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"Valuation of human actions for the protection of the Environment and application" in public health »

Memoir

Presented by: **OUAZANI Souheila**

To obtain a diploma

Master in Animal Ecology

Theme

Evaluation of water quality in the middle Wadi of Tafna using bacteria as bio-indicators

Assessed in : 29 /06/ 2025, by a jury of:

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أَلَمْ تَرَ أَنَّ ٱللَّهَ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَسَلَكَهُۥ يَنَٰبِيعَ فِى ٱلْأَرْضِ ثُمَّ يُخْرِجُ بِهِۦ زَرْعًا مُّخْتَلِفًا أَلْوَٰنُهُۥ ثُمَّ يَهِيجُ فَتَرَىٰهُ مُصْفَرًّا ثُمَّ يَجْعَلُهُۥ حُطَٰمًا إِنَّ فِى ذَٰلِكَ لَذِكْرَىٰ لِأُو۟لِى ٱلْأَلْبَٰبِ

﴿ الآية ٢١ ﴾

سورة الزمر

Thanks

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Dedication

I dedicate this work to my parents who instilled in my heart the love of knowledge, to those whose prayers were my support; and to those who slept with me, rejoiced in my joy, and supported me in my fatigue.

To my dear father, Abed El Hamid

whose name I carried with me the symbol of strength and generosity, you who taught me that the sky can only be touched with effort, thank you for all your support, which was a strength for me.

To my beloved mother, Fatiha

the spring of tenderness, my prayer companion at every dawn, my other spring, and my support when the soul tilts, you who planted hope in my heart and watered it with patience,

To my beloved brothers and sisters

Abe<d Rahim, Mohamad, Asma and Meriam

You are the support when the world narrows,
You are the hand that silently pats my heart,
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To those who shared the beginning of the story with me,
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Introduction

A general introduction

Water is defined as a transparent liquid that is colorless, tasteless, and odorless. It constitutes approximately 71% of the Earth's surface and is indispensable for all living organisms.

The molecular structure of water consists of hydrogen and oxygen atoms, with each molecule comprising two hydrogen atoms and one oxygen atom (H_2O). Water plays a vital role in numerous critical processes, including digestion, respiration, and growth, and is a suitable solvent for a wide range of substances. Water can be found in nature in three forms: liquid, solid (ice), and gaseous (water vapor).

Water sources are essential elements that contribute to meeting the needs of humans and societies for fresh water, whether for human consumption, agriculture, or industry. Several natural and man-made forms represent water sources, differing in their formation and use. The most important of these sources is surface water, groundwater, reservoirs or rainfall.

Although surface water is Algeria's primary source of drinking water, groundwater is becoming more and more popular. A vast amount of exploitable water is found in groundwater, which is why people are turning to it **(Chekroud, 2007).**

The risk of surface water contamination in Algeria is a significant environmental issue with a prolonged history, exacerbated by pollution and population growth that considerably impact water quality due to industrial pressures and demographic development **(Chekirou & *al*., 2017).**

Technological and industrial advancements have resulted in a new phenomenon: the development of water ice, a by-product of industrial waste and untreated sewage, has facilitated the proliferation of various bacteria and viruses that constitute a substantial threat to public health.

One of the most harmful forms of environmental pollution is bacterial contamination of water, which can result in the spread of infectious diseases such as viral hepatitis, cholera, and typhoid. This pollution happens when dangerous germs enter water sources through industrial waste, sewage, and unsanitary farming methods. There are major health concerns associated with drinking tainted water or using it for irrigation and cooking, particularly for young people and the elderly. To lessen this contamination and guarantee the security of water supplies, better sanitation systems, together with water treatment and disinfection, are important.

The introduction of harmful bacterial species into aquatic environments, which causes environmental damage and serious health risks, remains the main problem from a bacteriological standpoint.

This situation is especially concerning since it threatens public health everywhere counting microbial organisms is part of the bacteriological evaluation of water quality.

A universal technique for evaluating bacteriological water pollution is the counting of fecal contamination, which is indicated by the presence of a large number of fecal markers.

Fecal contamination and the likely presence of pathogenic bacteria are indicated by the presence of indicator organisms, such as fecal coliform **(Burton & *al*., 1987).**

La bactérie E. coli est rejetée dans l'environnement à travers les fèces à une concentration d'environ 108 UFC/g de fèces. Elle se retrouve dans les eaux environnementales par le biais des effluents, tels que les eaux usées, les lisiers ou les fumiers des animaux d'élevage ou par les déjections des animaux d'élevage ou des animaux sauvages (Abraham, 2018).

Water is therefore a noble element that needs to be preserved for coming generations **(Fleming & Kirkpatrick, 2008).**

- **Pathogenicity**

- Urinary tract infection
- Intestinal infection
- Sepsis

The prevention of waterborne illnesses and the preservation of public health thus depend heavily on the sensory, physical, chemical, and microbiological quality of water. Because of this, water safety regulations have been developed over time. The majority of these guidelines are explicitly applied in two stages, namely chemical and microbiological dangers, and have been included in health regulations **(Web n°1).**

Our research is primarily concerned with the water quality of the Wadi of Tafna in the Wilaya of Tlemcen and the identification of bacteria in contaminated water through the use of bacteriological quality as a bio-indicator.

- ***Object of study is to:***
  - Evaluate the bacteriological quality of water .
  - The causes of high levels of microorganisms reveals the existence of contamination, which must be investigated.

- ***There are two significant sections to the work:***

- Bibliographic analysis
- The experimental study.

Bibliographic synthesis

Chapter 1

Presentation of the bacteria

I. General information on Escherichia coli

Bacteria are single-celled, tiny creatures. They are some of the oldest known life forms on the planet. There are thousands of varieties, and they can be found all over the world in every kind of habitat. They inhabit the sea, the land, and the deep crust of the earth. Even radioactive waste can harbor certain germs. The resident flora, also known as the microbiome, is a large group of bacteria that live on and in the bodies of humans and animals (on the skin and in the respiratory tract, mouth, digestive tract, and genitourinary tract) without causing harm. The number of bacteria in the resident flora is at least equal to the number of cells in the body, and the majority of these bacteria are actually beneficial to humans, such as aiding in food digestion or preventing the growth of other, more harmful bacteria **(Bush, 2024).**

In dry and semi-arid areas, a Wadi is a river or stream that is frequently seasonal. These rivers are essential ecosystems that sustain a variety of microorganisms, including bacteria, which are essential for maintaining ecological balance, cycling nutrients, and occasionally causing pollution. There are several types of bacteria, both natural and from contaminated agents.

I.2. Types of Bacteria in the Wadi

These species are the most prevalent in Wadi waters:

I.2.1. The coliforms

Coliform bacteria are a group of rod-shaped, Gram-negative bacteria commonly found in soil, vegetation, and the intestines of warm-blooded animals. They are widely used as indicators of water quality because their presence suggests possible contamination by pathogenic microorganisms.

- Coliform bacteria are classified into three main groups:

Intestines of humans and warm-blooded animals serve as the principal habitat for coliform bacteria, which are also excreted in feces. Soil also includes them. *Escherichia coli* (*E. coli*), a type of fecal coliform, and total coliforms (which include all varieties) are some of the different measurements of coliform bacteria.

I.2.1.1. Differences between fecal and total coliforms in water

A. Total coliforms

Many warm-blooded creatures, including humans and animals, have intestines that contain a type of bacteria called total coliforms. They are typically seen as a sign of possible fecal contamination in water.

Numerous bacteria, including those found in soil and vegetation, may be of non-fecal origin and are included in total coliforms. They are not exclusive to a fecal source; rather, they serve as a generic indicator of the microbiological health of water **(Web n°2).**
This bacterial group is used as an indicator of the microbial quality of water, as it contains, in particular, bacteria of the following origins.

- Include bacteria from the environment (soil, water, and plants).
- Presence in water does not always indicate fecal contamination but may suggest contamination from decaying organic matter.

B. Fecal coliforms:

A particular subtype of total coliforms known as fecal coliforms is most frequently found in the feces of warm-blooded mammals, such as humans.

Fecal coliforms, like *Escherichia coli* (*E. coli*) and other bacteria that come from the intestines of mammals, are usually seen as better indicators of fecal contamination.

A subtype of total coliforms known as fecal coliforms, or thermotolerant coliforms, may ferment lactose at 44.5°C. *Escherichia coli* and, to a lesser degree, several species of the genera Citrobacter, Enterobacter, and Klebsiella are the most commonly found species linked to this bacterial group **(Elmund & *al*., 1999; Health Canada, 1991; Edberg & *al*., 2000).**

Although the presence of fecal coliforms is usually indicative of contamination of fecal origin, many fecal coliforms are not of fecal origin, coming instead from waters enriched with organic matter, such as industrial effluents from the pulp and paper or food processing sectors **(Barthe *&* *al*., 1998; OMS, 2000)**

Coliform Bacterial Sources in the Wadi

- ***Human Waste***: Septic system leaks and untreated sewage
- ***Animal waste:*** includes wildlife droppings and livestock discharge
- ***Runoff from agriculture:*** fertilizers made from manure. Pollutants from both urban and rural areas are carried into the Wadi via stormwater runoff.

❖ **Enterococci**

Enterococci are gram-positive, short- and medium-chain facultative anaerobic bacteria that cause infections that are difficult to treat in hospitals. They are a common cause of urinary tract infections, bacteremia, infective endocarditis, and, rarely, intra-abdominal infection and meningitis.

I. 2.2. *Escherichia coli* (*E. coli*)

Escherichia coli are Gram-negative bacteria normally found in the intestines of healthy people, but certain strains are responsible for infections in the digestive tract, urinary tract and many other parts of the body.

Fecal contamination contains *E. coli*, some strains of which can cause gastrointestinal disorders.

The species *Escherichia* is a member of the Enterobacteriaceae family, which gets its name from the frequent isolation of the digestive tube and/or mammal feces **(Greatorex & Thorne, 1994).**

- There are two types

E. coli ⟶ Humans' and other warm-blooded animals' digestive tracts are home to the bacteria *Escherichia coli*. Only a small percentage of *E. coli* strains are harmful to humans, while the rest are benign.

Enterohemorrhagic *E. coli* (*EHEC*) ⟶ is the cause of Shiga toxin. *EHEC* strains frequently result in food poisoning when raw or undercooked animal products (meat or dairy products) are consumed. Additionally, fresh produce that has come into contact with *EHEC* may be vulnerable.

Tableau 1: The different between the *E. coli* and *EHEC*

	E. coli	***EHEC***
Nature	Bowel or feces	Water. raw meat
Characteristics	**Nothing**	Shiga toxin
Symptoms	Nothing	Bloody diarrhea Stomach pain Fever

I.2.2.2. The causes

Escherichia coli is mostly introduced into water by:

- Untreated wastewater
- Runoff from agriculture (animal waste)
- Fecal contamination in humans or animals.

I.2.2.3. Survival and longevity

Unlike how it behaves in the intestines, *E. coli* usually does not proliferate once in the water; however, it can live for days or even weeks, depending on:

- ***Temperature:*** Like most mesophilic bacteria, *E. coli* thrives in a relatively narrow temperature range. Its optimal growth temperature is around 37°C, similar to body temperature. However, it can grow and divide in a wider range of temperatures, from around 10°C to 45°C and they can survive in 25°, but their movement is slow.
- ***UV light:*** Exposure to sunlight's UV rays reduces its survival.
- ***Microbial competition and predators:*** Some bacteria and protozoa consume *E. coli*, which reduces its persistence
- ***Nutrient presence:*** Its survival may be extended in settings high in organic materials, such as contaminated waterways.

I.2.2.4. Morphological characteristics

It is a Gram-negative, round-tipped bacillus measuring approximately 2 to 4 µm in length and 0.6 µm in width. It does not produce capsules or spores, and it can exist alone or in short chains (Figure 1), as well as occasionally in the form of extremely long filaments. **(Djelouat, 2008).**

Although it has cilia and is typically motile due to its peritrichous flagella, the strain's motility varies significantly depending on the seeding medium.

Certain bacteria produce mucous colonies by growing on solid substrate and developing capsules **(Le Minor & Richard, 1993).**

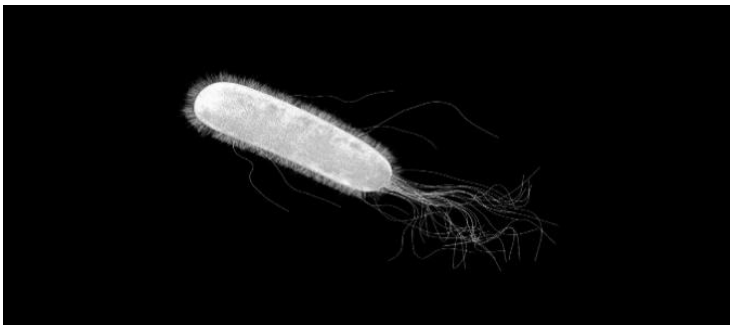


Figure1 :*Escherichia coli* form **(web n°6)**

I.2.2.5. Their transmission cycle

The life cycle of *E. coli* begins inside the human or animal intestine, and it multiplies there in an environment rich in organic matter and optimal temperature, then it is released to the external environment through feces **(Figure 2).** It reaches multiple environmental sources, such as surface water (rivers, valleys or dams).

They can live or thrive in soil or vegetables when irrigated with contaminated water.

E. coli can live in the environment for an equivalent period of time (days to weeks) depending on conditions such as temperature, sunlight, and the presence of organic matter.

The bacteria can be transmitted to humans or other animals in several ways, such as drinking contaminated water or eating contaminated food (such as vegetables or undercooked meat).

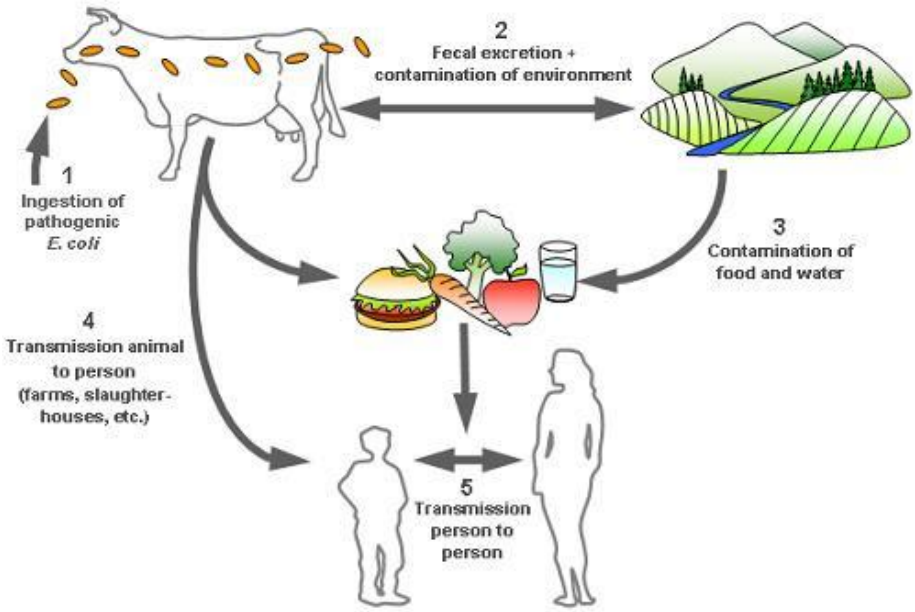


Figure2 : Transmission cycle **(web n°7)**

I.2.2.6. Effect on health

Urinary tract infections are the most common infections caused by *E. coli*, and people can also develop intestinal infections by eating contaminated food (such as undercooked ground beef), touching infected animals or drinking contaminated water.

When food contaminated with enteroh*emorrhagic Escherichia coli* is eaten, the first signs appear around the 3rd or 4th day, sometimes up to 10 days.

These include abdominal pain, cramps and diarrhea, which can rapidly turn bloody. A week after this digestive episode, a much more severe infection may occur: hemolytic uremic syndrome which is a condition that occurs when small blood vessels become damaged and inflamed. The damage may cause clots to form in blood vessels throughout the body. Clots can cause damage to your kidneys and other organs. Hemolytic uremic syndrome can lead to kidney failure, which can be life-threatening.

Anyone can develop hemolytic uremic syndrome. But it's more common in young children. Most often, it's caused by an infection with certain strains of *E. coli* bacteria.

This syndrome occurs in 10% of cases and manifests itself as fatigue, pallor and a decrease in urine, which becomes much darker.

Antibiotics can effectively treat *E. coli* infections occurring outside the digestive tract and most intestinal infections, but are not used to treat intestinal infections caused by any strain of these bacteria.

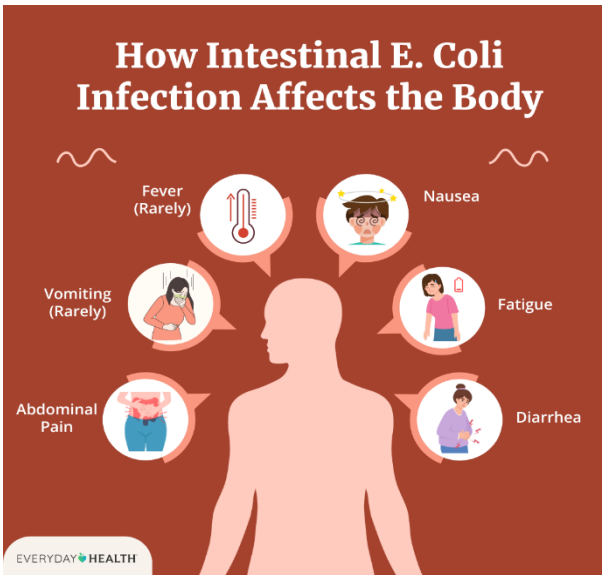


Figure3 : Effect of *E. coli* in body **(web n°8)**

Chapter2

Presentation of the study environment

II.1. Situation of the Tafna Wadi

Our present study takes place in two stations located on Oued Tafna. The first stop is in the village of Bourouah, and the second is in Hajrat el Gat.

