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MICROBIOLOGICAL QUALITY ASSESSMENT OF BEACH SAND IN MOSTAGANEM (SIDI MEJDOUB AS CASE STUDY).

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DEDICATION

I dedicate this work to my beloved father Mr. Joseph Cobbinah , my prayer warrior Mrs. Georgina Cobbinah, and my siblings Augustine, Joseph, Emmanuel and my twin brother Justin who have sacrificed everything for me to be where I am today. Words cannot express how grateful I am to you for everything you have done for me. Your love and support have helped me achieve my goals, and for that I am grateful. I also want to thank you for all of your prayers on my behalf.

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GOD BLESS US ALL.

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ABSTRACT

Sandy beaches are popular recreational destinations where beachgoers spend much of their time rather than in the water. Mediterranean areas, such as beaches, play an important role in human recreation; nevertheless, they expose humans to pathogenic bacteria and microorganisms directly or indirectly through anthropogenic activities resulting in pollution. The objective of this study was to assess the microbiological quality of Sidi Majdoub beach sand in Mostaganem (Algeria). The studied sand samples were taken from three different sites on this beach and then their microbiological quality was examined to detect the presence of contamination indicator microorganisms by isolating, identifying them by macroscopic and microscopic observation and performing some biochemical tests. . The results obtained indicate that the 3 sites have mediocre microbiological quality due to the presence of indicator microorganisms such as *Staphylococcus* spp., *Pseudomonas* spp., Enterobacteria, *Penicillium* spp., yeast (candida) and *Cladosporium*. The results indicate also that among the three sites studied, the site C was found to have the highest microbial density and *Staphylococcus* spp. was the most dominant bacteria detected. In this study Enterobacteriaceae and *Pseudomonas* were resistant to beta-lactam antibiotics such as Piperacillin, amoxicillin and clavulanic acid.

Keywords: Microbiological quality, Sand beaches, bacteria, fungi, antibiotics.

RESUME

Les plages de sable sont des destinations récréatives populaires où les baigneurs passent une grande partie de leur temps plutôt que dans l'eau. Les zones méditerranéennes, telles que les plages et les ports, jouent un rôle important dans les loisirs humains ; Néanmoins, ils exposent l'homme à des bactéries et micro-organismes pathogènes directement ou indirectement par le biais d'activités anthropiques entraînant une pollution. L'objectif de cette étude était d'évaluer la qualité microbiologique du sable de la plage de Sidi Majdoub à Mostaganem (Algérie). Les échantillons de sable étudiés ont été prélevés sur trois sites différents sur cette plage, puis leur qualité microbiologique a été examinée pour détecter la présence de micro-organismes indicateurs de contamination en les isolant, en les identifiant par observation macroscopique et microscopique et en effectuant des tests biochimiques. Les résultats obtenus indiquent que les 3 sites ont une qualité microbiologique médiocre en raison de la présence de micro-organismes indicateurs tels que *Staphylococcus* spp., *Pseudomonas* spp., Entérobactérie, *Penicillium* spp., levure (*candida*) et *Cladosporium*. Les résultats indiquent également que parmi les trois sites étudiés, le site C s'est avéré avoir la densité microbienne la plus élevée et *Staphylococcus* spp. était la bactérie la plus dominante détectée. Dans cette étude, les entérobactéries et les *Pseudomonas* étaient résistantes aux antibiotiques bêta-lactamines tels que la pipéracilline, l'amoxicilline et l'acide clavulanique.

Mots clés : Qualité microbiologique, Plages de sable, bactéries, champignons, antibiotiques

الملخص

الشواطئ الرملية هي وجهات ترفيهية شهيرة يقضي فيها مرتادي الشواطئ معظم وقتهم أكثر مما يقضونهم في الماء. ولمناطق البحر الأبيض المتوسط ، مثل الشواطئ و الموانئ ، دورًا مهمًا في الترفيه البشري ؛ ومع ذلك ، فقد يتعرض البشر في هذه المناطق للبكتيريا والكائنات الدقيقة المسببة للأمراض بشكل مباشر أو غير مباشر من خلال الأنشطة البشرية الملوثة.

تهدف هذه الدراسة إلى تقييم الجودة الميكروبيولوجية لرمال شاطئ سيدي مجدوب في مستغانم (الجزائر) حيث تم أخذ عينات الرمل المدروسة من ثلاثة مواقع مختلفة على هذا الشاطئ و تم فحص جودتها الميكروبيولوجية للكشف عن وجود كائنات دقيقة كمؤشر تلوث وذلك بعزلها وتحديدها عن طريق الملاحظة الماكروسكوبية والميكروسكوبية وإجراء بعض الاختبارات البيوكيميائية . .النتائج التي تم الحصول عليها تشير إلى أن المواقع الثلاثة ذات جودة ميكروبيولوجية رديئة بسبب وجود كائنات دقيقة مثل *Staphylococcus* spp و *Pseudomonas* spp.، و البكتريا المعوية و *Penicillium* spp.، و *Candida albicans* و *Cladosporium*. . كما تشير النتائج أيضًا إلى أنه من بين المواقع الثلاثة التي تمت دراستها، وجد أن الموقع C يحتوي على أعلى كثافة ميكروبية واكبر عدد من *Staphylococcus* spp. . و أن البكتيريا البكتريا المعوية و *Pseudomonas* كانت مقاومة للمضادات الحيوية ببراسكلبن واموكسيسلين وحمض كلافلانيك .

الكلمات المفتاحية: الجودة الميكروبيولوجية ، الشواطئ الرملية ، البكتيريا ، الفطريات ، المضادات الحيوية.

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LIST OF ABBREVIATIONS

AST: Antibiotic Susceptibility Test

Ak: Amikacin

AML: Amoxicillin

AUG: Amoxicillin -clavulanic acid

BCP: Bromocresol purple lactose

BTEX: Ethylbenzene and xylene

BN: Nutrient broth

CSOs: Combined sewer overflows

CD: Cercarial dermatitis

CFU: Colony Forming Unit

DNA: Deoxyribonucleic acid

EUCAST: European Committee on Antimicrobial Susceptibility Testing

FIO: Fecal Indicator organism

GI: Gastroenteritis illness

GM: Gentamicin

H_2O_2: Hydrogen peroxide

IE: Intestinal enterococci

McFad :McFarland

MH: Muller -Hinton

NaCl: Sodium Chloride

NA: Nalidixic acid

ND: Not detected

NOR: Norfloxacin

OX: Oxacillin

PDA: Potato Dextrose Agar

PRL : Piperacillin

R : Resistant

sp: Species

S : Susceptible /sensitive

TOB : Tobramycin

TTC: Thermo-tolerant coliform

VA : Vancomycin

UK : United Kingdom

UNEP : United Nations Environment Program

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INTRODUCTION.

As human populations grow and industrialization expands and diversifies, the problem of environmental pollution becomes more pressing. One of the world's common health threats is source of water even though water is a necessity for human survival. Water covers more than 70% of our planet's surface, (Sahu, 2019).

Beaches are comprised of unconsolidated sediment that forms the interface between water (oceans, lakes, and rivers) and land; they are typically made up of sand, mud, or pebbles. Sand beaches are popular recreational destinations. In some cases, particularly at higher latitudes, a large proportion of time is spent on the beach rather than in the water ("Beach Sand," 2021). Beaches along the Mediterranean coasts attract both tourists and locals for relaxation and water sports such as swimming, snorkeling, kite-surfing, jet-skiing, and paddle (Toubiana *et al.*, 2021). Coastal zones, such as beaches and harbors, play an important role in human recreation nevertheless, it exposes humans to pathogenic bacteria and microorganisms directly or indirectly as a result of various types of waste directed to coastal zones as a result of domestic, agricultural, livestock, and industrial activities (Furukawa *et al.*, 2011).

The Mediterranean Sea is a closed basin that renews itself once every 80 years; its area is estimated to be 1% of that of other seas and oceans, but its pollution accounts for 20% of global sea pollution. The United Nations Environment Program (UNEP) published a report in 1990 indicating that 93% of shells collected in the Mediterranean contain fecal spores exceeding the World Health Organization's maximum authorization due to population growth. The rapid expansion of the coastal cities, pollution, and an alarming influx of tourists are endangering the animals and plants that live there. Other sources of urban pollution include radiological and chemical pollution, heavy metals, oil, detergents, and fertilizers, all of which endanger not only marine animals and plants but also humans (swimmers, fishermen, and consumers).

The quality of bathing water and sand have become important factors in global competition among beach locations (Torres-Bejarano *et al.*, 2018) and are typically determined by using microbiological parameters to indicate fecal contamination, thermo-tolerant coliforms (TTC,

including *Escherichia coli(E.coli)*), and intestinal enterococci (IE), which could be altered by persistent populations of these bacteria in sand (Toubiana *et al*., 2021).

Beach sand may serve as a reservoir for a variety of microorganisms, including enteric pathogens. The fecal pathogens that may be present in this environment are believed to pose a significant risk to human health, particularly for those with advanced age, diabetes, immunodepression (temporary or permanent), children and respiratory problems (Brito.,2019)

Given the importance and frequency with which sand and water are used, as well as the fact that people spend more time on the sand than in water (Anonym, 2021) ,it is clear that, in addition to the need to maintain the quality of recreational waters, there is also a need to consider the quality of beach sands (Solo-Gabriele *et al.*, 2016) .

In regards to Khodir studies that was carried out in 2002 by the Blue Plan for the Environment and Development of the Mediterranean, under the umbrella of the United Nations program for the environment, the Mediterranean regions, particularly Algeria, will be confronted by 2050 horizon for a sharp decrease and significant pollution of water resources caused by anthropogenic pressure (agriculture, industrialization, urbanization, tourism)(Khodir.,2002)

The prospective study goal is to evaluate the microbiological quality of Mediterranean beaches in Mostaganem -Algeria; Sidi Majdoub to be precise. The main objectives for this study are as follows;

- To determine the microbial contamination of various beach sands in nearby beaches within a similar environment.
- To provide recommendations to help address the human health risks associated with pathogens presence in beach sands.

This work framed under chapters which includes; Introduction, Literature review which talks about marine pollution, its sources and health implications. The chapter for materials and methods comprises various analyses and their respective protocols, results and discussions also

gives the interpretations of the findings for the analysis conducted whiles Conclusion summers up the work and the final findings.

PART 1

LITERATURE REVIEW.

CHAPTER I:

SOURCES OF BEACH SAND AND SEAWATER POLLUTION.

I- Seawater pollution

Pollution is any form of contamination that has a negative impact on an ecosystem, including physical modification and the presence of invasive species.

