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# Isolation and Identification of Bacteria from Groundwater Sources after Floods in Derna City, Libya.

**Fatin S. Al_masouri[1], Mahmoud F. Gaballa[3], Idress Hamad Attitalla[*3], Maraia F. Elmhdwi[2]**

[1]Department of medical laboratories, Faculty of Medical Technology Derna, Libya

[2]Department of chemistry, Faculty of Science, Benghazi University. Benghazi, Libya

[3]Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University AL-Bayda, Libya

***Corresponding Author: Idress Hamad Attitalla**

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Floods, Derna City, groundwater, Daniel Hurricane

**ABSTRACT:**
Flooding is the most common type of disaster globally, responsible for nearly half of all natural disaster victims and economic losses. During and after flooding, human exposure to microorganisms and chemical pollutants. This study aimed to assess and compare the quality of groundwater in Derna City, Libya, between affected and unaffected areas. The results were also compared with international drinking water guidelines recommended by the World Health Organization (WHO) and Libyan standards. A total of 50 groundwater samples were collected in September 2023 from both affected and unaffected regions in Derna city to assess their microbiological and some physical-chemical properties. The results of the water samples showed that pH values ranged from (6.97 to 7.82) in unaffected areas and from (6.99 to 7.96) in affected areas, indicating neutral to slightly alkaline conditions. Temperature in unaffected areas ranged from (19.5°C to 22.9°C) while in affected areas it ranged from (18.7°C to 22.5°C), These values were within the limits recommended by (WHO). Electrical Conductivity (EC) values in unaffected areas ranged from (1680 to 5210µS/cm), while in affected areas they ranged from (390 to 5214µS/cm), These results indicate that most samples exceeded permissible limits. Total Dissolved Solids (TDS) also exceeded permissible limits in most samples, with values ranging from (1070 to 3320mg/l) in unaffected areas, and from (260 to 3389.1 mg/l) in affected areas. All samples in unaffected areas were colorless, except for samples (S15, S17, and S19). Similarly, in affected areas, all samples were colorless except for (S25, S38, S39, S44, S48, and S49). Microbiological analyses showed the Total Bacterial Count in unaffected areas ranged from (50 to >300 CUF/ml), while in affected areas it ranged from (10 to >300 CUF/ mL). Total Coliform Counts ranged from (0 to >300 MPN/100mL) in unaffected areas, and from (0 to 240 MPN/100 mL) in affected areas. E.coli levels from (10 to >300 MPN/100ml) in the unaffected areas, while it was from (0-90 MPN/100ml) affected areas. SPSS analysis showed it was fond that most of the samples in the unaffected areas were seriously contaminated to Total Count it was in proportion (68.5%), Total Coliform Bacteria (84.6%) and E.coli (81.7%), as for the affected areas Total Count (31.5%), Total Coliform (15.4%), and Fecal Coliform (18.3%) except of some sample for the steam station was a natural result sample (S50), (S35), (S47), (S8), and (S5). The results revealed that bore well water is not safe for human consumption. The presence of indicator bacteria suggests potential contamination with pathogenic bacteria. Major contributing factors include the flood, aging water infrastructure, pipeline leaks, poor sanitation, and inadequate waste management.

**INTRODUCTION:** Flooding is one of the most common natural disasters globally, responsible for nearly half of all disaster-related casualties and significant economic losses (EM-DAT. 2011). It can be defined as the overflow of water onto land that is usually dry. Flooding occur when water from sources such as rivers, lakes, or oceans surpasses their natural boundaries or due to heavy rainfall saturating the ground. These events greatly impact both the environment and human settlements (Wayback Machine. 2023). Flooding are triggered by various factors such as prolonged heavy rainfall, rapid snowmelt, strong winds over water, high tides, tsunamis, or dam failure. A notable example is the catastrophe in Derna, Libya, Storm Daniel made landfall in Libya on 10 September, bringing strong

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winds and heavy rainfall. The following day, two dams located upstream of Derna collapsed under the intense pressure caused by the heavy rainfall, resulting in the release of 30 million cubic meters of water that ripped through the city, home to approximately 100,000 inhabitants. the event involved two dams, The Derna Dam, the larger of the two, is located approximately 12 km from the city, while the smaller Mansour Dam is situated just 1 km from the city. Both dams are embankment dams with clay fill. historical imagery from Google Earth collected between 2009 and 2023 illustrates that the dams' reservoirs and the downstream river had been consistently at very low levels or completely empty. This is also confirmed by the adopted Shuttle Radar Topography Mission (SRTM) Digital Elevation Model (DEM) (SRTM Topography Database (NASA) via Watkins). where Storm Daniel released 350 mm of rain within 24 hours in the Wadi Derna basin. The enormous runoff overwhelmed two dams, causing them to collapse and releasing massive flood waves that destroyed entire neighborhoods and led to significant loss of life and infrastructure damage. This devastating event resulted, leaving behind a landscape of destruction and heartbreak (Annunziato, *et al*., 2023). which shows an empty riverbed upstream and downstream of the dams. The sudden surge of water resulting from the intense precipitation associated with Storm Daniel likely led to the overfilling of the Derna Dam and subsequent abrupt failure of the earth fill (Technical Data on Derna and Mansour Dams 2024). collapsed not being able to resist the huge volume of water experienced an unprecedented environmental and geographical disaster. Satellite imagery captured before and after the storm revealed the profound transformation of the city's landscape as illustrated (**Figure 1**). Derna was left devastated, with bridges completely destroyed and streets submerged in muddy water, resulting in the near-total paralysis of its infrastructure. drenched the area and brought flooding to the region. About 25 percent of the Libyan port city of Derna was destroyed by a massive torrent of water and mud after two dams located upstream from the city collapsed from pressure brought on by the heavy rains.

**Figure 1. City of Derna before (left, 19 June 2023) and after (right, 13 September) the Flood (©Google Earth).**

There is a critical need to evaluate well water quality immediately after flooding events to understand how system characteristics and user behaviors influence microbial contamination rates. Drinking water contamination in flood-impacted private wells is commonly evaluated, groundwater could be chemically, physically, or microbiologically contaminated. Each of which is linked to various sources and health related problems and consequences causing serious health problems. During flood and aftermath there are threats to health and safety. . Humans and other animals that get exposed to the water can get injured and are at risks of exposure to pathogenic microorganisms such as *Campylobacter* spp, *Giardia* spp, *Cryptosporidium* spp, and *Escherichia coli* which are threat to health. For example, according to the UN, diarrhea accounts for 80% of all diseases and over one third of deaths in developing countries, which are caused by the patients' consumption of contaminated water (Gasana, *et al*., 2002). Most of the gastrointestinal infections that may be transmitted through drinking water are transmitted via fecal–oral pathway.

