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The effect of wastewater on keratinophilic fungi diversity in sebkhet El -Mahmel (El-Mahmel district, khenchela)

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Dedication by wafa

To the greatest dad on the world you have always supported me with financial and emotional support, I am so grateful for your assistance. Your unwavering support has been the driving force behind my success, and I could not have made it this far without you.

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Abstract

The aim of the present work is to study the effect of wastewater on the distribution and growth of keratinophilic fungi in Sebkhet El Mahmel, Wilaya of Khenchela. We collected soil samples from three different areas, than we used hair-baiting technique with hair and feathers to grow the fungal colonies, than we isolated the fungus on potato dextrose agar (PDA) and they were incubated in the dark at room temperature for 7 to 14 days. At the end, we examined and identified fungi species depending on their macroscopic and microscopic characteristics using an optical microscope. We found nine different keratinophilic species in the Sebkha; Chrysosporuim SP, Penicillium SP1, Aspergillus SP1, Aspergillus SP2, Alternaria SP, Aspergilluis SP3, Mucors SP ant two unidentified species. Mucors SP was present only on the third area since it was near wheat field. These results confirms wastewater has an impact on the the biodiversity of keratinophilic fungi in Sebkhat El Mahmal.

Our study show that the wastewater have a positive effect on keratinophilic fungi diversity.

Key words: keratinophilic fungi; Sebkhat El Mahmel; Biodiversity; wastewater; Hair-baiting; PDA

Resume

L'objective de notre travail est d'étudier l'effet des eaux usées sur la distribution et la croissance des champignons kératinophiles à Sebkhet El Mahmel, Wilaya de Khenchela. Nous avons collecté des échantillons de sol dans trois zones différentes, puis nous avons utilisé la technique d'appâtage avec des cheveux et des plumes pour faire croître les colonies de champignons, puis nous avons isolé le champignon sur de la gélose au dextrose de pomme de terre (PDA) et ils ont été incubés dans l'obscurité à température ambiante pendant 7 à 14 jours. A la fin, nous avons examiné et identifié les espèces de champignons en fonction de leurs caractéristiques macroscopiques et microscopiques à l'aide d'un microscope optique. Nous avons trouvé neuf espèces kératinophiles différentes dans la Sebkha ; Chrysosporum SP, Penicillium SP1, Aspergillus SP1, Aspergillus SP2, Alternaria SP, Aspergilluis SP3, Mucors SP et deux espèces non identifiées. Mucors SP n'était présent que sur la troisième zone puisqu'elle était proche du champ de blé. Ces résultats confirment que les eaux usées ont un impact sur la biodiversité des champignons kératinophiles à Sebkhat El Mahmal.

Notre étude montre que les eaux usées ont un effet positif sur la diversité des champignons kératinophiles.

Mots clés : champignons kératinophiles ; Sebkhat El Mahmel; biodiversité; Eaux usées; Hair-bâting; PDA

الملخص

الهدف من هذا العمل هو دراسة تأثير المياه العادمة على توزع ونمو الفطريات الكيراتينية في سبخة المحمل بولاية خنشلة. جمعنا عينات من التربة من ثلاث مناطق مختلفة، ثم استخدمنا تقنية تطعيم الشعر بالشعر والريش لنمو المستعمرات الفطرية، ثم عزلنا الفطر على أجار دكستروز البطاطس (PDA) وتم تحضينها في الظلام في درجة حرارة الغرفة لمدة 7 إلى 14 يومًا. في النهاية، قمنا بفحص وتحديد أنواع الفطريات اعتمادًا على خصائصها المجهرية والميكروسكوبية باستخدام المجهر الضوئي. وجدنا تسعة أنواع مختلفة من الكيراتين في السبخة: Chrysosporuim SP، Penicillium SP1 ، Aspergillus SP1 ، Aspergillus SP2 ، Alternaria SP، Aspergilluis SP3 ، Mucors SP و نوعان غير معروفان. كانت Mucors SP موجودة فقط في المنطقة الثالثة لأنها كانت بالقرب من حقل القمح. تؤكد هذه النتائج أن مياه الصرف الصحي لها تأثير على التنوع البيولوجي للفطريات الكيراتينية في سبخة المحمل.