Marine pollution occurs when harmful effects are caused by the introduction of chemicals, particles, agricultural and residential waste. Land pollution is responsible for 80% of marine pollution. That of the air also contributes by carrying pesticides or dirt into the seawater. Pollution from land and air has been shown to be harmful to marine life and its habitat (Verma *et al.*, 2020).The introduction of substances into the marine environment (including coastal waters) by man, either directly or indirectly, leads to negative effects such as harm to living resources, hazards to human health, impediment to marine activities such as fishing.

One of the direct consequences of poor waste management and excessive toxic product discharge by industries is marine pollution. Fertilizers, pesticides, plastic bags, and other items left on dry land will eventually flow into the ocean via rivers, surface runoff, rainfall, or winds and also via physical contacts of vectors such as humans and animals (seagulls)(Ocean Pollution.,2020).

I-1- Forms of pollution

Marine pollution is caused by products that are disposed into the marine environment as a result of human activities, through industrial, urban, or agricultural means. There are three forms of pollution: biological, chemical, and physical pollution.

I-1-1- Biological pollution

Biological pollution is caused by the activities of foreign species in ecosystem, which disrupt the natural balance, reduce biodiversity, degrade habitats, change native genetic diversity, transmit exotic diseases to native species, and further cause danger to endangered plants and animals (Dave, 2009).

Biological pollutants are non-native, invasive plants, animals, insects, and other living organisms that reduce the quality of life in marine water while costing billions of dollars to control (Dave, 2009). Some examples of microbiological pollutants are ; bacteria (*Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and, *Vibrio harveyi*),fungi(Candida spp., *Aspergillus,Fusarium,Scopulariopsis,Scedosporium,Chrysosporium,Scytalidium Trichophyton*, *Microsporum* and *Epidermophyton*), parasitic nematodes and viruses (adenovirus, norovirus, enterovirus) (Abdelzaher *et al*., 2010 ; Stewart *et al*., 2008) .

I-1-2- Physical pollution

Physical pollution is the modifications of the physical properties of the environment (marine), such as temperature, color, turbidity, suspended solids, and so on by introduction debris into the environment.

I-1-3- Chemical pollution

Chemical pollution is defined as the release of substances made up of chemicals such as nitrates, phosphates, ammonia and other salt into the environment (marine) that are not naturally present or are found in amounts greater than their natural background values (Alpizar.,2019). The majority of the chemicals that pollute the environment are man-made, the result of various activities in which toxic chemicals are used for various purposes.

(Cribb.,2021).They mainly originate from industrial, domestic and agricultural activities. Chemical pollutant makes the water-bodies unsafe habitat for marine species and users as well. Chemical water pollution is caused by two categories of chemical pollutants;

a- Organic Pollutant;

Most organic pollutants are xenobiotic, which means they do not occur naturally in the environment. The majority of them are toxic even at low concentrations and may be carcinogenic. They enter the environment as a result of unintentional discharge (using fuels, solvents), industrial activities (e.g., chemical and petrochemical), agriculture (e.g., pesticides), and military operations (e.g., explosives and chemical weapons). Furthermore, polluted areas frequently contain a mixture of organic and inorganic pollutants. Some organic pollutants include solvents (e.g., tri-chloroethylene), explosives (e.g., trinitrotoluene (TNT) and cyclotrimethylene trinitrine), Polycyclic Aromatic Hydrocarbons, petroleum products (e.g., benzene, toluene, ethylbenzene, and xylene (BTEX), Polychlorinated biphenyl, and pesticides (e.g., atrazine) (Muhammad and Babura , 2016).

b- Inorganic Pollutant ;

Inorganic pollutants are found naturally in the earth's crust and atmosphere. Human activities such as industry, mining, motorized traffic, agriculture, and military actions also contribute to their release and concentration in the environment, resulting in toxicity(Pilon-Smits ,2006).

Inorganic pollutants are primarily heavy metals, making them more persistent than organic contaminants (Wei.,2006).They cannot be broken down, but they can be altered through reduction or oxidation. They can also migrate into various plant parts, where they can be accumulated and volatilized.

Metals or metalloid such as Cadmium(Cd), Copper (Cu), Mercury (Hg), Magnesium(Mg), Selenium(Se), and Zinc(Zn), radionuclides such as Cesium (Cs),Phosphorus (P), and Uranium(U), and plant fertilizers such as nitrate and phosphate.

II- Beach sand pollution

II-1- Sources of beach sand pollution

Sources of pollution affecting the world's beaches are diverse and thousands of activities of humans are in two classes;

- Point source pollution ;this form of source can be traced back to a single, specific location where the pollutant originates, such as a sewage pipe from a company, or the deep horizon oil drilling platform leak (Vijayavel *et al*., 2010).
- Non-point source pollution; it has a diffuse source and cannot be traced back to a specific location or time. Agricultural runoff, dust from strip mining, and urban storm water runoff are all examples.(Sauer *et al*., 2011) Non-point source pollution is currently the leading cause of water pollution with polluted agricultural runoff being the most significant form.

These two classes of sources of pollution can be divided into two categories of sources of beach pollution : land-based sources and sea-based sources. These two categories can also be classified based on the type of human activity, such as the disposal of domestic sewage, industrial and agricultural wastes, the deliberate and unintentional discharge of shipboard pollutants, interference with the marine environment caused by the exploration and exploitation of marine minerals, the disposal of radioactive waste caused by peaceful uses of nuclear energy, and military uses of the water-bodies (Mishra *et al*., 2023).

II-2- Land-based source of litter

Land-based litter load originates on the shoreline, for example, as a result of tourism, or the debris is transported from distant areas such as inland towns and industrial sites via rivers,

wastewater pipelines, and drains(Mira Veiga *et al.*, 2016).Land-based pollution is caused by anthropogenic activities such as agriculture, urban runoff, industrial effluent, ship-breaking industries, port activities, sedimentation, deforestation, and urbanization(Alam *et al.*, 2021).Land-based debris primarily enters the marine environment as formal, informal, and illegal debris carried by rivers, waste- and storm-water outlets, or wind-blown directly into the oceans(Verster and Bouwman, 2020).

Land-based pollution sources includes the following:(Sheavly.,2005)

II-2-1- Storm water discharges:

Storm drainage systems collect runoff water produced by heavy rains. The wastewater is discharged directly into nearby streams, rivers, or the ocean by the poor drainage which most likely contains Street litters which has been washed into storm drains directed to streams/rivers, which is linked to other water-bodies (ocean) (Pawar *et al.*, 2016). These two classes of sources of pollution can be divided into two categories of sources of beach pollution : land-based sources and sea-based sources. These two categories can also be classified based on the type of human activity, such as the disposal of domestic sewage, industrial and agricultural wastes, the deliberate and unintentional discharge of shipboard pollutants, interference with the marine environment caused by the exploration and exploitation of marine minerals, the disposal of radioactive waste caused by peaceful uses of nuclear energy, and military uses of the water-bodies (Mishra *et al.*, 2023).

II-2-2- Tourists and Locals:

The main source of beach litter is tourists; both local and out-of-town. While the exact percentage varies by beach, study found that beach users were responsible for approximately 70% of beach litter. Individual beach users attribute beach litter to beach users as a group, despite the fact that individuals are unlikely to admit littering

Beach goers frequently leave food and beverage packaging, plastic beach toys, and cigarette butts on the beach. Recreational fishing equipment is also frequently abandoned as litter. Tourists who go boating for fun or on cruise ships also contribute significantly to beach litter and marine debris. Food packaging, plastic bags, and fishing gear are frequently 'lost' overboard, either intentionally or unintentionally(Sheavly.,2005).

Beaches and Camping sites can be potential sources of marine litter. All beaches and camping grounds should have adequate waste bins, regular collections, recycling and properly disposed facilities (landfills)(Valavanidis and Vlachogianni, 2012).

II-2-3- Industrial activities

When waste items produced through industrial processes (production scraps, flawed products, and packaging material) are improperly disposed of on land, industrial facilities contribute to the float-able debris problem. Finished products can also become float-able debris if they are lost during loading and unloading at port facilities, or if they are lost while being transported by water or land. Plastic resin pellets, which are small spheres produced as the raw form of plastic, are one example. Pellets are used in the manufacturing of plastic products Some resin pellets may be released into the environment during the production, transportation, and processing of plastic resin pellets. Wind and storm water, like other types of trash, can carry these pellets to nearby bodies of water(Anyoms.,2002).

II-2-4- Solid Waste Disposal and Landfills:

Waste disposal activities can cause issues when trash is lost during collection or transportation, or when trash blows or is washed away from disposal facilities. This float-able material can be of any type, but it is most commonly garbage. Medical waste is of particular concern, but it appears to have been adequately controlled in recent years, compared to the late 1980s. Medical waste is defined by the Environment Protection Agency as infectious agent cultures and stocks; human blood and blood products; human pathological wastes, including those from surgery and autopsy; contaminated animal remains from medical research; wastes

from patients isolated with highly communicable diseases; and all used sharps (e.g., needles, scalpels, etc.) and certain unused sharps. Hospital solid waste includes administrative papers and records, bandage and catheter wrappers, intravenous (IV) bags and used vials, syringes and needles, and disposable items like tongue depressors and thermometer covers(Anyoms.,2002)

Another source of marine debris is illegal dumping of household or industrial waste into coastal and marine waters(Sheavly.,2005).

II-2-5- Overflows from Combined Sewer System

Combined sewers are the pipelines that transport both sewage and storm water. Sewage is carried in the same pipe system as storm water runoff in many areas of the country with older sewer systems. Unlike individual storm drains, combined sewer pipes transport waste to a sewage treatment plant rather than directly into a body of water. Sludge (solid waste materials) and wastewater are separated from sewage at the sewage treatment plant. The sludge is either dried and disposed of in a landfill or treated and sold as fertilizer. The treated wastewater is discharged, solid waste-free, into a river or other nearby waterway. Combined sewer overflows (CSOs) can occur as a result of too much volume during heavy rains. The CSOs and storm water are discharged into the nearest receiving waters.(Anyoms.,2002).

Street litter, sewage-related items (e.g., condoms, tampons, applicators), medical items (e.g., syringes), resin pellets, and other material that may have washed into storm drains or run off land, as well as industrial wastes from nonresidential sewer system users, are examples of floatable debris from CSOs which contains the marine environments.

II-3- Sea -based source of litter:

In the ocean, vessels of various sorts and structures are all potential vectors for the introduction of debris into the marine environment. Even if environmental regulations

are strictly followed, marine debris can still enter the marine environment from vessels at sea due to accidental loss, especially during bad weather conditions. All ships have the potential to harm the aquatic environment by improperly disposing of trash at sea. Litter from used items and passengers throwing litter on merchant ships, ferries, and cruise liners (plastic, paper, boxes, bottles, aluminum cans, etc.) (Valavanidis and Vlachogianni, 2012)

II-3-1- Merchant, military and research vessels

Ships' garbage may be accidentally released or blown into the water, or it may be purposefully thrown overboard. Large vessels with a large crew generate solid waste on a daily basis, which can end up as marine debris if not properly secured and stored which leads to pollution (Sheavly.,2005).

