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QUALITY & MUTAGENICITY OF WATER SUPPLY IN SYRIAN AND PALESTINIAN REFUGEE CAMPS IN LEBANON

by
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ABSTRACT
OF THE THESIS OF

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As a country hosting the highest refugee population per capita and per square kilometer in the world, Lebanon's refugee crisis is aggravating the already deteriorating infrastructure. Amidst political, economic, and governance structural challenges, the burden of disease among Syrian and Palestinian refugees in Lebanon from environmental degradation and poor WASH conditions is alarming. This study aimed to examine the quality and mutagenicity of water supplies in two Syrian and Palestinian refugee camps, by assessing the water physico-chemical, microbiological, and cytotoxic activity, using both an in vitro approach and certified water quality testing techniques. Representative water samples were collected between October and November 2022. Water mutagenicity was examined using the Ames Test with and without rat liver extracts. Results showed mutagenic activity in water supply from the Palestinian refugee camp in Dbayeh. In addition, several samples from both camps revealed potential cytotoxic effects. On the other hand, almost all samples showed high levels of fecal coliform. In addition, many physical (Turbidity, Electrical Conductivity, and Total Dissolved Solids) and chemical (Total Hardness, Calcium Hardness, Chlorides, Nitrite Nitrogen, Nitrate Nitrogen, and Ammonia Nitrogen) parameters exceeded the maximum admissible levels. Findings from this study provide new baseline data on important WASH components in two major vulnerable communities in Lebanon and provide a strong insight into the potential association between water safety and prevalent epidemics among targeted groups, particularly cancer incidence, developmental, and reproductive health indicators.

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ABBREVIATIONS

APHA	American Public Health Association
AUB	American University of Beirut
AWWA	American Water Works Association
BOD	Burden of Disease
DALYs	Disability-Adjusted Life Years
DBP	Disinfection Byproducts
EBML	Establishment of the Water of Beirut and Mount Lebanon
EDC	Endocrine Disrupting Chemical
FAO	Food and Agriculture Organization of the United Nations
hAR	Human Androgen Receptor
HDPE	High-Density Polyethylene
ICESCR	International Covenant on Economic, Social and Cultural Rights
IS	Informal Settlement
ITS	Informal Tented Settlements
LCRP	Lebanon Crisis Response Plan
MoE	Ministry of Environment
MSW	Municipal Solid Waste
S. typhi	Salmonella typhimurium
SPE	Solid Phase Extraction
UNICEF	United Nations International Children's Emergency Fund
UNRWA	United Nations Relief and Works Agency for Palestine Refugees in the Near East
US EPA	United States Environmental Protection Agency
WASH	Water, Sanitation, and Hygiene
WEF	Water Environment Federation
WHO	World Health Organization

CHAPTER I

INTRODUCTION

The right to access water is a basic human dignity integral to all. The International Covenant on Economic, Social, and Cultural Rights (ICESCR) recognizes the right to water as an undeniable basic human need (ICESCR, 2022). Everyone is entitled to access “sufficient, safe, acceptable, continuous, and affordable water for personal and domestic use” (UN-Water, 2022). Currently, the WHO estimates more than 2 billion people living in water-stressed countries, including 1.2 billion people lacking basic levels of water service, a situation that is likely to deteriorate mainly due to climate change and population growth (WHO, 2022b).

Deficient water, sanitation, and hygiene (WASH) significantly account for a large burden of disease across the globe. Using polluted water, whether for drinking, or in food preparation, personal hygiene, and removal of wastes, is associated with waterborne diseases and water-related vector-borne diseases. That includes the risk for adverse maternal and perinatal health outcomes, malnutrition, tumors, and many infectious diseases, including diarrheal diseases, Salmonella, Trachoma, Schistosomiasis, Malaria, and more (Campbell et al, 2015; Forouzanfar et al., 2016; Kaur et al., 2021; Lin et al., 2022; Prüss-Ustün, 2019; WHO, 2016; WHO, 2018a). Unsafe domestic water supplies may also contain excessive amounts of chemicals, toxins, and/or mutagens resulting in serious illnesses and potentially leading to death (WHO, 2019). The Lancet Commission on pollution and health reports that water pollution was responsible for 1.4 million premature deaths in 2019, and that women and children were more susceptible to dying from the consumption of polluted water compared to adult men (Fuller et al., 2022). In particular, refugees and displaced individuals are considered among the most vulnerable, as many of them do not have access to adequate WASH services (Calderón-Villarreal et al., 2022). Such vulnerability is associated with conditions of poor hygiene, inadequate qualitative and quantitative access to safe drinking water, and substandard or below-standard shelter environments (WHO, 2018b).

A. Problem statement

Lebanon currently hosts the highest refugee population per capita and per square kilometer in the world (UNHCR, 2022), with over 1.5 million Syrian and 200,000 Palestinian refugees (LCRP, 2022; UNHCR, 2022). The refugee crisis in Lebanon is aggravating the already deteriorating infrastructure, particularly following the impact of the COVID-19 outbreak since March 2020, the Beirut Port Explosion in August 2020, the recent cholera outbreak in October 2022, and the country's ongoing economic crisis, which is increasing the national water demand and challenging sustaining an adequate WASH (UNHCR, 2022). The unsuitable shelter conditions and inadequate WASH services and facilities in refugee and displaced camps in Lebanon are due to deteriorating water and waste management systems, hence posing a major health threat (UNICEF, UNHCR, & WFP, 2022). Over half of the Syrian refugee population in Lebanon is currently residing in overcrowded, below humanitarian standards, and/or collapsing shelter conditions (UNICEF, UNHCR, & WFP, 2022). Similarly, Palestinian refugees, residing in Lebanon or displaced from Syria to Lebanon, are also reported to be residing in substandard rundown shelter conditions (LCRP, 2022) and poor environmental health conditions (UNRWA, 2022a). However, data on WASH quality and information on domestic water supply quality and toxicity remains scarce despite their critical impact on health, particularly the association with higher cancer and endocrine disruptions risks (Gonsioroski et al., 2020; Wee & Aris, 2017; Wee & Aris, 2019; Xiao et al., 2017).

B. Significance and Objectives of the study

Despite recent declines in attributable mortality, inadequate WASH remains an important determinant of the global disease burden, especially among young children in vulnerable populations. This study is motivated by the unusually high influx of refugees to Lebanon, and the existing data gaps on water health risks and safety in these vulnerable groups. It aims to examine both the quality and the toxicity of water supplies in two refugee camps in Lebanon. The overarching goal of this research is to examine and compare the toxicity of water supplies in both Syrian and Palestinian refugee camps in Lebanon. The current study aims to:

Aim 1: Examine the mutagenicity of water supplies in refugee camps

Aim 2: Examine the water quality in refugee camps

Aim 3: Compare the cytotoxicity of water supplies in Syrian Refugee camps to those in Palestinian camps.

The proposed study is original and impactful in (1) being the first of its kind in approaching water quality in Lebanon and the region at the level of its biological activity, (2) its ability to generate new data on an important WASH component in two major vulnerable communities in the country, and (3) providing a strong insight into the association between water safety and prevalent epidemics among targeted groups, particularly cancer incidence, and developmental and reproductive health indicators.

CHAPTER II

BACKGROUND INFORMATION

A. WASH Burden of Disease: Quality, Mutagenicity & Toxicity of Water Supplies

1. Introduction to WASH Burden of Disease

Direct water household use, whether for drinking, food preparation, and/or hygiene, has direct impacts on human health (Schwarzenbach et al., 2010; Pal et al., 2018). Water-borne communicable and non-communicable diseases are associated with a major disease burden. Poor water and sanitation were estimated to account for 0.9% of global Disability-Adjusted Life Years (DALYs) in 2010 (Lim et al., 2012). In 2016, 829,000 WASH-attributable deaths and 49.8 million DALYs were estimated to be associated with diarrheal diseases and inadequate WASH practices (Lin et al., 2022; Prüss-Ustün, 2019; United Nations, 2022; Wolf et al., 2014).

Hounslow (2018) classifies water pollutants into three types; first are nutrients such as phosphates and nitrates; second are trace metals; and third are synthetic organics such as pesticides and industrial byproducts. Major sources of water pollution contribute to various water contaminants such as heavy metals from industrial wastes, chlorination disinfection byproducts (DBPs) from treatment processes, increased mineral content from seawater intrusion, fecal wastewater contamination from deteriorating sewage networks, and trace metals from deteriorating water supply infrastructure (Halwani et al., 2019; Hassoune et al., 2010; Korfali & Jurdi, 2007; Korfali & Jurdi, 2009; Lakkis et al., 2018; Massoud et al., 2010; Stocks et al., 2014). These chemical and microbiological contaminants are associated with poor water quality posing major public health concerns, including vector-borne diseases, gastrointestinal diseases, anti-microbial resistance, endocrine disruption, and much more (Boelee et al., 2019; Halder & Islam, 2015; Halwani et al., 2019; Massoud et al., 2010; Schwarzenbach et al., 2010).

2. *Microbial Hazards*

A high frequency of waterborne diseases, toxicities, and outbreaks continues to be associated with deficient water supply quality and management systems (Halder & Islam, 2015; Korfali & Jurdi, 2009). Risks of gastrointestinal and skin infections may arise from bathing in polluted water (Pal et al., 2018; WHO, 2021). In addition, several diseases, including cholera, dysentery, diarrhea, giardiasis, typhoid fever, Escherichia coli (E. Coli) infection, salmonellosis, parasitic infections, schistosomiasis, dracunculiasis, and hepatitis A, are all directly associated with the microbial quality of consumed water (Bridle, 2021; Griffiths, 2017; National Institute of Environmental Health Sciences, 2022; WHO, 2022b). This is consistent with several studies conducted in refugee camps across the globe (Roberts et al., 2001; Shultz et al., 2009). Studies have also indicated that several pathogens, such as Giardia, Vibrio Cholera, Cryptosporidium, parasites, such as Protozoa and Helminths, as well as viruses are often found in polluted water sources causing widespread health risks among users (US EPA, 2022a; WHO, 2022b).

3. *Chemical Hazards*

The World Health Organization divides chemical contaminants into five major source groups: naturally occurring, chemicals from industrial sources and human dwellings, chemicals from agricultural activities, such as those arising from manures, fertilizers, and pesticides, chemicals from water treatment or materials in contact with drinking water, such as coagulants, disinfection byproducts (DBPs), and piping material; and chemicals from pesticides used in water (WHO, 2022b). DBPs are formed in the case of overuse of water disinfectants, as disinfectant residuals in water distribution systems combine with organic material in water to form DBPs. Consumption of excess levels of DBPs is associated with increased adverse health effects, including cancer (US EPA, 2022a).

Heavy metals, such as arsenic, lead, chromium, mercury, iron, cadmium, copper, nickel, and zinc, may also accumulate in water due to natural and anthropogenic sources (Vareda et al., 2019). Bioaccumulation of these toxic metals causes neurotoxicity, immunological toxicity, reproductive and development toxicity, cardiovascular toxicity, genotoxicity, hepatoxicity, nephrotoxicity, skin toxicity, and carcinogenesis (Balali-Mood et al., 2021; Jaishankar et al., 2014; Kim et al., 2015; Mitra et al., 2022). A study on groundwater quality assessment in a Kurdish refugee camp in Iraq revealed high concentration levels of heavy metals potentially linked to wastewater leakage from the campsite (Mohammed et al., 2020). Another study in a Pakistani Hindu Refugee camp in India revealed an excessive level of lead and a high level of copper in drinking water (Nathan et al., 2014).

On the other hand, chemical contaminants in water may cause endocrine disruption. The endocrine system consists of all the body’s hormones regulating a multitude of biological functions and maintaining homeostasis across developmental, reproductive, immune, and nervous systems (US EPA, 2022c). Endocrine Disrupting Chemicals (EDCs) represent a group of persistent and bio-accumulative man-made or natural contaminants (WHO/UNEP et al., 2013) with estrogenic, androgenic, thyroidogenic, progestogenic, anti-estrogenic, and anti-androgenic activities (Gore et al., 2015). Sources of EDCs contaminating water are diverse, ranging from water disinfection byproducts to industrial and livestock activities or even therapeutic drugs released into sewages and then infiltrating essential water supplies (Gonsioroski et al., 2020). Studies conducted on EDCs in water show a correlation between EDC contamination and diseases or dysfunctions in the reproductive, metabolic, cardiopulmonary, immune, brain, and nervous systems, leading to increased incidences of diseases, cancers, and mortality from such disorders (Diamanti-Kandarakis et al., 2009; Gore et al., 2015; Vilela et al., 2017; WHO/UNEP et al., 2013). And due to the active endocrine system, EDCs are more likely to have effects even at very low doses (Gore et al., 2015; WHO/UNEP et al., 2013).

