coliform group. They appear in great quantities in the intestines and feces of people and animals. The presence of fecal coliform in a drinking water sample often indicates recent fecal contamination ,meaning that there is a greater risk that pathogens are present than if only total coliform bacteria are detected. *E. coli* is a subgroup of the fecal coliform group. Most E. coli are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, may cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination, meaning that there is a greater risk that pathogens are present. *E. coli* outbreaks receive much media coverage. Treating contaminated drinking water with a disinfectant or boiling the water destroys all *E. coli*, including O157:H7 (EHFS, 2004).

Escherichia coli is considered the most suitable index of faecal contamination because the organism does not generally multiply in drinking water systems. *E. coli* is the most common thermotolerant coliform present in faeces (typically greater than 90 per cent) (Edberg *et al*, 2000).

In most circumstances, populations of thermotolerant coliforms are composed predominantly of *E. coli*,(or, alternatively, thermotolerant coliforms) is the first organism of choice in monitoring programmes for verification, including surveillance of drinking-water quality..In addition, E. coli is far more sensitive to disinfection than are enteric viruses and protozoa.(ADWG, 2004; WHO,2006).

Alves *et al*., (2002) had evaluated the microbiological quality of different commercial mineral water brands, wells and reservoir supplies in surrounding areas of the city of Marlia, Brazil, to determine the amount of total and fecal coliforms. Eighteen samples of each source (mineral and reservoir supplies) were analyzed using Colilert Technique in cellophane. The results revealed that one sample of mineral water and one sample collected from the reservoir supply had been contaminated by a bacterium of the total coliform group, (one bacterium/100 ml of water). None of the water samples showed contamination by fecal coliforms.

Kassenga (2007) studied the health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. The author found that total and fecal coliform bacteria were present in 4.6% and 3.6%, respectively, of samples analyzed with a tendency for higher contamination rates in plastic-bagged drinking water. Microbiological quality of tap water was found to be worse compared with bottled water, with 49.2% and 26.2% of sampling points showing the presence of total coliform and fecal coliform organisms, respectively.

Another aspect determined by Schets *et al*., (2005) who studied "the *E. coli O157:H7* in drinking water from private supplies in Netherlands". The aim of his study was to examine *E. coli O157:H7* in drinking water and the sample included the microbiological quality of drinking water from 144 private water supplies in the Netherlands. Fecal indicators were enumerated by using standard membrane filtration methods. The presence of *E. coli O157:H7* was determined using a specific enrichment method. Eleven percent of the samples contained fecal indicators whereas *E. coli O157:H7* was isolated from 2.7% of the samples that otherwise met the drinking water standards. The *E. coli O157:H7* positive water supplies were located on campsites in agricultural areas with large grazer densities. Pulsed field gel electrophoresis (PFGE) analysis suggested that cattle might have been the cause of contamination. The main results indicate that compliance with microbiological quality standards obtained in routine monitoring does not always guarantee the absence of pathogens.

Yassin *et al*., (2006) found in Gaza strip that the major findings showed that the contamination level of total and fecal coliforms exceeded that of the World Health Organization (WHO) limit for water wells and networks. Furthermore, the contamination percentages in networks were higher than that in wells.

Also, Giannoulis *et al*., (2000) in their study found that the 36.8% of the source water samples was found in conformity with WHO guidelines, 42.1% of low risk, and 21.1% of intermediate risk while there were no samples of high or very high risk. The color-code classification for FC contamination was found as 36.8% A (blue, no risk), 42.1% B (green, low risk) and 21.1% C (yellow, intermediate risk).

Bharath *et al*., (2002) found that of the 344 water samples tested, 262 (76.2%) and 82 (23.8%) were domestic and imported brands, respectively. Eighteen (5.2%) of the 344 samples contained coliforms with a mean count of 6.38 ± 0.88 coliforms per 100 ml, while

5 (1.5%) samples contained *E. coli*. The prevalence of total coliforms in domestic brands of bottled water was 6.9% (18 of 262) as compared with 0.0% (0 of 82) detected in imported brands. The difference was statistically significant (*P*=0.004).

Chatterjee *et al*., (2007) in their study revealed that according to Central Pollution Control Board, India, total coliforms organism MPN/100 ml shall be 50 or less in drinking water source without conventional treatment but after disinfection (water class-A). The consumption of drinking water contaminated with pathogenic microbes of fecal origin is a significant risk to human health in the developing world, especially in remote rural areas and peri-urban `shanty' communities.

Lévesque *et al*.**,** (2008) who conducted study of "Assessment of microbiological quality of drinking water from household tanks in Bermuda". The tanks surveyed were selected randomly from the electoral register. Governmental officers visited the selected household (n = 102) to collect water samples and administer a short questionnaire about the tank characteristics, the residents' habits in terms of water use, and general information on the water collecting system and its maintenance. At the same time, water samples were collected for analysis and total coliforms and *E. coli* were determined by 2 methods (membrane filtration and culture on chromogenic media, Colilert kit). Results from the 2 methods were highly correlated and showed that approximately 90% of the samples analysed were contaminated with total coliforms in concentrations exceeding 10 CFU/100 mL, and approximately 66% of samples showed contamination with *E. coli*. Tank cleaning in the year prior to sampling seems to protect against water contamination. If rainwater collection from roofs is the most efficient mean for providing freshwater to Bermudians, it must not be considered a source of high quality drinking water because of the high levels of microbial contamination.Total and faecal coliforms in roof tanks are higher than their relative sources.

Various levels of total and faecal coliforms have also been found in water samples from 20 ground water wells located in the surrounding of the random waste water treatment pond of Beith Lahia, Gaza Strip (Melad, 2002).

2.3.3.3 Pseudomonas aeruginosa

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.

P. aeruginosa is a common environmental organism and can be found in faeces, soil, water and sewage. 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. *Pseudomonas aeruginosa* is sensitive to disinfection, and entry into distribution systems can be minimized by adequate disinfection. It cannot be used as an indicator of faecal contamination, but the presence of the organism in drinking water may indicate a serious deterioration in bacteriological quality. (WHO, 2006).

Pseudomonas aeruginosa is an opportunistic pathogen bacterium which is prevalent in water and can cause various diseases such as rash and otitis (especially for those who swim in the polluted water) (Itah and Ekpombok, 2004; Baumgartner and Grand, 2006).

Also *P. aeruginosa*, an opportunistic pathogen often associated with waterborne-disease transmission has a relatively high resistance to disinfection (McFeters, 1990).

P. aeruginosa (which has recently been introduced as a new indicator of water contamination) and number of phototrophic bactria (Okaghue et al., 2002, Wingender and Flemming, 2004, Lal and Kaur, 2006, Kassenga, 2007).

Pseudomonas and Aeromonas have been recovered from biofilms. In Barcelona, Spain, both *Pseudomonas and Aeromonas* were considered useful potential indicators of bacterial growth, although Pseudomonas was deemed a better indicator (Ribas *et al*., 2000). Stelzer et al. (1992) came to a similar conclusion after analyzing the drinking water supply in Germany.

In Mexico, De Victorica and Galvan (2001) documented an outbreak of *E. coli* and *P. aeruginosa*, where there was a primary infection by *E. coli* and a secondary infection by *P. aeruginosa* in 5 children. De Victoria and Galvan (2001) concluded that *P. aeruginosa* should be used as an indicator of waterborne diseases.

Hardalo and Edberg (1997) who conducted a study *Pseudomonas aeruginosa*: assessment of risk from drinking water. It can be recovered, often in high numbers, in common food, especially vegetables. Moreover, it can be recovered in low numbers in drinking water. A small percentage of clones of *P. aeruginosa* possesses the required number of virulence factors to cause infection. However, *P. aeruginosa* will not proliferate on normal tissue but requires previously organs. Further narrowing the risk to human health is that only certain specific hosts are at risk, including patients with profound neutropenia, cystic fibrosis, severe burns, and those subject to foreign device installation. Other than these very well-defined groups, the general population is refractory to infection with *P. aeruginosa*. Because of its ubiquitous nature, it is not only not practical to eliminate *P. aeruginosa* from our food and drinking water, but attempts to do so would produce disinfection byproducts more hazardous than the species itself. Moreover, because there is no readily available sensitive and specific means to detect and identify *P. aeruginosa* available in the field, any potential regulation governing its control would not have a defined laboratory test measure of outcome. Accordingly, attempts to regulate *P. aeruginosa* in drinking water would not yield public health protection benefits and could, in fact, be counterproductive in this regard.

Yoshpe *et al*., (1987), found in his study that there is a strong correlation between the presence of *P. aeruginosa* and TC and FC in seawater from beaches of central Israel, the result showed that the correlation between the presence of *P. aeruginosa* to that of TC and FC was 99.1 and 98.3%, respectively.

2.3.3.4 Fungi

Exposure to molds may cause human allergies and infections and the main route of exposure is inhalation of mold conidia from contaminated indoor air.

