



Faculty of Graduate Studies
M.Sc. Program in Water and Environmental
Sciences

**Removal Efficiency of Pathogenic Microorganisms
in Three Different Palestinian Wastewater
Treatment Plants**

كفاءة ازالة المجهريات الممرضة في ثلاث محطات معالجة للصرف
الصحي في المناطق الحضرية الفلسطينية

Master Thesis

Prepared by

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January 2020



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Requirement for the Master Degree in Water and
Environmental Sciences from the Faculty of Graduate
Studies at Birzeit University-Palestine

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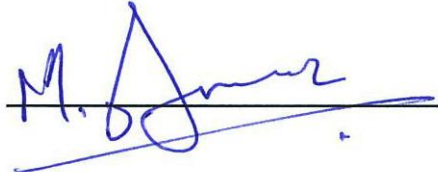
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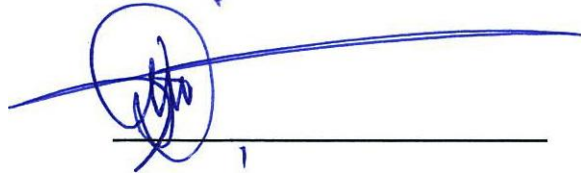
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The findings, interpretations and conclusions expressed in this study do not necessarily express the views of Birzeit University the views of individual members of the M.Sc. committee or the views of their respective employers.

Dedication

To My Beloved Country Palestine

My Hometown Jerusalem

I Dedicate my work to the soul of my Father.

To my mother.

*To my only sister **Usra**, to my brothers, **Subhi**, **Mahmoun**, **Manar** and **Murad**, and to my beloved family who all the time gave me love and continuous support all these past four years.*

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List of Abbreviations

Acronyms

BOD
COD
TN
TP
TSS
TDS
FC
EC
PH
BZU
CAS
CFU/g
PPM
MF
MBR
MBBR
MF
Ms/cm
PCBS
NGOs
EPA
WHO
PSI
PWA
WWTPs
DNA
RNA
PCR
PFU
qRT-PCR
RT-PCR
RPM
MCM
cDNA
CT

Definition

Biochemical Oxygen Demand
Chemical Oxygen Demand
Total Nitrogen
Total Phosphate
Total Suspended Solids
Total Dissolved Solids
Faecal Coliform
Electrical Conductivity
Potential Hydrogen
Birzeit University
Conventional Activated Sludge
Colony Forming Unit Per Gram
Part Per Million
Membrane Filtration
Membrane Bioreactor
Moving Bed Bioreactor
Membrane Filter
Millisiemens / Centimeter
Palestinian Central Bureau of Statistics
Non-Governmental Organizations
Environmental Protection Agency
World Health Organization
Palestinian Standards Institute
Palestinian Water Authority
Wastewater Treatment Plants
Deoxyribonucleic Acid
Ribonucleic Acid
Polymerase Chain Reaction
Plaque Forming Units
Qualitative Real- Time Polymerase Chain Reaction
Reverse Transcription Polymerase Chain Reaction
Round Per Minute
Million Cubic Meter
Complementary DNA
Cycle Threshold

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Abstract

Generating unconventional water resources under full Palestinian control, such as treated wastewater, is a way of by-passing the political *status quo*, alleviating water insecurity as well as providing alternatives to farmers in the face of growing aridity. However, all attempts on wastewater reuse were in vain due to many reasons and illegal irrigation practices with partially treated influent in certain areas still impose serious health hazards and environmental problems.

Recently the best method used and proved rapid detection of enteric viruses in wastewater was qualitative real time PCR and (qPCR), for its high sensitivity and specificity. Detection of enteroviruses needs specific virus strains depending on the chosen targeted DNA/RNA sequences using specific primers and probes. Occurrence and concentration of viruses in sewage can be affected by many factors such as water usage, season and incidence of infection among the population. Need for virus detection was important because they may cause public health hazards, enterovirus may cause respiratory tract infection and meningitis, rotavirus, adenovirus, and norovirus may cause gastroenteritis and adenovirus causes respiratory diseases, eye infection and sometimes causes meningitis.

The aim of my Thesis first to identify the efficiency of three different wastewater plants, particularly pathogen removal and specifically pathogenic human viral removal. It also aims to see if there was any correlation between the removal of enteric viruses in sewage and the removal of other microorganisms like bacteria and protozoa using faecal coliform as an indicator. The case study was concentrated mainly in Ramallah governorate, Al-Tireh Membrane Bioreactor (MBR), Al-Bireh Activated sludge (AS), and Moving Bed Bioreactor (MBBR) in Rawabi. Second, was reclaimed effluent safe enough to be used as unconventional water source, for example agricultural irrigation. The sampling was in march 2019 ,72 grab sample 0.5 liter collected at three different time intervals) and the sum of samples at the end was 24 composite samples procedure worked was polyethylene glycol (PEG) with NaCl for precipitation and concentration of influent and effluent samples, followed by extraction for both DNA and RNA nucleic acids using NucliSens system of all samples and lastly qualitative real Time qPCR (Taq-man) using the automated 7500 Real Time PCR system in Al-Caritas baby hospital in Bethlehem, using special

primers and probes for the four mentioned viruses, (q PCR) Qualitative Polymerase Chain reaction method works as a multiple amplification cycles by which template DNA was first denatured followed by annealing of oligonucleotide primers target specific sequences. Tag-Man probes were used which gave a fluorescent labelled at the 5'. The main outcomes of the assessment made for physical parameters TSS removal efficiency was 99%, 98% for Al- Bireh and Rawabi WWTP's respectively. For BOD removal 97%, 96%, 95% for Al- Bireh, Al- Tireh, and Rawabi WWTP's respectively. For the biological parameters log removal of fecal coliform (FC) indicator, 2.65 log removal of Al- Tireh, 1.75 log removal for Al- Bireh and 2.92 log for Rawabi. Detection of enteric viruses in the three WWTP's under investigation Al-Tireh effluent had 100% removal efficiency of the four different viruses enterovirus, adenovirus, norovirus and rotavirus, while Al-Bireh effluent was 33% and 33% removal adenovirus and rotavirus respectively. Finally for Rawabi effluent 100% contamination with adenovirus, 33% removal efficiency of norovirus, and 66% removal of rotavirus. That means there was no correlation between the fecal coliform indicator and virus removal indicator and to prevent public health hazards mentioned before further tertiary treatment should be added for Al- Bireh and Rawabi WWTP's. Disinfection alone with UV light and chlorine was not enough and this was confirmed from previous studies, for example adenovirus was resistant to chlorine, that means the removal efficiency was from the type of treatment and not from chlorine disinfection, in Al-Bireh there was no chlorine disinfection and the UV light not working and there was 100% removal of norovirus and 33% removal of adenovirus and rotavirus respectively. The pores of the membrane in Al-Tireh membrane bioreactor technology are very small (nominal pore size of the immersed hollow fiber was 0.04 micron) and does not allow the tiny viruses (0.01-0.3um sizes) to pass through, the submerged membrane in Al-Tireh act as a tertiary treatment since not only the physical and chemical parameters match with the guidelines but also the biological parameters are also met. Finally the Palestinian Water Authority (PWA), must take in charge changes in the guidelines of treated wastewater saving ground water and the environment from contamination, because as we have seen from the outcomes of this thesis, enteric viruses in treated and partially treated wastewater may threaten public health.

ملخص

ان توليد موارد المياه غير التقليدية تحت السيطرة الفلسطينية الكاملة، مثل مياه الصرف المعالجة، يشكل وسيلة لاجتياز الوضع السياسي الراهن، وتخفيف حدة انعدام الامن المائي وتوفير البدائل في مواجهة الجفاف. غير ان جميع المحاولات الرامية الى اعادة استخدام مياه الصرف الصحي ذهبت سدى بسبب ممارسات الري غير المشروعة التي تلقى معالجة جزئية في مناطق معينة والتي تفرز مخاطر صحية ومشاكل بيئية خطيرة. في الاونة الاخيرة كانت افضل طريقة مستخدمة وثبتت سرعة الكشف عن الفيروسات المعوية في مياه الصرف الصحي تفاعل البلمرة المتسلسل (Real-time PCR)، وذلك لحساسيته العالية وخصوصية هذه الطريقة.

يحتاج اكتشاف الفيروسات الى ادخال فيروسات محددة وفقا لتسلسلات الحمض النووي/الحمض الريبوزي ويمكن ان يتاثر وجود الفيروسات وتركيزها بعوامل كثيرة مثل استخدام المياه، والموسم، وحدثت الاصابات بين السكان، وكانت الحاجة الى الكشف عن الفيروسات مهمة لانها قد تسبب مخاطر صحية عامة، فانتيروفيرس يسبب التهابات في المسالك التنفسية والتهاب السحايا، روتا فيروس ، ادينوفيرس وونوروفيرس تسبب التهاب المعدة والامعاء والادينوفيرس يسبب امراض الجهاز التنفسي وامراض في العين واحيانا يسبب التهاب السحايا. الهدف من رسالتي هو كفاءة ازالة المجهرات الممرضة في ثلاث محطات تنقية مختلفة وعلى وجه التحديد ازالة الفيروسات الممرضة في المياه العادمة المعالجة. كما تهدف الى معرفة اذا كان هناك ارتباط بين ازالة الفيروسات المعوية في مياه المجاري وازالة الكائنات المجهرية الاخرى مثل البكتيريا والاوليات باستخدام بكتيريا قولونية برازية كمؤشر، وتركزت الدراسة في محافظة رام الله في محطة التنقية في البيرة (باستخدام تكنولوجيا الحماية المنشطة)، في محطة التنقية في الطيرة مفاعل الاغشية الحيوية Membrane (MBR) ومحطة تنقية روابي مفاعل السرير الحيوي المتحرك Moving Bed Bioreactor (MBBR) ، وهل المياه المعالجة آمنة للاستخدام كمياه غير تقليدية لري المزروعات. تم جمع 72 عينة عشوائية بحجم 0.5 لتر وكان مجموع العينات في النهاية 24 عينة مركبة للثلاث اوقات وكان هناك ثلاث خطوات للعمل الخطوة الاولى تركيز وترسيب العينة باستخدام مركب بولي ايثيلين جلايول وكلوريد الصوديوم والخطوة الثانية استخراج الاحماض النووية باستخدام جهاز NucliSens والخطوة الثالثة استخدام نظام تفاعل البلمرة المتسلسل الالي للكشف عن الفيروس -real Time PCR) دورات تضخيم متعددة من خلالها تم تغيير طبيعة الحمض النووي للقالب يليه تضخيم بادئات النكليوتيد المستهدفة بالتسلسلات المحددة. تم استخدام تحقيقات Tag-Man التي أعطت الفلورسنت المسمى في نهاية "5 . كانت نتائج الكفاءة الفيزيائية ازالة المواد العالقة 99% و98% لمحطة البيرة وروابي على التوالي. اما بالنسبة للخصائص الكيميائية الاكسجين الممتص حيويًا فكانت النتائج 97%، 96%، و95% للبيرة والطيرة وروابي على التوالي، اما بالنسبة للخصائص البيولوجية بكتيريا قولونية برازية فكانت كفاءة الازالة (2.65 log) للطيرة، (1.75 log) للبيرة و (2.92 log) لروابي. اما بالنسبة لنتائج الكشف عن

الفيروسات المعوية كانت كفاءة ازالة الاربع انواع الانتيروفيروس، ادينوفيروس، روتافيروس، ونوروفيروس 100% من محطة الطيرة، اما بالنسبة للمياه المعالجة في البيرة كانت كفاءة ازلتها 33% بالادينوفيروس و33% بروتافيروس، واخير المياه المعالجة في روابي كانت 100% ملوثة بالادينوفيروس وكفاءة ازالة 33% و66% بنوروفيروس وروتافيروس على التوالي. هذا يدل انه لا يوجد ارتباط بين مؤشر البكتيريا القولونية البرازية ومؤشر ازالة الفيروسات فلا بد من اتخاذ الاجراءات المناسبة كمعالجة مستوى الثالث لمحطة البيرة وروابي لمنع المخاطر الصحية التي تسببها هذه الفيروسات والتي ذكرت سابقا. لم يكن التطهير بمفرده باستخدام الأشعة فوق البنفسجية والكلور كافياً وهذا ما تم تأكيده من الدراسات السابقة، على سبيل المثال كان الفيروس الغدي (ادينوفيروس) مقاوماً للكلور، وهذا يعني أن كفاءة الإزالة كانت من نوع العلاج وليس من التطهير بالكلور، ولم يكن هناك في البيرة تطهير بالكلور أو ضوء الأشعة فوق البنفسجية، وكان هناك إزالة بنسبة 100 % انتيروفيروس، و100% ازالة لنوروفيروس، 33 % إزالة لادينوفيروس وروتافيروس على التوالي. مسام الغشاء في تقنية مفاعل الغشاء الحيوي في آل بيته في محطة الطيرة صغيرة جداً (حجم المسام الاسمي للألياف المجوفة المغمورة كان 0.04 ميكرون) ولا يسمح للفيروسات الصغيرة (0.01-0.3) ميكروميتر بالمرور من خلاله، في محطة الطيرة لا تتطابق المعايير الفيزيائية والكيميائية مع المواصفات فحسب، بل يتم استيفاء المعايير البيولوجية أيضاً. أخيراً ، يتعين على سلطة المياه الفلسطينية (PWA) ، أن تتحمل مسؤولية التغييرات في المواصفة الخاصة بالمياه العادمة المعالجة الخارجة من محطات التنقية، حفاظا على المياه الجوفية والبيئة من التلوث. لأننا كما رأينا من نتائج هذه الأطروحة ، فإن الفيروسات المعوية في المياه المعالجة الخارجة من المحطة والمعالجة جزئياً قد تهدد الصحة العامة.

Chapter One

Introduction

1.1 Background of the Research

Waterborne diseases are still reported globally and cause the death of 2.2 million people every year, 1.4 million of the deaths are children. Waterborne pathogens and related diseases are a major public health concern worldwide, since it's a burden on governments, economic loss of nearly I billion dollars mainly in US was estimated, this high costs needed for treatment and prevention (Castillo, 2015).

1.2 Epidemiology of pathogenic human viruses in treated

Wastewater

Enteric human viruses are shed in the feces and vomit and the more voluminous the fluid output the greater is the environmental contamination caused. Gastrointestinal human viruses tend to be strong enough to bear extreme and difficult conditions more than the respiratory viruses.

Two epidemiological patterns are seen:

1- Outbreaks occurs due to ingestion of contaminated food or water by many people. Transmission particularly occurs to ingestion of contaminated salads, uncooked shellfish, or through drinking unsafe river water.

2- In households without running water, and lacking the basic needs for hygiene, hand washing facilities, or toilets, outbreaks occurs particularly in places that face poverty and lacking education (Burrel, 2017).

1.3 Islamic perspectives for using Reuse and reclaimed water

Direct reuse of reclaimed water effluent was used in many parts of the world and particularly in a large number of Muslim countries that face severe aridity and shortage of fresh water supply. The Qura'n mentioned water 63 times, and the importance of water was emphasized by stating that all kinds of living things were created with water. The Qura'n mentioned water as a cleansing agent and divided it into three categories. Tahir, is pure natural water which can be used for religious purposes have not changed. In case of change odor, color or taste of water is negligible (Yasir), the Tahir character of water is not impaired. Tahir water becomes Tahir after religious washing and can be used for mundane purposes without any treatment but cannot be utilized again for religious purposes. Tahir and Tahir waters are liable to become mutanajjis water if they are defiled with impurities such as urine, feces etc. rendering them unfit both for the religious and mundane purposes.

The reuse of wastewater effluents seems perfectly legitimate from the Islamic religious viewpoint and has therefore to be examined in each specific case from the latest concerns of health, cost, and public acceptance.

Recently a body of Muslim scholars in Saudi Arabia have unanimously approved the reuse of Wastewater effluent for all purposes after proper treatment (Farooq, 1981).

In my study I send a letter to the Grand Al-Mufti of Jerusalem and Palestinian territories Mohammad Hussein asking him a question of the rule of Islam in using treated effluent in irrigation, the answer of my question was near to the previous study by Shaukat Farooq, that treated effluent could be used for irrigation after right treatment with modern techniques to get rid of suspended solids and pathogenic microbes (the question and answer from Grand Al- Mufti are illustrated in Appendix1).

Water scarcity is a major constraint for economic and social development and sustainability of the agricultural sector in arid and semi- arid areas such as Palestinian – Territories.

Water scarcity will become more critical on domestic and industrial sectors place higher and higher demand on water; Palestine will experience serious water deficit which will be about 271 MCM in year 2020 (PWA, 2005).

The reuse of treated wastewater and water demand management, particularly in irrigated agriculture are the most recommended alternative for alleviation of severe water shortage in Palestine, so wastewater reuse in agriculture is one of the strategic alternatives.

History of this phenomenon "Removal of Pathogens from wastewater treatment plants" began in Britain (1865) were the British, logo in that time, wastewater to the soil and rainwater to the rivers; it was followed by the United States of America in (1871), then France (1872), Germany (1876), Australia (1893), and Mexico (1904).

And in the Arab countries who used untreated wastewater for agricultural crop started in (1994) in Egypt, followed by other Arab countries that was interested in the quality of the Reuse and use it for agriculture.

In Palestine, sustainable development and Management of water, this what 'PWA' focuses on water management problems are already apparent in Palestine, water quality is deteriorating water supply and irrigation services are often rationed with consequences for human health, agriculture productivity and the environment water needs are increasing due to population growth and the expected increase in the standards of living while the amount of water resources available remains more or less fixed due to both natural and artificial constraints mainly due to Israeli imposed regulations and control.

Palestinians are facing many challenges in water sector and demonstrated how solutions could be found through applied innovative research and capacity building.

In Palestine, because of limited access to water resources and lack of financial support to establish or rehabilitate old sewage works and enhance services, urged some farmers to use partially treated wastewater leading to an uncontrolled agricultural wastewater use.

The main disadvantage of utilizing wastewater for agricultural purposes is the presence of pathogens, viruses, and parasites that can pose health risks for the farmers, soil, nearby communities, and to the consumers of the products irrigated with this water.

This study evaluated treatment technologies capable of meeting the Ministry of Agriculture (MoA) and Palestinian water Authority (PWA) effluent discharge limits associated with revised human health water quality criteria for treated water reuse. These technologies extended aeration activated sludge treatment (AS). Anaerobic stabilization ponds WWTPs Membrane bioreactors wastewater plants (MBR) constructed wetland's (CWs), Rotating Biological contractors (RBCs) WWTPs and trickling filter, and MBBR technology. These WWTPs technologies were distributed in west bank and Gaza, selecting the right technology depending on the location (area) and served population (CECP, 2016).

The big challenge in this study is the willingness of farmers to pay for reclaimed waste water (Abu –Madi, et.al. 2003) this study took place in Jordan and Tunisia showed the willingness of farmers to use treated waste water for irrigation of crops.

Another study aimed to the awareness and willingness of farmers to use treated and to pay for the treated effluent (reuse) for irrigation in Hebron district in Dura, the majority of consumers, who accepted to pay, voted for WTP almost half the price of fruits and vegetables irrigated with fresh water (Isaed, R., et al., 2008).

World health organization (WHO) and United Nations Environment Program (UNEP) have put restrictions on crops irrigated with treated wastewater reuse, there should be treatment before Reuse (Blumenthal et al., 2000). Achieving the Millennium Goals (MDGs) by reducing the number of people access to safe water by 50% in 2015.

Indirect reuse has been practiced for centuries around the globe.

History of Reuse consumed as an unconventional source of water started in the 16th century in Europe. Indirect reuse has been practiced for centuries around the globe.

In 1906, California the first claim in California monthly Bulletin was for using wastewater for irrigation, by this process saving the fertilizing properties. In 1942, wastewater reuse was used for steel processing for industrial reuse. In 1960's due to rapid population and increased humid climatic region, particularly in Florida there was development for reclamation and reuse regulations and guidelines in many states.

In 1918, California was a pioneer in developing water reclamation. In 1972, implementation of (CWA) Clean Water Act type of wastewater treatment that met the effluent standards.

Safe Drinking Water (SDWA) control on potable water supply and indirect control on the wastewater that will be discharged into streams.

Until 2004, United States used reclaimed water for irrigation landscapes.

In Washington DC. 2004, Guidelines for wastewater Reuse was published by U.S. Environmental Protection Agency and U.S. Agency for International development (Asano,et.al. 2007).The historical development of treated Wastewater Reuse world-wide through the year 1968 to the year 2004. (illustrated in tables in appendix 2).

In the United States Arizona and California were using reclaimed water for irrigation of lawns and gardens and used in industries as cooling water.

Bacteriophages are good alternative indicators for the presence of enteric viruses in raw and treated effluent.

Factors influencing fate and transport of bacteriophages, biotic and abiotic factors influencing survival of bacteriophages:

- ❖ Viral surface change
- ❖ PH of water
- ❖ Level of suspended solids in water column

Poor sanitation was one of the causative agents of waterborne diseases (WHO, 2017).

Parasites were quantified by USEPA Method 16231/2012, genotyping was performed using specific primers based on the 18 SrRNA gene for *Cryptosporidium* and *gdh* gene, *Giardia* was detected in 83.3% of the samples and *cryptosporidium* in 37.5%.

1.4 Pathogens of Concern

Four groups of microorganism found in treated and untreated wastewater are: Bacteria, Protozoa, Helminths eggs and Viruses.

Historically waterborne outbreaks of cholera in England in 1860's, *Escherichia Coliform* (*E Coli*), due *Escherich*, Typhoid fever linked to *Salmonella typhi*, *Giardia lamblia*, waterborne protozoa in 1960's, *Cryptosporidium parvum* a protozoan first isolated, Enteric pathogens particularly enteric bacteria, protozoa and viruses.

- ❖ Gastrointestinal illness routine stool examination including culturing for Salmonella, Shigella, Campylobacter.
- ❖ Many types of harmless and beneficial bacteria are found in the intestinal tract and are routinely shed in the feces. (Bacteria are microorganisms 0.2-10 um in length).

Enteric bacteria include:

- ❖ Shigella causes Shigellosis (S. sonnei cause bulk waterborne infections).
- ❖ Salmonella cause gastrointestinal illness (S.typhi causes typhoid).
- ❖ S. paratyphi causes paratyphoid fever.
- ❖ Ecoli member of Faecal Coliform group in intestinal tract.
- ❖ Giardia lamblia causes waterborne giardiasis.
- ❖ Viruses are obligate intracellular parasites able to multiply only within a host cell and are host specific. Viruses vary in size between (0.01-to 0.3um), and are composed of nucleic acid surrounded by an outer coat of protein. The genetic material (DNA & RNA) are coated by a protein that allows the viruses to live for a long period of time.

Enteroviruses: Are a big family branched into:

- 1- Polioviruses.
- 2- Echoviruses.
- 3- Coxsackieviruses.
- 4- Hepatitis A.

5- Rotaviruses.

6- Calciviruses (e.g. Noroviruses).

Most enteric viruses causes gastroenteritis or respiratory infections, others cause encephalitis, neonatal disease, myocarditis, aseptic meningitis, and jaundice.

