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**Surveillance of the Microbiological Quality in Indoor Public
Swimming Pools in Bethlehem, Palestine**

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M.Sc. Thesis

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Swimming Pools in Bethlehem, Palestine**

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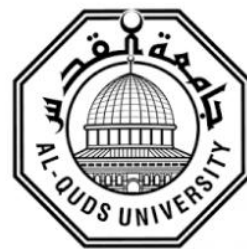
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Thesis Approval

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Dedication

I dedicate this humble work to my husband because of his constant support, to my parents, to my brothers and sisters, to everyone who has supported me throughout my career.

Declaration

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

Signed.....*Nihad..Alassa*

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Date :30/5/2021

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Abstract:

Swimming is an important recreational activity in most people, there is a high demand for the use of swimming pools, which makes them more susceptible to microbes and be a vector of infectious diseases, thus increasing the percentage of injuries and risks caused by their use. Accordingly, preventive measures and measures must be put in place to reduce the risk of these places. So we have done frequent microbiological tests of swimming pool water, where the biological factor is an indication of the health of the water and make it safer when using, the greatest damage is biological. In this research we dealt with the three most important microbial tests approved by the Palestinian Ministry of Health and global health, namely total heterotrophic count, pseudomonas and E-coli. The aims of research are investigate the microbiological quality of indoor swimming pools water by using conventional heterotrophice plate count and polymerase chain reaction, and to investigate the compliance of swimming pools water with environmental and puplic healthy agencies requierments, guidelines, and regulations.

The samples taken showed that the bacteria screening of all pool samples did not exceed the standards approved by the Palestinian Ministry of Health and World Health Organization. But check been showed that 8.3% of the sample is not matching the criteria adopted in the pools A, B and E for pseudomonas, and in pool C presented in 66% of the sample were not accepted but presented about 40% in pool D which were not acceptable to Palestinian criteria. As for the E. coli bacteria, they appeared in one sample each in A, B and C, while Pool E was clean and did not appear in any sample. Quite the opposite in Pool D which had the presence of *E.coli* in two samples.

And for the first time, we did a polymerase chain reaction of these samples, to confirm the presence of these types of bacteria, in addition to knowing the microbes that were present in the pool before. We also took samples from the backwash of the filters and were examined by polymerase chain reaction and we noticed the presence of numbers of bacteria common between the samples and thus confirm the passage of swimming pool water, so the result is interrelated between the pool and the filter and the relationship is strong between them in getting better results for swimming pool

مراقبة الجودة الميكروبيولوجية في حمامات السباحة المغلقة في بيت لحم .فلسطين

المشرف : الدكتور .عامر كنعان

اسم الطالب . نهاد كمال احمد العصا

ملخص

السباحة من أهم النشاطات الترفيهية التي تلقى أقبالا كبيرا من قبل الناس خاصة خلال فترة الصيف للاستجمام وقضاء أوقات ممتعه خلال فترة الصيف, حيث يكون استخدام المسابح في الذروة. هذا الاستخدام المفرط لحمامات السباحة يجعل البرك وسط ناقل للميكروبات وللأمراض المعدية, وبالتالي أعتبرنا المسابح مكان يسبب الخطر وينتج عنه أصابات في حالة الأستخدام غير السليم. لذلك تم وضع معايير وأجراءات وتدابير وقائيه من قبل وزارة الصحة الفلسطينية التي تساعد على تقليل التلوث الميكروبي داخل المسابح, حيث تقوم الصحة بعمل فحوصات دورية للمسابح للكشف عن انواع من الميكروبات التي يعتبر وجودها في الماء من الدلائل على عدم نظافتها. حيث يعتبر العامل الميكروبي من اهم العوامل التي تحدد مدى صحة وسلامة مياه المسابح.

الهدف من هذه الدراسة كان تقييم لمدى سلامة المسابح الداخلية من التلوث الميكروبي, كانت بأستخدام طريقتين الاولى الطريقه التقليديه لفحص البكتيريا حيث قمنا بفحص ثلاث انواع من البكتيريا وهي البكتيريا البرازيه والتي تدل على مدى نظافة الماء ,و بكتيريا الزيدومونس الذي يعطي مؤشر على كفاءة عملية التعقيم داخل المسبح وفحص للبكتيريا الكلية وذلك بزراعة البكتيريا في الاجار المناسب لها. أما الطريقه الثانيه فهي تفاعل البلمرة المتسلسل الذي يعتبر أكثر دقه وذلك لانه لا يحتاج الى وجود بكتيريا حية وإنما نستطيع الكشف عن وجودها بواسطة الجين الوراثي بعمل تضخيم وتحديد نوع البكتيريا الموجوده.

ومن خلال النتائج تبين أن معظم العينات قد توافقت مع المعايير الفلسطينيه والعالميه, لكن ظهر عدم توافق في عينه واحده من العينات في المسابح A, B, E, لوجود بكتيريا الزيدومونس, وتبين ان المسبح C قد ظهرت في ثمانية عينات, أما المسبح D فقد ظهرت في خمس عينات. أما البكتيريا القولونية فقد ظهرت في عينة واحده في كل من المسبح A,B,C, وظهرت في عينتين في المسبح D, وكان المسبح E خاليا من البكتيريا القولونيه. لكن البكتيريا الكلية كانت ظاهرة في جميع العينات لجميع المسابح.

نتائج PCR جاءت متوافقه مع البكتيريا التي تم زراعتها حيث أظهرت النتائج لكل مسبح القراءات لكل نوع بكتيريا ومدى توافقه مع سلامة المسبح وتلوثه بالميكروب, فهذه الطريقه تعطي نتائج أدق وأكثر تفصيلا لنوع وكمية الملوثات في المياه. أيضا أضفنا لهذا البحث نوع جديد من العينات حيث قمنا بأخذ مياه الغسيل العكسي للفلاتر التي في الاصل تكون ملوثة بالميكروب الموجود في المسبح وقمنا بعمل فحص ال PCR لنؤكد على ان نوع البكتيريا الموجود في المسبح يتواجد أيضا في الفلاتر, حيث أظهرت نتائج هذا الفحص للمسابح A,B توافقها مع نتائج مياه المسبح.

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Abbreviations & units

WHO	World Health Organization
CDC	Centers for disease control & prevention
MAHC	Model aquatic health code
EU-BWD	European bathing water directive
EPA	Environmental protection agency
NTU	Nephelometric Turbidity Unit
mg/L	Milligram per liter
mL/min	Milliter per minute
cfu/l	Colony forming unit
°C	Celsius
Cl₂	Chlorine
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid

Chapter 1

Introduction

Swimming and recreational water activities have long been recognized as extremely beneficial to health and personal well-being, (CDC, 2016; Thomson et al., 2003). It attracts a lot of people, especially in warm seasons (Mansoorian et al., 2015). Swimming, in particular, is acknowledged as one of the most beneficial forms of exercise that all ages enjoy and is also an important rehabilitation therapy (Department of Health, 2019; Kamioka et al., 2010). However, swimming pools may be supplied with fresh (surface or ground), marine or thermal water. They may be private, semi-public or public, and may be located indoors, outdoors or both. In addition, there are many specialist pools for a particular user type (e.g. paddling pools, learner or teaching pools) (WHO, 2006).

Many epidemiological studies have shown that there is a risk of exposure to recreational water contaminated with feces, as there are more than 90 million cases of gastrointestinal, respiratory, ear, eye and skin infections associated with swimming pools in the United States (Fadaei and Amiri, 2015). About two billion people around the world use water contaminated with faeces, according to the World Health Organization (2006) (Decision, 2006). Microbes that are introduced to the pools during swimming by release different microorganisms from bathers. The degree of risk is significantly related to the concentration of pathogens in the pool water and the duration of contact with pathogens (Fadaei and Amiri, 2015).

Microbiological contamination could be directly caused by the swimmers or indirectly caused through the contamination of filling water sources (Thomson et al., 2003). Many public health scientists intuitively believe that there could be a relation between the degree of fecal contamination and the risk acquiring certain illnesses during swimming activities. Infections are mainly associated with fecal contamination water, but non fecal human release into the pool water (e.g. from

skin, hair, mucus, saliva, fecal residues and urine) is a potential source of pathogenic and harmful microorganisms (Bonadonna and La Rosa, 2019).

A variety of microorganisms can be found in swimming pools, which may be introduced in the pool water in a number of ways. In many cases, the risk of illness or infection has been linked to fecal contamination of the water, due to feces released by bathers or a contaminated source water or, in outdoor pools, may be the result of direct animal contamination (e.g. from birds and rodents) (WHO, 2006; Yoder et al., 2008).

In addition, certain free-living aquatic bacteria and amoebae can grow in waters, in pool components or facilities (including filters, heating, ventilation and air-conditioning systems) or on other wet surfaces within the facility to a point at which some of them may cause a variety of respiratory, dermal or central nervous system infections or diseases, or on other surfaces used by swimmers, such as chairs, door handles, etc. (Mansoorian et al., 2015; WHO, 2006; Yoder et al., 2008).

Therefore, swimming pools are often associated with outbreaks or incidents of waterborne infections (Lee et al., 2002). The infectious agents isolated from swimming pools include a variety of pathogens embracing bacteria, viruses, protozoa and fungi (Dziuban et al., 2006; Schets et al., 2008; WHO, 2006). Many of the outbreaks related to swimming pools would have been prevented or reduced if the pool had been well managed as the first reason for the transmission of waterborne diseases is poor management of swimming pools and the quality of water used (Mansoorian et al., 2015).

A swimming pool is a body of water of limited size contained in a holding structure, and these pools are artificial ecosystems that imitate natural water where the water is not in contact with the soil as in surface water, they contain two compartments one for swimming and the second for treatment of water either physical or biological (Wei et al., 2018). The swimming pool water should be of potable quality (Saleem et al., 2019) and is treated with additional disinfectants (chlorine compounds, ozone, or other means). Although modern swimming pools have a re-circulating system so that the water can be filtered and disinfected

effectively, relevant research studies, show that neither treatment systems nor disinfectants can prevent the colonization of the pool water with hazardous pathogens (Braue et al., 2005; Esterman et al., 1987; Reali et al., 2004). But the colonization of these organisms may be in the filters during the process of treatment of swimming pools water, but it is eliminated by doing reverse washing of the filters (backwash). However, they give an indication of the type of contaminants that have been present in the water over a period of time. In addition, the surviving chlorine tolerant bacteria might also be antibiotic resistant, which was already documented for bacterial isolates from treated drinking water (Barben et al., 2005; Papandreou et al., 2000).

As a result of poor microbiological pool water quality, exposure to bathing waters may pose health risks for swimmers. They may contract illnesses such as gastro-enteritis from infections with bacteria, viruses or parasites of fecal origin (Cordoba et al., 2001; Esterman et al., 1987; Pond, 2006), but also skin disorders like cercarial dermatitis as a result of contact with the larvae of the parasite *Trichobilharzia* (WHO, 2013), or otitis externa due to an infection with *Pseudomonas aeruginosa* (Dallolio et al., 2013; Leonard et al., 2018; Leoni et al., 1999).

Since swimming pools are considered to be a strong and dangerous source of microbial contamination, sanitary standards, quality control, and microbiological safety of swimming pools must be established (Dziuban et al., 2006; Russo et al., 2020). So bathing water legislation is in place to protect swimmers from contaminants, although guideline values are not based on pathogen concentrations but on fecal indicator levels (Horák and Kolářová, 2001). Previous studies have demonstrated that bathing water that even complies with bathing water guideline values may contain pathogens (CDC, 2016; Decision, 2006; Graczyk et al., 2007), whether of fecal origin or not, and thus still pose an unacceptable health risk for swimmers (Arnold et al., 2016).

In the Netherlands, they are working to update and improve the quality of water used in swimming pools based on the guidance FFL German collected from a number of experts taught microbiology and toxicology technologists, specialists

in the construction of swimming pools, and for protection of the public from the diseases caused from the swimming pools there (Wei et al., 2018).

A study of water ingestion during active swimming in a swimming pool showed that non-adult swimmers (≤ 18 years) swallowed far more water than adult swimmers during a period of 45 minute of their swimming activity (37 vs. 16 mL) (Schets et al., 2008). The average volume of water ingested per minutes by children (≤ 18 years) during an average visit to a swimming pool of 81 min, is 0.63 mL/min; adult men ingested 0.50 mL/min, and women 0.34 mL/min (Dufour et al., 2006).

Despite the well-recognized health benefits associated with the attendance of swimming pools, the possible presence of pathogenic microorganisms in swimming pool water or any other recreational water facility may constitute a public health risk. Therefore, monitoring and management practices have been recommended by WHO, intended to be used as a basis for the development of international and national approaches for the control of hazards, to ensure water safety to pool users (Schets et al., 2011; Yoder et al., 2008) that have been converted into locally appropriate standards in several countries. Other Agencies, such as the South Australia Environmental and Public Health Service (Yoder et al., 2008) and the United Kingdom Health Protection Agency (WHO, 2006) have established standards for routine monitoring of public and semi-public pools and hot tubs for microbial parameters. In the United States, the Centers for Disease Control and Prevention (CDC) is working about safety and prevention in recreational water environments, also developing recommendations for pool inspectors to optimize the effectiveness of the recreational water illnesses prevention efforts. Since pool codes are created by local public health agencies, there are no uniform national standards in the US. However, the Model Aquatic Health Code (MAHC) of CDC's Healthy Swimming program is a resource providing uniform guidelines (Jillian Deans, 2006). In Europe, a European Bathing Water Directive (EU-BWD) from institutions working to protect swimmers by disseminating risks related to fecal contamination of pool water (Wei et al., 2018).

The swimming pools in Palestine considered a major recreational place because no marine water easily accessible. The Ministry of Health in Palestine give many

legislation which used to optimize the effectiveness of swimming pool. The goals of this research is using of polymerase chain reaction to detection of bacteria which used the first time in the research about Palestine. In addition the objective of these atudy are investigate the microbiological quality of indoor swimming pools water using heterotrophice plate count(HPC) and polymerase chain reaction(PCR). And to investigate the compliance of swimming pools water with environmental and public health agencies requirements, guidelines, and regulations.

Chapter two

Literature Review

Microbiological quality in swimming pools

The large turnout of the pools giving a clear indication of the height of microbial contamination of water, this indicates that the most important source to meet microbial is from the swimmers themselves, unlike pollutants originating from soil, air, plant, or animal (European Environmental Agency, 2019). There are specialized programs to track microbes in swimming pools and to give their safety and free of contaminants according to the microbiological, chemical and physical standards of each country according to its own economic and health conditions. For example, in China the approved temperature is (22 – 26⁰ C), pH is from 6.5 to 8.5, turbidity (5 NTU), free residual chlorine levels (0.3-0.5mg/l), and total bacteria count is 1000 cfu/ml, total coliform 18 cfu/L (Bautista et al., 2016). On the other hand, Germany takes into account the microbial criteria where total heterotrophic count less than 200 cfu/ml, the temperature and residual chlorine varies depending on the nature of the pool (range 27-33⁰ C) (2-5mg/L), respectively. The pH7.2-7.8 (Wei et al., 2018).

Many studies show that the microbiological pathogens can cause waterborne disease where estimate to cause more than 2.2 million death per year and many disease including diarrhea, gastrointestinal and other illnesses. Bacteria are considered one of the most important pathogens, most notably non-fecalley bacteria and some free water bacteria and amoeba, as this species grows inside swimming pools and on wet surfaces, and is considered a pathogen of the respiratory and nervous system (Pond, 2006). In addition to other types of pathogens such as *Salmonella*, *Shigella*, pathogenic E-coli, *Yersinia*, *Mycobacteria*, *Staphylococcus aureus*, *legionella*, *Dermatophytes* and

Pseudomonas aeruginosa. *Pseudomonas aeruginosa* which is considered one of the most dangerous pathogens affecting the lungs (WHO, 2006).

The microbiological tests of swimming pool are indicator of the sanitary of the water and if it suitable for using (Bautista-De los Santos et al., 2016). According to WHO (WHO, 2006), to assess the microbial quality of swimming pools, many parameters must be used to determine the sanitary quality and suitability for public use. There are several pathogens used as indicator of water safety, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Legionella* spp. *E.coli* used as faecal indicator, whereas *Pseudomonas aeruginosa* used as indicator organism for disinfection efficiency because it is very resistant to disinfection treatments, resulting from accumulating it in a form of biofilm and it has the ability to grow in moist and warm conditions with low nutrients (Murphy et al., 2018).

Other studies took 30 years by Centers for Disease Control and Prevention (CDC) and Environmental Protection Agency (EPA) to surveillance waterborne outbreaks, during that >11000 cases of illness and >70% of waterborne outbreaks were connected with swimming pools (Lumb et al., 2004).

2.1.1 Potential sources of microbes in swimming pool

The microbial safety of swimming pools is highly questionable in countries with poor regulatory framework due to the many existing possible microbial contaminant pathways. Microbial contamination of swimming pools can occur through: fecal contaminated source water or direct release from swimmers, birds, and animals into the water, non-fecal human shedding from vomiting, sneezing, mucous, spitting, or skin in the swimming pool, poor wastewater disposal, microbial biofilm formation along piped water networks, and infected users can directly contaminated pool water (Adetunde and Ninkuu, 2016; Arnold et al., 2016).

2.1.2 Transmission of microbial

Microbial infection is a great risk in swimming pools. Most infectious diseases occurrence must have a source that causes this infection, such as excretion of fecal material from an infected person or from a child or similar secretions from

the ears, eyes, respiratory system, skin, digestive system and urogenital system for people who have suffer from infection, and thus the swimming pool becomes the carrier medium, that is, it is transmitted through swimming pools and other related recreational facilities. Therefore, immediate disinfection of swimming pools must be done on a regular basis to eliminate outbreaks of transmissible and infectious diseases (Department of Health, 2019; U.S.EPA, 2014).

2.1.3 Managing water quality

2.1.3.1 Circulation and filtration

The process of water circulation and filtration is one of the most important factors that remove turbidity from swimming pool water, which includes separation of solid and suspended materials in the pool water, and the effectiveness of sterilization and filtration depends on the flow rates expressed in cubic meters per hour per square meter ($M^3/hr/m^2$), where its identification is considered one of the most important therapeutic steps to protect the health of pool users (Queensland Health, 2018).

2.1.3.2 Disinfection system

Disinfection is one of the most important reasons for microbiological safety, as there are many disinfection systems available to treat public swimming pools by decrease the waterborne infections, water become clear and potable quality, and inactivation of the microbial in the pools (Kanan and Karanfil, 2009). These system use many type of disinfection in several forms like chlorine compound, ozone, or other means. Chlorination is the most procedure for disinfection in public swimming pools. Chlorine compound may be used as gaseous form (Cl_2), liquid and solid form as hydrochlorous acid or as sodium or calcium hypochlorite. In water it is very important to keep the pH of the pool at the range (7.2-7.6) (Braue et al., 2005; Dziuban et al., 2006).

2.1.3.3 Backwashing

Each swimming pool has a filtration system that is used to remove plankton and microbes routinely, and as a result of its frequent use, it must be cleaned so that its effectiveness is high, and this process is called backwashing, where most of

the microbes that are stuck in the filter are washed out. Samples taken from the backwash are to confirm the presence of these microbes. There are some studies that have used backwash water to detect cryptosporidium and giardia and to determine the viability of the pool for use (Barben et al., 2005; Fadaei and Amiri, 2015; WHO, 2006).

2.1.3.4 Water quality standard

Making pool water clean and healthy is one of the most important things the EU has been doing for years. Therefore, it imposes provisions and restrictions on swimming pools to reach an excellent level of quality throughout the swimming season, due to the increase in the numbers of swimming pools and the increase in demand for them. Swimming pools are evaluated by conducting periodic water checks to find out the cause of pollution and reduce its recurrence, thus improving the level of water quality in swimming pools (European Environmental Agency, 2019; U.S.EPA, 2014; US EPA, 2003).

Based on this, several studies have been done that deal with the water quality and its relationship to the transmission of diseases. The quality of water, whether good or bad, is determined based on several indicators and measures (Table 2 and Table 1), the most important of which is the examination of the bacteria fecal and E. coli as a quality indicator (Favero, 1985).

However, the elimination of these microbes is not definitive, whether using disinfectants or advanced technological systems. Some reports in the U.S. have shown that pathogens are not eliminated definitively using disinfectants, as there have been some species and traits that are resistant to chlorine (Horák and Kolářová, 2001; Xue et al., 2013).