Some studies have suggested that exposure through inhalation of aerosolised molds from water can also occur. Marketed water may spread potentially allergenic, toxigenic and opportunistic fungal species to hospitals and private homes. Species of mold have been shown to produce unwanted flavors and odors in water. Mold spores and hyphal fragments may become aerosolised in indoor air when contaminated water passes through showerheads, taps or toilet cisterns which may result in respiratory exposure to potentially harmful species. Some mold species may survive disinfection and water treatment and could therefore contaminate the water reaching the consumer (Hageskal, *et al*., 2006).

The presence of fungi in drinking water and within biofilms of distribution systems has received limited attention. This is due, in part, to the fact that causal relationships between fungal occurrence and water quality remain uncertain. Nonetheless, waterborne fungi are likely associated with taste and odor problems, contamination in food and beverage preparation, and a variety of health-related effects (Bays *et al.*, 1970; Metzer *et al*., 1976).

Uncertain consequences of fungi in potable water have led to a limited number of investigations which show that fungi are present in a significant proportion of tap water samples; however, species abundance and diversity are extremely variable (Nagy & Olsen 1982; Rosenzweig *et al*.; 1986; Yamaguchi *et al*., 2007). This variation is often attributed to factors such as raw water source, water temperature patterns, treatment conditions, and maintenance of distribution systems. The nature and extent of fungi within distribution biofilms remain more obscure; only a single report has demonstrated conclusively that fungi are an integral biofilms component (Doggett, 2000). Although it is assumed that contamination is attributable to spore deposition within the biofilms matrix, there is limited evidence to support this premise.

Hageskal and co-workers in (2006) identified 94 mold species belonging to 30 genera in drinking water systems. The mycobiota was dominated by species of *Penicillium*, *Trichoderma*, and *Aspergillus*, with some of them occurring throughout the drinking water system. Several of the same species as isolated from water may have the potential to cause allergic reactions or disease in humans. Other species are common contaminants of food

and beverages, and some may cause unwanted changes in the taste or smell of water. The results of Hageskal and co-workers indicated that the mycobiota of water should be considered when the microbiological safety and quality of drinking water are assessed. Moreover, they recommended that molds in drinking water should possibly be included in the water supply and drinking water regulations (Hageskal *et al*., 2006) .

The microbiological contamination is also reported in water samples collected from the distribution system of big hospitals in Turkey. The results of Hapcioglu *et al*., showed that, in fifty-one samples, sixteen species of fungi were isolated, the most frequent being *Penicillium* spp. (24%), *Aspergillus* spp. (8%) and *Acremonium* spp. (5%). In thirteen samples more than one type of fungi was determined, Hapcioglu *et al*., (2005).

Cabral and Fernández., (2002) studied " Fungal spoilage of bottled mineral water ". The occurrence of filamentous fungi together with bacteriological parameters was assessed in 126 samples of still bottled mineral water of eight different commercial brands in Argentina. In spoiled samples with visible mycelium growth, the most frequently isolated fungal species were *Penicillium citrinum*, *P. glabrum*, other *Penicillium* species, *Cladosporium cladosporioides* and *Alternaria alternata*. In unspoiled samples, the genera found were *Penicillium*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Phoma*. Only three of the 126 samples failed to meet the required microbiological standards because they were found to contain faecal streptococci.

Chapter Three

Materials and Methods

Materials and methods

3.1 Study design

An experimental analytical design has been used with cross sectional method for data collection and water sampling for microbiological examination. In addition direct interviews were performed with the producers, distributors and sellers.

3.2 Population and samples

3.2.1 Study population

The population of this study included desalination plant, the water vending machines and water distribution vehicles in Gaza city that provide marketed drinking water for people. Furthermore, it included the common bottled drinking water produced at factories in the Gaza city. Each water sample was collected and analyzed in triplicate . In the present study vending machines are referred to the local distribution shop tanks.

3.2.2 Study sample

Convenient samples of desalination plant, vending machines, water distribution vehicles, and bottled drinking water in Gaza city have been taken. The water samples were identified, labeled and analyzed for microbial purposes.

3.2.3 Place of research

The study was limited to the samples selected from the desalination plant and bottled water factories working in Gaza city.

3.2.4 Study duration

The study duration was 3 months (June, July, and Aug.) to assess water quality.

3.2.5 Tools of the study

3.2.5.1 Questionnaire

Informative, operative, and hygienic data were collected through direct interviews with the producers, distributors and sellers. The questionnaire was divided into 3 sections. The first part is related to the water producing factories and desalination stations with inquires related to operations, sources of water, pre and post operation safety measures and quality control. The second part is related to distributors, while the third part is designed for the public seller points at the local shops. The second and third parts will include inquiries about storage, cleaning and hygienic issues. Detailed components of the proposed questionnaire are attached to the Annex (7).

3.2.5.2. Experimental and Laboratory Work

For desalinated water source, 3 samples of 500 ml each were collected in triplets from: the operative factory or station, the distribution containers, and from the vending machines at the shops. At each collection point, sterilization of the output end by direct flaming was performed before sampling. All samples were collected in sterilized containers. For each bottled water sample, the sample was collected for the same patch number in triplicates from the producing companies or from market. The collected 500 ml samples at each point were used to perform the afterwards microbiological examination.

Five hundred ml of water samples were used for the isolation of molds. The samples were filtered through specific membranes, and filters were incubated on Doxi's medium in darkness at 20 ±1 C° for up to 2 weeks. The number of colonies were determined (number of CFU per 100 ml).

Another five hundred ml water samples were used for the isolation of total bacteria. The samples were filtered through specific membranes and filters were incubated on nutrient agar medium at 37 ±1 C° for up to 48 hours. The number of colonies was determined (number of CFU per 100 ml).

For the isolation of total coliforms and *Pseudomonas aeruginosa* five hundred ml water samples were collected then membrane filtered, and the filters were incubated on MacConkey and cetrimide agar media respectively at 37 ±1 C° for up to 48 hours. For

fecal coliforms isolation, another 500 ml water samples were used, then membrane filtered and the filters were incubated on MacConkey medium at 44.5 ±1 C° for up to 48 hours.

Confirmatory identification was done for the isolated colonies to determine the genera incorporated in water contamination if present.

3.3 Equipments and instruments

- Ice box.

- 500 ml glass bottles.

- Bag.

- Autoclave.

- Volumetric flasks.

- 45 mm disposable pre-sterilized culture dishes (Petri dishes).

- Incubator.

- Vacuum filtration apparatus.

- 0.45 µm porosity membrane filters. (Schleicher & Schuell, ME 25/21 ST).

- Forceps." Sterilized"

- Media.(MacConkey Agar base, Nutrient Agar , Plate Count Agar, cetrimide media, Doxi medium) Annex (4).

3.4 Ethical consideration

An approval letter was obtained from Helsinki committee to carry out the study and make the necessary analysis that required in the study annex (1). Another approval letter was obtained from Coastal Municipalities Water Utility annex (2). However, the researcher explained the aim of the study for water distributors and sellers in Gaza city; and also the benefits of conducting such study.

The researcher did part from his experimental analysis on Public health laboratories in the ministry of health that approved the study annex (3). In addition, The researcher conduct

the second part of experimental analysis at Al Azhar University under the supervision of his main supervisor.

3.5 Sampling procedure

3.5.1 Sample bottle

Five hundred ml glass bottles were used to collect the samples. The glass bottles and covers were washed with tap water, and 0.1 ml of sodium thiosulfate per 100 ml of sample was added to glass bottles to neutralize any residual chlorine disinfectant. The glass bottles and the covers were sterilized at 121 C° for 15 minutes. The glass bottles with covers were tightly closed and only opened just before sampling.

3.5.2 Sample collection and transportation

Before samples collection, any external additions such as tap water filter or external pieces of plastic or rubber were removed so as to ensure no contamination of these accessories, the water was allowed to run for two minutes to insure that stagnant water is flushed from the pipes, then the tap water was flamed , to ensure that no pollutants which may affect the results, using a flame for a minute, after sterilization opening the pipe and water being left for two minutes to cool the exit of water so as to ensure that there is no killing for any microbe that might be occur present in the water to be tested, and then the sample was collected in the sample bottle and quickly closed tightly. The origin and the time of collection of the sample was written on the label, then the bottle was stored in the ice box under a temperature of up to 4 C°. Then the samples were transferred to the laboratory to perform the tests during a 2-6 hour. The sample volume was 500 ml.