Hepatitis A could be transmitted by drinking water and causes inflammation of liver (necrosis).

Noroviruses and other Calciviruses, viruses of this group are identified by molecular analysis, reverse transcriptase PCR and electron microscopy.

Noroviruses causes gastroenteritis. Rotaviruses causes acute gastroenteritis, especially in children, in developing countries, Rotavirus are causative agent of infants mortality, and they are transmitted via oral- faecal rout, and found in rivers, lakes, tap-water, and municipal wastewater.

Enteroviruses include polioviruses, coxachieviruses and echoviruses all these are found particularly in wastewater and drinking water.

In 1952 poliovirus outbreaks was reported in United States were 16 cases of paralytic disease was recorded.

Adenoviruses are 47 types, 40 and 41 type are causative agents of gastrointestinal illness especially in children, other types cause respiratory illness and common cold.

Adenoviruses are transmitted via oral- faecal rout and are resilient to disinfectants and are not removed by conventional treatments.

Bacteriophages are viruses that infect bacteria, Coliphages are better indicators for human enteric viruses than bacterial indicators (Asano *et.al.*, 2007).

Bacteriophages and enteric viruses are often attached to particulate matter in aquatic environment, (affected by heterogeneity of different bacteriophage and attachment of coliphage to particles).

Detachment rates are typically 100- 1000 times lower and this process is also affected by oxygen content, greater adsorption leads to increase in oxic environment.

Also bacteriophages depend on the soil /sediment also velocity of faecal indicator bacteria (FIB) attachment to particles.

- Human activities, swimming and boating affect viral loads.
- Affect of temperature on survival of bacteriophages (survival was more in lower temperature).

Enteric viruses are leading etiologic agents of waterborne disease outbreaks found due to:

1. Exposure to human waste.
2. Secondary effluent disinfected.
3. Primary wastewater effluent.

Waterborne pathogens if found in treated wastewater, low doses are required for illness.

Faecal indicator bacteria (FIB) faecal coliform, Ecoli, Enterococci, Bacteriophages are viruses dependant on host bacteria for replication (Korajkic, 2017).

1.5 Guidelines and regulations for Reclaimed wastewater

Nowadays, most countries of the world are not only facing scarcity of water due to climate change, but the big challenge is the highest cost of making water available at the right place at the right time with the required quality, not only climate change is a challenge, but also the increase in water demand is due to the big growth of the world population which is expected by 2050 to reach 11 billion (UNDP, 2003).

For that reason, there should be an alternative other than the conventional water resources to reach sustainability in life on the whole biosphere.

When Kretchmer talked about 70% of water resources consumed by crop irrigation, 20% was used for industry and 10% went to residences (Kretchmer et al., 2004).

A case study took place in Pakistan showed that the world total annual rainfall is around 110,000 billion cubic meter (bcm) and the renewable water resources was about 40,000 bcm, the annual water availability was 6,700m³per capita.

This study said that one third of world population will experience severe water scarcity within the next 20 years, 17 countries in Pakistan will not have enough water (UER, 2000).

An un-conventional alternative is the reclamation and reuse of treated wastewater mainly for crop irrigation, to satisfy further increases in demand, especially in regions in the world that suffer from mismatches between water supply and demand .requirement for stringent Regulation and Guidelines were essential on wastewater reclamation and reuse, to stop the detrimental effect on the ecosystem

and human health, since Wastewater Reuse consists of waterborne pathogens that would cause illness and mortality.

World health organization (WHO) and United Nations Environment Program (UNEP) put restrictions on crops irrigated with treated wastewater reuse, there should be treatment before Reuse (Blumenthal et al., 2000).

Faced with demand from a growing population and an expansion in irrigated farming, water resources are now under greater threat than ever and the distribution of these resources ever more complex. Among other factors, the 2014 report by the IPCC (Intergovernmental Panel on Climate Change) warns of greater pressure on water resources in the south of Europe and in the Mediterranean in the decades to come. These regions lie in particular in areas that already experience serious water stress.

Projected global water scarcity in 2025 (Adapted from IWMI, 2000). In the global scale, countries of North Africa and the Middle East, Pakistan, India, and the northern part of China are projected to face severe water scarcity.

In view of this situation, the reuse of treated water is growing sharply worldwide, with differences according to each country. It is forecast that the volume of recycled water will be more than double from 19.4 M m³/day in 2005 by 2020.

Thus, the techniques used to recycle treated used water are now well-known and most countries have adopted regulations for the reuse of treated water that are based on international regulations, in particular those of the World Health Organization (WHO). A comparison of criteria given by well-known authorities (maximum limits) for wastewater Reuse through irrigation illustrated in (Table1).

Table 1.1 Comparison of Criteria for wastewater reuse for irrigation

<i>Parameters</i>	<i>California (1978)</i>	<i>USEPA (1992)</i>	<i>WHO (1989)</i>	<i>France (1991)</i>	<i>Italy (1977)</i>
Regulation type	Law	Guidelines	Guidelines	guidelines	Law
Minimum treatment required	Advanced	Advanced	Stabilisation ponds	–	Secondary treatment
BOD5 (mg/l)	–	10	–	–	–
SS (mg/l)	–	5	–	–	–
Turbidity (NTU)	2	2	–	–	–
pH	–	–	–	As WHO	–
Total coliform, (MPN/100 ml)	2.2	0	–	–	2
Fecal colif. (MPN/100 ml)	–	–	1000	–	–
Helminths, (eggs/100 ml)	–	–	1	–	–
Res. chlorine, (mg/l)	Present	1	–	–	–
Salinity	–	–	–	–	SAR <10
Metals	–	–	–	–	Yes
Main treatment processes	Oxidation, filtration and disinfection	Filtration, disinfection	Stabilisation ponds or equivalent	–	–

Source: Al-Suwaiyan et al. (2003)

Among these authorities, California's standards are more stringent, since the fecal coliform should be nil or zero coliform while the WHO guidelines are more flexible since the faecal coliform could be 1000 CFU/100ml.

Table 1.2 Classification of the Treated Wastewater according to Quality (MOA, 2012).

Maximum limits for chemical and biological properties (mg/L) unless otherwise stated	Quality of Treated Wastewater			
	High quality (A)	Good quality (B)	Medium quality (C)	Low quality (D)
Biochemical Oxygen Demand BOD ₅	20	20	40	60
Total Suspended Solids TSS	30	30	50	90
Fecal coliform bacteria (colony/100 mL)	200	1000	1000	1000
Chemical Oxygen Demand COD	50	50	100	150
Dissolved Oxygen DO	< 1	< 1	< 1	< 1
Total Dissolved Solids TDS	1200	1500	1500	1500
Potential of Hydrogen PH	6-9	6-9	6-9	6-9
Fat, Oil and Grease	5	5	5	5
Phenol	0.002	0.002	0.002	0.002
Detergents MBAS	15	15	15	25
Nitrate Nitrogen NO ₃ -N	20	20	30	40
Ammonium Nitrogen NH ₄ -N	5	5	10	15
Total Nitrogen T-N	30	30	45	60
Chloride Cl ⁻	400	400	400	400
Sulfate SO ₄ ²⁻	300	300	300	300
Sodium Na ⁺	200	200	200	200
Magnesium Mg ⁺⁺	60	60	60	60
Calcium Ca ⁺⁺	300	300	300	300
Sodium adsorption ratio SAR	5.83	5.83	5.83	5.83
Phosphate Phosphorus PO ₄ -P	15-20	15-20	15-20	15-20
Aluminum Al	5	5	5	5
Arsenic As	0.1	0.1	0.1	0.1
Copper Cu	0.2	0.2	0.2	0.2
Iron Fe	5	5	5	5
Manganese Mn	0.2	0.2	0.2	0.2
Nickel Ni	0.2	0.2	0.2	0.2
Lead Pb	0.2	0.2	0.2	0.2
Selenium Se	0.02	0.02	0.02	0.02
Cadmium Cd	0.01	0.01	0.01	0.01
Zinc Zn	2	2	2	2
Cyanide CN	0.05	0.05	0.05	0.05
Chrome Cr	0.1	0.1	0.1	0.1
Mercury Hg	0.001	0.001	0.001	0.001
Cobalt Co	0.05	0.05	0.05	0.05
Boron B	0.7	0.7	0.7	0.7
Bacteria E. coli (Colony/100 mL)	100	1000	1000	1000
Nematodes (Eggs/L)	≤ 1	≤ 1	≤ 1	≤ 1
Maximum temperature	35	35	35	35
The degree of turbidity	5-10	5-10	5-10	5-10

Source of this table was taken from PSI and ministry of agriculture (2012).

Wastewater reuse covers four parameters;

- Chemical standards.
- Microbiological standards.

- Wastewater treatment processes.
- Irrigation techniques.

Looking at table one shows how California guidelines is more stringent when Biological Oxygen Demand (BOD) is zero, SS (suspended solids) also should be zero, for salinity and metals also zero, this for chemical parameters, and for microbiological analysis Faecal coliform and Helminths eggs should be also zero.

The status of Wastewater reuse in the Mediterranean basin the study illustrates that four Mediterranean countries have the minimum required water availability and by 2025 they will become 8 countries.

Wastewater reuse used as a source of irrigation this practice provides

- Low cost water source.
- Using the fertilizing properties found in wastewater reuse and by this lowering the demand for synthetic fertilizers.
- Eliminate the need for expensive tertiary treatment.
- Taking precautions before using wastewater reuse is necessary.

Israel was a pioneer in the development of wastewater reuse practices followed by Tunisia, Jordan and Cyprus.

In Israel 92% of Wastewater reuse is collected by municipal sewers, 72% is used for irrigation and 30% is used for water recharge.

Wastewater was not a major issue in Morocco but due to rapid population growth water consumption increased and this lead to transfer of freshwater from one

catchment area to another and the replacement of wastewater instead of freshwater for crop irrigation.

Volume of wastewater available for reuse would be increased by improvement of sewerage networks, within a few decades the share of wastewater reuse in the overall water resources, this solved the problem locally and scale for the towns that were isolated from the major water supply system.

The inadequate sanitation, collection and treatment of WW, is a risk to eutrophication of dams, on the other hand discharging of raw WW on a national affects the aesthetic value of the beaches due to odours and bad sanitation.

Liquid Sewage National Master Plan was currently due to the very fast demographic expansion, and poor sanitation lead to high incidence of waterborne diseases in Morocco, around 25% of the population are infected, and this explains the need for guidelines and regulations of WW Reuse in Morocco.

Many projects for WW were scheduled and a draft for guidelines and regulations of reuse was written in 1995 close to the California standards serving mainly arid and semiarid regions in Spain due to lack of rain during several months of the year lead that the coastal line was fed with treated and untreated wastewater.

WHO quality guidelines are less stringent than the California guidelines, the WHO guidelines rely on the monitoring of the intestinal nematodes, while the California guidelines rely on monitoring total coliform (Asano and Levine et al.,1996).

Under these conditions, the automatic treatment of used water and the reuse of water treated are required in order to optimize water resources. While the

treatment of Reused water protects the quality of water resources (whether they come from sources on the surface, springs or aquifers), REUSE provides an additional water resource, replacing water extracted in particular for agriculture, which consumes more water than any other activity.

Moreover, domestic waste water was rich in organic material, phosphate and nitrogen. In a context in which more and more inputs are used in agriculture, domestic waste water can help reduce fertilizer inputs by close to 30% (WHO, 2010).

Therefore, waste water will mainly be destined for reuse for agricultural irrigation in order to reduce the extraction of water resources for competing agricultural, domestic and industrial purposes and to enable farmers to improve their yields.

Thus, REUSE is an alternative solution to the scarcity of water that is becoming more and more common around the world. Of course, the need to implement this solution depends on local constraints on water resources, as well as on public policy, training and the awareness of populations. On the other hand, it does not require access to particular sophisticated water treatment techniques in addition to standard treatment: these techniques are well-known, easy to implement and inexpensive.

1.6 Economics of Wastewater Reuse

Doctor Maher Abu Madi and Doctor Rashed Al- Sae'd highlighted in their paper on the precious value of treated water mainly in the MENA region (Middle East and North Africa) due to scarcity problems of water in these regions, also direct

policy makers and stakeholders and experts on the right management and utilization of this precious unconventional source of water.

In the Mena region more than 89% are used for agriculture and 11% of water used for municipal and industrial purposes.

When talking about Treated wastewater it's a big issue, and three pillars should be in mind collection of this water by special sewage systems, the best treatment of wastewater and lastly reusing this water for irrigation.

Sustainability of treated wastewater by identifying the right utilization of this water, talking about right monitoring and management of this water market of this reclaimed water and an important thing to reach sustainability was to reduce the gap between the supply and demand for this water, also one of the recommendations they come to was make decisions of the location and the technology and the effluent quality after deciding on the end uses of this reclaimed water for restricted agriculture, unrestricted agriculture, discharging into to the sea, industrial use, potable or non- potable water.

Also stringent guidelines and standards in the MENA region hampered the use of treated water so another recommendation was to adopt flexible standards and in the same time complying with public health and environment protection.

Suggestion of using an indicator called (WRI) Waste Water Reuse Index which makes quantification of the Reuse as a percentage of the total generation of wastewater by dividing the amount of wastewater being actually reused by the total amounts of wastewater generated at country level. The benefit of this indicator was to highlight to the policy makers about the right way for

improvement of wastewater management, mitigation measures of the gap between supply and demand, reducing losses and by this reaching sustainability of treated wastewater (Abu-Madi, 2009).

Promotion of Wastewater/Sludge Reuse

The Jordanian experience in irrigation using the treated wastewater was a successful one, and it was first supported by breaking the fear of the farmer of using this treated wastewater. Awareness campaigns were concentrated to persuade the farmers to use it, the treated wastewater was sold freely at first and then the government increased the cost when the demand for treated wastewater increased.

From economical point of view the Jordanian farmers agreed with this idea that it was beneficial to them and they need more amounts of it, this cost effective unconventional source of water encourages the farmers to apply this experience in Jericho.

Identifying the type of crops that depend on treated wastewater quality which are not found in Palestine like the Dutch herb and can be introduced in Jericho and the need for more workshops for reclamation and Reuse.

Exchanging knowledge and experience with other farmers, persuading them of the cost effective benefit ratio of this treated water.

Jordanian Government helps and assists farmers in reusing the treated wastewater by offering the treated water for free for the first few years. Now, the water is provided by some fees to farms especially those near the WWTP. The treated

wastewater at Jericho WWTP has higher quality than the visited Jordanian WWTP so it will be better for crop- irrigation.

The outcome of this successful experience was these four recommendations:

- ❖ Government participation in assisting the farmers in reusing the wastewater, for their crop irrigation.
- ❖ Community participation in this experience
- ❖ Awareness campaigns for public to convince them of using this unconventional source of water, a way of compensation and not to lose more fresh water for irrigation.
- ❖ Work on reducing the price of treated wastewater.
- ❖ Reduce the purchasing cost of the treated wastewater (Seiba, 2016).

1.7 Aim of the Research

The major aim of this study was to determine the occurrence and levels of microbial indicators and pathogens in raw and treated wastewater and compare their persistence after chlorination in different wastewater treatment plants.

1.8 Research Goal and Objectives

1. Technical evaluation of the mechanism and wastewater treatment procedure on three treatment plants in Ramallah district in Palestine.
2. Laboratory testing of untreated and treated wastewater according to the international standards.

3. Specify the quality of treated wastewater in order to determine the utmost use of that treated water.
4. Using this treated wastewater in agricultural projects if its quality is in accordance with the international standards.
5. Determine the content of selective microbial pathogen, enteric viruses in three different wastewater treatment plants using real-time PCR data.
6. Determine the release of infectious viruses in the treated effluent.
7. Compare virus removal efficacy between Al-Tireh MBR facility and other two conventional wastewater treatment systems Al-Bireh activating sludge and MBBR in Rawabi.

1.9 Methodology

For successful achievement of the main objectives of this research study, the following research methodology steps were adopted sequentially:

Step one: Collecting all the available studies, technical reports and published data related to my proposal title “Removal of pathogens in different wastewater plants having different technologies” first in case studies in the world and then focussing on case studies in Palestine.

Sampling of composite raw influent and treated effluent from three wastewater plants in Ramallah district. Composite samples were taken every three hours for both the raw and the treated wastewater from the three different wastewater plants.

Wastewater samples were sent to by icebox at 4C⁰ and was preserved in refrigerator waiting for the chemical and biological analysis.

Physical parameters was measured onsite on the wastewater samples, parameters measured onsite was PH, EC (conductivity), turbidity, and temperature.

Step One: Chemical and biological analysis was done in PWA (Palestine Water Authority) central laboratory, the chemical analysis done was COD (Chemical Oxygen Demand) and BOD₅ (Biological Oxygen Demand).

Step Two: Biological analysis done in PWA Lab was the Faecal Coliform count was used as a biological indicator of waterborne pathogens in wastewater and this was used as a correlation indicator to the fourth step in the analysis.

Step Three: RT-qPCR (qualitative real time polymerase chain reaction technique) was done in the molecular Lab of Al- Caritas hospital RT-qPCR analysis looking for viruses present in the raw and the treated wastewater samples such as Enteroviruses, Adenoviruses, Noroviruses and lastly Rotaviruses to make comparison on the presence of the enteric viruses in the two samples to see the removal efficiency in the treated wastewater in the three different plant technologies and to see if there was a correlation between the removal of viruses and the log removal of Faecal Coliform indicator.

1.10 Research hypothesis

Since treated wastewater may contain harmful microbial pathogens that pose public health and environmental hazards, the use of treated wastewater and stabilized biosolids for agricultural purposes was a main issue of concern to national regulatory bodies responsible for public health and environmental

security. The findings of this study will provide science based knowledge and deep understanding that enable efforts to update the current environmental guidelines for sustainable beneficial uses of reclaimed water to reduce public and environmental risks.

- Two hypothetical questions in my thesis first was the three different wastewater plants with different technology which does not have tertiary treatment efficient enough to bring out treated wastewater free of pathogenic microorganisms?
- Second question fecal coliform (FC) count by membrane filtration technique was good to confirm that the treated effluent was free of pathogenic microorganisms like pathogenic viruses for example? That means there should be other indicators for presence of viruses like bacteriophages.

In other words if the treated effluent fecal coliform (FC) count was within the recommended guidelines is that means it is free of pathogenic viruses? Is there any correlation between the fecal coliform indicator and the viruses indicator???

1.11 Limitations of the analysis

There are two types of PCR (1) relative quantification and (2) absolute quantification.

For absolute quantification like Taq-man real-time PCR needs probes for every virus and they cost a lot and needs standards also costs, complementary DNA (cDNA) kit that change RNA to DNA.

The second option was the relative quantification real time PCR no need for expensive standards and emphasize how efficient the treatment to reduce the folds

of viruses. So one challenge was the fund for the PCR analysis, since we are looking for viruses in sewage the materials, (probes, etc.) used for PCR costs a lot of money, not only financial problems but also choosing the right method for detection of viruses.

Thanks a lot for Al-Quds University particularly Dr. Ziad Abdeen who tried to help by providing special filters called Corning Filters (430769), the filters pores size was 0.22 micrometer, but this was not the right procedure and not the right filters since from further studies and investigations and asking a specialist in viruses (Dr. Mousa Hindiyeh), he confirmed that I lost the viruses by filtration because the viruses sizes between (0.01-0.3um) are smaller than the Corning filter pores, we lost only 200cc each sample. Then I looked for the right procedure with the help and guidance of Dr. Musa Hindiyeh. (Pictures of making filtration by using Corning filters in Al- Quds University are illustrated in Figure (18) in Appendix 3).

And the second challenge was transportation and right preservation of the samples in refrigerator until making the analysis in the Laboratory, thanks a lot for Al-Quds University who kept the samples in their refrigerator, transportation was also a problem since it was not possible to make real time PCR in Birzeit University, analysis was done in Al-Caritas Baby Hospital in the molecular Lab department in Bethlehem.

Limitations concerning the molecular analysis for detection of viruses in sewage:

- Seasonal trend affect the number of viruses detected since the abundance of viruses fluctuated daily and seasonally.

- Also type of viruses vary if the samples were collected at different time of the year.
- Fluctuation in treatment efficiency also affects the presence of viruses in the final effluent.
- Adherence of the viruses to the solid particles may have been lost in the filtration step.

1.11 Thesis outline

Chapter1: In this chapter there was an introduction about wastewater reuse, talking about the epidemiology of pathogenic human viruses and the Islamic perspective for usage of reuse and reclaimed water and the history of Reuse worldwide, we also mentioned the aim of the research and the research goals and objectives. Guidelines and regulations were discussed, and economics of Reuse, also we made a summary to the methodology, and concentrated on the research hypothesis and limitations of the analysis.

Chapter2: There was an introduction of the different levels of treatment plants, and description of the three different technologies and the current situation in Ramallah and Al-Bireh regarding Reuse and wastewater plants.

Chapter3: In this chapter literature review and previous studies about removal of pathogens from wastewater plants with different technologies.

Chapter4: Overview from previous studies about methods and protocols done.

Also in this chapter identifying the right sampling and followed by the three steps done, preparation and concentration of the 18 samples, followed by extraction to

nucleic acids, and finally real time PCR analysis for detection of the strains of Enterovirus, Adenovirus, Rotavirus, and Norovirus, also discussing the routine tests in PWA Fecal coliform membrane filtration test, BOD₅, COD, using Oxi-Top method and Hach method respectively.

Chapter5: The results and discussion of the real time PCR illustrated in a table that shows the cycle threshold of every virus if the result was positive and a table discussing the results of the routine tests, BOD₅, COD, and fecal coliform for the influent and effluent done in PWA lab, also a table showing the assessment of the three parameters, physical, chemical and biological parameters, discussion of the results of viruses was documented according to the literature review mentioned before.

Chapter six: Conclusion and Recommendations

In the conclusion I reviewed the main outcomes of this thesis and the results of Enteric viruses in the three different wastewater plants and in the recommendation concentrating on water safety plan (WSP) and choosing the right technology in wastewater plants and planning from the beginning the end uses of the effluent and addition of tertiary treatment to increase the log removal efficiency of pathogens particularly viruses.

References

Appendix 1

Appendix 2

Appendix 3

Chapter Two

Study Area

2.1 Introduction

Wastewater treatment technologies with characterization suitable for use in developing countries and producing effluent safe for agricultural irrigation since it concentrated mainly on pathogen removal and nutrient conservation. Wastewater treatment technologies are divided into two categories:

- ❖ Natural systems: which does not depend on energy consumption which are more suitable for developing countries and includes stabilization ponds, wastewater storage and treatment reservoirs, septic-tanks, Imhoff-tanks, UASB-reactors, high-rate anaerobic ponds and constructed wetlands.
- ❖ The second category are the energy-intensive-systems which includes aerated lagoons, activated sludge systems, MBR (Membrane bioreactor) RBC (rotating bioreactor (Jimenez, 2012).

Treatment levels

➤ Pre-treatment

This level is composed of screens, grit chambers that provide removal of sand, floating, and coarse matter and some supplementary units as equalization tanks or flow-rate measurements.