In addition, many of the chemical contaminants may have genotoxic activity. Several organic pollutants in water can serve as genotoxic pollutants; including but not limited to EDCs, Nitrosamines, Organochlorine Pesticides, Polychlorinated Biphenyls, Polycyclic Aromatic Hydrocarbons, and Pharmaceuticals and Personal Care Products; which hold high bio-accumulative and persistence potencies (Guan et al., 2017; Wagner & Plewa, 2017). Genotoxicity can lead to DNA mutation, either directly or through a series of oxidative stress reactions and toxic pathways (Farina et al., 2013; Dong et al., 2022). Additionally, some chemicals in water, such as Nitrosamines, have tumorigenesis potency and hence can lead to epigenetic or genomic instability, DNA repair interference, and receptor-mediated signaling pathways' bioactivation, resulting in immunosuppression and defective cell proliferation (Dong et al., 2022). As well, high levels of metals contaminating water, such as copper, lead, cadmium, and nickel, have also been associated with mutagenic events (Dourado et al., 2017; Matos et al., 2017; Tlenshieva et al., 2022).

More recently, there is a growing interest in examining the cytotoxic potential of water as a more comprehensive approach to water quality assessment. In a recent study in Lake Sevan in Armenia, collected water samples demonstrated mutagenic activity when tested in vitro (Avalyan et al., 2017). In another study, drinking water sources collected from six different Chinese locations induced different levels of DNA damage in vitro and showed a mutagenic potential (Guan et al., 2017). Similar results were found in chlorinated drinking water samples collected during the wet season in Guelma, Algeria (Abda et al. 2015). Another study on water in the Asa River in Nigeria revealed pollutants that can induce chromosomal aberration and other cytotoxic and genotoxic effects; capable of inflicting adverse effects on humans using it (Akinboro et al., 2020). A bacterial genotoxicity study on raw, disinfected, and tap water from Wuhan city in China detected different types of genotoxic effects of the drinking water samples collected in the winter and summer seasons of the year 2009 (Zeng et al., 2015). Another river in Tucuman, Argentina was revealed to contain potentially genotoxic and mutagenic contaminants as a result of industrial effluent discharge (Gana et al., 2008). Additionally, a test on water collected from the Sava River in Croatia detected genotoxic compounds present in the collected samples, serving as toxicity indicators capable of inducing morphological modifications and mitotic and chromosomal aberrations (Radić et al., 2010). Similar results were found in 11 out of 14 samples from surface waters and sediments of the Seine river estuary in France, downstream of an industrial wastewater treatment plant, as these samples were revealed to be highly mutagenic and containing compounds that are genotoxic for human hepatocytes (Vincent-Hubert et al., 2012). A more recent study on water samples from the Corrente River in Brazil revealed toxic and mutagenic potencies of the water, while it is being used as the main source of water supply by the population (Batista et al., 2016). Another in vitro study on water samples from Sapucaia and Esteio streams in Brazil also reveals the genotoxic and mutagenic effects of these samples amidst their potencies of inducing DNA damage through a series of oxidative mechanisms (Picinini et al., 2022). A similar study on water from the Guaribas river in Brazil detected cytotoxic, mutagenic, and genotoxic potencies despite different seasons, amidst high levels of heavy metals and physio-chemical parameters exceeding Brazilian standards (De Castro e Sousa et al., 2017). An additional study on heavy metals present in the Ili river water in Kazakhstan confirmed genotoxic and histopathological effects on the fish present in the river and linked such pollution to anthropogenic contamination (Tlenshieva et al., 2022). As well, a study on 10 rivers in Kazakhstan detected an excess of maximum allowable concentrations for various heavy metals, as well as phytotoxic, cytotoxic, and mutagenic potencies in the water samples (Lovinskaya et al., 2021). However, despite the rising global trend in assessing the cytotoxic, genotoxic, and mutagenic potential of water, such research remains relatively non-existent in Lebanon.

B. Provision, Management, and Challenges to a Safe Sustainable Domestic Water Supply in Lebanon

The provision of sustainable access to safe domestic water supplies is challenged by several factors hitting the country. While there are prevalent international goals, targets, resolutions, and commitments, most refugees in Lebanon still face social burdens and inconsistent WASH services. Durable solutions are not options for host countries like Lebanon, due to restricted resources, capabilities, space, and economic capital (United Nations – Lebanon, 2022; World Bank, 2013). From surviving a fragile water supply system in Lebanon to a collapsing one, the vulnerable communities in Lebanon are currently challenged by the inability to afford to access safe water. The crumpling water supply infrastructure is currently also exacerbated by the skyrocketing oil prices, the worsening fuel shortages, the backdrop of multiple socioeconomic crises in the country, and the aftermath of the COVID-19 pandemic and the 2020 Port of Beirut explosion (UNICEF, 2022). Additionally, Lebanon is considered to have a high level of water stress (75 to 100%), indicated by the freshwater withdrawal as a proportion of total renewable freshwater resources (United Nations, 2022, p. 39).

Groundwater mismanagement is another challenge facing the Lebanese state. Lebanon holds an abundant number of groundwater wells owing to the increased demand for water and the proximity of dug wells to residential, agricultural, and business facilities, amidst the lack of formal controls and absent public water supplies (Shaban, 2020). The quality of groundwater is challenged amidst infiltrated pollutants, seawater intrusion, uncontrolled drilling of wells, and inadequate public water supply (MoE & UNDP, 2011). A 2020 report on the Naameh Landfill in Lebanon revealed that landfill leachate infiltration and sewage infiltration into groundwater have caused bacteriological contamination of aquifers, deteriorating the water quality (Citton et al., 2020).

On another note, on August 4th, 2020, a 2,750 tons of ammonium nitrate explosion at the Beirut Port not only injured thousands, killed hundreds, and displaced hundreds of thousands, but was also associated with major environmental concerns linked to air pollution, contaminant runoff into water supply systems, disaster waste, and chemical pollution from secondary damages (Al-Hajj et al., 2021; Ur Rehman et al., 2021). More recently, in early October 2022, the Lebanese Ministry of Public Health notified the WHO of the first cholera outbreak that started with two laboratory culture-confirmed cholera bacteria cases reported from the Northern region of the country, namely from two

Syrian refugees living in informal settlements (WHO, 2022c). Cholera is an acute diarrheal infection caused by the ingestion of food or water contaminated with the bacterium Vibrio cholera (WHO, 2022d). Given that the transmission of cholera is associated with inadequate access to WASH, refugee camps and informal settlements are considered at-risk spots when minimum standards of clean and safe WASH are compromised (CDC, 2022). Accordingly, challenges to the provision and management of safe sustainable domestic water supplies are plenty in Lebanon, and they disproportionately impact the residents of the country.

C. WASH Services & the Refugee Crisis in Lebanon

The Government of Lebanon estimates that 1.5 million Syrians are currently hosted within its borders (LCRP, 2022). The influx of Syrians who have fled the war in Syria since 2011 includes 879,598 registered as refugees with UNHCR (LCRP, 2022), other than the illegally residing Syrian refugees whose exact number is inaccurate. As for the Palestinians, an average of 210,000 Palestinian refugees are recorded, with an average of 180,000 Palestine Refugees in Lebanon and 30,000 Palestine refugees from Syria; dispersed between twelve official Palestinian refugee camps and other overcrowded settlements (UNRWA, 2022b).

The prevailing socio-economic crises, legal status, and shelter conditions highly impact the type and quality of WASH services available for the vulnerable population of refugees who bear a huge weight of the intertwined crises. Deficient WASH services are due to the deteriorated water and waste management infrastructures associated with water contamination at source and beyond, posing threats to the refugee’s public health (UNICEF, UNHCR, & WFP, 2022). Accordingly, 54.4% of the refugees residing in

Lebanon’s informal settlements (ISs) are vulnerable to water stressors (UNHCR, 2021). To illustrate, while the main water sources in Palestinian refugee camps are generally municipal networks and community wells, the quality of provided water varies tremendously, with some being non-potable (UN-Habitat, 2014).

Several types of unreliable water supplies are accessible across refugee camps and ISs in Lebanon, such as resorting to using bottled drinking water, water trucking, open surface water and/or unprotected springs. Each type of these water sources poses its own challenges to the refugee communities in Lebanon; increasing their vulnerabilities to inadequate water, sanitation, and hygiene. Vulnerability depends on several other factors, including housing conditions (residential, non-residential, or non-permanent) and location (in close proximity to the coastal areas, landfills, agricultural crops, rivers, industries, etc.).

Despite advancements in analytical chemistry techniques and ease of accessibility and use of several water quality assessment kits, there still exist huge data gaps on water health risks and safety in refugee camps and ISs in Lebanon. The Syrian and Palestinian refugee camps and informal settlements in Lebanon lack water quality monitoring. When examining the WASH conditions for refugees in Lebanon, a recent national research study revealed the presence of elevated levels of fecal indicator bacteria, namely E. coli and fecal coliforms, in rivers across Lebanon (Dagher et al., 2021), near Syrian refugee camps (Kassem & Jaafar, 2020). In another published report, Palestinian refugees from Syria and those residing in Lebanon were found to be unable of accessing clean water due to the poor water infrastructure and high water salinity (LCRP, 2022). Even studies on old drinking water systems in the Shatila Palestinian refugee camp in Lebanon revealed fecal and parasitic contamination (Khoury et al., 2016).

On the other hand, the burden of diseases and fatalities among Syrian and Palestinian refugees in Lebanon is rising, many of which are associated with health risks from environmental degradation and poor WASH conditions in informal settlements (LCRP, 2022). A recent nationwide study on the prevalence of non-communicable diseases (NCDs) among adult Syrian refugees in Lebanon revealed high risks of hypertension, diabetes, cardiovascular diseases (CVD), chronic respiratory diseases, and cancer among these Syrian refugees (Saleh et al., 2021). A previous 2014 cross-sectional research study conducted among Syrian refugees and Lebanese host communities to assess the burden of NCDs revealed that among the Syrian sample, more than 50% reported a member of the household having at least one of five NCDs (Doocy et al., 2016). As for the Palestinians, a study revealed a high prevalence and burden of NCDs among Palestinian refugees from Syria, with limited access to services (Chaaya et al., 2021). In addition, the rising prevalence of cancer among refugees in the country is alarming (Alawa et al., 2019; Alawa et al., 2020).

CHAPTER III

MATERIALS AND METHODS

A. Study Area

Pilot field visits were first planned in order to inspect and gather information on the refugees in target sites, water sources, conveyance, and general water distribution schemes. A survey was shared with the camp supervisor to confirm the practicality and feasibility of water sampling in the camp. The first field visit was conducted on December 28, 2021, for the Choueifat El-Oumara Syrian Informal Tented Settlements (ITSs) (Figure 1). While, the second field visit was conducted on February 7, 2022, for the Dbayeh Palestinian Refugee Camp (Figure 2). Each of the camps was selected given its ease of access and entry and the water sampling's feasibility and practicality.

The Dbayeh camp, one of the 12 official UNRWA-managed serviced Palestinian refugee camps in Lebanon, is located around 12 kilometers north of the Capital city Beirut (UNRWA, 2022c). The 84,300 square meters in size Dbayeh camp was established in 1952 and currently has 4,591 UNRWA registered persons (UNRWA, 2022c); the count may not represent the actual number as many of which might have left over the years amidst an absent governmental- and UNRWA-tracking system. On the other hand, the targeted Syrian Informal Tented Settlements study area is the Choueifat El-Oumara ITSs, located around 12 kilometers North of Beirut. No reliable source of data mentions the exact size of the Choueifat El-Oumara informal settlements.