3.5.3 Membrane Filter Technique

The membrane filter technique was used for measuring Coliform numbers (quantity) in the water sample (figure 3.1). This technique involves filtering a known volume (100 ml of drinking water samples) of water through a special sterile filter. These filters, made of nitrocellulose acetate, are 150 μm thick and have 0.45 μm diameter pores. A grid pattern is typically printed on these filter disks in order to facilitate colony counting. When the water sample is filtered, bacteria (larger than 0.45 μm) in the sample are trapped on the surface of the filter. The filter is then carefully removed, placed in a sterile petri plate on a pad saturated with agar-based medium, and incubated for 48 hours at 37 C°. It is assumed that each bacterium trapped on the filter will then grow into a separate colony. By counting the

colonies one can directly determine the number of bacteria in the water sample that was filtered. For the isolation of total coliforms and *Pseudomonas aeruginosa* five hundred ml of water samples were used then membrane filtered and the filters were incubated on MacConkey and cetrimide media respectively at 37 ±1 C° for up to 48 hours. While, for fecal coliforms isolation 500 ml of water samples were used, then membrane filtered and the filters were incubated on MacConkey medium at 44.5 ±1 C° and 37 ±1 C° for up to 48 hours. Five hundred ml of water samples were used for the isolation of molds. The samples were filtered through specific membranes, and filters were incubated on Doxi's medium in darkness at 20 ±1 C° for up to 2 weeks. The number of colonies was determined (number of CFU per 100 ml) along with the species diversity within each sample.

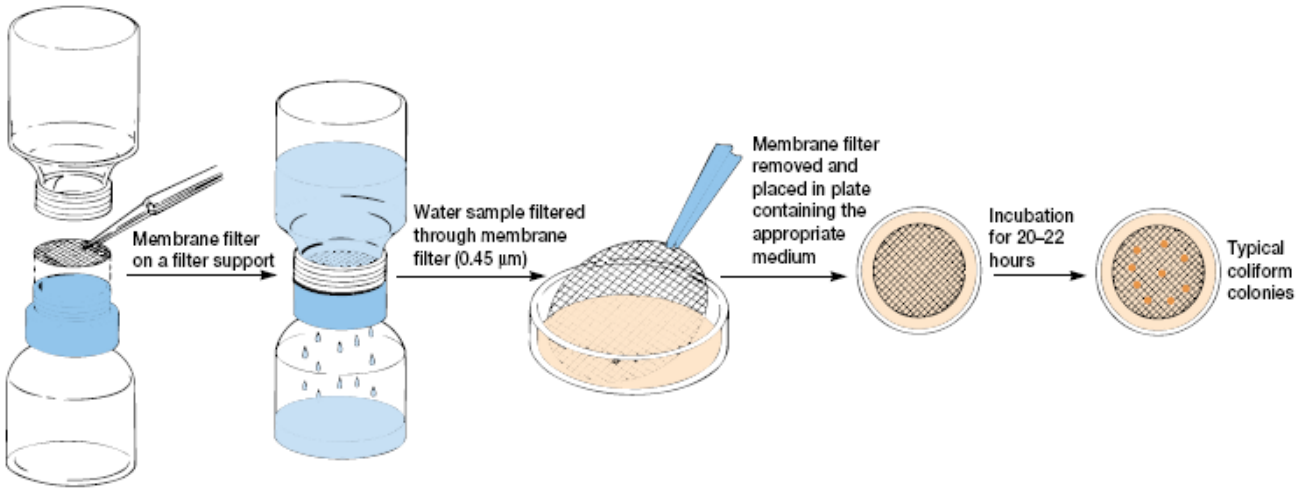


Figure 3.1 Membrane Filter Technique for the Direct Recovery of Coliform Bacteria from Water Samples (Laboratory exercises in microbiology, fifth edition, Prescott, 2002).

3.5.4 Procedure

1- Collect the sample.

2- Select the appropriate nutrient or culture medium.

3- Flame the forceps, and remove the membrane from the sterile package.

4- Place the membrane filter into the funnel assembly.

5- Flame the pouring lip of the sample container and pour the sample into the funnel.

6- Turn on the vacuum and allow the sample to draw completely through the filter.

7- Rinse funnel with sterile buffered water. Turn on vacuum and allow the liquid to draw completely through the filter.

8- Flame the forceps and remove the membrane filter from the funnel.

9- Place the membrane filter into the prepared Petri dish.

10- Incubate at the proper temperature and for the appropriate time period.

3.6 Analysis of drinking water samples

Total coliform determination:- A 100 ml of drinking water was vacuum filtered through a 0.45 μm sterile membrane filter (nitrocellulose acetate) and placed on MacConkey medium. The plate was incubated at 37 ±1 C° for 18-24 hr, Colonies were suspected to be Coliform were confirmed by using Gram stain and a portion of the colony was inoculated into lactose fermentation test tubes containing lauryl sulphate broth, also API 20E was used for identification.

Fecal coliform determination:- A 100 ml of drinking water was vacuum filtered through a 0.45 μm sterile membrane filter and placed on MacConkey medium. The plate were incubated at 44.5 ± 0.2 C° for 18-24 hr, and colonies were suspected to be Fecal Coliform confirmed by subculture on bile Esculin agar.

Pseudomonas aeruginosa determination:- A 250 ml of drinking water was vacuum filtered in through a 0.45 μm sterile membrane filter and placed on cetrimide media, the plate was incubated at 37 ±1 C° for up to 48 hours.

Fungi determination:- The samples were filtered through specific membranes, and filters were incubated on Doxi's medium in darkness at 20 ±1 C° for up to 2 weeks. The number of colonies was determined (number of CFU per 100 ml).

3.7 Bacterial identification

Identification of the obtained bacteria was based on the conventional taxonomy procedures as described in Bergey's manual of systematic bacteriology (Kreig and sueath *et al*, 1984), in addition of using prepared identification Kits (API systems) to confirm the identification (Anon., 1991).

3.7.1 Staining and microscopic examination

Simple and compound staining procedures were used for determining size and cell shape, Gram reaction. size, shape, color and other colony morphological features were also used to help in the identification.

3.7.2 Biochemical tests

After grouping the bacterial isolates to Gram-positive and Gram-negative bacteria, the following biochemical tests were used for bacterial identification to the level of genus/species : Methyl red, Voges-proskaur test "Barritt method" ,Citrate utilization "Simmon method", Indole reaction, Nitrate reduction, Urease test, Oxidase test "wet filter paper method", Fermentation of carbohydrates. All tests were done according to Bergey's manual of systematic bacteriology (Kreig and sueath et al, 1984).

3.7.2.1 Methyl red test

The methyl red test is employed to detect the production of sufficient acid during the fermentation of glucose. The liquid medium annex (5) was lightly inoculated from a young agar slope culture and incubated at 37 C° for 48 hrs. Five drops of the methyl red reagent (App. II) were added, mixed and the result was immediately recorded. Positive reaction gave a bright red color while the negative gave a yellow color.

3.7.2.2 Voges-Proskaur test

This test was used to detect the production of acetyl methyl carbinol ($CH_3CO_3CHOHCH_3$) or its reduction product, 2,3 butylene glycol ($CH_3CHOHCHOHCH_3$), which is produced from the fermentation of carbohydrates by many bacteria . The liquid medium, as used in methyl red test, was inoculated and incubated at 37 C° for 48 hr. annex (6)

One ml of 40% potassium hydroxide and 3 ml α- naphthol in absolute ethanol were added after incubation. A positive reaction is indicated by the development of a pink color shortly after 2-3 min, becoming in 30 min.

3.7.2.3 Citrate utilization test

This test demonstrates the ability of an organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen.

Simmon's solid citrate medium annex (5) was inoculated and incubated at 37 C° for 96 hrs. The indicator turns blue "with streak of growth" due to the alkaline positive reaction, while the slope remain green with no growth in the negative reaction.

3.7.2.4 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium. The liquid medium annex (5) was inoculated, and then incubated at 37 C° for 48 hrs. After incubation, 0.5 ml Kovac's reagent annex (6) was added, and shacked gently. A positive test is indicated by a red color in the alcohol layer.

3.7.2.5 Nitrate reduction test

This test determines the presence of the enzyme nitrate reductase which causes the reduction of nitrate. A liquid medium annex (5) was inoculated, and incubated for 96 hrs at 37 C°.A 0.1 ml of the test reagent annex (6) was added to the test culture. A positive test was indicated by a red color developing within a few minutes.

3.7.2.6 Urease test

Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by urease enzyme. Christensen's medium annex (5) was heavily inoculated over the entire slope surface and incubated at 37 C° for 24 hrs. If urea was decomposed (i.e. positive), the produced ammonia changes the color of the indicator to purple pink.

3.7.2.7 Mobility test

A semi-solid nutrient agar medium was used for testing the mobility of bacteria annex (5). The sterile medium was inoculated by stabbing the center of the medium, and then incubated at the optimum temperature for the organism. Mobility was manifested by a diffused zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffused growth through the medium, while others may show diffused spreading from one or two points only as nodular outgrowths along the stab.

3.7.2.8 Oxidase test

A strip of filter paper was soaked in a freshly prepared 1% solution of the oxidase reagent annex (6) and then used at once by rubbing a streak of culture on it with a platinum loop. A positive reaction is indicated by a coloration in 10-60 sec. and a negative reaction by absence of coloration or by coloration latter than 60 min.