تظهر دراستنا أن مياه الصرف الصحي لها تأثير إيجابي على تنوع الفطريات الكيراتينية.

الكلمات المفتاحية : الفطريات الكيراتينية؛ سبخة المحمل؛ التنوع البيولوجي؛ مياه الصرف الصحي؛ تطعيم الشعر؛ PDA.

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List of abbreviations

Ph : Potential of hydrogen

BOD : biological oxygen demand

PDA : potato dextrose agar

% : percent

Mm: millimeter

P(mm) : precipitation per millimeter

Cm : Centimeter

DO : Dissolved Oxygen

ds/cm : deciSiemens per meter

mg/L : milligramme per liter

°C : degree Celsius

h : hour

UV : Ultra violet

C : Chrysosporuim

m : meter

EC : Electrical conductivity

g : gramme

TDS : Total Dissolved Solid

LOI : Loss On Ignition

ha : Hectare

Sp : species plurimae

Tmax : Temperature maximale

Tmin : Temperature minimale

Tavr : Temperature Average

Wt : Weight

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Introduction

Wastewater is the result of human residential, artisanal, or industrial activities; it has an enormous impact on many organisms, due to its physic-chemical alterations that lead to major changes in the characteristics of environments, such as salinity, acidity and water temperature (**Islam and Tanaka, 2004**). The consequences of the pollution of aquatic environments are multiple, it lead to massive mortality of species, but also has less visible effects: eutrophication of environments, more or less long-term toxic effects, diseases or endocrine disturbances (**Wu, 1999).** The collection of polluted water in the soil may affect for example the availability of oxygen as an electron acceptor that makes denitrifying bacteria convert available nitrate into nitrogen liberated in the environment resulting pollutants like ammonia and ozone that can cause negative repercussions like affecting plant growth, human ability to breath and vision (**Rabah *et al.*, 2010**).

The soil is the primary source of microorganisms and biological variety, both of which are endangered by water pollution. Depending on the substrate type, this ecosystem offers the habitat and ideal conditions for the growth of a variety of organisms (**Munguia *et al.*, 2011**). Fungi, a common group in soil, have a variety of roles in the environment. They can be useful by establishing mutualistic relationships with plants, for example, or harmful, causing diseases in humans, animals or plants when they exist freely in nature. Keratinophilic fungi group etiological agents of human dermatomycosis (dermatophytes and non-dermatophytes). They are an environmentally vital class of fungi known for their unique ability to consume one of the most prevalent and highly stable proteins on earth that are resistant to decomposition by microbial organisms (**Filipello Marchisio, 2000**). In aquatic ecosystems, the most important ecological role of keratinophilic fungi is the self-purification of water in natural reservoirs and in various types of treatment plants, they contribute significantly to the mineralization of insoluble in water protein substrates (bird feathers and mammalian hair) by Keratinase enzymes which break down keratin into smaller compounds by cleaving the peptide bonds that hold the amino acids together in the keratin protein **(Bandh *et all.*, Jambholkar and yadav, 2023).**

In Algeria, there is a great diversity of fungi, although the knowledge of fungal diversity and distribution is still incipient. The importance of knowing the biodiversity and distribution not only of genera but also of fungal species lies in the fact that it can be a reference to understand the role that fungi play in ecosystems; likewise, this information can be useful to measure the impact of human activities related to the distribution of fungi on ecosystems. The

distribution of keratinophilic fungi in soil gain a huge interest since they were considered saprophytes emerging as new pathogenic microorganisms (**Lopez, 2014**).

The aim of our study is investigating the biodiversity of keratinophilic fungi in an aquatic ecosystem polluted by wastewater. Our work is divided into three main chapters:

— Chapter I: A literature review about keratinophilic fungi; definition, lifestyle, role and biodiversity in both aquatic systems and sediment surfaces on soil microbial colonies.