Table 1. Chemical parameters (WHO, 2006)

Parameter	Pool type	Criteria
Free chlorine	Indoor	Min 2.0 mg/l
Turbidity	Indoor	Max 1 NTU
Ph	Indoor	7.0 - 7.8
Temperature	Indoor	27-33°C

Table 2. Microbiological parameters (WHO, 2006)

Parameter	Guideline value
Escherichia coli	0 cfu/100ml
Pseudomonas aeruginosa	0 cfu/100ml
Heterotrophic colony count	Less than 100 cfu/ml

The control system of Palestinian Authority on Swimming pools depend on the many regulations and actions which preserve of the water to become more sanitary and safety for bathers.

Table 3. Microbiological requirements for swimming pool water according to Healthy & Human Services (WHO, 2006) and Palestinian standard (Shabieb, 2006)

Microbiological requirement	WHO standard	Palestinian Standard
Total heterotrophic counts	<100 cfu/ml	2000 cfu/1ml
E-coli	0 cfu/100ml	0 cfu/100 ml
Pseudomonas	0 cfu/100ml	0 cfu/100ml

Table 4. Recommended operational monitoring frequency according to Palestinian Ministry of Healthy and standard value (Shabieb, 2006)

Parameters	Frequency	Standard value
Residual chlorine	Once every two hours	1-2.5 mg/l
pH	Once every two hours	7.2-7.6
Turbidity	**	5 NTU
Pseudomonas	**	0 cfu/100ml
Total heterotrophic counts	Once every two weeks	2000 cfu/ml
E-coli	Once every two weeks	0 cfu/100 ml

** Not available

2.2 Microbiological identification techniques

2.2.1 Conventional heterotrophic plate counts (HPC)

Heterotrophic plate counts (HPC) is the descriptive term for a group of similar methods used routinely by water utilities for general microbiological monitoring of water samples. The method enumerates a variety of heterotrophic bacteria that are cultivable on semi-solid nutrient-rich media under defined incubation conditions (APHA et al., 2017; Garibyan and Avashia, 2013). The basic HPC method was proposed well over a century ago and was for a considerable time regarded as indicative of the hygienic quality of drinking water (Gensberger et al., 2015). However, during the 1980's and 1990's it was decisively

concluded that HPC measurements have no hygienic relevance. Increasingly, HPC was regarded as a process variable to monitor a range of events and/or processes relevant to the general microbiological quality of drinking water in treatment and distribution systems (Bartram, 2013; Gensberger et al., 2015). For most of the previous century, HPC was regarded as the best available technology for drinking water process monitoring, and HPC data contributed towards considerable advances in our understanding of drinking water microbiology (Bartram, 2013).

In the last two decades, a number of powerful quantitative and molecular methods have emerged for water analysis. Application of these new techniques showed that bacterial communities in drinking water were vastly more abundant and complex than what was previously understood from research based on cultivation-dependent methods (Chowdhury, 2012). Current evidence suggests that the drinking water microbiome consists of as many as 9,000 distinct taxa, with total numbers ranging between 1,000–500,000 bacteria mL⁻¹ (Berry et al., 2006).

2.2.1 Polymerase chain reaction(PCR)

The conventional culture techniques have distinctive advantages, but they have some limitations include needing to preserve the bacterial viability, identification of low numbers of microorganisms, transport conditions, labor intensiveness, needing expert personnel, an elongated period of time before outcomes and strict sampling (Centers for Disease Control and Prevention, 2018).

The PCR method lets amplification of genes and their RNA transcripts isolated from several sources such as water (Anand et al., 2014; Armitage, 1996). Each PCR test needs the presence of the DNA polymerase enzyme, extracted DNA sample, primers, and nucleotides.

I. Template extracted DNA is the identified target sequence that requires to be amplified. DNA polymerase is a key enzyme to replicate target sequences of DNA that attaches individual nucleotides together to generate the PCR product. Primer molecules are appropriate short and single-stranded sequences of DNA or RNA designed to particularly anneal to a desired target nucleic acid. Forward and reverse primer pairs have a length of 18–22 base pairs. For PCR amplification, DNA is extracted from a desired sample such as swimming pool water and added

to the reaction mix tube including primers, PCR buffer, deoxynucleotides (dNTP), MgCl₂ and DNA polymerase enzyme in an examination tube. The reaction tube then is located in a thermocycler that runs repeated cycles of DNA replication to take place in the following steps. Denaturation of DNA (Heating reaction tube to 94 °C) is for separation of the double strands DNA and yielding two single strands of DNA molecules (Tomo, 2017).

II. Annealing – At 50–65 °C, forward and reverse primers anneal to a particular site at each of the single-stranded DNA templates. Melting Temperature (T_m) of the primer pairs determines the *annealing* temperature.

III. Extension or elongation – at 72 °C, new complementary DNA strands synthesizes by the elongation of primers using DNA polymerase enzyme (Tomo, 2017).

PCR reaction carries out with automated thermal cyclers during each reaction step at the precise temperature and for a defined duration (Tomo, 2017).

Generally, the procedure is repeated 30–40 times. At the end, the reaction tube includes about $(2)^{30}$ molecules of the preferred PCR product (Tomo, 2017). Different manual and automated techniques are presented to evaluate the amplified PCR product which the usual is to recognize the product by size after movement through electrophoresis technique on an agarose gel or polyacrylamide gel. By ultraviolet light, PCR products is illuminated and according to the size of the amplified DNA sequence visualize as a single band (Tomo, 2017).

The main advantages of PCR technique are simple quantification, high sensitivity, accuracy, rapid analysis, reproducibility, quality control, and minimum contamination (Tomo, 2017). PCR also lets accurate detection of strains of different microorganisms with different phenotype. Enormous samples can be evaluated at the same time. It is a beneficial tool in the study of strictly anaerobic microorganisms, in which cell death could happen during sampling and transportation, where cell viability is not the important factor in the PCR method (Mendes Silva and Domingues, 2015). As PCR makes it possible to replicate DNA or RNA sequences, several million times, it uses as few as 1–100 cells.

Chapter Three

Materials and Methods

3.1 Study Area

This study conducted in Bethlehem. Five swimming pools (A, B, C, D, & E) were investigated, which are indoor pools. Each swimming pool has type of swimmers . This are all the indoor public pools existing just in Bethlehem.

Swimming pool	Type of swimmer
Swimming pool A	Recreation, womens for all ages
Swimming pool B	Recreation, womens for all ages
Swimming pool C	Education, children less than 10 years
Swimming pool D	Recreation and education, adults girl
Swimming pool E	Excercising, professional training, adults for both sexes

3.2 Sample collection

In this study water samples were collected from five swimming pools located in Bethlehem city. Sample collection was started in the beginning of July until the end of August of 2019, between 10 am and 3 pm. Two samples were obtained monthly; each sample is triplicate from three differient point of the pool that means every pools have 12 samples. Samples were collected from the main water body of pools at 30 cm from the water surface and 40 cm from the pool edge in 1.5 L glass bottles. Sodium thiosulfate salt was added in excess to quench residual chlorine in the sampled water. In total 60 water samples were obtained from each pool and delivered to microbial analysis in the lab as soon as they are collected. In addition 12 samples were collected from filters backwash water for further microbial analysis. At each sampling event, water temperature, pH, swimming pool load (number of bathers) were collected at the site. Samples were

transferred to the lab using an ice box. Water turbidity was measured at the lab as soon as the sample is in the lab.

3.3 Microbiological analysis

3.3.1 Conventional method (HPC)

Bacteriological samples were analyzed by the membrane filter technique, using 0.45µm pore size filters to determine the following parameters:

-total heterotrophic plate count, suitable agar is plate count agar (23.5 g) dissolved in 1000 ml deionized water by heating, then sterilize by autoclaving at 15 lbs pressure and 121 °C for 15 min.

-E. coli, suitable agar is m-Endo agar (51.05g) dissolved in 980ml of deionized water, heat until boiling to dissolve the media completely, and aseptically add 20ml of 95% ethanol(substitute of autoclaving).

-*Pseudomonas aeruginosa*, using molar helton agar which is suitable to detect this type of bacteria, dissolved 38 g in 1000ml of deionized water by heating then sterilize by autoclaving at 15 lbs pressure and 121°C for 15 min (method 8074 membrane filtration, DOC316.53.01224).

Every filtered paper of samples transferred to suitable agar plate and incubation at 35 °C for 24 hours, then counting colonies of bacteria.

3.3.2 Polymerase chain reaction method (PCR)

All samples (1000 ml) were collected from 5 swimming pools in plastic

bottles filtrated with 0.45µm pore size and stored at 4°C for further PCR analysis.

3.3.2.1 DNA extraction

Genomic DNA was extracted from bacterial cells found in water samples in triplicate using standard phenol extraction method followed by ethanol precipitation. The analyzed water samples were centrifuges and to the precipitate a lysis buffer was added. So, extraction was started by the addition of 300µl of DNA lysis buffer (50mM NaCl, 10mM EDTA, 50mMTris-HCL pH 7.4, 1% Triton X-100) to the precipitate followed by addition of 30µl of 10mg/ml proteinase K (Sigma-Aldrich, St. Louis, USA), the mixture then was incubated at 60°C for 2hr. this was followed by addition of 200 µl of phenol solution (pH 8) (Sigma Aldrich, St. Louis, USA) to each samples, extraction was performed by vortex for 1 min

then centrifugation for 3 min at 14000 rpm in micro centrifuge tube. The aqueous upper layer which contained the DNA was collected in a new 1.5 ml Eppendorf tube and DNA was precipitated using 5M NaCl to bring the final salt concentration to 0.2M NaCl. Directly this was followed by the addition of 700 µl of cold 100% ethanol. DNA was recovered after incubating the samples at -20 °C for overnight for maximum precipitation of DNA. On the next day, DNA was recovered from the solution after centrifugation for 10 min at 14000 rpm at 4 °C, the supernatant was discarded and a small pellet was air-dry to remove the remaining alcohol and then was suspended in 100 µl double distilled water (DDW) and kept at -20 °C for further use.

3.3.2.2 Polymerase Chain Reaction (PCR)

All PCR reactions were performed in a total reaction volume of 25µl using ready-mix PCR kit (Syntezza, Jerusalem, Israel). PCR amplification for each indicated system using 20 moles of each direct and reverse primers as they are indicated in Table 1, these primes are general for bacterial DNA amplification based on 16s ribosomal DNA. For each PCR reaction 5µl of the extracted DNA was added. The used temperature profile started at 95°C for 15 min, followed by 35 cycles of 95°C for 30s, 30s at the specified annealing temperature, and 72°C for 1 min, and then concluded with an elongation step at 72°C for 10 min.

Table 5. DNA sequence of the PCR primers that were used for microbiome amplification.

PCR system	Primers*	Tm (°C)
16S rRNA	Direct: CCTACGGGNGGCWGCAG Reverse: AGGACTACHVGGGTATCTAATCC	60

*All used primers synthesized using the below forward and reverse overhang adaptor.

- **Forward adaptor:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG

- **Reverse adaptor:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC

3.3.2.3 Agarose Gel Electrophoreses:

PCR products were run on a 1.5% agarose gel (1.5g agarose, 100ml 1X TAE and 10µl Ethidium bromide). The 50X TAE electrophoresis running buffer (242g Tris base, 57.1 ml glacial acetic acid and 100ml 0.5M EDTA (pH 8.0)). The Gene Ruler 50 bp DNA ladder (Thermo Scientific, # SM0371) was used for sizing PCR amplified products.

3.3.2.3 DNA sequence analysis using next generation sequence method.

For DNA sequence analysis of bacterial DNA that was amplified by PCR, this was done based on Nextera microbiome MiSeq DNA sequence protocol used in detecting micro-biome in water samples. This was done by two steps:

1- First PCR in which bacterial specific primers were used. In this PCR system the bacterial DNA (16s ribosomal gene was amplified) for all possible types of available bacterial cells.

2- Second PCR: In which an adaptor which consists of 8 nucleotide sequence were added to each samples, the addition of these adaptors enabling the exact labeling of each samples for easier sequence recovery later on.

After the second PCR all samples were mixed in one tubes (a library) and they were sent for DNA sequence analysis next generation DNA sequencing (NGS) Service Company (sequencing was done on MiSeq machine using 500 cycle kit from Illumina Co.).

3.3.2.5 Bioinformatics analysis

Raw Illumina sequencing data was generated from all analyzed PCR amplicons as FASTQ files of read1 (forward) and read2 (reverse) for each individual sample. These sequence reads were uploaded to Galaxy platform at (usegalaxy.org) for further sequence processing and analysis (Ref: Afgan et al). Initially raw sequences were filtered for quality control at a phred score of 20 equivalent to 99% confidence of each nucleotide, followed by merging forward and reverse reads, the amplified specific genes were selected according to their specific sequence length and sequence identity. The selected sequence reads from

each soil and plant leaves were analyzed for sequence homology above 97% using BLAST analysis tools in order to determine number of reads related to specific microbiome or fungi operational taxonomic unit.

Chapter four

Result and Discussion

Swimming pools A, B, C, D, and E are indoor swimming pools, and most of the bathers were female, especially swimming pools A, B and D. Pool C was used to give training courses for children from age 2 to 10 years, males and females. Pool E was also a teaching and training pool for students of different ages and both sexes. Swimmers' clothes were not uniform because every swimmer comes with his own swimming clothes, but the children did not wear swimming suits or caps mostly. The area of the pools ranged from 80 m² to 100 m², and the height was from 60 cm to 170 cm. All swimming pools use chlorine in the disinfection process, and it was checked manually whenever necessary, except pool E, which has an automatic system that check and adjust the level of chlorine as necessary (not less than 1 mg/L and not more than 2.5 mg/L). The pH is also related to the concentration of chlorine not less than 7.2 and not more than 7.6, its increase and decrease are directly proportional to the concentration of chlorine added. As for the turbidity factor, we can observe the turbidity of the water, which is associated with an increase or decrease in the concentration of chlorine depending on the number of swimmers and the extent to which the pool is managed as should be for water circulation or the efficiency of filtration. So the detection of bacteria associated with bathers' number and turbidity.

The microbiological quality of the swimming pools was considered acceptable/unacceptable according to the Healthy and Human Services (WHO, 2006) and the Palestinian standard (Shabieb, 2006), see table 3.

4.1 Results

4.1.1 Cultural method

The results of the measured water quality parameters during sampling and the results of bacteriological test are shown in tables 6, 7, 8, 9, and 10. A total of 60 samples of swimming pools were collected in two months and included 3 types of bacteria.

The microbiological results were varied for the same sample taken under the same conditions and also for other samples of the same pool. We observed the presence of the microbe despite the presence of chlorine in the standard concentration as well as the degree of acidity. The results showed a sharp rise in bacterial counts.

We note from table 6 the result of Pool A that the degree of acidity and chlorine was among the required standards, while the turbidity was the required standards except for samples A10, A11 and A12, where it was high. But the number of bathers that were detected when taking the sample showed that the bathers number are large in samples A4, A5 and A6, which give high number of colony of plate count agar (PCA). On the other hand, we see that a small number of swimmers are matched by high bacterial growth in A10, A11 and A12 samples for plate count agar (PCA), but we find that the turbidity was high, and in this sample give positive of *E.coli*. But in A2 *Pseudomonace aeruginosa* appeared in two colony. The difference in the bacteria reading of the sample taken under the same conditions is due to the different points from which the water was taken, where the bathers number concentrated in site than another like A5 less colony than A4 and A6.

Table 6. Measurement value of the assessed parameters in swimming pool A

Sample	Date	pH	Residual Chlorine ppm	Turbidity (NTU)	Bathers	<i>P. aeruginosa</i>	Plate Count Agar (PCA)	<i>E. coli</i>
A1	9 July	7.2	2.0	1.91	7	None	1	None
A2	9 July	7.2	2.0	1.74	7	2	4	None
A3	9 July	7.2	2.0	1.94	7	None	1	None
A4	12 July	7.4	3.0	1.21	70	None	28	None

A5	12 July	7.4	3.0	1.22	70	None	7	None
A6	12 July	7.4	3.0	1.23	70	None	Full	None
A7	2 August	7.3	2.5	1.71	15	None	3	None
A8	2 August	7.3	2.5	1.66	15	None	2	None
A9	2 August	7.3	2.5	1.73	15	None	4	None
A10	19 August	7.2	1.5	16.6	10	None	40	2
A11	19 August	7.2	1.5	18.5	10	None	30	None
A12	19 August	7.2	1.5	17.0	10	None	18	None

Measurement units: pH: no unit; Residual chlorine: mg/L; Turbidity: NTU; TC and FC: MPN/100mL; *Pseudomonas aeruginosa*, *E.coli* and PCA: cfu/mL.

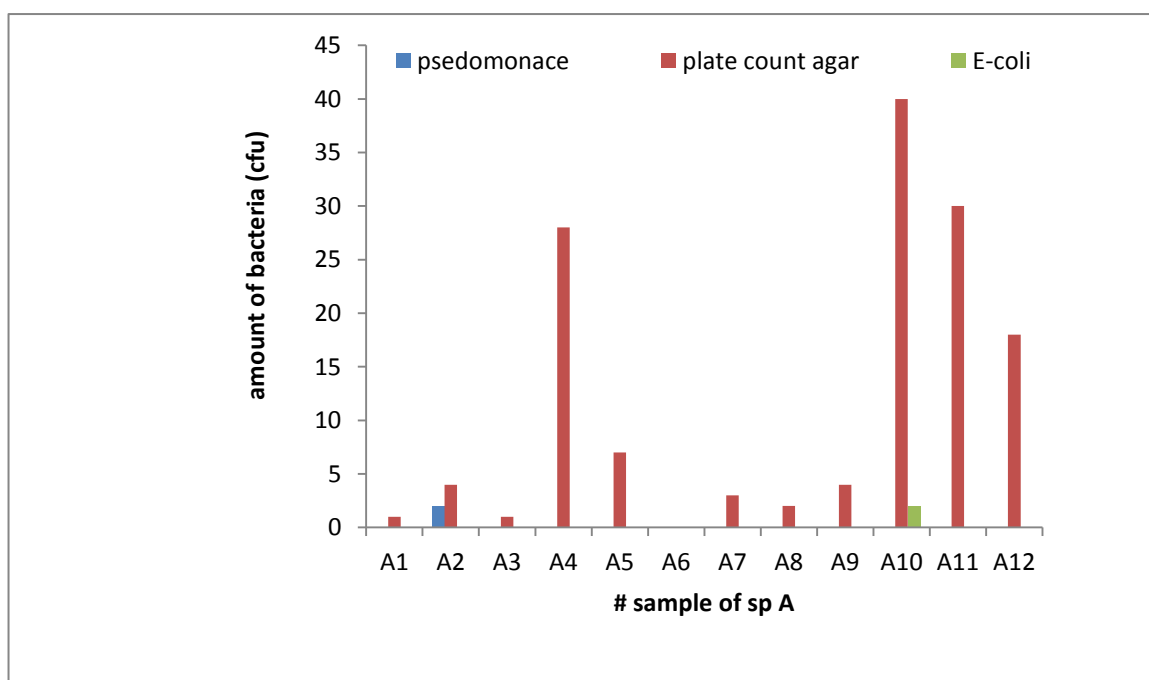


Fig 1. Amount of bacteria of swimming pool A

The samples of pool B are shown in Table 7 as well as figure. 2 showing the difference between the appearances of bacteria in the samples. From the results shown the acidity and chlorine were acceptable to the required standard without sample number B10,B11, and B12 which are acidic because the cholrine residual not adjusted,but the turbidity was required standard at all samples. Bather numbers was high at B1, B2 and B3 also the number of colony in plate count agar (PCA) are high

for these sample, but for B9 the PCA is higher, we observed the turbidity of the water is the maximum for all samples. The presented of *Pseudomonas aeruginosa* and *E. coli* bacteria only in one sample in B5 of *Pseudomonas aeruginosa* and B3 of *E. coli* bacteria. Figure 2 shows the difference between the three types of bacteria in Pool B.