II-3-2- Commercial fishing

When commercial fishermen fail to retrieve fishing gear or throw fishing gear or other trash overboard, they generate marine debris. Commercial fishing debris includes nets, lines and ropes, strapping bands, bait boxes, bags, gillnet or trawl floats, as well as galley waste and household trash. (Valavanidis and Vlachogianni, 2012).

Figure 1: Marine litter from commercial fisheries(Marine Litter, n.d.)

II-3-3- Exploration and offshore mineral, oil and gas production

Offshore oil and gas platforms are structures built in the ocean that serve as the foundation for oil and gas drilling. Because offshore oil and gas platforms are surrounded by water, any items that fall off intentionally or unintentionally become floatable debris released the marine environment. Items such as hard hats, gloves, plastic drill pipe thread protectors ,gallon storage drums, survey materials, and personal waste are examples of floatable debris generated by these platforms into the water-bodies(Vann.A,.2018.)

III-4- Climate changes

Rising temperatures are a direct result of anthropogenic activities that cause climate change. The increase in temperature influences the survival and persistence of specific microbial species in both the beach water and sand and also increases the occurrence of other pathogenic microbes capable of multiplying in water environments close to body temperature

(Sharma,2015). Also, increases in temperature increase the rate at which beaches are used for heat relief, particularly when there are unusual or extreme weather events(Smith, 1993 ; Moreno *et al.*, 2008),where there will be an expansion of reactionary activities like water sports and beach camping. As a result, direct human inputs may temporarily increase through shedding, resulting in increased pathogen loading on beaches (Shuval,2003) or even outbreaks of gastroenteritis illnesses(GI).

CHAPTER II:

IMPACT OF MICROBIOLOGICAL CONTAMINATION OF BEACH ON USER'S HEALTH.

1- Impact of microbiological quality of beach sand on human health

According to epidemiological studies, humans who are exposed to feces-contaminated water and sand on beaches are more likely to contract infections such as (GI), cercarial dermatitis (CD), and other illnesses by activities such as hand-to-mouth microbiota transfer, inhalation and shedding of non-fecal opportunistic pathogens such as dermatophytes by beachgoers (Bonilla *et al.*, 2007 ;Whitman *et al.*, 2009 ; Solo-Gabriele *et al.*, 2016). For example, swimming has numerous human health benefits as an aerobic exercise that boosts one's metabolism and supports an active lifestyle, as well as providing a medium for relaxation, a psychological benefit that improves people's mood. However, ailment can be contracted from recreational sites such as the beach due to contact with water-bodies, and sand with poor microbial quality can counteract the health and well-being of beach users(DeFlorio-Barker *et al.*, 2018).

GI refers to gastrointestinal inflammation caused by viral, bacterial, or parasitic infection (2010)and has any of the following descriptions : diarrhea (having more than three loose stools in a 24-hour period); vomiting episodes; nausea and stomach ache symptoms; nausea that affected regular activity; and stomach ache and fever symptoms. This definition was used to improve the sensitivity and identification of GI cases, to reduce losses due to inter-individual variations in the general perception of what constitutes a case of GI (Fleisher *et al.*, 2010).Children are thought to be more susceptible to swimming-related gastroenteritis than adults because they spend more time in the water, are more likely to swallow water (the primary mode of pathogen exposure), and have less developed immune systems (Wade.,2008 ; Creel .,2003). The germs (*Escherichia coli* (*E. coli*), *Enterococcus* spp and other fecal indicator organisms) responsible for gastroenteritis that are shed in infected people's feces . There are

small amounts of unseen feces present on everyone's skin and are rinsed into the water-bodies which allows germs to spread even when there is no fecal accident. People become ill when they drink germ-infested water while swimming or put contaminated hands or objects into their mouths. Contamination from animals, sewage, and runoff is also possible and more common in lakes and beaches.(Swimming_related_illness.Pdf, 2008.)

Cercarial dermatitis is an aliment also known as (swimmer's itch);it is caused the infestation of the skin by cercariae (larvae) of non-human Schistosome (flat worm) ; whose usual hosts are birds and small mammals. CD is acquired by skin exposure to fresh and, to a lesser extent, salt water. The cercariae penetrate intact human skin within a few minutes. CD occurs to swimmers and those with occupations that include water exposure. One develops the skin rash due to an allergic reaction to certain microscopic parasites (Fraser *et al.*, 2009) . The time from exposure to onset of symptoms varies from a few minutes to a maximum of 24 hours after exposure.

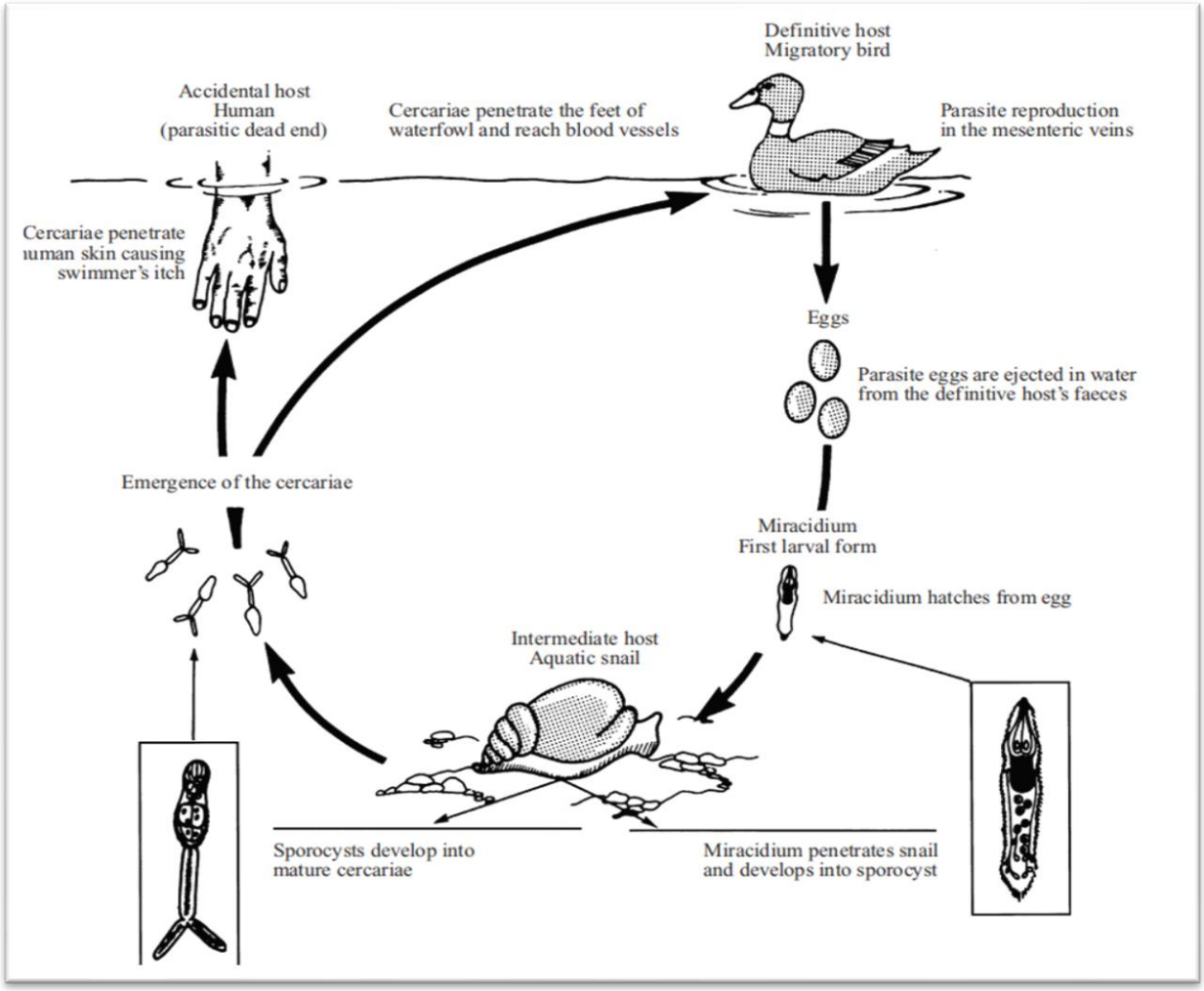


Figure 1 : Life cycle of Cercarial Dermatitis *(Lévesque et al., 2002)*

2- Microbiological quality of beach sand

The microbiological quality of beaches is based on the measurement of the microbiological conditions that are relevant to human and animal health. The assessment is to proof the existence of pathogenic organisms (*Pseudomonas* spp, *Staphylococcus aureus* ,*Salmonella* spp), as well as fecal indicator organisms (FIO),fungi (*Candida* spp. and, dermatophytes fungi*)* parasitic nematodes and viruses (adenovirus, norovirus, enterovirus)(Abdelzaher *et al.*,

2010).Marine bacteria differ physiologically from the non-marine in that, they are highly adapted to the unique conditions of the marine environment such as salinity, pH, reduced oxygenation, low temperatures, and often significant pressures(Ventosa *et al.*, 2014).Microorganisms are the most numerous organisms in aquatic ecosystems, with bacteria constituting the majority. Microbes in seawater that are thought to be indicative of fecal or sewage pollution have received a lot of attention. They include Coliform, *E. coli*, enterococci, *Pseudomonas aeruginosa*, *Clostridium perfringens*, salmonella, aeromonads, bifidobacterial, enterovirus, rotaviruses, coliphages and Bacteroides phages, and certain yeasts (Halley.,1997).

Many studies have found that beach sand can act as a reservoir for pathogens that are harmful to human health and indicator microbes that are released into surrounding waters via tidal action or run-off (Sabino *et al.*, 2014). In reality, bacterial cell numbers in sand can be significantly higher than in waters; for example, in the Great Lakes region of the United States, *E. coli* in sand can be found at levels 10 to 100 times higher than in adjacent waters, ranging from 10^3 to 10^4 CFU/g at enclosed beaches to $10^{1.5}$ to $10^{2.5}$ CFU/g at open beaches(Yamahara *et al.*,2007 ; Oshiro and Fujioka, 1995). This phenomenon may be of additional concern in high-latitude regions where bathers spend more time on the beach than in the water(World Health Organization, 2003).