The Tafna Valley is one of the most important valleys in Algeria in terms of economic and environmental importance, with an area of about 8,500 square kilometers (Km^2) and a length of 170 kilometers, with 150 kilometers of the valley being exploited by the Tlemcen state **(Figure 4).**

Tableau 2:coordinates of zenata Tlemcen Location of tafna wadi

Station	**Latitude**	**Longitude**	**Altitude**
Wadi Tafna	34°47' and 35°15' North	2°15 and 0°15' West	280 m.

The Tafna basin, which covers the entire wilaya of Tlemcen and measures 7245 km², is one of the main watersheds in northwest Algeria. It is situated between longitudes 2°15 and 0°15' west and latitudes 34°47' and 35°15' north. **(Houari Khemissi, 2020)**

It contributes to providing water for agriculture and drinking, in addition to its role in supporting biodiversity. However, it faces several challenges, such as pollution and overexploitation, which call for measures to preserve it and ensure its sustainability for future generations.

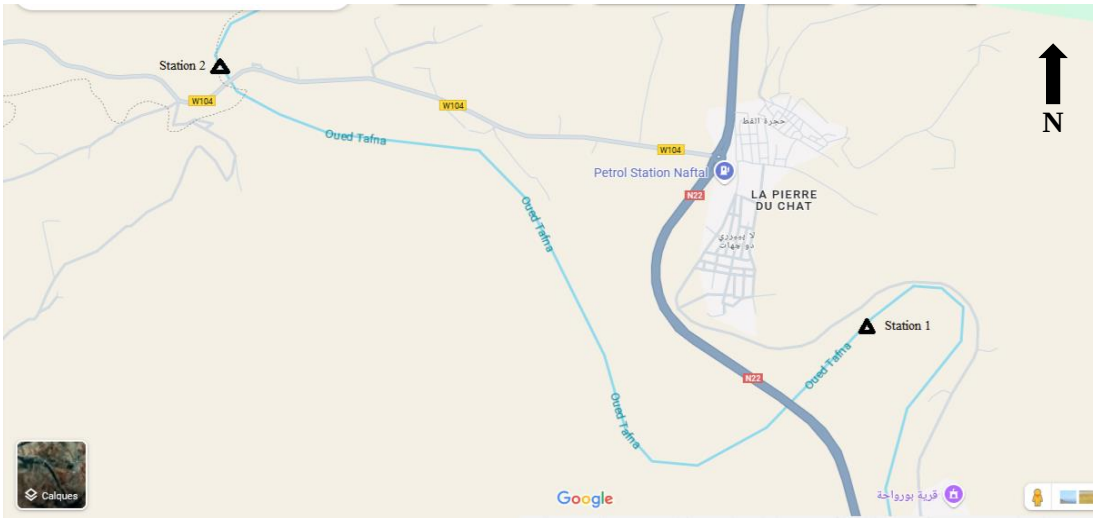


Figure4 : Cart of the situation of Tafna Vally (web n°9)

II.2. Wadi Tafna Detailed study

The Wadi of Tafna originates from the Atlas Mountains in the province of Saida, at an altitude of approximately 1400 meters above sea level. Its waters are formed by several small tributaries that collect in the highlands and then begin to flow in a northwesterly direction. During its journey, the valley passes through several terrains, from mountains to plains, before reaching the Mediterranean coast.

The Tafna flows into the Mediterranean Sea on the southern edge of the Alboran Sea at Rachgoun, on a steep coast with a very wide continental platform **(Figure 5)**.

The first original feature of this basin is that it belongs to two different structural domains: alpine or tello-rific in the eastern Traras and the Sebaa Chioukh; and atlasic or "Tlemcenian," as S. Elmi in 1985 puts it, in the Monts de Tlemcen and the western Traras **(Remaoun, 2003).**

Figure5 : Flow Path of the Tafna River toward in Rachgoun (wen n°10)

II.2.1. Geographical route

The Tlemcen Valley's geographical path is regarded as one of the most significant and expansive, given its traversal of multiple valleys. The specific routes and valleys involved are outlined below:The valley flows through four major provinces:

- Saida (upstream)
- Sidi Bel Abbes
- Tlemcen
- Ain Temouchent (downstream in the Mediterranean Sea, near Beni Saf)

The main tributaries that feed Tafna Wadi are:

- ✓ Oued Mouillah
- ✓ Oued Arisha
- ✓ Oued Terni.

II.3. The ecological importance and biodiversity of Tafna Wadi

In Northwest Algeria, the Tafna Valley is a significant ecological valley that sustains the biodiversity of the area and is vital to the ecological balance. In addition to being a significant supplier of water and natural resources, the valley traverses ecologically diverse regions, supporting various plant and animal species.

II.3. The ecological importance of the Tafna Wadi

II.3.1. A major source of fresh water

Overall, the Tafna watershed is divided into three parts **(Benest, 1990).**

- **Haute Tafna:** The Tafna river drains the southern slopes and high valleys of the Monts de Tlemcen. It receives the Sebdou river on its right bank and the Khémis Wadi River on its left bank. The Beni Bahdel dam retains water. Downstream, the Wadi of Tafna reforms from springs and resurgences, crossing the mountains of Tlemcen in steep gorges.

- **The Middle Tafna:** Once through the gorges, the Tafna flows into the Maghnia plain through a low valley.

This section of the river receives several tributaries, the most important of which are:

❖ **On the left bank:**

The Wadi of Mouillah, which originates at El Abed at an altitude of 1,250 meters. It then crosses into Morocco and is called the Isly Wadi. It serves as a wastewater collector for the town of Oujda. The river then returns to Algeria under the name Mouillah. Here, it drains an agricultural watershed and receives domestic and industrial effluent from Maghnia via the El-Abbes and Ouerdeffou wadis. The Mouillah and the Tafna Wadis converge at an altitude of 160 meters on the Maghnia plain where the Hammam Boughrara dam retains water. The temporary Wadi of Boukiou rises in the mountains of Traras and joins the Tafna in the Ghossel plain.

❖ **On the right bank**:

The Isser Wadi, its second tributary, is very important both for its long course (118 km) and its flow. It rises at Ain-Isser at an altitude of 900 m and flows into the Tafna upstream of the

village of Pierre du Chat on the Remchi plain at an altitude of 80 m. Its main tributaries are the Chouly and Sikkak Wadis (left bank) and Bouhadi Wadi (right bank). Its downstream limit coincides with the Sidi Abdelli dam. The Tafna also receives the Valley of Boumessaoud and Zitoun Wadi.

- **The lower Tafna:**

This is the lower course of the Tafna, stretching from the Tahouaret gorges towards the village of Pierre du Chat to Rachgoune beach in the Mediterranean Sea over a distance of 20 km. It is mainly fed by the Ed-Diab Wadi (left bank) **(Haddou, 2019).**

II.3.2. Soil conservation and desertification prevention

The valley moisturizes the soil in neighboring areas, preventing desertification and enhancing the fertility of agricultural land. Water flows during the winter contribute to the redistribution of fertile soil on its banks.

II.3.3. Impact on the local climate

The presence of water and vegetation surrounding the valley helps moderate temperatures and improve humidity in the area region this reduces the impact of dry winds, creating a more stable environment for living organisms.

II.3.4. The contribution to the hydrological cycle

It contributes to the hydrological cycle by aiding in groundwater replenishment, which maintains a steady supply of water in the region. By acting as a natural rainwater drainage channel, it reduces the likelihood of flooding and erosion.

II.4. The biodiversity of The Tafna Wadi

II. 4.1. Plant diversity

The Wadi of Tafna passes through different environments, making it rich in different and important plant diversity, such as:

- Green oak and pine trees, which cover the mountainous areas.
- Cork trees, which are economically and environmentally important.
- Reeds and aquatic plants on its banks, which contribute to maintaining water quality.
- Wild herbs and aromatic plants, such as thyme and wormwood, that grow in nearby areas.

II.4.2. Animal diversity

- **Birds**

There are white storks and herons, which use the valley as a rest stop during their migration, and wild ducks, which take advantage of the valley's wet areas.

- **Mammals**

Hares which depend on the vegetation available on the banks of the valley. Hedgehogs and jackals, which take advantage of the shelter provided by nearby trees and caves.

- **Reptiles and amphibians**

Water turtles, which live in quiet pools of water and frogs, which are a healthy indicator of water quality in the valley.

II.5. Ecological threats

The discharge of polluted industrial and agricultural water leads to the deterioration of water quality and the disappearance of some important organisms, and the use of pesticides and chemical fertilizers in the surrounding agricultural areas causes serious pollution due to erosion.

- **Overexploitation of water**

Excessive withdrawal of water from the valley for irrigation purposes leads to a decrease in its level, especially during the summer.

Agricultural projects rely heavily on its water, which can lead to drought in some areas during hot summer periods.

- **Deforestation and urbanization**

Logging and random construction on the banks of the valley threatens the ecological balance and leads to soil erosion.

Overgrazing also reduces vegetation cover, increasing the risk of desertification.

- **Climate change**

Fluctuating rainfall amounts due to climate change led to reduced water flows. Rising temperatures increase evaporation rates, reducing the amount of water available.

III.6. Bioclimatic Study

Bioclimatic variables are primary determinants that influence the natural environment of a place, since they directly impact vegetation, biodiversity, and agricultural and economic activity.

In this analysis, the bioclimatic characteristics of the Zenata region were based on climatic records covering a ten-year period (from 2013 to 2023). Key variables such as maximum and minimum temperatures and precipitation rates were analyzed to understand the environmental and climatic impacts on the region's bio systems.

III.6.1. Temperature

a- Minimum temperatures in the Zenata station (2013 to 2023)

The following table shows the distribution of minimum temperatures in the Zenata zone in 2013 to 2023

Tableau 3: Monthly minimum temperaturs in degreesCelsius (2013-2023)

Period 2013-2023	**JA**	**Fa**	**Ma**	**Ap**	**May**	**jun**	**jul**	**Au**	**Sep**	**Oct**	**Nov**	**Dec**
T°(m)	5,89	7,02	8,64	11,17	14,13	17,91	21,77	22,57	19,66	15,29	10,18	7,72

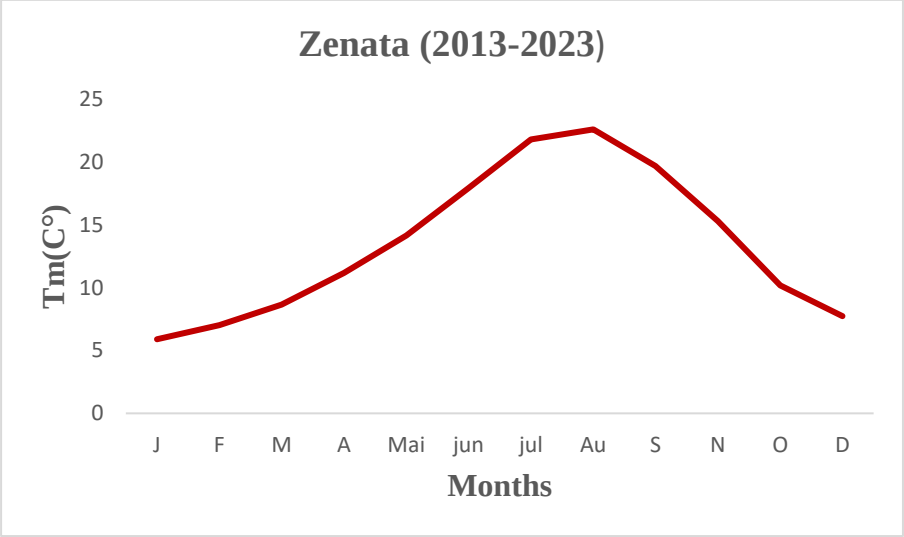


Figure6 : Graph of variation of monthly minimal Temperature for the period 2013-2023 in zenata station

This graph represents the evolution of minimal temperatures in Zenata from 2013 to 2023. The horizontal axis shows the months and the vertical axis represents the temperature at the annual average, in degrees Celsius.

We note that the temperatures of the months of December and January are the coldest, with values of 7.72°C and 5.82°C, respectively, which proves that we are in the winter season.

The temperature rises again from May to August, reaching a maximum of 22°C, indicating that this period transitions from spring to summer.

b- Maximal temperatures in the Zenata station (2013- 2023)

The following table shows the distribution of maximal temperatures in the Zenata zone in 2013 to 2023

Tableau4 : Monthly maximal temperatures in degrees Celsius (2013-2023)

Period (2013-2023)	**JA**	**Fa**	**Ma**	**Ap**	**May**	**Jun**	**Jul**	**Au**	**Sep**	**Oct**	**Nov**	**Dec**
T°(M)	18.77	20.2	22.51	24.34	28.67	32.77	36.02	37.62	32.41	30.56	23.78	20.51

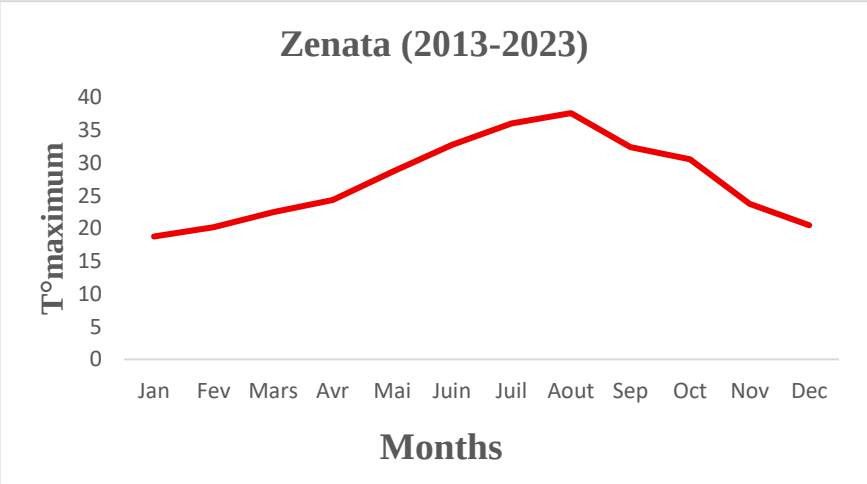


Figure7 : Graph of variation of monthly maximal temperature for the period 2013-2023 in zenata station

This graph (**Figure 7)** represents the evolution of maximum temperatures in Zenata station from 2013 to 2023

We can see from the figure that the average temperatures have generally seen an upward trend during these years, with some slight variations.

We observe that the temperature fluctuated between 20°C and 25°C from January to April.

From April to August, the temperature increased significantly, reaching a maximum of 37°C.

A slight decrease is observed again between August and December, reaching 20°C.

c- Average monthly temperatures in the Zenata station (2013 to 2023)

The table below shows how the average temperatures in the Zenata zone changed from 2013 to 2023

Tableau5 : Monthly average temperatures in degrees Celsius (2013-2023)

Period (2013-2023)	**JA**	**Fa**	**Ma**	**Ap**	**May**	**Jun**	**Jul**	**Au**	**Sep**	**Oct**	**Nov**	**Dec**
T (°C)	13,33	16,34	22,17	26,07	29,61	29,11	25,35	21,73	17,78	15,21	13,06	11,57

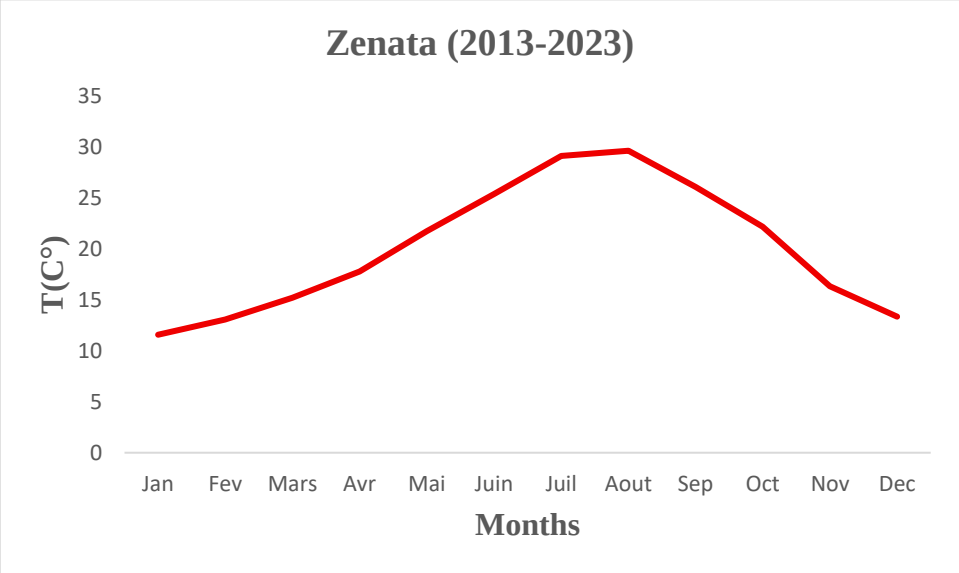


Figure8 : Graph of variation of monthly average temperature for the period 2013-2023 in zenata station

III.6.2. Precipitation

To get a general idea of the extent and distribution of heavy rainfall over time, we used the data provided for the amount of rainfall over the months for 2013-2023

The following table shows the amount of rainfall in the Zanata region from 2013 to 2023

Tableau6 : Average monthly precipitation (mm) for the period (2013-2023)

Period (2013-2023)	**JA**	**Fa**	**Ma**	**Ap**	**May**	**Jun**	**Jul**	**Au**	**Sep**	**Oct**	**Nov**	**Dec**
T (°C)	56,117	29,792	35,606	41,574	23,801	10,676	1,995	1,04	10,111	21,054	30,727	37,928

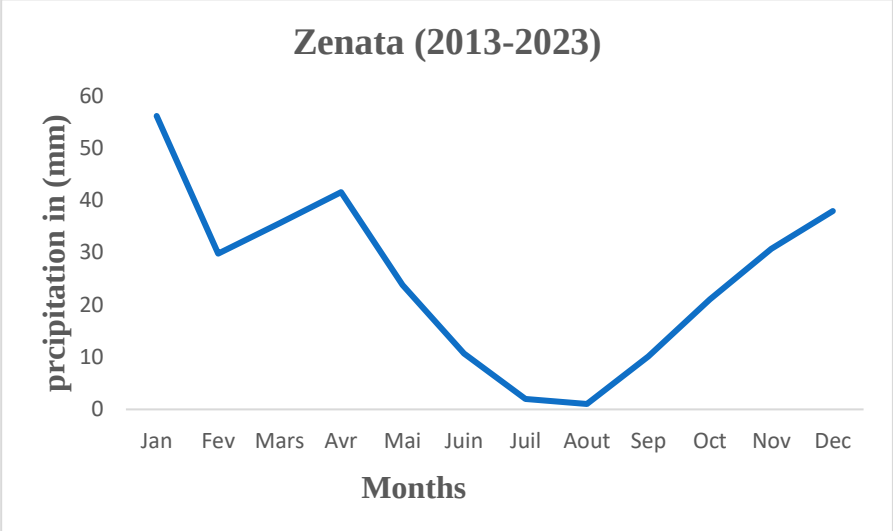


Figure9 : Average monthly precipitation variation curve of the period (2013-2023)

This graph shows the average amount of rainfall for each month of the year for Zanata from 2013 to 2023.