➤ **Primary treatment**

This level usually includes preliminary settling tank in which suspended solid matters and organic matters are removed partly. Advanced primary Treatment, this level is provided with precipitation with chemical addition or filtration and suspended solid matters and organic matter are removed at advanced level.

➤ **Secondary Treatment**

In addition to the degradable organic matters and suspended matters, nutrients (nitrogen and phosphorus) are removed.

➤ **Tertiary Treatment**

This level includes the advanced removal of the suspended solids and organic matters that cannot be removed during secondary treatment. Micro-screening or filtration was provided. Disinfection and nutrient removal are also defined as tertiary treatment.

Advanced Treatment includes the advanced removal of suspended and organic matters that can be removed with biological treatment, in order to reuse treated water.

The three wastewater plants in Ramallah district under study were:

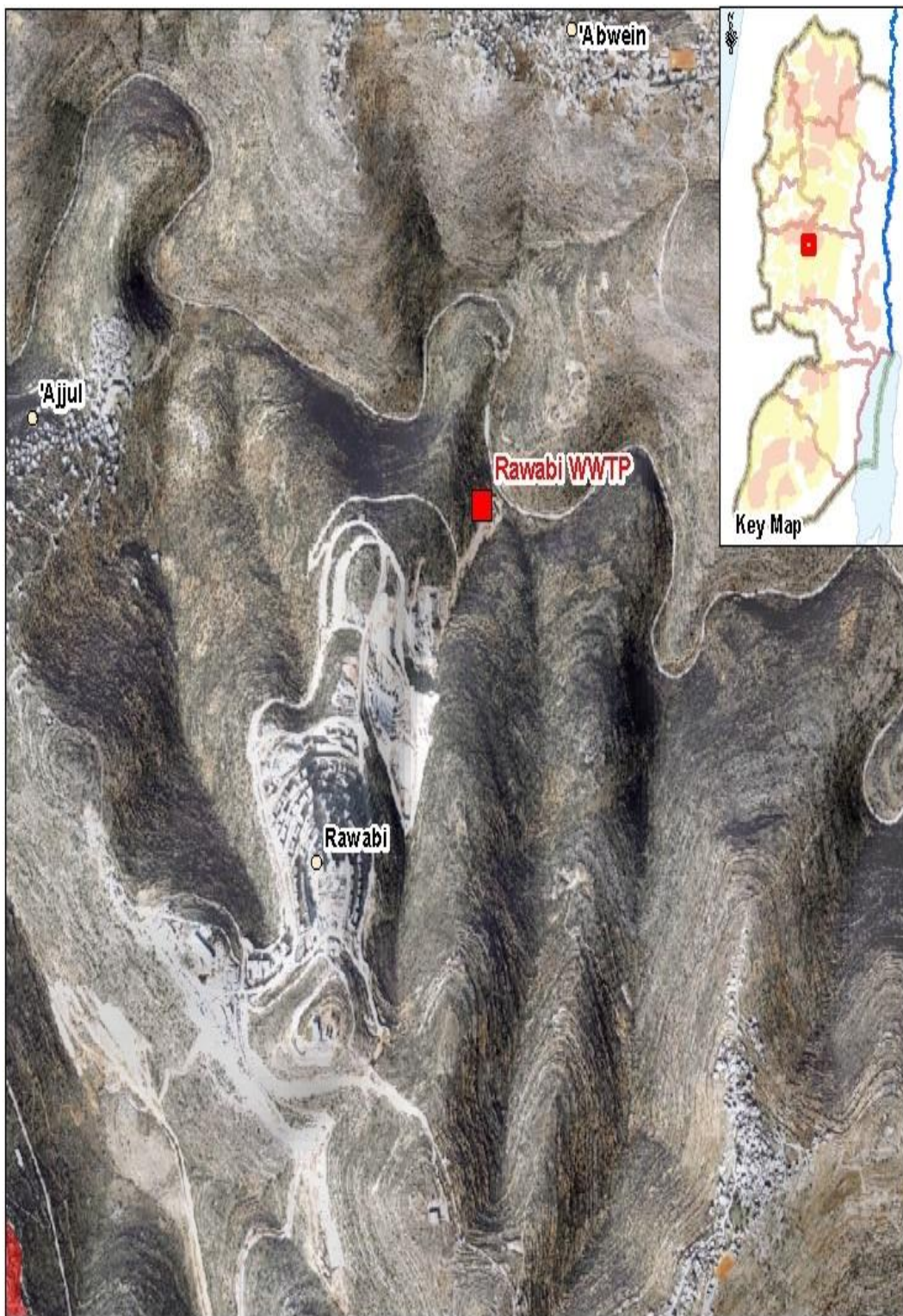
1. Al-Bireh Wastewater Treatment Plant. (ASWWTP).
2. Al-Tireh Wastewater Treatment plant. (Al- Tireh MBR).
3. Rawabi Wastewater Treatment plant. (RWWTP MBBR).

Map 2.1 A map of Ramallah Al- Bireh WWTP



Source PWA 2019

Map 2.1 B Map of the Rawabi WWTP



Source PWA 2019

Map 2.1 C Map of Al-Tireh WWTP



Source PWA 2019

2.2 Al-Bireh Wastewater Treatment Plant

Location:

Al-Bireh Wastewater Treatment plant was constructed in the year 2000 with the support of the German Government through the German Development Bank (KfW). The plant location is the Wadi Al-Ein, 2 km south east of Al-Bireh city, over 22 dunms of land (Figure2.1) with enough capacity to serve future expansion.

The total capital investment for the establishment of the WWTP was 13 million USD, excluding the purchase cost of land (Al-Bireh municipality 2010).

Fig 2.1 the different sectors of Al- Bireh treatment plant



Source of this figure taken from (Al- Sa'ed, 2007).

Wastewater sources and Characteristics

Al- Bireh WWTP was designed to be built in two phases first phase to serve 50,000 and second phase to serve 100,000 persons with the extended aeration treatment technology.

Currently the WWTP serves around 50,000 persons residing in Al-Bireh city, Qaddura- Camp, AL-Am'ari Camp and some neighborhoods of Ramallah City. It treats an average daily flow of 5,750 m³ of wastewater originating from domestic, commercial and industrial sources.

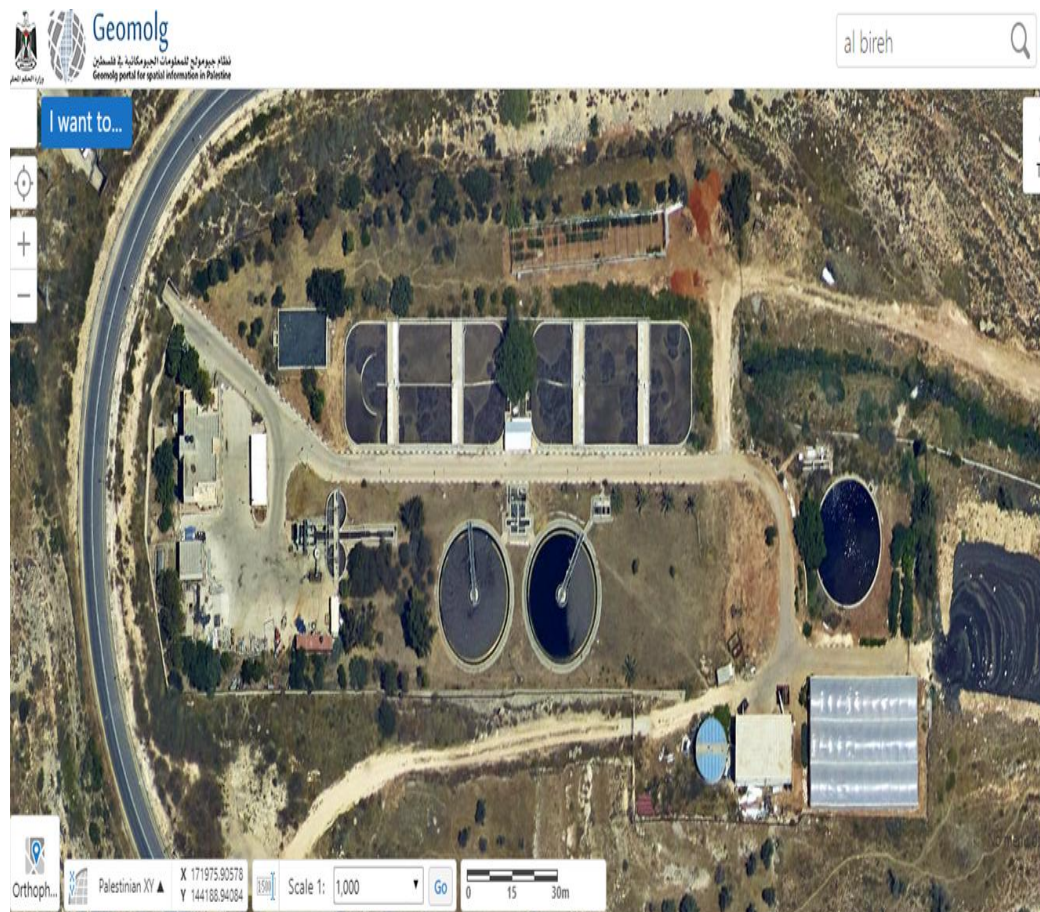
The technology used was activated sludge, in this process consist of Aeration tanks or basins containing a suspension of the wastewater and microorganisms, the mixed liquor in the aeration tanks are mixed vigorously by aeration devices which supplies oxygen to the microorganisms in the tank that feed on the nutrients in the aeration tanks and by this the microorganisms play major role in clearance of the wastewater plant from the organic matter.

Following the aeration step, the microorganisms are separated from the liquid by sedimentation tanks and the clarified liquid is secondary effluent. A portion of the biological sludge is recycled to the aeration basin to maintain a high mixed-liquor suspended solids (MLSS) level. The remainder is removed from the process and sent to sludge processing to maintain a relatively constant concentration of microorganisms in the system. Several variations of the basic activated sludge process, such as extended aeration and oxidation ditches, are in common use, but the principles are similar.

There should be a tertiary treatment that helps in getting rid of small microorganisms and waterborne pathogens that could not be removed by the secondary and this could be achieved by addition of chlorine as a disinfectant, or by UV disinfection, or by microfiltration and ultrafiltration.

In the design of Al-Bireh wastewater, there was UV disinfection in 2000, and currently, the UV installed in Al- Bireh WWTP are aged and non- functional the UV units were operational in the past 20 years.

Map 2.2 Aerial Photo of Al- Bireh Wastewater plant



Source of the map Ministry of Local Government 2019

2.3 Al-Tireh Wastewater Treatment Plant

the design was located in Al-Tireh Alban'a Ramallah St. Ramallah, Palestine, and started operation in 15/01/2014.

The owner of this design was Ramallah Municipality and was also funded by it, the project value was 4.0 million dollars for design built and phase and the project value for operation phase for 4 years was 220,000.00\$.

The capacity of the design was 2,000 m³/day and the current capacity was 1,650 m³/day, its energy consumption was 1850 kWh/day.

The plant working duration was 24hr./day , 7days/week and the population covered was 25,000 Person (Al-Tireh Residents). The plant total area was 2500.0 m² and the treatment process area was 8000.00m² the technology used was MBR (Membrane bioreactor), the influent type (Sewage input) was domestic sewage and the effluent type (treated sewage output) was Class A (BOD 10, TN 10, FC 10) the treated effluent could be used for non -restricted irrigation.

The MBR biofilm treatment plant was operated daily basis receiving 1600 cubic meters of water flowing from Al-Tireh, Al-Jadwal, Al-Irsa, Mussafah and downtown areas. The design capacity was 2000 cubic meters, serving currently about 18000 people. Specification of Zee Weed 500 Module the immersed hollow Ultrafiltration membrane used in Al-Tireh WWTP in the period of sampling (Appendix 3, Figure 24).

2.4 Rawabi Wastewater Treatment Plant

Map 2.3 Aerial Map of Rawabi Wastewater Plant



Source of the map was from Ministry of Local Government 2019

Process Description

The wastewater will flow through the following units before entering to the proposed biological reactor: 1-Fine screening (max. 5 mm opening). 2-Grit and Grease removal system.3-Equalization tank, volume = 30% of daily flow = 260 m³ (at expanded capacity).

4- The proposed biological process is the IFAS (Integrated Fixed Film Activated Sludge)

AGAR system, which utilizes attached growth on BC (Biomass Carriers) with suspended solids returned from the clarifier.

The AGAR technology will be installed in a biological reactor with a total operative volume of 480 m³.

5- The biological reactor will be divided into 3 process stages as following:

- The 1st stage will be an anoxic stage for pre-denitrification. This stage will be equipped with mechanical mixer/s.
- The 2nd stage will be an aerobic stage for BOD removal (activated sludge), and nitrification. Biomass carriers will be filled inside this stage.
- The 3rd stage will be an aerobic stage for nitrification. This stage will be equipped with mechanical mixers.
- The biological reactor will be followed by a secondary inclined plate (lamella) clarifier (or equivalent) for solids / liquid separation.

Aeration in stages 2 will be achieved by fine bubble diffusers, installed at the reactor bottom in the required pattern. Unique airlift hydraulics will assure mixing of biomass carriers, in the 2nd stage.

A wedge wire screen will be installed at the outlet of the 2nd stage in order to maintain the carriers inside the reactor, whereas mixed liquor may flow unobstructed. The wedge wire screen is continuously cleaned by the mixing flow of water, air bubbles and biomass carriers.

An internal re-circulation from the 2nd (aerobic) stage to the 1st (anoxic) stage will ensure sufficient de-nitrification.

The treated wastewater will flow from the biological reactor to a secondary gravitational clarifier for solids-liquid separation. Solids from the clarification stage are periodically removed for further treatment and dewatering.

Returned activated sludge will be re-circulated from the secondary clarifier to the 1st stage of the biological reactor.

In order to meet the 10/10 (BOD / TSS) requirement the secondary clarifier will be followed by a tertiary filtration & disinfection system.

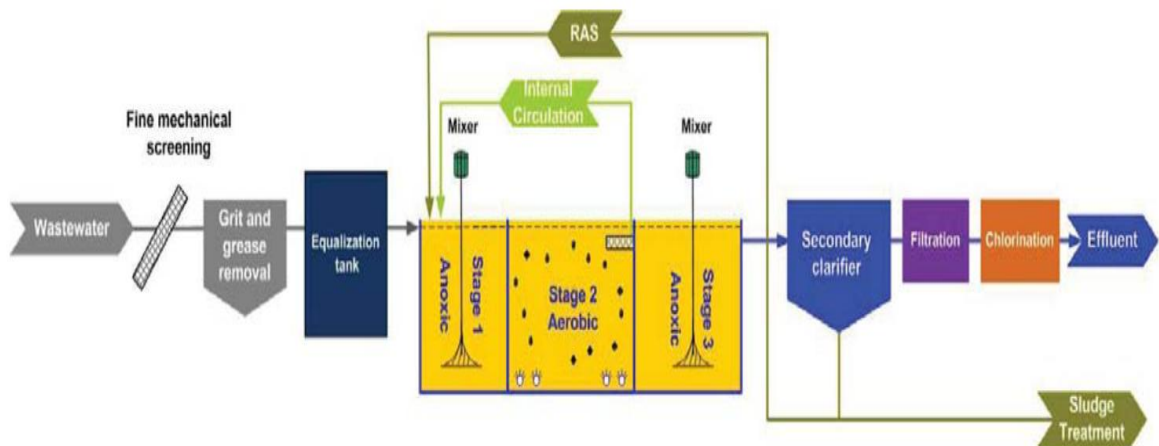
Figure 2.2: Rawabi wastewater treatment plant, containerized WWTP



Source of the figure my phone 2019

Figure 2.3: Identifying different stages of Rawabi WWTP

Schematic process flow diagram:



2.3 Current situation in Ramallah and Al- Bireh

Recommendations and outcomes of a study done by doctor Rashed Al- Sa'ed was to apply tertiary or advanced treatment to Al- Bireh Activated Sludge plant, since the UV. Disinfection was not in operation, and from the assessment done although there was removal efficiency for BOD₅ (< 20mg/l), however there was presence of pathogenic bacteria, protozoa, and helminthes ova.

Fecal Coliform indicators vary from one country to another due to variation in wastewater treatment technologies, variation in climatological conditions, and the different technologies used for an end use of reclaimed treated water with compliance to guidelines of different countries. (Angelakis et al.,1999)

For example Jordan Fecal Coliform indicator are < 1000 CFU/100ml, Israel are <250, Kuwaite <1000CFU/100ml.

The outcomes of this assessment was that the analysis of the samples over four months showed high numbers of Fecal Coliforms and worm eggs in the raw sewage samples, and 31% of the raw samples consist of Salmonella. The plant efficiency in Fecal Coliform and Fecal Streptococci was 99.64% and 93.44% respectively. The end uses of the reclaimed water was for unrestricted irrigation meeting the WHO guidelines Fecal Coliform < 1000CFU/100ml, and helminthes eggs < one (Al- Sa'ed, 2007).

The results of the assessment done by doctor Rashed Al-Sa'ed in 2012 comparing the removal efficiency of the chemical parameters COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand), Total Nitrogen (TN), Total Phosphate (TP), between the influent and effluent met the Palestinian wastewater treatment requirements for restricted irrigation, but still for reaching sustainability of Al- Bireh wastewater treatment plant and Reuse schemes needs adequate administrative and operational management.

Among the seven urban wastewater treatment plants (WWTP) in Palestine, Al-Bireh was the only new urban (WWTP) in operation since 2000, while the rest are overloaded or recently upgraded. (Al-Sa'ed, 2012)

Table 2.1: list of UWWTP in Ramallah/Al-Bireh Governorate West Bank

UWWTP location site	Capacities (PE)	Types of treatment	Technical difficulties during operation mentioned by UWWTPs managers
Al- Bireh	50000	Activated sludge process. Tertiary treatment: 1- Disinfection. 2- UV. Sludge treatment: filter press+ centrifuge	<ul style="list-style-type: none"> ➤ Lack of spareparts ➤ Break of control system ➤ UV disinfection not used ➤ Sludge disposable and handling
Al- Tireh	25000	Membrane Biological Reactor	<ul style="list-style-type: none"> ➤ Inlet BOD₅ ➤ Rainy weather ➤ Sudden variability ➤ Sometimes availability of spareparts
Rawabi	875m ³ /day	MBBR	NA

Source (<https://washdata.org/data>, WHO/UNICEF JMP, 2015)

100 % of the population did have access to improved sanitation, including 63 % of the population, who were connected to a collective sewer in 2015. But only 47 % of wastewaters were treated, on the other hand the existing treatment plants do not have the capacity to treat all of the produced wastewater, causing severe water pollution.

Thus, there is a big need to extend the sanitation systems and especially the collective ones; including the building of UWWTPs. In parallel, there is a big need to train the operators of the actual and future UWWTPs in the aim to ensure the sustainability and efficiency of investments.

But the development of the sector highly depends on external financing. The PWA and JWSSCs hasn't the technical and financial means and skills for

ensuring efficiency, its mission related to sanitation. In particular, PWA hasn't enough capacities for following analysing and assisting technically the waste water sector.

The major reason of these difficulties was the low wastewater treatment tariff and the non-efficient wastewater bill collection which affects cost recovery and thus sustainable operation; especially for sanitation. But there was a great disparity between all the Joint Water and Sanitation Services. Each Joint Water and Sanitation Service Council has its own method for recovering the sanitation cost. At Ramallah, the situation seems convenient by recovering taxes as a proportionally to the inhabited surface. The main problem in many Palestinian municipalities is the ability and the affordability of families to pay for sanitation.

In 2011, PWA reported that the average financial recovery rate for a sample of 11 operators of various sizes was 75 %. Irregular and low, the collected revenues do not allow to ensure a sufficient resource to the operator. This poor collection of bills does not seem to be linked to a culture of non-payment of services or an inability to pay households, but rather to poor management of communities, insufficient follow-up, lack of adaptability of payment terms, lack of coercive measures in case of non-payment.

Thus, local operators find themselves unable to pay their debts to the Palestinian wholesale water supplier (the West Bank Water Department - WBWD), which then finds itself in a difficult situation (chronic debt). This indebtedness has repercussions on Palestinian national finances through the so-called net lending mechanism against which the Palestinian Authority is trying to fight.

On the other hand, irregular incomes for operators has a bad impact on the quality of service provided to the population. Since priority is given to the payment of wages, operators often find themselves unable to cope with the recurrent costs of basic maintenance and keep up of their infrastructure. The level of quality of the service is negatively impacted thereby compromising the credibility of the supplier to the users. (<https://washdata.org/data>, WHO/UNICEF JMP, 2015)

From table (2.2) the major difficulties mentioned for Al-Bireh WWTP/ near Ramallah are:

1. Lack of spare parts.
2. Break of control systems.
3. UV disinfection not used.
4. Sludge disposal and handling.

And for Al- Tireh/Ramallah:

1. Inlet BOD₅ (Biological Oxygen Demand).
2. Rainy weather.
3. Sudden variability of load.
4. Sometimes availability of spareparts, and for the Regulations.

As far as we can understand, there are two guidelines and references, used in the West Bank.

The first one was signed by the JWC (Joint Warfare Centre) - Palestinian and the Israelis sides - based on that guideline the Israeli side provides the Palestinian side the approved and the permit to construct the WWTP all over the West Bank even if the area is Zone A, or B or C. So, those guidelines are requested and used to

obtain the JWC approval. Although, and upon the donor interferes in the JWC approval process, some changes are allowed like the Total Nitrogen (TN) is accepted to be 50 mg/l for Nablus West and Hebron and Tubas for example. It seems to be called the JWC like 20/30/50 - BOD/TSS/TN. This is the first base of the regulation.

The second guideline (PS742), which was approved in 2015, by the Palestinian authority, indicates more restricted values for the chemical parameters (20/30/30 - BOD/TSS/TN).

So the constraints of the regulation in terms of treated water quality is defined, including the expectation in terms of sampling for the monitoring and follow up.

Table 2.2 Maximum limits of treated WW

Chemical Oxygen Demand (COD)	50	50	100	150
Dissolved Oxygen (DO)	>1	>1	>1	>1
Total Dissolved Solids (TDS)	1200	1500	1500	1500
pH	6-9	6-9	6-9	6-9
Fat, Oil, & Grease	5	5	5	5
Phenol	0.002	0.002	0.002	0.002
MBAS	15	15	15	25
NO ₃ -N	20	20	30	40
NH ₄ -N	5	5	10	15
TOTAL -N	30	30	45	60

Source (<https://washdata.org/data>, WHO/UNICEF JMP, 2015)

Table 2.3 Sample conditions

Wastewater treatment plants	Repetitive sampling		Evaluation period
	Type	Control authorities	
Mechanical treatment process	Routine tests: 2 samples /month physical &chemical properties : 2 samples /month Nematodes: 2 samples /month الایسیریتسیا کولای 2 samples /month FC : 8 samples /month (Individual)	Routine tests: 8 samples /month (composite sample) physical &chemical properties : 3 samples /day (Individual) Nematodes: 4 samples /month (composite sample) الایسیریتسیا کولای 8 samples /month (Individual) FC :8 samples /month (Individual)	3 months A
Natural treatment process	Routine tests: 1samples /month physical &chemical properties : 1 samples /month Nematodes: 1samples /month الایسیریتسیا کولای 1 samples /month FC : 1 samples /month	Routine tests: 4 samples /month (composite sample) physical &chemical properties : 3 samples /day (Individual) Etc	6 months B

(<https://washdata.org/data>, WHO/UNICEF JMP, 2015)

Chapter Three

3.1 Literature review

Literature review on this study, is abundant all over the world, five wastewater treatment plants in and around Rome were under investigation using a quantitative Taq-Man Real time PCR (Polymerase chain reaction) for detection of enteric viruses on samples of influent and effluent, waterborne enteric viruses were analyzed; adenoviruses, enteroviruses and noroviruses and compared to classical bacterial indicators of fecal contamination. The concentration of adenoviruses was the highest, in both raw and treated water.