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**MATERIALS AND METHODS:**

**Description of the Study Area:** The Derna city is located in the north-east of Libya in an important locality on the eastern coast of Libya, which is also part of the south shoreline of the Mediterranean basin and its geographical coordinates are located between the latitudes of 32°37' and 32°48' north and the longitudes of 22°30' and 22°55' east (Zurqani *et al*., 2018). The farms are spread out over a large region of the city, and the zone has been heavily impacted by pollutants from a cement factory, a water desalination plant, agricultural chemicals, and recently, floods. Groundwater wells are the primary water source in Libya, including the city of Derna; as the city is boarded from the south by a chain of rocky hills the city has expanded along the shore line. (**Figure 2).**

**Water Samples:** 50 samples of groundwater were collected from the Derna city from un-affected and affected areas "after cleaning the wells" from the Daniel flood, Derna city 2024.


**Figure 2. Map of the Locations of the Studied Area (Google Earth).**

**Groundwater Samples Collection:** Firstly, data collection was performed to obtain the pertinent information needed for this research. The samples for primary information were all gathered from the groundwater wells in the study area. The sampling of data collection and laboratory extraction of primary data started on 25 January 2024 and was completed on 25 August 2025. In accordance with the procedures indicated. Separate bottles were designated for chemical and biological analyses, following standardized procedures to ensure sample integrity during collection and transport. The samples were transported from Derna to Benghazi in insulated cool boxes containing ice, and all samples were delivered to the laboratory within 6 hours of collection. Analyses were conducted at the " Water Quality Monitoring Department" of the Man-made River Project, Central Laboratory in Al-Hawari". The physical- chemical and microbiology parameters of the water samples were determined in accordance with the standard method (Bartram *et al*., 1996).

**Groundwater Analysis:**

**Physical – chemical Parameters in the Laboratory:**

**Temperature (°C):** The temperatures of the samples at the point of collection was measured using a mercury in glass thermometer, was measured using an HI 98517 (Hanna Instruments, Smithfield, RI, USA) and was calibrated and set to 25 °C.

**Electrical Conductivity (EC):** Electrical conductivity was measured using a YSI multiparameter water quality meter (Model: 3100C, Xylem, Westchester County, NY, USA).

**Total Dissolved Solids (TDS):** The TDS is a device for measuring TDS is a water mater, it is used to measure the volume of ionized substances, were measured using a YSI multiparameter water quality meter (Model: 3100C, Xylem, Westchester County, NY, USA).

**pH (Value):** The pH-meter model pH-meter model 210A Orion**,** was measured using an HI 9024-C (Hanna Instruments, Smithfield, RI, USA), and the calibration of the pH meter used the standard buffer solutions of pH 4, 7, and 10.

**Color:** Color was determined using a standard visual comparator method and compared against platinum cobalt (Pt- Co) color standards, which is a widely accepted method for water quality assessment.

**Bacteriological Tests:**

**Total Bacterial Count Test:**

Groundwater samples were cultured on Nutrient Agar to isolate and identify general heterotrophic bacteria. This medium Nutrient Agar was chosen for its ability to support the growth of wide range of non-fastidious microorganisms (cappuccino *et al*., 2019).

**Coliform Bacteria:**

Divided into two groups:

- Total Coliform Bacteria.
- (Fecal) Coliform Bacteria.

Were carried out by method Most Probable Number (MPN) MTFM Technique. This method is also called as multiple tube fermentation technique. This technique was used to detect the total coliforms. The test was performed sequentially in three stages namely the presumptive, confirmed, completed tests (Figueras and Borrego, 2010).

**Presumptive Test:** Prepare MacConkey broth media of single and double strength in test tube with Durham's tube for the detection of formation gas. the tubes are covered with cotton and then sterilized in an autoclave at 121℃ for 15 minutes. Incubate the inoculated tubes or bottles at 37℃ for 24 hr. After 24hr shake each tube gently and examine: if there is no gas, acid, after the first 24 hr. test tubes in a Durham tube or bottles for the presence of color change with gas add another 24 hr. then check the tubes again. If there is no change, a negative result is recorded. If there is a change in color and gas in the dirham tube "**positive result**", Then we move to the confirmatory test

**Confirmed Test**: Gently shake the tubes positive in the presumptive test to redistribute the growth bacteria through the tubes. Using a sterile lobe with a diameter of 3- 3.5 mm, transfer one or more lobes from the tube positive to Brilliant Green Lactose bile (BGB)2% broth. Shake the tubes well until the sample is mixed with the agar**.** Tube incubate the Brilliant Green tubes at 37℃ for 24-48hr. The appearance of gas in the Durham tube at any time during the incubation period is considered a positive result. The numbers of tubes in each set showing gas production, were counted and the most probable count number/100 ml of the water sample was Calculated by comparing with (McCrady chart), following the standard methods for examination of water given by APHA.

**Completed Test:** Since some positive results from the confirmation test may be false positives, it is recommended to perform complete tests. To do this a sample from each tube positive for the confirmation test is placed on EMB or Endo agar plate. In this process, a sample from each BGLB- positive tube is passed onto a selective medium such as eosin methylene blue agar. Both plates are incubated at 44.5± 0.2 ℃ for 24 hr. after incubation, all plates are examined for the presence of typical colonies. *E.coli* produces colonies with a greenish metallic sheen, distinguishing them from non-coli colonies. The presence of typical colonies at high temperatures (44.5℃) indicates the presence of *E.coli*.

**Isolation and Identification of Bacteria:**

**Isolation and Purification of Bacterial Isolates:** The samples were initially cultured on Nutrient agar medium and incubating at 37℃ for 24 hr. after the incubation period, bacterial colonies of various shapes, sizes, and colors were observed. Five ba6cterial colonies were selected colonies that showed great similarity in morphological characteristics and were the most frequently on the culture plates were selected. For initial diagnosis and more accurate bacterial species identification, these selected colonies were re-cultivated on other selective and diagnostic differential media, such as: Blood agar: for detecting hemolytic activity and Mac Conkey agar for isolated Gram-negative.

**Gram Staining Technique:** Is a method of staining used to classify bacterial species into two large groups: gram positive bacteria and gram negative bacteria.