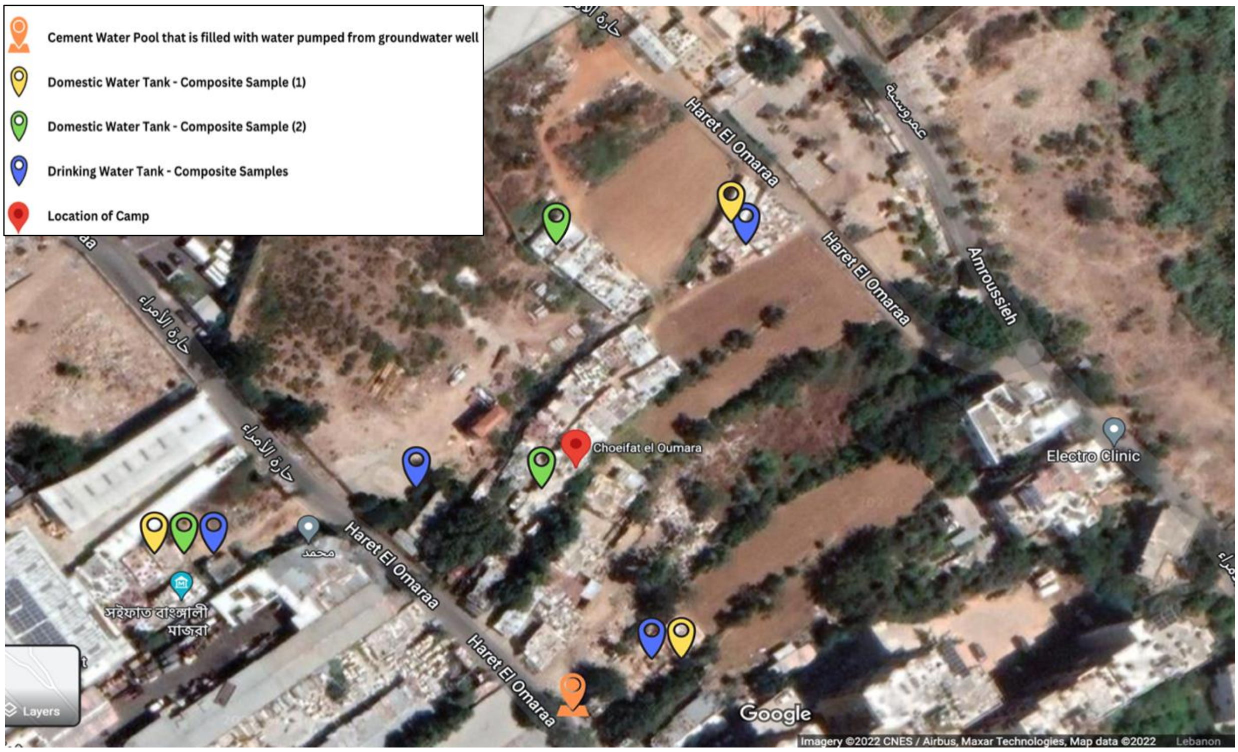

Figure 1 Geographic diagram with its legend showing the water sample collection scheme within the Choueifat El-Oumara Syrian ITS

(Source of Map: Google Earth, 2022a)

Figure 2 Satellite Image of the Dbayeh Palestinian Refugee Camp

(Source of Map: Google Earth, 2022b)

B. Assessment Site Survey

An assessment site survey template was developed in order to collect information on: the number of people residing on the site, the duration of the site's inhabitation, the presence or absence of a central water supply system for potable water distribution, the type of water distribution system network, the presence or absence of a major water storage tank from which water is distributed, the type of primary and complementary sources of water and their usage purposes, the potential water treatment and/or quality monitoring means, and major water complaints (as reported by the camp supervisor and some camp residents while checking the sites). Further details on the used survey are found in the Appendix I.

C. Water Samples Collection and Transportation

All water samples were collected in triplicates. Obtained replicates from each source were combined to construct one representative sample divided into three containers: 60L-volume stainless steel buckets collected for the mutagenicity test, 4L-volume aluminum-foiled glass bottles collected for endocrine-disruption testing (data not included in this thesis), and 1L-volume High-Density Polyethylene (HDPE) plastic bottles collected for the water quality parameters and stored on-site at 4˚C using a portable cooler. Containers were then transported to the Environmental Health Department Laboratories at the Faculty of Health Sciences at the American University of Beirut for testing. For most of the water quality parameter measurements, water samples were tested immediately upon arrival to the lab, while other tests were conducted within 24 hours of collection time.

The 60L-volume water collected from the Palestinian camp consisted of 30L from the main water tank and 30 L from different domestic water tanks across the camp (10 L from a tank in each street; with a total of three streets). Similarly, the 60L-volume water collected from the Syrian ITSs consisted of 30L from the main pool and 30L from different drinking water tanks across the camp. Chosen domestic water tanks were collected from the start, middle, and end of the camp area in both targeted sites, in order to test for water quality.

D. Water Quality Testing

The collected water samples were analyzed for their physicochemical and microbiological parameters using certified prepared reagents under EPA standards of the HACH water quality testing and analytical instruments manufacturing company, and the procedures followed the standard methods recommended by American Water Works Association (AWWA), American Public Health Association (APHA), and Water Environment Federation (WEF) (APHA, AWWA & WEF, 2017). A blank was run for every batch of samples during spectrophotometry analysis. Duplicate testing was conducted for all the parameters in order to ensure quality, and the average concentration was recorded.

Tested parameters were selected based on the WHO's standard protocol guidelines for drinking water quality analysis (WHO, 2008). Laboratory analysis testing included examining water microbial quality using total and fecal coliform, water physical quality, including turbidity, electrical conductivity, and total dissolved solids (TDS), and chemical properties, including pH, Alkalinity, Total Hardness, Calcium Hardness, Total Chlorine, Free Chlorine, Chlorides, Nitrite, Nitrate, Ammonia Nitrogen, Orthophosphate, and Sulfate. Table 1 lists the analytical methods adopted to quantify the identified water testing parameters, as per HACH water quality testing methodology and standards methods by AWWA, APHA & WEF (2017).

Table 1 List of analytical methods used for water analysis

Analytical Parameter	**Standard Analytical Method**	**Type of Analytical Equipment and Main Reagents**
Turbidity	Nephelometric Method	HACH Turbidimeter 2100 p
Electric conductivity	Electrical Conductivity Method	HQ40D HACH Conductivity, pH, DO Meter
Total Dissolved Solids	Electrical Conductivity Method	HQ40D HACH Conductivity, pH, DO Meter
pH	Potentiometric method	HQ40D HACH Conductivity, pH, DO Meter
Alkalinity	Standard Acid Titration Method using Sulfuric Acid (0.02N)	Burette Titration apparatus and Bromcresol green-methyl red indicator
Total Hardness	Standard EDTA Titrimetric Method	Hardness Buffer (ammonium acetate), Erichrom Black T indicator (Manver 2 indicator powder pillow), EDTA, and Burette Titration Apparatus
Calcium Hardness	Standard EDTA Titrimetric Method	Murexide indicator (CalVer 2 powder pillow), Erlenmeyer flask, EDTA, and Burette Titration apparatus
Free Chlorine	DPD Method	DPD Free Chlorine Reagent Powder Pillows and DR 2800 HACH Spectrophotometer
Total Chlorine	DPD Method	DPD Total Chlorine Reagent Powder Pillows and DR 2800 HACH Spectrophotometer
Chlorides	Standard Mercuric Nitrate Titration Method	Acidified indicator, Diphenylcarbazone powder pillow, standard Mercuric Nitrate 0.0141N solution), Erlenmeyer flask, and Burette Titration apparatus

Ammonia-Nitrogen	Direct Nesslerization Method	DR 2800 HACH Spectrophotometer, Mineral Stabilizer, Nessler Reagent, and Polyvinyl Alcohol Dispersing Agent (PVA)
Nitrite-Nitrogen	Diazotization Method	NitriVer 3 Nitrite Reagent Powder Pillow (sulfanilic acid) and DR 2800 HACH Spectrophotometer
Nitrate-Nitrogen	Cadmium Reduction Method	NitraVer 5 Nitrate Reagent Powder Pillow and DR 2800 HACH Spectrophotometer
Orthophosphate	Ascorbic Acid Method	PhosVer 3 Phosphate Reagent Powder Pillows and DR 2800 HACH Spectrophotometer
Sulfates	SulfaVer 4 Turbidimetric Method	SulfaVer 4 Reagent Powder Pillows and DR 2800 HACH Spectrophotometer
Total and Fecal Coliform	Membrane Filtration Technique	Millipore Filtration Apparatus, M-Endo Broth, and M-FC Broth

E. Extraction of Organic Water Pollutants

Figure 3 Water Extraction Apparatus for the 60L water samples

Extraction of organic water pollutants was conducted by customizing a setup consisting of silicone tubing, a vacuum pump, SPE columns, and Büchner flasks (Figure 3). The 60L-volume water was passed through a Dionex™ SolEx™ (Spectatridge, California, United States) C18 Silica-Based solid phase extraction (SPE) to adsorb organic pollutants as described (Guan et al., 2017). SPE columns were preconditioned with 5mL of methanol and 5mL ddH$_2$0 prior to their use. Each sample was then extracted with a 40 mL C18 column at a flow rate of 25 mL/min; with frequent pauses to avoid the overheating of the vacuum pump. The used C18 columns have a maximum sample load of 6 mL or 1 g sorbent weight. Columns were dried in a vacuum freeze-dryer overnight, and then elution was conducted with 1 mL of methanol, 1 mL of acetone, 2 mL of dichloromethane, and 2 mL of hexane. The eluent solution was then dried using a nitrogen stream with a water bath at 40°C through nitrogen purging given that the sample size was too little to be dried by rotary evaporation. Finally, the extracts were re-dissolved in 6 mL DMSO and stored at -20°C for further analysis and later use.

F. Mutagenicity Test

Water mutagenicity was examined using the *Ames MPF™ 98/100 AQUA Microplate Format Mutagenicity Assay* kit according to the manufacturer’s instructions (Xenometrix, Allschwil, Switzerland). Briefly, the bacterial reverse mutation test uses already mutated *Salmonella typhimurium* (*S. typhi*) strains to detect substitution, addition, or deletion point mutations of one or a few DNA base pairs (Ames et al., 1975; Maron & Ames, 1983; Gatehouse et al., 1994). The used S. typhi bacterial strains are customized to have point mutations in the histidine operon, rendering the bacteria incapable of producing the histidine amino acid, which hinders their growth unless supplied with histidine (Xenometrix, 2018). We used two different strains of S. typhi: *TA100* is for the detection of base substitution mutations and *TA98* is for the detection of frameshift mutations. A chemical that can revert the already-present mutations in the S. typhi strains and restore the functional capability of the His$^-$ bacteria to synthesize the essential amino acid is considered mutagenic.

First, overnight cultures of *TA100*, *TA98*, and a negative control were prepared. The overnight cultures were composed of the growth medium, the cells pellet and ampicillin, while the negative control growth was only composed of the growth medium. Overnight cultures were incubated in 2 mL micro-centrifuge tubes with a screw cap to allow aeration, in an environmental shaker set at 37°C with 250 rpm (Thermo Scientific™ MaxQ™ 4000 Barnstead Lab-Line A-Class, Massachusetts, United States), and for a total of 16 hours. On the second day, the OD_{600} values of the overnight cultures were measured using a spectrophotometer. Table 2 lists the results of the actual optical density of the overnight cultures.

Table 2 Actual optical density of the overnight cultures

Culture	**OD_{600} (x10) overnight**	**Acceptable Range***
TA98	7.11	>2.0
TA100	6.35	>2.0
Negative	0.01	≤0.05

Given that the OD_{600} values for the cultures were greater than 2.0, and that the OD_{600} value of the negative control was less than 0.05, this proved that overnight culturing was successful and sufficient for the downstream testing. Both *TA100* and *TA98* bacteria were exposed to 6-serial dilutions of the collected water extracts from both refugee camps (0.25, 0.5, 1.0, 1.5, 2.0, and 2.5L equivalent water source) (Figure 4 & Table 3) in media containing sufficient histidine to support approximately two cell divisions.

In order to account for metabolic bioactivation, the same samples have to be tested for their ability to cause bacterial reverse mutations in the presence of a rodent liver fraction (S9) treated with an enzyme-inducing agent (Ames et al., 1975; Maron & Ames, 1983) (Figure 5 and Table 4). Therefore, a 30% S9 mix was prepared by combining 0.575 ml S9 – Buffer salts, 0.025 ml S9 – G-6-P, 0.1 ml S9 – NADP, and 0.3 ml S9 fraction. The positive control for the sample without S9 was prepared by a mixture of 31.5 µl of the 100 µg/ml stock solution of 2-nitrofluorene (2-NF) l in DMSO and 31.5 µl of the 5 µg/ml stock solution of 4-nitroquinoline N-oxide (4-NQO) in DMSO; and were added to the positive control well containing 1.1 ml sterile water on the 24-well dilution plate (for both *TA98* and *TA100* cultures). As for the positive control for the sample with S9, it was prepared by adding 63 µl of 62.5 µg/ml stock solution of 2-aminoanthracene (2-AA) in DMSO solution to the positive control well of the 24-well dilution plate containing 1.1 ml sterile water.

Cells preps were added to the dilution plates (both *TA100* and *TA98* bacteria). These exposure plates were incubated for 90 minutes at 37°C for 250 rpm in an incubator shaker (Infors AG CH-4103 Bottmingen, Switzerland). After incubation, 2.6 ml of the pH-indicator medium was added to each well of the 24-well plates via an 8-channel repeating pipettor with only 4 tips. Afterward, 50 µl aliquots of these cultures were transferred by an 8-channel pipettor, aliquoted in the 384-well plates covered with sterile gas-permeable plate sealers (Figure 6) and kept in a sealable plastic incubation bag at

37°C dry incubator (Velp Scientifica Cooled Incubator, Usmate Velate MB, Italy) for 2 days.