Table 7. Measurement value of the assessed parameters in swimming pool B

Sample	Date	pH	Residual Chlorine ppm	Turbidity (NTU)	Bathers	<i>P.aeruginosa</i>	Plate Count Agar (PCA)	<i>E- coli</i>
B1	9 July	7.2	3	1.34	32	None	6	None
B2	9 July	7.2	3	1.76	32	None	10	3
B3	9 July	7.2	3	1.79	32	None	1	None
B4	12 July	7	1	0.67	8	None	None	None
B5	12 July	7	1	3.03	8	2	6	None
B6	12 July	7	1	0.64	8	None	3	None
B7	2 August	7	3	0.71	10	None	4	None
B8	2 August	7	3	2.99	10	None	5	None
B9	2 August	7	3	3.87	10	None	18	None
B10	19 August	Acidic	0.1	1.99	0	None	2	None
B11	19 August	Acidic	0.1	2.02	0	None	1	None
B12	19 August	Acidic	0.1	2.04	0	None	4	None

Measurement units: pH: no unit; Residual chlorine: mg/L; Turbidity: NTU; TC and FC: MPN/100mL; *Pseudomonas aeruginosa*, *E.coli* and PCA: cfu/mL.

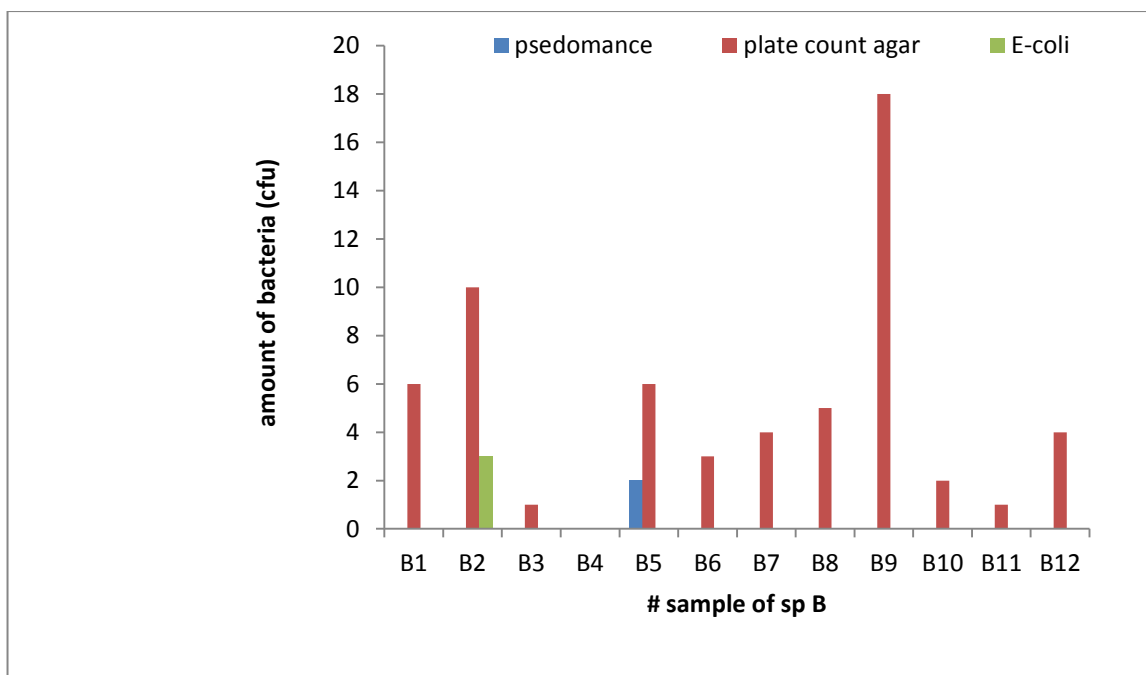


Fig 2. Amount of bacteria of swimming pool B

The result of pool C in table 8 show that the acidity, residual chlorine and turbidity were acceptable to the required standard. In this pool, the swimmers are children who can't control themselves, for that the children swimming pools more contaminate, so we must special work. We observed from the table 8 the number of bather high at sample C4, C5, and C6 and the *Pseudomonace aeruginosa* presented at this sample (C4 & C5), also *E.coli* appearance at C5. But plate count agar (PCA) presented at all samples and the high one at C10 which no bathers and all the factors are acceptable, so this contamination from children who uses this pool before take sample. See figure 3 showing the appearance of these types at different rates.

Table 8. Measurement value of the assessed parameters in swimming pool C

Sample	Date	pH	Residual Chlorine ppm	Turbidity (NTU)	Bathers	<i>P. aeruginosa</i>	Plate Count Agar (PCA)	<i>E- coli</i>
C1	9 July	7.4	2.0	1.95	14	1	2	None
C2	9 July	7.4	2.0	1.92	14	None	1	None
C3	9 July	7.4	2.0	2.01	14	None	1	None
C4	12 July	7.3	3.0	1.67	20	1	2	None
C5	12 July	7.3	3.0	1.40	20	1	4	1
C6	12 July	7.3	3.0	1.74	20	None	3	None
C7	2 August	7.2	3.0	0.97	10	3	3	None
C8	2 August	7.2	3.0	1.15	10	None	5	None
C9	2 August	7.2	3.0	1.22	10	3	3	None

C10	19 August	7.4	3.0	0.68	0	2	15	None
C11	19 August	7.4	3.0	1.07	0	2	6	None
C12	19 August	7.4	3.0	0.7	0	2	10	None

Measurement units: pH: no unit; Residual chlorine: mg/L; Turbidity: NTU; TC and FC: MPN/100mL; *Pseudomonas aeruginosa*, *E.coli* and PCA: cfu/mL.

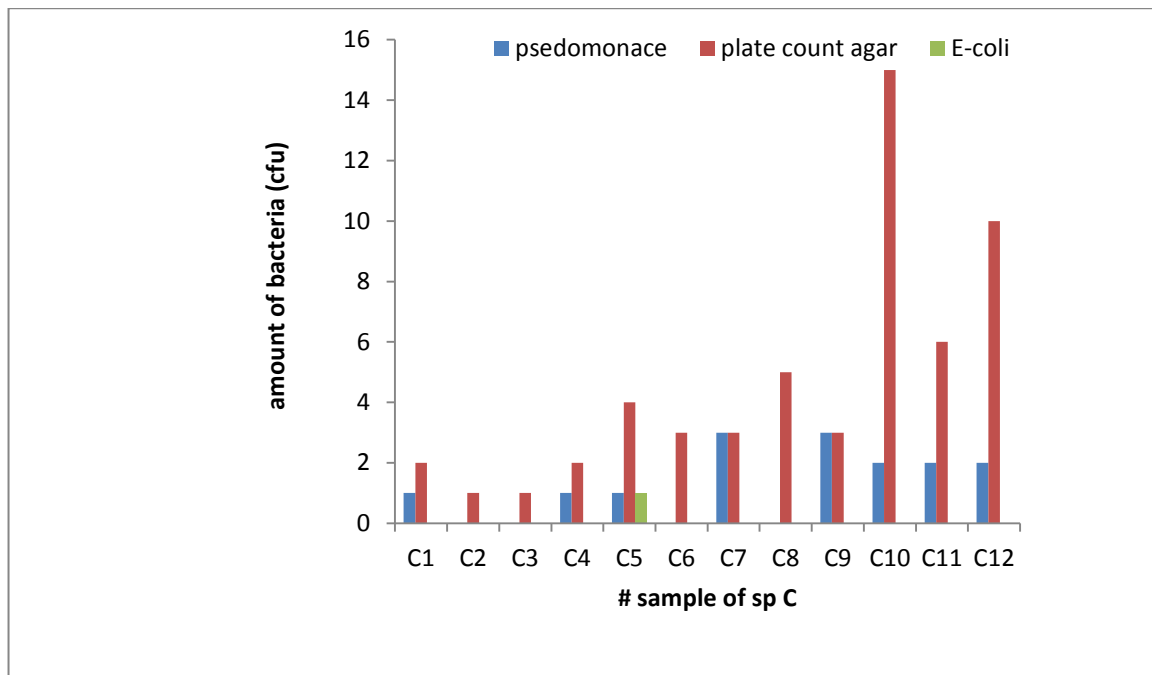


Fig 3. Amount of bacteria of swimming pool C

The pool D result show at table 9 . In this pool the bathers are just women, who are the physiology of women varies greatly in terms of the type of exudate and its nature, in addition women use creams and fragrances more often than in men and children, so the amount of contamination high than other. We observed the the acidity and residual chlorine acceptable to require standerd, but the turbidity high at D10, D11 and D12 especially no bathers which means maybe the water circulation not efficiency or the water filtration not effective, and at D7, D8 and D9 the turbidity is highest. Overall samples give positive plate count agar (PCA) without D5, and *Psedomonace aeruginisa* presented in D1, D2, and D3 also *E.coli* presented at D1and D2, that returne to the number of bathers and at the same time the PCA also high, even that the turbidity is high, so the level of contamination high at this sample, but the explaining of D3 is the different of the point when take the sample.

Table 9. Measurement value of the assessed parameters in swimming pool D

Sample	Date	pH	Residual Chlorine ppm	Turbidity (NTU)	Bathers	<i>P. aeruginosa</i>	Plate Count Agar (PCA)	<i>E- coli</i>
D1	9 July	7.0	1.0	4.66	30	7	35	70
D2	9 July	7.0	1.0	4.52	30	2	7	5
D3	9 July	7.0	1.0	4.55	30	3	4	None
D4	12 July	7.2	2.5	2.97	12	None	4	None
D5	12 July	7.2	2.5	3.05	12	2	None	none
D6	12 July	7.2	2.5	3.00	12	1	2	None
D7	2 August	7.6	3.0	11.00	12	None	17	None
D8	2 August	7.6	3.0	12.80	12	None	5	None
D9	2 August	7.6	3.0	14.10	12	None	12	None
D10	19 August	7.2	2.3	3.86	0	None	4	None
D11	19 August	7.2	2.3	4.46	0	None	11	None
D12	19 August	7.2	2.3	4.35	0	None	6	None

Measurement units: pH: no unit; Residual chlorine: mg/L; Turbidity: NTU; TC and FC: MPN/100mL; *Pseudomonas aeruginosa*, *E.coli* and PCA: cfu/mL.

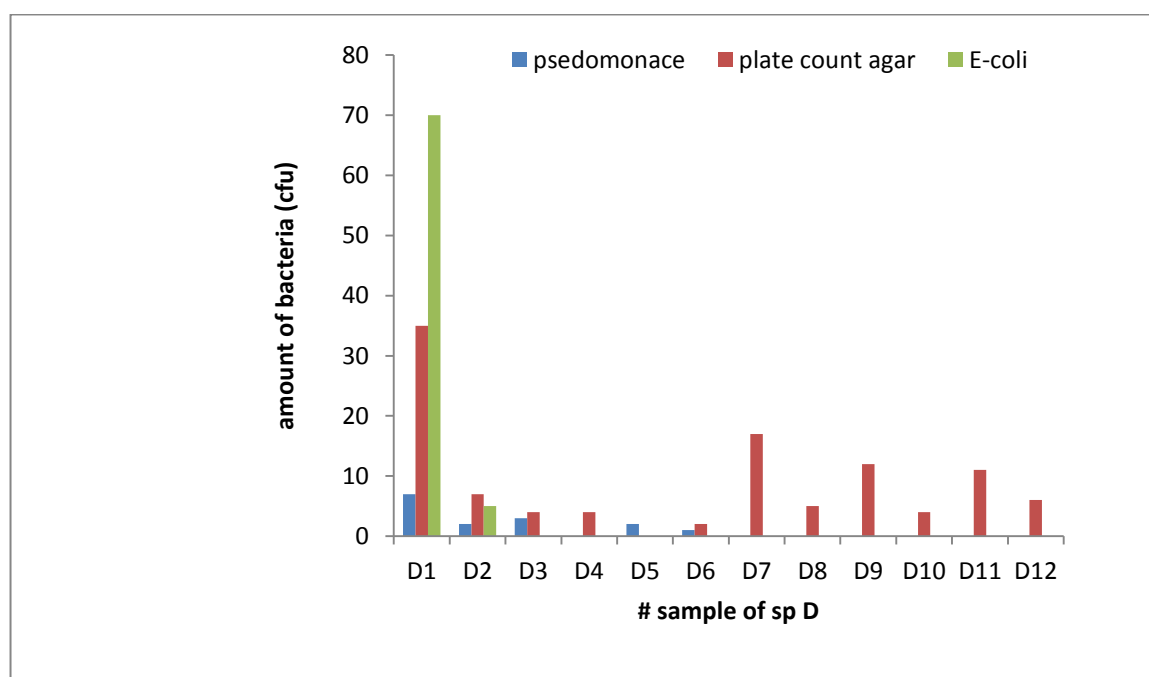


Fig 4. Amount of bacteria of swimming pool D

Finally, pool E users are from professional bathers who applied the swimming standard. In table 10 show the acidity and chlorine are acceptable of required standard, also the turbidity is low expected the E7, E8 and E9 were high and the bather number of the same sample also high which gives apposite sample of PCA and negative for *Pseudomonas aeruginosa* and *E.coli*. *Pseudomonas aeruginosa* presented in E.2 and *E.coli* is negative at all samples. We see diagram 5 showing the appearance of the three types of bacteria.

Table 10. Measurement value of the assessed parameters in swimming pool E

Sample	Date	pH	Residual Chlorine Ppm	Turbidity (NTU)	Bathers	<i>P. aeruginosa</i>	Plate Count Agar (PCA)	<i>E- coli</i>
E1	9 July	7.2	1.5	0.62	15	None	9	None
E2	9 July	7.2	1.5	0.70	15	2	2	None
E3	9 July	7.2	1.5	0.67	15	None	2	None
E4	12 July	7.2	1.5	0.96	10	None	15	None
E5	12 July	7.2	1.5	1.17	10	None	5	None
E6	12 July	7.2	1.5	1.42	10	None	17	None
E7	2 August	7.2	1.5	6.13	40	None	12	None
E8	2 August	7.2	1.5	4.29	40	None	10	None
E9	2 August	7.2	1.5	7.53	40	None	6	None
E10	19 August	7.2	1.5	2.22	20	None	8	None
E11	19 August	7.2	1.5	2.00	20	None	7	None
E12	19 August	7.2	1.5	2.14	20	None	3	None

Measurement units: pH: no unit; Residual chlorine: mg/L; Turbidity: NTU; TC and FC: MPN/100mL; *Pseudomonas aeruginosa*, *E.coli* and PCA: cfu/mL.

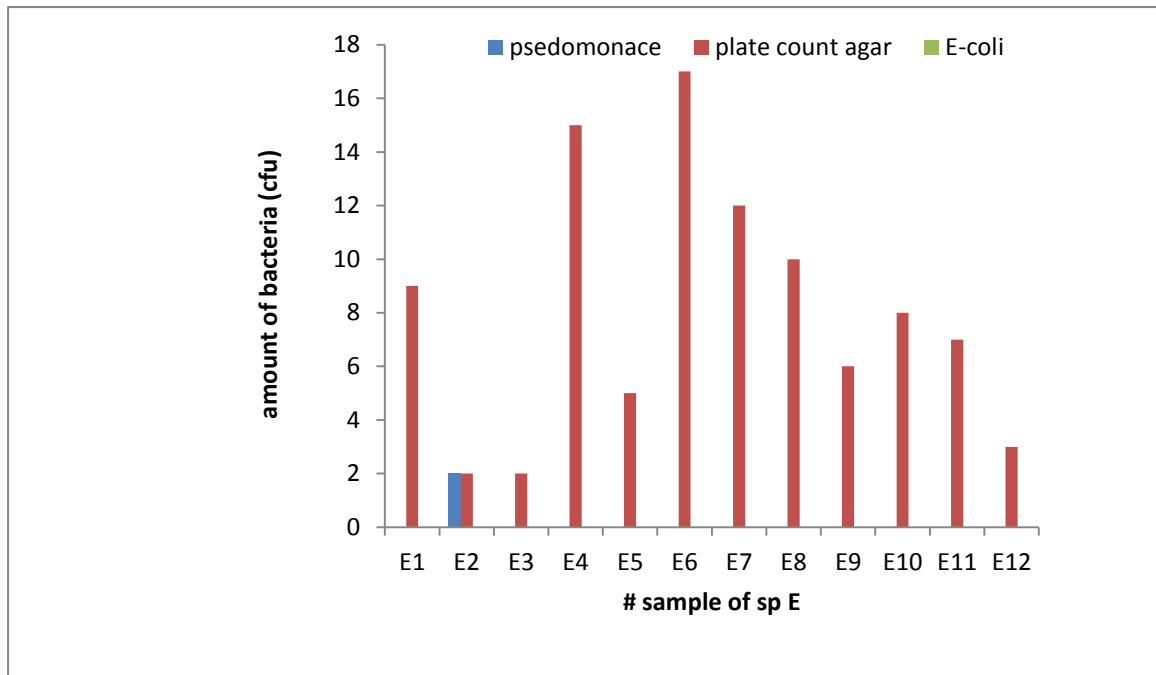


Fig 5. Amount of bacteria of swimming pool E

4.1.2 PCR results

Each time successful amplification was tested at 1.5% for bacteria. Where Figure 5 showed the results of electrophoresis of the bacterial 16S rRNA system.

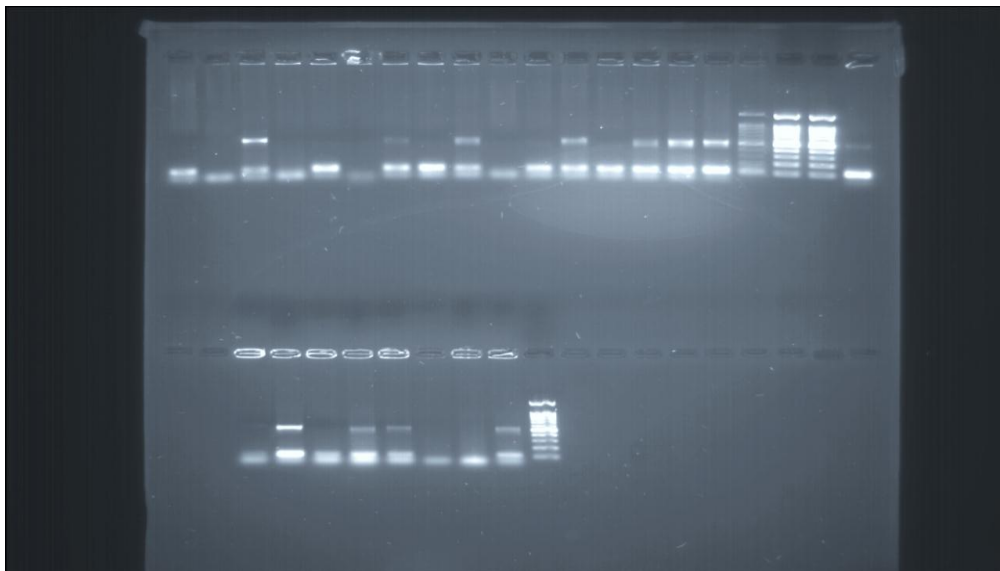


Fig 6. Agarose gel electrophoresis analysis of amplified microbiome 16s rDNA from random pools water samples collected.

The use of the amplification process in this research was for the purpose of confirming the result of our cultural method and clarifying the nature, source and concentration of pollutants, so the process of evaluation of the pool was based on a

strong reference, explaining the preparation of bacteria and their types in a detailed way. As a result, we note that all pools are positive of bacteria and different types, most of which are due to the origin of bacteria from the soil or water itself.

Note in Figure 7 that the all pools have shown bacteria readings are different, but keep the Pool D is the most polluted high resolutions bacteria and the Pool E is the most system for low readings bacteria have we so agree with what has been explained in the cultural method.