A study in 2012 in Al- Bireh oxidation ditch, using molecular techniques (DGGE) denaturing gradient gel electrophoresis. 16s r RNIA cloning and sequencing showed several filamentous bacteria like Nocardia (Swaileh et.al., 2012).

Human enteric viruses, such as noroviruses (NoVs), adenoviruses (AdVs), and enteroviruses (EVs), are excreted at high concentrations in the feces of infected individuals (upto 10^{11} virus/gfeces), with or without symptoms, and are transmitted via the fecal-oral route including contaminated food and water. Recent investigations based on qPCR have revealed that some viruses as (NoRs) norovirus and (SaVs) saprovirus increase in winter, their epidemic period, whereas enteric viruses as AdVs and EVs are constant all over the year (Harramoto, 2016).

Infectious agents found in wastewater are bacteria, protozoa, viruses and helminths eggs. World health Organization (WHO) said that improving water

quality will reduce the global government burden for treatment of waterborne diseases by 4% (Castillo, 2015).

Public health and environment are nowadays threatened due to exposure of large numbers of viruses and bacteria excreted from human faecal matter, since current sewage treatment technologies in different wastewater plants have no efficient removal of these pathogenic microorganisms (Symonds, 2009).

Faecal matter contain large number of viruses and current bacterial indicators used for monitoring wastewater quality do not correlate with the presence of pathogenic viruses.

Adenoviruses and Enteroviruses are used as indicators for faecal pollution in the environment.

PCR (polymerase chain reaction technique) was used for detection of adenoviruses, entetroviruses, hepatitis B viruses, herpesviruses, morbilliviruses, noroviruses, papillomaviruses, picobirnaviruses, Enteronoroviruses.

Adenoviruses and picobirnaviruses are detected in 100% of raw sewage samples so they are potential markers of faecal contamination. Millions of viruses and bacteria are excreted in human fecal matter and current methods of sewage treatments do not always effectively remove these organisms. Majority of treated wastewater as well as untreated sewage drains into the marine environment, threaten environment and public through swimming and sea food consumption.

EPA (Environment Protection Agency) mandates the use of faecal coliform and enterococci to assess wastewater quality. Monitoring with these bacteria was simple and inexpensive, but faecal associated bacteria are not ideal indicators of

faecal pollution that is bacterial indicators have no correlation with the presence of viruses in wastewater.

Human pathogenic viruses associated with faeces are generally more robust than enteric bacteria and are not easily eliminated from wastewater by the current methods of wastewater treatment plants.

Adenoviruses are more resilient to tertiary treatment and U.V. disinfection than are bacterial indicators of faecal pollution (Symonds, 2009).

Giardia & *Cryptosporidium* was detected in surface water sources used by public after treatment (Sato et al 2013).

These two parasites are of most important concern in water supplies contamination since both are resistant to disinfection processes of chlorination usually used in Decentralized wastewater treatment plants (DWTP S) (WHO, 2017).

There are many spp. Of *Giardia* and *Cryptosporidium* and it was necessary for human identification of the genotypes that causes human disease.

Giardia intestinalis sub-genotype B111 was the causative agent of Giardiasis outbreak in Bergen Norway between 2004 and 2005 and the source of contamination was leakage of sewage from residential area another *Cryptosporidium hominis* outbreak was reported in Sweden. Sensitivity analysis highlights the urgency for addressing strategies for source water protection and set targets for water treatment (Robertson, 2005).

Water safety plan (WSP) measures should be taken to improve the epidemiological surveillance of waterborne diseases in Brazil to improve water quality at water catchment areas to improve health protection

Quantitative real Time PCR assay proved to be a useful and rapid tool in detection of WPV₁-SOAS strain (Wild poliovirus type₁, South Asian strain), in 2013 there was ongoing silent circulation of this virus in south Israel, the endemicity of this virus was Afghanistan and Pakistan. Bacteriophage MS₂ was used as an internal control, by this method silent circulating asymptomatic wild poliovirus was detected in south Israel. There was excellent specificity of this analysis since there was no cross reaction between the viral genomes and SOAS/MS₂ assay (Hindiye, 2013).

The spread of Norovirus genogroup into the environment, irrespective of the wastewater treatment process, coincides with its national clinical predominance over Norovirus genogroup1.

This study provides important evidence that municipal wastewater plants not only achieve pathogen removal but can also be the source of environmental pathogen contamination (Water Health, 2012).

Another study presents a comparative technical and financial analysis between two activated sludge systems (ASS) with different reclamation stages. One as plant followed by slow sand filters (SF) and another by two membrane technologies (MT), an ultrafiltration (UF) and a reverse osmosis (RO) stage. Results obtained an effluent quality of both systems revealed that MT produced

high quality water source suitable for unrestricted irrigation (Al- Sae'd et. al., 2008)

After reading literature review that evaluates the performance of AL- Bireh wastewater treatment plant (AWWTP), data were gathered for influent and effluent COD; (Chemical Oxygen Demand); TSS (total suspended solids); TN (total nitrogen); TP (total Phosphorus). Despite seasonal variation AWWTP effluent (reuse) requirement for restricted irrigation were met (Al -Sa'ed et.al., 2008)

Doctor Subhi Samhan said in his study Pathogenic Removal in UASB- septic Tanks in Al- Bireh Oxidation Ditch WWTP that the reuse of treated effluent represents a national interest and considered as an important component of the overall maximization of water resources in Palestine, the results of the study is that there was high removal efficiency by the oxidation ditch, in terms of parasitology presence *Entameba histolytica*, *Trichomonas* in the influent, that is the effluent should not be discharged to Wadis without treatment (Samhan, S., 2007).

A study in 2003, composite sewage samples were collected from three locations in Ramallah / Al- Bireh district and analyzed for several chemical and physical parameters, g COD per capita / day was very high, that is UASB (up flow Anaerobic sludge Blanket should be modified to overcome the sewage high solids contents and low temperature during winter time.

By law, the Palestinian water Authority (PWA) is responsible for all regulatory, planning, and monitoring, research, and training functions. Wastewater should be

collected, treated, and reused. Effluent reuse practices protect not only the limited water resources, but also enrich the quality and quantity of groundwater and surface water (Samhan et.al., 2011).

Fate of pathogenic microorganisms and indicators in secondary activated sludge wastewater treatment plants.

This study was undertaken to investigate the removal of pathogenic microorganisms and their indicators in a laboratory scale biological treatment system, that simulated the secondary treatment process of a wastewater treatment plant (WWTP) four groups of microorganisms including bacteria, viruses, protozoa and helminthes as well as the selected indicators were employed in the investigation the results demonstrated that approximately 2-3 log₁₀ removal of the microbial indicators was achieved in the treatment and that the log removal of clostridium-perfringes spores was low due to their irreversible adsorption to sludge flocs.

A study took place in northwestern Ireland (plant A-D) in four secondary wastewater treatment plants. Final effluent and biosolids was observed; of how efficient the plant is in removing the pathogens. The percolating biofilm system at plant D resulted in better effluent quality than in the extended aerated activated sludge systems (plants A and B); primary biosolids produced at plant D may pose higher health risk to the locals.

Bacterial indicators such as fecal coliforms and enterococci are commonly used for water quality. A paper received in May 1979 illustrated that bacterial indicators did not show accurate reflection for virological quality of water, since

bacteria are more sensitive to environmental changes and disinfectants than enteric viruses. Echovirus type 7 was the most predominant followed by poliovirus1, echovirus 6, and coxasachie-virus B2, B5 &B6, these viruses were yielded from 45 (29%) of a total of 155 different sources of samples, groundwater, swimming pool and surface water (Marzouk et al., 1979).

Enteric human pathogenic viruses are generally more resistant than enteric bacteria to current methods of wastewater treatment.

Specifically, enteroviruses (EVs) and adenoviruses (Advs) have been suggested as indicators for monitoring the human fecal contamination of water and for determining the efficacy of disinfection treatment.

A research article focused on the study about wastewater Reuse for irrigation in Morocco (A case study of Settat and Soualem Regions). This study aimed do evaluate the potential risk just that humans and animals are exposed, when wastewater (raw and treated) are reused for irrigation. The analysis of vegetable samples has revealed that 50% of crops from farmland were contaminated by helminth eggs, among there were Taenia species. Ascaris species Toxocara species and strong legs. The outcome of this study is that consumers have to apply good disinfected effluent for crops to reduce their contamination (Hajjami. et. al., 2013)

A study by the Palestinian Water Authority in 2003 focusses on the PWA and the challenges. That the water sector is witnessing the worse destruction due to the Israeli invasion .It mentioned the German support for the water sector. It also discussed the looting of Palestinian water by the Israelis who impose the water

deprivation policy as a terrorising tool .It also added that the Palestinian territories once had plenty of water and springs which have been seized and controlled by the Israelis who, in turn, sold it to the Palestinians.

Palestinian studies such as that which was conducted by Dr. Subhi Samhan that I had discussed earlier. Another thesis was presented by Wafaa Kareem under the supervision of Dr, Hafiz Shaheen at Al-Najah National University .This study made on Al Bireh Plant, the results of the analysis have proven that this treatment plant is in a good operational condition and in serving the purpose which it was intended to do. It was able, throughout the consecutive treatment stages, to get rid of water pollutants and produce treated water suitable for some purposes like agriculture and irrigation, cleaning of roads and public places .The final stage of the plant was operating properly, i.e. if Ultraviolet disinfection was working it will make it fit for direct human consumption (Kareem W., 2006).

Some of the by-product of the solid purification can also be used in fertilization without having any risks of causing any harm.

The treatment plant has conducted many tests on the treated water to ensure that it could be used safely and it concluded that it was safe especially in irrigation without having any pathological or harmful effects. The plant has achieved the two objectives that it had set which is the prevention of the environmental pollution and the reuse of the treated water which helped in the mitigation of the water crisis which plagues Palestinian Territories (Kareem W., 2006).

Bacterial pathogens are no more good indicators of faecal contamination since they have no correlation with the pathogenic resilient viruses that are currently

used as indicators for faecal contamination, since they are transmitted via oral faecal route. The outcomes of this study were detection of adenoviruses and picobirnaviruses in 100% of raw sewage and 25% and 33% of final effluent samples, enteroviruses, noroviruses were detected in 75% and 58% of raw sewage respectively and its presence 8% in final effluent sample and are proposed as potential markers of faecal contamination (Symonds et al., 2009). It is expected that viral load in the human population differs for each virus and that the abundance of these viruses fluctuated daily and seasonally in the raw sewage.

Detection of infectious and non-infectious human adenoviruses (HAdV) and Enteroviruses (EV) in raw and treated effluent from WWTPs and domestic sewage, 22 samples were analysed, polyethylene glycol PPT (PEG) method was used followed by integrated cell culture. PCR (ICC- PCR), viral genome amplification was confirmed by sequencing, HAdV-dB and HAdVD were detected in 5 samples, EV was detected in 45.5% of the samples.

From previous studies many conclusions confirmed that conventional wastewater treatment processes did not achieve complete removal of viruses, presence of infectious HAdV and EV in treated effluent emphasizes the need of wastewater treatment surveillance.

Faeces of an infected person can contain more than 10 virus particles for poliovirus and other enteroviruses although when released to the environment a number of infectious virus particles decline.

Enteric viruses are resistant and can survive for long period of time depending on pH and temperature.

Morocco had a moderate and subtropical climate, reclaimed water was an important water resource. Most studies on viruses in sewage clarify that HAdV and EV with DNA genome include 52 types with seven spp. (A-G) associated with a number of clinical syndroms:

- Gastroenteritis.
- Respiratory diseases.
- Haemorrhage cystitis.
- Conjunctivitis.

HAdV 40 & 41 have been documented as important causative agents of acute viral gastroenteritis in children.

EV(enterovirus) genus belongs to piconarviridae family includes poliovirus, coxachievirus A&B echovirus and other enteroviruses, most EV are asymptomatic and some of them can cause a wide variety of illness, aseptic meningitis, dermatomyositis, polymiositis, and dilated cardiomyopathy. Rotavirus and astrovirus are difficult to multiply by cell culture

(Amadiouni H. et.al. 2012).

Parasites were quantified by USEPA Method 16231/2012, genotyping was performed using specific primers based on the 18SrRNA gene for Cryptosporidium and gdh gene, Giardia was detected in 83.3% of the samples and cryptosporidium in 37.5%.

Giardia intestinalis A&B were present as well as Cryptosporidium hominis & cryptosporidium parvum. Extraction of genomic DNA from Giardia cysts and Cryptosporidium oocyst was performed using the commercial QIA amp DNA and we could mention the steps of the analysis of identification of parasites in the methodology.

Chapter Four

Methodology

4.1 Overview

Three important steps in the analysis should be taken in consideration, first the write sampling, collection of a composite sample not a grab sample and if there was an auto-sampler was more representative. Since the auto-sampler was not available, when the samples were collected they were taken in three different time intervals that was every three hours taking in consideration the peak value in the early morning and in the afternoon between three and four o'clock, collection of samples was in March 2019.

The second important step was the protocol used for concentration and preparation of the samples the sum of samples we are working on in the molecular lab are 18 wastewater samples, nine influent samples and nine effluent samples, preparing the samples for the next two steps extraction of the DNA and RNA and the final step Real- Time PCR analysis.

When reading this paper we notice a very similar methodology of what we have done, this paper talks about evaluation of four different systems for extraction of RNA from stool suspension using MS-2 Coliphage as an exogenous control for real time inhibition.

The automated extractor NucliSENS EasyMag was used for extraction of RNA, and three other systems were used QIAamp Viral Kit, King Fisher and MagNA

pure LC2 automatic extractor, MS2 rRT-PCR inhibition varied between the four used methods, inhibitors of RNA extraction are haemoglobin (Hb), immunoglobulins, bilirubin, triglycerides, complex polysaccharides, organic and phenolic compounds, glycogen, fats, metabolic products. The four previously mentioned methods were used for extraction of 93 stool samples spiked with MS2 coliphage.

MS2 was added as an external control because it was absent from human clinical samples. MS2 also measures the efficiency of RT-PCR reaction. The results of this paper confirm that RNA extracted with magnetic bead-based system contained fewer inhibitors than column-based system (Shulman, 2012).

Also, Routine chemical and biological parameters were done in PWA central Lab in Ramallah, the routine tests were BOD₅ (Biological Oxygen Demand) using Oxitop method and COD (Chemical Oxygen Demand) using Hach kits and the work done according to APHA (American public health association standard methods for examination of water and wastewater).

4.2 Sampling

Three different wastewater treatment technologies Al-Bireh activated sludge. 1- Al-Tireh (MBR) membrane bioreactor. 2- Rawabi (MBBR) moving bed bioreactor wastewater treatment plants were under investigation.

1. Influent (raw wastewater and treated effluent 0.5 liter bottle triplicate sample was taken every three hours from the three different wastewater plants the sum of samples 24 composite samples collected from each wastewater plant and the

sum of grab samples from the three wastewater plants was 72 influent and effluent samples, collected at three different time intervals.

2. Then the influent of every three different time interval 8.30, 11.30, 2.30 was collected in 2 liters sterilized bottle to be a representative composite sample triplicate composite influent sample and triplicate composite effluent samples (2-liters bottle) i.e. three composite influent and three composite effluent for every wastewater plant the sum 18 (2-liter bottles) for all wastewater plants.
3. Then transported by ice box at 4⁰C to Al-Quds Laboratory Al- Abraj.
4. Six additional samples composite influent and effluent (three composite sample for the influent of each wastewater plant was transported to PWA laboratory by ice box at 4⁰C for chemical analysis (BOD₅ and COD) and biological analysis & (detection of Fecal Coliform by Membrane Filtration Count Technique for calculation of the log Faecal removal.)
 - Physical parameters (temperature, PH, turbidity and EC.) for every sample was taken onsite in the wastewater plant on the day of sampling.
 - Chemical parameters was made in PWA central LAB for BOD₅ and COD.
 - Biological analysis was made also in PWA lab by serial dilution using membrane filtration Fecal count technique for the influent (I made 1/500 four serial dilutions for three samples and for the effluent (I made 1/100 four serial dilutions) to calculate at the end the Fecal Log removal. The results of these three parameters are ready with me from the first week of sampling.
 - For further biological analysis samples were send to real-time PCR polymerase chain reaction department in Caritas Baby Hospital for further

molecular analysis and detection of viruses in influent and effluent of the three wastewater plant.

4.3 Protocol for the analysis of viruses in wastewater

4.3.1 Materials and Equipments used in sewage processing

1. Sterile 0.5, 1, and 2 L screw cap bottles for storage of unprocessed sewage at 4°C and at -20 °C.
2. Wide-mouthed screw cap 2 L plastic bottles: 12 cm diameter × 22.5 cm tall, mouth >6 cm in diameter.
3. Magnetic stirrers and magnetic stirring bars
4. Vortex mixers.
5. Rotary shakers for containers ≤500 and ≤50 mL.
6. Test tubes: 1.5, 2.0, 15, 50, 250, and 500 mL tubes suitable for centrifugation and for freezing.
7. Centrifuge with head suitable for spinning 500 mL or 250 mL centrifuge bottles at 10,000 ×
8. Centrifuge suitable for spinning tubes ≤50 mL at 1400 × *g*.
9. Micro centrifuges for 1.5 and 2.0 mL micro centrifuge test at up to 14,000 × *g*.
10. Chemical fume hood or biological–chemical biosafety level 2 (BSL-2) laminar flow hood for initial stages of processing.
11. Biosafety level 2 (BSL-2) laminar flow hood.
12. 4 °C refrigerator(s) for temporary storage of large bottles containing volumes up to 2 L.

13. -20 °C freezers.
14. -70 °C freezers.
15. Polyethylene glycol 6000 (Sigma or Merck).
16. AR grade NaCl.
17. 5mg Diphenyle Thiocortoze in 500 ml chloroform(lot #907025)
18. Chlorine for disinfection, Chlorine tablets containing 1.7 g sodium.
19. PBS: Dulbecco's calcium- and magnesium-free phosphate-buffered saline (NaCl 8 g, KCl 0.2 g, Na HPO 1.15 g, KH PO 0.2 g, 1 L water; pH= 7.3–7.4).
20. PBS–Tween 80: PBS containing 0.1 % Tween 80.
21. PSF stock: *Penicillin* 50 mg/mL, *Streptomycin* 50,000 U/mL, and *Fungizone* 0.5 mg/mL.
22. Mycostatin stock [6250 U/mL].
23. PSMY stock: *Penicillin G* 50,000 U/mL, *Dihydro-Streptomycin* 50 mg/mL, and *Mycostatin* 6250 U/mL.
24. 3 % DIFCO (Becton, Dickinson and Company, NJ, USA) beef extract in water (pH 7.2). (Shulman L. M., 2015)
25. NucliSENS easyMag semiautomatic extractor (bioMérieux, Marcy l'Etoile, France) using the Specific B extraction protocol with EasyMag.
26. External RNA control: shielded RNA or MS2 coliphage as an external control for the presence of inhibitors of PCR or RT-PCR that might be co-extracted with the RNA.
27. AB Applied Biosystem 7500 Real Time PCR automated system used for Real Time PCR (ABI 7500 instrument (Life Technologies, Thermo Fisher

Scientific, Waltham, MA,USA) using 8-well microtiter strips for maximum flexibility

4.3.2. A Step one: Preparation and concentration of influent and effluent samples:

1. Composite samples were collected and transported to molecular Lab by ice box and the temperature in ice box was maintained during sample transport to the laboratory to be approximately 4⁰C.
2. 40grams of polyethylene glycol 6000 (Sigma or Merk) and 8.5grams of NaCl was weighed on electronic balance in special plastic cups.
3. 18 Sterile special plastic two liter bottles are marked with a marker from A to R and also the influent and effluent samples are marked from A to R.
4. Influent and effluent samples were vigorously agitated for homogenization of the samples.
5. 500ml (0.5liter) of the samples (influent & effluent samples) was poured into the marked bottles i.e.500ml of sample A poured in plastic bottle marked with A and B in B and so on.
6. A big magnetic stirrer and the weighed 40gr.of Polyethelene glycol (PEG) and 8.5gr. of NaCl was added to each bottle, the same procedure done for the 18 samples, then the 18 bottles are put on an electronic stirrer for 45 minutes, for homogenization of the whole contents in plastic bottle.
7. After 45 minutes of agitation on the electronic stirrer the 18 bottles are stored overnight for the next day to allow formation of a net like matrix every organic

particle sit on it, and keeping them to settle overnight, settlement of all the solids with viruses adherence to the organic particles, and the salt NaCl was added to make a pH suitable for the negative charged viruses to make a strong ionic strength with the buffer (polyethelene granules).

8. Quietly the upper suspension was thrown from the plastic bottle in the sink without moving the bottle because we need the pellet down, 300 ml was taken from the precipitate (ppt. pellet) and was put in special plastic wide-mouthed screw cap 2 L plastic bottle (500ml bottles suitable for centrifugation).
9. Every two bottles are put on balance and if need balancing distilled water was added, every six bottles are put in 5000 RPM (round per million) for one hour.
10. After centrifugation water was thrown from the plastic bottle and the pellet on the edges of the bottle was rinsed with P.B.S. (Phosphate buffer solution Tween PBS–Tween 80: PBS containing 0.1 % Tween 80, which act as a solvent soap that helps the pellet to be rinsed and get out from the bottle using a plastic pipette and 5ml of chloroform was also added to 50ml test tube with all the previous contents.
11. The tubes are closed firmly with the cap and put in balance in a vortex mixer for 20minutes. (Caution glass pipettes are used because chloroform may dissolve the plastic).
12. Every time we worked with six tubes because the centrifuge cannot tolerate more than six tubes .
13. The test tubes are then moved from the vortex to the 3000 rpm (round per million) with temperature 7⁰C.

14. The virus suspension was clarified by centrifugation to new marked conical test tubes.

15. (1400g 20min, 4⁰C) after vigorously shaking it in 3% beef extract (manufactured protein from cow), pH 7.2. Both supernatant was pooled and the antibiotics was added, Precipitate with 3% beef extract was vortexed for 20 minutes and then send to centrifuge for 20 minutes. (Preparation of beef extract 3g in 100 distilled water dissolve and adjust pH 7.5 by addition of (HCl), hydrochloric acid, and then autoclaving for 20 minutes).