3.7.2.9Carbohydrate fermentation

The ability of bacteria to ferment particular sugar was tested. Fructose, glucose, arabinose, raffinose, D-ribose, xylose, sorbitol, trehalose, maltose, lactose or galactose was individually added to the nutrient broth to a final concentration of 1 %. Acid production was detected by change the color of 0.01% phenol red indicator, while gas production was detected by the collection of bubbles in a small inverted tube (Durham tube) immersed upside down in the medium.

3.7.3 Confirmatory identification using API system

API-20E is a standardized identification system for Enterobacteriaceae. During incubation, each metabolic reaction produces color changes either spontaneously or revealed by the addition of certain reagent. The reactions were visually recorded and the final identification was obtained referring to the identification tables (Anon., 1991).

3.7.4 Fungi

Fungal isolates were purified investigated and microscopically.

3.8 Data entry and analysis

The Statistical Package for the Social Sciences (SPSS) version 11 was used in data entry, statistical analysis and treatments. Descriptive and frequencies were used in the clarification of the collected data.

N.B.: An experimental analytical design was used for water analysis regarding bacterial presence depending on their media.

3.9 Limitations

The limitations of the present study seem to be very little because the water samples were collected as purchased water from the market.

However, the most critical issues that arose during sample collection were that we found:

1. Some of desalination plants administrators refused to give water sample for unknown reasons.
2. Some desalination plants have no water distribution vehicles.
3. Some water distribution vehicles are not specified for particular desalination plants.
4. Some water distribution vehicles have no license numbers (without plate. Number).
5. Limited amount of the marketed bottled water during data collection period.

Chapter four

Results

4. Results:

The data of the present work were collected through two procedures: questionnaire and experimental analysis. The questionnaire provided data and information about the nature, origin, and operational system of the desalination plant, water distributions cars, and vending machines. While, in the experimental part of this work the researcher collected the water samples and followed the proper methodology and techniques for filtration and identification of the different microbiological contaminations.

4.1 Descriptive analysis of the different water containing systems

4.1.1 Desalinated drinking water

In the present work, 57 desalinated water samples were collected from water desalination system (Table 4-1). Twenty (35 %) water samples were collected from desalination plants, 12 (21%) from the water distribution cars, and 25 (44%) from the water vending machines that found at the shops. As mentioned in table 4-1, (55 %) of the desalination plants are producing water without any formal authorization or operation license. Nearly two-thirds of the desalination plants were established and operated in the last five years, with private wells is the source of water for 85 % of these desalination plants. About (60 %) of the desalination plants of the present work are operating with a daily production capacity of more than 20 cubic meters.

Regarding the chemical and microbiological monitoring and analysis of the input and the output water, (95 %) of the desalination plant operation directors reported the performance of these chemical and microbiological tests on water before and after desalination. In addition, about (90%) of the desalination plants are using plastic tanks for storing the desalinated output water, while only 10 % are using stainless steal container for the same purpose.

Table (4-1): Types of Water Stations Sources

Type of water resource	**No.**	**%**
• Water Stations	20	35%
• Water Distribution Cars	12	21%
• Vending Machines	25	44%
Total	**57**	**100**
Water Stations license	**No.**	**%**
• Yes	9	45%
• No	11	55%
Total	**20**	**100**
Water Stations Age	**No.**	**%**
• Less than one year	4	20%
• 1-5 year	13	65%
• More than 5 years	3	15%
Total	**20**	**100**
Stations Water Resources	**No.**	**%**
• Municipal well	1	5%
• Private well	17	85%
• Agricultural well	1	5%
• Other	1	5%
Total	**20**	**100**
Stations Daily Capacity	**No.**	**%**
• 5-10 m3	2	10%
• 11-20 m3	6	30%
• more than 20 m3	12	60%
Total	**20**	**100**
Water Test before and after Production	**No.**	**%**
• performed Outside Station	19	95%
• Not Performed	1	5%
Total	**20**	**100**
Tanks used for water storage	**No.**	**%**
• Plastic	18	90%
• Stainless steel	2	10%
Total	**20**	**100**

4.1.2 Water Distribution Cars

Table (4-2) showed that 12 (21%) samples were taken from water distribution cars in Gaza city. From which, there are (83.8%) sole water from specified stations. Eight of them (66.7%) doesn't make hygienic check for water safety. Seven (58.3%) did cleaning for water tanks at non-specified time, and five (41.7%) didn't make tank cleaning. However, five of them (41.7%) use Cl_2 for water disinfectant, and seven of them (58.3%) use other types of disinfectant including soap.

Table (4-2): Water Distribution Cars

Sole station for tanks	**No.**	**%**
• Yes	10	83.8%
• No	2	16.2%
Total	**12**	**100**
Hygienic check	**No.**	**%**
• Yes	4	33.3%
• No	8	66.7%
Total	**12**	**100**
Period of Hygienic Check	**No.**	**%**
• weekly	1	8.3%
• monthly	2	16.7%
• other	1	8.3%
• Not performed	8	66.3%
Total	**12**	**100**
Tank Cleaning	**No.**	**%**
• Yes	7	58.3%
• No	5	41.7%
Total	**12**	**100**
Period of Tank Cleaning	**No.**	**%**
• no specified time for cleaning	7	58.3%
• Not performed	5	41.1%
Total	**12**	**100**
Type of Sterilization	**No.**	**%**
• Cl_2	5	41.1%
• Other (soap)	7	58.3%
Total	**12**	**100**
Full Drainage	**No.**	**%**
• Daily	6	50%
• At the end of the stock	6	50%
Total	**12**	**100**

4.1.3 Vending Machines

There were 25 samples (44%) collected from different vending machine that found on front of the shops.

In the following table (4-3) the results showed that the most used vending machines made of stainless steal (92%) and 2 other samples were collected from plastic vends (8%). Four samples (16%) were performed the hygienic check for the used drinking water on monthly basis, but 21 samples (84%) were not performed the hygienic check for the used drinking water. Ninety six percent of the vending machine (23 vends) were restricted for sterilization and cleaning as reported by the operators of the vending machines, and most

of them (52%) did this on irregular time (not specified) but 40% of them (10 vends) did this on monthly basis.

Also, the table (3-4) represent that six samples (24%) used Cl_2 for disinfection of water, and 19 samples (76%) were used other disinfectant materials. About the location of the vends and its exposure to sun light, 3 samples (12%) were protected from direct sunlight and 22 samples (88%) were not protected from direct sunlight as reported by the operators.

Table (4-3): Vending Machines Characteristics and Treatment

Type of vending machines used for storage	**No.**	**%**
• Plastic	2	8%
• Stainless steal	23	92%
Total	**25**	**100**
Vending hygienic check in health	**No.**	**%**
• Yes	4	16%
• No	21	84%
Total	**25**	**100**
Period of vending check	**No.**	**%**
• Monthly	4	16%
• Not performed	21	84%
Total	**25**	**100**
Sterilization and Cleaning of Vends	**No.**	**%**
• Yes	24	96%
• No	1	4%
Total	**25**	**100**
Period of cleaning and Sterilization (Vend)	**No.**	**%**
• Weekly	1	4%
• Monthly	10	40%
• Other	13	52%
• Not performed	1	4%
Total	**25**	**100**
Type of Sterilization	**No.**	**%**
• Cl_2	6	24%
• Other	19	76%
Total	**25**	**100**
Full Drainage	**No.**	**%**
• Daily	1	4%
• Weekly	1	4%
• At the end of the stock	23	92%
Total	**25**	**100**
Location of Vend	**No.**	**%**
• protected from dust and sunlight	3	12%
• not protected from dust and sunlight	22	88%
Total	**25**	**100**

4.2 Microbial Presence in Drinking Water

4.2.1 Microbial Contamination in Desalination Plant

As shown in the following table (4-4) the results revealed that the most prevalent type of bacterial contamination was total coliforms. Twelve samples (60%) were contaminated with total coilforms ranging from (1 -50 cfu/100ml) and only 8 samples were not contaminated among the selected samples of the desalination plant.

Also, the results found that six samples (30%) were contaminated with fecal coliforms ranging between (1-30 cfu/100ml); where 14 samples (70%) were not contaminated.

Nine samples (45%) out of 20 samples were contaminated with *Pseudomonas aeruginosa* ranging between (1 -200 cfu/250ml) eleven sample (55%) were not contaminated.

While the results indicated that 5 samples (25%) were contaminated with mold ranging between (1-10 cfu/100ml); and 4 samples (20%) were contaminated with yeast ranging between (1-50 cfu/100ml).