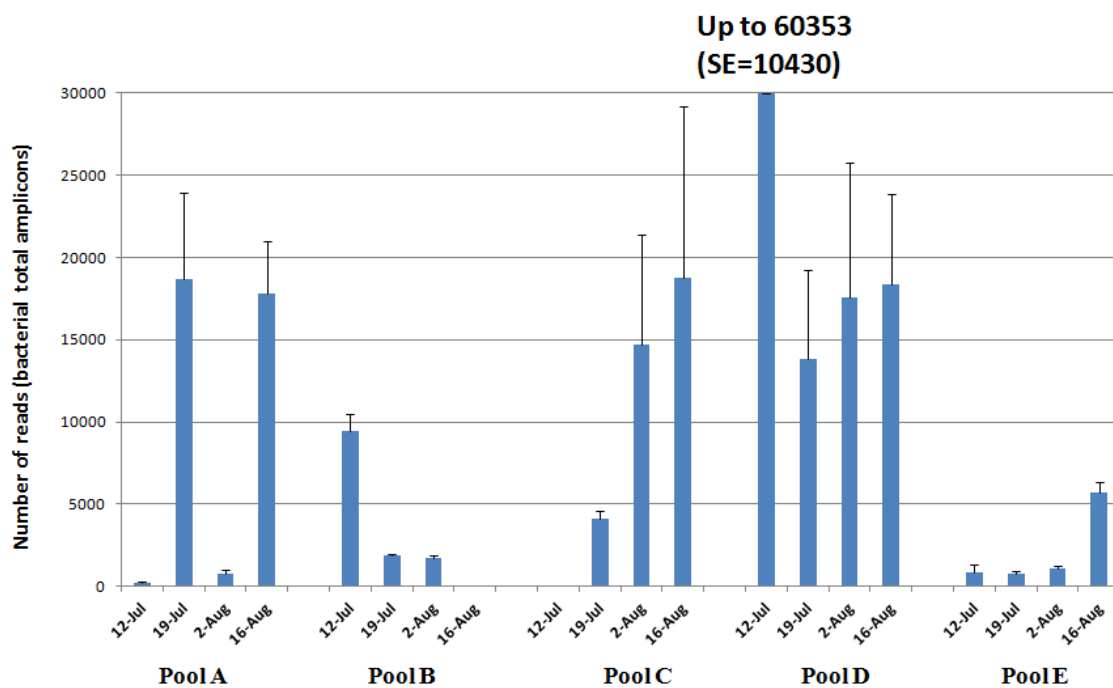
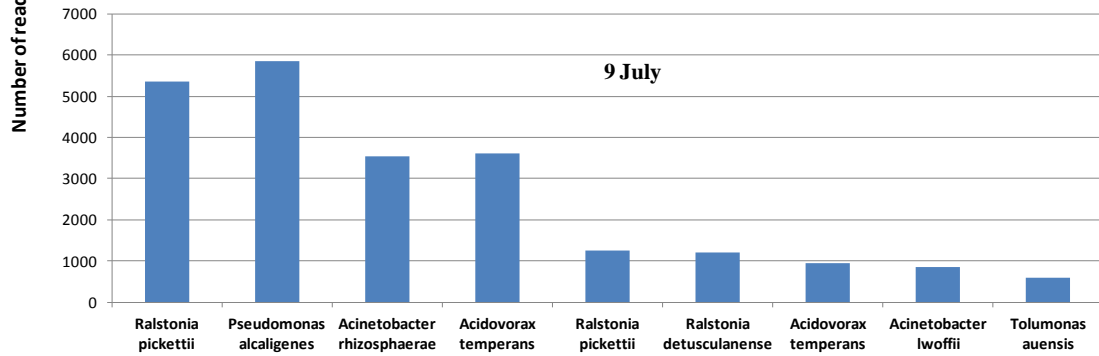
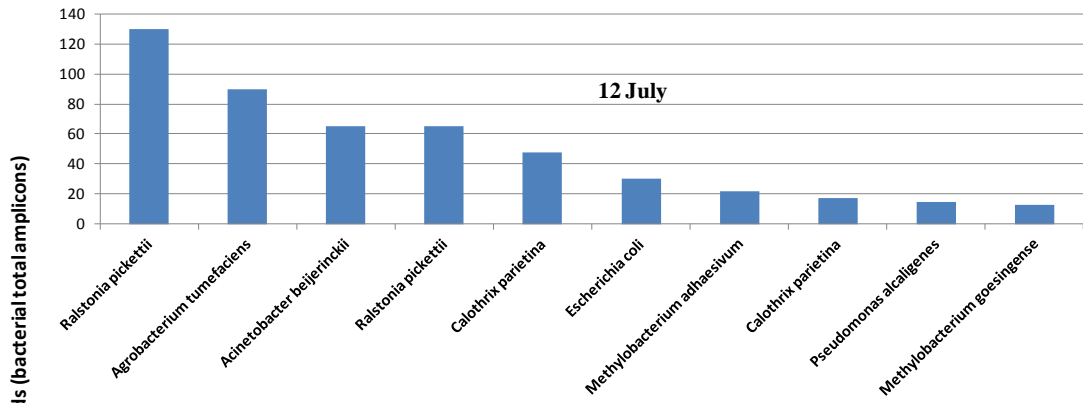


Fig 7: A histogram showing the total number of bacterial reads representing the total number of bacterial amplicons in studies pool over the indicated sample collection time.

Analysis of the samples and to clarify the kinds of bacteria that currency shown by Figure 8 on stages four to sample the Pool A, we note the emergence of a few *Escherichia coli* and *Pseudomonas aeruginosa*, the most visible are of the original water and soil is considered non-harmful.



Number of reads (bacterial total amplicons)

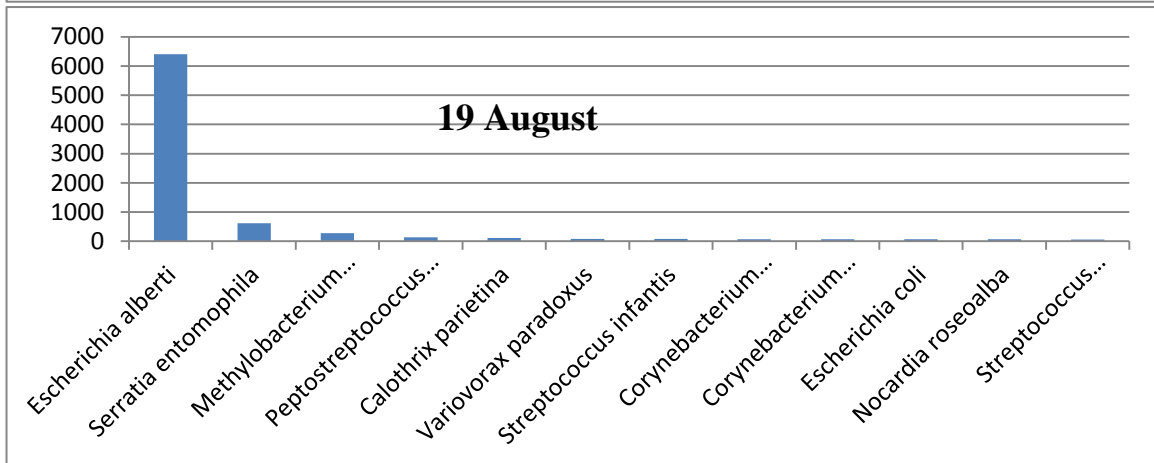
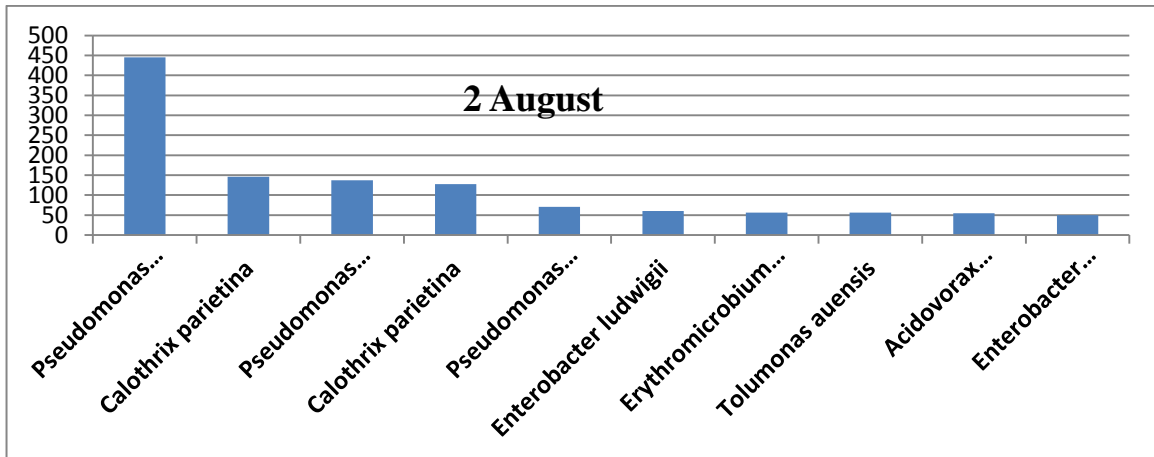


Figure 8: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from water samples that were collected at the above indicated time. **(Pool A)**

Another analysis of pool B gives a low reads of *E. coli* and not appearance of *Pseudomonas aeruginosa* see that in figure 9

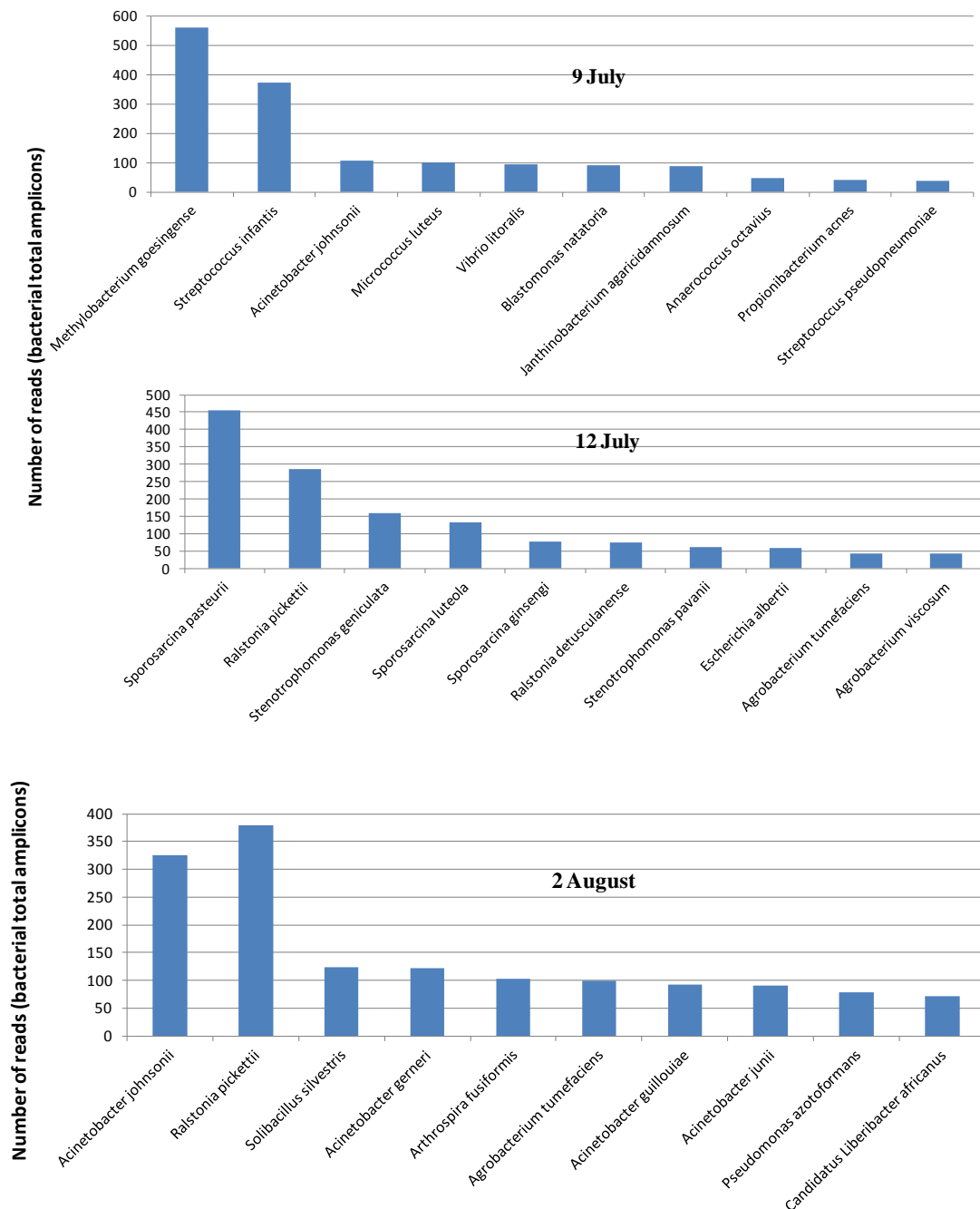


Figure 9: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from water samples that were collected at the above indicated time. **(Pool B)**

Analysis of Pool C samples in Figure 10 gives us high readings for the presence of *E. coli* bacteria in the 12 July samples and in the 2 August samples, but *Pseudomonas aeruginosa* appeared with few readings in 12 July and 2 August, in 19 August all of bacterial species were of soil and plant species.

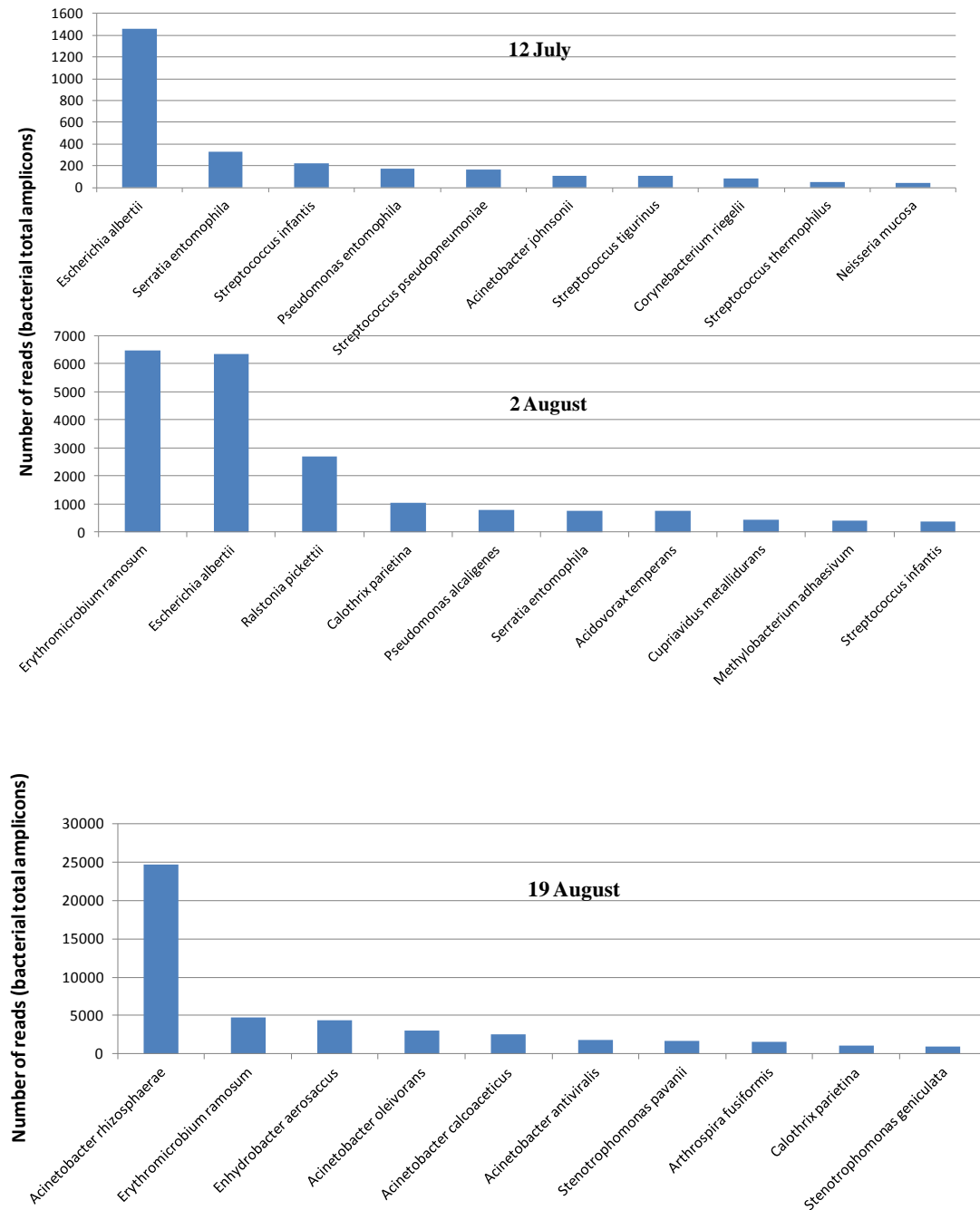


Figure 10: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from water samples that were collected at the above indicated time. (Pool C)

Pool D shows a detail of the readings of bacteria that appeared by analysis (figure 11), and is considered one of the most polluted among the pools the appearance of bacteria with very high readings, was the appearance of *Pseudomonas aeruginosa* in medium to small amounts, but the large readings of bacteria are of natural source.

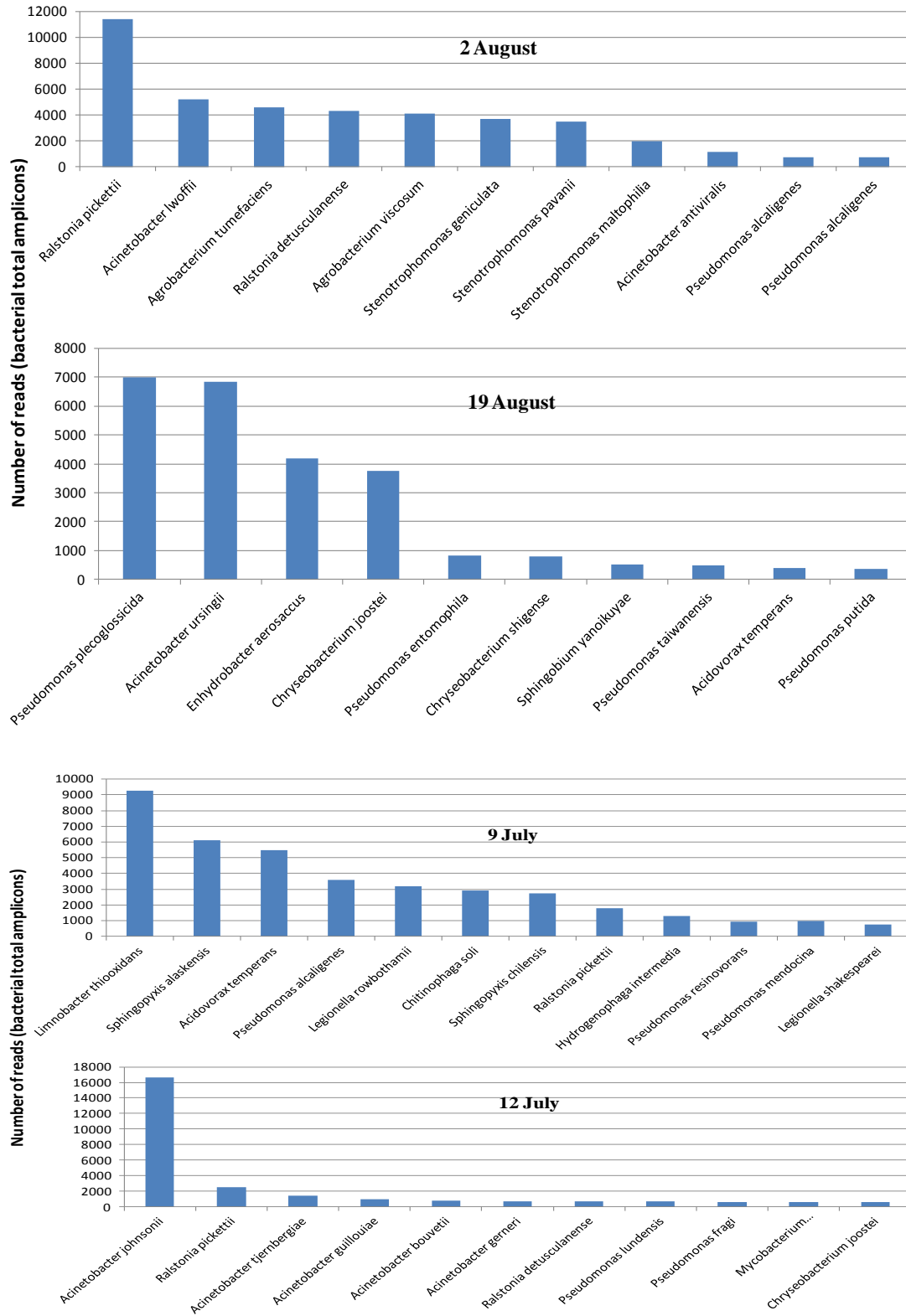
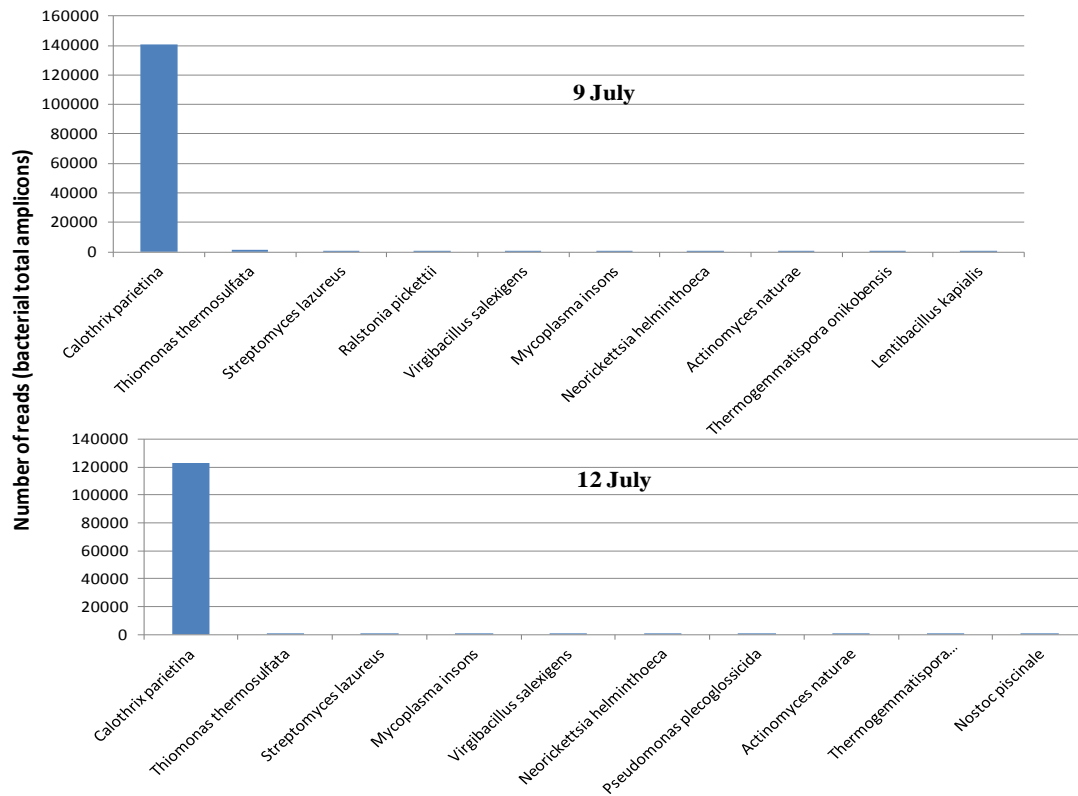