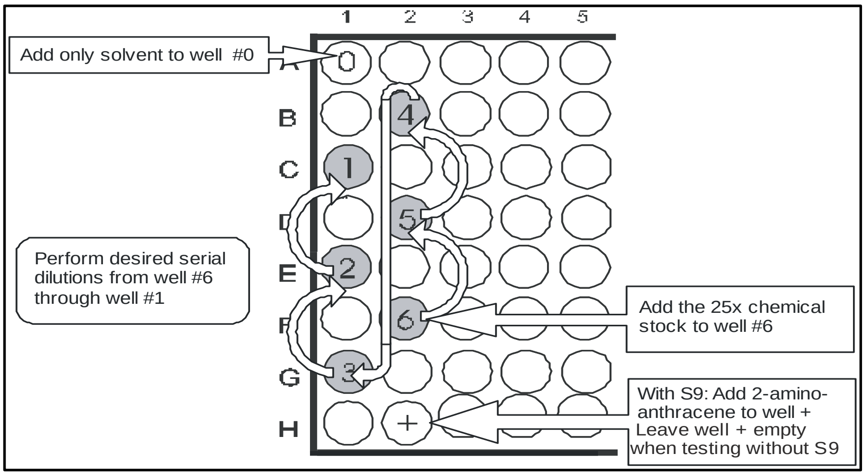

Figure 4 Dilution Plate Preparation (Xenometrix, 2018)

Table 3 Dilution Pipetting Scheme (Xenometrix, 2018)

Well	**Add sample**	**Add water**	**Transfer to next well, mix**	**Final concentration**	**Final Volume**
0	-	1.2 ml	-	Solv. Cont.	1.2 ml
1	-	1.2 ml		3.13%	2.4 ml
2	-	1.2 ml	1.2 ml ↑	6.25%	1.2 ml
3	-	1.2 ml	1.2 ml ↑	12.5%	1.2 ml
4	-	1.2 ml	1.2 ml ↑	25%	1.2 ml
5	-	1.2 ml	1.2 ml ↑	50%	1.2 ml
6	2.4 ml	-	1.2 ml ↑	100%	1.2 ml

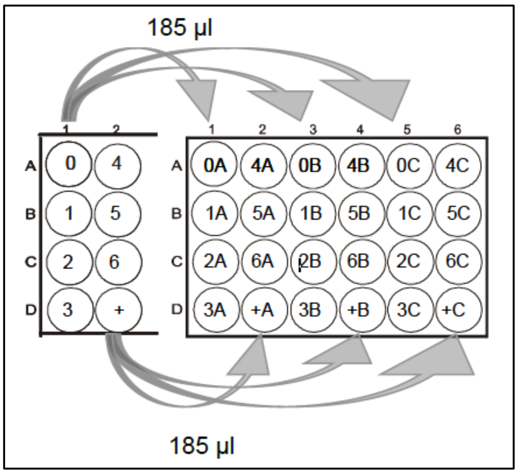

Figure 5 Exposure Plate Preparation (Xenometrix, 2018)

Table 4 Exposure Media Preparation (Xenometrix, 2018)

	TA98		TA100	
	–S9	+S9	–S9	+S9
Sterile water	15 µl	-	15 µl	-
10X exposure medium working solution	25 µl	25 µl	25 µl	25 µl
Sample	185 µl	185 µl	185 µl	185 µl
Bacteria	25 µl	25 µl	25 µl (1 :2)	25 µl (1 : 2)
S9 mix	-	15 µl	-	15 µl

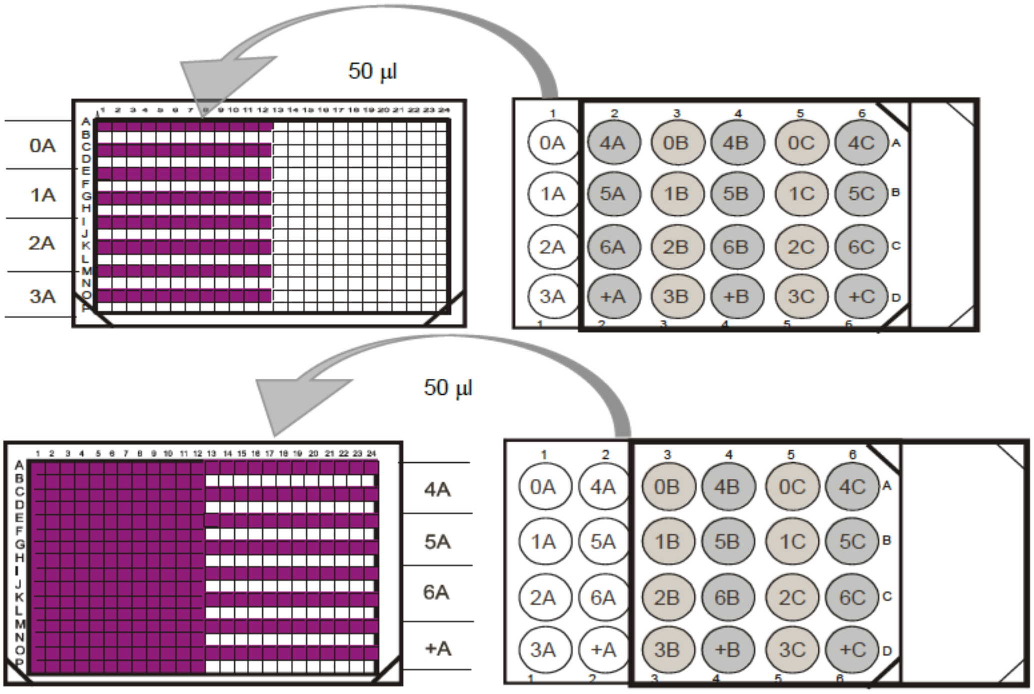

Figure 6 Transfer Scheme of Exposed Cultures from 24-well Plate to 384-well Plates (Xenometrix, 2018)

During incubation, the cells that had undergone reversion to histidine grow into colonies. Bacterial metabolism reduces the pH of the medium, changing the well's color from purple to yellow, noting that small greyish dots in the wells represent formed colonies and hence identifying positive wells. The number of wells containing revertant colonies, identified colorimetrically, were counted for each dilution (equivalent to a dose) and compared to a negative (water) control. Each dose was tested in triplicate to allow for statistical analysis of the data.

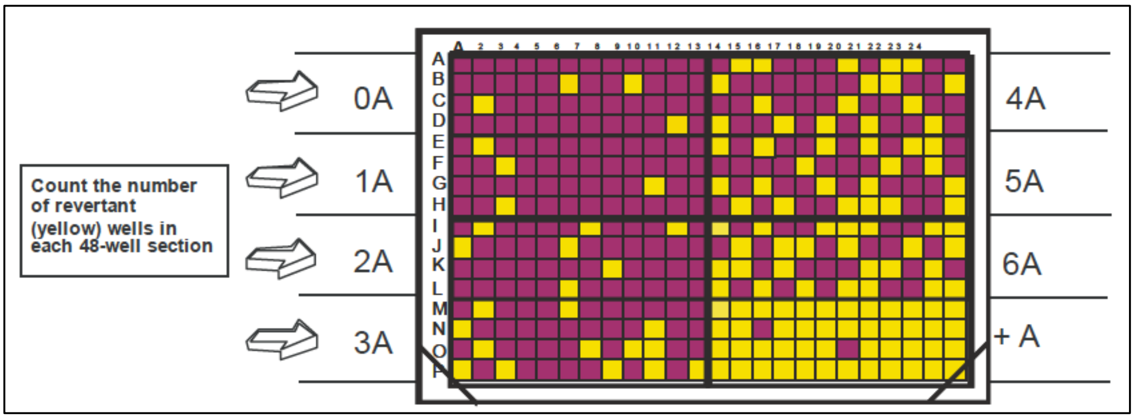

Figure 7 Plate Scoring Scheme (Xenometrix, 2018)

G. Statistical Analysis for Mutagenicity Test Results

Statistical analyses of data were performed through a Binomial test in order to compare the cytotoxicity of sampled water supplies from the different refugee camps. The statistical method serves to analyze the mutagenic potential of the samples, where samples are considered mutagenic if they show a dose-dependent ('Fold increase over baseline ≥2) and statistically significant increase ('Binomial B-value ≥0.99) in revertant colony numbers upon exposure to the tested samples, when compared to the solvent controls (Xenometrix, 2018). A binomial B-value greater than or equal to 0.99 indicates that mutagenic chances are extremely low (≤ 1%) and accordingly the test result would be considered negative. And in cases of B value ≤ 0.01, this means that the data points are significantly smaller than the mean number of spontaneous revertants, accounting for potential cytotoxic effects.

CHAPTER IV

RESULTS AND DISCUSSION

A. Assessment Survey Results

The water body in the Choueifat El-Oumara Informal Tented Settlements (ITSs) is a groundwater source pumped to an exposed cement pool that is used for non-potable domestic water sources. The water body in the Dbayeh camp is a water source pumped from the Establishment of the Water of Beirut and Mount Lebanon (EBML) to a closed cement tank used as a source of non-potable domestic water. Both water body sources in Choueifat IS and Dbayeh camp, are near a highly populated residential area, surrounded by agricultural activities along the coastal line (Figures 1 and 2). This exposes the pool water and groundwater aquifer, in the case of Choueifat IS, and the groundwater wells, in the case of Dbayeh camp, to anthropogenic sources of pollution such as residential pollution and leakage of wastewater (sewage coming from cesspools, septic tanks, and mismanaged sewer systems), agricultural runoffs (including fertilizers, pesticides, and animal manure), seawater infiltration, and potential leachate from nearby dumpsites.

1. Choueifat El-Oumara Syrian Informal Settlements

The Choueifat El-Oumara ITSs site has been inhabited by Syrian refugees for around fifteen years. There are currently fifty families residing on the site, each composed of six to ten family members. No central water supply system for potable nor for non-potable water distribution exists. Instead, residents are provided with a water allowance by UNICEF to purchase potable water trucking services. Procured water is directly filled into individual tanks every 2 to 4 days, depending on the season and the needs. Domestic water is pumped from a groundwater well into a partially exposed cement pool (Figure

8). This water is used for cooking/washing food, personal hygiene, and domestic hygiene. While all residents generally use domestic water from the same main water source (the cement pool) to pump into their storage tanks, however, some households use a common storage water tank with their neighbors, while others have a separate water storage tank for their families. Accordingly, some tents have individual 300L volume potable water tanks and 10,000L volume non-potable water tanks. In other parts of the ITS site, four tents share a common 10,000L non-potable water tank. Residents' major complaints about the water quality revolve around power shortage (preventing water pumping), the presence of rodents, fungi around and inside the water tanks, and the prevalence of skin allergies among infants. No water treatment is employed prior to distribution.