Table (4-4): Microbial Contamination in Desalination plants:

Microbial Contamination		**No. of stations**	**Percent %**
Total coliform	No contamination	8	40%
	Contaminated	12	60%
	Total	**20**	**100**
Fecal coliform	No contamination	14	70%
	Contaminated	6	30%
	Total	**20**	**100**
Pseudomonas aeruginosa	No contamination	11	55%
	Contaminated	9	45%
	Total	**20**	**100**
Mold	No contamination	15	75%
	Contaminated	5	25%
	Total	**20**	**100**
Yeast	No contamination	16	80%
	Contaminated	4	20%
	Total	**20**	**100**

4.2.2 Microbial Contamination in Water Distribution Cars

As shown in the following table (4-5) the results revealed that six samples (50%) out of 12 samples were contaminated with total coliforms ranging between (1-20 cfu/100ml) in the water distribution cars. Where five samples (41.7%) out of 12 samples were contaminated with fecal coliforms ranging between (1-10cfu/100ml).

Six samples (50%) out of 12 were contaminated with *P. aeruginosa* ranging between (1-20 cfu/250ml); and six samples (50%) were contaminated with yeast ranging between (1-20cfu/100ml) in the water distribution cars.

While ten samples (83.3%) out of 12 samples were contaminated with molds ranging between (1-15cfu/100ml).

Table (4-5): Microbial Contamination in Water Distributions Cars

Microbial Contamination		**No. of cars**	**Percent %**
Total Coliform	No contamination	6	50%
	Contaminated	6	50%
	Total	**12**	**100**
Fecal coliform	No contamination	7	58.3%
	Contaminated	5	41.7%
	Total	**12**	**100**
Pseudomonas aeruginosa	No contamination	6	50%
	Contaminated	6	50%
	Total	**12**	**100**
Mold	No contamination	2	16.7%
	Contaminated	10	83.3%
	Total	**12**	**100**
Yeast	No contamination	6	50%
	Contaminated	6	50%
	Total	**12**	**100**

4.2.3 Microbial Contamination in Vending Machine

As shown in the following table (4-6) the results indicated that fifteen samples (60%) out of 25 samples were contaminated with total coliforms ranging between (1-50cfu/100ml) in the vending machine that found at the shops. While 4 samples (16%) were contaminated with fecal coliforms.

But there were twelve samples (48%) were contaminated with *P. aeruginosa* ranging between (1-50cfu/250ml).

While there were 14 samples (56%) were contaminated with molds ranging between (1-20cfu/100ml); and 4 samples (14%) were contaminated with yeast ranging between (1-15cfu/100ml).

Table (4-6): Microbial Contamination in Vending Machine

Microbial Contamination		**No. of vends**	**Percent %**
Total Coliform	No contamination	10	40
	Contaminated	15	60
	Total	**25**	**100**
Fecal coliform	No contamination	21	84
	Contaminated	4	16
	Total	**25**	**100**
Pseudomonas aeruginosa	No contamination	13	52
	Contaminated	12	48
	Total	**25**	**100**
Mold	No contamination	11	44
	Contaminated	14	56
	Total	**25**	**100**
Yeast	No contamination	21	84
	Contaminated	4	14
	Total	**25**	**100**

4.2.4 Microbial Contamination in Bottled water

As shown in the following table (4-7) the results indicated that the twelve samples that taken from the bottled water were free from total and fecal coliforms and yeast. While four samples (33%) were containing (>100 CFU/ml) which exceed the WHO standards that must be (maximum allowed amount must be less than 100CFU/ml).

However one sample was contaminated with *P. aeruginosa* (3 cfu/250ml) and two samples were contaminated with mold (10cfu/100 ml) and (15cfu/100ml).

Table (4-7): Microbial Contamination in Bottled water

No.	Source of sample	TC	FC	TBC	Ps.a.	Mold	Yeast
1.	Bottled water A (Room Temp.)	N	N	>100	N	N	N
2.	Bottled water A (dark)	N	N	>100	N	N	N
3.	Bottled water A (refrigerator)	N	N	>100	3	10	N
4.	Bottled water A (sun)	N	N	N	N	N	N
5.	Bottled water B (Room Temp.)	N	N	10	N	N	N
6.	Bottled water B (dark)	N	N	10	N	15	N
7.	Bottled water B (refrigerator)	N	N	2	N	N	N
8.	Bottled water B (Sun)	N	N	>100	N	N	N
9.	Bottled water C (Room Temp.)	N	N	5	N	N	N
10.	Bottled water C (dark)	N	N	15	N	N	N
11.	Bottled water C (refrigerator)	N	N	10	N	N	N
12.	Bottled water C (sun)	N	N	8	N	N	N

4.2.5. Microbial Contamination in Bottled water After Three Months of Storage

As shown in the following table (4-8) the results found that four samples (33%) were containing TBC (> 100 cfu/ml); and two samples (16.6 %) contaminated with total coliforms (3-10cfu/100ml) and mold (10-15cfu/100ml).

Table (4-8): Microbial Contamination in Bottled water After Three Months of Storage

No.	Source of sample	TC	FC	TBC	Ps.a.	Mold	Yeast
1.	Bottled water A (Dark)	N	N	80	N	N	N
2.	Bottled water A (Sun)	N	N	N	N	N	N
3.	Bottled water A (New)	3	N	>100	N	10	N
4.	Bottled water A (Room Temp.)	N	N	50	N	N	N
5.	Bottled water B (refrigerator)	N	N	20	N	N	N
6.	Bottled water B (Sun)	N	N	>100	N	10	N
7.	Bottled water B (New)	10	N	>100	40	15	N
8.	Bottled water C (dark)	N	N	>100	N	N	N
9.	Bottled water B (Room Temp.)	N	N	5	N	N	N
10.	Bottled water C (refrigerator)	N	N	2	N	N	N
11.	Bottled water C (sun)	N	N	N	N	N	N
12.	Bottled water C (Room Temp.)	N	N	N	N	N	N

Chapter Five

Discussion and recommendations

5.1 Discussion:

5.1.1 Total coliforms

The results showed that marketed drinking water in Gaza city was highly contaminated with various types of bacterial contamination. The desalination plants, water distribution cars, and vending machines were contaminated with Total coliforms bacteria that exceeded the WHO standards for this types of bacteria. 12 desalination plant 60% out of 20 desalination plant were contaminated with Total coliforms (1-50 cfu/100ml); 6 water distribution cars 50% out of 12 were contaminated (1-20 cfu/100ml); and 15 vends (60%) out of 25 vending machines contaminated with Total coliforms (1-50 cfu/100ml).

These results represent that our marketed drinking water in Gaza city were highly contaminated and concern immediate intervention to treat and manage these critical problems. According to our results regarding the source of raw water that treated in the RO plants, it seems that privet wells were better than the municipal wells in term of salinity. Furthermore, many of the water providers didn't make hygienic treatment for water resources that accumulated these types of bacteria.

In addition, the most critical issue in this subject is the distributors non-compliance to water standard that must be followed in our country or any relevant water standard such as WHO water standards. However some of water distribution systems for any desalination plant, water distribution cars, or vending machines have no governmental license for conducting such work which exacerbates the contamination problems.

Our results seem to be consistent with the study of Yassin *et al*., (2006) who found that the contamination level of total and fecal coliforms exceeded that of the (WHO) limit for water wells and networks. Furthermore, the contamination percentages in networks were higher than that in wells.

Al-Khatib and Orabi (2004) who studied "drinking water contamination in rain-fed cisterns in three villages in Ram-Allah" found that 87% of tested samples of drinking-water were highly contaminated and in need of coagulation, filtration and disinfection based on the WHO guidelines for drinking-water, and 10.5% had low contamination and were in need of treatment by disinfection only. Only 2.5% of the tested samples were not contaminated and were suitable for drinking without treatment. The main cause of drinking-water contamination was the presence of cesspits, wastewater and solid waste dumping sites near the cisterns.

In addition, Alves et al (2002) found that one sample collected from the reservoir supply had been contaminated by a bacterium of the total coliform group. Furthermore, another study by Senta et al (2007) who found that total coliforms were detected in 24 samples and values were from 2 to >240/100 ml, and 16 samples had values above maximal allowed concentration.

However, in another study by Lévesque et al **(**2008) found that approximately 90% of the samples analyzed were contaminated with total coliforms in concentrations exceeding 10 CFU/100 ml. And Yamaguchi et al (2007) found that 12 (20.0%) bottled mineral water out of 60 samples were positive for total coliform, compared with only 3 (5.0%) out of 60 samples from tap water.

5.1.2 Fecal coliforms

Fecal coliform group which is a sub-group of total coliform that consequently found in water containing system and sometimes mentioned by another names (thermotolerant coliforms and / or *E.coli*). This type of coliforms was found in the marketed drinking water in Gaza city and exceeded the percentage of WHO standards for its presence which indicate that it must be (Zero) when using Membrane Filtration Technique.

Six (30%) out of 20 desalination plant were contaminated with fecal coliforms (1-30 cfu/100ml); 5 water distribution cars (41.7%) out of 12 were contaminated with fecal coliforms (1-10 cfu/100ml); and 4 vends (16%) out of 25 vends were contaminated with fecal coliforms (1-10 cfu/100ml).

These results indicated that we need urgent attention since the results didn't meet the WHO standards for fecal coliform bacteria present in drinking water. The marketed drinking water didn't commit to the standards for cleaning, washing, sterilization techniques that must be followed for safe drinking water.