The horizontal axis (X) represents the months of the year from January to December and the vertical axis (Y) shows the amount of rainfall in millimeters (mm).

By observing the plot, the months of the year can be divided into wet and dry seasons as shown below:

Tableau7 : Annual rainfall distribution (Dry and rainy months)

Rainiest Months	Moderate rainfall months	Lowest rainfall months
January (**59** mm)	February, March, April, November, December (**30** to **40** mm)	May, June, July, August, September, October (**20** mm)

III.6.3. Ombrothermic diagram of Bagnouls and Gaussen (1953)

Bagnouls and Gaussen (1953) developed a climatic classification that meets the needs of plant ecology, which allows for the determination of the duration of the dry period. To do this, they devised a method of comparing rainfall curves (ombrothermic curves) and temperature curves (thermal curves); this resulted in ombrothermic diagrams. For these authors, a dry month is one where the average total precipitation is double the average temperature expressed in degrees Celsius(°C) With:

$$P \leq 2T$$

P: average monthly precipitation in (mm)

T: Average temperature of the same month in (°C)

The rainfall and temperatures are represented on a diagram. The months of the year are plotted on the x-axis, the left y-axis shows the average monthly precipitation (in mm), and the right y-axis shows the average monthly temperatures (°C), with a scale double that of the precipitation. We actually obtain two overlapping diagrams.

The periods of aridity are those where the rainfall curve is below the temperature curve **(Ramade, 2003).**

Drought periods are those when the rainfall curve is below the temperature curve **(Ramade, 2003).**

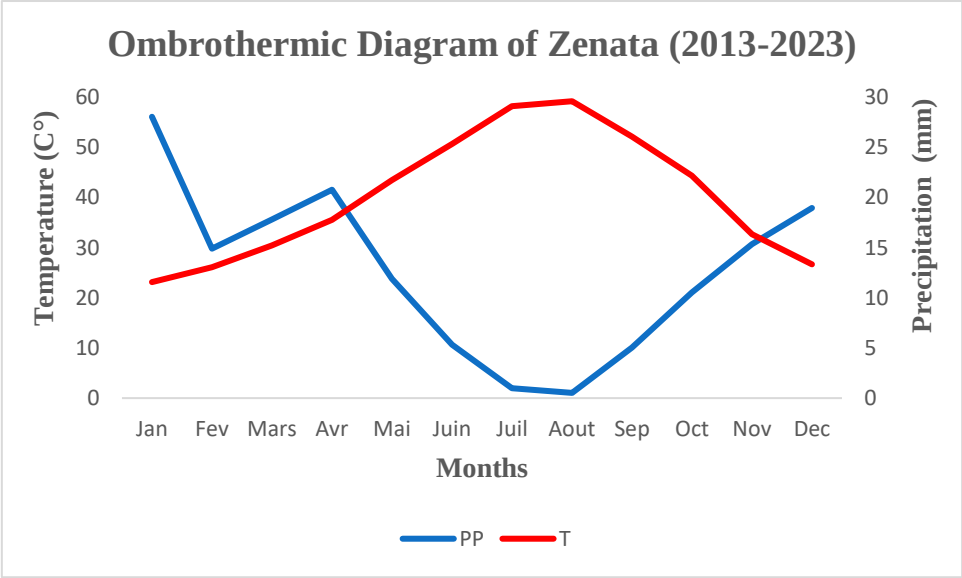


Figure10 : Ombrothermic diagram of zenata station form 2013-2023

The Ombrothermic diagram represents the distribution of both temperature and precipitation over the months for the year 2013-2023 of Zenata station.

We observe that temperatures start to rise gradually between April and May to reach 30°C in June, July, and August, and then start to decrease again from September to December.

As for rainfall, it records the highest amounts in winter, especially in January (56 mm) and December (40 mm), and drops sharply in summer, recording the lowest value of 0 mm in June, July and August and less than (20 mm) in September.

We conclude that the dry period in these years extends from the end of April to the beginning of November (**7 months**). The wet period extends over the rest of the year.

III.7. The Emberger index (1932)

The Emberger index (1932) defines the climate's degree of humidity. It takes into account the annual precipitation**(P)**, the average temperature maximum of the warmest month **(M)** and the average temperature minimum of the coldest month **(m)**. As with Gaussen's xerothermic index, it is particularly well-suited to Mediterranean regions, where it can be used to distinguish between different climatic stages. In these regions, Emberger noted that thermal amplitude **(M-m)**, and therefore evaporation, is an important factor in plant distribution. We know that, for the same average temperature, evaporation is greater the higher the thermal

amplitude. The rainfall factor taken into account is the product of the number of rainy days per year **(n)** multiplied by the average annual accumulation

Louis Emberger also proposes the calculation of a quotient, an empirical expression of rainfall efficiency. This rainfall quotient, or Emberger's climatic index, is used to define the five different types of Mediterranean climate, from the most arid to the most mountainous. (web n°11)

This quotient is defined by the formula:

$$Q = \frac{2000P}{M^2 - m^2}$$

P: precipitation

M: Maximum temperature of the hottest month (K°)

m: Minimum temperature coldest month(K°)

Note*:* For the calculation, the numbers are multiplied by (Temperature in °K=T°C+273).

Tableau 8: Rainfall-thermal quotient

Period	Precipitation (mm)	M (°K)	M (°K)	Q2	Bioclimatic phase
(2013-2023)	**300.421**	**310.62**	**278.89**	**32.12**	**semi-arid with mild winter**

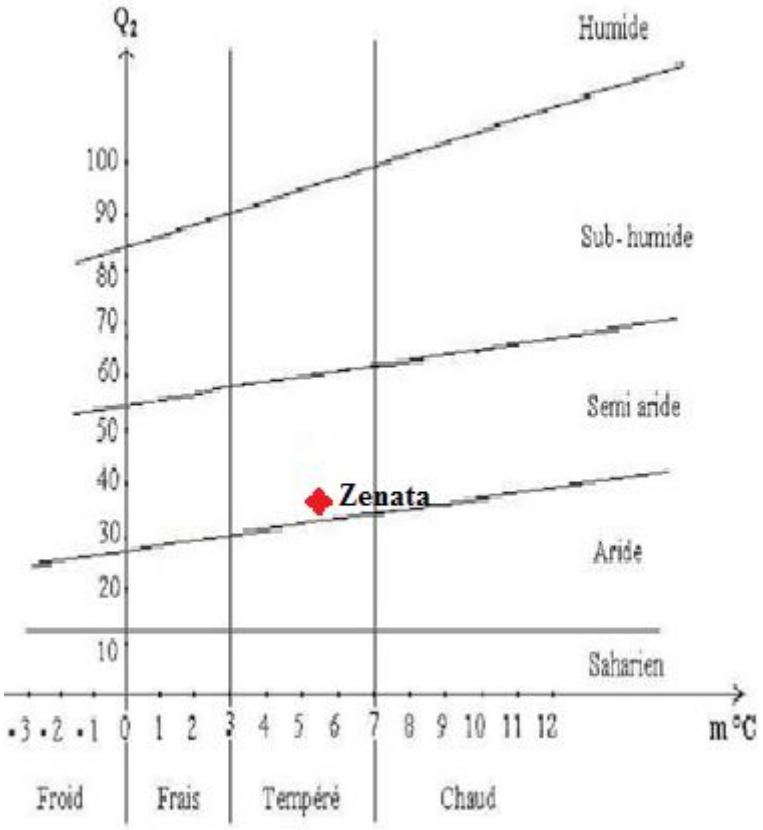


Figure11 : Position of zenata region of the period (2013-2023) on Emberger climagram

The calculation of EMBERGER's quotient allows us to classify our study area in the bioclimatic tier as a semi-arid tier superior to temperate winter.

The experimental study

Chapter3

Materials and methods

III. Characteristics of the area studied

Our present study takes place in two stations located on Oued Tafna. The first stop is in the village of Bourouah, and the second is in Hajrat El Gat.

III.1. The first station

The area is almost far from the village of Hajrat El Gat; access to it is via steep slopes; it is considered an open and wide area, and there is a significant variety of plants.

We took the sample in two different water conditions, fast-flowing water and stagnant water.

a) Fast-flowing water

- In the case of fast-flowing water, the area was measured at 4 meters in width, the air temperature registered at12°C, and the depth was determined to be 30 centimeters.

- The station exhibited minimal wind speed and an absence of direct solar radiation, characteristics attributable to its proximity to a substantial amount of vegetation. Additionally, the water exhibited a turbid appearance, characterized by a dark brown hue, and was devoid of conspicuous large stones **(Photo 1).**

Photo1 : Fast -Flowing water in the first station (original)

b) Stagnant water

- The condition of the stagnant water, where the height of the area from our standing area to the other area was 17 meters, the air temperature was 13 °C, and the depth was 16 cm, the water was not clear.

- The area received strong sunlight, lacked any type of vegetation, and consisted of either clay or fine sand soil **(Photo 2).**

Photo 2: Stagnant water in the first station (original**)**

III.2. The second station

Also, in this station we took the sample in two different water conditions, fast-flowing water and stagnant water.

a) Fast-flowing water

- This area is considered relatively closed because it is located under a bridge, which requires descending through small slopes. Additionally, there is an agricultural area surrounding it, and the waterway contains a significant amount of heavy waste, such as tires and plastic. However, it is also regarded as a relatively rich area with trees, rocks, and various birds, including ducks that use it as a resting spot.
- The water is not clear and has a strong current with a height of 10 meters from our stand to the end with a temperature of 16°C
- The immediate area lacks vegetation, but there are various rocks of different sizes. Additionally, the site receives little to no sunlight **(Photo 3).**

Photo3 : Fast -flowing water in the second statin (original)

b) Stagnant water

- The water is stagnant, with the area height from our position to its terminus measuring 15 meters and a temperature of 16°C.

There are only small grasses in the area and no big plants. There are, however, some rocks of different sizes that get strong sunlight. The water is still and a light yellow color **(Photo 4).**

Photo 4 : Stagnant water in the second station (original)

III.2. Materials and methods used

In this section, we describe the tools and techniques used to carry out this study on the bacteria present in the Wadi studied. A rigorous methodological approach was adopted to guarantee the reliability of the results obtained.

Firstly, water samples were taken according to a standardized protocol, taking into account the spatio-temporal variations of the watercourse.

Next, various physicochemical analyses were carried out to characterize the environmental conditions influencing bacterial diversity.

Finally, the microbiological methods used to identify and quantify bacteria are described in detail.

All the procedures followed are designed to ensure optimal data collection and interpretation.

III.2.1. On the ground

III. 2.1.1. Environment Equipment

- **Materials**

To ensure that there were no contaminants present that could contaminate the sample, they were collected in sterile vials of 500 ml in a temperature of 130 °C. In order to protect the bottles and preserve the water temperature while collecting the sample, they were then put in a heat-insulated bag **(Figure 12).**

To calculate the height of the area and the depth of the water, we used a tape measure.

Remarks ! After we take the sample, we immediately store it in the refrigerator.

Tape measure to measure the width and depth of the Wadi

Sterile vials of 500 ml were used for collecting the water sample

A heat-insulated bag to protect the temperature of sample water

Refrigerator to preserve the sample at 4 °C

Figure12 : Equipment used on the ground (original photos)

- **Methods**

The method of collecting samples in the valley is considered an easy and simple method. We first record the date, the weather conditions, and the environmental factors in the area, including plants, trees, stones, animals, and any human influences that are present. We calculate the width and depth of the location, then submerge the sterile glass flask to a depth of 10 centimeters, open it underwater downstream, and close it immediately after it fills up, ensuring that the flask remains submerged throughout the process **(Photo 5).**

Photo5 : The jars is full of wadi water (original)

III.2.2. In the laboratory

III.2.2.1. Laboratory Equipment

At this stage we have two different types of parameters, physicochemical and bacterial.

III.2.2.1.1. Materials and methods of parameters physicochemical

We used the multi-parameter 340i (WTW) **(photo 6)** but with different metal rods: one for dissolved oxygen and the second for conductivity and salinity.

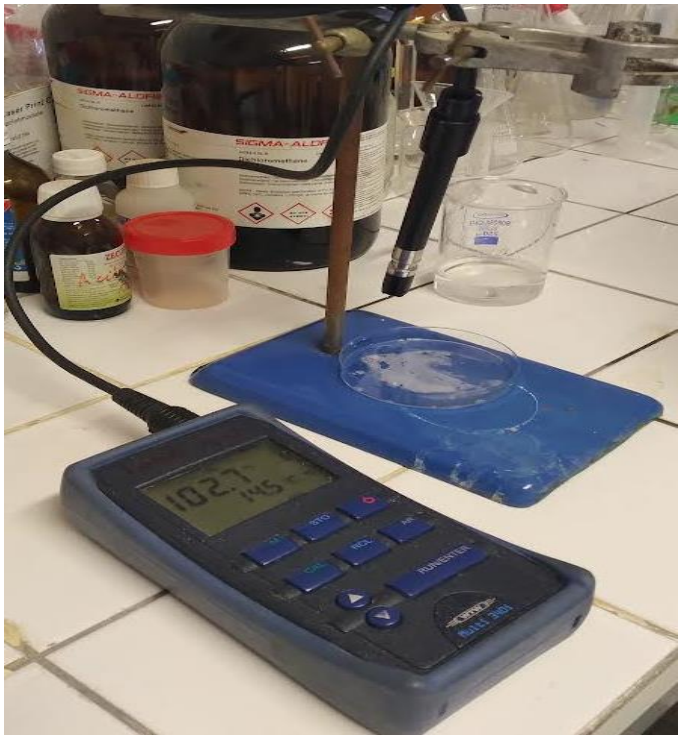


Photo6 : Multi parameter 340i (WTW) (original)

a) Dissolved oxygen (O_2)

At this point, we put the sample water in a Becher, dip the metal rod into the grater, and wait until the numbers stop.

b) Conductivity and salinity

At this point we also used the multi-parameter 340i (WTW) with the same route technique but this time we changed the metal rod to another one to measure conductivity and salinity.

c) pH and redox

We used Benchtop Meters (Adwa pH meter AD 1030) to measuring pH in sample water (**Photo 7**). We turn on the device, insert the rod into the water sample, wait until the numbers stabilize, and then take the result.

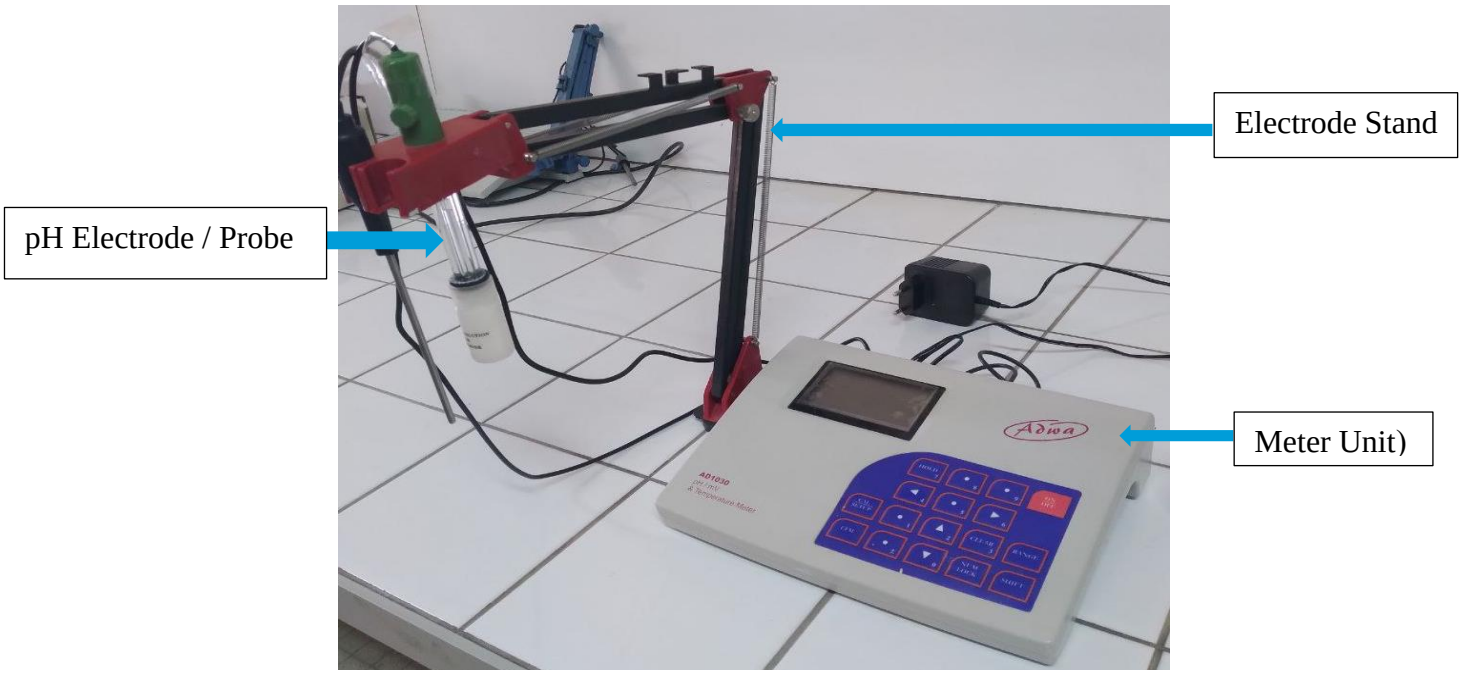


Photo7 : Adwa pHmeter 1030(original)

III2.2.1.2. Materials and methods of measuring the organic matter

a) Materials

✓ a funnel, a glass conical flask and Beaker, filter paper (to remove heavy impurities) **(photo 8),** and a photometer (Wagteche 7100) to measure the organic matter nitrite and nitrate (**photo 9**).