**Biochemical Characterization**: Biochemical test is among the most essential tools used for the accurate identification and isolation of bacteria, as they rely on the ability of microorganisms to produce specific enzymes or utilize certain chemical compounds. In this study, a set of standard biochemical tests was employed, which are widely recognized diagnostic tools in clinical microbiology. Aiding in their classification as outlined in (Berge'sManual of Determinative Bacteriology and Manual of Clinical Microbiology) published by the American Society for Microbiology (ASM). Furthermore, the obtained results were compared with standard reference tables found in (Jean F. McFaddin's 2000) Biochemical Tests for Identification of Medical Bacteria to ensure accurate matching and identification of bacterial species based on their biochemical profiles.

**Catalase Test:** The catalase test is important because it is used to detect the presence of the catalase enzyme in bacteria. This helps differentiate between different types of bacteria based on their ability to break down hydrogen peroxide into water and oxygen.

**Citrate Utilization Test:** The citrate test is used to determine if a bacterium can use citrate as its sole carbon and energy source. It relies on the bacteria's ability to produce the enzyme citrate permease, which transports citrate into the cell for metabolism.

**Coagulase Test:** The coagulase test is a bacterial test used to determine wither bacteria can produce an enzyme called coagulase, which converts fibrinogen in plasma into fibrin, leading to plasma clotting.

**Triple Sugar Iron (TSI):** To detect the ability of bacteria to ferment three sugars (glucose, lactose, sucrose), and to produce hydrogen sulfide ($H_2O$) and/or gas. The test uses a specialized agar slant medium test tube to provide both aerobic (on the slant surface) and anaerobic (in the butt) environments. A pH indicator (phenol red), sodium thiosulfate, and an iron salt (like ferrous ammonium sulfate).

**Oxidase Test:** The oxidase test was used to determine whether a bacterium produces the enzyme cytochrome c oxidase. which is part of the bacterial electron transport chain, In the presence of cytochrome c oxidase, a colorless reagent (such as tetraethyl-p-phenylenediamine) is oxidized and turns dark purple or blue within seconds. If the bacterium does not have the enzyme, there is no color change.

**Sulfide-Indole-Motility (SIM):** The SIM test stands for sulfide, indole, and motility test. it is a combination test used to identify and differentiate gram-negative enteric bacteria based on three characteristics, sulfide reduction: detects the ability of bacteria to produce hydrogen sulfide ($H_2O$). The hydrogen sulfide gas reacts with iron salts in the medium to from a black precipitate, indicating a positive result. Indole production: detects the ability to produce indole from the amino acid tryptophan. After adding Kovac's reagent, a red or pink layer on the surface indicates positive indole test. Motility: tests whether bacteria can move through the semi-solid medium. Motile bacteria spread away from the stab line, making the medium turbid.

**Urease Test:** The urease test is a biochemical test used to determine whether bacteria produce the enzyme urease, which breaks down urea into ammonia ($NH_3$) and carbon dioxide ($CO_2$). This reaction increases the alkalinity of the medium due to the released ammonia, causing a color change in the medium depending on the pH indicator used. Use a medium containing urea and a pH indicator (such as phenol red).

**Statistical Analyses**:

SPSS software version 26 was used to analysis the obtained data and the resulting statistics presented to identify if there are differences between the parameter affected and unaffected areas process for the Descriptive statistics, according to the methods described by (chase,1967)

## RESULTS AND DISCUSSION

The results were compared against by the World Health Organization (WHO) and local Libyan specification. The results are presented in the following tables, and discussed individually as follows:

**Physio-chemical Analysis:**

**pH:** groundwater obtained from unaffected areas, as shown in **Table (1)** the lowest pH value was recorded at well sample (S20) with a pH of (6.97), while the highest value was observed at well sample (S1) with a pH of (7.82). For the affected areas, the results lowest pH value well sample (S30) (6.99), whereas the highest pH value was recorded at well sample (S24) (7.96) resented in **Table (2).** The pH values of groundwater samples collected from both affected and unaffected areas were found to be within the acceptable limits recommended by the World Health Organization (2017) and the

Libyan drinking water standards (2008), ranging between 6.5 and 8.5 Show in **Table (3)**. No significant differences were observed between the affected and unaffected areas, as all pH values remained stable within the natural range. It should be noted that the samples from the affected areas were collected after well cleaning and disinfection procedures, which likely contributed to maintaining stable pH levels and preventing any deviations outside the permissible limits. at A similar finding was reported by (Brhane *et al*., 2017), who studied the groundwater quality in the Adiabat region of Ethiopia and indicated that the pH distribution ranged between (6.5 and 8.5).

**Temperature (°C):** The groundwater temperature was measured as presented in **Table (1)** for the unaffected area, where the lowest temperature was recorded in sample (S19) (19.5°C) while the highest temperature was observed in sample (S11) (22.9°C). and in the affected areas, as showed **Tables (2)**, the lowest temperature was recorded in sample (S50) (18.7°C), whereas the highest was found in the sample (S26) (22.5°C), the results of this study indicate that the groundwater temperatures in both affected and non-affected areas fall within the limits recommended by (WHO) and comply with the standards adopted in Libya as show in **Table (3)**. This finding is similar to that of a previous study by (Mostafa *et al*.,2013)**.** who revealed that the variation in groundwater temperature may be due to dissimilarities in the collection time, which fluctuates from one period to another.

**Electrical Conductivity (EC):** The Electrical Conductivity of water obtained from the studied wells in unaffected erase, as presented in **Table (1),** show that the lowest EC value was recorded in sample (S16) groundwater well (1680 µs/cm), while the highest value was observed in the sample (S20) groundwater well (5210 µs/cm). all results were found to exceed the permissible limits which were acceptable based on the WHO's, except for samples (S12, S14, S16, S17, S18, S19). It was within the permissible limits according to the Libyan specifications, in contrast, for the affected areas, as shown **Table (2)**, the lowest EC value was recorded in sample (S50) groundwater well (390 µs/cm), whereas the highest value was found in sample (S39) groundwater well (5214 µs/cm). all results exceeded the permissible limits; even after sterilization, therefore, the water from these wells generally unsuitable for drinking purposes, except for sample (S27, S35, S36, S42, S48, S50), which were acceptable based on the It was within the permissible limits according to the Libyan specifications limit for drinking water. Which are within the permissible limits of the world health organization (1500 µs/cm). and the Libyan standard specifications for drinking water, which must not be exceeding (2300 µs/cm) as show in **Table (3)**. these results indicate the strong mineralization of the groundwater wells. This phenomenon might be due to the discharge of sewage water from the residential area towards the groundwater wells in the city. (Qaseem, *et al*., 2018) evaluated the groundwater quality in Iraq and showed that the high EC value was due to the high level of dissolved chemical ions in the groundwater wells; the flow of water through calcite rocks resulted in high total dissolved salts (TDS).