Figure 11: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from water samples that were collected at the above indicated time. **(Pool D)**

Swimming pool E as shown in Figure 12 is the cleanest due to the low readings of bacteria, as it has not recorded any case infected with *E. coli* or *Pseudomonas aeruginosa* and keeps the types of bacteria that have appeared are of the types of fresh water.



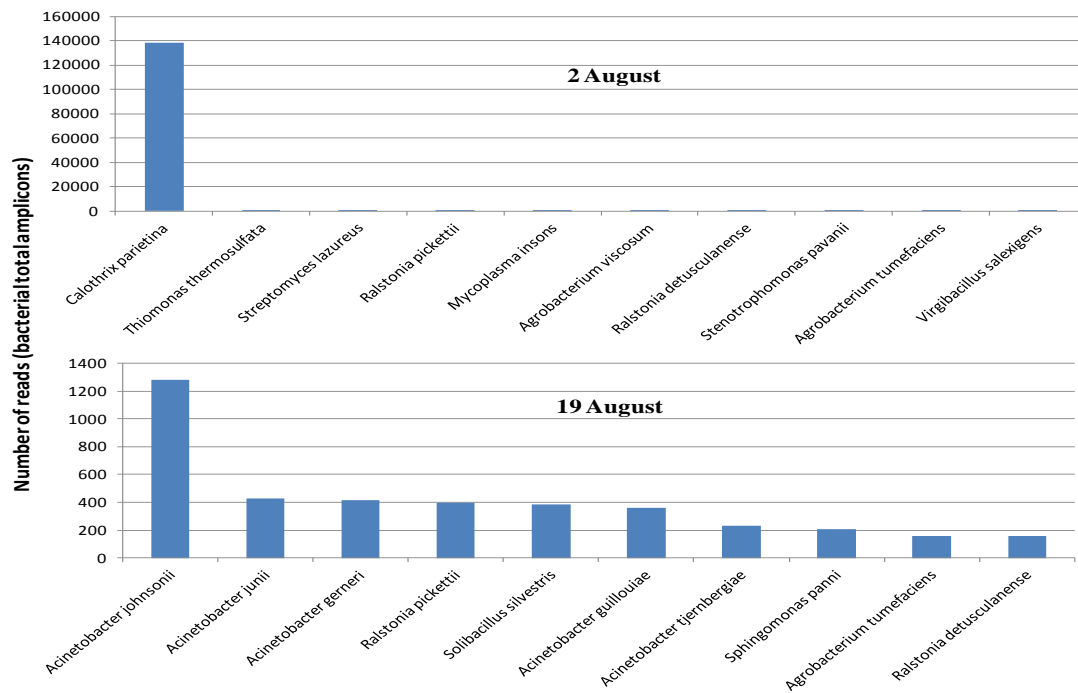


Figure 12: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from water samples that were collected at the above indicated time. **(Pool E)**

The result of backwash samples:

Backwash gives a general survey of the pool and the type of microbes that passed the pool and were disposed of it confirms the passage of this type of bacteria, but this analysis shown especially bacteria.

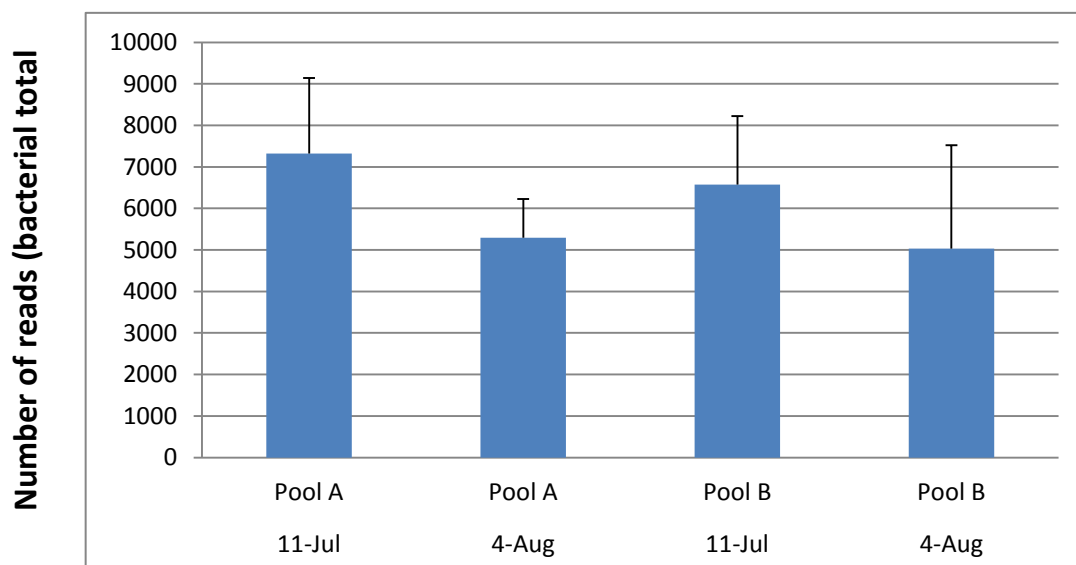


Figure 13: A histogram showing the total number of bacterial reads representing the total number of bacterial amplicons recovered from pool A and B backwash samples at the indicated time periods.

The bacteria readings were high in pools A and B as we note figure 13, and this is considered normal because the water is originally contaminated, but the types of bacteria that existed inside this pool are shown in Figure 14 of Pool A, which shows the appearance of *E. coli* and *pseudomonas* both times to take samples. This examination supports the samples of Pool A, which gave readings of the appearance of these types of bacteria, which confirms their presence inside the pool and their passage with filters. In the case of Pool B, neither *E. coli* nor *pseudomonas* appeared in them (Figure 15), while the *E. coli* bacteria appeared in very small numbers in the examination of Pool B water samples (figure 9).

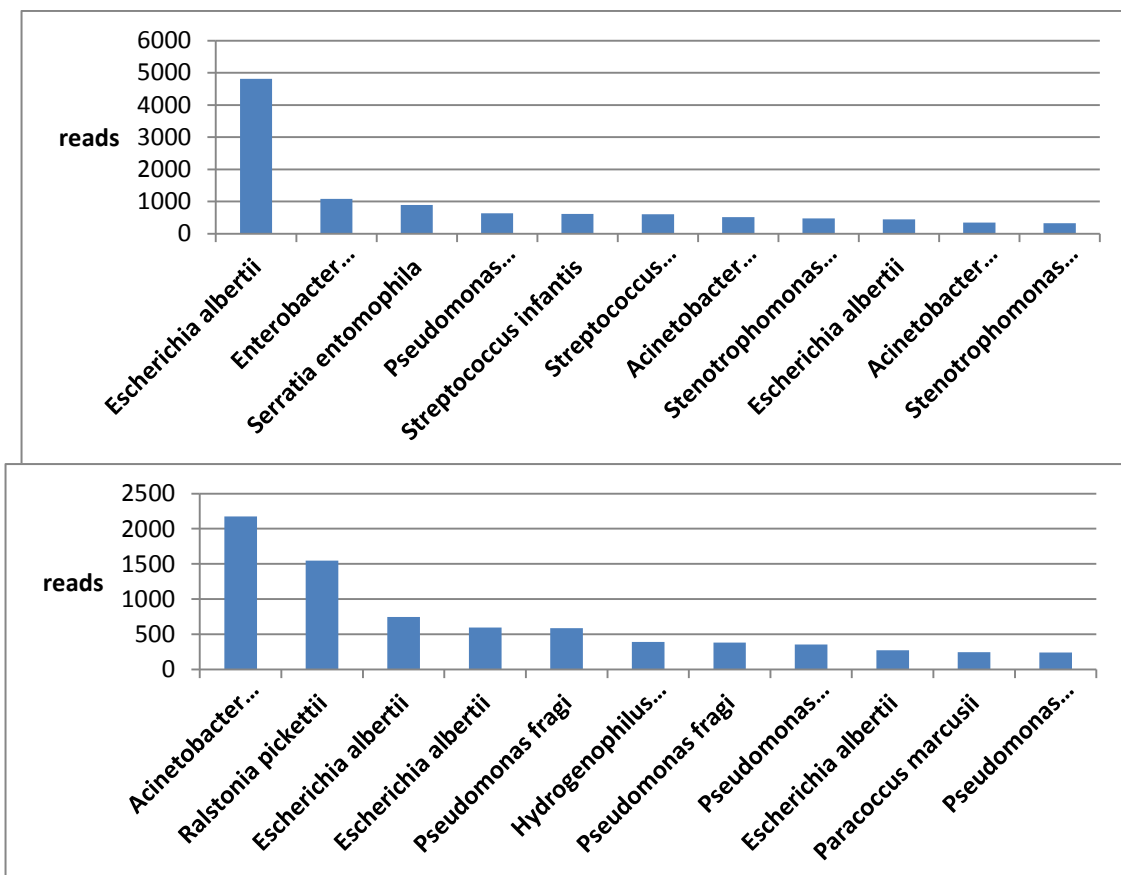


Figure 14: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from backwash water samples that were collected at the above indicated time. **(Pool A)**

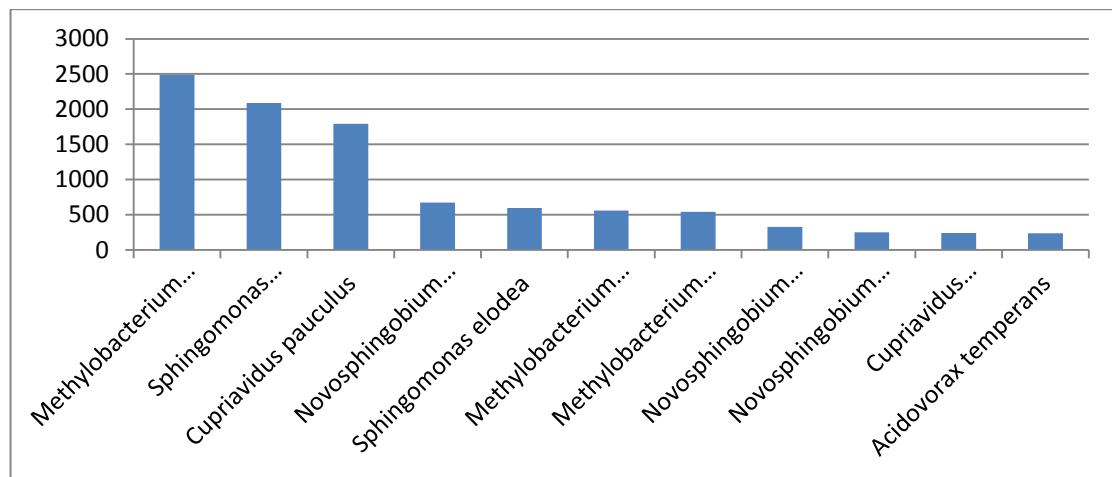
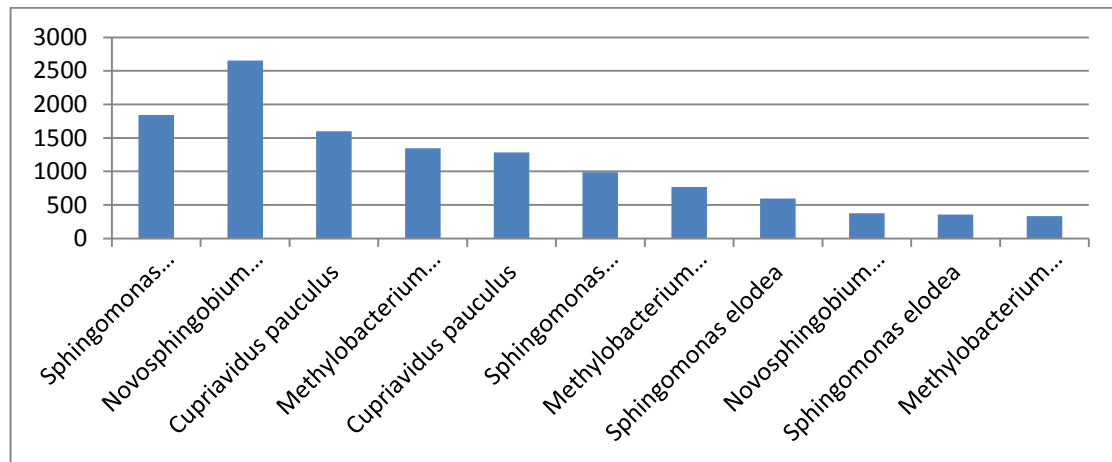


Figure 15: The most common bacterial species (only the most 10 abundant species were shown) that were found based on DNA sequence analysis from backwash water samples that were collected at the above indicated time. **(Pool B)**

Discussion

In this study, we observed that physical and chemical parameters may be higher or lower than approved levels and may be completely identical, so this cannot be considered a major cause of microbial contamination. There are studies that have linked water quality to microbiological indicators and their association with physical and chemical factors irregular chlorine, lack of filtration and high numbers of swimmers from the causes of swimming pool pollution (Wei et al., 2018). There are other factors that may increase the pollution of swimming pools and the rise of the microbial rate in them, for example swimmers enter the pools without bathing immediately before entering which is an important factor in the increase of microbes and the work of chemical pollution or the like, this swimming pool may be full of germs that there are people who suffer from certain secretions that are potentially harmful and pathogenic, in addition to creams and similar substances used in the hair and body, which will go directly to the water and affect its nature and purity. We also add to this not to wear special clothes for swimming, especially in adult users because this type of clothing is specially designed to not leak secretions during swimming such as vaginal discharge and feces, which results in the worst types of bacteria that cause diseases and carriers, but the biggest reason is from the swimmer himself for poor management of himself during swimming pool as well as the pool supervisor it is imperative to clarify this to users and give them tips.

Previous studies have shown that mismanaged swimming pools could transmit waterborn disease. The main study in Greec carried out 374 swimming pool inspections according WHO standared, including 48 swimming pools in the West Bank of Palestine and all samples were unacceptable to the WHO standared and all samples contaminated at least one microbial indicater, 37% were contaminated with two or more indicators and only 34.1% had acceptable water (Papadopoulou et al.,2007)

According to the principles followed by the Palestinian Health and the World Health Organization, the pH in the pools ranged from 7 to 7.4 and is acceptable in all cases, except Pool B when taking the sample 10, 11 and 12, the water was acidic and not able to swimming and used on this day for swimming

purposes until it is calibrated with chlorination, we notice the high acidity was offset by a sharp decrease in chlorine, where the chlorine by adjusting it works to adjust the pH they are related to each other. This sharp decrease in acidity leads to irritation of the skin and eyes and the appearance of skin spots for swimmers if used. For turbidity, it exceeded the recommended limit in Pool A in samples 9, 10, and 11, that means the filter circulation not efficiency or can related it to number of bathers like pool D in samples D7 and D9, that loads 40 bathers, and the efficiency of chlorine that affects the turbidity, the remaining samples were compliant with the standards and did not exceed them (Table 5).

Based on the data from the bacterial culture process, we found that the numbers of bacteria larger when they were grown in plate count agar in all pools, but the bacteria *E. coli* and *Pseudomonas aeruginosa* range from appearance to absence.

Pool A: the appearance of *Pseudomonas aeruginosa* was in only one sample (8.3% of the samples), as well as *E. coli* bacteria in one sample (8.3% of the samples), while 100% of plate count agar of the samples appeared to contain bacteria in general (table 6).

Pool B: the appearance of *Pseudomonas aeruginosa* and *E. coli* bacteria is about 8.3% of samples, quite the opposite in plate count agar gives 8.3% of negative samples (table 7).

Pool C: the appearance of *E.coli* is about 8.3% of samples, but *Pseudomonas aeruginosa* appeared elevated about 66% of infected sample, and stays the plate count agar higher counts (table 8).

Pool D: it was an appearance of *E.coli* about 16.6% of samples, while *Pseudomonas aeruginosa* 40% of samples, while the plate count agar stay higher (92% of samples) (table 9).

Pool E: it was clean of *E.coli* and had 8.3% of *Pseudomonas aeruginosa*, but 100% of samples were appearance of plate count agar (table 10).

Here we emphasize the emergence of these bacteria by polymerase chain reaction, based on microbial sequencing data it was clearly shown that there is a differences in bacterial content of each of the studied pools, in general the most contaminated pool was (pool D), and the most clean pool was (pool E). The following is analytical description of the bacterial load in each pool and the kinetic of bacterial cell accumulation during the study period.

Pool A: Two high beaks were seen at two different intervals and each following a significant drop in bacterial load. These beaks were seen to be after an intense use of this swimming pool. Most of the found bacterial species were of soil of plant leaf origin (*Ralstonia*, *Agrobacterium*, *Pseudomonas sp.*, *Serratia entomophila*, and others). Also it was noted the presence of some bacterial cells that are limited to fresh water (*Calothrix*). The presence of these bacterial cells is obvious from the surrounding environment (soil and plants). On the other hand at the end of the season; *E. coli* was reported to be among the highest bacterial cells.

Pool B: Although the total number of bacterial count was less than pool A, but still the same type of bacterial cells was reported to be in this pool. Of the most important pathogenic bacteria species that were reported are (*Vibrio sp.*, *Corynebacterium*, *Neisseria*, and *mycoplasma*), these pathogenic bacterial cells were reported in a low numbers. This indicates that they are non-dangerous to cause infection, but it is worth to examine their source.

Pool C: This pool was arranged as the second most contaminated pool, even the contamination was increasing in consequence collection time. Bacterial count started by less than 5000 reads and ended by about 20,000 reads. Similarly, most the revealed bacterial species were of soil and plant species, and less occurrence of medically important species. *E. coli*, was never seen in this pool, which indicates a clean source of water.