Fortunately, we found the results of our study consistent with the results of Yassin et al (2006) who found that the contamination level of fecal coliforms exceeded that of the (WHO) limit for water wells and networks.

In addition, the study of Kassenga (2007) found that fecal coliform bacteria were present in 3.6% of samples analyzed with a tendency for higher contamination rates in plastic-bagged drinking water. In addition, Lévesque et al (2008) found approximately 66% of samples showed contamination with *E. coli.*

In another study by Senta et al (2007) who found that Fecal coliforms were detected in 17 samples out of 34 samples from private wells and values were from 2 to >240/100 ml.
But our results inconsistent with the study of Alves (2002) The results revealed that one sample of mineral water and one sample out of 18 samples were collected from the reservoir supply had been contaminated by a bacterium of the total coliform group, (one bacterium/100 ml of water). But None of the water samples showed contamination by fecal coliforms using Colilert Technique in cellophane.

5.1.3 *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa was found in different marketed drinking water in Gaza city including desalination plant; water distribution cars, and vending machine by a variety of percentages and by a notable samples of water that consider a great attention and management. 9 desalination plant (45%) out of 20 were contaminated with *P. aeruginosa* (1- 200 cfu/250ml); 6 water distribution cars (50%) out of 12 water distribution cars contaminated with *P. aeruginosa* (1-20 cfu/250ml); and 12 vends (48%) out of 25 vending machine contaminated with *P. aeruginosa* (1-65 cfu/250ml).
The researcher hypothesized that the presence of *P. aeruginosa* in drinking water is an indicator of water quality deterioration and its presence is considered as another indicator for another types of bacterial contaminant. And the presence of bacteria and other types in the drinking water related to non compliance to appropriate cleaning system, and non – hygienic procedures that followed by the distributors.

In a study by Schillinger and Knorr (2004) who studied "drinking water quality", they found statistically significant associations between operator accessibility and presence of fungi, Pseudomonas spp., and *Pseudomonas aeruginosa*, and between presence of fungi and the servicing interval.

There is another study conducted by Baumgartner et al (2006) studied bacteriological quality of drinking water from dispensers (Coolers) and possible control measures". The authors found 35 (21.6%) of 162 water samples (10 ml) from coolers yielded *P. aeruginosa*, suggesting potential growth of *P. aeruginosa* in the dispensers. Pulsed-field gel electrophoresis typing and antibiotic susceptibility testing found 19 *P. aeruginosa* isolates from the coolers and bottles to be identical, indicating that a single strain originated from the bottled water rather than the surroundings of the coolers.

5.1.4 Fungi (Molds and Yeast):

Another aspect that measured in water samples called Fungi including (Molds & Yeast) were found by alarming numbers in water sample including the three main collected points of marketed drinking water desalination plant; water distribution cars; and vending machines. Five desalination plant (25%) out of 20 desalinations were contaminated with mold (1-10 cfu/100 ml); 10 water distribution cars (83.3%) out of 12 cars contaminated with Mold (1-15cfu/100ml); and 14 vends (56%) out 25 vending machine were found contaminated with molds (1-20 cfu/100ml).

Four desalination plant (20%) out of 20 were contaminated with yeast (1-50 cfu/100 ml); 6 water distribution cars (50%) out of 12 were contaminated with yeast (1-20 cfu/100ml); and 4 vends (14%) out of 25 vending machine were contaminated with yeast (1-20 cfu/100ml).

The mold and yeast need long period to grow, and its occurrence depends on the period of water storage. This supported by the more period of water storage, will increase the susceptibility of fungi growth. Our results indicated that there were molds and yeast in the collected samples, so this related to long period of storage and non-compliance to frequent or regular cleaning.

In a study performed by Hinzelin and Block (1985), they found that of 38 samples, (50%) and (81%) were yeast and filamentous fungi contaminated respectively. The concentrations ranged between 1 and 28 yeasts per litre and between 2 and 65 filamentous fungi per litre.

And Another study by Nagy and Olson (1982) found that the mean number of filamentous fungal colony-forming units per 100 mL of drinking water was 18 in the unchlorinated and 34 in the chlorinated system. The majority of filamentous fungi isolated were saprophytic Deuteromycotina. The four most frequently occurring genera were *Penicillium, Sporocybe, Acremonium*, and *Paecilomyces*. In the chlorinated system, only physicochemical parameters correlated with observed fungal frequencies, whereas in the unchlorinated system, none of the parameters exhibited significant correlations with fungal numbers.

Furthermore, Yamaguchi et al (2007) who studied " Yeast and filamentous in Drinking water" found that yeasts were detected in (36.6%) and (11.6%) of the bottled mineral on water dispensers and tap water samples from municipal system, respectively.

Hageskal and co-workers in (2006) found that the mycobiota of water should be considered when the microbiological safety and quality of drinking water are assessed. Moreover, they recommended that molds in drinking water should possibly be included in the water supply and drinking water regulations.

5.2 Main results:

- The results revealed that the most prevalent type of bacterial contamination was total coliforms. Twelve samples (60%) were contaminated with total coilforms ranging from (1-50 cfu/100ml) and only 8 samples were not contaminated among the selected samples of the desalination plant.
- The results found that six samples (30%) were contaminated with fecal coliforms ranging between (1-30 cfu/100ml); where 14 samples (70%) were not contaminated.
- Nine samples (45%) out of 20 samples were contaminated with *P.aeruginosa* ranging between (1-200 cfu/250ml) eleven sample (55%) were not contaminated.
- Five samples (25%) were contaminated with mold ranging between (1-10 cfu/100ml); and 4 samples (20%) were contaminated with yeast ranging between (1-50 cfu/100ml).
- Six samples (50%) out of 12 samples were contaminated with Total coliforms ranging between (1-20 cfu/100ml) in the water distribution cars. Where five samples (41.7%) out of 12 samples were contaminated with fecal coliforms ranging between (1-10cfu/100ml).
- Six samples (50%) out of 12 were contaminated with *Pseudomonas aeruginosa* ranging between (1-20 cfu/250ml); and six samples (50%) were contaminated with yeast ranging between (1-20 cfu/100ml) in the water distribution cars.
- Ten samples (83.3%) out of 12 samples were contaminated with molds ranging between (1-15cfu/100ml).
- The results indicated that fifteen samples (60%) out of 25 samples were contaminated with total coliforms ranging between (1-50cfu/100ml) in the vending machine that found at the shops. While 4 samples (16%) were contaminated with fecal coliforms.
- But there were twelve samples (48%) were contaminated with *P.aeruginosa* ranging between (1-50cfu/250ml).
- There were 14 samples (56%) were contaminated with molds ranging between (1-20cfu/100ml); and 4 samples (14%) were contaminated with yeast ranging between (1-15cfu/100ml).
- Twelve samples those taken from the bottled water were free from total and fecal coliforms and yeast. Four samples (33%) were containing (>100 cfu/ml) which

exceed the WHO standards that must be (maximum allowed amount must be less than 100 cfu/ml).

- One sample contaminated with *P. aeruginosa* (3 cfu/250ml) and two samples were contaminated with mold (10, 15 cfu/100 ml).
- Four samples (33%) were containing TBC (> 100 cfu/ml); and three samples (25%) contaminated with total coliforms (3-10cfu/100ml) and mold (10-15cfu/100ml) in the stored bottles.

5.3 Recommendations:

- WHO standards and guidelines for safety of drinking water should be adopted and strictly observed.
- Water sources must be licensed.
- Periodical analyses and checks for desalination plants should be performed to guarantee the application of the WHO standards and regulations for safe drinking water.
- Water distribution cars and vending machines must comply with the WHO standards for drinking water.
- The distribution systems must be monitored by the Palestinian Water Authority.
- Municipal wells (current or new) should be used as a source of water for the desalination plants instead of private or agricultural wells to grantee the safety of drinking water.
- Follow up by the municipality for all water sources in its area of jurisdiction and responsibility.
- Regular checks and monitoring for cleaning procedures and sterilization techniques should be done by the responsible authorities.
- Designation of specified water source for all distributors to grantee water quality and safety.
- Make direct connection with the municipalities and Palestinian Water Authority to be updated and informed by the standards of drinking water and its safety to the consumers.
- Periodical instructions and information regarding drinking water should be available to water distributors.
- Providing a clear system for cleaning, sterilizing, and managing the drinking water according to the Palestinian Water Authority rules and regulations.

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Annex (1)

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

Date: 15/8/2008

التاريخ: ١٥/٨/٢٠٠٨

Name: Shady Al Tartory

الاسم: شادي الترتوري

I would like to inform you that the committee has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

Microbiological Assessment of Marketed Drinking Water in Gaza City.

In its meeting on August 2008
and decided the Following:-
To approve the above mention research study.