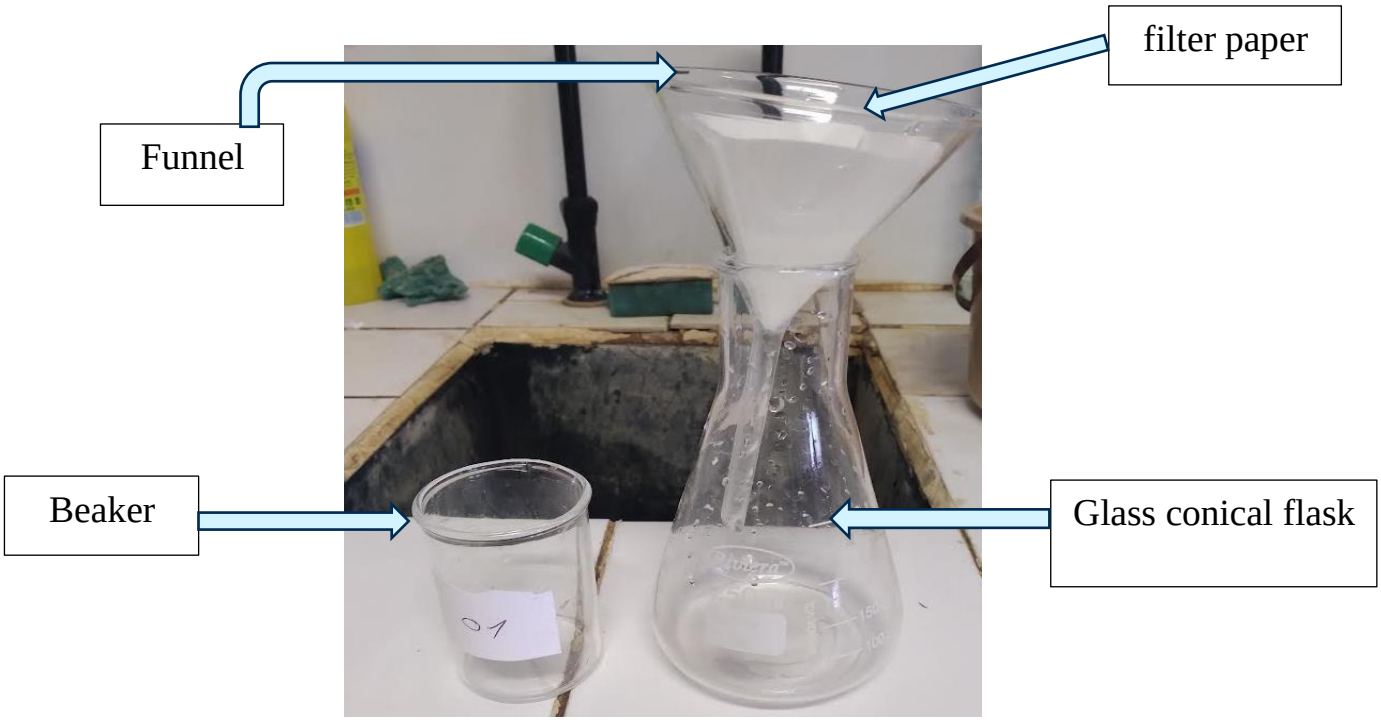


Photo8 : Filtre methode (original)

Photo9 : Photometer (wagtache 7100) (original)

b) Methods

We filter the water with a distillation technique for 10 minutes to avoid impurities in the water, after which we measure the nitrates and nitrites by following their protocol.

- **Nitrite protocol**

- **Test procedure**
  - ✓ Fill tube to 10 ml of sample
  - ✓ Add a 'Nitricol' tablet, crush and stir to dissolve Wait 10 minutes to allow color development **(photo 10)**;
  - ✓ Select Phot 24 for results in mg/l N, or Phot 64 for results in mg/l NO_2
  - ✓ Read the result (see instrument operating instructions;
  - ✓ To convert from mg/l N to mg/l NO2, multiply by 3.3.

Photo10 : material use in nitrate test (original)

- **Nitrate protocol**

Chromotropic acid is the reaction between nitrates and reagents that produces a yellow color in the sample.

- **Reagents required**
  - Multi-parameter bench photometer;
  - HI 93766-0;
  - Nitrate reagent;
  - 1tube;
  - 1 bag;

✓ Select the nitrate program using the up and down arrows.
✓ Open the reagent tube and add exactly 1.0 ml of sample, holding the tube at 45 degrees.
✓ Carefully close the tube and mix by tilting about ten times. This is the blank.
✓ Place the tube in the reading system and press the ZERO key.
✓ A "SIP" message appears, followed a few seconds by a "SIP" message;
✓ Remove the tube from the instrument and add a bag of HI 93766-0 reagent.
✓ Close the tube and mix with rocking movements about ten times.
✓ Press the TIMER button. A stopwatch will count down 5 min, after which a "SIP" message appears.
✓ followed by a display in mg/l of nitrous nitrogen (NO-N).

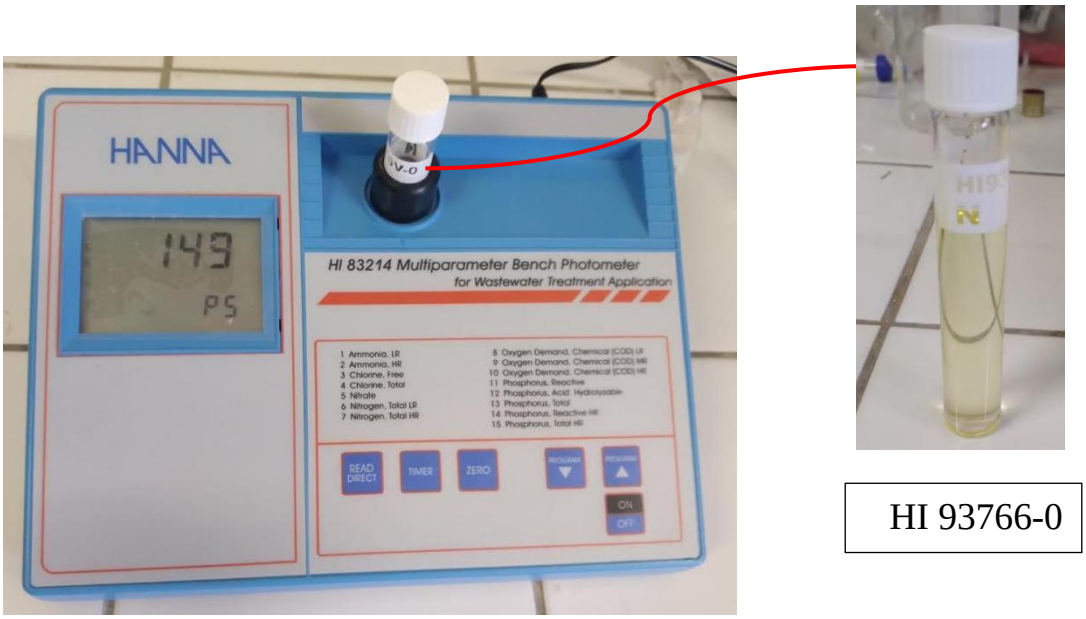


Photo11 : Multi -parameter bench photometer (original)

III.2.2.1.3. Bacteriological parameter

The analysis of bacteria present in the Wadi requires the use of specific laboratory equipment to ensure accurate and reliable identification of the microorganisms. This equipment plays an essential role in the various stages of the process, from bacterial cultivation to biochemical and molecular characterization.

This study mobilized several tools, including incubators for bacterial colony growth.

All this equipment made it possible to guarantee a thorough and rigorous analysis of the samples collected, thus contributing to the reliability of the results obtained.

a) Materials

In order to guarantee the result and achieve experimental conditions, the:

- Glass tubes are used to prepare the bacterial culture area **(Photo 12)**

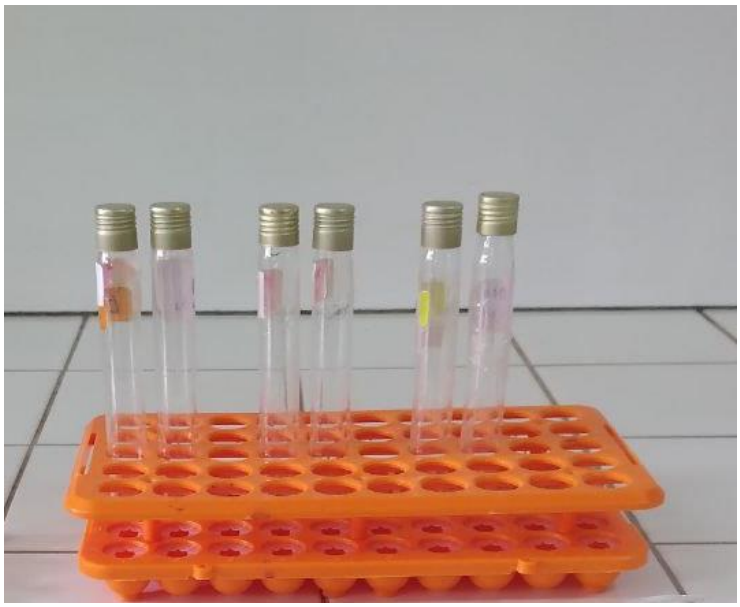


Photo12 : Glass tubes (original)

- Glass cloche tubes to monitor the release of gas during bacterial fermentation of lactose **(Photo 13).**

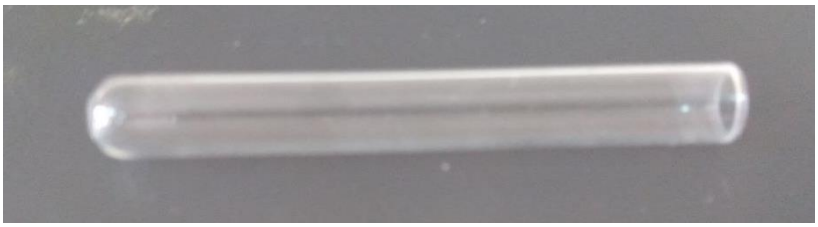


Photo13 : Durham glass cloche tube (original)

- 1000-milliliter graduated measuring cylinder **,**a conical glass flask and a sterile needle (5ml) **(Photo 14)**.

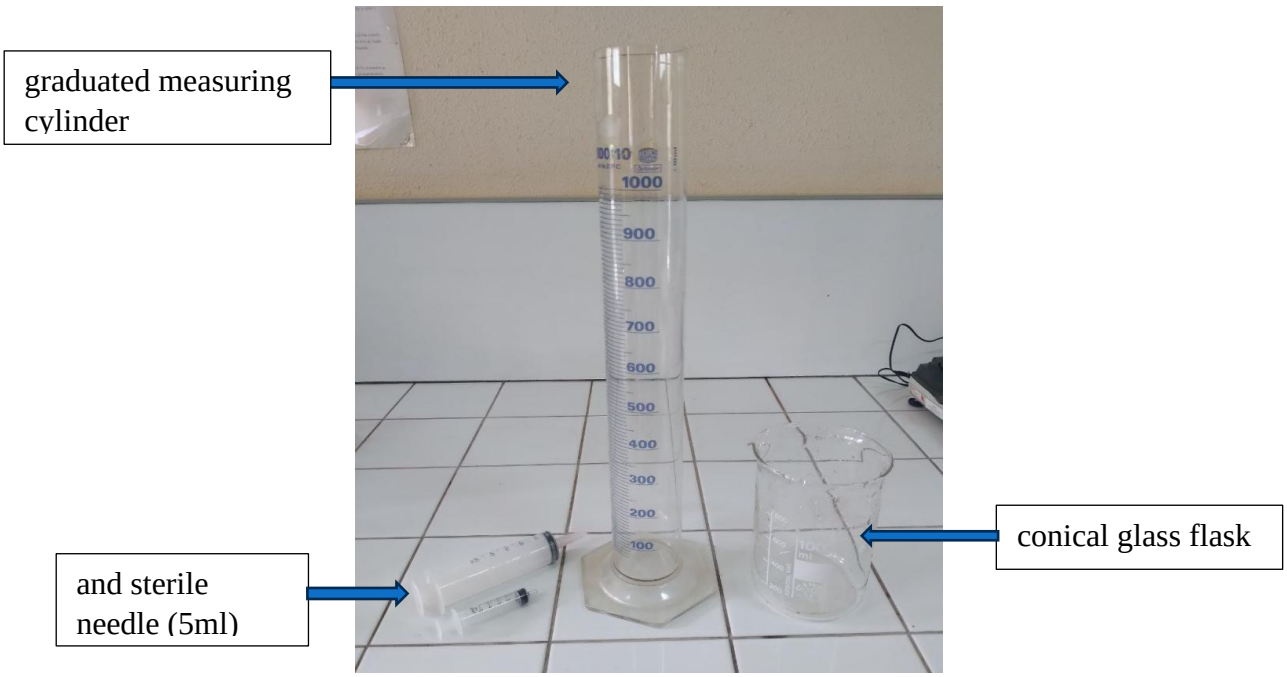


Photo14 : Material for measuring water and the culture medium (original)

- Magnetic baron for mixing solid media with water (**Photo 15).**

Photo15 : Magnetic baron (original)

- Balance, magnetic agitator, lactose broth with Bromcresol purple for coliform total and Schubert broth for coliform fecal **(Photo 16).**

Photo16 : Material for preparation the culture medium (original)

- Tryptone and NaCl for *E. coli* detection

Photo17 : The product use to detected *E. coli*(original)

b. Method of coliforms

In this part we have two methods, one for bacteria total coliforms and two for fecal coliforms;

- **Total coliforms**

The coliform bacteria are a type of Gram-negative bacteria that resemble batonets and have the ability to ferment lactose while producing gas and acid at a temperature of 35 to 37 °C, usually within 24 to 48 hours.

- **Technique in liquid media**

In the application phase, we used the BCPL test:

- ✓ a glass vial of 100 ml in D/C (double quantity)
- ✓ 5 test tubes D/C (double quantity)
- ✓ 5 test tubes S/C (simple quantity)

In D/C We measured 15.6 g of Lactose broth containing Bromcresol purple in 600 ml of distilled water and heated the mixture to 121 degrees; additionally, in S/C, we measured 13 g of Lactose broth.

We distribute it as follows:

- ✓ 50 ml in the glass vial;
- ✓ 10 ml in the glass test tubes.

We put in both the vial and test tube cloche of Durham to detect the emission gas. Then we seal them tightly and put them in the sterilization unit for 20 minutes at 130 degrees and then let them cool completely for 15 minutes to put the test samples with sterile needles in them to avoid the death of the existing bacteria.

Place the quantity of samples after they have cooled down as follows:

in D/C test tube ⟶ **10 ml from the samples**

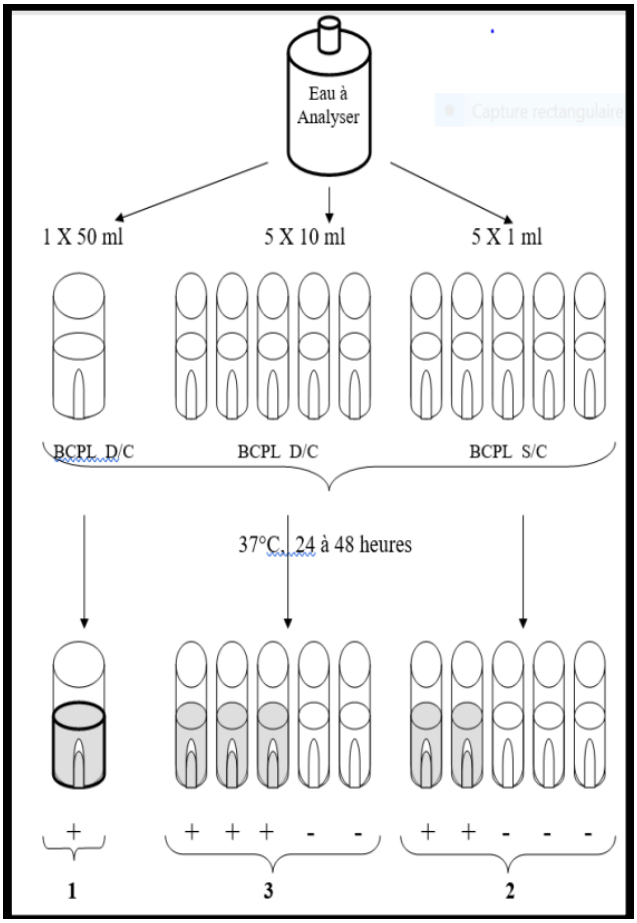
in D/C glass vial ⟶ **50 ml from the sample**

in S/C test tube ⟶ **10 ml from the sample**

We then incubate the sample at 37 degrees for 24 to 48 hours **(figure 13).**

- **Result*:***

The result appears to be a color change from violet to yellow with the appearance of gas release.



After 24 or 48 hours of incubation, we observe in the positive tubes a color change from violet to yellow with the release of gas, which indicates the process of bacterial fermentation.

After the result is visible, we sort the positive tubes to calculate them in a table of Mac Grady for finding the positive number of MPN table.

Inoculum	Test de présomption	Nombre caractéristique
1 X 50 ml	+	**1**
5 X 10 ml	+ + + - -	**3**
5 X 1 ml	+ + - - -	**2**

Example of the number of positive tubes

Figure13 : Protocol used for the total coliforms

- **Fecal coliforms**

At this point, we take one tube with a positive result from D/C, one tube from S/C and a vial if available. And in this stage, we use Schubert Broth to detect fecal coliforms; in D/C we measured 12.4 g in 200 ml of distilled water, and in S/C we measured 3.1 g in 100 ml.