Although some coliform are undoubtedly of environmental origin, they have traditionally been regarded as the primary indicator of fecal pollution (Geldreich and Clarke, 1966) *E. coli* is widely regarded as the sine qua non of fecal pollution, and its presence in temperate seawater is unmistakable proof of fecal pollution. Fecal coliform counts of 0 to 10000 per 100ml have been obtained in UK coastal waters known to be affected by nearby sewage discharges as acknowledgment that there can be significant variation in counts due to weather conditions, tidal state, and other factors (Gameson *et al* 1970); and, not least, the methods used to detect them (Agg and Stanfield 1979).

Fecal streptococci are found in human feces, but in smaller numbers than fecal coliforms(Pipes .W.O., 1982), however, they are more resistant to environmental decay than fecal coliform such as *E. coli* and have been shown to be more reliable indicators of the presence of enteric viruses in seawater than fecal coliform (Dufour.A.,1984 ; Fattal .B. *et al.* 1983). Again, the methods used to detect them will have a significant impact on the counts, with each method tending to select different members of this group (Stanfield *et al*. 1978).

3- Strategies to combat the existences of microbes in beach sand and seawater .

There is a need to implement measures that may help in minimizing the persistence of indicator microbes and some pathogens ;(Solo-Gabriele *et al.*, 2016)

- Practical beach management and tourist education should be carried on about dangers of pollution (littering) .
- Provision of sufficient number of animal-proof trash cans will limit disease-carrying wildlife, the spread of damaged, discarded food and waste, and the vectors connected with it on the beach.
- Removing local sources of air pollution (in the case of airborne fungal spores) could aid in the control of fungi in beach sands.
- Proper drainage system should be constructed and redirected away from the marine environments.

1- Background

The analytical study was carried out within the Research Laboratory of "Microbiology" in the Department of Biology of University of Abdelhamid Ibn Badis-Mostaganem over an internship period ranging from March to May 2023. The objective of this work concerns the microbiological quality of Mediterranean beach sand as well as the isolation and identification of fungi and bacteria from three different points of the Mostaganem beach (Sidi Majdoub).

2- Presentation of the wilaya of Mostaganem

Mostaganem is a port city in Algeria and the 27th wilaya (city) in the Algerian territorial administration. The city is located in the north-west part of Algeria, 350 km west of Algiers (the capital city) and 80km east of the city of Oran. The wilaya Mostaganem has a population of about 130,000(2020 data). The city is enclosed on the north by the Mediterranean Sea, on the west by the provinces of Oran and Mascara, on the east by the province of Chlef, and on the south by the province of Relizane. Mostaganem covers an area of 2269 km^2 and a coastal outlet of 124 km long (Caïd *et al*., 2019; Kies and Kerfouf, 2014).

Mostaganem has economic assets whose exploitation offers promising prospects for economic development in agricultural, maritime, industrial, tourism, and fisheries fields (*Découvrir Mostaganem*, n.d.). Despite its advantageous assets, the wilaya of Mostaganem is subject to a number of constraints that are impeding its socioeconomic development, such as coastal zone pollution. (Kies *et al*., 2018)

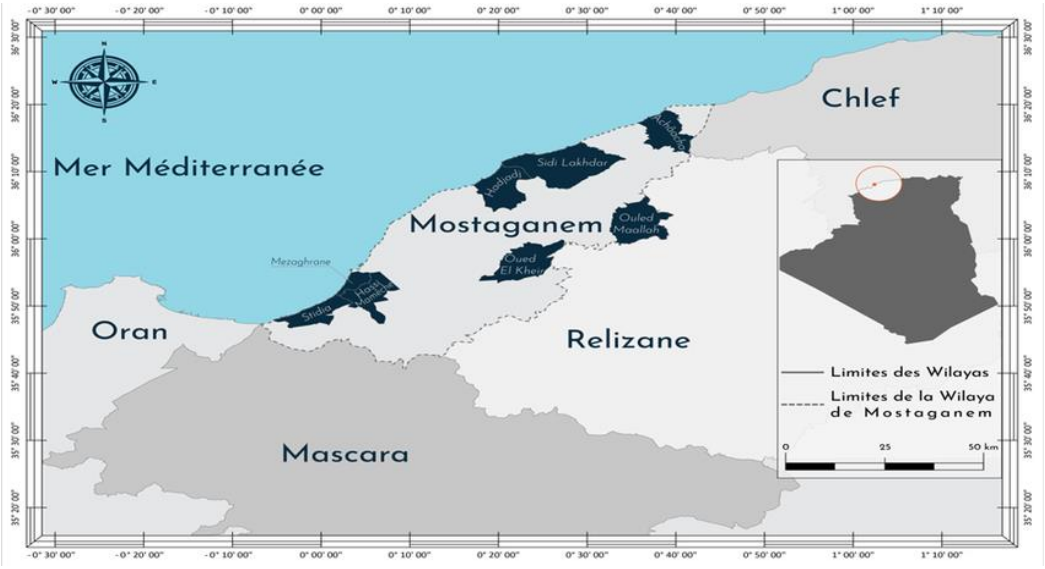


Figure 3: Geographical location of Mostaganem *(Caïd et al., 2019)*.

3- Study site (Sidi Mejdoub beach)

The beach of Sidi Mejdoub is located about 4.0 km east of the port of Mostaganem. Many houses are located at the dimension level. The area is under constant threat all year because of its ease of access to tourists. Sidi Mejdoub is constantly exposed to the significant contributions of the emissary main sewer of the city of Mostaganem**(Figure 3 and 4).**

Figure 4: Site Sidi Mejdoub-Mostaganem.

Figure 5 : Earth satellites of site Sidi Mejdoub

4- Procedure for Handling Sampling.

Sand samples were collected from three points (sites) of Sidi Mejdoub beach: Point A, Point B and Point C, making three samples in all **(Figure 4 and 5)**. The wet sand sampling on each point from the beach was carried out using a disinfected little garden shovel, 3 cm below the surface. The samples were placed in a sterilized, labeled plastic container. The samples were collected from various points in the morning, transported to the laboratory, and analyzed within a 4-hour period **(Figure 6)**.

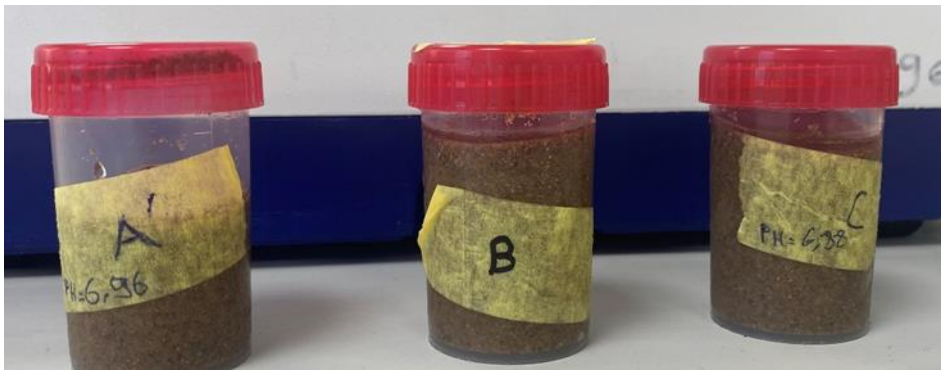


Figure 6: Sidi Majdoub beach sand samples

5- METHODS

5-1- FUNGAL ANALYSIS.

5-1-1-Isolation and enumeration of fungal.

The aim of the analysis was to enumerate the fungi present in the wet beach sand and also detect the dominant genre.

A serial dilution of 10^{-1} to 10^{-4} was made from 1g of wet beach sand which was suspended into 9mL of physiological water for the sample collected from each point. The mixture was vertically shaken to form a uniform mixture with the aid of a vortex. 0,1ml (100uL) of the suspension (dilution of 10^{-2} to 10^{-4}) was spread onto Petri dishes containing Potato-Dextrose - Agar (PDA) with the help of a micropipette. Two Petri dishes were used for every dilution. The Petri dishes were incubated at 25°C for five to seven days . After the 7th day , the developed colonies were purified by subculturing the colonies onto a Petri dish containing PDA.

5-1-2 Purification of fungi isolates.

A colony was selected from the previous inoculated Petri dishes and streaked in other Petri dishes containing the PDA culture media for the respective fungi isolated. The Petri dishes are incubated at 25°C for five to seven days.

5-1-3 Identification of fungal isolates.

I- Macroscopic observation of fungal isolates

The macroscopic characters of the fungal colonies and culture, namely: appearance, texture, pigmentation, color of the mycelium, the color of the colonies on the reverse side of the Petri dish, the type of growth (aerial or radial growth) is determined with the naked eye.

II- Microscopic observation of fungal isolates

The microscopic study /direct microscopic examination was based on the determination of the morphological characters of the hyphae which includes the type of thallus, the color of the hyphae, the structure of the conidiophores, etc. as well as the form of reproduction (endogenous origin or exogenous spores, appearance of spores, mode of formation of conidia, presence of chlamydospores, etc.) of isolates under the microscope observation (X40). In the present study, the cello tape impression technique was used for microscopic identification.

A drop of distilled water was placed in the center of a microscope slide, and the surface of the colony was gently touched with the adhesive side of cello tape using a tweezers. The fungal-imprinted side of the tape was placed on the microscope side with the distilled water, then covered with a cover glass and observed under the microscope(X40).

5-3- BACTERIOLOGICAL ANALYSIS

5-3-1 Detection and isolation of the bacteria present

The aim of the experiment was to detect the presence of bacteria (Enterobacteria, *Staphylococcus*, *Pseudomonas*) in the wet beach sand.

1g of each sample from the three points (A,B,C) was suspended into 9mL of physiological water for the serial dilution of 10^{-1} to 10^{-2}. The mixture was vertically shaking to have a

uniform mixture with the aid of a vortex. 1mL of the suspension was poured onto Petri dishes, the various culture media; Bromocresol purple lactose(BCP),Chapman, King A and King B were then mixed with the bacteria suspension in the Petri dishes using the technique “swirl on eight” .

a- For the detection of *Staphylococcus* ,1mL of the sample from stock solution for each point (A,B,C) was inoculated in the Chapman culture media using the plate method ;that is, the culture media was added onto the sample. After plating the inoculum , the three Petri dishes were incubated at 37°C for 24h.

b- The detection of *Pseudomonas* from the samples collected was done by plating 1mL of inoculum with dilution serial 10^{-2} in King A culture media using the pour plate technique. This same procedure was used to inoculate the inoculum in King B culture media .The Petri dishes were incubated at 30°C for 24h to 48h.

c- 1mL inoculum from test tubes containing the stock solution and solution of dilution 10^{-1} was plated in the BCP culture media using the plate method to detect the presence of Enterobacteria from the various samples collected. The six Petri dishes were incubated at 37°C for 24h.

5-3-2- Purification of bacteria isolates.

A colony was selected from the previous inoculated Petri dishes and streaked in other Petri dishes containing the various culture media for the respective bacteria isolated. The Petri dishes are incubated at the temperatures of 37°C and 30°C for 24h.