و ذلك في جلستها المنعقدة لشهر أغسطس ٢٠٠٨
و قد قررت ما يلي:-
الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member
عضو

Member
عضو

Chairperson

Ministry of Health
Helsinki Committee

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

Gaza Etwam – Telefax 972-7-2878166

Annex (2)

Al-Quds University
Jerusalem
School of Public Health

جامعة القدس
القدس
كلية الصحة العامة

2008/7/15

الأخ المهندس / منذر شبلاق المحترم
مدير مصلحة بلديات مياه الساحل
تحية طيبة وبعد،،،

Coastal Municipalities Water Utility
Gaza Emergency Water Project
31. 07. 2008
Received
Ref.: 2282
by: Noha

الموضوع: مساعدة الطالب شادي الترتوري

يقوم الطالب المذكور أعلاه بإجراء بحث بعنوان:

"Microbiological Assessment of Marketed Drinking Water in Gaza city "

كمتطلب للحصول على درجة الماجستير في الصحة العامة-مسار صحة البيئة و عليه نرجو التكرم للإيعاز لمن ترونه مناسب لتسهيل مهمة الطالب في جمع البيانات اللازمة ،علماً بأن المعلومات ستكون متوفرة لدى الباحث و الجامعة فقط.

و اقبلوا فائق التحية و الاحترام،،،

د. بسام أبو حمد
منسق عام برامج الصحة العامة

Coastal Municipalities Water Utility
(CMWU) / GEWP
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Annex (3)

Al-Quds University
Jerusalem
School of Public Health

جامعة القدس
القدس
كلية الصحة العامة

2008/7/15

الأخ/د. فؤاد العيسوي المحترم
مدير عام الرعاية الأولية – وزارة الصحة
تحية طيبة وبعد،،،

الموضوع: مساعدة الطالب شادي الترتوري

يقوم الطالب المذكور أعلاه بإجراء بحث بعنوان:

"Microbiological Assessment of Marketed Drinking Water in Gaza city "

كمتطلب للحصول على درجة الماجستير في الصحة العامة-مسار صحة البيئة و عليه نرجو التكرم للإيعاز لمن ترونه مناسب لتسهيل مهمة الطالب في جمع البيانات اللازمة وعمل الفحوصات مع إمكانية استخدام الأجهزة المتوفرة لديكم . علماً بأن المعلومات ستكون متوفرة لدى الباحث و الجامعة فقط.

و اقبلوا فائق التحية و الاحترام،،،

د. بسام أبو حمد
منسق عام برامج الصحة العامة

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Annex (4)

MacConkey Agar base (Himedia Laboratories Limited Mumbain, 400 086, India)	
Formula	**g/L**
Peptic digest of animal tissue	17:00
Proteose Peptone	3.0
Bile salt	1.5
Sodium chloride	5
Crystal violet	0.001
Neutral red	0.03
Agar	13.50
PH (at 25 C°) 7.1 ± 0.2.	

Nutrient Agar (Himedia laboratories limited Mumbain, 400 086, India)	
Formula	**g/L**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.5
Yeast extract	1.5
Agar	15.00
PH (at 25 C°) 7.4 ± 0.2.	

Plate count Agar himedia laboratories Limited Mumbain, 400 086, India	
Formula	**g/L**
Casein enzymic hydrolysate	5.00
Yeast extract	2.50
Dextrose	1.00
Agar	15.00
PH (at 25 C°) 7.0 ± 0.2.	

Czapek Doxi Agar	
Formula	**g/L**
Sucrose	30.00
Sodium nitrate	3.00
Dipotassuim sulphate	1.00
Magnesium sulphate	0.50
Potassium chloride	0.50
Ferrous sulphate	0.01
Agar	15.00
PH (at 25 C°) 7.2 ± 0.2.	

Cetrimide Agar	
Formula	**g/L**
Peptic digest of animal tissue	20.00
Potassium Sulphate, anhydrous	10.00
Magnesium Chloride, anhydrous	1.40
Cetrimide	0.50
PH (at 25 C°) 7.2 ± 0.2.	

Annex (5)

methyl red-Voges Prockaver medium (Glucose phosphate peptone water)	
Formula	
Peptone	5.00g
Dipotassium hydrogen Phosphate (K_2HPO_4)	5.00g
Water	1 Liter
Glucose, 10 percent solution	50.0g
Sterilized separately	
PH to 7.6	

Citrate Utilization Medium (Simmon's Citrate Medium) Simmon's citrate medium is a modification of koser's medium with agar and an indicator added.	
Formula	
Sodium chloride, NaCl	5.0g
Magnesium sulphate, $MgSO_4$	0.2g
Ammonium dihydrogen phosphate, $NH_4H_2PO_4$	1.0g
Potasium dihydrogen phosphate, KH_2PO_4	1.0g
Sodium citrate, Na_2C6H_3-$O7._2H_2O$	5.0g
Agar	20.0g
Bromothymol blue	0.08g
Distilled water	1.0L
PH should be 6.8	

Indole Medium	
Formula	**g/L**
Peptone (brand containing sufficient tryptophan)	50.0g
Sodium chloride, NaCl	5.00g
Distilled water	1L
Adjust the PH to 7.4	

Nitrate Reduction Medium	
Formula	
Peptone nitrate, KNO3 (nitrate free)	0.2g
Peptone	5.0g
Distilled water	1L

Urease Medium (Christensen's Medium)	
Formula	
Peptone	1.0g
Sodium chloride, NaCl	5.0g
Dipotassium hydrogen phosphate	2.0g
Phenol red (1 in 500 aqueous solution)	6.0ml
Agar	20g
Distilled water	1 L
Glucose, 10 percent solution, sterile	10 ml
Urea, 20 percent solution, sterile	100 ml
PH 6.8 – 6.9	

Annex (6)

Methyl red	
Formula	
Methyl red	0.1g
Ethanol	300 ml
Distilled water	200 ml

Voges. Proskaur	
Formula	
Reagent (1)	40 percent potassium hydroxide.
Reagent (2)	5 percent - naphthol in absolute ethanol.

Indole (Kovac's reagent)	
Formula	
Amyl or isomyl alcohol	150 ml
P-Dimethyl aminobenzadehyde	10 ml
Conc. Hydrochloric acid	50 ml

Nitrate Reduction	
Formula	
Solution A	Dissolve 8.0 g of sulfanilic acid in 1 L of 5 N acetic cid
Solution B	Dissolve 5.0 g of -naphthylamine in 1 L of 5 N acetic acid

Oxidase	
Formula	
1 percent solution of tetramethyl-P-phenylenediamine dihydrchloride.	

RNase solution	
Formula	
10 mg/ml RNae (sigma) in acetate buffer (0.1 M sodium acetate and 0.3 mM EDTA, PH 4.8) . The RNase solution eas preheated at 80 Cº for 10 min to inactivate only DNase enzymes possibly present with the RNase.	

Annex (7)

استبيان عن تقييم مياه الشرب المباعة في مدينة غزة(من الناحية البيولوجية)

يهدف هذا الاستبيان إلى معرفة مدى التلوث ومصدره في مياه الشرب المباعة من محطات التحلية ومن سيارات النقل التابعة لها ومن خزانات المبيع الموجودة في المحلات التجارية الموجودة في مدينة غزة, بالإضافة إلى الشركات الخاصة بتعبئة المياه المحلاة. لذا نرجو الإجابة بصراحة ولا تترك أي إجابة فارغة. تذكر أن المعلومات التي تقدمها لنا سرية ومجهولة.

Annex (7-A)

اسم المحطة:.....				
العنوان:.....				
الترخيص الصحي للمحطة:	❐ نعم	❐ لا		
عمر المحطة:	❐ أقل من 1 سنة	❐ من1 - 5 سنوات	❐أكثر من 5 سنوات	
مصدر المياه المستخدمة في التحلية؟	❐ شبكة بلدية	❐ بئر خاص	❐ بئر زراعي	
هل مصدر المياه مرخص من الجهات المعنية؟	❐ نعم	❐ لا		
ما هي آلية إنتاج المياه المحلاة ؟	❐ التناضح العكسي	❐ التقطير	❐ التبخير	
الطاقة الإنتاجية للمحطة في الساعة ؟	❐ 1 كوب /الساعة	❐ 3 كوب / الساعة	❐ 10كوب / الساعة	
الطاقة الإنتاجية للمحطة في اليوم ؟	❐ 5-10كوب	❐11-20كوب	❐ أكثر من 20 كوب	
إجراءات و معايير لفحص المياه قبل و بعد عملية الإنتاج؟	❐ داخل المحطة	❐ خارج المحطة	❐ لا تجرى	
ما هي الفحوصات التي يتم إجراءها؟	❐ كيميائي	❐ ميكروبي	❐ الاثنان معا	
هل يتم تخزين المياه المنتجة ؟	❐ نعم	❐ لا		
إذا كانت الإجابة بنعم كم يوم؟	❐ 1-7 أيام	❐ 7-14 يوم	❐ أكثر من ذلك	
الخزانات المستخدمة في التخزين؟	❐ بلاستيك	❐ فيبرجلاس	❐ ستانلستيل	
هل يتم تنظيف الخزانات المستخدمة في التخزين؟	❐ نعم	❐ لا		
إذا كانت الاجابه بنعم ما هي الفترة الزمنية التي تعتمد في تنظيف الخزانات؟	❐ يوميا	❐ أسبوعيا	❐ شهريا	
هل يتم تعقيم للمياه,,,؟	❐ نعم	❐ لا		
إذا كانت الإجابة بنعم فما هي المواد المستخدمة في عمليه التعقيم؟	❐ أشعة فوق بنفسجية	❐ كلورين	❐ الاثنان	
هل توجد رقابة رسمية من قبل الدوائر المتخصصة لمراقبة جودة و صلاحية المياه قبل توزيعها؟	❐ نعم	❐ لا		
إذا كانت الإجابة بنعم فما هي آلية الرقابة؟	❐ بشكل دوري	❐ بشكل مفاجئ	❐ الاثنان معا	
عند ضخ المياه المحلاة إلى سيارات التوزيع هل تقومون بعملية تعقيم لمخارج و مداخل عملية الضخ؟	❐ نعم	❐ لا		