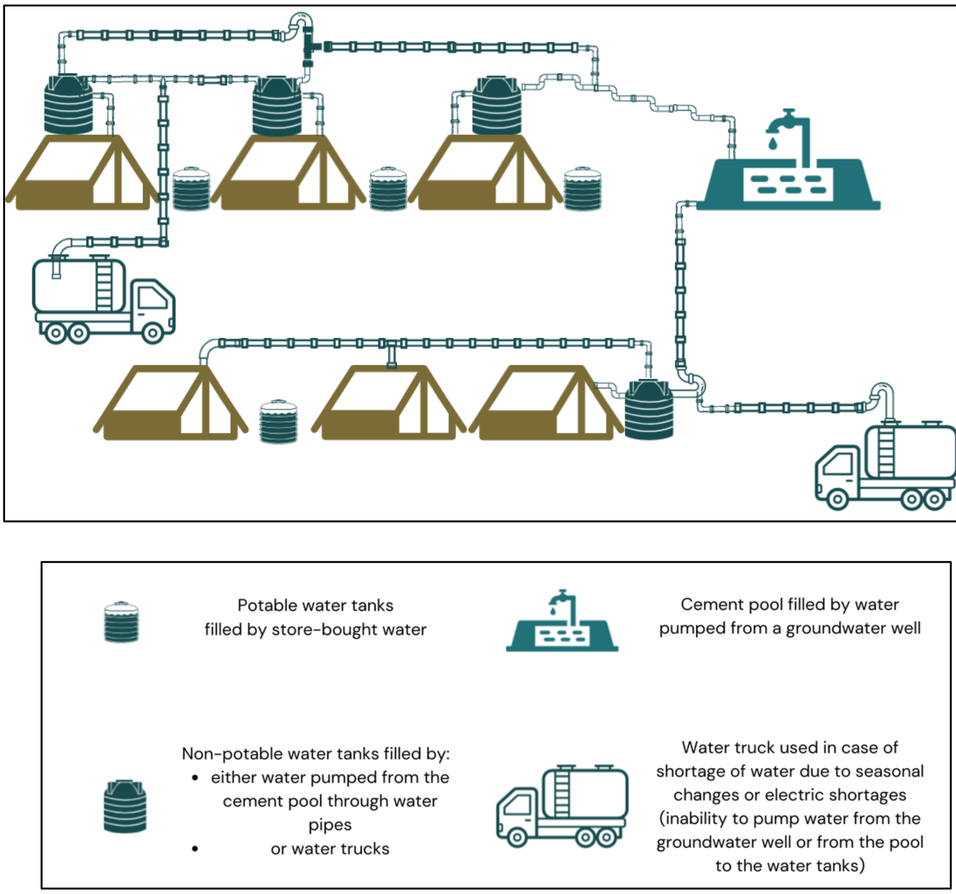

Figure 8 Schematic diagram with its legend showing the water distribution scheme within the Choueifat El-Oumara Syrian ITSs

2. Dbayeh Palestinian Refugee Camps

The Dbayeh Palestinian Refugee camp ISs site has been inhabited since 1952. Residing in the camp are currently around 540 Palestinian families, with an average of seven members per family, in addition to 60 Syrian families, with an average of five members per family. A central water supply system exists ever since the establishment of the camp; however, the same piping and water distribution scheme is still used to this day, with no renovations to the piping systems. Several pipes are worn out, causing wastewater to contaminate the water network. In addition, several families do not have access to water through the existing deteriorating network. Each residence within the Dbayeh camp has its own water storage tank. Residents tend to use water pumped from groundwater wells as their primary source of water supply, with the second option being self-paid water trucks. This water is used for cooking/washing food, personal hygiene, and other domestic purposes. Occasionally, the water is chlorinated by residents by adding chlorine tablets to the water storage tanks. Additionally, due to electricity shortages, water distribution and pumping to tanks have been challenging. For drinking, camp residents rely on various other sources of water.

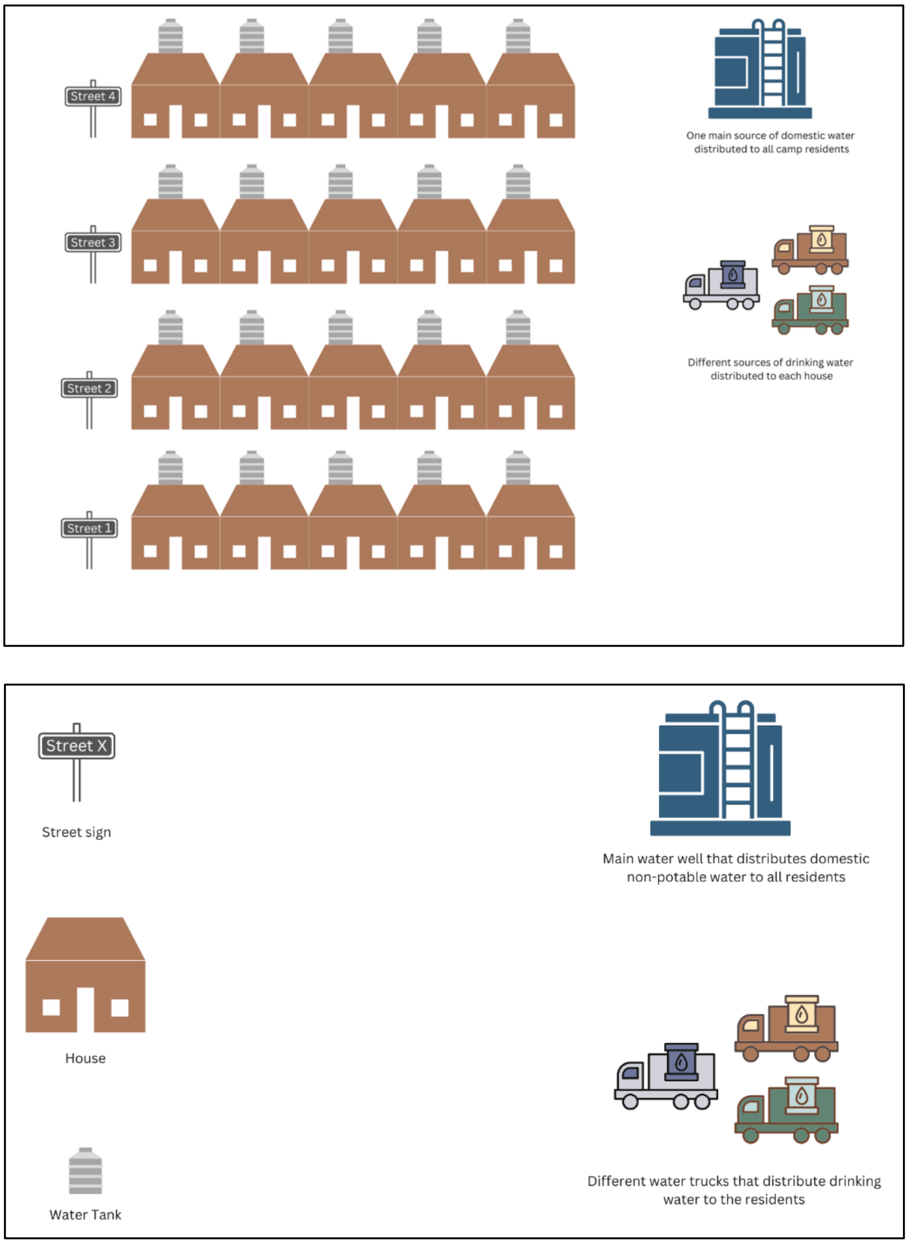

Figure 9 Schematic diagram with its legend showing the water distribution scheme within the Dbayeh Palestinian Refugee Camp

B. Water Quality Results

1. Physical and Microbiological Quality

Results show positive fecal and total coliforms in both the Syrian camp water supply in Choueifat and the Palestinian refugee camp water supply in Dbayeh. . These results are in accordance with previous studies reporting rivers' contamination with fecal coliform water across Lebanon (Geara-Matta et al., 2010; Houri & El Jeblawi, 2007). Water quality deterioration is mainly due to the mismanagement of discharged wastewater. A recent nationwide assessment of water quality in fourteen rivers in Lebanon revealed heavily contaminated water samples in fecal coliform and E. coli (Dagher et al., 2021). Fecal contamination can amount to wastewater with industrial effluents from several industrial and small-scale manufacturing facilities, hence resulting in alarming chemical pollution into waterways and water bodies from the generated wastewater.

In addition, turbidity was relatively high in all collected samples which may be caused by the highly porous nature of the well (limestone characteristic), or due to the deterioration of the used pipes (as reported by the Dbayeh Camp supervisor). Turbidity may also be a reflection of the suspended solid matter that comes from domestic, agricultural, and industrial activities. In addition, high levels of TDS are evident in the Choueifat Camp water supply and may be due to the presence of inorganic salts such as calcium (Ca) and magnesium (Mg), organic matter, natural (rocks and geologic formation), seawater infiltration, buffering effect in the well, and industrial and agricultural runoff. Further testing is required in order to confirm the sources of pollution. The high TDS levels as indicated usually reflect the presence of inorganic salts such as calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulfates in addition to small amounts of organic matter, which may be objectionable to consumers (WHO, 2022b). TDS levels in water usually originate from natural sources such as rocks, bedrock, soil, plankton, silt, seawater intrusion, sewage, urban runoff, and industrial wastewater. At TDS levels lower than 600 mg/l, the taste of water is acceptable; however, it may become significantly unpalatable for consumers at levels exceeding 1000 mg/l (WHO, 2022b). On the other hand, TDS levels greater than 1200 mg/l are associated with excessive scaling in water pipes, heaters, boilers, and household appliances (WHO, 2022b), Still, no direct health hazards are associated with the ingestion of water containing high levels of TDS. However, their presence may be associated with irritation of the gastrointestinal tract (WHO, 2022b).

As for electrical conductivity in the water, it is evident in all collected water samples in both camps, except in the purchased drinking water samples of the Choueifat ISs. This parameter is primarily caused by the minerals present in the water and can be possibly attributed to seawater infiltration, potentially due to the proximity of these camps to the coastal areas.

2. *Chemical Quality*

pH was leaning towards alkalinity in all collected samples, and this can also be possibly attributed to seawater infiltration. Alkalinity was also high, reaffirming the almost-alkaline pH, which may be explained by the natural formation of the groundwater well (Calcium and Magnesium increase alkalinity and they originate from the groundwater’s geologic limestone formation in both camps) and by the buffering effect which turns water from a weak acid into an alkali, through Ca and Mg reacting with carbon dioxide (CO_2) and forming carbonates and bicarbonates. The limestone nature of the aquifers may cause a higher deposition of minerals in the water (Shaban, 2020). At the same time, alkalinity can result from agricultural runoff, which may include phosphate and potassium, and from sewage contamination and surrounding industries (U.S. Geological Formation, 2018).

Hardness, which includes carbonate and non-carbonate, was also found to be high in all collected water samples in both camps, except in the purchased drinking water samples of the Choueifat ISs. Non-carbonate hardness is attributed to chlorides and sulfates and, in this case, is probably present as a result of the potential sources of pollution from sewage and seawater infiltration, and to a lesser extent from organics present in the industrial effluent and agricultural runoff. As for sulfates, all tested samples consisted of acceptable levels.

While Ammonia-Nitrogen, Nitrate-Nitrogen, and Nitrite-Nitrogen were relatively within acceptable levels based on LIBNOR Standards in almost all samples, excess levels could be due to the bacterial and/or fecal contamination from the sewage as well as the fertilizers that are being used in the agricultural sector (Berger et al., 2022; Sekar et al., 2021; LIBNOR, 2016; Mateo-Sagasta et al., 2017; WHO, 2022b). Orthophosphate levels were of acceptable values in all tested water samples in both camps. In addition, nitrates were found to be partially present, due to the same types of pollution as ammonia, potentially originating from fertilizer-use runoff, septic tank leakage, sewage, and erosion (US EPA, 2022c). On the other hand, chlorides level were excessive in the pool and composite water tank samples, probably emerging from seawater infiltration mostly and from sewage.

Total Chlorine and Free Chlorine levels were relatively low in all tested samples. The likely sources of chlorine contamination are water additives used to control microbes. That can also be linked to the cholera outbreak and the precautions that several citizens in Lebanon started using, which are adding chlorine to the water to kill all microbiological bacteria, namely V. cholera in this case.

Seawater intrusion in coastal areas is common in Lebanon. A previous study revealed that coastal regions in Lebanon, namely those located between Choueifat, Jiye, and Rmaiyleh are subjected to seawater intrusion (Bakalowicz, 2009), rendering the water and soil inadequate for the cultivation of many crops (MoE & UNDP, 2011). This seawater intrusion level is further aggravated by the over pumping of water over a long period of time (MoE & UNDP, 2011). These factors may explain the excessive level of some chemicals and the noncarbonated hardness levels that are associated with high water salinity and increased mineral content.

On the other hand, the deterioration of the distribution networks may be impacting the quality of the distributed water. As well, intermittent distribution of piped water supplies may lead to pressure imbalance and inflow of foreign matter in case of breakage of pipes, leaching from pipes due to stagnation, and contamination from wastewater. All these interfere with the water quality and safety and accordingly the risk of exposure to waterborne diseases.

Additionally, the groundwater wells in both camps are surrounded by residential areas as well as industries in the case of the Choueifat Syrian ISs, so such wells are susceptible to sewage water infiltration, solid wastes, and industrial waste. Furthermore, agricultural activities from the neighboring environment may also lead to runoff from fertilizers and pesticides.

Below are the water quality results detailing the physical, chemical, and microbiological parameters of collected water samples (Tables 5 & 6). Cells in Tables 5 & 6 highlighted with an Asterix (*) exceed the Maximum Admissible Levels/Concentrations of the Libnor national standard for drinking water (2016), highlighted with a Yen sign (¥) exceed those of the WHO Guidelines Drinking water quality guidelines Standards (2022b), and underlined (___) exceed those of the US EPA Primary Drinking Water Regulations (2022c) (Table 7).