5-3-3- Identification of the bacteria isolates.

The goal of the experiment was to identify the bacteria isolates with respect to their morphology, Gram stain and the various biochemical properties of the isolated bacteria.

A colony was selected from the previous inoculated Petri dishes and purified in another Petri dishes containing the various media for the respective bacteria isolated. The bacterial

identification was carried out by macroscopic and microscopic observation, biochemical tests which includes the catalase test ,oxidase test ,salinity test and Antibiotic Susceptibility Test(AST).

5-3-3-1- Macroscopic and Microscopic study of the bacteria isolates.

The macroscopic characters of the bacteria isolates and culture , namely: appearance and the color of the colonies on the reverse, are determined with the naked eye.

The microscopic study was based on the determination of the morphological characters of the bacteria as well as the Gram stain of isolates under the microscope (X100). In the present study, the selected colonies were stained and used for microscopic identification.

5-3-3-2- Gram Stain.

The Hans Christian Gram stain (Gram, 1884) is a differential stain that separates bacteria into two large groups: Gram (+) or Gram (-). This analysis is based on the difference in the structure of the wall and its composition.

A heat-fixed smear of selected colonies on microscope slide was stained for two minutes with crystal violet (first stain); it is then rinsed with distilled water and fixed with Lugol solution for thirty seconds (was done twice). The smear is distained with 80% alcohol (thirty seconds) then washed with distilled water until the dye stops escaping. The latter is then subjected to a minute counterstaining with fuchsin. The slide is rinsed with distilled water and then dried over a Bunsen burner flame. The smear thus prepared is observed under a light microscope under immersion. “Gram positive” bacteria appear dark purple / violet while “Gram negative” bacteria are stained pink/rose .

5-3-3- Biochemical tests.

I- Catalase Test

The method consists of depositing a drop of hydrogen peroxide on a slide in which a sample of the colony to be studied were emulsified to test for the catalase enzyme capable of hydrolyzing the molecule of hydrogen peroxide (H_2O_2) into oxygen and water.

Equation for reaction $2H_2O_2 \rightarrow 2H_2O + O_2$

II- Oxidase Test

A colony that is well isolated and representative of the fresh culture was chosen and it was rubbed on an oxidase disc placed on a lame with the help of inoculation needle to detect the bacteria that possess the enzyme cytochrome oxidase. Observe for the appearance of a purple / blue color within 30 seconds.

III- Antibiotic Susceptibility Test (AST)

The antibiotic susceptibility testing was performed using the Kirby Bauer disc diffusion method (Bauer *et al.*, 1966) on Mueller-Hinton agar plates (Reller *et al.*, 2009). The antibiotic discs (Bio-Rad Antibiotic Disks, Marnes la Coquette, France)(Strauss *et al.*, 2020) used included amikacin (Ak-30 µg), Piperacillin (PRL-30 µg), amoxicillin (AML-30 µg), norfloxacin (NOR-10 µg), vancomycin (VA-30 µg), amoxicillin-clavulanic acid (AUG-30 µg), tobramycin (TOB -10 µg), gentamicin (GM-10 µl), oxacillin (OX-1 µg) and Nalidixic acid (NA-30 µm).

The bacterial suspension was prepared in sterile nutrient broth (BN) from a young and pure culture on an appropriate isolation media. The turbidity of the suspension was measured using a spectrophotometer with a 1-cm light path and absorbance reading of 0.08 to 0.1 at 625nm which is equivalent to 0.5 McFarland (McF) Standard.

A sterilized cotton swab was immersed in the suspension and the swab was swirled against the sides of the tube to remove excess liquid before it was been rubbed on the entire surface of the Mueller-Hinton(MH) agar dish three times by rotating the dish approximately 60° between streaks to ensure even distribution. Finally, the swab was run around the edge of the agar to remove any excess moisture with the caution of minimizing aerosols by avoiding hitting the sides of the plate with the swab.

The various selected antibiotics discs were placed firmly on the surface of the dried inoculated MH agar with the aid of sterile tweezers (maximum four discs on a 9 cm diameter Petri dish).A light amount of pressure was exerted on the disc to ensure full contact of the disc with the MH agar with the help of the sterile tweezers. Incubation was done for 24h under the temperature of 37 °C to observe the zone of inhibition.

I- Salt Tolerance Test

Bacteria isolates from a pure culture was streaked in a nutrient agar media of 6,5% and 8% of Sodium Chloride agar with pH of 7.2 to determine the ability of the organism to grow in high concentrations of salt (NaCl).

A selected colony was streaked in a nutrient agar media containing the respective quantity of NaCl and incubated for 24h under the temperature of 37°C .

CHAPTER 2

RESULTS AND DISCUSSIONS.

1- FUNGI ANALYSIS

1-1- Isolation and enumeration of fungi.

The fungi were isolated on PDA media which was incubated for a period of 7days at 25°C. The results indicated that, there was an outnumbered fungi isolates in point C compared to point B and A, which makes point C more contaminated.

The count of the fungal microflora presents in the different samples collected was carried out by counting the number of colonies present in the media after incubation.(**Figure 7 and Table 1**)

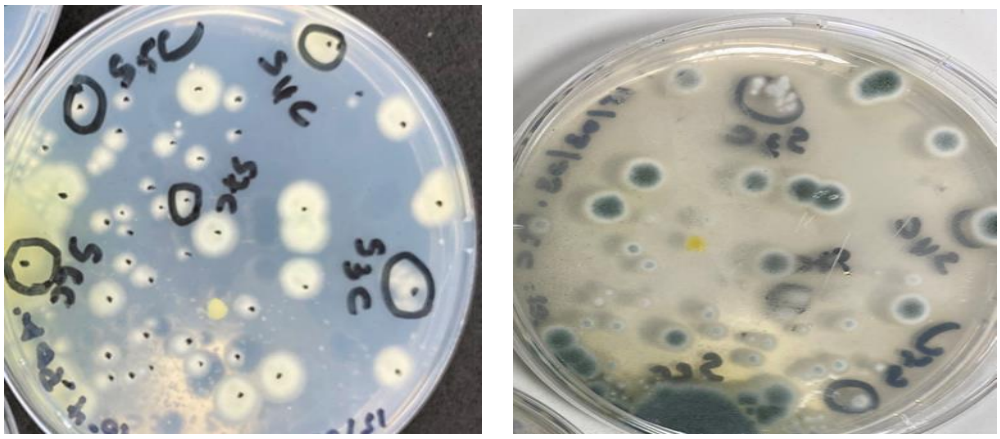


Figure 7: Front and back view of one dish of fungi isolation on PDA media.

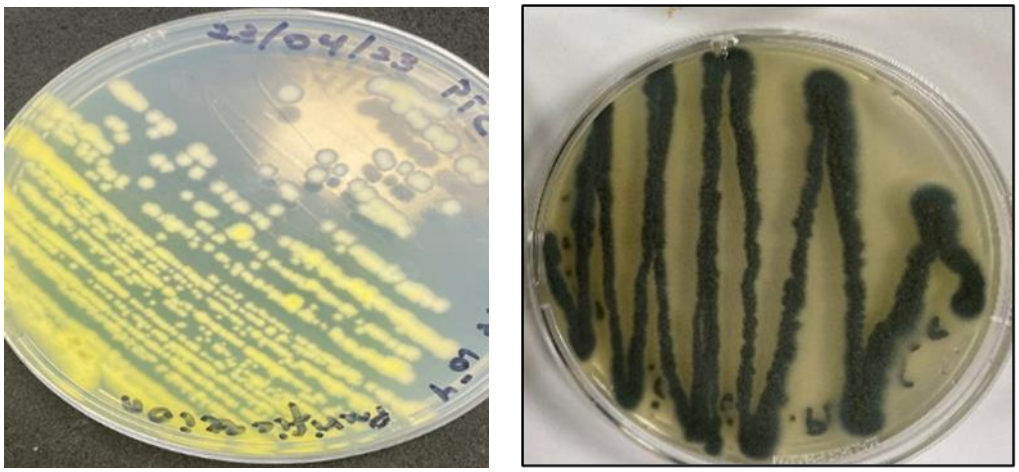
Table 1: Fungal counts of the three samples isolated on PDA medium

Samples	**Dilution**	**Number of colonies**	**UFC/g of sand**
PT A	10^{-3}	9	9×10^{5}
PT B	10^{-2}	4	4×10^{4}
PT C	10^{-4}	43	4.3×10^{5}

1-2- Characterization and identification of fungal isolates.

The identification of the fungus's genus, was performed by macroscopic and microscopic observations. Macroscopic study of the purified fungal isolates cultured on PDA and sabouraud media showed colonies with moderate growth, a woolly texture with a short or powdery aerial mycelium. Colonies in culture generally ranged in color from white, green to yellowish with a colorless, beige or greenish gray underside of the plates. The microscopic identification was based on the study of the spore formations ,conidiophore and hyphae.*(*Salvamani and Nawawi, 2014*)*.

Two isolates (02) **S3C** and **S7C** have green colonies with a dark brown *back view* and a yellowish pigment diffused in the medium. Under an optical microscope it appears with a septate mycelium and phialides in brush and conidia in chain, which allowed us to classify them as *Penicillium* (**Figure 8**).



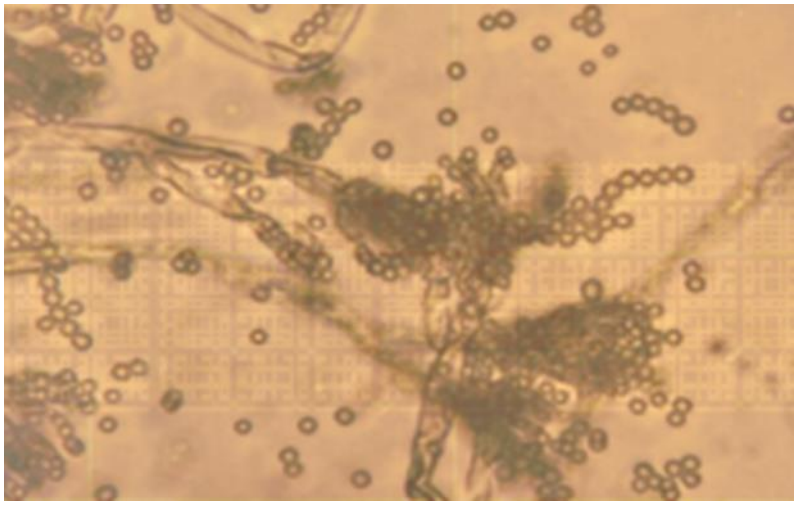


Figure 8: Macro and microscopic morphology of the genus *Penicillium(*X40).