Annex (7-B)

الجزء الخاص بنقاط البيع للجمهور				
اسم المحل.....				
هل يتم اعتماد محطة معينه لتعبئه المياه ؟	❐ نعم	❐ لا		
اسم المحطة التي يتم التعامل معها في تعبئة المياه				
الخزانات المستخدمة في التخزين؟	❐ بلاستيك	❐ فيبرجلاس		❐ ستانلستيل
هل تقومون بفحص الخزانات من الناحية الصحية؟	❐ نعم	❐ لا		
إذا كانت الاجابه بنعم ما هي الفترة الزمنية التي تعتمد لفحص الخزانات؟	❐ يوميا	❐ أسبوعيا		❐ شهريا
هل تقومون بإجراء عمليات التنظيف و التعقيم لهذه الخزانات؟	❐ نعم			❐ لا
إذا كانت الاجابه بنعم فما هي الفترة الزمنية لعملية التنظيف والتعقيم؟	❐ اسبوعيا	❐شهريا		❐ غير ذلك
ما نوع المعقمات اللاتي يتم استخدامها ؟		❐ كلورين		❐ غير ذلك
هل تتم عملية تفريغ كامل لمحتوى الخزان ؟	❐ يوميا	❐ أسبوعيا		❐ عند انتهاء المخزون
موقع الخزانات:- (محمية من الغبار والشمس)	❐ محمية	❐ غير محمية		
عند ضخ المياه المحلاة من سيارات التوزيع إلى مستودعات البيع هل تقومون بعملية تعقيم لمخارج و مداخل عملية الضخ (end to end)؟			❐ نعم	❐ لا

Annex (7-C)

الجزء الخاص بسيارة التوزيع:-				
رقم السيارة				
هل يتم اعتماد محطة معينه لتعبئة المياه ؟	❐ نعم	❐ لا		
اسم المحطة التي يتم التعامل معها في تعبئة المياه				
هل تقومون بفحص الخزانات من الناحية الصحية؟	❐ نعم	❐ لا		
إذا كانت الاجابه بنعم ما هي الفترة الزمنية التي تعتمد لفحص الخزانات؟	❐ يوميا	❐ أسبوعيا		❐ شهريا
هل تقومون بإجراء عمليات التنظيف و التعقيم لهذه الخزانات؟	❐ نعم			❐ لا
إذا كانت الاجابه بنعم فما هي الفترة الزمنية لعملية التنظيف والتعقيم؟	❐ اسبوعيا	❐شهريا		❐ غير ذلك
ما نوع المعقمات اللاتي يتم استخدامها ؟		❐ كلورين		❐ غير ذلك
هل تتم عملية تفريغ كامل لمحتوى الخزان ؟	❐ يوميا	❐ أسبوعيا		❐ عند انتهاء المخزون

الخلاصة

نقص المياه أصبح من أهم المشاكل في العالم حاليا , خاصة في مناطق مثل قطاع غزه حيث أن القطاع يعانى مـن نقص حاد في المياه. كمان أن الجودة الميكروبية للمياه في قطاع غزه قليله " ضعيفة" جدا, ولهذا السبب قمنا بعمـل هذه الدراسة والتي تهدف لاستبيان الجودة الميكروبية لعدد من أنواع المياه المباعة في سوق مدينة غزه.

ضمنت الدراسة عشرون عينة جمعت من محطات التحلية , 12 عينه من سيارات توزيع الميـاه و 25 عينـه مـن حاويات البيع و 12 عينه من المياه المعبئة في مدينه غزه. التجارب المخبريه استخدمت لاختبار الجودة الميكروبيـة للمياه وتم ذلك باستخدام تقنية الترشيح خلال فلتر من اسيتات النيتروسليولوز و ذلك على بيئات غذائية " مكـونكى , سترميد و دوكسى " لعزل كل من البكتريا المعوية (الكليه والبرازيه) ويكتريا سيدوموناس أبروجينوزا والفطريـات (الاعفان والخمائر) بالتوالي.

أظهرت نتائج عينات المياه المأخوذة من محطات التحليه أن 12 عينه (60%) من أصـل عشـرين عينـه ملوثـه بالبكتريا المعويه (1-50 مستعمره لكل 100 مل), (50%) من العينات المجموعة من سيارات التوزيع أي 6 عينات من أصل 12 عينة كانت ملوثه بالبكتريا المعويه بمعدل (1-20 مستعمره لكل 100 مل).

المياه المجموعة من حاويات التوزيع أظهرت نسبة عاليه من التلوث كما في محطات النحلية حيث كانت 15 عينـه من أصل 25 (60%) ملوثه بالبكتريا المعويه وبمعدل (1-50 مستعمره لكل 100 مل) .

بالنسبة لوجود بكتريا معويه برازيه في عينات المياه المختلفة , وجد أن 6 عينات من أصل 20 عينه من محطـات التحليه والتي تمثل (30%) ملوثه بالبكتريا المعويه البرازيه , بمعدل (1-30 مستعمره لكل 100 مل) و 5 عينات من أصل 12 عينه والتي تمثل (41.7%) من المياه المجموعة من سيارات التوزيع ملوثه بالبكتريا المعويه البرازيه بنسبة (1-10 مستعمره لكل 100 مل) و 4 عينات من أصل 25 (16%) من المياه المجموعة من حاويات التوزيع.

أما بالنسبة لبكتريا سيدوموناس أبروجينوزا في العينات المفحوصة , أظهرت النتائج بأن 9 عينـات والتـي تمثـل (45%) من أصل 20 عينة من العينات المجموعة من محطات التحليه كانت ملوثه بمعدل (1-200 مستعمره لكـل 250 مل) ,و 6 عينات والتي تمثل (50%) من أصل 12 عينه من عينات المياه المجموعة من سـيارات التوزيـع كانت ملوثه بمعدل (1-20 مستعمره لكل 250 مل).

أما حاويات البيع فأظهرت النتائج أن 12 عينه والتي تمثل (48%) من أصل 25 عينه من العينات المجموعة كانت ملوثه ببكتريا سيدوموناس أبروجينوزا وبمعدل (1-65 مستعمره لكل 250 مل).

أما عينات المياه التي تحتوى على الفطريات (الاعفان والخمائر) فأظهرت النتائج انه في محطـات التحليـه أن 5 عينات (25%) من أصل 20 عينه و 4 عينات (20 %) من أصل 20 عينه من العينات ملوثه بالأعفـان بمعـدل (1-10 مستعمره لكل 100 مل) والخمائر (1-50 مستعمره لكل 100 مل) بالتتـابع , 10 عينـات (83.3%) , 6 عينات (50%) من أصل 12 عينه كانت ملوثه بالأعفان بمعدل (1-15 مستعمره لكل 100 مل) والخمائر (10-20 مستعمره لكل 100 مل) بالتتابع في عينات المياه المأخوذة من سيارات التوزيع. وأخيرا في حاويات البيع , وجد أن 12 عينه (56%) و 4 عينات (16%) من أصل 25 عينه كانت ملوثه بالأعفان (1-20 مستعمره لكل 100 مـل) والخمائر (1-20 مستعمره لكل 100 مل) بالتوالي.

أما نتائج عينات المياه ه المجموعة من زجاجات المياه المعبئة أظهرت أن جميع العينات خاليه من البكتريا المعويـه والبكتريا المعويه البرازيه بالنسبة للعدد الكلى للبكتريا أظهرت النتائج وجود 4 عينات (33%) من أصل 12 عينـه كانت ملوثه بأعداد كبيره من البكتريا (> 100 مستعمره / 1 مل) , بينما وجدت عينتان (16.6%) من أصل 12 عينه تحتوى على أعداد من بكتريا سيدوموناس أبروجينوزا وخلت جميع العينات من الخمائر والاعفان.