16. Now in this step antibiotics are added to the conical test tube:

- 0.2 ml Neomycin 2.5% was added to each conical test tube.
- 0.2ml P.S.F. (Fungizone 0.5 mg/ml) to remove and kill the fungi.
- 0.3ml of PSMY stock (penicillin at 50mg/ml, streptomycin at 50,000u/ml, and mycostatin at 6.5u/ml) were added to the 50ml conical test tube all these antibiotics are added to kill the bacteria and fungi and keep the viruses alive.

The supernatant inoculated in a new marked conical test tube containing the supernatant and the beef extracted supernatant and the antibiotics added and then the test tubes are stored in -20⁰C for the next step which is the extraction of the DNA and RNA from the viruses.

4.3.2. B. Step two: Extraction of DNA and RNA nucleotides

Extraction of DNA and RNA was not made manually while otherwise it was extracted by automated extraction system called NucliSens Easy Mag

NucliSens lysis buffer was used in this system for the release of total nucleic acid from the influent and effluent samples which we have worked on in the previous preparation and concentration step (4.3.2), based on BOOM chemistry using magnetic extraction reagents and the NucliSens mini MAG.

The 18 samples of the influent and effluent (1ml) was added to NucliSens lysis buffer containing MS2 coliphage at a concentration of 10,000 PFU/mL. which contain guanidine thiocyanate. Any viral particle or cell in the sample will be disrupted releasing all the nucleic acids that may be present. RNases & DNases in the specimen will be inactivated.

Safety measures should be taken when handling with extraction procedure.

Extraction: RNA was extracted from waste water using semi-automatic extraction-NucliSENS easyMag semi-automatic extractor (bioMerieux, Marcy l'Etoile, France) using the easyMag extraction kit, 200 ml aqueous solution extracted and eluted into 55 ml. Extractions were performed according to manufacturers' instructions. The RNA was stored at 270 uC pending analysis and between analysis.

Figure (4.1): Viral Detection by Real Time PCR in Al-Caritas baby hospital in Bethlehem.



Figure (4.2) Applied Biosystem 7500 system used for real time PCR



Extraction steps as illustrated in Figure 4.5 in four steps:

1. The buffer lysis was added in the system and 1ml of the sample was also added, during incubation the targeted nucleic acids was captured by the magnetic silica particles.
2. The NucliSens EASY MAG magnetic device attracts all the magnetic silica, enabling the system to make purification of the nucleic acids through several washing steps.
3. In this step heat was needed for releasing the nucleic acids from the silica.
4. Finally, the magnetic silica particles are separated from the eluate by the magnetic device. (illustrated in Figure 17 in Appendix 3).

4.3.2. C. Step three qReal Time PCR Taq-Man

The real time PCR procedure was done in the automated Applied Biosystem 7500 Fast Real PCR system, a fully optimized and the results are of high quality in short time 35 minutes.

The system was used since 10 years and have powerful gene expression.

Applied Biosystem 7500 was used for detection and quantification of nucleic acids in standards 96-well formats. It contains thermal cycling system, powerful software, optimized reagents.

Reagents and disposables include Taq-Man Fast universal PCR master Mix, and disposable 96-well plates. (Applied Biosystems 7500 Fast, 7500 and, n.d.)

Real- Time PCR System for detection of one copy of template in 5ul reaction for a single reporter Taq-Man assay with 99.7% confidence.

Optimization of qRT-PCR (qualitative Real- Time Polymerase Chain reaction) specific for the viruses of interest.

1. Standard qRT-PCR conditions are followed for a reaction mix of 25 μL ; 5 μL of RNA is added to a 20 μL reaction mixture containing AgPath-ID.
2. One-Step RT PCR reagents (Life Technologies, NY, USA), primers and probe specific for the viruses of interest, Enterovirus, Adenovirus, Norovirus, Rotavirus and MS-2 primer and probes.
3. RT at 48 °C for 30 min.
4. Taq polymerase activation at 95 °C for 10 min.
5. Cycles of strand separation at 95 °C for 15 s, and elongation at 60 °C for 1 min.
6. Data collection is only for the 60 °C elongation step.
7. Interpretation of the assay results and the number of reactions for any given RNA was dependant on these initial results.
8. Evaluate different concentrations of the newly designed VP-1 non-degenerate primers (300, 600, and 900 nM) and probe (200 and 300 nM) to optimize the multiple qRT -PCR in a multiplex using 150 nM each of MS2 external control primer and 50 nM MS-2 probe.

The viruses in sewage we are looking for 1- Enterovirus (single stranded RNA), Adenovirus (DNA), Norovirus (RNA), Rotavirus (double stranded RNA).

Real- Time PCR are four molecular rooms:

- First room was a clean room for extraction of the (template) nucleic acids of the four viruses we are looking for using 7500 Real-Time PCR system.

- Clean room for addition of primers that are similar to the targeted virus we are looking for, at certain temperature (48⁰C) and the cycles continued for 30 minutes.

The primers added in a tube together with the enzyme polymerase and addition of magnesium, the forward primer goes and sit on the site similar to it, the polymerase enzyme find the primer and add more nucleotides for replication of DNA.

- For Enterovirus, Rotavirus and Norovirus the polymerase enzyme could not see the primers for that reason addition of reverse transcriptase that change the RNA to cDNA at temperature 30⁰C- 50⁰C the amplification step continues.
- The last step was the analysis of the stains of virus , for conventional PCR, gel electrophoresis was used, and for Real-Time PCR, a probe was added which act as a fluorescent label (no need for Gel) , Tag-Man probe, the system make a signal or fluorescent when catches the virus.
- For a Mastermix all the primers are put in the tube the primers, forward and reverse and the probe, in this case the system catches every virus according to a certain wavelength, and make fluorescence at this specific wavelength.

Semi-quantitative RealTime RT-PCR (rRT-PCR): The ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) was used for the amplification and detection of Enterovirus, norovirus rotavirus and adenovirus by Taq-Man technology as previously. Briefly, 5 ul of nucleic acid was added to the AgPath Mastermix (Ambion, Applied Biosystems Inc, Foster City, California), which contained the published concentrations of primers and probes and 5-

carboxy-X-rhodamine succinimidyl ester (ROX) as an internal reference dye. rRT-PCR was performed under the following conditions: 30 min at 48 °C, 10 min at 95 °C, and 50 cycles of 15 s at 95 °C and 1 min at 60 °C.

4.4 Biological procedures done in PWA central Lab

4.4.1- Introduction

Fecal Coliform Membrane Filtration Procedure

Fecal Coliform Membrane Filter procedure, the Agar media prepared was enriched with lactose medium since Fecal Coliforms are lactose fermenters and the suitable incubator for them to live was $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ and incubation period was 24 hours. Fecal Coliforms are a group of warm blooded animals present in the gut and feces of warm blooded animals, and are used as indicators of fecal pollution. Colonies produced by fecal coliform bacteria on M_FC (GREENBERG, Standard Methods for Water and Wastewater, 19th edition 1989).

Calculation of Coliform Density, the count used in membrane filters within 20 to 80 colonies and not more than 200 colonies.

Colonies are counted according to the following equation.

$$\text{Fecal coliform colonies/100ml} = \frac{\text{fecal coliform colonies counted} \times 100}{1 \text{ ml sample filtered}}$$

Quantitative information may provide an indication of the magnitude of fecal contamination in water. Colonies produced by fecal coliform bacteria on M- FC medium are blue colonies and the non-fecal colonies are grey to cream colored. The number of colonies as mentioned before in paragraph guidelines and

regulations in chapter one should not exceed (1000 CFU /100ml) according to the ministry of agriculture guidelines.

4.4. 2-Preparation of Difco-Fecal Coliform Agar in PWA central Lab

1. 52grams of Difco m Fecal Agar was suspended in 1 liter of purified water.
2. Mixing the suspension thoroughly, then heating with frequent agitation by putting a stirrer in the flask.
3. Boiling for one minute to completely dissolve the powder.
4. Addition of 10ml of a 1% solution of rosailic acid in 0.2N NaOH.
5. Continue heating for one minute. Do not autoclave.
6. Wait 5 minutes to cool then pour the contents of the flask in small sterile petri dishes.

4.4. 3- Membrane Filter procedure

1. Filtration units made of autoclavable plastic was used in PWA central Lab.
2. The filter set made of a plastic funnel fastened to a porous base.
3. Sterile membrane filter (cellulose nitrate filter pore size 0.45um was used).
4. The design should permit the membrane filter to be held securely on the porous plate of the receptacle without mechanical damage and allow the fluid (influent and effluent samples) to pass through the membrane during filtration.

5. Mark the sterile prepared petri dishes with the Difco- Fecal agar for the influent sample and the effluent samples respectively for the three wastewater plants.
6. Serial dilution 1/100 for the effluent samples was prepared.
7. Serial dilution 1/500 for the influent samples was prepared.
8. The sterile filtration set was prepared and pouring 100ml of the sample after installation of the sterile membrane filter with sterile forceps and fixing the sterile plastic funnel on the set.
9. Filter the samples using partial vacuum.
10. Upon completion of final rinse and filtration process the vacuum was disengaged, the funnel was unlocked and removed and immediately and carefully the membrane filter with sterile forceps was placed on the selected media with rolling motion to prevent and avoid entrapment of air.
11. The same procedure was repeated for all labeled and serial diluted samples.
12. The membrane filter set is sterilized with hot water between the different samples to prevent cross contamination.
13. Sterilization of the forceps using a beaker half full of ethanol or hot water could be used between the different samples also to prevent contamination.
14. Petri dishes with the selected culture media and the membrane filters are then incubated in an incubator at 44.5⁰C temperature for 24 hours, petri dishes should be put upside down that is the culture media up and the cover down to prevent water vapor to accumulate on the colonies and it will be then not easy to read the colonies.

15. Results and colony counting should be read next day.
16. Colonies produced by fecal coliform bacteria on M-FC agar are blue in color, non-fecal colonies are grey to cream color.

4.5 Chemical procedure made in PWA central Lab

4.5. 1- Biological Oxygen Demand (BOD₅)

This was done using **OXiTop** method (WTW)

BOD₅ measurement with OXiTop measuring system was based on pressure measurement (difference measurement). The measuring was made by pressure measurement via piezoresistive electronic pressure sensors.

BOD₅ determination with the OXiTop measuring system was possible in the undiluted sample.

Required instruments and accessories:

1. OXiTop measuring system.
2. Inductive stirring system.
3. Incubator thermostatic box (temperature 20⁰C).
4. Sample bottles brown (nominal volume 510ml).
5. Stirring rods.
6. Stirring rod remover.
7. Suitable overflow measuring beakers.
8. Rubber quivers.

Operation and measurement:

Table 4.1 illustrating sample volume and the measuring range and the factor multiplied with.

Sample volume (ml)	Measuring range	Factor
432	0-40	1
365	0-80	2
250	0-200	5
164	0-400	10
97	0-800	20
43.5	0-2000	50

Sample preparation and measurement:

1. Collected composite samples should be agitated vigorously for homogenization of the sample.
2. The selected sample was taken according to the table for example for the effluent we take 432ml of the sample the measuring range should be between 0-40 and the result after 5 days was multiplied by the factor 1, for the influent we take 97ml , the measuring range of the result was between 0-800 and the result after 5 days was multiplied by factor 20.
3. The required homogenized volume was put in a special brown bottle.with a stirrer for continuous homogenization of the sample.
4. A rubber quiver was inserted in the neck of the bottle.

5. 2 tablets of sodium hydroxide was put into the rubber quiver with a tweezers (caution the tablet must never come into the sample).
6. The OXitop was screwed tightly on top of the sample bottle and starting measurement by pressing S and M simultaneously until the display shows zero.
7. The bottles with samples are put in incubator for 5days at 20⁰C.
8. During the 5 days the sample was continuously stirred.
9. The OXITop automatically store one value every 24 hours for 5days.
10. After 5 days to have the current value press the M key.

Calculation

Convert the displayed current value after 5 days into the BOD₅with the following equation

Digits of the measured value* Factor = BOD₅

Chemical Oxygen Demand was also done for the influent and effluent in PWA lab using HACH principle

Log removal= $\frac{-\log \text{conc. effluent}}{\text{Conc. of influent}}$

Chapter Five

Results and Discussion

5.1 Results

Table 5.1 The Cycle Threshold of the four viruses in influent and effluent samples

Sample	Enterovirus	Adeno virus	Norovirus	Rota virus
A Effluent of Al- <u>Bireh</u>	Negative	39.1	Negative	31.7
B Effluent of Al- <u>Bireh</u>	Negative	Negative	Negative	Negative
C Effluent of Al- <u>Bireh</u>	Negative	Negative	Negative	Negative
D Effluent of Al- <u>Tireh</u>	Negative	Negative	Negative	Negative
E Effluent of Al- <u>Tireh</u>	Negative	Negative	Negative	Negative
F Effluent of Al- <u>Tireh</u>	Negative	Negative	Negative	Negative
G Effluent of <u>Rawabi</u>	Negative	36.0	Negative	29.9
H Effluent of <u>Rawabi</u>	Negative	38.6	Negative	Negative
I Effluent of <u>Rawabi</u>	Negative	35.4	33.6	21.4
J Influent of Al- <u>Tireh</u>	Negative	31.0	Negative	27.6
K Influent of Al- <u>Tireh</u>	Negative	30.0	32.5	27.9
L Influent of Al- <u>Tireh</u>	Negative	29.7	35.0	25.4
M Influent of Al- <u>Bireh</u>	41.6	26.1	30.9	26.2
N Influent of Al- <u>Bireh</u>	46.8	25.9	28.6	28.3
O Influent of Al- <u>Bireh</u>	38.9	26.2	34.2	28.1
P Influent of <u>Rawabi</u>	Negative	35.7	34.4	32.8
Q Influent of <u>Rawabi</u>	Negative	35.2	Negative	35.5
R Influent of <u>Rawabi</u>	Negative	33.6	Negative	17.8

Real- Time PCR using automated 7500 Real Time PCR system

Qualitative real time polymerase chain reaction (qPCR) technique using Taq-man Chemistry was performed and analyzed at Caritas baby hospital. The qPCR was performed on extracted samples to check for enteric human viruses present in the raw and treated wastewater samples. The viruses were Enteroviruses,

Adenoviruses, Noroviruses, and Rotaviruses. From each treatment unit two samples were collected (influent and effluent) to make comparison on the presence of the enteric viruses to identify the removal efficiency of viruses in the treated wastewater in the three different wastewater plants. This will allow having an answer to the hypothesis question if there was a correlation between the removal efficiency of viruses and the removal of fecal coliform indicator.

Degenerate primers reverse and forward primers and probe for each of the targeted viruses were added to the master mix tube and later 5ul nucleic acid was added of the extracted nucleic acid from the previous step. The detection of the amplified amplicon was performed on the Applied Biosystem 7500. Specific primers and probes are added for the different types of viruses. For adenovirus qPCR was performed with Taq-man Polymerase, while for enterovirus, rotavirus, and norovirus reverse transcriptase was used first to generate the cDNA (Complementary DNA) at temperatures 50⁰C for 30 minutes prior to the amplification step. The virus specific probes had fluorescent label at the 5'-end. The generated fluorescent signal were detected by CCD camera in the 7500 machine.

Table 5.1 identifies the cycle threshold of the four viruses in the influent and treated effluent by Real-Time PCR using automated 7500 Real-Time PCR system, the composite collected samples are labelled from A to R every WWTP technology have three effluents and three influent samples, the three samples are similar in components and the same conditions of sampling.

Illustrating the results in table 5.1 it was clear that the automated system (AB 7500 thermal system) detected the enterovirus in the influent of Al- Bireh in three different cycle threshold (Ct) number 41.6, 46.8, 38.9 respectively as shown in as shown in figure (5.1). The Ct value is the cycle that the fluorescent level starts to go above the background levels. For the effluent in Al-Bireh sample labelled A, the system detected the adenovirus at Ct 39.1 and the rotavirus at Ct 31.7.

The results of Al-Tireh influent as shown in the table was negative for enterovirus in the three samples (J-K-L), for adenovirus there was detection of the virus in the three samples, norovirus was detected in two samples and rotavirus was positive in the three samples and that was also clear in the graph in Fig.(5.3). And for the effluent of Al-Tireh labelled (D-E-F) the table showed 100% removal efficiency of viruses since the results are negative.

For the influent of Rawabi it was clear from the table that influents labelled (P-Q- results were negative in for enterovirus detection, and for adenovirus the PCR system detected the adenovirus in three samples at three different Ct (35.7-35.2-33.6), and for norovirus it was positive in one sample from the three samples, and for rotavirus it was detected and positive in the three samples. For the effluent samples (G-H-I) of Rawabi from the (table 5.1) it was clear that there was no detection of enterovirus in the three samples, and for adenovirus there was positive detection in the three samples, and for norovirus one positive and two negative, and for rotavirus two effluent samples were positive and one negative.

Talking about the removal efficiency, in Al-Tireh WWTP there was 100% removal, for Al-Bireh WWTP there was 33% removal efficiency for adenovirus

and 33% removal of rotavirus. In Rawabi effluent (G-H-I), as illustrated in table 5.1 there was no removal of adenovirus and the removal efficiency of norovirus was 33%, and the removal efficiency of rotavirus in effluent of Rawabi was 66%, and this was also illustrated in Figure (5.4). It was clear that the PCR system detected the adenovirus in influent of Rawabi marked in black in graph and also positive detection of adenovirus in effluent marked in red color in the figure.

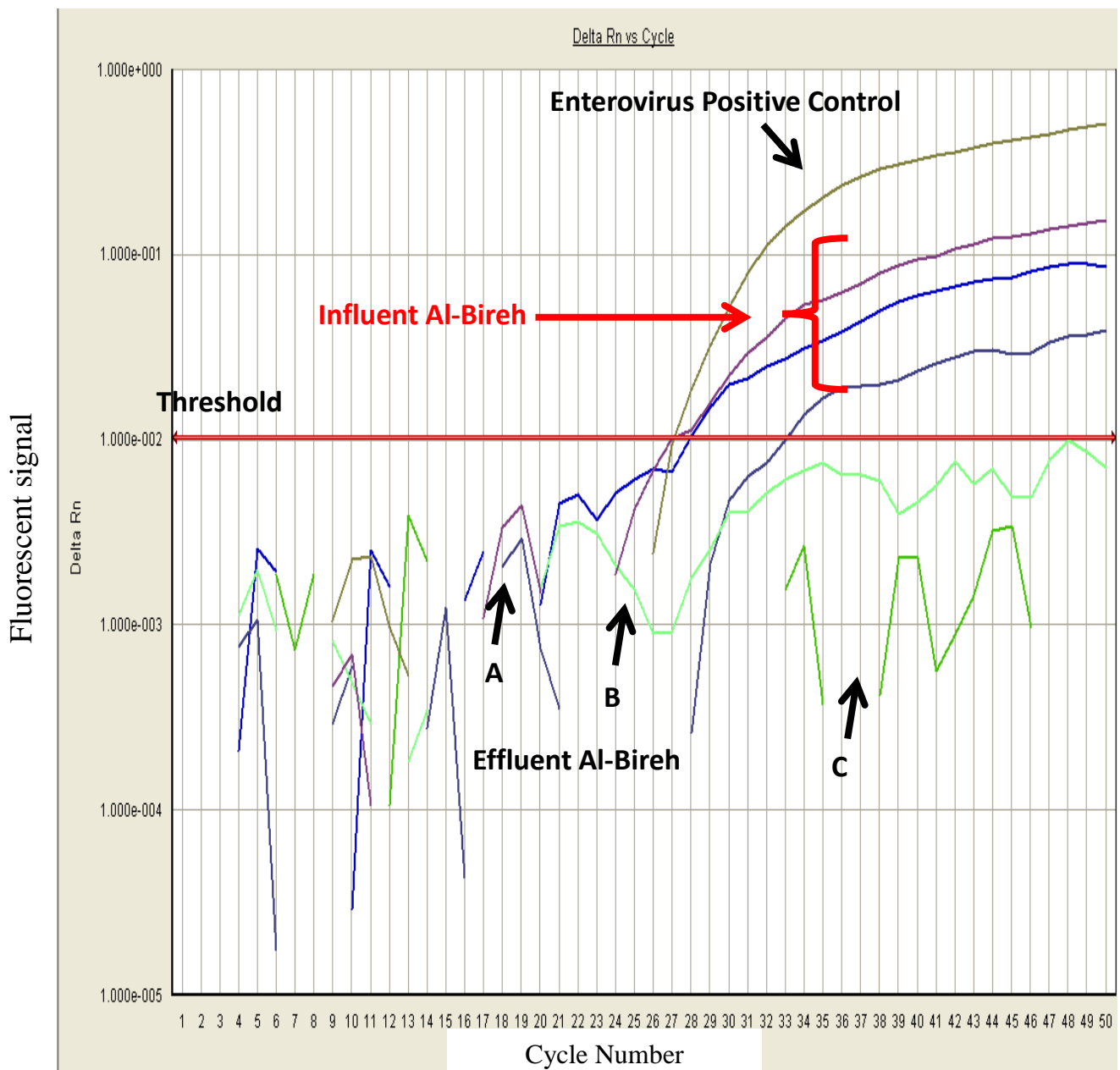


Figure (5.1) : RED: Al-Bireh Influent Enterovirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Al-Bireh effluent Enterovirus Real Time PCR negative Real Time PCR amplification curve