**Total Dissolved Salts (TDS):** The lowest TDS concentration among the unaffected groundwater wells was recorded in the sample (S19) at (1020 mg/L), while the highest concentration was observed in the sample (S20) at (3320 mg/L), as presented in **Table (1)**. In the affected areas, the lowest TDS value was recorded in sample (S50) at (260 mg/L), whereas the highest value was found in the sample (S39) at (3389.1 mg/L), as in show in **Table (2).** It is noteworthy that all groundwater samples exhibited TDS concentration exceeding the (500 mg/L) threshold recommended by WHO for drinking water quality, as presented in **Table (3)**. except for sample (50) where the value was (260 mg/L) within the permissible limit. Indicating salinity or the accumulation of dissolved ions in the groundwater. It was also observed that elevated TDS values persisted even in samples collected from affected areas after well maintenance and disinfection, confirming that this parameter is more closely related to geological formation and recharge sources rather disinfection processes. While disinfection effectively reduces microbial contamination, it does not lower the concentration of dissolved salts and minerals. Similar findings were reported by (Mahmood *et al*., 2018) who conducted a study in the city of Kirkuk in Iraq and showed that TDS ranged from 759 mg/L to 1368 mg/L.

**Color:** The color of the water has no special significance, pure water characterized by being colorless. In some cases, the color of this water is due to the presence of some organic materials, or the presence

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of some element ions, present from the study results obtained from unaffected areas in **Tables (1)** it is clear that: all samples are colorless except sample (S15, S17, S19) which have color. the result obtained from the affected areas as shown in **Tables (2)** it was colorless except of samples (S44, S48, S49) which were colored. some groundwater samples showed noticeable changes in color even after well cleaning and disinfection. This alteration is most likely related to the presence of dissolved metals, particularly iron and manganese, which can oxidize and cause reddish or brownish discoloration. or an apparent color when suspended materials. Similar findings were reported in Misurata city, Libya, where newly drilled well showed a reddish color due to high concentrations of iron compared to other water sources (Alhadded *et al.*, 2023). This indicates that color changes in groundwater are not only linked to microbial contamination but may also persist after disinfection because of the geochemical composition of the aquifer.

**Table (1): Physio-chemical Analysis of Groundwater Samples form Unaffected Areas All the Test Reapplies Three.**

| Color | PH | TDS | EC | TEM | Sample. NO |
|---|---|---|---|---|---|
| Colorless | 7.2 | 2530.56 | 3954 | 22.1 | S22 |
| Colorless | 7.55 | 2053.76 | 3209 | 21.9 | S23 |
| Colorless | 7.96 | 1743.36 | 2724 | 22.4 | S24 |
| Color | 7.91 | 1659.52 | 2593 | 22.4 | S25 |
| Colorless | 7.56 | 2284.8 | 3570 | 22.5 | S26 |
| Colorless | 7.71 | 1013.28 | 1677 | 22.4 | S27 |
| Colorless | 7.43 | 1752.96 | 2739 | 22.5 | S28 |
| Colorless | 7.33 | 2031.36 | 3174 | 22.1 | S29 |
| Colorless | 6.99 | 2941.25 | 4525 | 20 | S30 |
| Colorless | 7.35 | 2284.1 | 3514 | 20.5 | S31 |
| Colorless | 7.49 | 2337.4 | 3596 | 20.5 | S32 |
| Colorless | 7.27 | 2308.8 | 3552 | 20.4 | S33 |
| Colorless | 7.39 | 2674.75 | 4115 | 20.9 | S34 |
| Colorless | 7.16 | 1490.45 | 2293 | 20.8 | S35 |
| Colorless | 7.26 | 1095.5 | 1993 | 20.4 | S36 |
| Colorless | 7.44 | 2763.8 | 4252 | 20.1 | S37 |
| Color | 7.26 | 2797.6 | 4304 | 20.4 | S38 |
| Color | 7.2 | 3389.1 | 5214 | 20.4 | S39 |

**Table (2): Physio-chemical Analysis of Groundwater Samples form Affected Areas, All the Test Reapplies Three.**

| Color | PH | TDS | EC | TEM | Sample. NO |
|---|---|---|---|---|---|
| Colorless | 7.2 | 2530.56 | 3954 | 22.1 | S22 |
| Colorless | 7.55 | 2053.76 | 3209 | 21.9 | S23 |
| Colorless | 7.96 | 1743.36 | 2724 | 22.4 | S24 |
| Color | 7.91 | 1659.52 | 2593 | 22.4 | S25 |
| Colorless | 7.56 | 2284.8 | 3570 | 22.5 | S26 |
| Colorless | 7.71 | 1013.28 | 1677 | 22.4 | S27 |

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| | | | | | |
|---|---|---|---|---|---|
| Colorless | 7.43 | 1752.96 | 2739 | 22.5 | S28 |
| Colorless | 7.33 | 2031.36 | 3174 | 22.1 | S29 |
| Colorless | 6.99 | 2941.25 | 4525 | 20 | S30 |
| Colorless | 7.35 | 2284.1 | 3514 | 20.5 | S31 |
| Colorless | 7.49 | 2337.4 | 3596 | 20.5 | S32 |
| Colorless | 7.27 | 2308.8 | 3552 | 20.4 | S33 |
| Colorless | 7.39 | 2674.75 | 4115 | 20.9 | S34 |
| Colorless | 7.16 | 1490.45 | 2293 | 20.8 | S35 |
| Colorless | 7.26 | 1095.5 | 1993 | 20.4 | S36 |
| Colorless | 7.44 | 2763.8 | 4252 | 20.1 | S37 |
| Color | 7.26 | 2797.6 | 4304 | 20.4 | S38 |
| Color | 7.2 | 3389.1 | 5214 | 20.4 | S39 |

**Table (3): Comparison between WHO and Libyan Guidelines for physical Water Quality Indicators**.

| | | | | | |
|---|---|---|---|---|---|
| Colorless | 7.21 | 1941.25 | 3300 | 22.4 | S40 |
| Colorless | 7.41 | 1284.1 | 3100 | 19.4 | S41 |
| Colorless | 7.48 | 1037.4 | 2070 | 20.2 | S42 |
| Colorless | 7.3 | 1308.8 | 3790 | 21.3 | S43 |
| Color | 7.45 | 1274.74 | 3550 | 19.9 | S44 |
| Colorless | 7.39 | 1490.45 | 3570 | 21.2 | S45 |
| Colorless | 7.07 | 1195.5 | 3260 | 21.3 | S46 |
| Colorless | 7.34 | 1763.8 | 3540 | 19.8 | S47 |
| Color | 7.73 | 1097,6 | 1940 | 20.6 | S48 |
| Color | 7.3 | 2389.1 | 4470 | 20.4 | S49 |
| Colorless | 7.43 | 260 | 390 | 18.7 | S50 |