— Chapter II: Ecological characteristics of the study area "Sebkhat El Mahmal ", its geography, hydrology and climatology. In addition, the materials and methods used to collect samples and their processing.

— Chapter III: The results we got and the discussion

Chapter I

1. The impact of wastewater on aquatic ecosystem

The discharge of wastewater into rivers, oceans, or lagoons causes a deterioration in the quality of their waters. The toxic substances contained in wastewater can have serious consequences for aquatic environments. Chemicals can cause biological disturbances that result in perturbations in the reproduction, growth, or immune systems of aquatic organisms (**Mazzitelli *et al.*, 2018**).Nutrients in wastewater cause the enrichment of aquatic ecosystems, which promotes the rapid development of algae that can suffocate seagrass beds and coral reef. These algae also cause a decrease in oxygen levels in the waters causing the death of certain aquatic organisms (**Rabalais, 2002**). The state where there is a low amount of dissolved oxygen that is too low to support living things is called hypoxia (less than 2 mg/L of oxygen), and anoxia where there is 0 mg/L dissolved oxygen (**Gunderson, 1998**). However, hypoxia can have other origins: water temperature increase (oxygen being less soluble in hot water), water stagnation, discharge of deoxygenated water, eutrophication, (**Rabalais *et al.*, 2010**). Salinity is a decisive parameter too; many aquatic organisms are not able to survive at a salinity greater than 3 grams of salt per liter of water (**Telesh and Khlebovich, 2010**).

2. The impact of wastewater on soil microbial communities

Soil contamination is one of the greatest concerns nowadays because of its clear effect on soil ecosystems, especially microbial communities (**Chen et al**., **2015; Mekki and Sayadi, 2017**). Organic wastes from wastewater ameliorate many parameters of soil, such as aeration, increasing organic carbon storage, the capacity of holding water and cation exchange (**Lal, 2004**). However, it is known that wastewater contains a large variety of potentially harmful pathogens and pollutants. Its effect differs from one microbial biomass to another, depending on its physicochemical parameters. For example, when the soil is soaked with wastewater, its temperature increases, resulting in a decrease in oxygen, which in fact motivates denitrifying bacteria to reduce the available nitrate into nitrogen that enters the atmosphere, causing negative effects (**Adesemoye et al., 2006**).

3. The effect of wastewater on soil fungi

Wastewater has an enormous physical and biological as well as chemical impact on soil fungi. The irrigation with waste water cause a metals pollution and those metals could become toxic at high concentrations (**Siddiquee et al., 2015**). Some types of fungi

populations can resist this (**Iram *et al.*, 2013**). As already mentioned, wastewater may contain nitrogen , which is an essential nutrient for biological growth for all living organisms, such as plants and microorganisms such as fungi (**Mlitan *et al.*, 2015**).

4. Keratinophilic fungi

4.1. Definition

Keratinophilic fungi are an ecologically significant group of fungi known for their ability to degrade one of the most abundant and highly stable proteins on earth "keratin", which is resistant to degradation by other microorganisms (**Filipello Marchisio, 2000**). These microorganisms cannot be noticed by the naked eye, unlike macro fungi like mushrooms. The word keratinophilic means, Keratin loving, as their name indicates they grow on or have affinity for keratin, such in hair, feathers, nails and skin. However, many fungi that grow on natural hair for example do not actually degrade the keratin but rather use the non-keratin lipid fraction of the hair. Thus, only fungi that actually use keratin should be considered as keratinophilic fungi (**Rahul and RC, 2003**). These fungi could outstand other fungi in their characteristic that they can consume keratinous proteins as a source of nitrogen as well as carbon even in the presence of sugar, showing a major variety in their way of nutrition and physiology (**Kunert, 2000; Malek *et a*l., 2013**).