Pool D: This was the most contaminated pool over all the study period, bacterial count started by more than 60,000 reads at the beginning of the study and ended by 20,000 reads; both reads are higher than the occurrence of bacterial cells in all other studied pools. According to the indicated bacterial species most of these species were of natural source (soil and plants), as well as fresh water

species. It could be the location and the cleaning times of this pool affecting their way of contamination.

Pool E: This pool was the most clean concerning bacterial count, and most of the identified bacterial species are of fresh water type. No occurrence of E. coli; which indicates no contamination from sewage source.

Conclusion and recommendation

In conclusion, the prevention of risks from swimming pools you need to adjust several aspects and make them interconnected with each depends on the other, the design and construction of the pond must follow the established requirements when you start the installation. As well as the requirements for the quality of the water used and the physical, chemical and biological standards of the pool water and their periodic follow-up. The most important public health and safety requirements that explain to users the conditions to be followed before using the pool and when entering it, and thus improve the attitudes that in turn reduce the risk of transmission of infection and associated risks as a result of the knowledge and awareness of users to ensure a safer and healthier environment inside the pools, for example take a shower before entering the pool significantly reduces the entry of contaminants.

In addition, the assessment of the microbial safety of the water in the swimming pools examined relied heavily on the presence of *E. coli* and *Pseudomonas aeruginosa* in the pool water samples, which turned out that it was necessary to update the general management of the pools and properly treat the water to prevent the transmission of diseases. We note that the results in this research showed that the Pool E is the cleanest, and the reason for this is that the pool has followed all the guidelines of Public Health and safety and applied all methods to reduce the risk of microbes and their transmission, and type of the bathers who is competitive swimmers, but we add that the only difference between this pool and the rest of the swimming pools that have been examined is the use of a computer to check the level of chlorine in the pool water, which means that it is set electronically, the level of chlorine when it decreases in the pool water is adjusted immediately, it does not allow the microbe to remain in the pool water for a period of time but is eliminated immediately if it is in the water. As for the rest of the swimming pools, the tester was manually during a certain period of time not less than two hours except in cases where the number of swimmers is very large, we explain that during the period in which the examination of the pool may produce bacteria that take their time in the pool until the level of chlorine is adjusted.

We recommend greater control on swimming pools, swimmers and supervisors inside swimming pools by applying the procedures to be followed before and during swimming, as well as the adoption of electronic examination of chlorine level and acidity level and make it within the requirements of Public Health and safety and thus significantly reduce the appearance and transmission of microbes.

After the study, it was indicated that all the five swimming pools sampled were contaminated especially with *Pseudomonas*, *Staphylococcus sp.*, *Enterococci*, legionella, Total and *Faecal* coliform. Also the study revealed the difference in bacteriological quality of swimming pool water and the relation of personal hygiene of swimmers and the number of bacteria isolated in the pool. The effective management can control potential adverse health consequences that can be associated with the use of unsafe recreational water environments. So the Guidelines are intended to be used as the basis for the development of approaches to controlling the hazards that may be encountered in recreational water environments, there is a need to develop improved prevention and control to provide safe recreational waters and to prevent the illness from contaminated recreational waters that requires a comprehensive approach which includes the control and remediation of environmental contamination, improved training of pool managers and maintenance staff, and enhanced health education activities.

The facility operators play a key role and are responsible for the good operation and management of the recreational water environment, and can play an important role in ensuring pool safety through public education and providing appropriate and targeted information to pool users. National legislation may include different sets of regulations that will apply to swimming pools. Regulation may control, for example, the design and construction of pools, their operation and management and control of substances hazardous to health.

Appendix A

Type of bacteria	Reads
Ralstonia pickettii	130
Agrobacterium tumefaciens	90
Acinetobacter beijerinckii	65
Ralstonia pickettii	65
Calothrix parietina	48
Escherichia coli	30
Methylobacterium adhaesivum	22
Calothrix parietina	17
Pseudomonas alcaligenes	15
Methylobacterium goesingense	13
Ralstonia pickettii	5356
Pseudomonas alcaligenes	5841
Acinetobacter rhizosphaerae	3549
Acidovorax temperans	3604
Ralstonia pickettii	1250
Ralstonia detusculanense	1222
Acidovorax temperans	962
Acinetobacter lwoffii	850
Tolumonas auensis	591
Pseudomonas alcaligenes	445
Calothrix parietina	146
Pseudomonas plecoglossicida	137
Calothrix parietina	128
Pseudomonas balearica	71
Enterobacter ludwigii	60
Erythromicrobium ramosum	56
Tolumonas auensis	56
Acidovorax temperans	55
Enterobacter hormaechei	50
Escherichia alberti	6402
Serratia entomophila	618
Methylobacterium goesingense	276
Peptostreptococcus stomatis	141
Calothrix parietina	113
Variovorax paradoxus	78
Streptococcus infantis	76
Corynebacterium durum	74
Corynebacterium accolens	72
Escherichia coli	69
Nocardia rosealba	65
Streptococcus pseudopneumoniae	61

Appendix

Type of bacteria	Reads
Methylobacterium goesingense	563
Streptococcus infantis	375
Acinetobacter johnsonii	109
Micrococcus luteus	102
Vibrio litoralis	94
Blastomonas natatoria	92
Janthinobacterium agaricidamnosum	89
Anaerococcus octavius	47
Propionibacterium acnes	41
Streptococcus pseudopneumoniae	38
Sporosarcina pasteurii	455
Ralstonia pickettii	285
Stenotrophomonas geniculata	160
Sporosarcina luteola	134
Sporosarcina ginsengi	78
Ralstonia detusculanense	76
Stenotrophomonas pavanii	62
Escherichia albertii	59
Agrobacterium tumefaciens	44
Agrobacterium viscosum	43
Acinetobacter johnsonii	325
Ralstonia pickettii	379
Solibacillus silvestris	123
Acinetobacter gernerii	122
Arthrospira fusiformis	102
Agrobacterium tumefaciens	99
Acinetobacter guillouiae	93
Acinetobacter junii	90
Pseudomonas azotoformans	79
Candidatus Liberibacter africanus	71

Appendix C

Type of bacteria	Reads
Escherichia albertii	1461
Serratia entomophila	327
Streptococcus infantis	222
Pseudomonas entomophila	170
Streptococcus pseudopneumoniae	169
Acinetobacter johnsonii	110
Streptococcus tigurinus	110
Corynebacterium riegellii	85
Streptococcus thermophilus	50
Neisseria mucosa	45
Erythromicrobium ramosum	6490
Escherichia albertii	6350
Ralstonia pickettii	2678
Calothrix parietina	1051
Pseudomonas alcaligenes	784
Serratia entomophila	756
Acidovorax temperans	755
Cupriavidus metallidurans	452
Methylobacterium adhaesivum	420
Streptococcus infantis	377
Acinetobacter rhizosphaerae	24653
Erythromicrobium ramosum	4724
Enhydrobacter aerosaccus	4388
Acinetobacter oleivorans	3011
Acinetobacter calcoaceticus	2556
Acinetobacter antiviralis	1829
Stenotrophomonas pavanii	1626
Arthrospira fusiformis	1565
Calothrix parietina	1110
Stenotrophomonas geniculata	1009

Appendix D

12-Jul	Reads
<i>Limnobacter thiooxidans</i>	9251
<i>Sphingopyxis alaskensis</i>	6116
<i>Acidovorax temperans</i>	5496
<i>Pseudomonas alcaligenes</i>	3577
<i>Legionella rowbothamii</i>	3166
<i>Chitinophaga soli</i>	2918
<i>Sphingopyxis chilensis</i>	2720
<i>Ralstonia pickettii</i>	1798
<i>Hydrogenophaga intermedia</i>	1274
<i>Ralstonia detusculanense</i>	1213
<i>Pseudomonas resinovorans</i>	947
<i>Sphingomonas oligophenolica</i>	750
<i>Legionella shakespearei</i>	745
<i>Pseudomonas mendocina</i>	493
<i>Acinetobacter guillouiae</i>	464
<i>Oxalobacter vibrioformis</i>	379
<i>Acinetobacter gernerii</i>	360
<i>Mycobacterium frederiksbergense</i>	353
<i>Caulobacter crescentus</i>	349
<i>Hyphomicrobium aestuarii</i>	298
<i>Delftia tsuruhatensis</i>	291
<i>Limnobacter litoralis</i>	255
<i>Hydrocarboniphaga daqingensis</i>	253
<i>Paracoccus carotinifaciens</i>	248
<i>Calothrix parietina</i>	244
<i>Sphingopyxis panaciterrae</i>	237
<i>Variovorax boronicumulans</i>	213
<i>Rhodobacter gluconicum</i>	210
<i>Chryseobacterium joostei</i>	200
<i>Pseudomonas pseudoalcaligenes</i>	188
<i>Pseudoxanthomonas mexicana</i>	187
<i>Acinetobacter lwoffii</i>	175
<i>Paracoccus marcusii</i>	165
<i>Delftia lacustris</i>	165
<i>Novosphingobium mathurense</i>	158
<i>Methylobacterium goesingense</i>	154
<i>Shinella granuli</i>	146
<i>Methyloversatilis universalis</i>	142
<i>Erythromicrobium ramosum</i>	138
<i>Sphingopyxis ginsengisoli</i>	134
<i>Phenylobacterium mobile</i>	133
<i>Novosphingobium acidiphilum</i>	109
<i>Pseudomonas lundensis</i>	102

<i>Ralstonia insidiosa</i>	94
<i>Blastomonas natatoria</i>	92
<i>Tenacibaculum litopenaei</i>	89
<i>Acinetobacter tjernbergiae</i>	87
<i>Thiobacillus thiophilus</i>	77
<i>Leptothrix discophora</i>	68
<i>Chryseobacterium shigense</i>	67
<i>Sphingomonas sanxanigenens</i>	66
<i>Caulobacter henricii</i>	66
<i>Pseudomonas xanthomarina</i>	65
<i>Hydrogenophaga pseudoflava</i>	63
<i>Pseudoxanthomonas japonensis</i>	61
<i>Pseudomonas azotoformans</i>	60
<i>Sphingomonas panni</i>	59
<i>Acidovorax wohlfahrtii</i>	57
<i>Segetibacter aerophilus</i>	55
<i>Sphingomonas hunanensis</i>	53
<i>Novosphingobium lentum</i>	52
<i>Acinetobacter beijerinckii</i>	52
<i>Thiomonas thermosulfata</i>	50
<i>Ochrobactrum pecoris</i>	48
<i>Arthrospira fusiformis</i>	48
<i>Escherichia albertii</i>	44
<i>Brevundimonas staleyii</i>	43
<i>Amaricoccus kaplicensis</i>	43
<i>Enhydrobacter aerosaccus</i>	42
<i>Nocardia roseoalba</i>	41
<i>Acinetobacter antiviralis</i>	41
<i>Legionella taurinensis</i>	40
<i>Kaistobacter terrae</i>	40
<i>Phenylobacterium lituiforme</i>	38
<i>Sphingopyxis witflariensis</i>	36
<i>Acinetobacter indicus</i>	35
<i>Rhodovibrio sodomensis</i>	33
Microvirus Enterobacteria phage PhiX174	33
<i>Caulobacter tundrae</i>	32
<i>Caulobacter vibrioides</i>	30
<i>Acinetobacter xiamenensis</i>	30
<i>Sphingomonas insulae</i>	29
<i>Serratia entomophila</i>	29
<i>Methylobacterium marchantiae</i>	29
<i>Legionella waltersii</i>	29
<i>Thalassospira tepidiphila</i>	27
<i>Roseospira visakhapatnamensis</i>	27
<i>Pseudomonas oleovorans</i>	27

<i>Acinetobacter johnsonii</i>	27
<i>Sphingopyxis taejonensis</i>	26
<i>Ectothiorhodospira haloalkaliphila</i>	26
<i>Aminobacter aminovorans</i>	26
<i>Niastella koreensis</i>	25
<i>Hydrogenophaga defluvii</i>	24
<i>Flavobacterium succinicans</i>	24
<i>Pseudomonas guineae</i>	23
<i>Paracoccus homiensis</i>	22
<i>Neisseria mucosa</i>	22
<i>Ammonifex thiophilus</i>	22
19-Jul	Reads
<i>Acinetobacter johnsonii</i>	16654
<i>Ralstonia pickettii</i>	2497
<i>Acinetobacter tjernbergiae</i>	1421
<i>Acinetobacter guillouiae</i>	1023
<i>Acinetobacter bouvetii</i>	765
<i>Acinetobacter generi</i>	738
<i>Ralstonia detusculanense</i>	716
<i>Mycobacterium frederiksbergense</i>	658
<i>Chryseobacterium joostei</i>	575
<i>Pseudomonas lundensis</i>	519
<i>Pseudomonas fragi</i>	441
<i>Pseudomonas mendocina</i>	371
<i>Pseudomonas lutea</i>	338
<i>Agrobacterium tumefaciens</i>	257
<i>Pseudoxanthomonas mexicana</i>	221
<i>Escherichia albertii</i>	185
<i>Ectothiorhodospira haloalkaliphila</i>	176
<i>Arthrosira fusiformis</i>	143
<i>Erwinia mallotivora</i>	132
<i>Chryseobacterium shigense</i>	102
<i>Hydrogenophaga pseudoflava</i>	101
<i>Acinetobacter beijerinckii</i>	100
<i>Shinella granuli</i>	88
<i>Yersinia nurmii</i>	81
<i>Serratia entomophila</i>	81
<i>Ancylobacter aquaticus</i>	76
<i>Ralstonia insidiosa</i>	74
<i>Yersinia ruckeri</i>	69
<i>Bdellovibrio bacteriovorus</i>	67
<i>Pseudomonas viridiflava</i>	63
<i>Methylobacterium goesingense</i>	63
<i>Enhydrobacter aerosaccus</i>	62
<i>Calothrix parietina</i>	60
<i>Chitinophaga ginsengisoli</i>	59

<i>Pseudomonas azotoformans</i>	58
<i>Brevundimonas staleyi</i>	57
<i>Stenotrophomonas geniculata</i>	54
<i>Corynebacterium callunae</i>	51
<i>Pseudomonas putida</i>	48
<i>Caulobacter vibrioides</i>	46
<i>Agrobacterium viscosum</i>	42
<i>Rhodococcus qingshengii</i>	39
<i>Blastomonas natatoria</i>	36
<i>Pseudomonas xanthomarina</i>	34
<i>Moraxella caviae</i>	31
<i>Pseudomonas alcaligenes</i>	30
<i>Stenotrophomonas pavanii</i>	29
<i>Acinetobacter antiviralis</i>	28
<i>Veillonella parvula</i>	27
<i>Caulobacter crescentus</i>	27
<i>Shinella yambaruensis</i>	26
<i>Pseudoxanthomonas japonensis</i>	26
<i>Mycobacterium hackensackense</i>	26
<i>Sphingobium yanoikuyae</i>	25
<i>Chryseobacterium indologenes</i>	25
<i>Xanthobacter polyaromaticivorans</i>	24
<i>Pseudomonas abietaniphila</i>	23
<i>Acinetobacter schindleri</i>	23
<i>Aggregatibacter aphrophilus</i>	22
<i>Ochrobactrum pseudogrignonense</i>	21
<i>Acinetobacter haemolyticus</i>	21
<i>Neisseria mucosa</i>	20
<i>Geothrix fermentans</i>	20
<i>Rathayibacter caricis</i>	18
<i>Acinetobacter lwoffii</i>	18
<i>Shewanella baltica</i>	17
<i>Pseudomonas graminis</i>	17
<i>Cupriavidus metallidurans</i>	17
<i>Streptococcus vestibularis</i>	16
<i>Sphingomonas panni</i>	16
<i>Rhizobium alamii</i>	15
<i>Pseudomonas parafulva</i>	15
<i>Phenylobacterium lituiforme</i>	15
<i>Mycobacterium smegmatis</i>	15
<i>Bradyrhizobium liaoningense</i>	15
<i>Variovorax paradoxus</i>	14
<i>Thiomonas thermosulfata</i>	14
<i>Streptococcus gordonii</i>	14
<i>Stenotrophomonas maltophilia</i>	14
<i>Pseudomonas entomophila</i>	14

Microvirus Enterobacteria phage PhiX174	14
Haemophilus parainfluenzae	14
Fusobacterium periodonticum	14
Streptococcus infantis	13
Enterobacter soli	12
Actinomyces naturae	12
Acinetobacter gyllenbergii	12
Sphingobacterium faecium	11
Pseudomonas taiwanensis	11
Pseudomonas benzenivorans	11
Streptococcus pseudopneumoniae	10
Rhodococcus baikonurensis	10
Pseudomonas plecoglossicida	10
Pectinatus cerevisiiphilus	10
Flavobacterium soli	10
Erwinia papaya	10
Erwinia billingiae	10
Enterobacter hormaechei	10
Sphingomonas oligophenolica	9
2-Aug	Reads
Ralstonia pickettii	11384
Acinetobacter lwoffii	5211
Agrobacterium tumefaciens	4600
Ralstonia detusculanense	4277
Agrobacterium viscosum	4118
Stenotrophomonas geniculata	3691
Stenotrophomonas pavanii	3475
Stenotrophomonas maltophilia	1962
Acinetobacter antiviralis	1151
Agrobacterium larrymoorei	859
Pseudomonas alcaligenes	707
Acinetobacter indicus	364
Pseudomonas resinovorans	325
Achromobacter insolitus	277
Ralstonia insidiosa	207
Xanthomonas oryzae	178
Calothrix parietina	153
Shinella fusca	144
Chitinophaga ginsengisoli	129
Brevundimonas diminuta	112
Methylobacterium goesingense	109
Escherichia albertii	109
Agromyces salentinus	89
Caulobacter crescentus	85
Shinella yambaruensis	81

<i>Ectothiorhodospira haloalkaliphila</i>	69
<i>Caulobacter vibrioides</i>	68
<i>Serratia entomophila</i>	66
<i>Phenylobacterium lituiforme</i>	63
<i>Brevundimonas terrae</i>	63
<i>Achromobacter arsenitoxydans</i>	55
<i>Thermomonas brevis</i>	49
<i>Luteimonas lutimaris</i>	49
<i>Pseudomonas entomophila</i>	48
<i>Oxalobacter vibrioformis</i>	40
<i>Acinetobacter johnsonii</i>	33
Microvirus Enterobacteria phage PhiX174	32
<i>Thiomonas thermosulfata</i>	31
<i>Aurantimonas manganoxydans</i>	29
<i>Agrobacterium albertimagni</i>	29
<i>Sphingobium yanoikuyae</i>	28
<i>Acidovorax temperans</i>	26
<i>Pectinatus cerevisiiphilus</i>	25
<i>Stenotrophomonas koreensis</i>	24
<i>Stenotrophomonas chelatiphaga</i>	23
<i>Microbacterium paludicola</i>	22
<i>Brevundimonas olei</i>	22
<i>Pseudomonas plecoglossicida</i>	21
<i>Pseudomonas lundensis</i>	21
<i>Comamonas kerstersii</i>	21
<i>Ochrobactrum rhizosphaerae</i>	19
<i>Acinetobacter gernerii</i>	19
<i>Thermomonas dokdonensis</i>	17
<i>Sphingomonas oligophenolica</i>	17
<i>Pedobacter kwangyangensis</i>	17
<i>Acinetobacter psychrotolerans</i>	17
<i>Niabella soli</i>	16
<i>Hydrogenophilus denitrificans</i>	15
<i>Corynebacterium appendicis</i>	15
<i>Mycetocola lacteus</i>	14
<i>Lautropia mirabilis</i>	14
<i>Agrococcus terreus</i>	14
<i>Actinomyces naturae</i>	14
<i>Pseudochrobactrum saccharolyticum</i>	13
<i>Dokdonella fugitive</i>	13
<i>Acinetobacter tjernbergiae</i>	13
<i>Agrobacterium undicola</i>	12
<i>Streptococcus infantis</i>	11
<i>Pseudomonas pseudoalcaligenes</i>	11
<i>Mesorhizobium camelthorni</i>	11