**Bacteriological Analysis:**

**Total Bacterial County**: In unaffected areas, the (T.C) results ranged from the lowest result was observed in the samples (S5) groundwater well (50 CFU/ml), and the highest occurrence was in the most than

samples groundwater wells (>300 CFU/ml).as show in **Table (11)**. Regarding the samples collected from areas affected by the floods as presented in **Table (12)**, the lowest positive bacterial count was recorded in samples (S50) only from the Steam station in Derna. with groundwater well (10 CFU to CFU/ml). The highest occurrence recorded in the most than samples groundwater wells (>300 CFU). Samples results are compared to the **Table (4).** classification of water based on microbial quality. As showed that the mean unaffected areas 208.14 were higher compared to the flood-affected areas of 165.38 see **Table (5)**. as **(Figure 4)** shows in statistical comparison of the mean total bacteria count, between both affected and unaffected areas. this can be attributed to fact that the affected wells underwent cleaning and disinfection procedures after the disaster, which contributed to reducing the bacterial load. To verify the presence of statically significant differences between the two groups, an Independent Samples t-test was performed. The obtained value was p= 0.243, as show in **Table (6).** Indicating that there was no statistically significant difference between the mean bacterial counts in affected and unaffected areas. As **(Figure 3)** illustrates the percentage distribution of the total bacteria count between proportion was observed in affected areas, the higher proportion was observed in unaffected areas (68.5%), while the affected areas showed a comparatively lower percentage (13.5%). And. Nevertheless, the descriptive differences between the two groups may be related to the disinfection measures applied in the affected areas. these findings are consistent with a previous study conducted in (Chennai, *et al*.,2015). in India, where researchers observed a sharp increase in (TC) immediately after the flood, followed by a notable decrease after restoration and sanitation measures were implemented. This supports the hypothesis that post-disaster interventions- such as well disinfection. And maintenance-play a critical role in reducing microbial load and restoring groundwater quality. so we cannot say for sure whether this sample is fit to drink or not, it is taken as a general indicator of water quality and not as an indicator of pollution. Most of the water samples the exceeded the limit standards of WHO (0/50 mL) drinking water standards.

**Table (4): Classification of Water Based on Microbial Quality.**

| Mean count | Category | Comments |
|---|---|---|
| **0** | A | **-Excellent** |
| | | **-** |
| **01-Oct** | B | **-Acceptable:** but make regular sanitary checks. |
| **Oct-50** | C | **-Unacceptable** |
| **More than 50** | D | **-Grossly polluted:** look for alternative source |

**Tables (5): Descriptive Statistics Total Bacteria Count both of Affected and Unaffected Areas.**

**Statistics**

| Total bacteria count | | Affected | Unaffected |
|---|---|---|---|
| N | Valid | 29 | 21 |
| | Missing | 0 | 8 |
| Mean | | 165.38 | 208.14 |
| Median | | 130 | 300 |
| Std. Deviation | | 130.139 | 126.35 |
| Range | | 299 | 300 |
| Minimum | | 1 | 0 |
| Maximum | | 300 | 300 |
| Percent | | 31.50% | 68.50% |

**Table (6): Statistical Significance, Independent Samples Test for Total Bacteria Count.**

**Independent Samples Test**

| | Levene's Test | t-test for Equality of Means |
|---|---|---|

| | | for Equality of Variances | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| total.count | Equal variances assumed | 1.549 | 0.219 | 1.183 | 48 | 0.243 | 42.76355 | 36.14704 | -29.91494- | 115.44203 |
| | Equal variances not assumed | | | 1.198 | 45.108 | 0.237 | 42.76355 | 35.6893 | -29.11365- | 114.64074 |

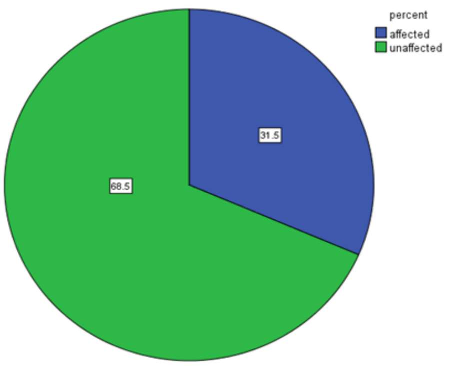


**Figure 3: Percentage Total Bacteria Count between Affected and Unaffected Areas.**

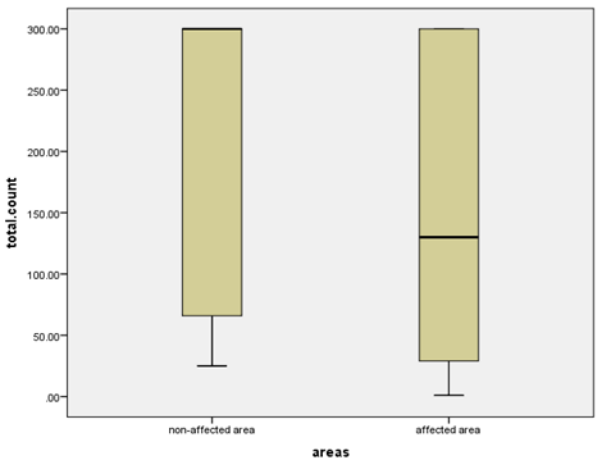


**Figure 4. Comparison Total Bacteria Count in Groundwater Samples between Affected and unaffected Areas.**

**Total Coliform Bacteria TC:** The presence of Total Coliforms (TC) in the groundwater wells ranged between (0.00–>300 MPN/100 ml). the lowest positive result was recorded in the samples (S5, S8), (0.00 MPN/100mL). in contrast, the highest counts were found in most groundwater wells (>300 CFU) in the unaffected areas as show **Table (11)**. For the samples collected from areas affected by the floods in the Derna city as presented in **Table (12)**, the lowest, positive result were observed in some samples (S35, S47, S50) groundwater well (0.00 MPN/100ml), while the highest count was recorded in samples (S24) groundwater wells (240 MPN/100ml). Total Coliform was detected in some groundwater wells in different numbers of propagations on the basis of their location in the study area from affected and unaffected areas and exceeding the WHO's recommended safe limit for drinking water. the statistical analysis in the **Table (7)**. Showed, with a mean of 71.86 and standard deviation 88.969for unaffected areas, with a mean of 21.79 standard deviation 44.531 for affected areas. of the sample taken from the unaffected areas were higher than those collected from the affected areas were collected after cleaning and disinfection procedures had been carried out, which may have contributed to a reduction in the concentration of the targeted elements or contaminants. Based on the Independent Samples Test **Table (8)** the significance value was found to be 0.012, which is less than the standard alpha level 0.05. this indicates that the difference in means between the samples from affected and unaffected areas is statistically significant. As **(Figure 5)** shows the percentage of total coliform bacteria between affected (15%) and unaffected areas (84.6%),where higher level of contamination were observed in unaffected areas compared to affected ones, the box plot **(Figure 6)** indicates greater variation with outliers in the unaffected areas, while the affected areas