One strain is characterized by white colonies with a velvety appearance and appears under the microscope as oval and elongated cells with a nucleus. Some of these cells are budding, some filaments are also present. These characters indicate that these isolates are yeast which can be belonged to candida albicans **(Figure 8).**

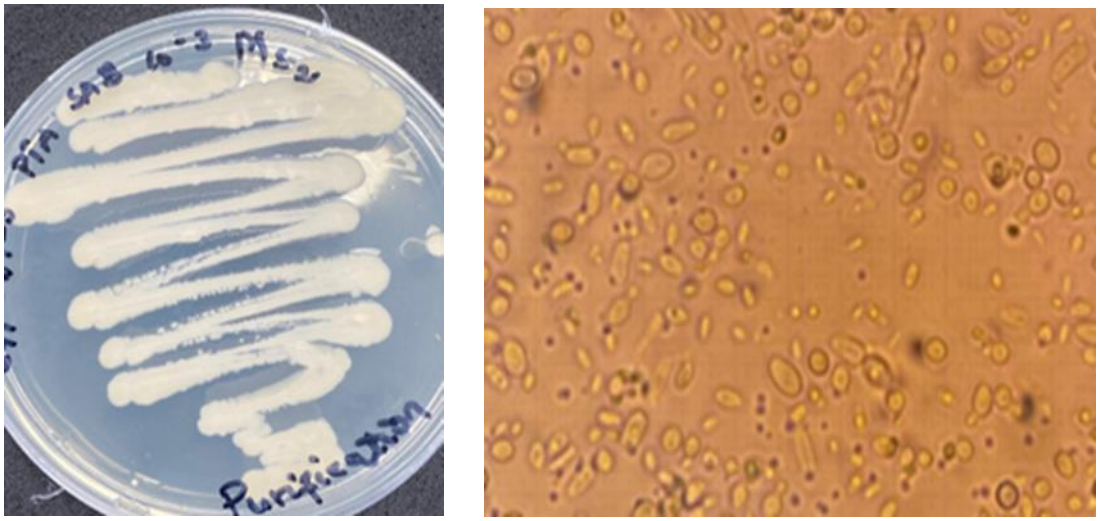


Figure 9: Macro and microscopic morphology of the isolated yeast(X40).

One (01) isolate with powdery white mycelium that turn olive green to blackish over time. The reverse side is olive-black and the conidiophores are more or less distinct from the vegetative hyphae, being erect, straight or flexuous, branching only in the apical region, the conidia are produced in chains. A presence of chains of conidia which disarticulate easily. These characteristics correspond to the genus Cladosporium (**Figure 10**).

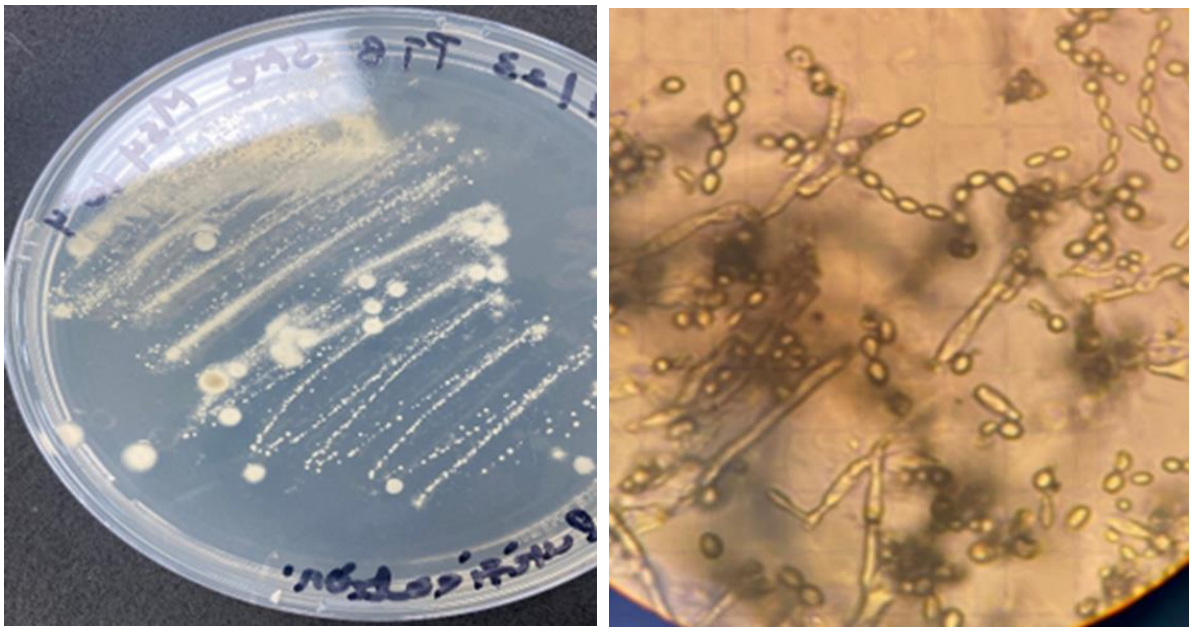


Figure 10: Macro and microscopic morphology of the genus *Cladosporium(*X40).

One (01) isolate with white color colonies that turn brown over time and blackish green color. It has sterile mycelium (devoid of spores). As these fungi do not produce spores, it is impossible to use traditional methods of morphological comparison to classify them. However, molecular techniques can be applied to determine them (**Figure 11**).

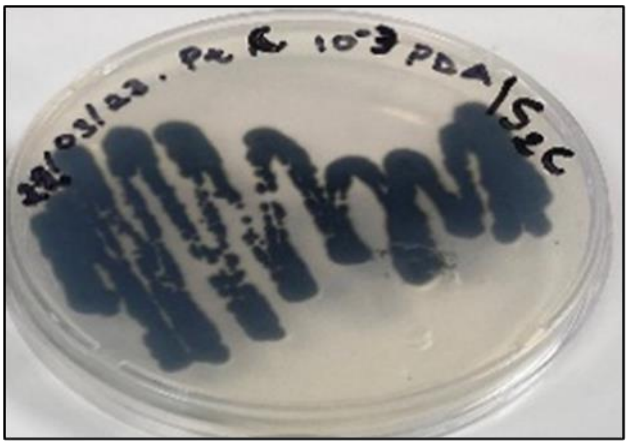


Figure11: Macroscopic aspects of colonies of fungi with sterile mycelium.

The study of the macroscopic and microscopic aspects of the isolated fungi from the sea sand of Sidi Majdoub showed the presence of 4 genera: *Penicillium*, *Cladosporium* , a yeast and one isolate with sterile mycelium. The latter are vegetative forms without spores, either because their culture medium does not favor sporulation, or they therefore belong to the group of fungi with sterile mycelium.

As mentioned above, isolate S3C and S7C was identified as *Penicillium* spp (Samanthi.,2019) indicated that the dominant fungal species isolated from wet beach sand includes *Aspergillus*, *Penicillium* spp, *Cladosporium* and Yeast -like fungi (Mancini.,2005; Moazeni *et al*., 2023).

Beaches can be a source of infection for superficial and deep mycosis due to *Aspergillus*, *Cladosporium*, and *Penicillium* species presence (Moazeni *et al.,* 2023). The majority of the filamentous fungi isolated from "Casa Caiada" and "Bairro Novo" beaches belonged to the previous genera.(Gomes *et al.*, 2008).

Several studies in the literature indicate the presence of these saprophytic genera in sea sand. (Gonzalez *et al*., 1998) mentioned a predominance of *Cladosporium cladosporoides* in the sands of three Mexican beaches. (Izquierdo *et al*., 1986). Sixteen species of fungi were isolated from beach sand along the northeastern Mediterranean coast of Spain, the majority of which

belong to the genera *Penicillium*, *Aspergillus* and *Cladosporium*. The study carried out on the sands of two beaches in Casablanca (Morocco) by (Abdallaoui *et al.*,2007) shows that seventy fungi were isolated from fifty-six samples, nineteen of which are yeasts (ten C. albicans and nine others) , five Trichophyton rubrum, four Scytalidium dimidiatum, twenty-five Aspergillus sp., thirteen Penicillium sp., two Cladosporium and two Scedosporium. In another study, five genera of filamentous fungi collected from the sea water of the port of Oran were identified with *Penicillium* , *Aspergillus*, *Cladosporium*, *Alternaria* and *Acremonium* (Maamar, 2015).

2- BACTERIOLOGICAL ANALYSIS

2-1- Enumeration of isolated bacteria

The results obtained from the enumeration of the isolates showed on **Table 2 and figure11** indicate that *Staphylococcus* has the highest number of colonies from all the selected sites compared to *Pseudomonas* and enterobacteria. Also, it was discovered that site /point A harbors all the isolates (*Staphylococcus*, enterobacteria and *Pseudomonas*) making the point more contaminated whereas the number of bacteria in point **C** was uncountable.

Table 2: Enumeration of bacteria in sand samples of Sidi Mejdoub

Isolates	**Sample**	**Dilution**	**Number of colonies**	**UFC/g of sand**
Pseudomonas (King B)	A	10^{-2}	24	$24x10^{4}$
	B	10^{-2}	0	0
	C	10^{-2}	uncountable	uncountable
Staphylococcus	A	Stock solution	60	600
	B	Stock solution	59	590
	C	Stock solution	70	700
Enterobacteria	A	10^{-1}	40	$40x10^{3}$
	B	10^{-1}	0	0

	C	10^{-1}	uncountable	uncountable
	A	Stock solution	0	0
	B	Stock solution	2	20
	C	Stock solution	2	20

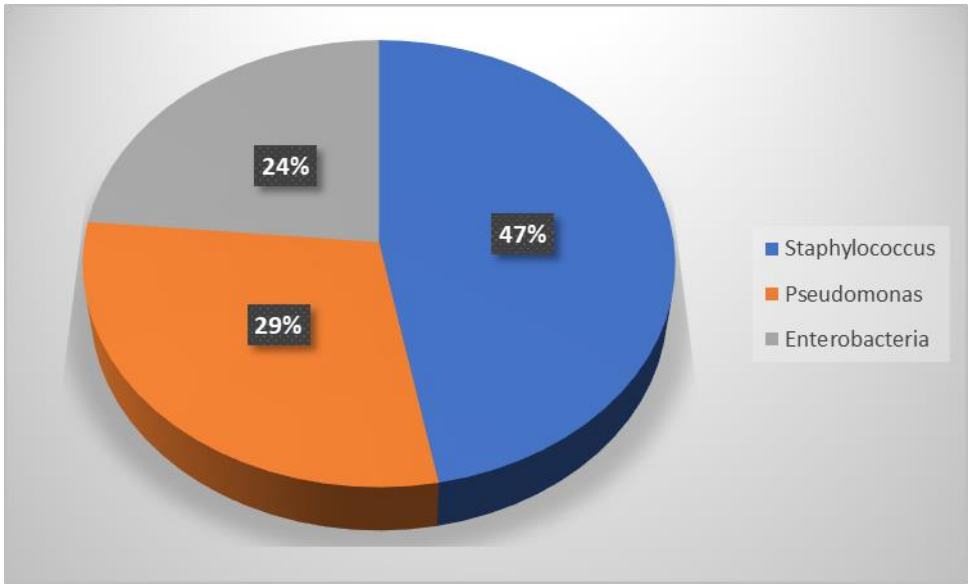


Figure 12: Pie chart demonstrating the percentages of the bacteria isolates identified

2-2- Identification of isolated bacteria

On the Chapman culture medium (**figure 13 a**), the colonies have a macroscopic appearance characteristic of the *Staphylococcus* genus. Colonies are small fermenting or non-fermenting mannitol. However, some colonies fermented mannitol, as did *S.aureus* (gave a yellow tint: mannitol +) (Denis *et al*., 2011). The Gram stain showed Gram-positive cocci (**figure. 13 b**)

isolated), grouped in diplococci or in short chains, and in clusters having the shape of bunches of grapes (Avril, 1992).