Enhydrobacter aerosaccus	11
Chitinophaga soli	11
Burkholderia ubonensis	11
Streptococcus tigurinus	10
Pseudomonas stutzeri	10
Pedomicrobium ferrugineum	10
Bradyrhizobium pachyrhizi	10
Bartonella rochalimae	10
Thermogemmatispora onikobensis	9
Streptococcus pseudopneumoniae	9
Sporosarcina pasteurii	9
Luteibacter anthropi	9
Brevundimonas staleyii	9
Tolomonas auensis	8
Rhizobium alarii	8
Pseudomonas parafulva	8
Pseudomonas azotoformans	8
Myroides odoratimimus	8
Burkholderia brasiliensis	8
Agromyces ramosus	8
Acinetobacter bouvetii	8
Streptomyces lazareus	7
Sphingomonas insulae	7
Pseudomonas benzenivorans	7
Gallionella ferruginea	7
Curtobacterium pusillum	7
Caulobacter segnis	7
Bradyrhizobium liaoningense	7
Arthrospira fusiformis	7
16-Aug	
Pseudomonas plecoglossicida	6983
Acinetobacter ursingii	6833
Enhydrobacter aerosaccus	4182
Chryseobacterium joostei	3754
Pseudomonas entomophila	837
Chryseobacterium shigense	785
Sphingobium yanoikuyae	518
Pseudomonas taiwanensis	489
Acidovorax temperans	399
Pseudomonas putida	359
Acinetobacter guillouiae	345
Calothrix parietina	281
Acinetobacter gernerii	260
Acinetobacter baumannii	216
Pseudomonas alcaligenes	177
Acinetobacter antiviralis	173

<i>Acinetobacter baylyi</i>	161
<i>Moraxella caviae</i>	144
<i>Chryseobacterium indologenes</i>	135
<i>Acinetobacter rhizosphaerae</i>	130
<i>Enterobacter hormaechei</i>	128
<i>Pseudomonas mosselii</i>	108
<i>Acinetobacter johnsonii</i>	105
<i>Ralstonia pickettii</i>	101
<i>Enterobacter amnigenus</i>	97
<i>Brevundimonas terrae</i>	80
<i>Ralstonia detusculanense</i>	74
Microvirus Enterobacteria phage PhiX174	72
<i>Cupriavidus pauculus</i>	62
<i>Pseudomonas lundensis</i>	60
<i>Stenotrophomonas retroflexus</i>	59
<i>Acinetobacter xiamenensis</i>	53
<i>Streptococcus infantis</i>	51
<i>Acinetobacter tjernbergiae</i>	51
<i>Pseudomonas mendocina</i>	49
<i>Methylobacterium mesophilicum</i>	46
<i>Pseudomonas resinovorans</i>	42
<i>Acinetobacter beijerinckii</i>	42
<i>Sphingomonas oligophenolica</i>	41
<i>Pseudomonas monteilii</i>	38
<i>Enterobacter asburiae</i>	38
<i>Streptococcus pseudopneumoniae</i>	35
<i>Erwinia billingiae</i>	30
<i>Streptococcus tigurinus</i>	28
<i>Chryseobacterium aquifrigidense</i>	28
<i>Acinetobacter oleivorans</i>	28
<i>Sphingomonas insulae</i>	26
<i>Pseudomonas panipatensis</i>	26
<i>Psychrobacter halophilus</i>	25
<i>Novosphingobium hassiacum</i>	25
<i>Pseudomonas teessidea</i>	24
<i>Chryseobacterium caeni</i>	23
<i>Escherichia albertii</i>	22
<i>Acinetobacter gyllenbergii</i>	22
<i>Variovorax boronicumulans</i>	21
<i>Enterobacter nickellidurans</i>	21
<i>Thiomonas thermosulfata</i>	20
<i>Chryseobacterium luteum</i>	20
<i>Acinetobacter soli</i>	20
<i>Novosphingobium nitrogenifigens</i>	19
<i>Stenotrophomonas pavanii</i>	18

<i>Caulobacter crescentus</i>	18
<i>Acinetobacter psychrotolerans</i>	18
<i>Acinetobacter bouvetii</i>	18
<i>Chryseobacterium oranimense</i>	17
<i>Janthinobacterium agaricidamnosum</i>	16
<i>Acinetobacter calcoaceticus</i>	16
<i>Fusobacterium gonidiaformans</i>	15
<i>Agrobacterium tumefaciens</i>	15
<i>Novosphingobium subterraneum</i>	14
<i>Methylobacterium longum</i>	14
<i>Agrobacterium viscosum</i>	14
<i>Rhodoferax antarcticus</i>	13
<i>Pseudomonas stutzeri</i>	13
<i>Pseudomonas moraviensis</i>	13
<i>Burkholderia brasilensis</i>	13
<i>Stenotrophomonas geniculata</i>	12
<i>Pseudomonas pseudoalcaligenes</i>	12
<i>Pseudomonas metavorans</i>	12
<i>Cupriavidus campinensis</i>	12
<i>Chryseobacterium vrystaatense</i>	11
<i>Acinetobacter haemolyticus</i>	11
<i>Sphingomonas dokdonensis</i>	10
<i>Pseudomonas clemancea</i>	10
<i>Pseudomonas benzenivorans</i>	9
<i>Pseudomonas balearica</i>	9
<i>Methyloversatilis universalis</i>	9
<i>Methylobacterium radiotolerans</i>	9
<i>Tolumonas auensis</i>	8
<i>Psychrobacter phenylpyruvicus</i>	8
<i>Luteimonas terricola</i>	8
<i>Flavobacterium succinicans</i>	8
<i>Clostridium akagii</i>	8
<i>Chryseobacterium formosense</i>	8
<i>Virgibacillus salexigens</i>	7
<i>Serratia entomophila</i>	7
<i>Nitrincola lacisaponensis</i>	7
<i>Neisseria mucosa</i>	7
<i>Klebsiella oxytoca</i>	7

Appendix E

12-Jul	Reads
<i>Calothrix parietina</i>	140407
<i>Thiomonas thermosulfata</i>	1467
<i>Streptomyces lazareus</i>	211
<i>Ralstonia pickettii</i>	123
<i>Virgibacillus salexigens</i>	69
<i>Mycoplasma insons</i>	55
<i>Neorickettsia helminthoeca</i>	45
<i>Actinomyces naturae</i>	29
<i>Thermogemmatispora onikobensis</i>	27
<i>Lentibacillus kapialis</i>	26
<i>Ralstonia detusculanense</i>	23
<i>Atopobium fossor</i>	23
<i>Nostoc piscinale</i>	21
<i>Trichodesmium tenue</i>	12
Microvirus Enterobacteria phage PhiX174	12
<i>Desulfuromusa succinoxidans</i>	11
<i>Thiohalorhabdus denitrificans</i>	10
<i>Maricaulis indicus</i>	10
<i>Thermogemmatispora foliorum</i>	7
<i>Mesorhizobium camelthorni</i>	7
Candidatus <i>Liberibacter africanus</i>	7
<i>Thermodesulfovibrio thiophilus</i>	5
<i>Thermodesulfatator atlanticus</i>	5
<i>Roseomonas terpenica</i>	5
<i>Peptococcus niger</i>	5
<i>Oscillatoria corallinae</i>	5
<i>Aurantimonas manganoxydans</i>	5
<i>Arthrospira fusiformis</i>	5
<i>Sporosarcina pasteurii</i>	4
<i>Agrobacterium viscosum</i>	4
<i>Agrobacterium tumefaciens</i>	4
<i>Polaribacter butkevichii</i>	3
<i>Mycoplasma timone</i>	3
<i>Luteimonas terricola</i>	3
<i>Trichodesmium havanum</i>	2
<i>Stenotrophomonas retroflexus</i>	2
<i>Microcoleus antarcticus</i>	2
<i>Leptolyngbya laminose</i>	2
<i>Gallionella ferruginea</i>	2
<i>Francisella hispaniensis</i>	2
<i>Escherichia albertii</i>	2
<i>Comamonas kerstersii</i>	2
<i>Chroococcus minutus</i>	2

<i>Acinetobacter antiviralis</i>	2
<i>Zoogloea resiniphila</i>	1
<i>Veillonella atypical</i>	1
<i>Variovorax paradoxus</i>	1
<i>Thermobaculum terrenum</i>	1
<i>Sulfobacillus yellowstonensis</i>	1
<i>Sphingomonas oligophenolica</i>	1
<i>Sphingomonas ginsenosidimutans</i>	1
<i>Slackia faecicanis</i>	1
<i>Shinella yambaruensis</i>	1
<i>Salinicoccus luteus</i>	1
<i>Ralstonia insidiosa</i>	1
<i>Pseudoxanthomonas sacheonensis</i>	1
<i>Pseudomonas clemancea</i>	1
<i>Pseudomonas azotoformans</i>	1
<i>Prevotella melaninogenica</i>	1
<i>Plesiocystis pacifica</i>	1
<i>Phormidium murrayi</i>	1
<i>Oxalobacter vibrioformis</i>	1
<i>Oleomonas sagaranensis</i>	1
<i>Novosphingobium acidiphilum</i>	1
<i>Nitrosococcus watsoni</i>	1
<i>Methylobacillus glycogenes</i>	1
<i>Luteibacter anthropic</i>	1
<i>Janthinobacterium agaricidamnosum</i>	1
<i>Halanaerobacter chitinivorans</i>	1
<i>Erythromicrobium ramosum</i>	1
<i>Erythrobacter aquimaris</i>	1
<i>Ehrlichia ovina</i>	1
<i>Ectothiorhodospira haloalkaliphila</i>	1
<i>Devosia hwasunensis</i>	1
<i>Desulfuromonas svalbardensis</i>	1
<i>Cyanobacterium aponinum</i>	1
<i>Corynebacterium simulans</i>	1
<i>Cohnella damuensis</i>	1
<i>Caulobacter crescentus</i>	1
<i>Candidatus Scalindua brodae</i>	1
<i>Brevundimonas staleyi</i>	1
<i>Aurantimonas litoralis</i>	1
<i>Ammonifex thiophilus</i>	1
<i>Amaricoccus macauensis</i>	1
<i>Amaricoccus kaplicensis</i>	1
<i>Alkalibacterium subtropicum</i>	1
<i>Aeromicrobium ponti</i>	1
<i>Actinopolyspora salina</i>	1
<i>Acinetobacter psychrotolerans</i>	1

Acinetobacter oleivorans	1
Acidovorax temperans	1
Acidiphilium symbioticum	1
Calothrix parietina	5636
Thiomonas thermosulfata	42
Streptomyces lazareus	8
Ralstonia pickettii	4
Virgibacillus salexigens	3
Actinomyces naturae	2
Stenotrophomonas pavanii	1
19-Jul	Reads
Calothrix parietina	123144
Thiomonas thermosulfata	772
Streptomyces lazareus	106
Mycoplasma insons	62
Virgibacillus salexigens	49
Neorickettsia helminthoeca	25
Pseudomonas plecoglossicida	23
Actinomyces naturae	22
Thermogemmatispora onikobensis	21
Nostoc piscinale	16
Atopobium fossor	10
Maricaulis indicus	9
Microvirus Enterobacteria phage PhiX174	8
Desulfuromusa succinoxidans	8
Thermogemmatispora foliorum	7
Thiohalorhabdus denitrificans	6
Sporosarcina pasteurii	6
Pseudomonas alcaligenes	5
Lentibacillus kapialis	5
Amaricoccus kaplicensis	5
Trichodesmium tenue	3
Candidatus Scalindua brodae	3
Acinetobacter gerneri	3
Thermodesulfator atlanticus	2
Roseomonas terpenica	2
Ralstonia detusculanense	2
Pseudoxanthomonas sacheonensis	2
Pseudomonas taiwanensis	2
Oscillatoria corallinae	2
Ochrobactrum rhizosphaerae	2
Halanaerobacter chitinivorans	2
Ectothiorhodospira haloalkaliphila	2
Wautersiella falsenii	1
Variovorax boronicumulans	1
Thermobaculum terrenum	1

<i>Streptococcus infantis</i>	1
<i>Sphingobacterium faecium</i>	1
<i>Shinella fusca</i>	1
<i>Serratia entomophila</i>	1
<i>Rickettsia asiatica</i>	1
<i>Rhodobacter apigmentum</i>	1
<i>Pseudomonas putida</i>	1
<i>Pseudomonas mosselii</i>	1
<i>Pseudomonas monteilii</i>	1
<i>Polaribacter butkevichii</i>	1
<i>Peptococcus niger</i>	1
<i>Nitrospina gracilis</i>	1
<i>Mycoplasma timone</i>	1
<i>Microcoleus antarcticus</i>	1
<i>Micrococcus thailandicus</i>	1
<i>Microbispora rosea</i>	1
<i>Methylostratum kenyense</i>	1
<i>Methylobacterium goesingense</i>	1
<i>Luteimonas terricola</i>	1
<i>Luteibacter anthropi</i>	1
<i>Gallionella ferruginea</i>	1
<i>Francisella hispaniensis</i>	1
<i>Escherichia albertii</i>	1
<i>Dokdonella fugitiva</i>	1
<i>Desulfotomaculum thermoacetoxidans</i>	1
<i>Desulfofrigus oceanense</i>	1
<i>Cycloclasticus oligotrophus</i>	1
<i>Cupriavidus pauculus</i>	1
<i>Corynebacterium minutissimum</i>	1
<i>Cohnella damuensis</i>	1
<i>Clostridium akagii</i>	1
<i>Clostridium aestuarii</i>	1
<i>Chryseobacterium taichungense</i>	1
<i>Caulobacter crescentus</i>	1
<i>Arthronema africanum</i>	1
<i>Anaerobacillus alkalilacustre</i>	1
<i>Alkalibacterium subtropicum</i>	1
<i>Agrobacterium tumefaciens</i>	1
<i>Acidovorax wohlfahrtii</i>	1
<i>Acidovorax temperans</i>	1
<i>Calothrix parietina</i>	122445
<i>Thiomonas thermosulfata</i>	680
<i>Streptomyces lazareus</i>	81
<i>Mycoplasma insons</i>	58
<i>Virgibacillus salexigens</i>	34
<i>Neorickettsia helminthoeca</i>	20

<i>Thermogemmatispora onikobensis</i>	16
<i>Nostoc piscinale</i>	15
<i>Actinomyces naturae</i>	15
<i>Ralstonia pickettii</i>	12
Microvirus Enterobacteria phage PhiX174	12
<i>Atopobium fossor</i>	10
<i>Lentibacillus kapialis</i>	9
<i>Halanaerobacter chitinivorans</i>	7
<i>Trichodesmium tenue</i>	6
<i>Desulfuromusa succinoxidans</i>	6
<i>Sporosarcina pasteurii</i>	4
<i>Ralstonia detusculanense</i>	4
<i>Trichodesmium havanum</i>	3
<i>Peptococcus niger</i>	3
<i>Maricaulis indicus</i>	3
<i>Limnobacter thiooxidans</i>	3
<i>Limnobacter litoralis</i>	3
<i>Thiohalorhabdus denitrificans</i>	2
2-Aug	Reads
<i>Calothrix parietina</i>	138416
<i>Thiomonas thermosulfata</i>	1124
<i>Streptomyces lazareus</i>	186
<i>Ralstonia pickettii</i>	142
<i>Mycoplasma insons</i>	95
<i>Agrobacterium viscosum</i>	53
<i>Ralstonia detusculanense</i>	49
<i>Stenotrophomonas pavanii</i>	46
<i>Agrobacterium tumefaciens</i>	45
<i>Virgibacillus salexigens</i>	40
<i>Stenotrophomonas geniculata</i>	33
<i>Neorickettsia helminthoeca</i>	31
<i>Stenotrophomonas maltophilia</i>	27
<i>Thermogemmatispora onikobensis</i>	25
<i>Actinomyces naturae</i>	24
<i>Nostoc piscinale</i>	21
Candidatus <i>Liberibacter africanus</i>	20
<i>Mesorhizobium camelthorni</i>	19
<i>Acidovorax temperans</i>	15
<i>Atopobium fossor</i>	11
<i>Trichodesmium tenue</i>	10
<i>Shinella granuli</i>	9
Microvirus Enterobacteria phage PhiX174	9
<i>Maricaulis indicus</i>	9
<i>Lentibacillus kapialis</i>	9
<i>Desulfuromusa succinoxidans</i>	9
<i>Thermogemmatispora foliorum</i>	8

<i>Peptococcus niger</i>	8
<i>Agrobacterium larrymoorei</i>	8
<i>Thermodesulfatator atlanticus</i>	6
<i>Pseudomonas alcaligenes</i>	5
<i>Amaricoccus kaplicensis</i>	5
<i>Streptococcus tigurinus</i>	4
<i>Ralstonia insidiosa</i>	4
<i>Polaribacter butkevichii</i>	4
<i>Phenylobacterium lituiforme</i>	4
<i>Parapedobacter koreensis</i>	4
<i>Oscillatoria corallinae</i>	4
<i>Erythromicrobium ramosum</i>	4
<i>Bartonella rochalimae</i>	4
<i>Acinetobacter gerneri</i>	4
<i>Streptococcus infantis</i>	3
<i>Sporosarcina pasteurii</i>	3
<i>Sphingopyxis chilensis</i>	3
<i>Shinella fusca</i>	3
<i>Serratia entomophila</i>	3
<i>Roseomonas terpenica</i>	3
<i>Pseudoxanthomonas sacheonensis</i>	3
<i>Luteimonas terricola</i>	3
<i>Ectothiorhodospira haloalkaliphila</i>	3
<i>Blastomonas natatoria</i>	3
<i>Acinetobacter antiviralis</i>	3
<i>Achromobacter arsenitoxydans</i>	3
<i>Trichodesmium havanum</i>	2
<i>Thermodesulfovibrio thiophilus</i>	2
<i>Rhizobium alamii</i>	2
<i>Pseudomonas resinovorans</i>	2
<i>Phormidium murrayi</i>	2
<i>Microcoleus vaginatus</i>	2
<i>Leptolyngbya laminosa</i>	2
<i>Hydrogenophilus halorhabdus</i>	2
<i>Hydrogenophilus denitrificans</i>	2
<i>Heliorestis baculata</i>	2
<i>Halanaerobacter chitinivorans</i>	2
<i>Gemella haemolysans</i>	2
<i>Gallionella ferruginea</i>	2
<i>Francisella hispaniensis</i>	2
<i>Escherichia albertii</i>	2
<i>Enhydrobacter aerosaccus</i>	2
<i>Chitinophaga ginsengisoli</i>	2
<i>Caulobacter vibrioides</i>	2
<i>Candidatus Scalindua brodae</i>	2
<i>Brevundimonas terrae</i>	2

Arthronema africanum	2
Alkaliphilus crotonatoxidans	2
Alkalibacterium subtropicum	2
Variovorax boronicumulans	1
Thiohalorhabdus denitrificans	1
Symploca atlantica	1
Symbiobacterium toebii	1
Streptococcus sanguinis	1
Streptococcus pseudopneumoniae	1
Stenotrophomonas chelatiphaga	1
Sphingopyxis ginsengisoli	1
Sphingomonas melonis	1
Sphingomonas japonica	1
Snowella rosea	1
Serratia marcescens	1
Salinicoccus luteus	1
Ruegeria lacuscaerulensis	1
Rhodobacter apigmentum	1
Psychrobacter phenylpyruvicus	1
Pseudomonas taetrolens	1
Pseudomonas stutzeri	1
Pseudomonas putida	1
Pseudomonas plecoglossicida	1
Pseudomonas luteola	1
Pseudomonas clemancea	1
Pseudomonas azotoformans	1
16-Aug	
Acinetobacter johnsonii	1282
Acinetobacter junii	428
Acinetobacter gerneri	416
Ralstonia pickettii	395
Solibacillus silvestris	387
Acinetobacter guillouiae	363
Acinetobacter tjernbergiae	230
Sphingomonas panni	206
Agrobacterium tumefaciens	158
Ralstonia detusculanense	157
Agrobacterium viscosum	134
Stenotrophomonas geniculata	95
Acinetobacter antiviralis	90
Acinetobacter baylyi	81
Candidatus Liberibacter africanus	78
Stenotrophomonas pavanii	76
Mesorhizobium camelthorni	72
Enhydrobacter aerosaccus	60
Acinetobacter rhizosphaerae	58