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recorded lower values with a narrower range, suggesting that cleaning and disinfection efforts after the flood may have contributed to reducing bacterial contamination levels. The possible of high coliform count non-affected area could be the proximity of certain boreholes to pit latrines and poor sanitary completion of boreholes may have led to contamination of groundwater. Total coliforms can also originate from environmental sources such as soils or from biofilms. Although information on the depth of the sampled boreholes was not available, another possible cause of microbial contamination is the depth of the borehole. During the study, it was observed that some of the boreholes are electrical such that the water is pumped into pipes for distribution**,** Rusty pipes affect the quality of water by allowing seepage of microbial contaminants into the borehole and Therefore, during the flood of Derna city floods some groundwater wells and The presence of Faecal coliform bacteria indicates contamination of water while Faecal waste may contain other harmful or disease-causing organisms including bacteria, viruses, or parasites. If a large number of Faecal coliform bacteria (over 300 colonies/100 mL of water sample) are found, it is possible that pathogenic organisms may also be present in the water. samples. Meanwhile, seasonal floods provide a suitable transmission where the water can be easily contaminated. When flood occurs, the transmission of becomes much evident, as it is transmitted TC must not be found in the water used by humans in accordance with the drinking water quality standards by the WHO. The current findings are consistent with those discussed by (Mkandawire *et al.*,2007) who examined TC in the groundwater in the Blantyre district of Malawi; the mean values of TC were 250 MPN/100 TC. This unexpected result may be attributed to post flood interventions such as well disinfection, changes in groundwater flow.

**Table (7): Descriptive Statistics of Total Coliforms Bacteria Different Between Affected and Unaffected Areas.**

**Statistics**

| Total coliform | | Affected | Unaffected |
|---|---|---|---|
| N | Valid | 29 | 21 |
| | Missing | 0 | 8 |
| Mean | | 21.79 | 71.86 |
| Median | | 6 | 28 |
| Std. Deviation | | 44.531 | 88.969 |
| Range | | 240 | 300 |
| Minimum | | 0 | 0 |
| Maximum | | 240 | 300 |
| Percent | | 15.6 | 84.4 |

**Table (8): Statistical Significance, Independent Samples Test. Total Coliform Bacteria.**

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| total.coliform | Equal variances assumed | 16.55 | 0 | 2.618 | 48 | 0.012 | 50.06404 | 19.12465 | 11.61136 | 88.51672 |
| | Equal variances not ssumed | | | 2.372 | 27.274 | 0.025 | 50.06404 | 21.10226 | 6.7861 | 93.34198 |

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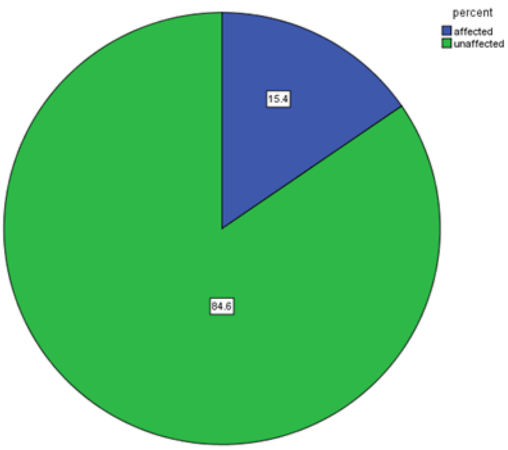