We do the same process, but this time the incubation is at 44 degrees.

- a glass vial of 100 ml in D/C (double quantity)
- 5 test tubes of 10 ml D/C
- 5 test tubes of 10 ml S/C (simple quantity)

After the first result, we put a few drops of the positive tubes obtained from the lactose broth medium with bromocresol purple into the tubes containing the previously sterilized Schubert Broth solution so that the whole work is between a Benzene Bec flame and incubated at 44 degrees for 48 hours **(figure 14-a).**

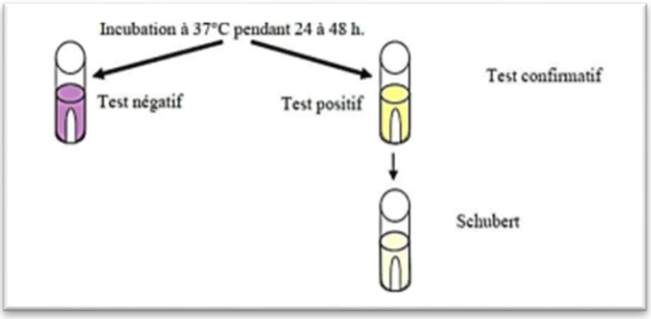


Figure14 : a- Confirmative test of fecal coliforms

- ***Escherichia coli***

We use a tryptone medium, which is often used in the final step of a series of tests to detect bacteria, particularly *E. coli*. This test determines whether bacteria can break down the amino acid tryptophan present in the medium and produce a substance called indole. For our study, we weighed 3.74 grams of tryptone. 2,5 grams of salt in 250 ml of distilled water. We mixed the solution at 121°C, distributed it to test tubes, and sterilized it for 20 minutes. Then, we added the samples and incubated them for 24 to 48 hours. After incubating the bacteria in this medium, we added Kovacs reagent. If a red ring appeared on the surface of the medium, it meant that the bacteria produced indole (a positive result), indicating that the bacteria were active and performing chemical reactions. This result means the presence of *E. coli.*

- **Kovacs reagent**

(p-dimethylaminobenzaldehyde). Kovacs reagent is a biochemical reagent consisting of isoamyl alcohol and para-dimethylaminobenzaldehyde (DMAB).

It also contains concentrated hydrochloric acid. It is used in tests to check if an organism can break down tryptophan into indole and α-amino propionic acid with the help of bacteria that have tryptophanase.

The formation of a red ring, soluble in ether, chloroform, and alcohol, indicates the production of indole. The indole produced forms a red ring **(Photo 23)** that is soluble in ether, chloroform, and alcohol. It was invented by the Hungarian-Swiss chemist Ervin Kovacs. Indole production is used in a test designed to distinguish among members of the Enterobacteriaceae family **(Webn°7).**

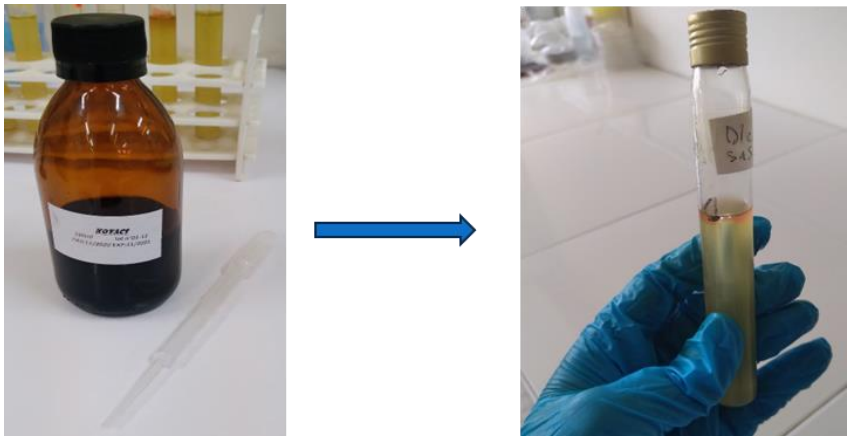


Photo 18: Kovacs test (original)

III.2.2.4. Bacteriologies Method

- **Dilution Method and Materials**

  - **Material**

Sterilization**:** oven (minimum temperature 170°C); autoclave; pressure cooker

- Laboratory incubator
- Microbiology material: Mac Conkey agar **(Photo 17)**;
- Sterile lab swab 1 ml;
- Pipettes (single-use)
- Glass teste tubes
- Sterile petri dishes and physiological water.

Photo19 : Material of the enumeration method (original)

  - **Method**

In this study, water samples were collected from various locations in the valley and analyzed using Petri dish culture techniques to evaluate bacterial contamination levels.

A series of dilutions were prepared for each sample and cultured on suitable nutrient media. The method is as follows:

First, we place two glass test tubes, each containing 9 ml of sterile water, between the flames of bec benzene. Then, we insert a sterilized needle into the sample water bottle, take 1 mL of the sample, and put it into the first test tube. This dilutes the sample to 10^{-1} (1/100)

We repeat this process by taking 1 ml of the initial 10^{-1} dilution to create a 10^{-2} (2/100) dilution. We repeat this process depending on the amount of water contamination **(Photo 18).**

In the second method, we used MacConkey gel to support the growth of bacterial colonies, ensuring sample spacing with the zigzag swabbing technique. We took 0.10 mL from 10^{-1} and 10^{-2} sample and distributed it in a zigzag line using a sterile laboratory swab (EcoVilon). Then, we put it in an incubator at 37 °C for 48 hours **(Figure 17).**

After the incubation period, we counted the number of visible colonies (colony-forming units, or CFUs) on each plate. This number was then used to calculate the bacterial concentration in the original sample in CFUs/ml.

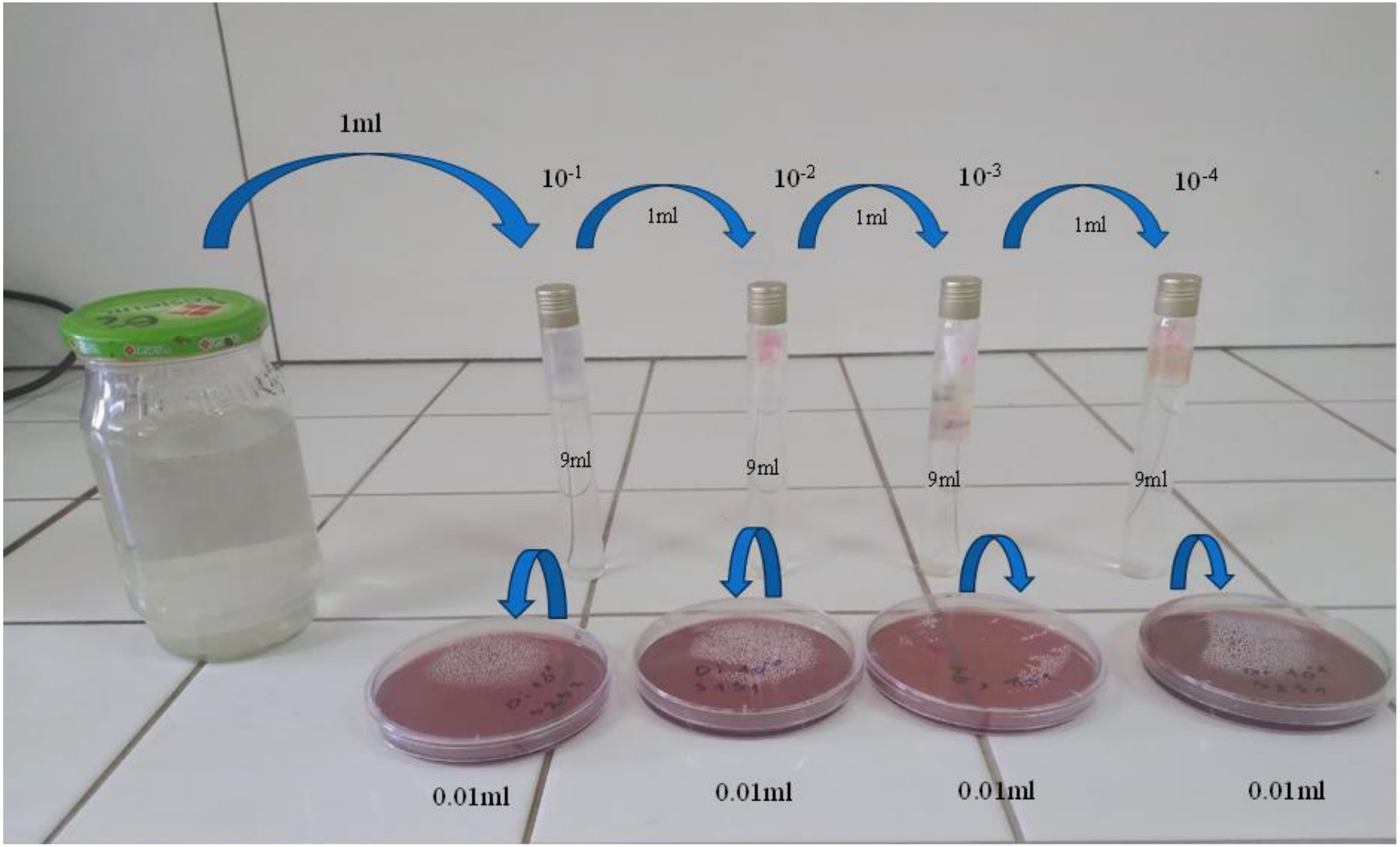


Photo 20: Dilution Methode (original)

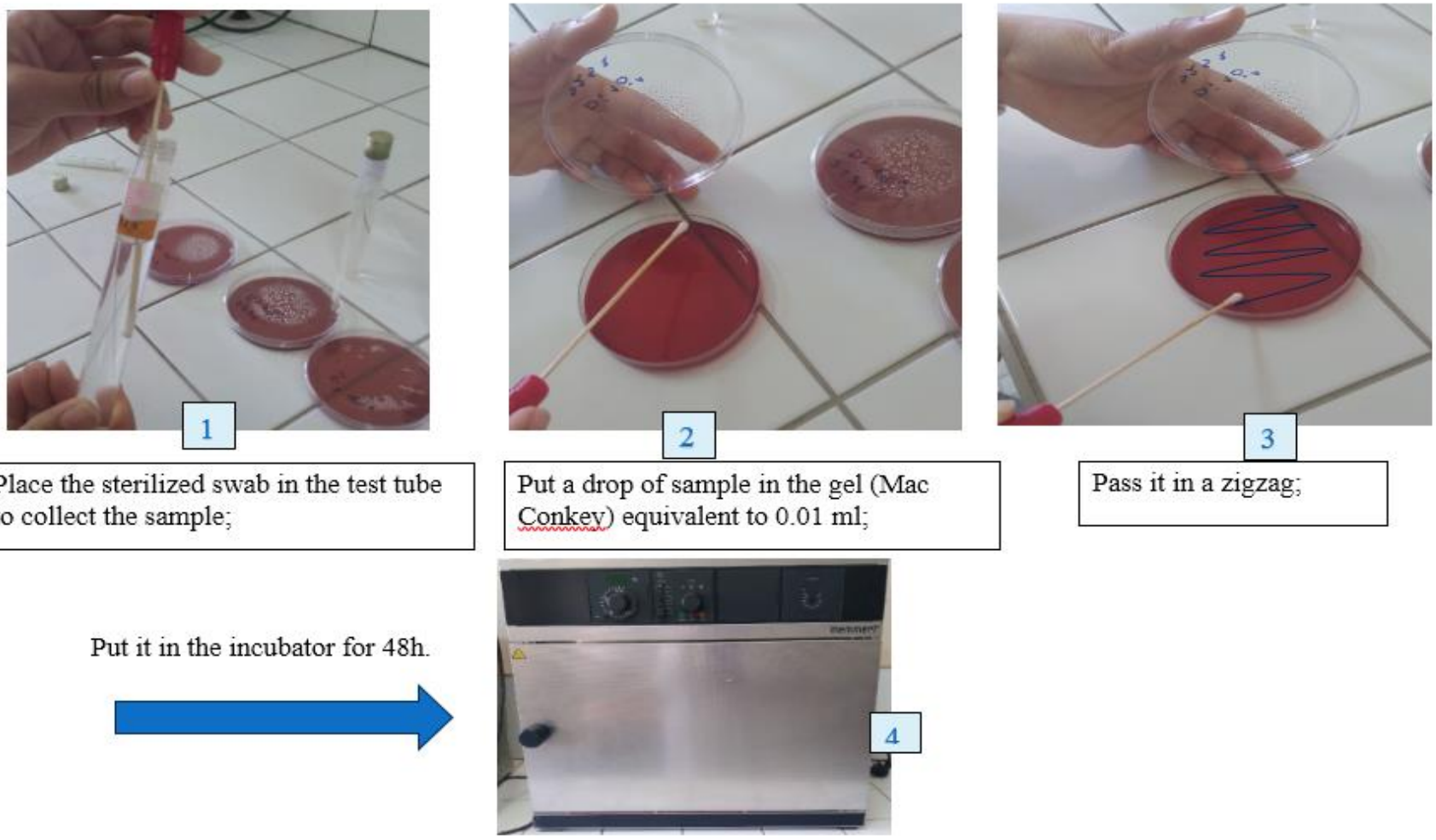


Figure15: Enumeration Methode (original photos)

III.2.2.5. Bacteria coloration

Gram staining is a basic and important method of microbiological analysis used to identify and classify bacteria as Gram-positive or Gram-negative. This staining process is based on differences in cell wall composition among bacterial species. Some bacteria permanently absorb the primary violet pigment, while others lose it after treatment with alcohol and acquire an alternative pigment that gives them a pink color.

A. Materials

- Light microscope (x100) **(Photo 19)**
- Blades and slats
- Platinum strap

- **For coloration**

- Fuchsine coloration
- Lugol
- Violet gentian
- Alcohol

Photo 19: Light microscope (Original)

B. Method

We ignite the fire of benzene, take the platinum belt, and place a small bacterial colony on a glass blade containing a drop of water. We stir in a circular motion to distribute the bacteria. Then, we color in the following order:

- Alcohol
- Violet gentian 1min
- Lugol 1min
- Alcohol 15 second
- Sterile water
- Fuchsine 1min
- Sterile water

We then proceeded to dry it and examined it under a microscope. The gram-negative sample appeared pink, while the gram-positive sample appeared purple (**Figure 17**).

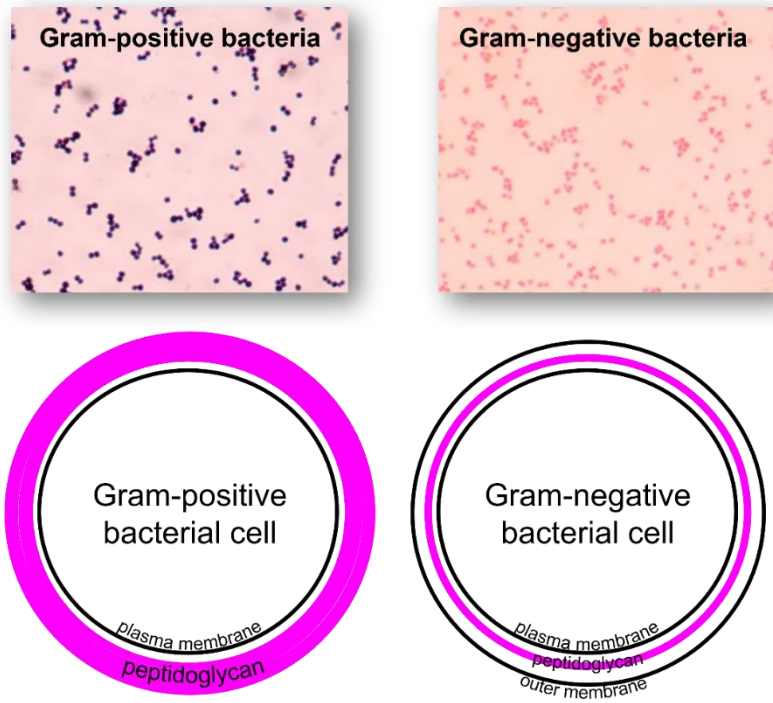


Figure 16: The different between gram negative and gram positive

After obtaining the results, we count the colonies in a Petri dish. There should be between 30 and 300 colonies to ensure accurate counting. If there are fewer than 30 colonies, the sample may not be representative. If there are more than 300 colonies, it may be difficult to count them accurately, and they may overlap.

If the core appears to be too contaminated to obtain an appropriate colony count, the sample should be diluted to determine the approximate number of bacteria present. We then calculate it with the following equation:

$$\frac{\text{No. of Colonies} \times \text{Dilution Factor (10x)}}{\text{Volume plated (ml)}} = \text{CFU/ml}$$

III.2.2.6 Collecting Copepods and Daphnia

Clean glass or plastic container (100-500 ml)

Plastic pipette or small plastic tube (plastic pipette/dropper)

Magnifying glass or microscope

Clean water (for rinsing or transferring)

Test tubes or petri dishes for collecting organisms

- Observation and separation:

Use a magnifying glass or microscope to observe daphnids (recognizable by their transparent bodies and hopping movements) and copepods (fast-swimming organisms with long antennae). Use ethanol to limit their movement .

If necessary, they can be separated, and each species can be placed in a different test tube using a pipette gently - not massaging, as this is incorrect in this context.

Results and discussion

This chapter presents the findings from the analysis of field samples collected from the valleys in the Zenata study region to determine the extent of water contamination.

Focusing on the values recorded at each study site and comparing them with the standards imposed by environmental and health authorities, the results were presented in accordance with the primary goals of the study and divided into physical, chemical, and biological outcomes.

These findings are intended to establish a solid scientific foundation that aids in comprehending the valleys' present environmental state and assessing the degree of risk they pose to the ecosystem and public health.

Tableau 9: The Field trip date

The field trip number	The date	The weather conditions
1	17/02/2025	Dry period with low temperature
2	06/04/2025	Heavy rainfall
3	17/052025	Moderate precipitation

We notice that in each field trip, the weather and the surrounding abiotic factors differed, which makes us put forth the possibility that it may be a reason for the change in the results.