<i>Comamonas kerstersii</i>	52
<i>Escherichia albertii</i>	51
<i>Acinetobacter soli</i>	51
<i>Acinetobacter beijerinckii</i>	51
<i>Stenotrophomonas maltophilia</i>	42
<i>Ectothiorhodospira haloalkaliphila</i>	42
<i>Lysinibacillus parviboronicapiens</i>	35
<i>Acinetobacter schindleri</i>	35
<i>Calothrix parietina</i>	29
<i>Brevundimonas terrae</i>	28
<i>Acinetobacter bouvetii</i>	28
<i>Bacillus endophyticus</i>	27
<i>Variovorax paradoxus</i>	21
<i>Serratia entomophila</i>	20
<i>Limnobacter thiooxidans</i>	20
<i>Agrobacterium larrymoorei</i>	20
<i>Ralstonia insidiosa</i>	19
<i>Sphingomonas oligophenolica</i>	14
<i>Caulobacter vibrioides</i>	14
<i>Bacillus thioparans</i>	14
<i>Brevundimonas diminuta</i>	13
<i>Chryseobacterium soli</i>	12
<i>Bacillus oryzae</i>	12
<i>Acinetobacter gyllenbergii</i>	12
<i>Sphingomonas hankookensis</i>	11
<i>Shinella fusca</i>	9
<i>Pectinatus cerevisiiphilus</i>	9
<i>Hydrogenophaga defluvii</i>	9
<i>Cupriavidus metallidurans</i>	8
<i>Shinella yambaruensis</i>	7
<i>Propionibacterium acnes</i>	7
<i>Pedomicrobium manganicum</i>	7
<i>Microvirus Enterobacteria phage PhiX174</i>	7
<i>Leptolyngbya laminosa</i>	7
<i>Cardiobacterium hominis</i>	7
<i>Anoxybacillus flavithermus</i>	7
<i>Acinetobacter calcoaceticus</i>	7
<i>Variovorax boronicumulans</i>	6
<i>Thermovenabulum ferriorganovororum</i>	6
<i>Streptococcus pseudopneumoniae</i>	6
<i>Streptococcus infantis</i>	6
<i>Sphingomonas dokdonensis</i>	6
<i>Pseudomonas alcaligenes</i>	6
<i>Erythromicrobium ramosum</i>	6
<i>Chitinophaga ginsengisoli</i>	6
<i>Acinetobacter oleivorans</i>	6

<i>Sphingomonas elodea</i>	5
<i>Kingella oralis</i>	5
<i>Ehrlichia ovina</i>	5
<i>Bacillus humi</i>	5
<i>Anoxybacillus rupiensis</i>	5
<i>Agromyces salentinus</i>	5
<i>Acinetobacter ursingii</i>	5
<i>Thiomonas thermosulfata</i>	4
<i>Streptococcus tigurinus</i>	4
<i>Rothia dentocariosa</i>	4
<i>Rathayibacter caricis</i>	4
<i>Pseudomonas stutzeri</i>	4
<i>Pseudomonas oryzihabitans</i>	4
<i>Pedomicrobium ferrugineum</i>	4
<i>Lysinibacillus xylanilyticus</i>	4
<i>Luteimonas terricola</i>	4
<i>Candidatus Liberibacter solanacearum</i>	4
<i>Bacillus alkalitelluris</i>	4
<i>Acinetobacter xiamenensis</i>	4
<i>Veillonella dispar</i>	3
<i>Thermogemmatispora onikobensis</i>	3
<i>Streptococcus fryi</i>	3
<i>Sphingomonas melonis</i>	3
<i>Sphingomonas mali</i>	3
<i>Pseudomonas resinovorans</i>	3
<i>Prevotella nanceiensis</i>	3
<i>Prevotella melaninogenica</i>	3
<i>Polaromonas jejuensis</i>	3
<i>Novosphingobium subterraneum</i>	3
<i>Novosphingobium acidiphilum</i>	3
<i>Neisseria lactamica</i>	3
<i>Lautropia mirabilis</i>	3
<i>Hyphomicrobium hollandicum</i>	3
<i>Hyphomicrobium aestuarii</i>	3

Appendix backwash

11-Jul	Reads	4-Aug	Reads
<i>Escherichia albertii</i>	4812	<i>Acinetobacter johnsonii</i>	2173
<i>Enterobacter hormaechei</i>	1080	<i>Ralstonia pickettii</i>	1547
<i>Serratia entomophila</i>	895	<i>Escherichia albertii</i>	746
<i>Pseudomonas entomophila</i>	638	<i>Escherichia albertii</i>	599
<i>Streptococcus infantis</i>	615	<i>Pseudomonas fragi</i>	587
<i>Streptococcus pseudopneumoniae</i>	605	<i>Hydrogenophilus halorhabdus</i>	392
<i>Acinetobacter johnsonii</i>	520	<i>Pseudomonas fragi</i>	382
<i>Stenotrophomonas retroflexus</i>	479	<i>Pseudomonas azotoformans</i>	357
<i>Escherichia albertii</i>	451	<i>Escherichia albertii</i>	276
<i>Acinetobacter johnsonii</i>	344	<i>Paracoccus marcusii</i>	247
<i>Stenotrophomonas acidaminiphila</i>	326	<i>Pseudomonas aeruginosa</i>	243
<i>Streptococcus tigurinus</i>	259	<i>Ralstonia detusculanense</i>	233
<i>Corynebacterium riegelii</i>	258	<i>Pseudomonas lundensis</i>	226
<i>Calothrix parietina</i>	250	<i>Sphingobium yanoikuyae</i>	222
<i>Pseudomonas plecoglossicida</i>	248	<i>Acinetobacter johnsonii</i>	210
<i>Enterobacter amnigenus</i>	199	<i>Serratia entomophila</i>	203
<i>Bacillus thermoamylovorans</i>	175	<i>Streptococcus parasanguinis</i>	201
<i>Streptococcus thermophilus</i>	157	<i>Pseudomonas lundensis</i>	176
<i>Acinetobacter beijerinckii</i>	149	<i>Acinetobacter tjernbergiae</i>	175
<i>Methylobacterium goesingense</i>	139	<i>Streptococcus infantis</i>	171
<i>Staphylococcus pettenkoferi</i>	135	<i>Streptococcus pseudopneumoniae</i>	155
<i>Neisseria flavescens</i>	124	<i>Sporosarcina pasteurii</i>	144
<i>Neisseria mucosa</i>	116	<i>Streptococcus pseudopneumoniae</i>	137
<i>Neisseria lactamica</i>	108	<i>Streptococcus infantis</i>	133
<i>Enterobacter hormaechei</i>	103	<i>Pseudomonas putida</i>	132

<i>Rothia dentocariosa</i>	98	<i>Serratia entomophila</i>	121
<i>Pseudomonas plecoglossicida</i>	97	<i>Calothrix parietina</i>	113
<i>Enterobacter amnigenus</i>	93	<i>Caulobacter vibrioides</i>	113
<i>Pseudomonas mendocina</i>	92	<i>Streptococcus vestibularis</i>	107
<i>Streptococcus vestibularis</i>	88	<i>Yersinia ruckeri</i>	97
<i>Corynebacterium appendicis</i>	87	<i>Stenotrophomonas geniculata</i>	89
<i>Calothrix parietina</i>	76	<i>Hydrogenophilus hirschii</i>	89
<i>Janthinobacterium agaricidamnosum</i>	75	<i>Ralstonia pickettii</i>	83
<i>Actinobacillus parahaemolyticus</i>	73	<i>Calothrix parietina</i>	80
<i>Calothrix parietina</i>	72	<i>Corynebacterium tuberculostearicum</i>	72
<i>Runella limosa</i>	69	<i>Acinetobacter gernerii</i>	71
<i>Muricauda lutimaris</i>	69	<i>Pseudomonas tropicalis</i>	67
<i>Stenotrophomonas terrae</i>	68	<i>Granulicatella adiacens</i>	65
<i>Veillonella dispar</i>	67	<i>Acinetobacter bouvetii</i>	64
<i>Acinetobacter tjernbergiae</i>	67	<i>Yersinia nurmii</i>	63
<i>Ralstonia pickettii</i>	66	<i>Streptococcus sanguinis</i>	58
<i>Pseudomonas alcaligenes</i>	65	<i>Hydrogenophilus halorhabdus</i>	57
<i>Serratia entomophila</i>	64	<i>Sphingomonas panni</i>	57
<i>Kingella oralis</i>	64	<i>Rothia mucilaginosa</i>	56
<i>Streptococcus parasanguinis</i>	61	<i>Serratia entomophila</i>	50
<i>Ralstonia pickettii</i>	61	<i>Neisseria mucosa</i>	50
<i>Aggregatibacter segnis</i>	59	<i>Sporosarcina luteola</i>	49
<i>Streptococcus infantis</i>	58	<i>Cupriavidus metallidurans</i>	49
<i>Acinetobacter bouvetii</i>	57	<i>Calothrix parietina</i>	47
<i>Veillonella parvula</i>	55	<i>Faecalibacterium prausnitzii</i>	45
<i>Modestobacter marinus</i>	55	<i>Tolumonas auensis</i>	44
<i>Abiotrophia defectiva</i>	55	<i>Caldicellulosiruptor kristjanssonii</i>	44
<i>Granulicatella</i>	54	<i>Streptococcus</i>	43

elegans		tigurinus	
Escherichia coli	51	Pseudomonas balearica	43
Streptococcus pseudopneumoniae	50	Lactobacillus iners	42
Peptoniphilus asaccharolyticus	49	Hydrogenophilus denitrificans	42
Pseudomonas mendocina	48	Faecalibacterium prausnitzii	42
Tolomonas auensis	47	Leuconostoc citreum	41
Pseudomonas parafulva	46	Lactococcus raffinolactis	39
Haemophilus parainfluenzae	46	Streptococcus tigurinus	39
Acinetobacter seohaensis	46	Corynebacterium durum	39
Acinetobacter tjernbergiae	45	Corynebacterium appendicis	37
Streptococcus sanguinis	44	Actinobacillus parahaemolyticus	36
Staphylococcus pseudolugdunensis	43	Methylobacterium goesingense	36
Veillonella atypica	42	Methylobacterium hispanicum	35
Pseudomonas benzenivorans	42	Corynebacterium appendicis	35
Microbispora rosea	42	Sporosarcina ginsengi	34
Corynebacterium durum	41	Sporosarcina ginsengi	34
Acinetobacter lwoffii	41	Yaniella halotolerans	33
Pseudomonas entomophila	39	Pseudomonas otitidis	31
Acinetobacter lwoffii	38	Acinetobacter tjernbergiae	29
Cupriavidus metallidurans	34	Paracoccus carotinifaciens	29
Corynebacterium matruchotii	34	Bacillus psychrosaccharolyticus	29
Blautia obeum	34	Sphingomonas azotifigens	29
Streptococcus oralis	33	Bacillus thermoamylovorans	28
Staphylococcus succinus	33	Yersinia nurmii	28
Methylobacterium goesingense	33	Pseudomonas plecoglossicida	28
Streptococcus gordonii	32	Arthrospira fusiformis	27
Microvirus	32	Yersinia ruckeri	26

Enterobacteria phage PhiX174			
Bartonella rochalimae	32	Methylobacterium goesingense	26
Anaerococcus octavius	28	Corynebacterium accolens	25
Acinetobacter gernerii	28	Sphingomonas melonis	25
Ralstonia detusculanense	27	Janthinobacterium agaricidamnosum	25
Corynebacterium kutscheri	27	Chitinophaga ginsengisoli	25
Enterobacter soli	26	Neisseria mucosa	24
Muricauda lutimaris	25	Anaerococcus octavius	24
Acinetobacter gernerii	25	Pseudomonas benzenivorans	23
Tannerella forsythia	24	Corynebacterium appendicis	22
Corynebacterium coyleae	24	Corynebacterium kutscheri	22
Pseudomonas panipatensis	23	Stenotrophomonas pavanii	21
Shewanella upenei	22	Enterobacter hormaechei	21
Ralstonia detusculanense	22	Enhydrobacter aerosaccus	21
Prevotella copri	22	Clostridium hiranonis	21
Neisseria mucosa	22	Brevundimonas diminuta	21
Streptococcus equinus	21	Acinetobacter lwoffii	21
Enterobacter aceae	21	Wautersiella falsenii	21
Aerococcus viridans	21	Variovorax paradoxus	21
Pseudomonas putida	19	Propionibacterium acnes	21
Acinetobacter rhizosphaerae	19	Neisseria elongata	21

Appendix backwash B

11-Jul	Reads	4-Aug	Reads
Sphingomonas azotifigens	1843	Methylobacterium radiotolerans	2488
Novosphingobium nitrogenifigens	1655	Sphingomonas azotifigens	2090
Cupriavidus pauculus	1597	Cupriavidus pauculus	1792
Methylobacterium radiotolerans	1344	Novosphingobium nitrogenifigens	675
Cupriavidus pauculus	1282	Sphingomonas elodea	596
Sphingomonas azotifigens	989	Methylobacterium mesophilicum	560
Novosphingobium nitrogenifigens	769	Methylobacterium longum	539
Methylobacterium radiotolerans	768	Novosphingobium subterraneum	328
Sphingomonas elodea	597	Novosphingobium capsulatum	248
Novosphingobium subterraneum	377	Cupriavidus campinensis	240
Sphingomonas elodea	357	Acidovorax temperans	237
Methylobacterium longum	332	Sphingomonas azotifigens	235
Novosphingobium acidiphilum	286	Burkholderia brasilensis	149
Acidovorax temperans	284	Methylobacterium radiotolerans	148
Methylobacterium mesophilicum	270	Sphingobium yanoikuyae	147
Cupriavidus campinensis	253	Cupriavidus pauculus	141
Sphingobium yanoikuyae	242	Novosphingobium acidiphilum	134
Methylobacterium mesophilicum	233	Acinetobacter junii	98
Methylobacterium longum	226	Novosphingobium nitrogenifigens	85
Burkholderia brasilensis	196	Clostridium saccharoperbutylaceticum	85
Cupriavidus campinensis	178	Burkholderia phenoliruptrix	84
Sphingomonas leidyia	165	Blastomonas natatoria	84
Novosphingobium capsulatum	154	Acinetobacter gerneri	84
Sphingomonas azotifigens	145	Sphingomonas oligophenolica	79
Methylobacterium radiotolerans	136	Sphingomonas sanxanigenens	75
Novosphingobium acidiphilum	134	Caulobacter crescentus	72
Novosphingobium aromaticivorans	132	Escherichia albertii	70

<i>Caulobacter crescentus</i>	132	<i>Enhydrobacter aerosaccus</i>	61
<i>Burkholderia brasiliensis</i>	131	<i>Calothrix parietina</i>	60
<i>Novosphingobium subterraneum</i>	126	<i>Erythromicrobium ramosum</i>	58
<i>Acidovorax temperans</i>	121	<i>Sphingomonas leidyia</i>	53
<i>Novosphingobium capsulatum</i>	117	<i>Acinetobacter johnsonii</i>	52
<i>Erythromicrobium ramosum</i>	106	<i>Novosphingobium subterraneum</i>	50
<i>Sphingomonas sanxanigenens</i>	101	<i>Magnetospirillum bellicus</i>	49
<i>Sphingomonas leidyia</i>	86	<i>Sphingomonas panni</i>	47
<i>Burkholderia phenoliruptrix</i>	85	<i>Novosphingobium aromaticivorans</i>	47
<i>Sphingomonas oligophenolica</i>	83	<i>Ralstonia pickettii</i>	45
<i>Escherichia albertii</i>	78	<i>Bergeyella zoohelcum</i>	43
<i>Blastomonas natatoria</i>	76	<i>Tolomonas auensis</i>	42
<i>Calothrix parietina</i>	72	<i>Acinetobacter tjernbergiae</i>	42
<i>Cupriavidus pauculus</i>	69	<i>Chryseobacterium soli</i>	39
<i>Sphingobium yanoikuyae</i>	69	<i>Sulfuricurvum kujiense</i>	37
<i>Sphingomonas sanxanigenens</i>	63	<i>Dechloromonas hortensis</i>	36
<i>Acinetobacter johnsonii</i>	53	<i>Methylobacterium gregans</i>	35
<i>Burkholderia phenoliruptrix</i>	53	<i>Sphingomonas elodea</i>	34
<i>Calothrix parietina</i>	51	<i>Calothrix parietina</i>	33
<i>Sphingomonas oligophenolica</i>	49	<i>Cupriavidus metallidurans</i>	32
<i>Blastomonas natatoria</i>	45	<i>Rhodoferrax antarcticus</i>	31
<i>Sulfuricurvum kujiense</i>	43	<i>Cupriavidus laharis</i>	30
<i>Chryseobacterium soli</i>	43	<i>Escherichia albertii</i>	29
<i>Novosphingobium aromaticivorans</i>	39	<i>Novosphingobium hassiacum</i>	29
<i>Novosphingobium nitrogenifigens</i>	38	<i>Acinetobacter lwoffii</i>	29
<i>Novosphingobium hassiacum</i>	37	<i>Methylobacterium mesophilicum</i>	27
<i>Enhydrobacter aerosaccus</i>	37	<i>Acidovorax temperans</i>	26
<i>Novosphingobium yangbajingensis</i>	36	<i>Serratia entomophila</i>	26
<i>Methylobacterium mesophilicum</i>	35	<i>Desulfovibrio desulfuricans</i>	26
<i>Cupriavidus laharis</i>	34	<i>Deinococcus caeni</i>	26
<i>Novosphingobium indicum</i>	33	<i>Bacteroides graminisolvens</i>	25
<i>Novosphingobium subterraneum</i>	32	<i>Deinococcus reticulitermitis</i>	24
<i>Methylobacterium gregans</i>	32	<i>Sphingobium yanoikuyae</i>	23
<i>Burkholderia ubonensis</i>	31	<i>Hydrogenophaga defluvii</i>	23
<i>Acinetobacter tjernbergiae</i>	31	<i>Bradyrhizobium</i>	23

		liaoningense	
Erythromicrobium ramosum	31	Acinetobacter indicus	23
Calothrix parietina	30	Sphingomonas phyllosphaerae	22
Sphingomonas panni	30	Hydrogenophilus denitrificans	22
Deinococcus reticulitermitis	29	Novosphingobium capsulatum	21
Tolomonas auensis	27	Pseudoxanthomonas mexicana	21
Sphingomonas phyllosphaerae	27	Enterobacter hormaechei	21
Clostridium saccharoperbutylaceticum	27	Burkholderia ubonensis	21
Cupriavidus metallidurans	26	Acinetobacter seohaensis	21
Sphingomonas elodea	25	Sphingomonas dokdonensis	20
Sphingomonas panni	23	Enterobacter amnigenus	20
Novosphingobium mathurensis	22	Arcobacter defluvii	20
Dechloromonas hortensis	22	Methylobacterium hispanicum	19
Cupriavidus metallidurans	22	Methylobacterium longum	18
Methylobacterium longum	20	Chryseobacterium soli	18
Rhodospirillum antarcticum	20	Ralstonia pickettii	17
Ralstonia pickettii	20	Citrobacter freundii	17
Novosphingobium hassiacum	20	Novosphingobium acidiphilum	16
Sphingopyxis chilensis	19	Acinetobacter johnsonii	16
Sphingopyxis alaskensis	19	Pseudomonas alcaligenes	15
Fusobacterium gonidiaformans	19	Phenylobacterium mobile	15
Deinococcus radiodurans	19	Novosphingobium indicum	15
Novosphingobium capsulatum	18	Leptolyngbya laminosa	15
Acidovorax temperans	18	Enterobacter soli	15
Sphingomonas dokdonensis	18	Clostridium fallax	15
Hydrogenophaga defluvii	18	Rheinheimera texasensis	14
Sulfuricurvum kujiense	18	Novosphingobium yangbajingensis	14
Methylobacterium gregans	18	Janthinobacterium agaricidamnosum	14
Dechloromonas hortensis	18	Flavobacterium succinicans	14
Streptococcus infantis	17	Dechloromonas agitata	14
Hydrogenophilus denitrificans	17	Cupriavidus campinensis	13
Bacteroides graminisolvens	17	Blastomonas natatoria	13
Runella limosa	17	Ralstonia insidiosa	13
Clostridium saccharoperbutylaceticum	17	Bacillus badius	13

Hydrogenophaga defluvii	16	Erythromicrobium ramosum	12
Cupriavidus laharis	16	Thiomonas thermosulfata	12
Magnetospirillum bellicus	15	Streptococcus infantis	12
Flavobacterium succinicans	15	Skermanella aerolata	12

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