**Figure (5): Percentage Total Coliform Bacteria between Affected and Unaffected Areas.**

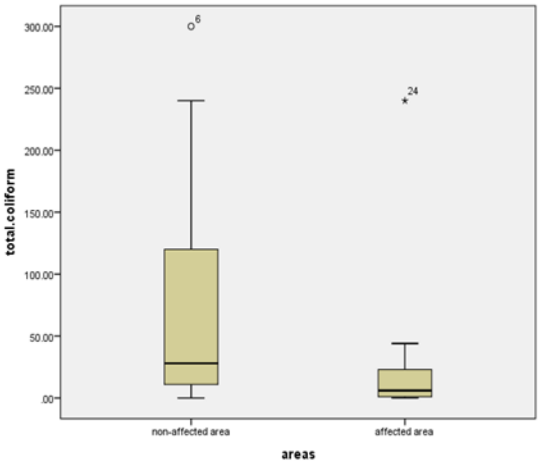


**Figure (6): Comparison in Total Coliform Bacteria Groundwater Samples between Affected and unaffected Areas.**

***Escherichia coli* (*E.coli*):** The prevalence of *E. Coli* in the measured groundwater samples ranged from (0.00 – >300 NPM/100 ml ). The lowest positive result was observed in the samples (S5, S8) groundwater well (0.00). Meanwhile, the highest occurrence of *E. coli* was detected in the samples (S3, S6, S9, S12) from groundwater wells located in unaffected areas, with counts exceeding (>300) areas as show **Table (11)**. For samples collected from flood-affected areas in the Derna city, as presented in **Table (12)**, the lowest positive result was similarly recorded in most samples (0.00 ). The highest occurrence of *E. coli* was found in the samples (S24), exceeding (90). The statistical results showed that the mean concentration of *E.coli* in the unaffected areas was 94.00 whereas the mean in the affected areas only 4.48. as show **Table (9)**, the T-test revealed a statistically significant difference between the two groups, with a p-value =0.001, which is less than the threshold of 0.05. this confirms that the difference in *E.coli* levels between affected and unaffected areas is statistically meaningful. as shown **Table (10)**, As shown in **Figure (6),** the percentage of *E.coli* contamination was calculated between flood-affected and unaffected areas. The results revealed that contamination in unaffected areas reached 81.7%, whereas it was only 18.3% in affected areas, indicating the effectiveness of post-flood disinfection and cleaning efforts in reducing microbial pollution. **Figure (7)** provides a comparative bar chart illustrating the distribution of *E.coli* across both regions. The majority of highly contaminated samples were detected in unaffected areas, while fewer contaminated samples were found in treated affected areas. These findings support the conclusion that unaffected groundwater sources may be more vulnerable to microbial contamination due to the lack of preventive measures. The results indicate that the presence of *E. coli* in groundwater wells from unaffected areas was higher compared to affected areas, suggesting **(Akhreim *et al.*, 2024)**. that the prevalence of *E. Coli* ranged from moderate to high levels in unaffected areas. Most groundwater wells in these areas exceeded the WHO's recommended safe limit for drinking water. On the other hand, most wells in the affected areas remained within the permissible limits set by WHO. Nevertheless, the presence of *E.coli* in groundwater remains a significant public health concern. This microorganism affects groundwater wells because the sewage systems in the city are not working correctly to prevent the discharge of Faecal contamination from infected humans or animals from reaching the groundwater wells. *E.coli* is the most commonly applied indicator of Faecal contamination. study conducted by **(Dada *et al.*, 2019)**, in a unflooded rural area of Nigeria assessed the microbial quality of groundwater from domestic wells. The findings revealed high levels of *E.coli* and coliform bacteria in these wells, despite the absence of any flooding or natural disaster **(Hassan *et al.*, 2025).** The authors attributed the contamination to a lack of regular disinfection, proximity of wells to pit latrines and

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surface pollutants, and low public health awareness. The study concluded that wells that had not undergone any form of treatment or protection were more prone to fecal contamination, even under stable environmental conditions. These finding are in line with previous studies. For instance, **(Rashed *et al.*, 2008)** and **(Ahmed *et al.*, 2004),** reported a significant reduction in *E.coli* levels following proper disinfection and cleaning of groundwater wells after flood events. Similarly, the **(Gaballa, 2017),** emphasizes the importance of well disinfection in emergency settings to control microbial contamination. therefore, the lower *E.coli* levels observed in the affected areas in this study may be attributed to the post-flood cleaning and disinfection efforts implemented in Derna city.

**Table (9): Descriptive Statistics of *E.coli*.**

**Statistics**

| E.coli | | Affected | Unaffected |
|---|---|---|---|
| N | Valid | 29 | 21 |
| | Missing | 0 | 8 |
| Mean | | 4.48 | 94.00 |
| Median | | .00 | 20.00 |
| Std. Deviation | | 44.955 | 123.231 |
| Range | | 90 | 300 |
| Minimum | | 0 | 0 |
| Maximum | | 240 | 300 |
| Percent | | 18.3 | 81.7 |

**Table (10): Statistical Significance Independent Samples Test *E.coli*.**

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | T | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| total.coliform | Equal variances assumed | 78.649 | .000 | 3.877 | 48 | .00 | 89.51724 | 23.08739 | 43.09693 | 135.93755 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Equal variances not assumed | | | 3.307 | 20.540 | .003 | 89.51724 | 27.07182 | 33.14140 | 145.89308 |

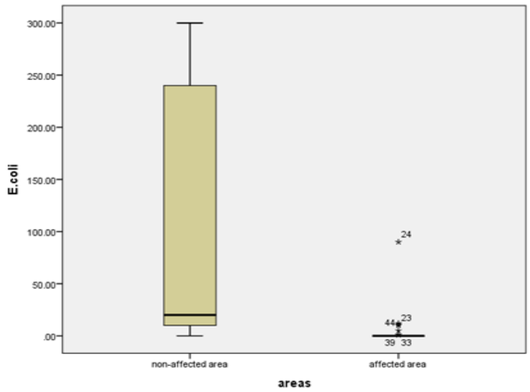


Figure (7): Different between affected and unaffected areas for *E.coli*

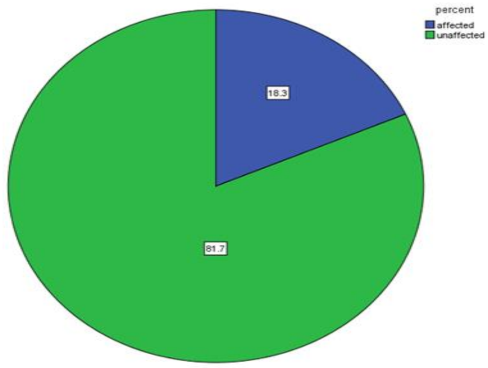


Figure (6): Percentage *E. coli* between Affected and unaffected areas.

**Table (11): Bacteriological Analysis of Groundwater Samples from Unaffected areas. all the test reapplies three**

| Sample No. | Sample Source | Total Count (T.C) \ 1 ml | Total coliform (T.C.F)\100ml | *Escherichia Coli (E.coli)* \100ml |
|---|---|---|---|---|
| S1 | Groundwater from the Eastern Coast area\ Derna | 200 | 11 | 43 |
| S2 | Groundwater from the Eastern Coast area\ Derna | 250 | 7 | 14 |
| S3 | Groundwater from the Eastern Coast area\ Derna | >300 | 240 | >300 |
| S4 | Groundwater from the Eastern Coast area\ Derna | >300 | 150 | 240 |
| S5 | Groundwater from the Eastern Coast area\ Derna | 50 | 0 | 0 |
| S6 | Groundwater from the area 400 in the Coast\ | It cannot be counted | >300 | >300 |

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| | | | | |
|---|---|---|---|---|
| | Derna | | | |
| S7 | Groundwater from the area 400 in the Coast\ Derna | >300 | 43 | 93 |
| S8 | Groundwater from the area 400 in the Coast\ Derna | >300 | 0 | 0 |
| S9 | Groundwater from the area 400 in the Coast\ Derna | >300 | 150 | >300 |
| S10 | Groundwater from the area 400 in the Coast\ Derna | >300 | 15 | 28 |
| S11 | Groundwater from the area 400 in the Coast\ Derna | >300 | 93 | 240 |
| S12 | Groundwater from the area 400 in the Coast\ Derna | >300 | 210 | >300 |
| S13 | Groundwater from the area 400 in the Coast\ Derna | 70 | 7 | 11 |
| S14 | Groundwater from the Eastern Coast area\ Derna | >300 | 120 | 41 |
| S15 | Groundwater from the Eastern Coast area\ Derna | >300 | 16 | 10 |
| S16 | Groundwater from the Eastern Coast area\ Derna | 250 | 35 | 11 |
| S17 | Groundwater from the Eastern Coast area\ Derna | 146 | 4 | 1 |
| S18 | Groundwater from the Eastern Coast area\ Derna | 68 | 23 | 7 |
| S19 | Groundwater from the Eastern Coast area\ Derna | 183 | 40 | 20 |
| S20 | Groundwater from the Eastern Coast area\ Derna | 95 | 17 | 5 |
| S21 | Groundwater from the | 266 | 28 | 10 |