Table 5 Mean Values of the Physical and Microbiological Parameters of the Water Supply in the Syrian Refugee camp of Choueifat (n= 3) and the Dbayeh Camp study area (n= 2)

	Physical Parameters			**Microbiological Parameters**	
	Turbidity (NTU)	**Conductivity** (µs/cm)	**TDS** (mg/L)	**Fecal coliform** (CFU/ 100ml)	**Total coliform** (CFU/ 100ml)
Choueifat Syrian Informal Settlements					
FIRST SAMPLING DATE: **Tuesday 6 September 2022**					
Pool	0.53¥	2690*¥	1250*¥	1*¥	too numerous to count*¥
Composite Water Tanks	0.29	2300*¥	1150*¥	19*¥	too numerous to count*¥
Drinking Water Tanks	0.42	308	150	2*¥	too numerous to count*¥
SECOND SAMPLING DATE: **Monday 17 October 2022**					
Pool	0.71¥	2730*¥	1350*¥	68*¥	too numerous to count*¥
Composite Water Tanks (1)	0.38	2660*¥	1340*¥	15*¥	too numerous to count*¥
Composite Water Tanks (2)	0.37	2730*¥	1370*¥		too numerous to count*¥

Drinking Water Tanks	0.58¥	330	170	33*¥	too numerous to count*¥
THIRD SAMPLING DATE: **Monday 24 October 2022**					
Pool	0.78¥	2650*¥	1330*¥	0	too numerous to count*¥
Composite Water Tanks (1)	0.59¥	2730*¥	1370*¥	0	too numerous to count*¥
Composite Water Tanks (2)	0.61¥	2700*¥	1360*¥		too numerous to count*¥
Drinking Water Tanks	0.53¥	290	150	0	too numerous to count*¥
Dbayeh Palestinian Refugee Camp					
FIRST SAMPLING DATE: **Tuesday 13 September 2022**					
Composite Water Tanks	0.99¥	794*	370	0	too numerous to count*¥
Main Storage Tank	0.64¥	881*	410	2*¥	12*¥
SECOND SAMPLING DATE: **Monday 24 October 2022**					
Composite Water Tanks	0.88¥	700*	365	0	too numerous to count*¥
Main Storage Tank	0.56¥	800*	480	0	too numerous to count*¥

* exceed the Maximum Admissible Levels/Concentrations of the Libnor national standard for drinking water (2016)

¥ exceed those of the WHO Guidelines Drinking water quality guidelines Standards (2022b)

___ exceed those of the US EPA Primary Drinking Water Regulations (2022c)

Table 6 Mean Values of the Chemical Parameters of the Water Supply in the Syrian Refugee camp of Choueifat (n= 3) and the Dbayeh Camp study area (n= 2)

	Chemical Parameters											
	pH	**Alkalinity** (mg/L as $CaCO_3$)	**Total Hardness** (mg/L as $CaCO_3$)	**Calcium Hardness** (mg/L as $CaCO_3$)	**Total Chlorine** (mg/L as Cl_2)	**Free Chlorine** (mg/L as Cl_2)	**Chlorides** (mg/L as Cl-)	**Nitrite Nitrogen** (mg/L as NO_2^--N)	**Nitrate Nitrogen** (mg/L as NO_3^--N)	**Ammonia Nitrogen** (mg/L as NH_3-N)	**Orthophosphate** (mg/L as PO_4^{3-})	**Sulfate** (mg/L as SO_4^{2-})
	Choueifat Syrian Informal Settlements											
FIRST SAMPLING DATE: **Tuesday 6 September 2022**												
Pool	7.8	260	850*¥	700*¥	0	0	625*¥	0.017*	9.65	0.055	0.5	123
Composite Water Tanks	7.7	260	800*¥	700*¥	0	0	600*¥	0.016*	1.6	0.045	0.69	117
Drinking Water Tanks	8.1	50	250	200	0.025	0	25	0.006	2.25	0	0.55	15.5
SECOND SAMPLING DATE: **Monday 17 October 2022**												
Pool	7.7	288	800*¥	600*¥	0	0	650*¥	0.007	9.35	0.055	0.81	123
Composite Water Tanks (1)	7.6	282	800*¥	640*¥	0	0	657*¥	0	8.2	0.105	0.79	122
Composite Water Tanks (2)	7.7	286	775*¥	625*¥	0	0	650*¥	0.01	8.4	0.15	0.78	120
Drinking Water Tanks	8.1	103	150	100	0	0	40	0.008	1.8	0.13	0.67	15.5
THIRD SAMPLING DATE: **Monday 24 October 2022**												
Pool	7.8	280	775*¥	585*¥	0.05	0.02	613*¥	0.013	11.15*	0.275	0.38	117
Composite Water Tanks (1)	7.8	272	700*¥	565*¥	0.03	0.02	625*¥	0.009	9.2	0.29	0.59	121

Composite Water Tanks (2)	7.9	290	725*¥	625*¥	0.06	0.04	600*¥	0.007	9.4	0.29	1.06	130
Drinking Water Tanks	8.1	40	100	60	0.1	0.05	55	0.009	1.7	0.23	0.16	12
Dbayeh Palestinian Refugee Camp												
FIRST SAMPLING DATE: **Tuesday 6 September 2022**												
Composite Water Tanks	7.6	240	350	250	0.1	0.1	75	0.021*	8.1	0.26	0.36	39.5
Main Storage Tank	7.5	240	350	300	0.03	0.025	75	0.012	7.05	0.11	0.26	41
SECOND SAMPLING DATE: **Monday 24 October 2022**												
Composite Water Tanks	7.9	245	300	250	0.035	0.03	70	0.011	4.1	0.23	0.32	40
Main Storage Tank	7.8	280	310	265	0.045	0.04	75	0.007	4.45	0.26	0.36	39

Table 7 National & International Guidelines for Water Supplies with Maximum Admissible Concentrations/Levels (WHO, 2022b; US EPA, 2022c; LIBNOR, 2016)

Parameter	Unit	WHO Guideline (2022b)	US EPA (2022c) Regulations	Libnor Standards (2016)
pH	pH Unit	Acceptable range: 6.5-8.5	Acceptable range: 6.5-8.5	Acceptable range: 6.5-8.5
Alkalinity	mg/L as $CaCO_3$	n/a	n/a	n/a
Total Hardness	mg/L as $CaCO_3$	500	n/a	500
Calcium Hardness	mg/L as $CaCO_3$	300	n/a	300
Total Chlorine	mg/L as Cl_2	0.6 - 1	4	0.5
Free Chlorine	mg/L as Cl_2	Acceptable range: 0.2 – 0.5	4	Acceptable range: 0.2 – 0.5

Chlorides	mg/L as Cl^-	250	250	250
Nitrite Nitrogen*	mg/L as NO_2^--N	0.912	1	0.0152
Nitrate Nitrogen*	mg/L as NO_3^--N	11.3	10	10.17
Ammonia Nitrogen	mg/L as NH_3-N	0.5	n/a	0.5 (as ammonia)
Orthophosphate	mg/L as PO_4^{3-}	n/a	n/a	n/a
Sulfate	mg/L as SO_4^{2-}	250	250	250
Turbidity	NTU	0.5	0.3	<1
Conductivity	µs/cm	1000	500	1500
TDS	mg/L	1000	500	1000
Fecal coliform	CFU/ 100ml	0	0	0
Total coliform	CFU/ 100ml	0	0	0

n/a: not assigned

* Conversion factors used as per WHO (2022b): 1 mg/l as nitrate = 0.226 mg/l as nitrate-nitrogen; 1 mg/l as nitrite = 0.304 mg/l as nitrite-nitrogen.

C. Mutagenicity Test Results

When comparing the Palestinian refugee camp in Dbayeh with the Syrian refugee camp in Choueifat, it is evident that only one domestic water supply composite sample in the Palestinian refugee camp has a potential mutagenic concentration, while those in the Syrian refugee camp do not have such mutagenic potency (Table 10). Mutagenicity results showed that almost all water samples are not mutagenic, with the exception of one sample obtained from the Dbayeh Palestinian refugee camp, which showed a positive result with the *TA98* cell line in the presence of S9 water samples (Tables 8 & 9). The positive results with *TA98* with post-mitochondrial rodent liver fraction (S9) mean that the water samples from the Dbayeh camp have the potential of causing frameshift mutations following metabolic bioactivation. Further testing of these alarming findings should be conducted particularly that the camp is supplied with water from the Establishment of the Water of Beirut and Mount Lebanon (EBML) which also supplies neighboring residential and commercial areas. Noting that frameshift mutations are produced by either the addition or deletion of DNA bases creating altered DNA synthesis and mutated DNA sequences and resulting in radical changes in the following protein sequence such as protein truncation (Gardiner et al., 2015; Pelley, 2012). Such mutations can lead to the inactivation of tumor-suppressor genes (Ballhausen et al., 2020; He & Liang, 2015), antibody deficiency and autoimmune disease formation (Schipp et al., 2016), and high tumor mutational burden (Chae et al., 2019).

Similar to our study’s mutagenicity tests, a Salmonella bioassay test on potential mutagenicity in river water samples in China revealed frameshift mutagens and linked such mutagenicity to the presence of aromatic amines and/or polycyclic hydrocarbons in the water body, mainly due to industrial runoff (Liu et al., 2009). Another more recent test on river water in Armenia revealed mutations in stamen hair indicating the potential mutagenic and clastogenic potential of contaminated water (Khosrovyan et al., 2022). Similar to our findings, an Ames test on distributed pipe water samples in Italy detected chemicals capable of inducing frameshift mutations, as they induced point mutations in the *TA98* strain Salmonella bacteria, while results in the *TA100* strain's were borderline and observed at the highest tested dose (Feretti et al., 2020). Another Ames test of water samples collected in the summer season from the River Yamuna in India revealed the maximum mutagenic response with the *TA98* strain, both with and without metabolic activation (Aleem & Malik, 2003). A more recent Ames test on suspended particulate matter in the Seine river estuary detected mutagenic *TA98* strains with S9 mix, confirming frame-shift mutagens in the collected water samples (Vincent-Hubert et al., 2012). Similar tests on water samples reveal cytotoxic, genotoxic, and mutagenic effects of organic and inorganic agents found in water samples obtained from a river in Brazil (Nunes et al., 2011). Nunes et al. (2011) linked such effects to industrial discharges and domestic urban sewages contaminating the water samples. Accordingly, our positive results of potential water mutagenicity may be linked to similar contaminating sources.

On the other hand, studies focused on refugee camps around the globe have also linked water contamination to mutagenic and genotoxic adverse impacts. Groundwater, in Rohingya refugee camps in Bangladesh, was revealed to be contaminated with elevated manganese concentrations and such contamination was linked to increased risks of cognitive development in the refugee children who are residing in the camp and using the water for domestic use across a prolonged period (Rahman et al., 2021). Manganese is considered to have weak mutagenic effects in vitro and possible clastogenic effects in vivo (Assem et al., 2011), hence water contaminated with elevated manganese levels can have significate health risks to its consumers (Gerber et al., 2002).

In addition, most tested samples resulted in potential cytotoxic effects in *TA100* cells, especially at high test sample concentrations (Table 9). Such results can be associated with cytotoxic base substitution activities from most sampled water. Cytotoxicity may include altering cell proliferation and cell death (Krewski et al., 2019), interfering with DNA transcription and replication, controlling the cell cycle (Diaz-Moralli et al., 2013), and potentially inducing cell damage (Akbari & Krokan, 2008) and nuclear alterations (Dos Santos et al., 2021). However, not every cytotoxic agent has mutagenic potency.