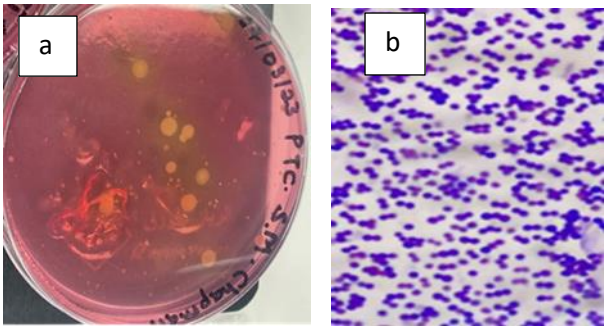


Figure 13: Macroscopic appearance of Staphylococcal colonies on Chapman agar (a) and microscopic observation of *Staphylococcus* after Gram staining (x1000)(b).

Macroscopic observation of the isolates of *Pseudomonas* cultured on King B medium (appendix) showed that all the whitish to yellowish round colonies are small to medium in size, opaque, mucous slightly domed with a circular edge except the stumps (5.6 ,9,14) are colonies forming a sheet covering the box. They are capable of producing a fluorescent yellow-green pigment that can be diffused in the medium.

The isolates obtained on King B agar (**figure 14-a**), are belonged to the genus *Pseudomonas.* They form a large and complex heterogeneous group of organisms belonging to the family Pseudomonadaceae, characterized by the production of the pigment pyoverdine which diffuse in cultures on King B agar coloring the medium greenish yellow, these characteristics are specific to fluorescent species of the genus *Pseudomonas* (Singleton, 1999). The Gram stain showed Gram-negative rods (**figure 14-b**). A Gram stain showed *Staphylococcus* cells as Gram-positive cocci, isolated, grouped into diplococci, in short chains and in clusters. *E.coli* and *Pseudomonas aeruginosa* cells appear Gram-negative rod-shaped.

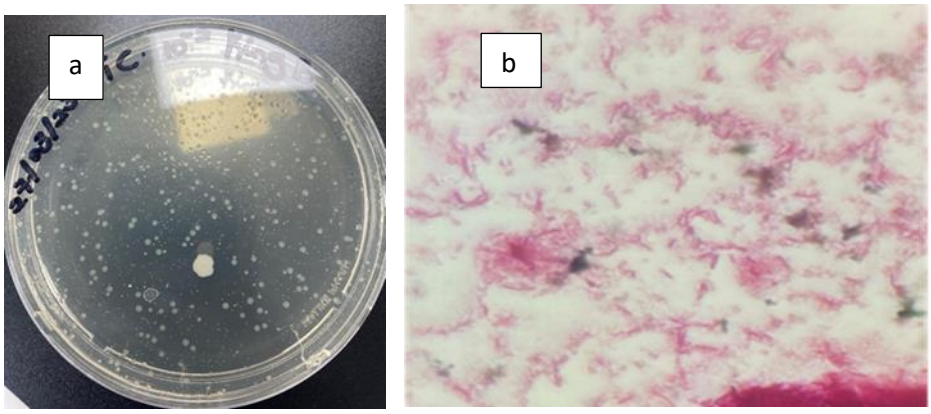


Figure 14: Macroscopic appearance of a *Pseudomonas* isolates on King B agar(a) and microscopic observation of *Pseudomonas* cells after Gram staining (x1000(b))

On BCP medium (**figure 15 - a**)we obtained a lactose positive medium-sized yellow colonies and lactose negative small gray colonies surrounded by blue-violet medium belonging to the group of enterobacteria. The Gram stain showed Gram-negative rods (**figure 15 - b**) .

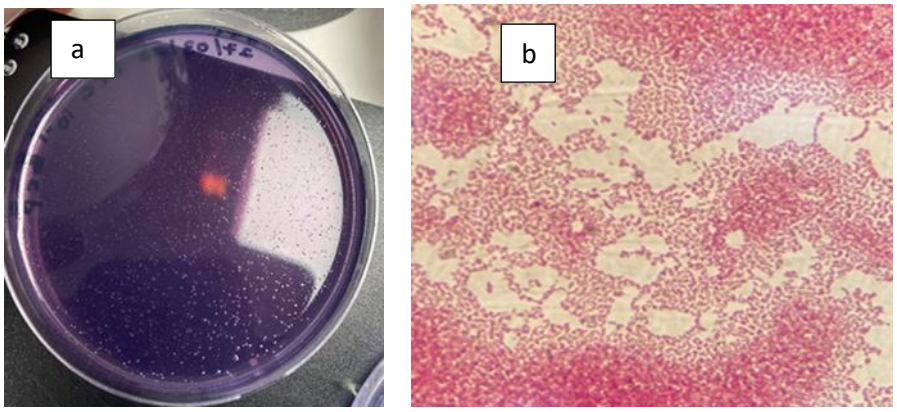


Figure 15: Macroscopic appearance of an enterobacteria colonies on BCP medium (a) and a microscopic observation of enterobacteria cells after Gram staining (x1000(b)).

On BCP medium (**figure 16- a**) lactose negative large and rough gray colonies surrounded by blue-violet medium are found. The cells are Gram positive, rod shaped and producing endospores which make them belong to the genus *Bacillus*. (**Figure 16-b**).

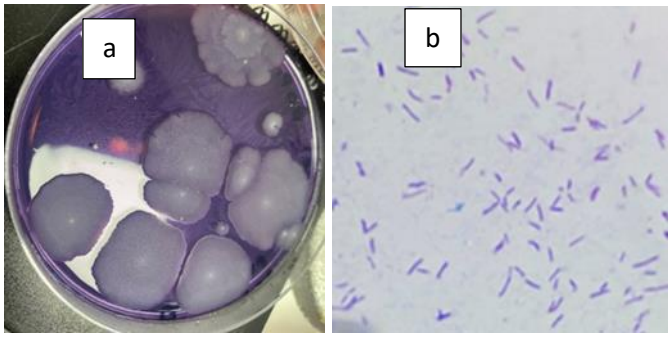


Figure 16: Macroscopic appearance of *Bacillus* colonies on BCP medium (a) and microscopic observation of *Bacillus* cells after Gram staining (x1000(b).

The results showed in **Table 3** indicate that all *Staphylococci* and Enterobacteria possess oxidase and do not possess catalase, and that all Enterobacteria tolerated the concentrations of salinity used, while *Staphylococci* and *Pseudomonas* showed varying in the tolerance of these concentrations.

As mentioned in literature , the Enterobacteria and *Pseudomonas* has the same morphological features but they are differentiated based on the biochemical tests ;Pseudomonas spp shows oxidase positive but the Enterobacteria shows oxidase negative (Jeong *et al.*, 2023). The effect of salts on bacteria varies according to their adaptability and salt tolerance. And if bacteria fail to adapt to the high ionic strength of salts, they cannot survive due to the inability to adsorb and absorb nutrients, replicate their DNA and bio-synthesize macromolecules (Le Rudulier et al., 2002).

Table 3: Catalase ,oxidase and salinity tests of isolated bacteria.

MEDIUM AND BACTERIA	PIONT OF ISOLATION	COLONY NUMBER	TEST OXIDASE	TEST CATALASE	SALINITY TEST	
					6,5% NaCl	8% NaCl
CHAPMAN (staphylococcus)	C	1	Negative	Positive	-	-
	C	2	Negative	Positive	+	-
	A	3	Negative	Positive	+	-
	A	4	Negative	Positive	+	-
	A	5	Negative	Positive	+	+
	B	6	Negative	Positive	+	+
	B	7	Negative	Positive	+	+
	B	8	Negative	Positive	+	+
King B (Pseudomonas)	A	1	Positive	Positive	+	+
	C	2	Positive	Positive	+	-
	C	3	Positive	Positive	-	-
	C	4	Positive	Positive	-	-
	A	5	Positive	Positive	+	-
BCP (Enterobacteria)	C	1	Negative	Positive	+	+
	A	2	Negative	Positive	+	+
	B	3	Negative	Positive	+	+
	C	4	Negative	Positive	+	+

+ = growth - = no growth

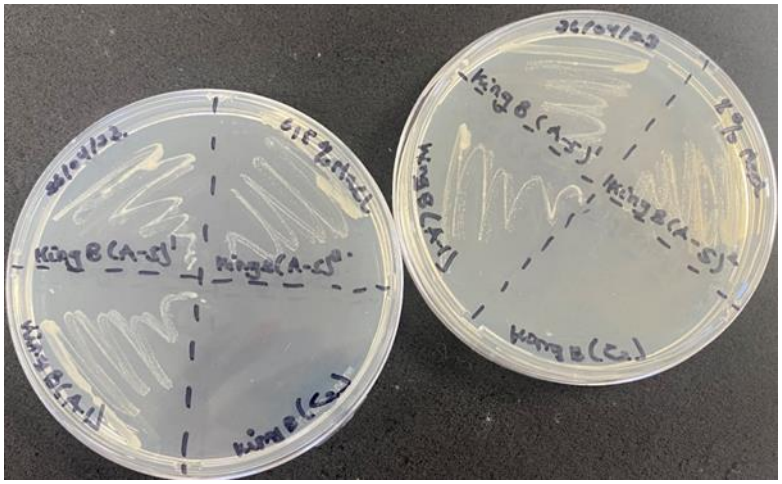


Figure 17: 6, 5% and 8% NaCl tolerance of some sand beach isolates studied.

2-3- Antibiotic Susceptibility Test (AST)

The results were interpreted following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2021 and were recorded as susceptible(S), intermediate(I), resistant(R) or Not- detected(ND) with respect to the diameter of the zone inhibition **Figure (17)** the different form of inhibitory zones obtained.