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Eastern Coast area\ Derna

**Table (11): Bacteriological Analysis of Groundwater Samples from Affected Areas, all the test reapplies three.**

| ***Escherichia Coli*** **(E.coli) \100ml** | **Total coliform (T.C.F)\100ml** | **Total count (T.C) \ 1 ml** | **Sample source** | **Samples No.** |
|---|---|---|---|---|
| 0 | 23 | 300 | Groundwater from the Jubaila area \Derna | S22 |
| 10 | 43 | 300 | Groundwater from the Jubaila area \Derna | S23 |
| 90 | 240 | >300 | Groundwater from the Jubaila area \Derna | S24 |
| 0 | 43 | 130 | Groundwater from the Jubaila area \Derna | S25 |
| 11 | 23 | 100 | Groundwater from the Jubaila area \Derna | S26 |
| 0 | 3 | 170 | Groundwater from the Jubaila area \Derna | S27 |
| 0 | 23 | 300 | Groundwater from the Jubaila area \Derna | S28 |
| 0 | 15 | 80 | Groundwater from the Jubaila area \Derna | S29 |
| 0 | 1 | 130 | Groundwater from the country \ Derna | S30 |
| 0 | 1 | 300 | Groundwater from the country \Derna | S31 |
| 0 | 1 | 85 | Groundwater from the country \Derna | S32 |
| 1 | 22 | 200 | Groundwater from the country \Derna | S33 |
| 0 | 1 | >300 | Groundwater from the country \Derna | S34 |
| 0 | 0 | 70 | Groundwater from the country \Derna | S35 |
| 0 | 5 | 300 | Groundwater from the country \Derna | S36 |
| 0 | 1 | 100 | Groundwater from the country \Derna | S37 |
| 12 | 33 | >300 | Groundwater from the country \Derna | S38 |
| 1 | 12 | 300 | Groundwater from the country \Derna | S39 |
| 0 | 2 | 91 | Groundwater from the country \Derna | S40 |
| 0 | 5 | 60 | Groundwater from the country \Derna | S41 |
| 0 | 41 | >300 | Groundwater from the country \Derna | S42 |
| 0 | 3 | 130 | Groundwater from the country\Derna | S43 |
| 5 | 23 | >300 | Groundwater from the country \Derna | S44 |

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| | | | | |
|---|---|---|---|---|
| 0 | 14 | 162 | Groundwater from the country \Derna | S45 |
| 0 | 44 | >300 | Groundwater from the country \Derna | S46 |
| 0 | 0 | 120 | Groundwater from the country \Derna | S47 |
| 0 | 6 | 229 | Groundwater from the country \Derna | S48 |
| **0** | **4** | **300** | Groundwater from the country/ Derna | S49 |
| **0** | 0 | 10 | Steam station/Derna | S50 |

threat to public health due to their ability to cause multiple infectious diseases, including diarrhea, urinary tract infections, respiratory illnesses, and wound infections **(Khalil *et al.,* 2023).** Notably, contamination also detected in some wells located in areas that were not visibly affected by the flood, suggesting that pollutants may have spread through subsurface seepage or mixing between floodwater and shallow groundwater layers. These findings are consistent with the study conducted by (**Donovan *et al.* 2008).**

**Bacterial Isolates and Identification:** The analysis of groundwater samples collected from both flood-affected and unaffected areas in Derna revealed varying levels of bacterial contamination. through microbial culturing and biochemical testing, five pathogenic bacterial species were successfully isolated and identified: *Escherichia coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa,* and *proteus*., as shown **in Table (12).** These bacterial species are well-recognized indicators of fecal contamination and represent a serious

**Table (12): Characterisation of Some Bacteria Isolates from Groundwater Samples**

| **Bacteria Isolate** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| Gram-stain | -ve rod | +ve | -ve | -ve | -ve |
| Indole | +ve | -ve | -ve | -ve | -ve |
| Citrate | -ve | +ve | +ve | +ve | -ve |
| Oxidase | -ve | -ve | +ve | -ve | -ve |
| Catalase | -ve | +ve | +ve | +ve | -ve |
| Butt | Y | Y | Y | Y | Y |
| Slant | Y | Y | R | R | R |
| H2S | -ve | -ve | -ve | -ve | +ve |
| Gas | +ve | -ve | -ve | +ve | -ve |
| Motility | +ve | +ve | +ve | +ve | +ve |
| ureas | -ve | | -ve | +ve | -ve |
| Tentative Identification | ***E. coli*** | ***Staphylococcus. aureus*** | ***Pseudomonas. aeruginosa*** | ***Proteus* spp** | ***Salmonella* spp** |

**Conclusion:** This study is one of the few that examined the impact of flooding on groundwater quality in the Derna. Through the laboratory analysis of the 50 samples it was revealed that most values exceeded the permissible limits for drinking water quality, regardless of whether the wells were located in

flood-affected and unaffected areas, even after maintenance efforts. Notably, only samples (S50) collected from the AL-Bukharuiya (stem) station in Derna, complied with both local and international standards across all tested parameters, including physicochemical and microbiological characteristic. regarding the physicochemical parameters, both temperature and pH levels were within the natural range in groundwater samples from both flood-affected and unaffected areas. However, when comparing the electrical conductivity (E.C) values with international guidelines- particularly those of the World Health Organization (WHO), which recommends a maximum acceptable level of (1500μS/cm) it was found that all samples exceeded this limit. According to the Libyan national standards, which allow up to (2300μS/cm), some samples were still within acceptable limits, but a significant portion surpassed this threshold as well. In contrast, the total dissolved solids (TDS) levels in all samples exceeded the acceptable limit of (500-1000mg/L), indicating widespread deterioration in water quality, particularly with respect to dissolved minerals and contaminants. Regarding the bacteriological analysis, the total bacterial count in most groundwater samples exceeded the permissible limits in both flood-affected and unaffected areas. Additionally, *E. coli* and total coliforms were detected in nearly all samples, indicating widespread fecal contamination. This contamination is primarily linked to the flood event, exacerbated by the aging water distribution system, pipeline leakages, poor sanitation, and inadequate waste management. The deteriorated sanitary infrastructure in Derna city appears to be a major contributing factor

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