Table 8 Mutagenicity results for collected water samples in *TA98* cell line

Choeifat Syrian IS Water Sample

TA98 -S9 **Assay Date:** **21/10/2022**

Conc. ()	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	6.17	3.13	9.29			
3.13	3	10.00	1.00		1.08	**0.9974**	
6.25	3	5.67	1.53		0.61	0.4131	
12.5	3	8.33	2.08		0.90	0.9546	
25	3	4.33	1.53		0.47	0.1027	
50	3	3.67	2.89		0.39	0.0342	
100	3	3.00	3.61		0.32	0.0080	Cytotoxic effect?
2-NF	6	45.17	1.17		**4.86**	**1.0000**	**Mutagenic Conc.**
Limit for "Pass":					*3.00*	*0.99*	

Choeifat Syrian IS Water Sample

TA98 +S9 **Assay Date:** **21/10/2022**

Conc. ()	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	1.67	1.03	2.70			
3.13	3	2.00	1.00		0.74	0.7648	
6.25	3	1.00	1.00		0.37	0.2601	
12.5	3	4.67	3.79		1.73	0.9998	
25	3	2.67	2.08		0.99	0.9354	
50	3	0.67	1.15		0.25	0.1202	
100	3	0.67	1.15		0.25	0.1202	
2-AA	6	48.00	0.00		17.78	1.0000	Mutagenic Conc.
Limit for "Pass":					*3.00*	*0.99*	

Dbayeh Palestinian Refugee Camp Water Sample

TA98 -S9 **Assay Date:** **21/10/2022**

Conc. (%)	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	6.17	3.13	9.29			
3.13	3	11.33	1.53		1.22	0.9999	
6.25	3	6.67	1.53		0.72	0.6988	
12.5	3	6.00	3.46		0.65	0.5124	
25	3	3.33	0.58		0.36	0.0174	
50	3	0.67	0.58		0.07	0.0000	Cytotoxic effect?
100	3	1.33	1.53		0.14	0.0000	Cytotoxic effect?
2-NF	6	45.17	1.17		4.86	1.0000	Mutagenic Conc.
Limit for "Pass":					*3.00*	*0.99*	

Dbayeh Palestinian Refugee Camp Water Sample

TA98 +S9 **Assay Date:** **21/10/2022**

Conc. (%)	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	1.67	1.03	2.70			
3.13	3	2.00	1.00		0.74	0.7648	
6.25	3	3.33	3.06		1.23	0.9879	

12.5	3	3.00	2.00	1.11	0.9707	
25	3	6.67	4.04	**2.47**	**1.0000**	**Mutagenic Conc.**
50	3	1.33	2.31	0.49	0.4374	
100	3	2.33	4.04	0.86	0.8703	
2-AA	6	48.00	0.00	**17.78**	**1.0000**	**Mutagenic Conc.**
Limit for "Pass":				*3.00*	*0.99*	

Table 9 Mutagenicity results for collected water samples in *TA100* cell line

Choeifat Syrian IS Water Sample

TA100 -S9 **Assay Date:** **21/10/2022**

Conc. (%)	**n**	**Mean # positive wells**	**SD**	**Base-line**	**Fold increase over baseline**	**Binomial B-value**	**Mutagenic Conc. / Cytotoxic Effect**
0	6	21.50	2.59	24.09			
3.13	3	18.33	5.69		0.76	0.0651	
6.25	3	12.00	4.00		0.50	0.0000	Cytotoxic effect?
12.5	3	11.33	2.08		0.47	0.0000	Cytotoxic effect?
25	3	5.67	4.93		0.24	0.0000	Cytotoxic effect?
50	3	0.67	0.58		0.03	0.0000	Cytotoxic effect?
100	3	1.67	1.53		0.07	0.0000	Cytotoxic effect?
4-NQO	6	46.83	0.75		1.94	**1.0000**	
Limit for "Pass":					*2.00*	*0.99*	

Choeifat Syrian IS Water Sample

TA100 +S9 **Assay Date:** **21/10/2022**

Conc. (%)	**n**	**Mean # positive wells**	**SD**	**Base-line**	**Fold increase over baseline**	**Binomial B-value**	**Mutagenic Conc. / Cytotoxic Effect**
0	6	7.00	2.37	9.37			
3.13	3	9.67	3.21		1.03	0.9736	
6.25	3	9.00	3.61		0.96	0.9336	
12.5	3	10.67	2.89		1.14	**0.9950**	
25	3	4.33	2.31		0.46	0.0325	
50	3	2.00	3.46		0.21	0.0001	Cytotoxic effect?

100	3	1.33	1.53		0.14	0.0000	Cytotoxic effect?
2-AA	6	47.00	1.67		**5.02**	**1.0000**	**Mutagenic Conc.**
Limit for "Pass":					*2.00*	*0.99*	

Dbayeh Palestinian Refugee Camp Water Sample

TA100 - S9 **Assay Date:** **21/10/2022**

Conc. (%)	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	21.50	2.59	24.09			
3.13	3	14.67	2.08		0.61	0.0003	Cytotoxic effect?
6.25	3	12.67	3.06		0.53	0.0000	Cytotoxic effect?
12.5	3	13.67	1.53		0.57	0.0000	Cytotoxic effect?
25	3	6.33	2.52		0.26	0.0000	Cytotoxic effect?
50	3	0.00	0.00		0.00	0.0000	Cytotoxic effect?
100	3	0.00	0.00		0.00	0.0000	Cytotoxic effect?
4-NQO	6	46.83	0.75		1.94	**1.0000**	
Limit for "Pass":					*2.00*	*0.99*	

Dbayeh Palestinian Refugee Camp Water Sample

TA100 +S9 **Assay Date:** **21/10/2022**

Conc. (%)	n	Mean # positive wells	SD	Base-line	Fold increase over baseline	Binomial B-value	Mutagenic Conc. / Cytotoxic Effect
0	6	7.00	2.37	9.37			
3.13	3	5.00	1.73		0.53	0.0931	
6.25	3	5.33	2.31		0.57	0.1428	
12.5	3	8.67	1.15		0.93	0.9002	
25	3	1.33	1.53		0.14	0.0000	Cytotoxic effect?
50	3	0.00	0.00		0.00	0.0000	Cytotoxic effect?
100	3	0.00	0.00		0.00	0.0000	Cytotoxic effect?
2-AA	6	47.00	1.67		**5.02**	**1.0000**	**Mutagenic Conc.**
Limit for "Pass":					*2.00*	*0.99*	

Legend:

- Fold increase over baseline values ≥ 2.0 appear in bold red
- A binomial B-value ≥ 0.99 indicating spontaneous mutation appears in bold red
- Data points with Fold increase ≥ 2 and Binomial B-value ≥ 0.99 are labeled "Mutagenic Conc.”
- Data points that are significantly smaller (B ≤ 0.01) than the Mean number of Spontaneous Revertants are labeled "Cytotoxic effect?"

Table 10 Mutagenicity results summary for collected water samples

Sample	**Mutagenic data points**		**Overall Result for strain *TA98* (at the tested doses)**	**Overall Result for strain *TA100* (at the tested doses**
	w/o S9	**w S9**		
Choeifat Syrian IS Water	No	No	Probably not mutagenic	Probably not mutagenic
Dbayeh Palestinian Refugee Camp Water	No	Yes (*TA98*)	Probably mutagenic	Probably not mutagenic

CHAPTER V

LIMITATIONS, CHALLENGES, CONCLUSIONS & RECOMMENDATIONS

Several limitations and challenges were faced throughout the preparation and execution phases of the thesis work. The following are the main highlights of the limitations and challenges. First, the SolEx C18 columns were used instead of Amberlite XAD-2 columns due to the unavailability of XAD-2 given that the suppliers no longer ship the XAD-2 columns to Lebanon. Accordingly, we had to adjust the preconditioning criteria of the used XAD columns. Second, the season of the year (wet/dry season) impacts the water quality, making it important to take samples and measure the parameters to optimize the strategies implemented all year long. Accordingly, given that we only tested during the dry season then we could have missed wet season water replenishment and quality analysis. Had the samples been collected in the wet season, there might have been dilution factors potentially resulting in lower concentrations of pollution compared to the dry seasons. And third, the small water sample size may not accurately represent the water supply in all the Syrian and/or Palestinian refugee camps.

Despite the mentioned limitations and challenges, findings from this study may be used to inform and direct stakeholders and funding agencies by providing new evidence to guide WASH programs and interventions. According to national and international guidelines (Table 7), the water bodies in the Choueifat Syrian ISs and the Dbayeh Palestinian camps may pose a health risk to its residents. However, further testing is advised to confirm our findings. That is especially given that the Dbayeh refugee camp which has a primary potable water source from the Establishment of the Water of Beirut and Mount Lebanon (EBML), which provides water for “about half of the Lebanese people located in the Lebanese capital Beirut and the regions of Mount Lebanon” (EBML, 2023). Hence, this water safety issue is of nationwide concern as it does not only impact the refugees hosted in Lebanon but also the Lebanese citizens themselves who are supplied from the same source. The deterioration in water quality is also becoming an observed pattern in informal urban areas and settlements in the country, where there is a lack of adequate shelter conditions, and inadequate WASH services.

Our findings, in addition to published findings in the literature, point out that durable solutions for refugees and sustainable solutions for the water crisis in Lebanon are inevitable. Our results in this study call for a large-scale water monitoring scheme, or an adopted policy on water monitoring in refugee camps, nearby neighborhoods, and other urban informal settlements, to address the need for improved shelter and sanitation infrastructure across the country. The recent cholera outbreak that started in a Syrian refugee camp in Lebanon can be a direct example of how severe a weak water management infrastructure can be. The cholera outbreak demonstrated that the impact of poor access to safe water and poor WASH on refugees goes beyond the boundaries of the camps themselves to reach the entire hosting nation, hence further burdening the deteriorating environment and the healthcare system in Lebanon.

Given that temporality is a factor that cannot be ignored, one must acknowledge the fact that, while both camps showed poor water quality, the Palestinian refugee camp in Dbayeh has been used for a much longer time than the Choueifat Syrian Refugee camp. Therefore, the worn out pipes and deteriorated water distribution system in the Dbayeh Refugee Camp have most probably impacted the difference in water quality and toxicity results from the more recently established and inhabited Choueifat Refugee Camp. Results from this study may be used as a baseline to influence decision-making at the level of international donors and local authorities, particularly in adopting a more comprehensive all-inclusive sustainable refugee management framework that takes into account WASH standards, until all refugees and displaced groups safely return to their countries of origin.

Additionally given that weak water distribution infrastructure, intermittent water distribution schemes, and worn-out water pipes can lead to chemical imbalances and biological interferences, proper monitoring and management of the water sector in the country is crucial. As water supply systems deteriorate, water pumping operations are shut down, costs of trucked water are on the rise, governmental investments for Water Establishments’ operations are off the table, and the country’s water monitoring and management systems have turned from minimal to null amidst an unprecedented national socioeconomic crisis (UNICEF, 2022).

Meanwhile, it is important to identify water quality monitoring sampling points for continuous assessment and evaluation of water quality and safety of the water tanks. This will facilitate comparing this study’s water quality and toxicity results to those of other informal urban areas and/or refugee camps on a larger scale. In addition, there is a need to conduct further testing of additional parameters such as bromate, bromide, and iodide in order to confirm contamination through seawater infiltration, and potassium in order to confirm and trace contamination by agricultural runoff. In addition, parameters should be monitored at multiple time points in order to account for seasonal variability (wet and dry).

This study has also highlighted the importance of an *in vitro* approach to test for water cytotoxicity and biological activity, in order to better assess the water's health impact. Water mutagenicity in particular can become a tool for assessing water supply on a national level. More specifically, national testing of water mutagenicity can help in the advanced assessment of WASH burden of disease, forecasting of potential epidemic outbreaks, and avoidance of such events.

In the future, we recommend the development and implementation of a water safety plan to profile the sources and to document and manage exposure to sources of pollution for all the refugee camps and settlements. The plan should be based on a holistic management and risk assessment strategy covering all stages of the drinking-water supply chain from source to end-user, guaranteeing the safety of drinking water (WHO, 2009). Accordingly, the plan should include the examination and identification of all possible pollution sources and characteristics, as well as the eventual efficient improvement of the water supply system assessment, process management, stakeholder engagement, and operational monitoring (WHO, 2009). Another important recommendation from this study is developing a concise policy brief that can use our methodology and findings to target multilateral management approaches to the water supply safety issues at hand.

APPENDIX I

Assessment Survey Used During the First Field Visit at Each Site

American University of Beirut - الجامعة الأمريكية في بيروت

Faculty of Health Sciences - كلية العلوم الصحية

Department of Environmental Health - إدارة الصحة البيئية

Survey - تقييم

Assessment of the Refugee Camp Site for Feasibility of Water Sampling

تقييم موقع المخيم لأخذ عينات من المياه

Dear Sir / Madam;

The following survey is conducted as part of the graduation project required to obtain a Master's Degree in Environmental Health at the Faculty of Health Sciences from the American University of Beirut.

This study aims at assessing the feasibility of collecting water samples from this camp.

Please note that this survey is intended for academic purposes only. All information is confidential and your name will not be displayed in the final report. Also, note that no direct benefit is provided to the respondent filling/answering this survey. However, your participation is highly appreciated to enrich our field studies and facilitate the process of our study site selection.

Thank you for your cooperation.

حضرة السيد / السيدة؛

المسح التالي يُجرى كجزء من مشروع التخرج المطلوب للحصول على درجة الماجستير في الصحة البيئية في كلية العلوم الصحية من الجامعة الأمريكية في بيروت.

وتهدف هذه الدراسة إلى تقييم جدوى جمع عينات مياه من هذا المخيم.

يرجى ملاحظة أن الغرض من هذا الاستطلاع هو أكاديمي فقط. كما وأنّ جميع المعلومات سرية ولن يتم عرض اسمك في التقرير النهائي. الرجاء أخذ بعين الاعتبار أيضا أنه لم يتم تقديم أي فائدة مباشرة إلى المجيب الذي قام بالرد على هذا الاستطلاع. ومع ذلك، فإن مشاركتكم تقدر تقديرًا عاليًا لإثراء دراساتنا الميدانية وتسهيل عملية اختيار موقع الدراسة.