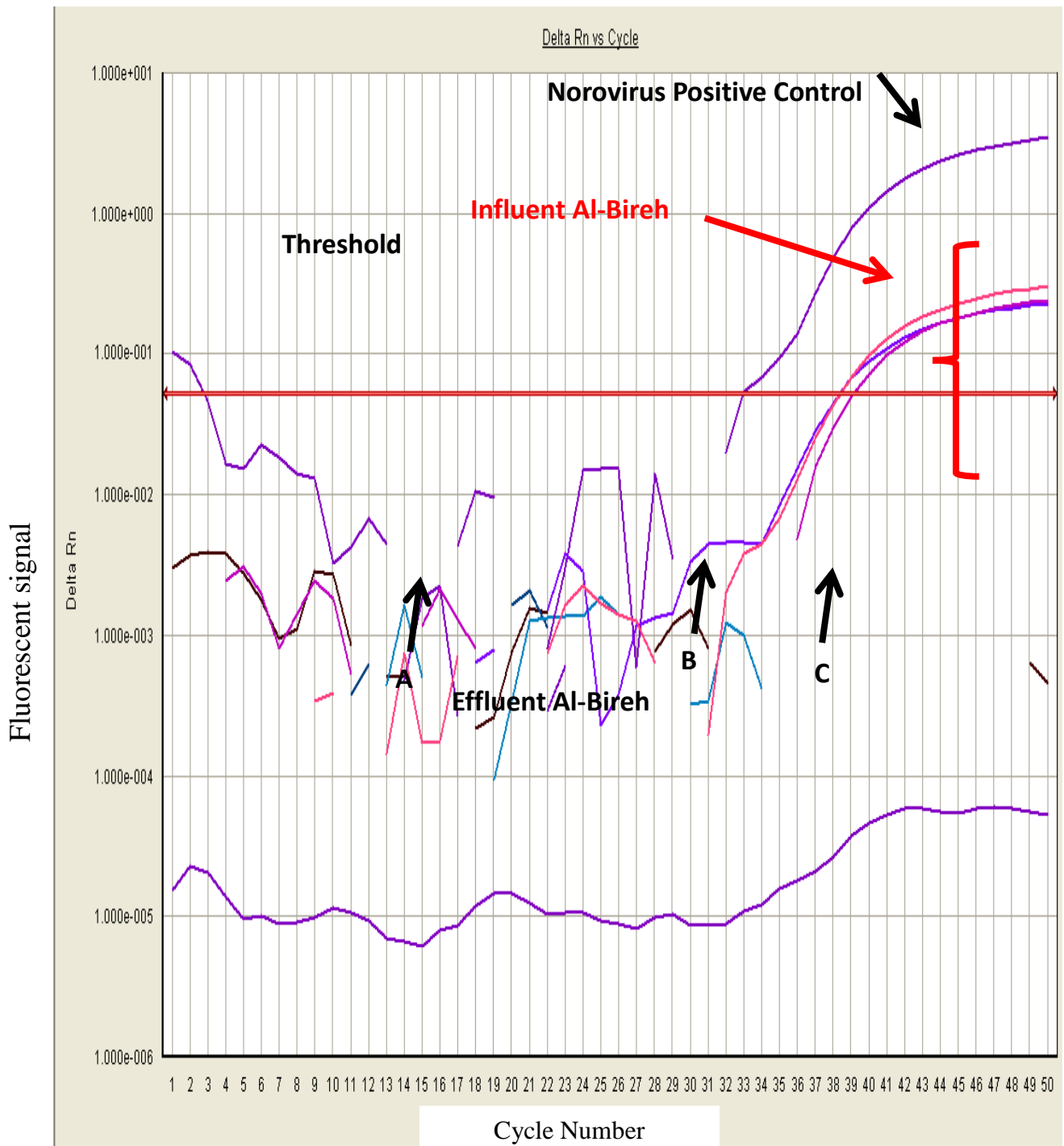


Figure (5.2) : RED: Al-Bireh Influent Norovirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Al-Bireh effluent Norovirus Real Time PCR negative Real Time PCR amplification curve

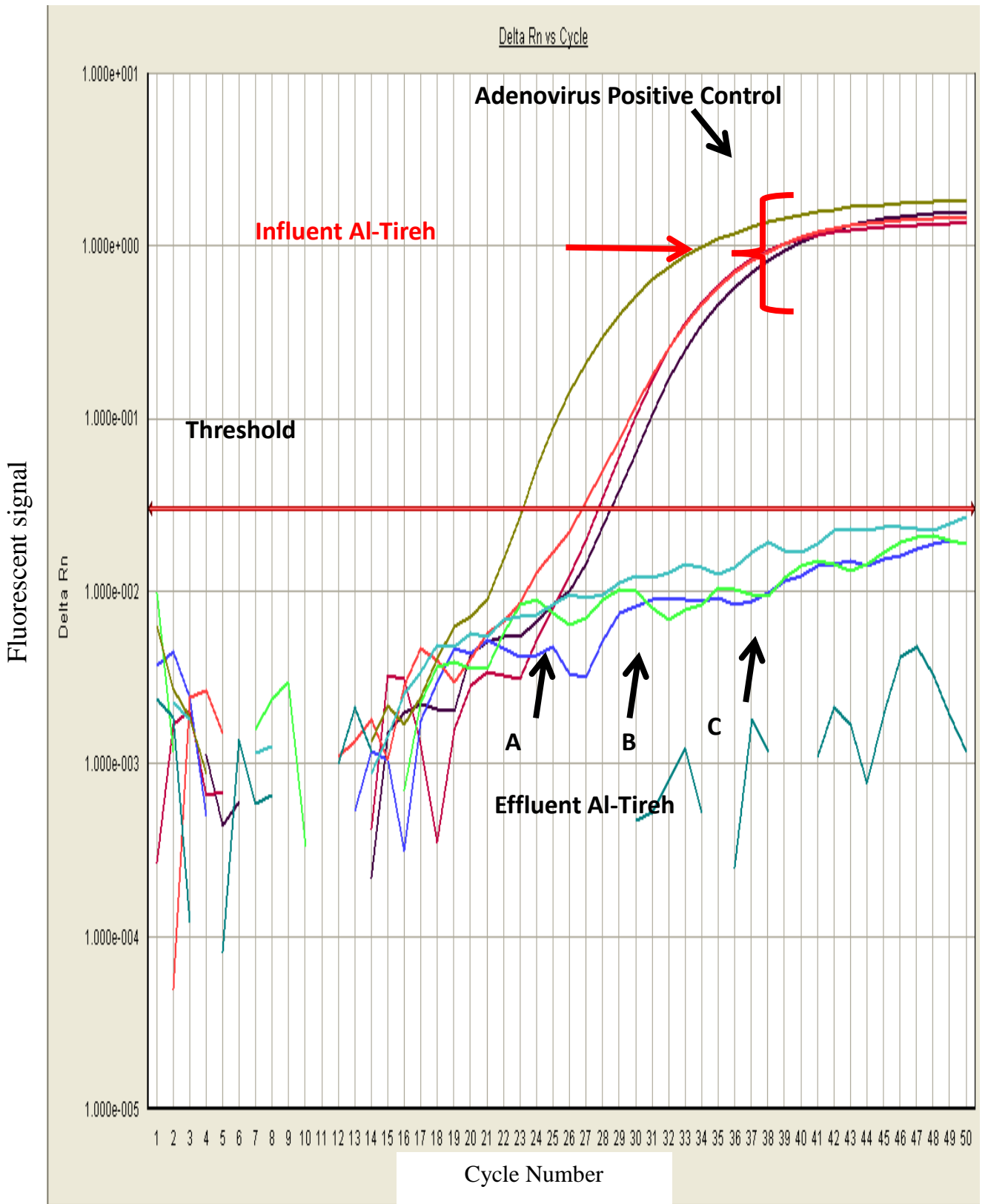


Figure (5.3): RED: Al-Tireh Influent Adenovirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Al-Tireh effluent Adenovirus Real Time PCR negative Real Time PCR amplification curve

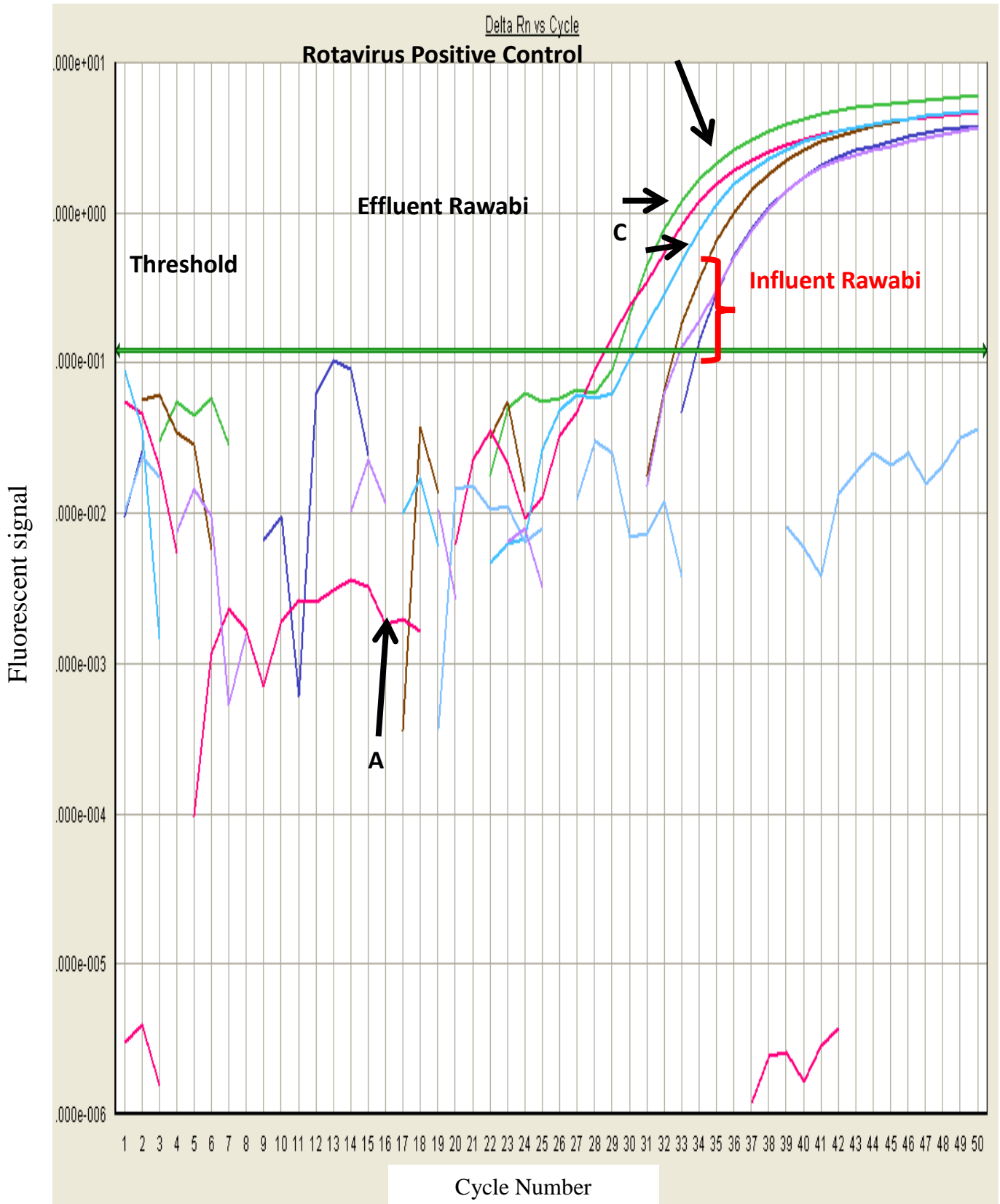


Figure (5.4): Rawabi Influent Rotavirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Rawabi effluent Rotavirus Real Time PCR positive Real Time PCR amplification curve

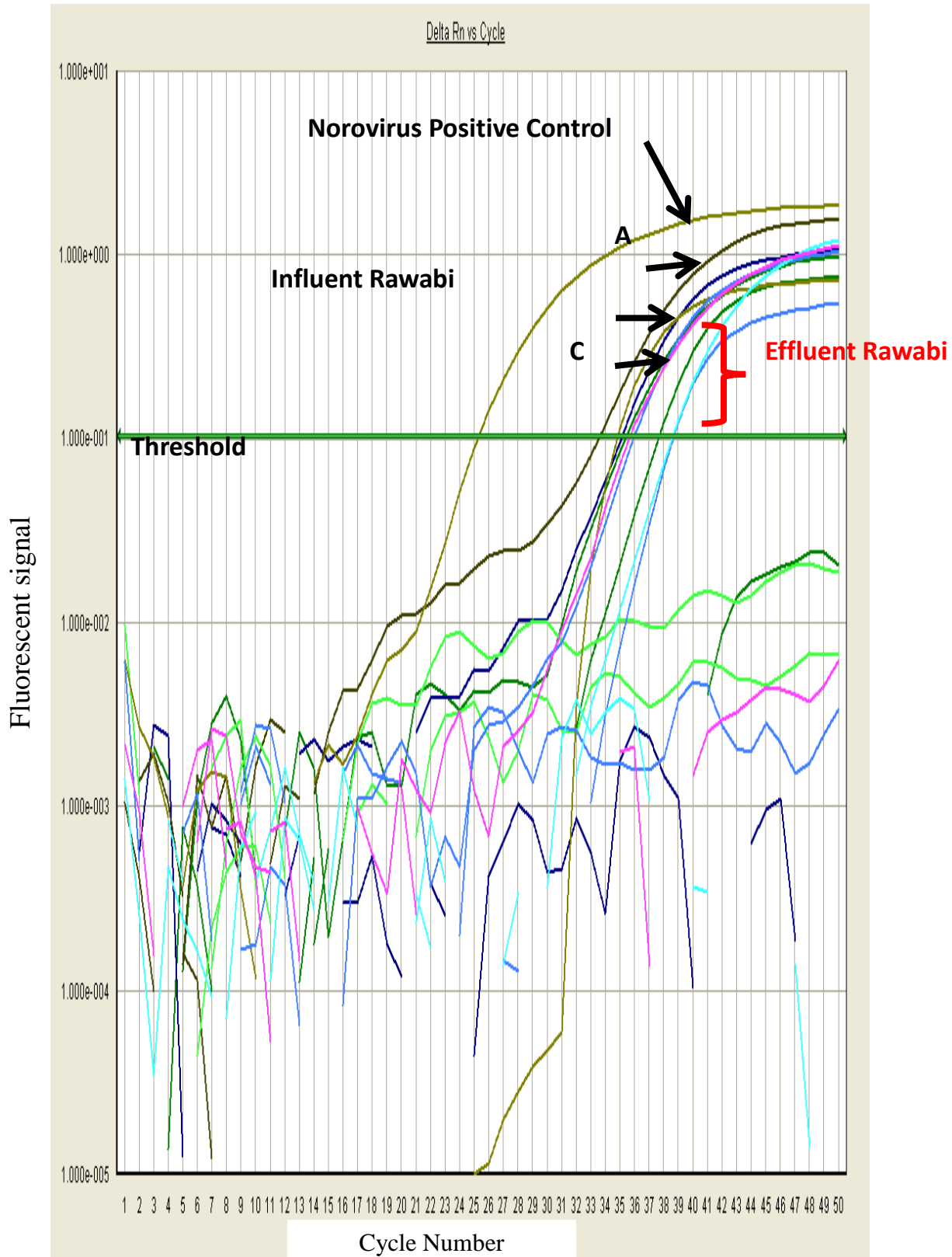


Figure (5.5): RED: Rawabi Influent Norovirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Rawabi effluent Norovirus Real Time PCR positive Real Time PCR amplification curve

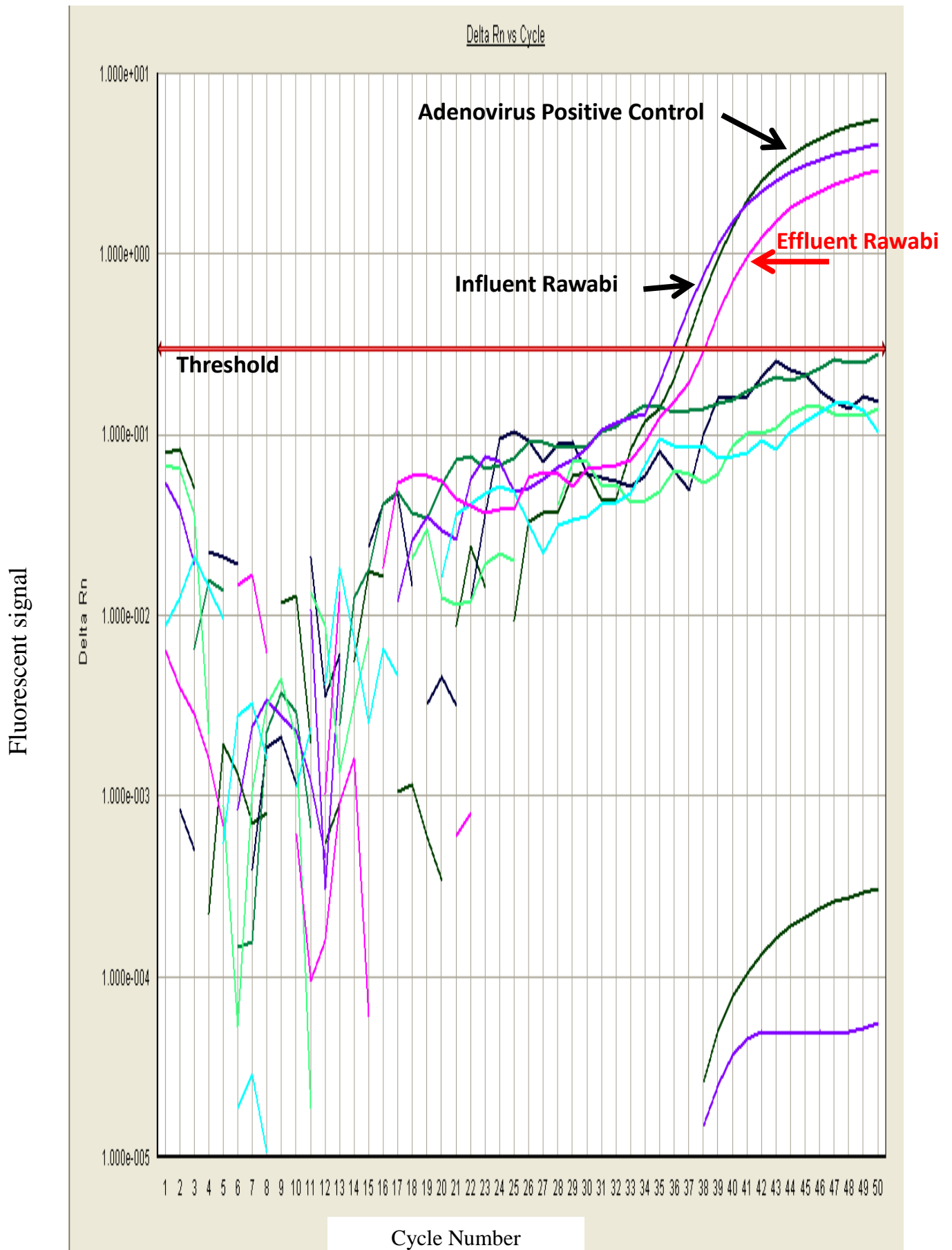


Figure (5.6): RED: Rawabi effluent adenovirus Real Time PCR positive amplification curve of Waste Water Treatment Plant. Black Rawabi influent adenovirus Real Time PCR positive Real Time PCR amplification curve

Human Viral Infections in 2019

- Caritas Baby Hospital

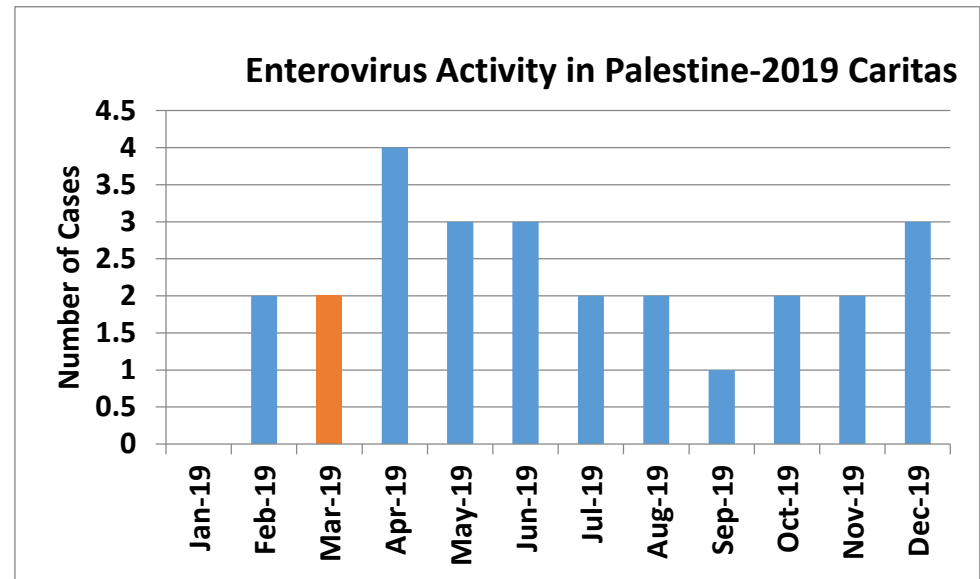
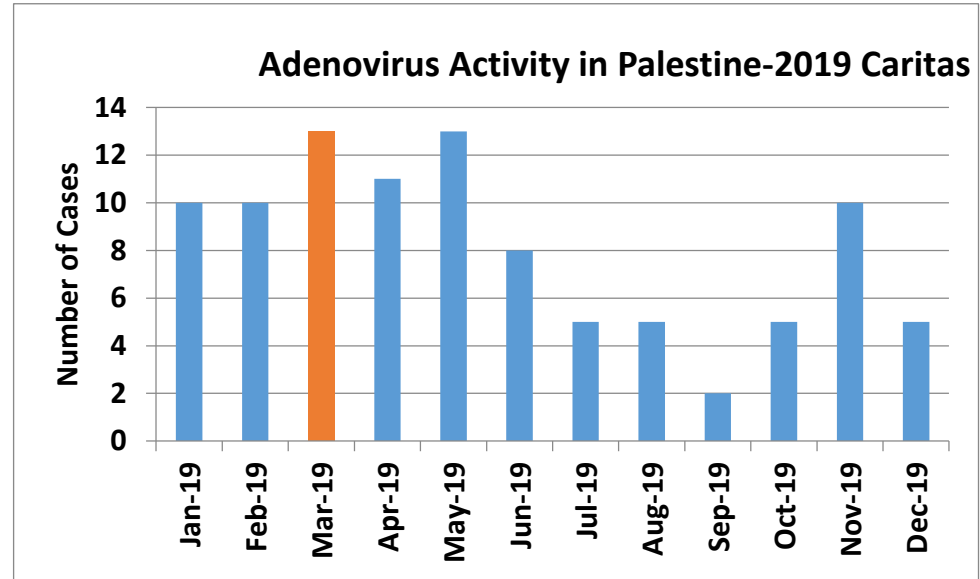
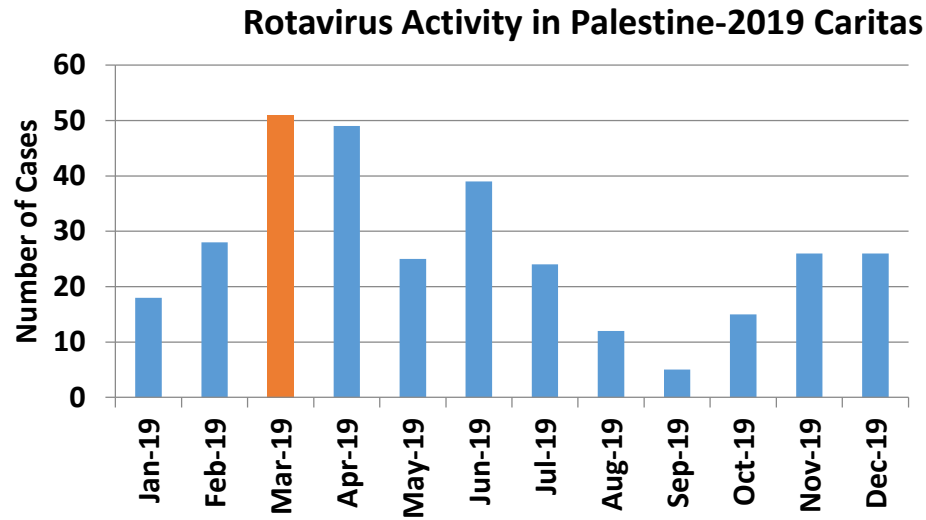


Figure (5.7): Data assessment from Caritas baby hospital on the same period of sampling March 2019 illustrating the number of cases of human viral infection rotavirus 50 cases, adenovirus 13 cases, and enterovirus 2 cases.