During the initial field trip, the weather was characterized by a dry spell with low temperatures. Whereas in the second and third field trips, the rains were so heavy that the shape, quantity and depth of the valley changed.

VI.1. The physicochemical parameters result

In this phase of the study, we measured some of the types of chemicals possibly present in the water, like nitrate and nitrite and the physical characteristics, like salinity, pH, and O_2.

Tableau 10: Result of measuring the physicochemical parameter

- **February Field trip**

	Sation 1		Station 2	
	FF	SW	FF	SW
Salinity	1.1g/l	1.1 g/l	1.1 g/l	1.1g/l
Conductivity	2.40 mS/cm	2.43 mS /cm	2.50 mS/cm	2.50 mS/cm
pH	7.60	7.81	7.40	7.45
Redox	-23.6 mv	-33.90 mv	-17 mv	-18.4
O2	6.53 mg/L (68%)	6.40 mg /L (66.1%)	6.80 mg/L (72.1 %)	7.01 mg/L (70.1%)

- **April Field trip**

	Station 1		Station 2	
	FF	SW	FF	SW
Salinity	2.05 g/l	2.60 g/l	2.09	2.30
Conductivity	5.12 mS/cm	5.06 mS/cm	4.57 mS/cm	4.47 mS/cm
pH	8.05	8.07	7.98	7.96
Redox	-49.60 mv	-50.8 mv	-46.4 mv	-43.7 mv
O2	7.60 mg/L (80%)	7.03 mg/L (80%)	7.33 mg/L (75%)	7.20 mg/L (75.7%)

- **May Field trip**

	Station 1		Station 02	
	FF	SW	FF	SW
Salinity	0.9g/l	0.9 g/l	0.9 g/l	0.9g/l
Conductivity	2.10 mS/cm	2.10 mS /cm	2.09mS/cm	2.08 mS/cm
pH	8.01	7.84	7.76	7.80
Redox	-45mv	-39mv	- 37.5mv	-37.3
O2	5.35mg/L (67%)	5.31 mg /L (64.3 %)	5.60mg/L (74.1%)	5.74mg/L (70.5%)

The tables above show the physical and chemical values of the water collected at two different locations in the valley in two different states:

FF: Fast-flowing water
SW: Stagnant water

We can see from the following tables that the pH and O_2 are good to moderate and do not indicate any risk of decrease or increase.
However, we note that the salinity is different each time, as it was high in February and April, and low in May month because the rain that fell which caused a decrease in salinity.

A previous study was identified that yielded results on the salinity levels in the area. These results were found to be comparable to the results obtained in this study.

Similar to that of electrical conductivity. Salinity values ranged from 0.9 g/L at the Isser station in mid-March to 4.7 g/L at the estuary station in early March. Levels during spatial variation ranged from 1.3 to 2.53 g/L. Salinity levels increased steadily from upstream to downstream **(Meziane, 2022).**

The evolution of salinity is similar to that of conductivity in the sense that it is maximum at low water and minimum at high water. Conductivity increases from upstream to downstream**. (Dahmani & *al*., 2002).** They have a close relationship and can stem from either natural or

human sources. Both natural and human origins can closely link them. Controlling the salinity of irrigation water is essential. A high salinity value indicates a high concentration of ions in solution **(Bouaroudj, 2012).** Electrical conductivity is a good indicator of water mineralization. Low conductivity means there are a lot of soluble and ionizable salts present. Salinity is a key stress factor for fecal pollution bacteria in salty environments **(Chedad & *al*., 2007).**

- **Nitrate and nitrite results**

Tableau 11: The result of nitrate and nitrite

- ***February Field trip***

	Station 1		Station 2	
	FF	SW	FF	SW
Nitrite NO2	1.10mg/l	1.25 mg/l	1.45 mg/l	1.20 mg /l
Nitrate NO3	6.6 mg/l	6.80 mg/l	7.80 mg/l	9.20 mg/l

- ***April Field trip***

	Station 1		Station 2	
	FF	SW	FF	SW
Nitrite NO2	0.57 mg/l	0.46 mg /l	0.66 mg/l	0.63 mg/l
Nitrate NO3	15.06 mg/l	16.39 mg/l	15.94mg/l	15.06 mg/l

- ***May Field trip***

	Station 1		Station 2	
	FF	SW	FF	SW
Nitrite NO2	0.46 mg/l	0.38 mg /l	0.59 mg/l	0.66mg/l
Nitrate NO3	7.08mg/l	6.64mg/l	6.64mg/l	16.89 mg/l

As can be seen from the following tables, the percentage of nitrite is significantly high in February, reaching 1.45 in the second region, while the rest exceed 0.60. This value is higher than the permissible limit of 0.1 for valley waters.

Similarly, the level of nitrate is also high, reaching an average of 16.89 in May. Nevertheless, it does not exceed the established limit of the 50 mg/L standard **(OMS, 2013).** The OMS standard (2004) of 0.1 mg/l for nitrite values has been exceeded.

These results suggest the presence of agricultural or human activity, or the discharge of untreated sewage. The proposed nitrite classes are: Good water quality is defined as 0 to 0.01 mg/L, average quality is defined as 0.01 to 0.1 mg/L, and poor to very poor quality is defined as 0.1 to 3 mg/L and NO_2 > 3 mg/L **(Chaibi, 2014).**

In **2005**, **Slim** and ***al.,*** discovered that a lot of nitrates in surface waters is connected to either more algae growing in those areas or to the process of denitrification, which changes nitrate (NO_3) into nitrogen (N_2) when organic matter is present.

Nitrites are transient and unstable during nitrification or denitrification, so their presence in the natural environment is low **(Potelon & Zysman, 1998).**

Nitrates and nitrites are the two most abundant forms of nitrogen found in water. Although they are naturally present in small quantities in surface water, very high concentrations of nitrates and nitrites can be toxic to aquatic animals **(Rodier, 2009).**

Therefore, we can generally conclude that the water quality at the Studie stations is stations is average. Seasonal variations in nitrate levels are significant and linked to the development of phytoplankton **(Rejsek, 2002).** Water containing nitrites is considered suspect because it is often associated with a deterioration in microbiological quality. However, water that comes into contact with certain types of soil may contain nitrites regardless contamination.

VI.2. The bacteriological study results

VI.2.1. Total coliforms

- ***Lactose broth with Bromocresol***

We have prepared the medium in which we will place the sample (Lactose broth with Bromocresol purple) and we sterilize it at 130° for 20 min **(Photo 20).**

Photo 21: Lactose broth with Bromocresol in tube vial (original)

- We added the samples as follows into the solution:

in D/C test tube ⟶ ***10ml from the samples***

in D/C glass vial ⟶ ***50ml from the sample***

in S/C test tube ⟶ ***1ml from the sample***

The following image shows the positive results indicating the presence of total coliforms **(Photo 21).**

Photo22 : Change the color of lactose broth (original)

VI.3. MPN (most probable number)

First, we record the number of tubes that show bacterial growth (total coliforms) in each dilution. Then, we refer to a table to compare the results and determine the approximate number of bacteria in the original sample.

This method is widely used in the analysis of drinking and surface water due to its effectiveness in detecting indicators of fecal contamination, such as *E. coli*, even at low concentrations. It provides indirect yet reliable quantification, especially when direct colony counting is difficult.

The MPN is a procedure to estimate the population density of viable microorganisms in the test sample. The preferred and most cost-effective method for detecting coliforms is the Most Probable Number (MPN). This technique allows statistical evaluation of the number of microorganisms present in a sample and measures the metabolically active viable proportion, which can be used to estimate the total population or a specific group of microorganisms. A series of inverted Durham tubes containing double and single robust lactose broth is infected and tested in measured amounts of water. The coliforms create acids and gases by fermenting the lactose in the soup. The number of positive tubes in relation to the MPN table is used to estimate the MPN of coliforms present in 100 milliliters of water **(Shubham Verm & *al.*, 2025).**

The Most Probable Number (MPN/NPP) test is used to estimate the concentration of microorganisms (such as Escherichia coli) in a non-sterile water sample, based on the number of positive tubes. After entering the number of positive tubes into the MPN table, the following values are obtained:

Characteristic number (Nombre caracteristique) This is the numerical value that represents the most probable estimate of the colony-forming units (CFU/ml) in the sample.

Lower confidence limit (Limite de cofinance Inferior) This indicates the lowest likely number of bacteria in the sample, within a given statistical confidence level (usually 95%). **Upper confidence limit (Limite de cofinance Supérieure)** This indicates the highest likely number of bacteria in the sample within the same confidence interval.

tableau12 : Results of test lactose broth with bromocresol of the first station in February

- **FF: fast flowing water**

5 MPN limit lower <0.5; Superior 13

Inoculum	Test of presumption	Characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	3
5 x1ml	+	1

SW: stagnant water

3 MPN limit lower <0.5; Superior 8

Inoculum	Test of presumption	Characteristic number
5 x 50ml	-	0
5 x10ml	+ +	2
5 x1ml	+	1

- **For sample (1), the test results showed**

Referring to the table, we find that the most likely number matches the distribution with the pattern (031), which corresponds to a value of 5 MPN 5/100

These results indicate minor contamination in the sample, possibly from a partially contaminated water source with organic or human waste. This requires additional monitoring.

- **For sample (2) the test results showed**

It showed 021, corresponding to a value of 3Npp 3/100. This result shows a slight contamination, but it cannot be ignored if the water is intended for direct human use.

Tableau13 : Result of test Lactose broth with Bromocresol of the second station in February

- **FF: Fast flowing water**

9 MPN: limit lower 2; Superior 21

Inoculum	Test of presumption	Characteristic number
5 x 50ml	+	1
5 x10ml	+ + +	3
5 x1ml	+	1

- **SW: stagnant water**

5 MPN: limit lower <0.5; Superior 13

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	3
5 x1ml	+	1

- **For sample (1), the test results showed**

The (131) MPN table corresponds to an estimated value of 9/ 100 This value indicates significant sample contamination and suggests the presence of a direct or indirect source of coliform contamination in the water.

- **For sample (2) the test results showed**

The (031) pattern corresponds to an MPN value of 5/100 This indicates minor contamination, which may not pose a significant risk but suggests that organic contaminants may have seeped into the water source.

Table14 : Result of test Lactose broth with Bromocresol of the first station in April

- **FF: Fast flowing water**

54 MPN: limit lower 18; Superior 140

Inoculum	Test of presumption	Characteristic number
5 x 50ml	+	**1**
5 x10ml	+ + + + +	5
5 x1ml	+ +	2

- **SW: stagnant water**

54MPN: limit lower 18; Superior 140

Inoculum	Test of presumption	Characteristic number
5 x 50ml	+	1
5 x10ml	+ + + + +	5
5 x1ml	+ +	2

We can observe that each of the two areas in the valley contains a result of 152 in MPN table, which corresponds to 54/100 of bacteria. This is considered a very high amount, and it is evidence of human or animal fecal pollution, or even sewage discharge, in the area. This may affect aquatic life, as well as surrounding animals, such as dogs, livestock, ducks, and birds, that consider the area a drinking source.

Tableau15 :Results of test Lactose broth with Bromocresol of secend station in April

- **SW: stagnant water**

160 MPN: limit lower 39; Superior 450

Inoculum	Test of presumption	Characteristic number
5 x 50ml	+	1
5 x10ml	+ + + + +	5
5 x1ml	+ + + +	4

- **SW: stagnant water**

5 MPN: limit lower <0.5 ; Superior 13

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	3
5 x1ml	+	1

We can see that the percentage of coliforms is much higher in Area 1, reaching 160/100 compared to Area 2, which reached 5/100 UFC. This indicates a high risk of bacterial contamination, even though it was raining heavily at this stage.

Tableau16 : Results of Lactose broth with Bromocresol of the first station in May

- **FF: Fast flowing water**

4 MPN: limit lower <0.5; Superior 11

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ +	2
5 x1ml	+ +	2

- **SW: stagnant water**

5MPN: limit lower <0.5; Superior 13

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	3
5 x1ml	+	1

Although the coliform content is slightly lower in both areas than in the previous one, there is still evidence of pollution so that they amounted to the first 4/100 and in the second 5/100

Tableau17 : Results of Lactose broth whith bromocresol of secend station in May

- **FF: Fast flowing water**

3MPN:limit lower <0.5 ; Superior 8

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	1
5 x1ml	+	2

- **SW: stagnant water**

2MPN :limit lower <0.5 ; Superior 6

Inoculum	Test of presumption	characteristic number
5 x 50ml	-	0
5 x10ml	+ + +	2
5 x1ml	+	0

We can observe that the result of the May sample is much lower than the other months, only 3/100 and 6/100

We note that of all the results, the April and February samples in particular recorded the highest bacterial values, and May is low. This is because the rains were very heavy in this month, which caused the water to dilute the bacteria, suggesting that there is a bacterial bloom nearby, based on the consistency of the results in both areas. I found some study of the Galma Wadi where a higher value was recorded than ours. We observe that the maximum value is 1400 CT/100 ml in site 1 during the month of May, while the minimum value is recorded in site 2 of 460 CT/100 ml during the month of April **(Boutebba, 2022).**

Total coliform counts increase during dry periods. The rise may be due to the presence of organic matter and increased temperature, both of which accelerate bacterial multiplication and development. Conversely, total coliform levels are low during cold periods, possibly due to rainwater diluting the water supply **(Mouaz & Bentchich, 2017).**

As qualitative indicators, total coliforms provide information on the potability and environmental condition of the water resource in question **(Belousova & Proskuriva, 2008; Shashikanth & *al*., 2008).**

These fecal contamination indicator germs are present because of animal or human waste, the presence of mammals like sheep and dogs, and the discharge of untreated domestic wastewater into the wadi **(Mouaz & Bentchich, 2017).**

Total coliforms are of animal and human origin, and their presence in water indicates recent contamination by fecal matter **(Chevalier, 2002).**

Total coliforms are not a sign of pollution; their origin may be environmental (soil, vegetation, water) **(Berrouane & *al*., 2018).** Their absence does not necessarily mean that the water is not pathogenic **(Abdi & *al*., 2014).**

VI.4. Fecal coliforms

VI.4.1. Schubert Broth

When the result of the first sample, Lactose Broth with Bromcresol Purple, appears, we put a few drops of it in a solution in a tube containing Schubert Broth.

- Incubate it at approximately 44°C for 24 to 48 hours.
- Observe for discoloration or gas production, which indicates the presence of *E. coli* or coliforms.
- Environmental conditions such as a temperature above 15°C, neutral pH (pH=7), and the presence of assimilable organic carbon (AOC) are ideal conditions for coliform reproduction **(Ainsworth R, Water S, WHO 2004).**

Result

If Positive Discoloration of the medium (yellow if it contains a pH indicator) and/or gas bubbles in the Durham tube; Negative No discoloration or gas production **(Photo 22).**

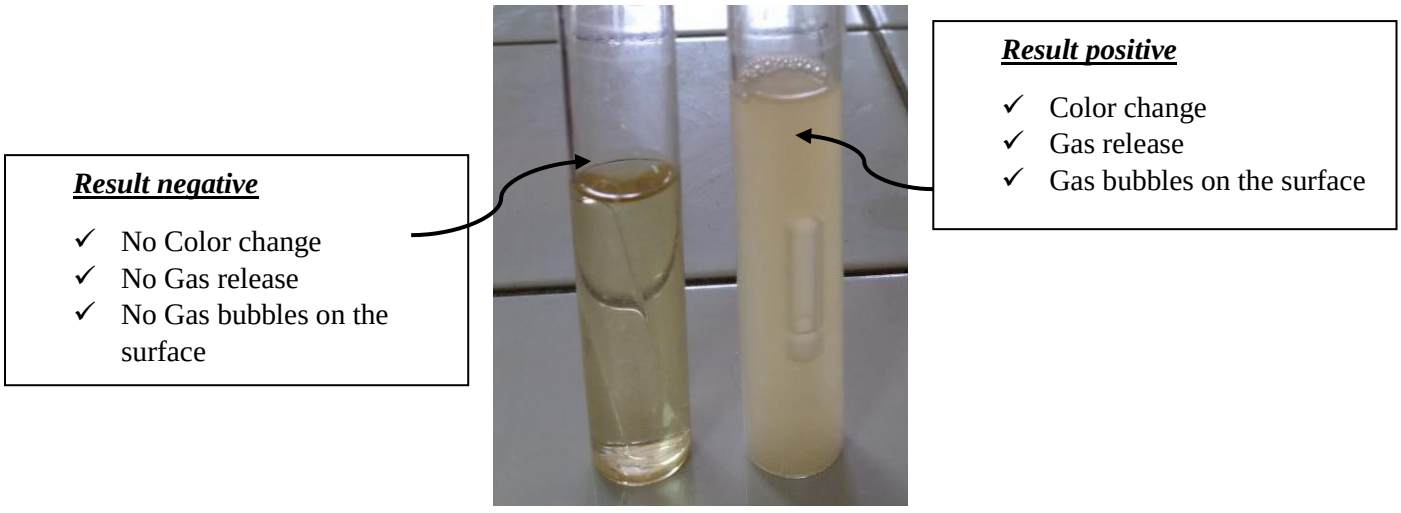


Photo23 : The different between positive and negative Schobert broth (original)

Fecal coliform, also known as thermotolerant coliforms are restricted to bacteria capable of fermenting lactose at 44.5 –45.5°C with the production of gases **(Shubham Verm & *al*., 2025).**

We then calculated the number of fecal coliforms in each region for each month. It turns out that the percentage of coliforms was high in April, reaching 240 UFC; this level is considered evidence of fecal contamination or *E. coli* **(Figure 18).**

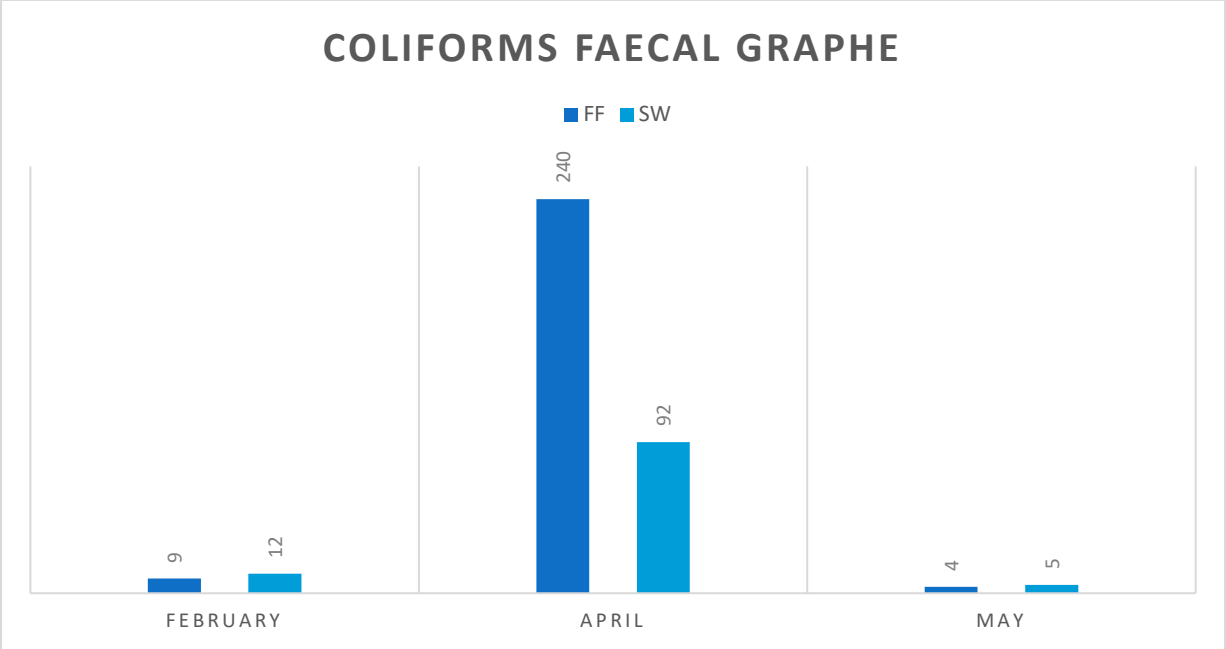


Figure 18: Graph of coliforms faucal in 3 months

There is fecal contamination of this wadi, on the one hand by the presence of numerous mammals (sheep, cattle, goats, etc.) and, on the other, by domestic wastewater discharges from the commune surrounding this river.