A: Although the zone appears to be relatively small and just a few colonies seem to be forming next to the plate, the area does seem to have a zone. If confidence was desired, this uncertainty would necessitate repeat testing. Here, there is no distinct zone**.**

B: There is a highly distinct zone here, with only a few minor abnormalities along the zone's perimeter. The diameter of the bacteria-free area would be measured, and the measurement's length would be used to calculate the zone's size.

C: It was observed in this image that there are several bacteria coming up to the disc, despite the background bacterial grass being significantly brightened. No detectable antibacterial effect exists.

Tables 4, 5, and **6** contain the AST (Antibiotic Susceptibility Test) results of the isolated bacteria strains, where the recorded zone was compared to the required diameter measurements of EUCAST to deduce the right interpretation for the antimicrobial effect.

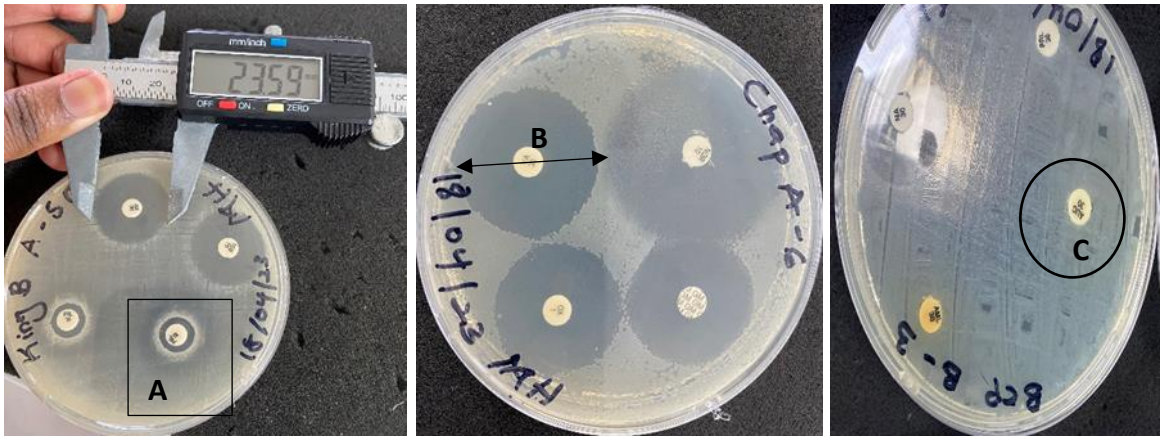


Figure18*:* Results indicating the interaction of the antibiotic disc gradient and the test bacterial inoculum produces inhibitory zones discovered by EUCAST after an overnight incubation.

It is visible from **table 4** that all the eight (08) isolate from the family *Staphylococcus* were susceptible to vancomycin, gentamicin and norfloxacin antibiotics based on their inhibition zones. Also ,it is clear that norfloxacin has the highest antibiotic effects to the bacteria isolates with inhibition diameter of the 40,63mm ,hence it can be concluded that among the three-antibiotic mentioned above ,norfloxacin can be prioritized during for the treatment of staphylococcus related infections since it has shown the highest susceptibility diameter. On the other hand, antibiotic gentamicin recorded the lowest inhibition zone of 17,54mm which signifies that the isolates of the family of staphylococcus are resistant. This means that the effects of antibiotic gentamicin are very low compared to norfloxacin as demonstrated on isolate **S3.** Meanwhile some previous studies demonstrates that antibiotic

gentamicin and vancomycin are ≥ 50% susceptibility to the staphylococcus family(Akanbi *et al*., 2017 ; Skórczewski *et al*., 2014)

Table 4: Results for Antibiotic susceptibility test for *Staphylococcus.*

	ANTIBIOTICS		
ISOLATES CODE	**NORFLOXACIN (NOR 10µg)**	**VACOMYCIN (VA 10µg)**	**GENTAMICIN (GM 10µg)**
S – 1	32,50mm	28,28mm	20,10mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 2	32,68mm	30,32mm	24,73mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 3	40,63mm	33,99mm	17,54mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 4	38,30mm	38,49mm	22,92mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 5	38,66mm	29,12mm	29,99mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 6	35,26mm	30,05mm	27,57mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S- 7	38,14mm	26,60mm	28,92mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)
S – 8	33,86mm	28,07mm	25,75mm
	S (≥ 17mm)	S (≥ 12mm)	S (≥ 18mm)

S:Susceptible /Sensitive

All the four (4) isolates of the family Enterobacterial were susceptible to antibiotic Acid-Nalidixic whilst isolate **E2A** was resistant to antibiotic Piperacillin and susceptible to amoxicillin -acid clavulanic antibiotic .On the contrary, there was no inhibition zone detected for isolates **E1C, E3B** and **E4C** which signifies that the mentioned isolates are automatically resistant to Piperacillin and amoxicillin-acid clavulanic antibiotics. Previous studies have identified gram negative bacteria such as *Pseudomonas* to be resistant to beta-lactam antibiotics which includes Piperacillin, penicillin, amoxicillin/clavulanic acid (Gad *et al.*, 2007).Based on the recorded results acid -Nalidixic is appreciated for treating Enterobacterial infections compared to Piperacillin and amoxicillin -acid clavulanic. A study by Ramesh *et al* .,2010 state that the resistance of antibiotics by bacteria isolates could be as results of the widespread usage of antibiotics in humans, veterinary medicine ,aquaculture and agriculture in various part of the globe. Then again ,further studies can be done in the future to identify why some isolates had no zone detection whilst others did (**table 5**).

Table 5: Results for Antibiotic susceptibility test for Enterobacterial.

	ANTIBIOTICS		
ISOLATES CODE(POINT)	**PIPERACILLIN (PRL - 30µg)**	**ACID- NALIDIXIC (NA -30µg)**	**AMOXICILLIN – ACID CLAVULANIC(AUG 30µg)**
E – 1 (C)	0	24,65mm	0
	ND	S (≥ 14mm)	ND
E – 2 (A)	10,98mm	28,18mm	23,00mm
	R (<17mm)	S (≥ 14mm)	S (≥19mm)
E – 3 (B)	0	24,65mm	0
	ND	S (≥ 14mm)	ND
E – 4 (C)	0	24,81mm	0
	ND	S (≥ 14mm)	ND

R:Resistant. S:Susceptible /Sensitive . ND: Not detected /resistant.

From table (06) it is indicated that all the five isolates of *Pseudomonas* family were susceptible to Amikacin and tobramycin antibiotics except isolate **P5A** which was not detected .In another study , antibiotic amikacin was found to be the most effective treatment against both clinical and environmental strains of *P. aeruginosa(Gad et al., 2007 ;Carol et al., 2013)*. Also for antibiotic Piperacillin, three of the isolates were resistance to the antibiotic as well as **P2C** and **P3C** which were not detected. Previous studies have identified gram negative bacteria such as *Pseudomonas* to be resistant to beta-lactam antibiotics which includes piperacillin,penicillins,amoxicillins/clavulanic acid *(Gad et al., 2007)*

Table 6: Results for Antibiotic susceptibility test for *Pseudomonas*

	ANTIBIOTICS		
ISOLATES CODE (POINT)	**PIPERACILLIN (PRL 30µG)**	**AMIKACIN (AK 30µG)**	**TOBRAMYCIN (TOB 10µG)**
P – 1(A)	10,36mm	25,14mm	23,13mm
	R (< 18mm)	S (≥15mm)	S (≥18mm)
P- 2(C)	0	29,94mm	24,61mm
	ND	S (≥15mm)	S (≥18mm)
P – 3 (C)	0	33,10mm	29,35mm
	ND	S (≥ 15mm)	S (≥ 18mm)
P- 4(A)	9,44mm	23,59mm	22,88mm
	R (< 18mm)	S (≥ 15mm)	S (≥ 18mm)
P -5(A)	10,91mm	25,59mm	0
	R (<18mm)	S (≥ 15mm)	ND

R:Resitant. S:Susceptible /Sensitive . ND: Not detected /resistant

CONCLUSION.

Anthropogenic pollution of both seawater and beach sand is prevalent. It endangers both the environment and the user's health. Contamination is frequently related with inappropriate waste handling. The harmful compounds in garbage make the sea environment unsafe for users like swimmers, causing health concerns such as gastroenteritis illnesses and skin infections.

The goals of this study were to extract and identify microbiological contaminants in wet beach sand and to give a few recommendations for reducing pollution and health hazards.

The samples were gathered from three different sites on the Sidi Mejdoub beach in Mostaganem and analyzed for two months.

The isolation method employed in this study was dilutions in sterile distilled water. After purification, the fungal and bacterial strains were identified by examining the macroscopic and microscopic morphology of the various colonies formed. Biochemical assays such as catalase, oxidase, AST, and salt tolerance were also used to identify the bacteria strains.

Fungi isolated from sea sand were determined the be genera : *Penicillium* spp., Yeast, and *Cladosporium* while the isolated bacterial genera were classified into *Staphylococcus* spp., *Pseudomona*s spp., *Bacillus* spp., and Enterobacteria. The presence of filamentous fungus and harmful bacteria was discovered in the wet beach sand samples collected throughout the research. . Point C had the greater microbe concentration, indicating a high amount of contamination. *Staphylococcus* spp. is the most common microbe, accounting for 47% of isolates. The AST was done to examine the bacterial response in the presence of antimicrobial agent. In our study, it was concluded that Gram negative bacteria such as enterobacteria and *Pseudomonas* were resistant to beta-lactam antibiotics like piperacillin, penicillin, amoxicillin/clavulanic acid .

Finally, the presence of fungus and bacterial species in three wet beach sand samples from the Mediterranean beach (Sidi Majdoub) indicates low microbiological quality. To increase sand quality and eliminate health concerns, education and sanitary measures must be addressed. Despite preliminary research indicating severe health impacts from polluted sand

exposure, several authors' proposed quality requirements are still proving challenging to implement.

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ANNEX

Potato Dextrose Agar		**Mueller Hinton Agar**	
Composition		**Composition**	
Glucose	40g	Peptone	35g
Potatoes	400g	Meat extract	4g
Distilled water	2l	Starch	3g
Agar	40g	Agar	40g
		Distilled water	2l
pH	5.6 ± 0.2 at 25°C	pH	6.8 ± 0.2 at 25°C
NaCl agar 6,5%		**NaCl agar 8%**	
Composition		**Composition**	
Peptone	1g	Peptone	1g
NaCl	16g	NaCl	18g
Yeast extract	0,6g	Yeast extract	0,6g
Distilled water	2l	Distilled water	2l
Agar	4g	Agar	4g
pH	7,2 at 25°C	pH	7,2 at 25°C