Waste Water Assessment Parameters

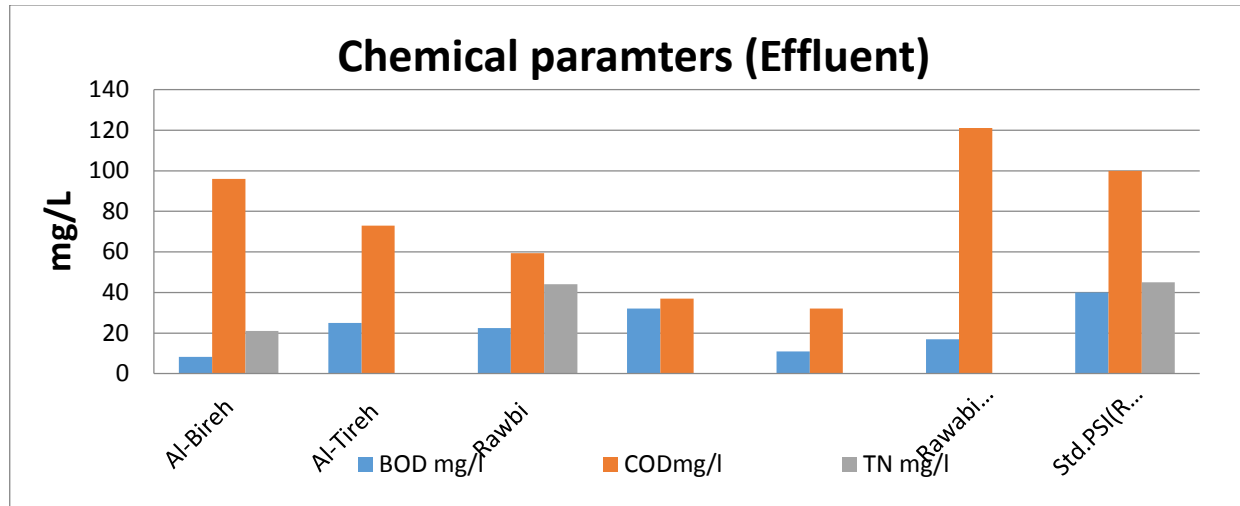


Figure (5.8): Wastewater chemical parameters BOD, COD, TN

- **Briefly**
 - **6 Samples were tested**
 - **Chemical standards**
 - Biological Oxygen Demand
 - Chemical Oxygen Demand
 - Total Nitrogen (TN)
 - **Microbiological standards**
 - Log Efficiency = $-\log \text{effluent/influent}$

Waste Water Assessment Parameters

- Removal Efficiency**

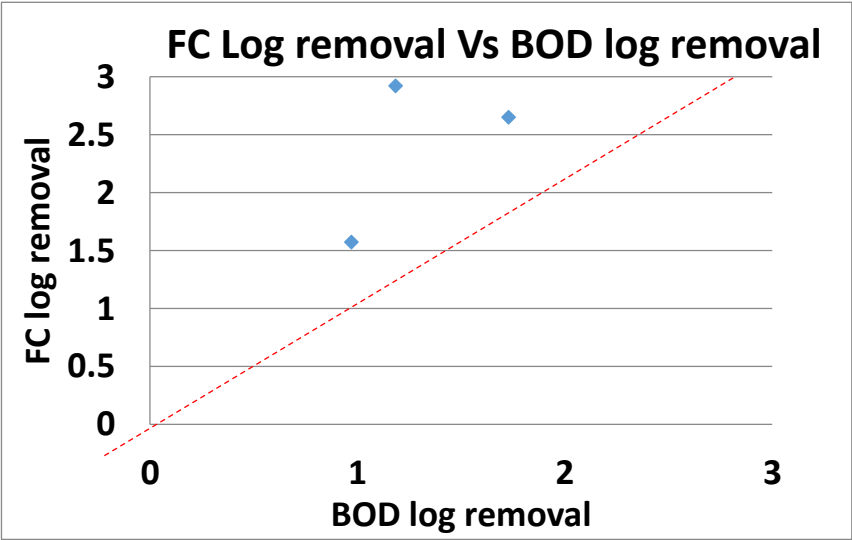
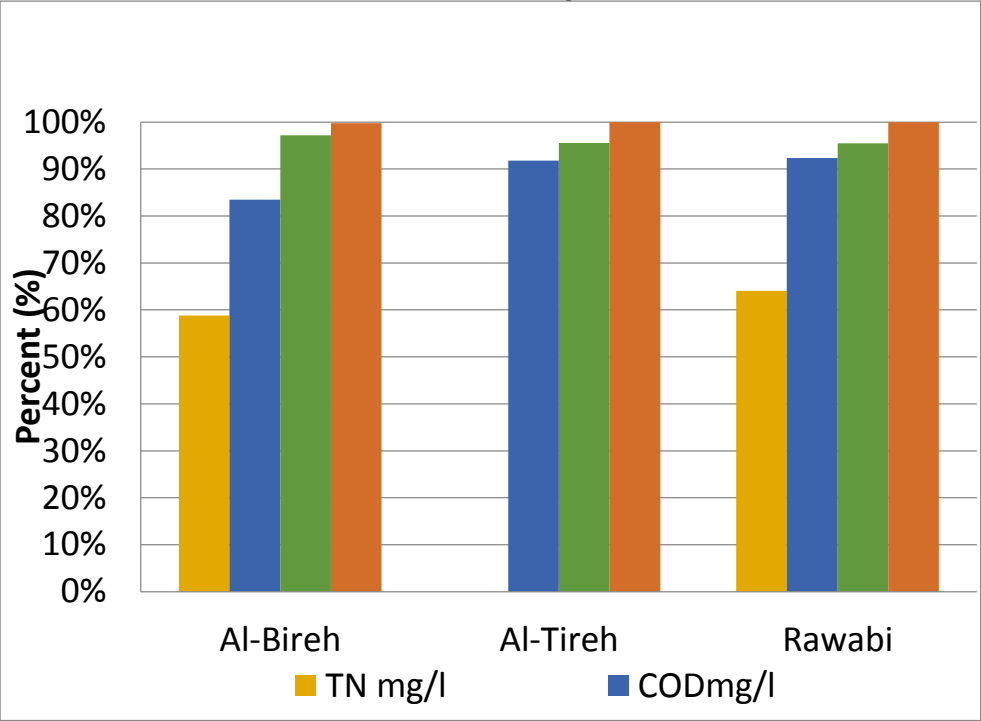


Figure (5.9) of removal efficiency of the three WWTP's of TN, COD ad BOD, and the fecal log removal

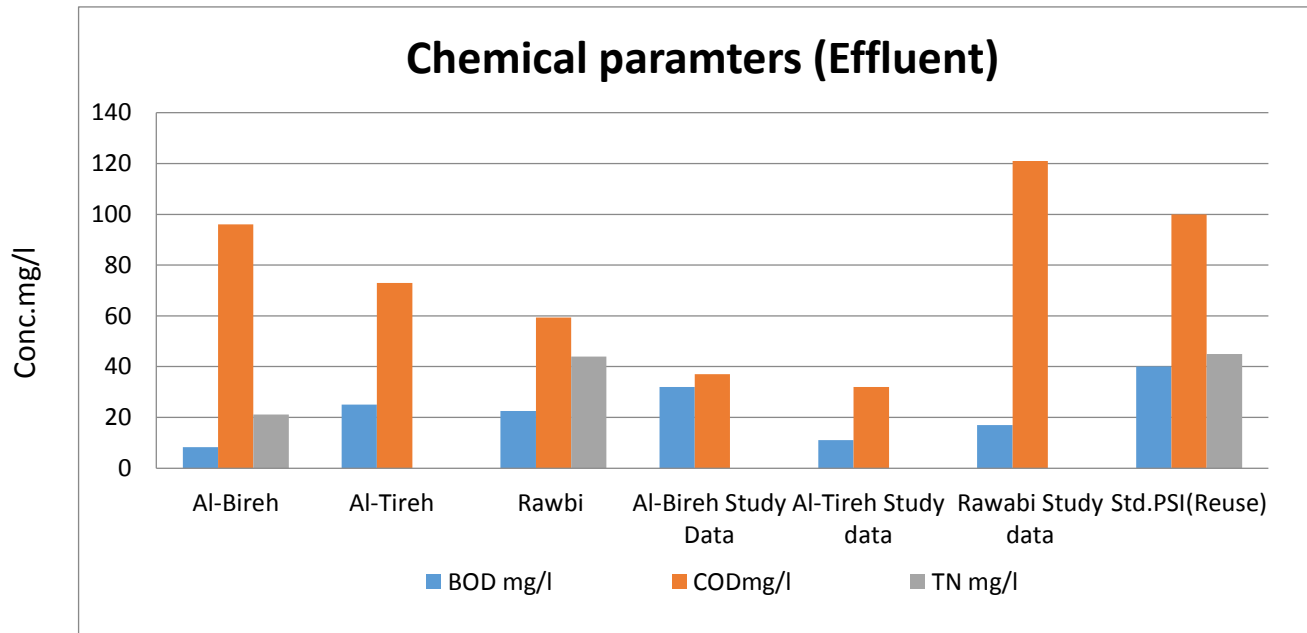
Figure 5.10 Removal efficiency of three WWTP's of TN, COD, BOD, FC

parameters	Bireh		Tireh		Rawabi		data of my own(Al- Bireh)		data of my own (Al- Tireh)		data of my own(Rawabi)		Removal efficiency (Log removal)		% efficiency					
	influent	effluent	influent	effluent	influent	effluent	influent	effluent	influent	effluent	influent	effluent	Std.PSI(Reuse)	Bireh	Tireh	Rawabi	Bireh	Tireh	Rawabi	
Physical													Meduim /quality							
Inlet flow [m3]	6882		1650		NA	NA												100%	100%	
EC. [ms/cm]	1.71	0.98	NA	NA	NA	NA												43%		
PH		7.58	8.5	8.47	NA	nA							6 to 9						0%	
TSS[mg/l]	6.88	0.1		18	132	3							50mg/l					99%	98%	
Temp.		17.3	25.3	18.5									35						27%	
TDS			361us/cm		496mg/l	511mg/l							1500mg/l							
Turbidity(NTU)				12.52NTU									5 to 10							
Chemical	Bireh in	Bireh out	Tireh in	Tireh out	Rawabi in	Rawabi out														
BOD mg/l	288	8.2	556	25	493	22.5	300	32	590	11	260	17	40 mg/l	BOD Log removal	0.97	1.73	1.18	97%	96%	95%
CODmg/l	582	96	890	73	776	59.3	620	37	915	32	600	121	100mg/l		1.22	1.46	0.70	84%	92%	92%
TN mg/l	51.2	21.1			122.3	44							45mg/l					59%	64%	
Biological																				
FecalColiform(FC)		NA		NA		Nilcfu/100ml	2250000	60000	1750000	3900	250000	300	1000	FC Log removal	1.57	2.65	2.92	97%	100%	100%
population	50,000		25,000		5000															
capacity/design	5750 m ³		2500m ³		1000m ³									BOD Log removal	0.971971276	1.729459	1.184524427			
														FC Log removal	1.574031268	2.651973	2.920818754			

Table 5.2 Removal efficiency of three WWTP's of TN, COD, BOD, FC

Chemical	Al-Bireh	Al-Tireh	Rawbi	Al-Bireh Study Data	Al-Tireh Study data	Rawabi Study data	Std.PSI(Reuse)	% removal efficiency		
								Al-Bireh	Al-Tireh	Rawabi
BOD mg/l	8.2	25	22.5	32	11	17	40	97%	96%	95%
CODmg/l	96	73	59.3	37	32	121	100	84%	92%	92%
TN mg/l	21.1		44				45	59%		64%
Fecal Coliform(FC)								Log1.75	Log2.65	log2.92

Figure 5.11 Removal efficiency of three WWTP's of TN, COD, BOD, FC



5.2 Discussion

- From the results previously mentioned it was obvious that there was detection of Enterovirus in the influent of Al- Bireh , and it was also clear that activated sludge of Al-Bireh succeeded in 100% removal of Enterovirus and Norovirus respectively from the effluent and 33% removal efficiency of Rotavirus and 33% removal of Adenovirus in the effluent of Al- Bireh, this proved that activated sludge technology was efficient in removal of viruses from the effluent, but needs additional tertiary treatment. And previous studies emphasizes that human pathogenic viruses associated with feces are generally more robust than enteric bacteria and are not easily eliminated from wastewater by the current methods of wastewater treatment plants, and that the adenoviruses are more resilient to tertiary treatment and UV disinfection than are bacterial indicators of fecal pollution (Symonds, 2009).
- However, for the results of the influent of Al-Tireh the cycle threshold when it was 29 and 30 that means there was high numbers of adenovirus and when the Rotavirus in the influent was 25.4 that means there are high numbers of viruses and the PCR system quickly detected them and when the number of cycle threshold was high big number of cycle threshold that means the number of viruses are low and the Real- Time PCR system took longer time for detecting them.
- The results especially in Al- Tireh were reliable and satisfied our expectations, and answered my hypothesis questions mentioned before, from previous

studies and our results MBR technology proved to be efficient removal of viruses more than the conventional activated sludge, since the submerged membrane with nominal pores 0.04 μm blocked the passage of small viruses (0.01-0.3 μm) to pass through and the answer to the second question was that there was no correlation between the Fecal Coliform indicator and log removal of viruses, since fecal coliform are good indicators of bacterial removal, protozoans, helminthes eggs and COD and BOD removal, and that was clear from the assessment made for these parameters, for example efficient removal of fecal coliform and COD and BOD in Rawabi and Al- Bireh does not necessarily mean efficient removal of viruses, in these two technologies there was efficient removal of Fecal Coliform ,BOD, COD, TN, but not efficient removal of viruses, that means there should be another indicator for viral removal and this indicator could be Bacteriophages like MS2, but in our procedure we used RNsP as an external control. This was emphasized from my literature review and previous studies, bacteriophages are viruses that infect bacteria, Coliphages are better indicators for human enteric viruses than bacterial indicators (Asano et.al.2007). Bacteria are more sensitive to environmental changes and disinfectants than enteric viruses (Marzouk et.al.1979).

- High Dilution in wastewater may hamper detection of the viruses, but the PEG polyethylene granule method with addition of NaCl in the preparation and concentration was a very long procedure but was the right choice to make us successful in detection of viruses, since addition of these materials with

continuous stirring and homogenization of the sample for one hour allowed the formation of net like matrix that every organic particle to sit on with the addition of salt NaCl that binds with the negatively charged viruses increase the ionic strength, all the particles together with the viruses adhering to it settled down after putting the plastic bottles overnight, in 4⁰C, this method allow not a single virus to be lost and also when we washed the pellet with Beef extract in order not to lose any single virus at the end three nice layers are formed the down red layer the chloroform, the middle layer the bacteria and debris and fungi and the upper layer consist of the viruses.

And this was emphasized from previous studies, log removal for indicators and viruses were higher from MBR than conventional secondary treatment. (Francy D.S., 2012).

- The antibiotics are added to kill all the bacteria and fungi , because we need only the viruses.
- RNsP (non structural protein) was used as an indicator of the presence of viruses, all the samples were spiked with it, to confirm that the extraction step was done accurately.

Chapter Six

Conclusion and Recommendations

6.1 Conclusion

The aim of the my thesis was evaluation of Removal efficiency of pathogenic microorganisms of three different Palestinian WWTP's by using qualitative real-time PCR (Polymerase chain reaction). Sampling was in March 2019, (72 grab samples, 0.5 liter) were collected from the three different WWTP's Al-Bireh activated sludge, Al-Tireh membrane bioreactor, and Rawabi Moving Bed Bioreactor, influent and effluent samples, the sum of samples was 24 composite samples.18 influent and effluent samples were send to molecular lab and qualitative real-time PCR was the procedure chosen to look for enteric viruses in effluent and to see if the three WWTP's was efficient in removal of these pathogenic enteric viruses and compare the three different technology in removal efficiency of pathogenic viruses, and to answer the hypothesis question was there any correlation between the fecal coliform indicator used for detection of pathogenic bacteria and removal of viruses. From the results we see that there was no correlation between the fecal coliform indicator and removal of viruses, that was emphasized from results when fecal coliform was zero in Rawabi the PCR system made a fluorescent signal at certain cycle threshold. The best technology that proved 100% removal efficiency of all four enteric viruses adenovirus, entererovirus, norovirus and rotavirus due to its submerged membrane with nominal pore size 0.04 micron that acted as tertiary treatment and blocked the

passage of small 0.01-0.3 μm viruses through the membrane, while the other two treatments Rawabi and Al-Bireh there was detection of enteric viruses and it was mentioned in recommendations that there should be additional tertiary treatment to be efficient enough in removing these pathogenic viruses. From the results of the assessment for physical and chemical parameters and fecal coliform there was physical and chemical and fecal coliform removal but no 100% removal of enteric viruses. And these viruses are pathogenic human viruses and are hazardous on public health. These viruses are frequently winter viruses and this was also emphasized from data assessment from Al-Caritas baby hospital in March 2019 the same period of sampling, Figure (5.7) illustrates the number of cases of human viral infection for these viruses, 50 cases Rotavirus, 13 cases Adenovirus and 2 cases Enterovirus and this confirms also the presence of these viruses in this period in sewage because they are gastrointestinal viruses and transmission as we mentioned previously oral -fecal transmission.

6.2 Recommendations

- Water safety plan (WSP) measures should be taken to improve the epidemiological surveillance of waterborne diseases and improving water quality at water catchment areas to improve health protection.
- Policy makers and regulatory councils should put a strategy for monitoring and regulating reuse and reclamation to be used as unconventional water resources.
- Membrane treatment units as Ultrafiltration (UF), Microfiltration, or even Nanofiltration (NF) are recommended as a post treatment stage to enhance the removal efficiency of viruses in Al- Bireh and Rawabi WWTP's.
- Enforcement and follow up of regular monitoring to the different WWTP's particularly those in Ramallah governorate under investigation and other WWTP's in the west bank, by regular assessment weekly or monthly or seasonally for the three parameters physical, chemical, and microbiological parameters.
- Protection of aquatic ecosystems, especially in Gaza strip by making mitigation measures for reducing toxic and hazardous pathogens entering waterway, from contaminated treated or partially treated wastewater.
- Policy makers and stakeholders should support Central labs in universities and institutions particularly PWA, through capital investments and upgrading testing methods, training of lab staff, this would promote safe effluent quality used as unconventional source for irrigation.

- Awareness campaigns to the public particularly farmers convincing them about the cost effective benefits of reclaimed water as a precious source of water if the quality of the reclaimed was within the standards, and regulations.
- Starting step forward in awareness by a question send to the Grand Mufti of Jerusalem and Palestine territories about the rule of Islam in the use of treated water in agriculture. The answer to this question was scanned in (Appendix 1), the answer was that we could use it after removal of all the organics and also the pathogenic microorganisms with modern technologies and the quality of the treated water should meet the guidelines and regulations.
- In the phase of construction and design of big and costly wastewater plants chose the right technology according to the end uses of the reclaimed water, for unrestricted irrigation, for restricted irrigation, for industrial use or only to be safe on environment.
- Concentrate on workshops on Reclaimed water, exchanging experience and knowledge from foreign countries about the right management of reclaimed water.
- Safety measures should be taken especially among the employees in the Wastewater plants in close contact with the wastewater, that this water contains hazardous pathogens that threatens their health.

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Appendix 1

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

STATE OF PALESTINE
DAR AL - IFTA' AL - FALASTEENIYYA
Public Administration

دولة فلسطين
دار الإفتاء الفلسطينية
الإدارة العامة

التاريخ: 23 هيفر 1441 هـ
الموافق: 22 تشرين الأول 2019 م

الرقم: 2287/2019/11/2

تاريخ تقديم الطلب: 2019/9/30 م
موضوع الفتوى: حكم استخدام المياه العادمة المعالجة لري المزروعات
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سماحة المفتي العام للقدس والديار الفلسطينية حفظه الله السلام عليكم ورحمة الله وبركاته



السؤال: هل تجوز إعادة استخدام المياه العادمة المعالجة لري المزروعات، مثل الأشجار المثمرة، والبقوليات، علماً أن هذه المياه غير صالحة للشرب، أو للطهو بعد المعالجة؟

أرجو إعطائي فتوى شرعية بذلك
ولكم الشكر

الحمد لله رب العالمين، والصلاة والسلام على أشرف الخلق سيدنا محمد الأمين، وعلى آله وصحبه أجمعين، وبعد؛

السائل الكريم: فهد فتحي صيام

السلام عليكم ورحمة الله وبركاته

بالإشارة إلى سؤالك المثبت نصه أعلاه؛ فقد بحث مجلس الإفتاء الأعلى في فلسطين مسألة استخدام المياه العادمة بعد معالجتها، وجاء في قراره رقم: 68/1 بتاريخ 2008/5/6 م، "أن المياه العادمة هي مزيج من الماء النقي وما خالطه من أوشاب وقاذورات، أحالته إلى ماء آسن، على أن هذا الصنف من الماء يظل طهوراً ما دام كثيراً وهو الغالب؛ لأن الماء الكثير الغالب لا ينجسه شيء، إلا باختلال واحد من أوصافه الثلاثة، وهي: الطعم واللون والرائحة، فأما خلل في واحد من هاتيك الصفات يفضي إلى أن يستحيل الماء إلى نجاسة، لا ينبغي استعمالها في التطهير، ولا بأس باستخدامه في سقاية المزروعات، وقد يكون الماء خالياً من أيما خلل في أوصافه الثلاثة، لكنه ربما خالطه شيء من القذر أو العفونة أو المواد الصلبة، فإنه حينئذ بالرغم من طهارته، وأنه مطهر لغيره، لكنه غير صالح للشرب، لما يحتمل أن يؤول شربه إلى مرض يصيب الإنسان، وذلك لما خالطه من أوشاب الجراثيم، حفاظاً على سلامة الأبدان، التي اعتبرتها الشريعة من الضرورات الخمس، على أن الماء الآسن يمكن معالجته بالوسائل العلمية التقنية، لتصفيته تماماً مما خالطه من قاذورات وأوساخ وجراثيم، فإذا تحقق ذلك بات الماء صالحاً للاستخدام".

وعليه؛ فإن معالجة هذه المياه بالوسائل الحديثة، والتقنيات العلمية المناسبة لتخليص المياه النجسة مما علق بها، وتقليل الميكروبات فيها، والتخلص من عفونتها يجعلها صالحة لري المزروعات، وأعمال البناء، وبذلك توفر كميات هائلة من المياه النقية التي تصرف في حياة الزراعة بمختلف أنواعها، ولا ينصح بشرب هذه المياه، أو استخدامها للطهي إن لم يُتأكد أنها خلصت من كل ما شابها من النجاسة، مع التنبيه إلى ضرورة مراعاة التعليمات الصحية التي تقررها الجهات المتخصصة في هذا الشأن، والله تعالى أعلم.