شكراً لكم على تعاونكم.

Geographic Location:

Date of Interview:

Time of Interview: Start: End:

الموقع الجغرافي:

تاريخ المقابلة:

وقت المقابلة: البدء: النهاية:

1. What is the number of people residing on the site?
   ما هو عدد الأشخاص المقيمين في الموقع؟

2. How long has the site been inhabited?
   منذ متى والموقع مأهول؟

3. Is there a central water supply system for potable water distribution?
   هل هناك نظام مركزي لإمدادات المياه لتوزيع المياه الصالحة للشرب؟

4. What Is the type of water distribution system network (material)?
   ما هو نوع شبكة توزيع المياه؟

5. Is there a major water Storage Tank from which water is distributed (capacity)?
هل يوجد خزان مياه رئيسي يتم توزيع المياه منه؟

6. What is the primary source of water and what is the percentage of distribution?
    a. Unsafe surface water source (rivers/lakes/irrigation or drainage channels)
        i. Groundwater wells
        ii. Springs
        iii. Unprotected spring(s)
    b. Water trucking paid for by NGO
    c. Water trucking paid for by residents

ما هو المصدر الرئيسي للمياه وما هي النسبة المئوية للتوزيع؟
أ. مصدر المياه السطحية غير الآمنة (الأنهار/البحيرات/قنوات الري أو الصرف)
i. آبار المياه الجوفية
ii. نبع ماء محمي
iii. نبع ماء غير محمي
ب. نقل المياه بالشاحنات الذي تدفع عنه المنظمات غير الحكومية
ج. شاحنات المياه التي يدفع عنها السكان

7. Do the sources differ in availability along the year? (based on the seasons)
هل تختلف مصادر المياه من حيث توافرها على مدار السنة؟ (استنادًا على الفصول)

8. What is the water used for …
    a. Drinking
    b. Cooking/washing food
    c. Personal hygiene
    d. Domestic hygiene

ما هو الماء المستخدم من أجل:
أ. الشرب
ب. الطبخ/غسل الطعام
ج. النظافة الشخصية
د. النظافة المنزلية

9. Is the source of water treated before distribution? If so, what is the type of treatment?
هل يتم معالجة مصدر المياه قبل التوزيع؟ إذا كان الأمر كذلك، فما هو نوع العلاج؟

10. Is the water Quality Monitored and by Whom?
هل نوعية المياه مراقبة ومِن مَن؟

11. What type of Monitoring is done?
أي نوع من المراقبة يتم؟

Monitoring - المراقبة	**Type of test -** نوع الاختبار	**Frequency -** تواتر إجراء الاختبار
Microbiological - ميكروبيولوجي		
Physical - طبيعي		
Chemical - كيميائي		

12. What are the Major water complaints relating to the provided water?
ما هي شكاوى المياه الرئيسية المتعلقة بالمياه المقدمة؟

13. Does the community use additional types of complementary water sources, and if so, what are they?
وهل يستخدم المجتمع المحلي أنواعا إضافية من المصادر التكميلية للمياه، وإذا كان الأمر كذلك فما هي؟

14. Are these complimentary water sources monitored/ By whom and what type of monitoring?
هل يتم رصد مصادر المياه التكميلية هذه؟ من جانب من وما نوع الرصد؟

APPENDIX II

Ames Test Photo Results

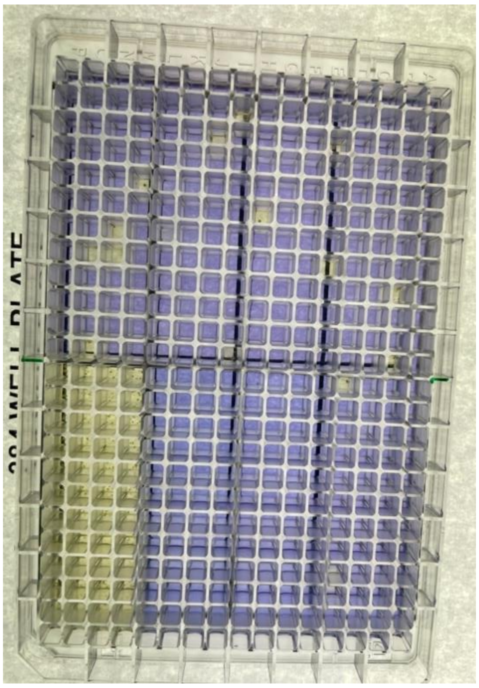
Figure 10 TA98 with S9 - Dbayeh

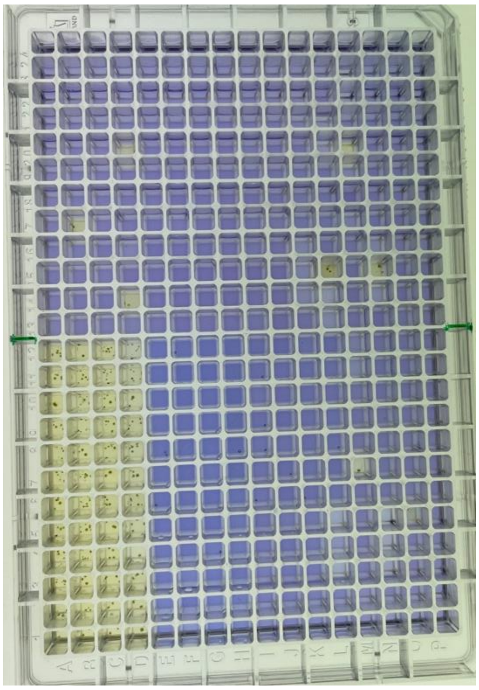
Figure 11 TA98 without S9 – Choueifat

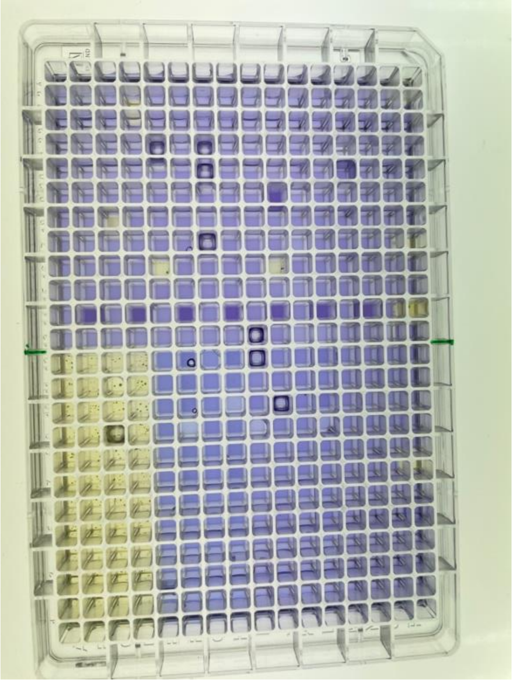
Figure 12 TA98 with S9 - Choueifat

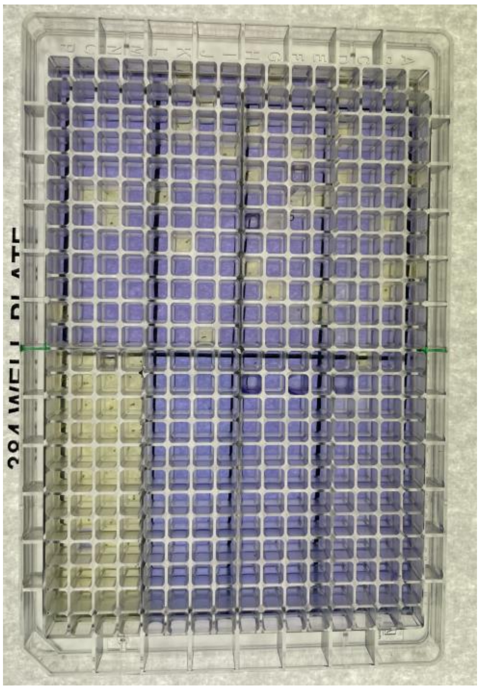
Figure 13 TA98 without S9 – Dbayeh

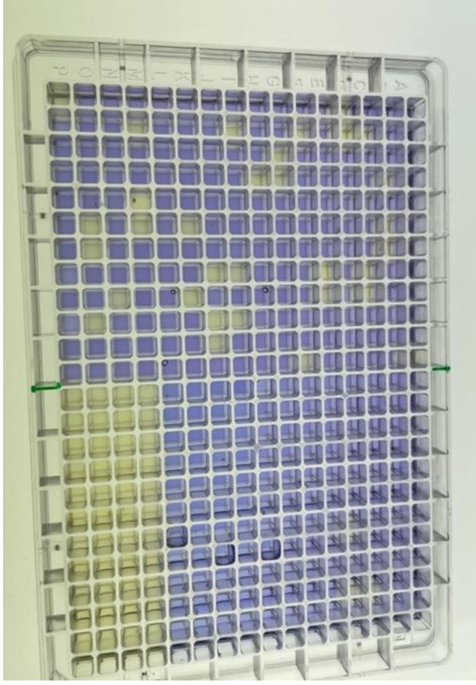

Figure 14 TA100 with S9 – Dbayeh

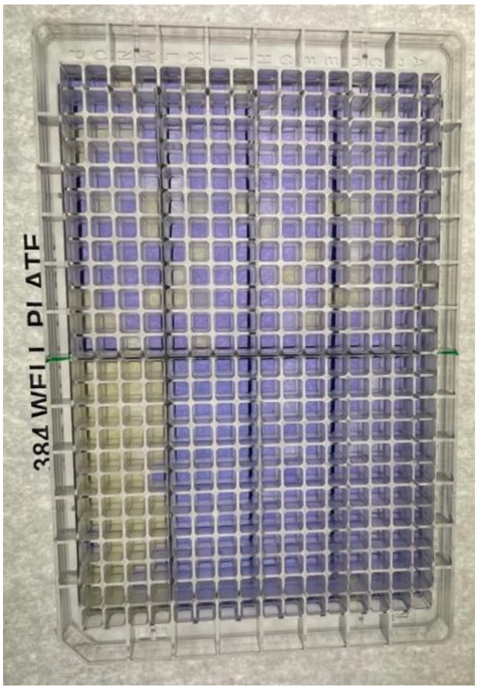

Figure 15 TA100 with S9 – Choueifat

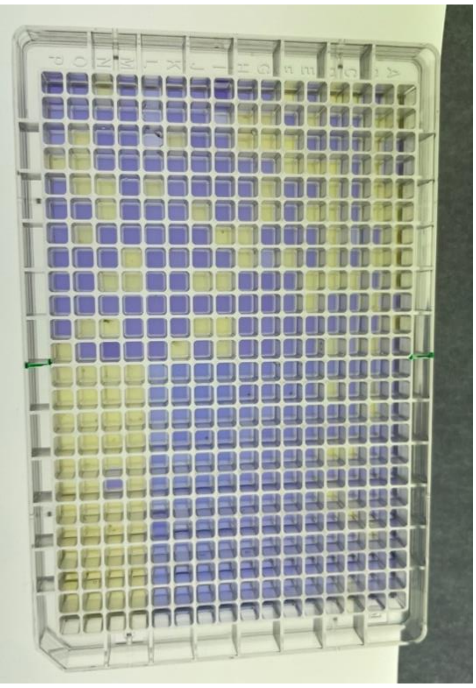

Figure 16 TA100 without S9 - Dbayeh

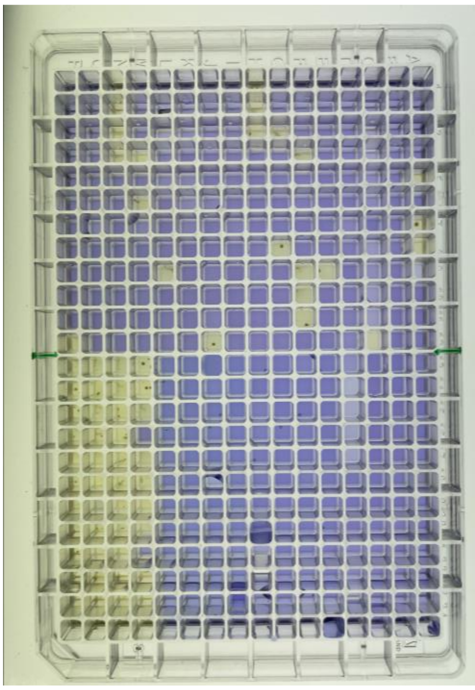

Figure 17 TA100 without S9 – Choueifat

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