The presence of high concentrations of fecal coliforms in the Beni Aza Wadi indicates fecal contamination from two sources. First Numerous mammals, such as sheep, cattle, and goats, as well as domestic wastewater discharges from the surrounding commune **(Abdelmalek & *al.*, 2012).**

As indicators of water quality, coliform bacteria have been used to monitor potability since 1920 due to their economic viability and ease of detection. Coliform is naturally present in the environment, but fecal coliform and *E. coli* are derived from human and animal waste. Thus, the presence of both of them on a material or in water indicates that the water is contaminated by human or animal waste **(Shubham Verm & *al.*, 2025).**

Jacinta and *al*., have noted in **2007** in their work that the surface water quality of many lakes and rivers suffers from the presence of high levels of fecal coliform. These bacteria are an indicator of fecal contamination.

If the water is constantly contaminated with fecal pollution, as almost all surface waters are. The situation is no longer an alarm signal but rather an assessment of the extent of fecal pollution. Most of this contamination comes from urban wastewater discharges **(Abdelmalek & *al*., 2012).**

VI.5. Identification of *E. coli*

A few drops of Kovacs reagent are added to each positive tube of Schubert medium, which shows indole production by forming a red ring, a typical reaction for *E. coli*. Enumeration is carried out using the MPN method. According to **Kiehl & *al*., 2005**.

The following image shows the sample which was negative in 3 months even though the coliform result was positive **(Photo 24).** The results confirmed the presence of fecal contamination and coliforms total but note *E. coli*.

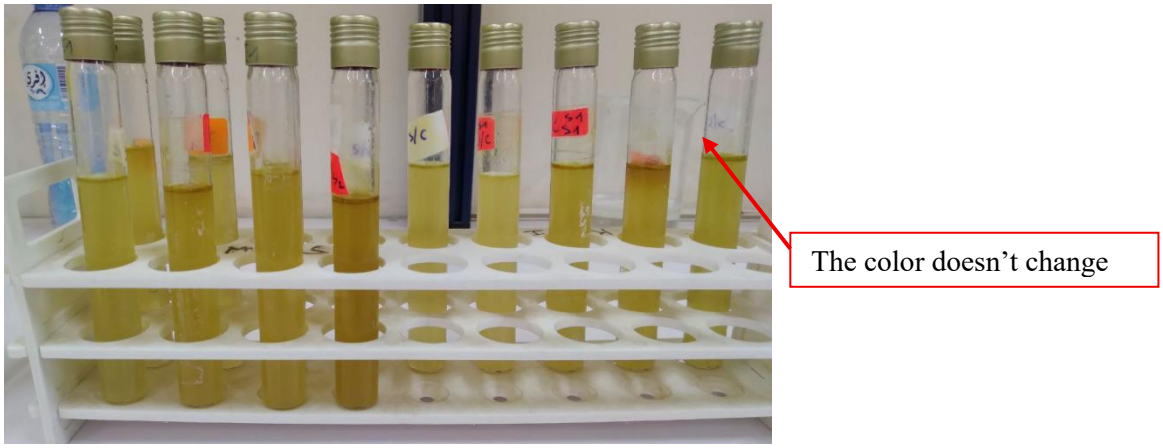


Photo24 : Negative result *E. coli* (original)

After all but one tube came back negative in the tryptone solution, we repeated the confirmatory test using the implantation method on MacConkey agar.

The results are significant evidence that *Escherichia coli* was present at the station only in April and May and negative in February which reinforces our theory that there is a recent or upcoming fecal coliform in the area.

The results show that the values far exceed the standards requiring the total absence of fecal coliforms. The highest values were recorded at Site 1 in May (1,400 CT/mL), and the lowest were recorded at Site 2 (Heliopolis) in April (240 CT/mL). These results confirm fecal contamination at these stations due to the high temperatures recorded during the dry season, which favor the rapid multiplication of various bacteria and fecal germs **(Boutebba & *al*., 2022).**

Back in **1893**, *E. coli* was the first bacterial species to be considered an indicator of fecal contamination. then, in 1907, researchers observed that E. coli is the dominant bacterium in human feces, while other species of Enterobacteriaceae may originate elsewhere **(Bengarnia, 2016).**

The presence of *E. coli* indicates that the contamination is of fecal origin and recent. According to **(Edberg & *al*., 2000),** *E. coli* is the best indicator of recent contamination of the aquatic environment by human or warm-blooded animal feces**.**

VI.5.1 The positive result in the petri dishes

This section displays the results recorded for each sample and shows the variation in bacterial density between positive results. We only recorded positive results for *E. coli* in April and May. It also displays qualitative observations about colony morphology, odor, and the presence of positive *E. coli* bacteria in the original sample.

- **The positive results from April**

The April Valley water sample was cultured on MacConkey medium. After incubation, pink to purple colonies were observed, indicating the presence of lactose-fermenting bacteria and providing conclusive evidence of ***E. coli***. A greenish-yellow colony resembling an ***Aspergillus flavus*** mushroom was observed on one side of the Petri dish. This type of bacteria is considered possible contamination of the sample **(Photo 27).** Although fungal contamination occurred in the dish, the ***E. coli*** colonies were clear in color and shape.

Staining confirmed their identity. The bacteria appeared pink, confirming that they are Gram-negative. Magnifying the sample under a microscope revealed that the bacteria were bacillus-shaped, consistent with the characteristics of *E. coli*.

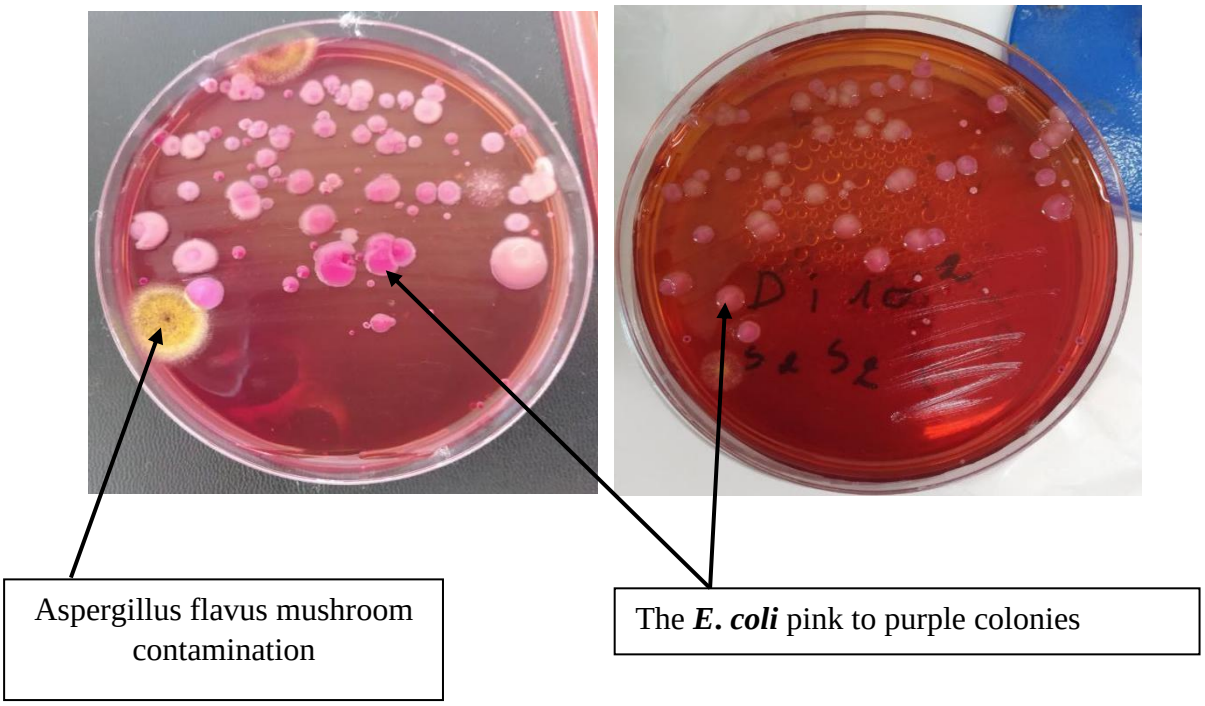


Photo25 : The positive *E. coli* from April sample (original)

In the future, it is recommended that laboratory working conditions be improved to avoid accidental contamination that may affect the accuracy of the results or that a special space be provided for the bacteria to prevent interference and spoilage.

- **The positive results from May**

Although the *E. coli* colonies are small in size compared to the April sample, we can clearly observe them. They exhibit the characteristic features of *E. coli* and appear pink -colored.

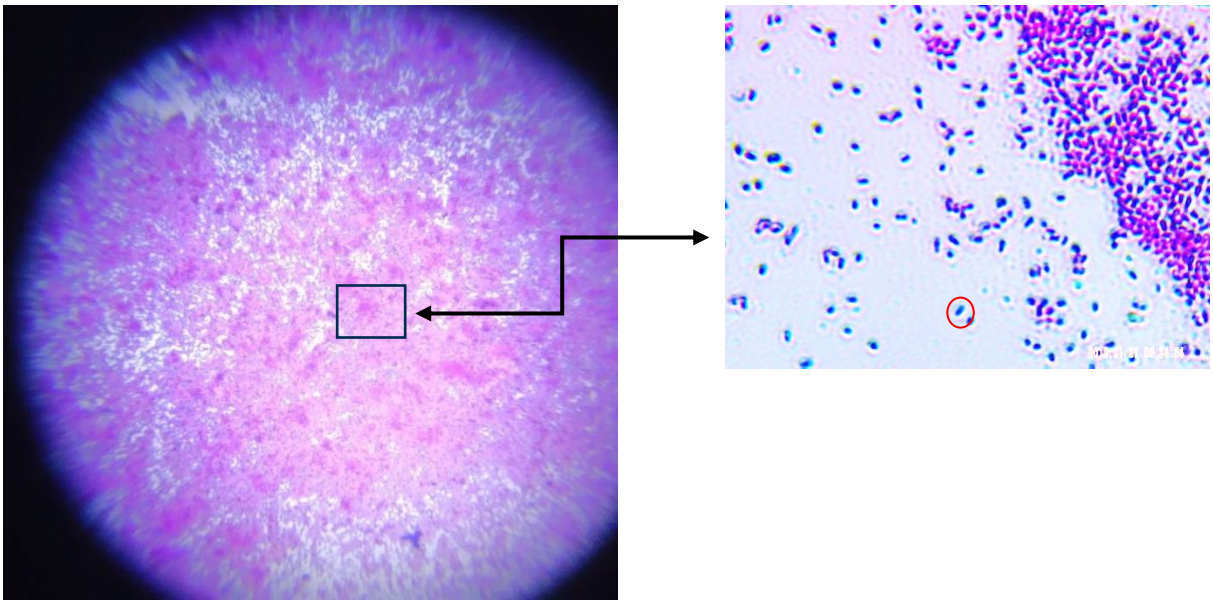
Photo26 : Positive *E. coli* from May sample (original)

The result was positive only in **the fast-flowing water** sample, which showed very small colonies, but nevertheless we cannot deny the presence of contamination.

VI.5.1.2. Samples under a light microscope

Under the microscope, we observe all the characteristics of *Escherichia coli*. It appears pink, indicating a negative Gram stain, and its cylindrical shape is clearly visible under a light microscope, as the image (5, 6) shows, (Note, it's pink under the microscope, but it looks purple in the photo I took with my phone.)

- **Results from April sample**



- **Results from May sample**

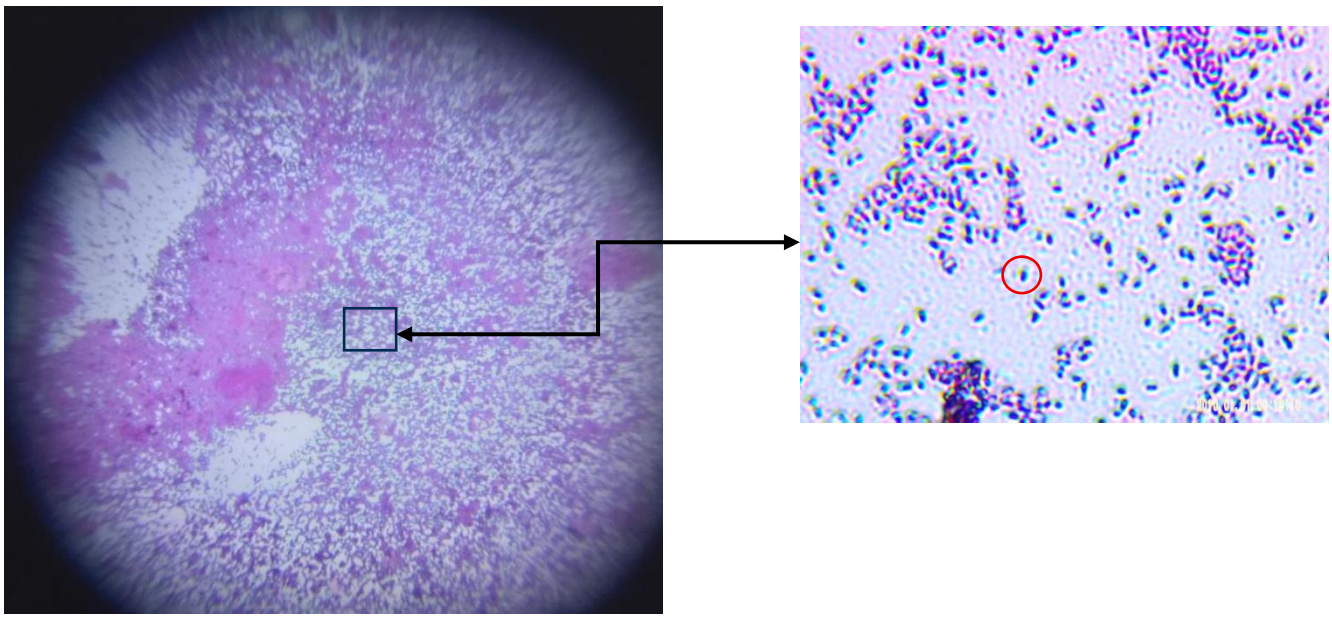


Photo27 :form of E. coli Ander microscope (original)

VI.5.1.3. Calculating the number of colonies in CFUs/ml

After incubating the sample at 37°C and showing the result, we counted the number of colonies and calculated CFUs/ml

- **April sample**

Table 18: The calculating the number of colonies in the first station

Station 1	No. Colonies	No. Dilution	Vol. palet	Unit in CFU/ml
S1	*50*	10^1	0.01ml	50000
S1	40	10^2	0.01ml	400000
S2	30	10^1	0.01ml	30000
S2	**20**	10^2	0.01ml	20000

S1: fast flawing water

S2: stagnant water

Table 19: The calculating the number of colonies in the second station

Station 1	No. Colonies	No. Dilution	Vol. palet	Unit in CFU/ml
S1	60	10^1	0.01ml	60000
S1	34	10^2	0.01ml	340000
S2	60	10^1	0.01ml	60000
S2	30	10^2	0.01ml	300000

We can observe that the *E. coli* score in two stations is very high, over taken to 100/ml. This means that there is a very high level of pollution.

May sample

Table20: The calculating the number of colonies in the first station

Station 1	No. Colonies	No. Dilution	Vol. palet	Unit in CFU/ml
S1	0	10^1	0.01ml	0
S1	0	10^2	0.01ml	0
S2	141	10^1	0.01ml	141000
S2	**57**	10^2	0.01ml	570000

Table 21: The calculating the number of colonies in the second station

Station 1	No. Colonies	No. Dilution	Vol. palet	Unit in CFU/ml
S1	0	10^1	0.01ml	0
S1	0	10^2	0.01ml	0
S2	0	10^1	0.01ml	0
S2	**0**	10^2	0.01ml	0

At this stage, we see that all the samples in S1 are negative except for S2, which shows a high number. This means there is contaminated water leaking in this area.