والله يقول الحق وهو يهدي السبيل

الشيخ محمد حسين

المفتي العام للقدس والديار الفلسطينية



نسخة:

- الملف العام.
- الملف الخاص.
- ن. ب. ي. ح.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
سماحة مفتي القدس والديار الفلسطينية
الشيخ محمد حسين حفظه الله

السلام عليكم وبعد :

المذنبوع : فتوى بخصوص استخدام
المياه المعالجة في ري المزروعات
أرجو إفادتنا بالرأي والفتوى حول استخدام
بعض المزارعين المياه المعالجة في حطاب
التنظيف لري المزروعات ومنها :
(أ) الأشجار المثمرة مثل التين والتفاح وغيرها
(ب) البقوليات والنعنع والتينوس وغيرها
سبباً أن هذه المياه لا تعتبر صالحة
للسرّب أو الطهر بعد المعالجة .
أفيدونا والله يحفظكم .

الطالب
فهد فتحاً صياح
0502976542

Appendix 2

Table1 Historical Development of treated Wastewater Reuse world- wide through 1968

Period	Location	Events
~ 3000 BC	Crete, Greece	Minoan civilization: use of wastewater for agricultural irrigation.
97 AD	Rome, Italy	The City of Rome has a water supply commissioner, Sexus Julius Frontinus.
1500 ~ 1700 ~	Germany United Kingdom	Sewage farms are used for wastewater disposal.
1800–1850	France, England, United States	Legal use of sewers for human waste disposal in Paris (1880), London (1815), and Boston (1833) instituted.
1850–1875	London, England	Cholera epidemic is linked to polluted well water by Snow.
1850–1875	England	Typhoid fever prevention theory developed by Budd.
1850–1875	Germany	Anthrax connection to bacterial etiology demonstrated by Koch.
1875–1900	France, England	Microbial pollution of water demonstrated by Pasteur. Sodium hypochlorite disinfection by Down to render water "pure and wholesome" advocated.
1890	Mexico City, Mexico	Drainage canals are built to take untreated wastewater to irrigate an important agricultural area north of the city, a practice that still continues today. Untreated or minimally treated wastewater from Mexico City is delivered to the Valley of Mexico where it is used to irrigate about 90,000 ha of agricultural lands, including vegetables.
1906	Jersey City, NJ	Chlorination of water supply.
1906	Oxnard, CA	The earliest reference related to a public health viewpoint of water quality requirements for the reuse of wastewater appears in the <i>Monthly Bulletin, California State Board of Health</i> , February, 1906 on the Oxnard septic tank system of sewage disposal.
1908	England	Disinfection kinetics elucidated by Chick.
1913–1914	United States and England	Activated sludge process is developed at the Lawrence Experiment Station in Massachusetts and demonstrated by Ardern and Lockett in England.
1926	United States	In Grand Canyon National Park treated wastewater is first used in a dual water system for toilet flushing, lawn sprinkling, cooling water, and boiler feed water.
1929	United States	The City of Pomona, CA initiated a project utilizing reclaimed water for irrigation of lawns and gardens.
1932–1985	San Francisco, CA	Treated wastewater is used for watering lawns and supplying ornamental lakes in Golden Gate Park.

Source of this table. (Asano *et.al.*, 2007)

Table 2 Historical Development of treated Wastewater Reuse world -wide through 1968 continued

Period	Location	Events
1955	Japan	Industrial water is supplied from Mikawajima wastewater treatment plant by Tokyo Metropolitan Sewerage Bureau.
1968	Namibia	Direct potable reuse begun at Windhoek's Goreangab Water Reclamation Plant.

^aAdapted in part from Metcalf and Eddy (1928); Ongerth and Jopling (1977); Barty-King (1992); Okun (1997); Cooper (2001); Angelakis et al. (2003).

Source of the table (Asano *et.al.*, 2007)

Table 3 Historical Development of treated Wastewater Reuse world- wide through 1968

Period	Location	Events
1960	Sacramento, CA	California legislation encourages wastewater reclamation and reuse in the State Water Code.
1962	Los Angeles County, CA	A major groundwater recharge project by surface spreading is initiated at the Whittier Narrows spreading basin.
1965	San Diego County, CA	Santee recreational lakes, supplied with reclaimed water, are opened for swimming, and put-and-take fishing.
1972	Washington, DC	U.S. Clean Water Act to restore and maintain water quality is passed.
1975	Fountain Valley, CA	Groundwater recharge by direct injection of reclaimed water into aquifers is started by the Orange County Water District (known as Water Factory 21).
1977	Pomona, CA	Pomona Virus Study, conducted by Sanitation Districts of Los Angeles County, is published.
1977	Irving, CA	Irving Ranch Water District initiates a major landscape irrigation project with a dual water system delivering reclaimed water.
1977	St. Petersburg, FL	Another major urban water reuse system is initiated in St. Petersburg, Florida.
1978	Sacramento, CA	California Wastewater Reclamation Criteria (Title 22 regulations) are promulgated by the Department of Health Services to be enforced by nine Regional Water Quality Control Boards.
1982	Tucson, AZ	Initiates a metropolitan water reuse program mandating use of reclaimed water in golf courses, school grounds, cemeteries, and parks.
1984	Los Angeles, CA	Health Effects Study by Los Angeles County Sanitation Districts is published.
1987	Monterey, CA	Monterey Wastewater Reclamation Study for Agriculture by Monterey Regional Water Pollution Control Agency is published.
1987	Sacramento, CA	Report of the Scientific Advisory Panel on Groundwater Recharge with Reclaimed Wastewater is published by the State of California Interagency Water Reclamation Coordinating Committee.
1992	Washington, DC	U.S. Environmental Protection Agency and U.S. Agency for International Development publish <i>Guidelines for Water Reuse</i> .
1993	Denver, CO	Potable Water Reuse Demonstration Plant— Final report (pilot plant operation began in 1984) is published.
1996	San Diego, CA	City of San Diego Total Resource Recovery Health Effects Study is published by Western Consortium for Public Health.

Source of table (Asano *et.al.*, 2007)

Table 4 Historical Development of treated Wastewater Reuse world -wide through 1968 continued

Period	Location	Events
2003	Sacramento, CA	Water Recycling 2030: Recommendations of California's Recycled Water Task Force, Department of Water Resources and State Water Resources Control Board, State of California is published.
2004	Washington, DC	U.S. Environmental Protection Agency and U.S. Agency for International Development published <i>Guidelines for Water Reuse</i> .

^aAdapted in part from Metcalf and Eddy (1928); Barty-King (1992); Ongerth and Jopling (1977); Okun (1997); Asano (1998); Cooper (2001); U.S. EPA (1992); State of California (2002a); U.S. EPA and U.S. AID (2004).

Source of the table (Asano et.al. 2007)

Appendix3 illustrates the pictures of my own of the day of sampling on March 21/3/2019

Figure 1 day of sampling in Rawabi



Source of picture my I-phone on 21/3/2019

Figure 2 Sampling in Al- Bireh



Making the turbidity and pH onsite

Figure 3 Sampling in Al-Bireh



Figure 4 Sampling in Al-Tireh



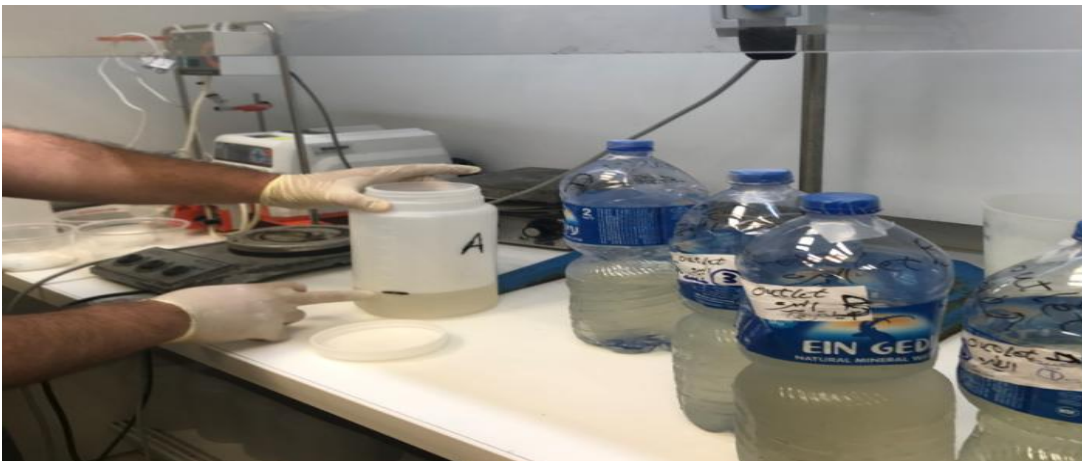
Source my I-phone on 21/3/2019

Figure 5 First step in preparation and concentration of the samples



Source from Lab: (Shulman L. M., 2015) addition of 40gm of polyethylene granules and 8.5gm of NaCl

Figure 6 Marking special bottles for pouring the samples and materials



Source the Lab: (Shulman L. M., 2015) 500ml of samples poured in the plastic bottle

Figure 7 point 6 in step one in preparation and concentration of the samples



Source of the picture the LAB: (Shulman L. M., 2015) the samples are stirred on an electronic stirrer and with a magnetic stirrer inside each bottle

Figure 8 place of analysis of preparation and concentration of the samples and the extraction procedures



Figure 11 illustrates step10 in the preparation step



Source the Lab: (Shulman L. M., 2015)

Figure 12 The pellet rinsed with tween PBS & 5ml chloroform are added to the conical test tube



Source the Lab: (Shulman L. M., 2015)

Figure 13 illustrating step 10 rinsing the pellet on the walls by tween 80 PBS soap



Source the Lab: (Shulman L. M., 2015)

Vortex for vigorous shaking of test tubes at 3000rpm

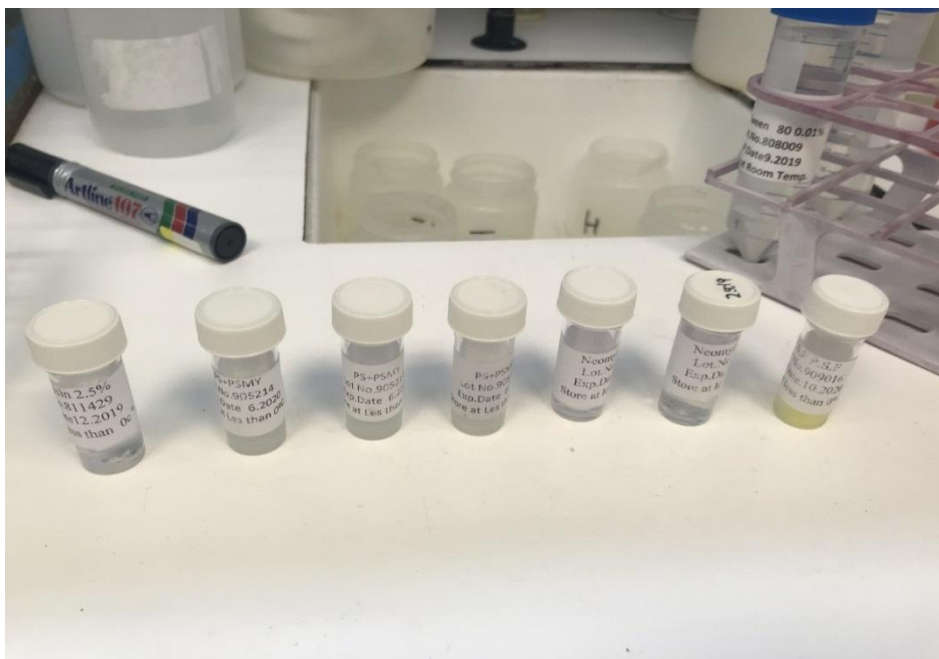


Figure 14 a centrifuge for test tubes at 3000rpm



Source the Lab: (Shulman L. M., 2015)

Figure 15 Addition of antibiotics as in point 16 in the preparation step



Source the Lab: (Shulman L. M., 2015)

Figure 16. Applied Biosystem 7500 Real Time PCR (polymerase chain reaction)

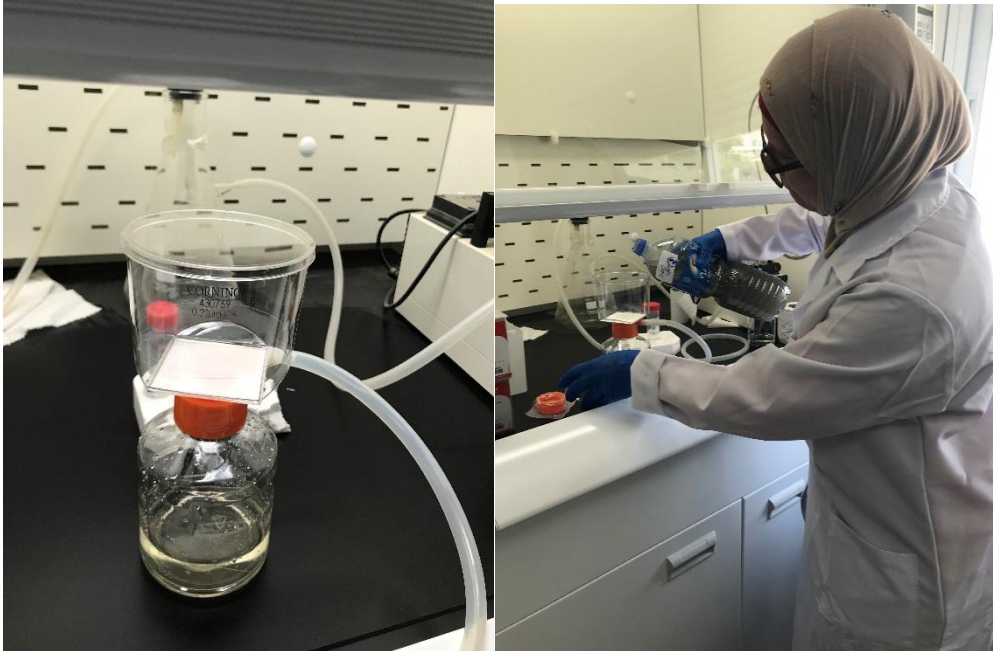


Figure 17 NucliSens Easy Mag the automated system used for Extraction Step



Source of this system the Lab (Shulman L. M., 2015)

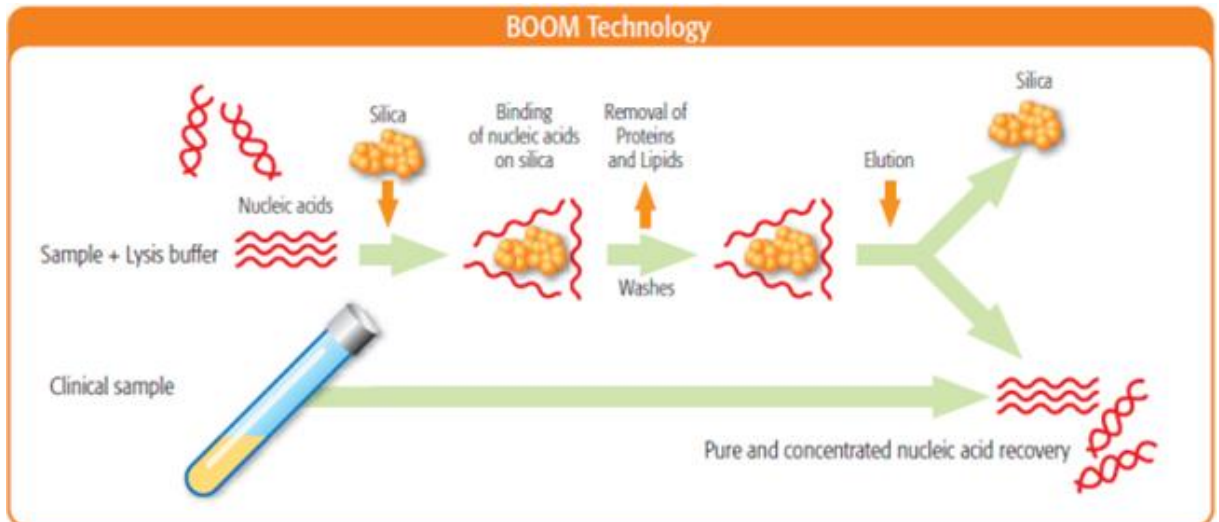
Figure 18 Corning filters used in Al-Quds University the step when we lost the viruses



Filters put in with phosphate buffer

Figure 19 Identifying the steps of extraction Source the Lab: (Shulman L. M., 2015)

Figure 19 BOOM Technology using magnetic silica



Source from internet (<https://www.biomerieux-diagnostics.com/nuclisensr-easymagr>, n.d.)

Figure 20. Preparation of Difco-Fecal Coliform Agar



Source PWA Lab

Figure 21 Membrane filtration unit for detection of Fecal Coliform



Source PWA central Lab

Figure 22 OxiTop for BOD₅ measurement incubator for 5 days



Figure 23 results of fecal coliform in Al- Bireh, Al- Tيره and Rawabi incubated Plates for 24 hours at 44° C

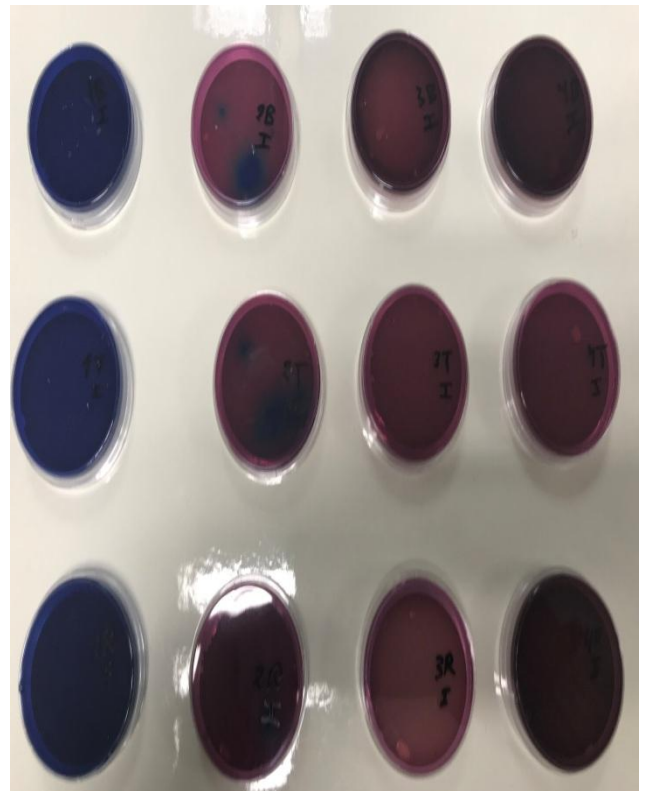
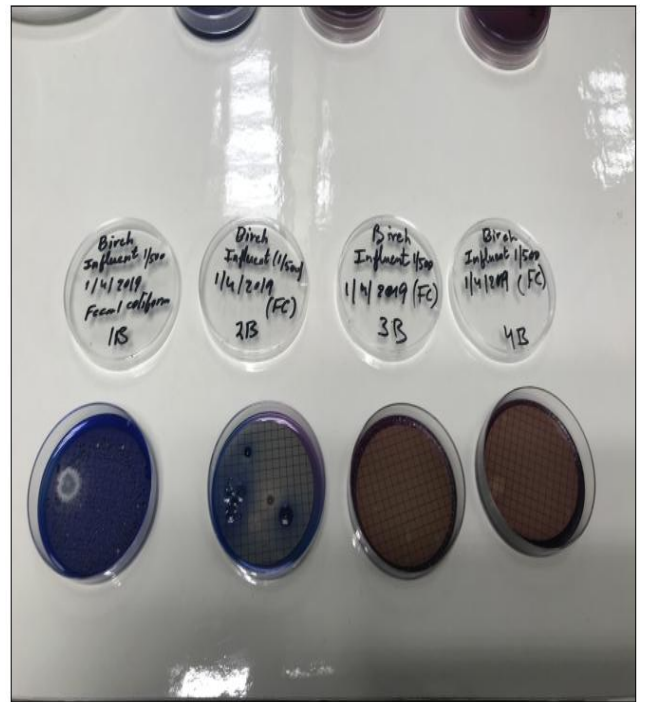
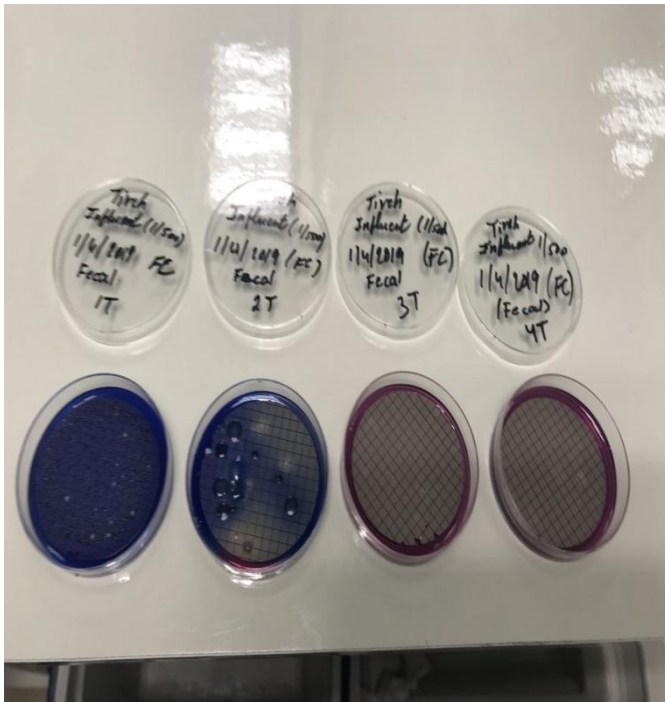


Figure 24 Specifications of ZeeWeed 500 Module
The Immersed Hollow fiber Ultrafiltration membrane in Al-Tireh WWTP

GE Power & Water
Water & Process Technologies

Fact Sheet

ZeeWeed* 500D Module

Immersed Hollow-Fiber Ultrafiltration Technology



Module Type	WW	DW
Application	Membrane Bioreactor	All Other Applications
Nominal Membrane Surface Area	340 ft ² (31.6 m ²)	340 ft ² (31.6 m ²) 440 ft ² (40.9 m ²)
Module Dimensions		
Height	2,198 mm (86.4")	
Width	844 mm (33.2")	
Depth	49 mm (1.9")	
Module Weight		
Max. Shipping Weight <small>(crated)</small>	26 kg (58 lb)	28 kg (62 lb)
Lifting Weight <small>(varies with solids accumulation)</small>	26 - 74 kg (58 -163 lb)	30 - 74 kg (66 -163 lb)
Membrane Properties		
Material	PVDF	
Nominal Pore Size	0.04 micron	
Surface Properties	Non-ionic & Hydrophilic	
Fiber Diameter	1.9 mm OD / 0.8 mm ID	
Flow Path	Outside-In	
Operating Specifications		
TMP Range	- 55 to 55 kPa (- 8 to 8 psi)	-90 to 90 kPa (-13 to 13 psi)
Max. Operating Temperature	40°C (104°F)	
Operating pH Range	5.0 - 9.5	
Cleaning Specifications		
Max. Cleaning Temperature	40°C (104°F)	
Cleaning pH Range	2.0 - 10.5	
Max. Cl ₂ Concentration	1,000 ppm	

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