In Station 2, we note that all the results are negative. And this does not negate the fact that there is fecal contamination.

VI.5.2 The Crustacea founded in water sample

Despite the highest recorded level of fecal pollutants in April, a group of organisms was observed in the water after it was stored for less than 15 days. These organisms were later identified as Daphnia and copepods.

a. Daphnia

Class: Branchiopoda

Order: Cladocera

Its classification as Daphnia is based on the presence of all the physical characteristics associated with that species.

Photo28 : The Daphnia found in the water sample (original)

a.1. A study of the correlation between Daphnia and bacteria

The Daphnia is a very efficient filter feeder of bacteria and small algae species in freshwater thanks to its filter mesh. The size of the mesh varies according to the species. In a 1985 paper, Nagata analyzed the mesh size of Daphnia and their filtering rates of natural bacteria. For example, *Daphnia longispina* has a filter mesh measuring between 0.17 µm and 0.36 µ. has a filter mesh measuring between 0.56 and 1.8 µm. *Daphnia galeata* has a filter mesh measuring between 0.32 and 1.0 µm. He concluded that many species of Daphnia are highly efficient bacteria feeders **(Walsh, 2022).**

Among cladocerans, filter-feeding species like Daphnia can eat a variety of tiny food particles in the water, including bacteria, by using their front limbs to catch them. They can collectively filter considerable volumes in short periods of time. As important species, Daphnia can affect

the number of tiny organisms in water, changing their size and makeup by either eating bacteria directly or by feeding on small creatures that eat bacteria **(Burnet & *al*., 2017).**

Indistinctive feeding behavior of the Daphnia. Several experiments showed that Daphnia do appear automatically in the ponds, though bringing in Daphnia brings a stable system sooner **(Ruud & *al*., 2017)**

In an experiment in which a group of Canadian scientists tracked the fate of *E. coli* bacteria, the bacteria were detected in the digestive system of Daphnia.
Clusters of *Escherichia coli* cells were observed in the guts of all five Daphnia individuals after 15, five, and two minutes of incubation. The fluorescent clusters of *E. coli* were localized in the filtering chamber, which contains the thoracic appendages, as well as in the foregut, midgut, and hindgut **(Photo 30) (Burnet & *al*., 2017).**
The presence of Daphnia in the Wadi waters is an important environmental indicator because these small crustaceans inhabit aquatic environments with specific water quality. In contrast, bacteria indicate fecal pollution.

The experiments showed that Daphnia in ponds are very effective in reducing the number of coliform bacteria within a hydraulic retention time of as low as 4 days, but only in a plug flow regime of ponds in series **(Kampf & *al.*, 1999; 2004).**

Daphnia are filter feeders that consume small particles suspended in water, such as microalgae, bacteria, and organic matter. Some studies have shown that Daphnia can reduce the concentration of *E. coli* and coliform in water by consuming it. This is why Daphnia are sometimes used in bioremediation studies. However, the efficiency with which Daphnia ingest bacteria depends on factors such as bacterial density, size, and water quality. Therefore, while the presence of Daphnia in contaminated water may contribute to reducing *E. coli* populations, it is not a complete substitute for pollution remediation.

Daphnia, are mainly indistinctive filter feeders. The intensive biological filtration process removes loose bacteria like fecal Coli effectively, but also prevent Algae to grow **(Kampf, 1999; 2004).**

Contrary to the expectations the ponds stay clear with low algae numbers, because of the indistinctive feeding behaviour of the Daphnia. Several experiments showed that Daphnia do appear automatically in the ponds, though bringing in Daphnia brings a stable system sooner **(Ruud & *al*,.2017).**

b. Copepods

Copepods vary considerably in size, typically measuring 1 to 2 mm in length. They have a teardrop-shaped body and large antennae. Like other crustaceans, they have a protective exoskeleton; however, they are so small that, in most species, this thin armor and their entire bodies are almost totally transparent. Most copepods have a single, bright red compound eye in the center of their transparent head. Subterranean species may be eyeless. Like other crustaceans, copepods have two pairs of antennae, with the first pair often being long and conspicuous **(Walsh, 2022).**

Class: Copepoda

Order: Cyclopoida

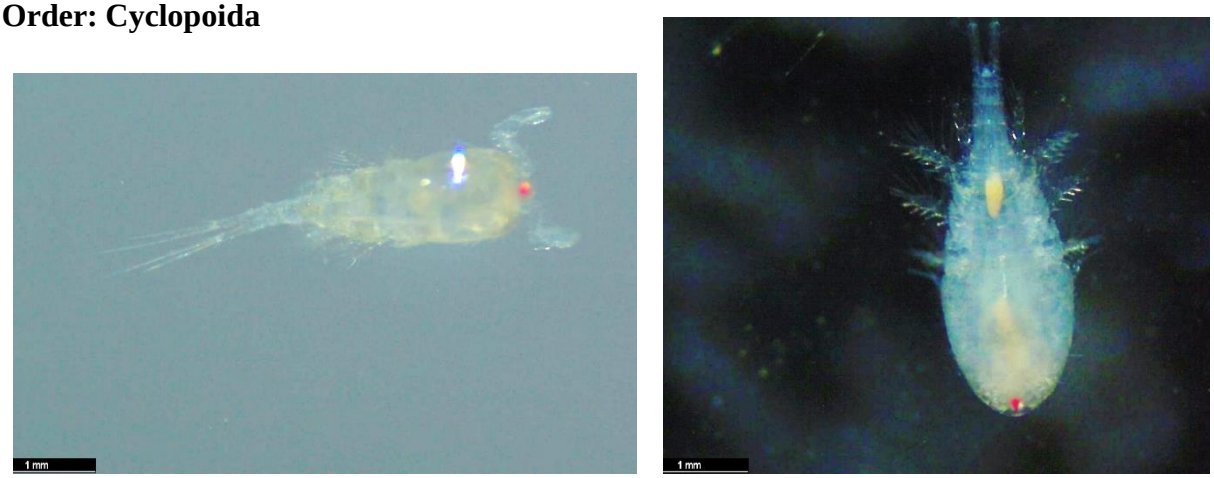


Photo29 :copepod fond in water sample(original)

Class: Copepoda

Order: Harpacticioda

Photo30 :copepod *Harpacticoid* found in water sample (original)

Copepods are considered one of the organisms that feed on bacteria, but they do not depend on them much, especially *E. coli*, unlike Daphnia. However, copepods are known for their tolerance of toxins and pollutants, particularly toxic metals in water.

The potential of using copepods as model organisms in ecotoxicology and environmental genomics has been recognized for some time. For some countries marine copepod species have been utilized in bioassays for regulatory toxicology **(Sheikh Raisuddin; & *al*., 2007).**

The removal efficiency of indicator and pathogenic microorganisms in constructed wetlands was analyzed, and the microorganisms' removal functions performed by copepods were determined. The results showed that the constructed wetlands effectively reduced *Escherichia coli*, fecal streptococci, total coliforms, and fecal coliforms; the Salmonella spp. removal efficiency was relatively low, and the *Clostridium perfringens* removal was the least. At copepod concentrations of 3.0 × 102/L and 6.0 × 102/L, high die-off rates were observed for indicator and pathogenic microorganisms compared to the control group, and indicator and pathogenic microorganisms in samples with higher concentrations of copepods decreased much more rapidly than those in samples with lower concentrations. These results suggest that predation by copepods is an important mechanism for the removal of bacteria in constructed wetlands **(Song & *al*., 2008).**

Conclusion

Conclusion

In conclusion, this research has attempted to shed light on the topic of assessing water quality in the Tafna Valley using bacteria as bioindicators. This has been achieved by addressing the issue of water pollution and its causes in the region, as well as the risk of waterborne disease outbreaks among the population if contaminated water is consumed for agricultural or livestock grazing purposes.

The health costs associated with disease outbreaks resulting from the consumption of contaminated water, awareness campaigns targeting populations living in substandard sanitary conditions are necessary.

Using the empirical research method, analyzing field and laboratory data, and reviewing the methods adopted in the study, we obtained important results which the April sample showed a slight increase in salinity, reaching 2.60, which exceeds the normal limit for valley water. . In particular, we found an increase in bacterial contamination, with the presence of coliform bacteria total coliform and fecal coliform in the water as *E. coli* was found by the Petri dish culture technique In April, 400,000 CFU/ml were found in running water and 340,000 CFU/ml in stagnant water. In May, 141,000 CFU/ml were found in the first sample and 570,000 CFU/ml in the second. These results are considered larger than normal and indicate significant water pollution. This result supports the hypothesis of fecal contamination in the area. This could be due to untreated sewage or grazing in the area. The presence of coliform bacteria is considered a biomarker for the onset of contamination. Alternatively, the valley water may have been contaminated by nearby factors, such as the presence of a residential area and signs of grazing. In addition to these findings, a slight increase in the number of nitrates and nitrites was found.

The salinity of the water may be due to the nearby agricultural areas or the infiltration of rainwater carrying fertilizers. This calls for taking preventive measures and intensifying studies in this field to limit the spread of pollution to other places or find solutions to mitigate the pollution before it gets worse.

These results emphasize the importance of this topic in health and environmental contexts, and pave the way for more in-depth future research. In addition to our study of the appearance of crustaceans such as daphnia and copepods after a period of water storage, which gives us hope that we can improve the quality of this water, these results open up new avenues for research.

In this scientific context, I propose further studies that

- include an intensive search for the cause of these pollutants and tracking their main source.
- as well as expanding the study of the bacteria present in it and identifying their types, sources, and health and environmental effects,
- identity of the bacteria will also be defined to know their morphological nature more clearly.

In conclusion, we hope that this study will contribute to enriching scientific knowledge, and that it will be a useful reference for researchers and those interested in this field.

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website list

- **web n°1:** https://datastream.org/fr-ca/guide/les-bacteries-coliformes
- **web n°2:** https://herli.com/les-coliformes-et-escherichia-coli-dans-leau-potable/
- **web n°3:** https://www.inspq.qc.ca/eau-potable/coliformes-fecaux
- **web n°5:** https://www.bonobosworld.org/fr/glossaire/geographie/climatogramme-d-emberger
- **web n°5:** https://store.microbiotech.dz/wp-content/uploads/2024/06/fiche-technique-kovacs.pdf?srsltid=AfmBOoo6m9AbwUhlBR3-mK4QN-yc3TRv5a_2siN2Yw3nMDK4DQIqbjp1
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- **effect of E. coli in body** https://www.everydayhealth.com/
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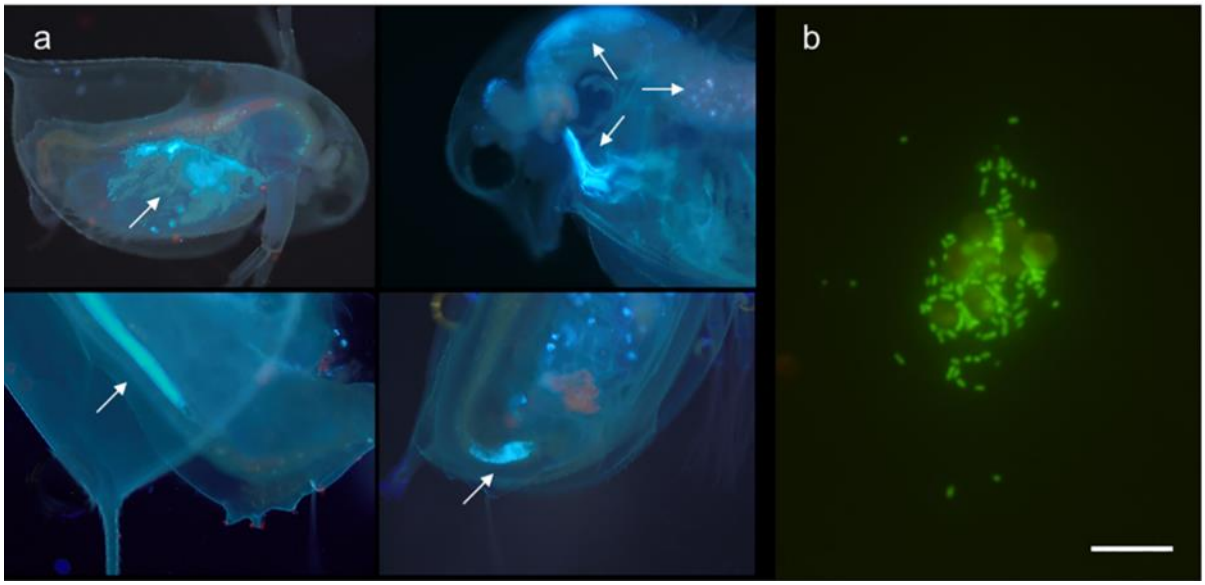
- **E.coli form** https://foodlawlatest.com/2014/07/09/fsa-released-revised-e-coli-o157-control-of-cross-contamination-guidance/
- **Figure daphnia and e.coli** from journal How does the cladoceran Daphnia pulex affect the fate of Escherichia coli in water? https://pubmed.ncbi.nlm.nih.gov/28178322/

Annexes

Annex 1: Table of MPN (Most Probable Numbers)

1 X 50 ml	5 X 10 ml	5 X 1 ml	Nombre caractéristique	Limites de confiance	
				Inférieure	Supérieure
0	0	0	<1		
0	0	1	1	⩽0,5	4
0	0	2	2	⩽0,5	6
0	1	0	1	⩽0,5	4
0	1	1	2	⩽0,5	6
0	1	2	3	⩽0,5	8
0	2	0	2	⩽0,5	6
0	2	1	3	⩽0,5	8
0	2	2	4	⩽0,5	11
0	3	0	3	⩽0,5	8
0	3	1	5	⩽0,5	13
0	4	0	5	⩽0,5	13
1	0	0	1	⩽0,5	4
1	0	1	3	⩽0,5	8
1	0	2	4	⩽0,5	11
1	0	3	6	⩽0,5	15
1	1	0	3	⩽0,5	8
1	1	1	5	⩽0,5	13
1	1	2	7	1	17
1	1	3	9	2	21
1	2	0	5	⩽0,5	13
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	18	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	59
1	4	3	28	9	85
1	4	4	35	12	100
1	4	5	43	15	120
1	5	0	24	8	75
1	5	1	35	12	100
1	5	2	54	18	140
1	5	3	92	27	220
1	5	4	160	39	450
1	5	5	>240		

Annex 2: Observation of E. coli cell in daphnia in her digestive system



الملخص

تقييم جودة المياه في وادي تافنة الأوسط باستخدام البكتيريا كمؤشرات حيوية

أُجريت دراستنا على وادي تافنة الذي يعتبر من أهم المجاري المائية في تلمسان، وذلك لرصد الحالة الراهنة لنوعية المياه المتدفقة في هذا الوادي، حيث تم أخذ عينتين في محطتين مختلفتين الأولى في قرية بورواحة والثانية قرب قرية حجرة القط، وذلك خلال الفترة من فبراير وأبريل ومايو من عام 2025.

تغطي الدراسة التحليل الفيزيائي الكيميائي (درجة الحرارة، التوصيلية، العكارة، الملوحة، الأكسجين، النتريت والنترات) وتقييم التلوث البكتيري من خلال دراسة القولونيات البرازية والإشريكية القولونية.

أظهرت نتائج التحاليل الفيزيائية والكيميائية أن درجة حرارة هذا المجرى المائي عادية، ودرجة الحموضة قلوية، وهناك ارتفاع طفيف في الملوحة والنتريت والنترات وضعف جودة النقل بالإضافة إلى وجود تلوث بكتيري، حيث أظهرت النتائج التي سجلناها وجود تلوث بكتيري في المحطتين خلال جميع الأشهر.

الكلمات المفتاحية: وادي تافنة، التلوث، التحليل الفيزيائي الكيميائي، التحليل البكتيري، الإشريكية القولونية

Résumé

Évaluation de la qualité de l'eau dans l'oued moyen de Tafna en utilisant des bactéries comme bioindicateurs

Notre étude a été menée sur la vallée de la Tafna, qui est considérée comme l'un des cours d'eau les plus importants de Tlemcen, afin de surveiller l'état actuel de la qualité de l'eau qui coule dans cette vallée et deux échantillons ont été prélevés à deux stations différentes, à savoir le village de Bourouaha et la seconde entre le village de Hajra El Gat pendant la période de février, avril et mai de l'année 2025.

Cette étude porte sur l'analyse physico-chimique (température, conductivité, turbidité, salinité, oxygène, nitrite et nitrate) et l'évaluation de la contamination bactérienne par l'étude des total coliformes, des coliformes fécaux et *d'Escherichia coli.*

Les résultats des analyses physico-chimiques montrent que la température de ce cours d'eau est normale, le pH est alcalin, il y a une légère augmentation de la salinité, des nitrites et des nitrates et une mauvaise qualité de transport en plus de la présence d'une pollution bactérienne, car les résultats que nous avons enregistrés ont montré une pollution bactérienne dans les deux stations pendant tous les mois .

Mots clés : Oued Tafna, Pollution, Analyse physico-chimique, Analyse bactérienne, *E. coli*

Abstract

Evaluation of water quality in the middle Wadi of Tafna using bacteria as bio-indicators

Our study was conducted on the Tafna Valley, which is considered one of the most important waterways in Tlemcen, to monitor the current status of the quality of the water flowing in this valley, and two samples were taken at two different stations, namely the village of Bourouaha and the second between the village of Hajra El Gat, during the period of February, April, and May for the year 2025.

This study is related to physicochemical analysis (temperature, conductivity, turbidity, salinity, oxygen, nitrite, and nitrate) and assessment of bacterial contamination by studying total coliforms, fecal coliforms, and *Escherichia coli*.

The results of the physical and chemical analyses show that the temperature of this waterway is normal, the pH is alkaline, there is a slight increase in salinity, nitrite, and nitrate, and there is a poor quality of transport in addition to the presence of bacterial contamination, as the results we recorded showed bacterial contamination in both stations in all months.

Keywords: Wadi of Tafna, Pollution, Physical and chemical analysis, Bacterial analysis, *Escherichia coli*.