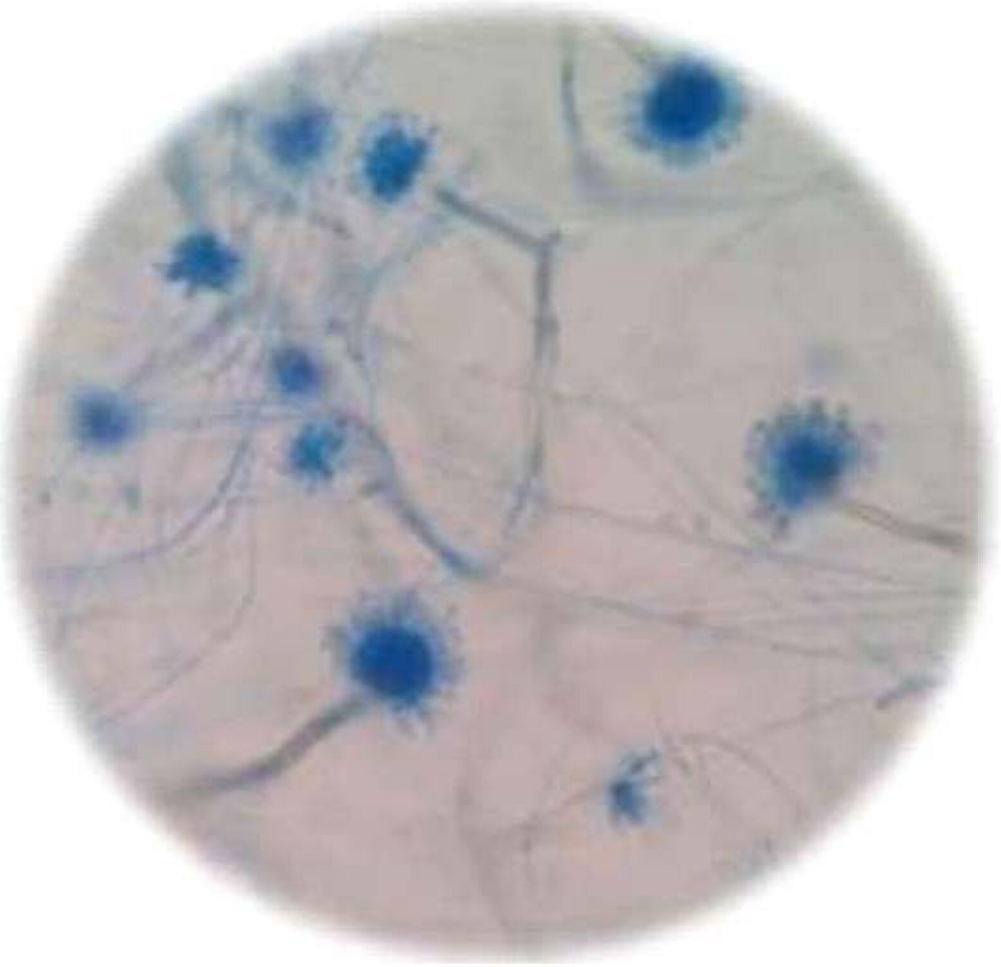

Figure .1. *A photomicrograph of a type of keratinophilic fungi, Aspergillus flavus (**Sosa et al., 2020**).*

4.2. Structure

The fungal structure consists of (**Córdoba *et al.*, 2021**):

- A complex called thallus or mycelium, which in turn is made up of multiple filaments or hyphae, or less often, unicellular structures or yeasts.
- A hypha (a word from Ancient Greek ὑφή (huphḗ)), which is a variable longitudinal tube formed by a rigid cell wall, in which the protoplasm flows. The diameter varies from 1 to 30 microns, ends in a point, constitutes the extension zone and represents the growth region. Hyphae have the ability to anastomose at contact points, mainly in higher fungi, and thus can exchange cytoplasm and nuclei. The ramifications are successive, giving the colony a circular shape reminiscent of tinea corporis.

- Fungal walls, they are an essential component in the fungal cell, providing rigidity to the fungus and protecting it from osmotic shock.

- Vesicles situated at the apex of the hyphae that form an inner membrane complex and contain enzymes that synthesize the wall or break it down; there are also particles called chitosomes, whose function is not definitively known.

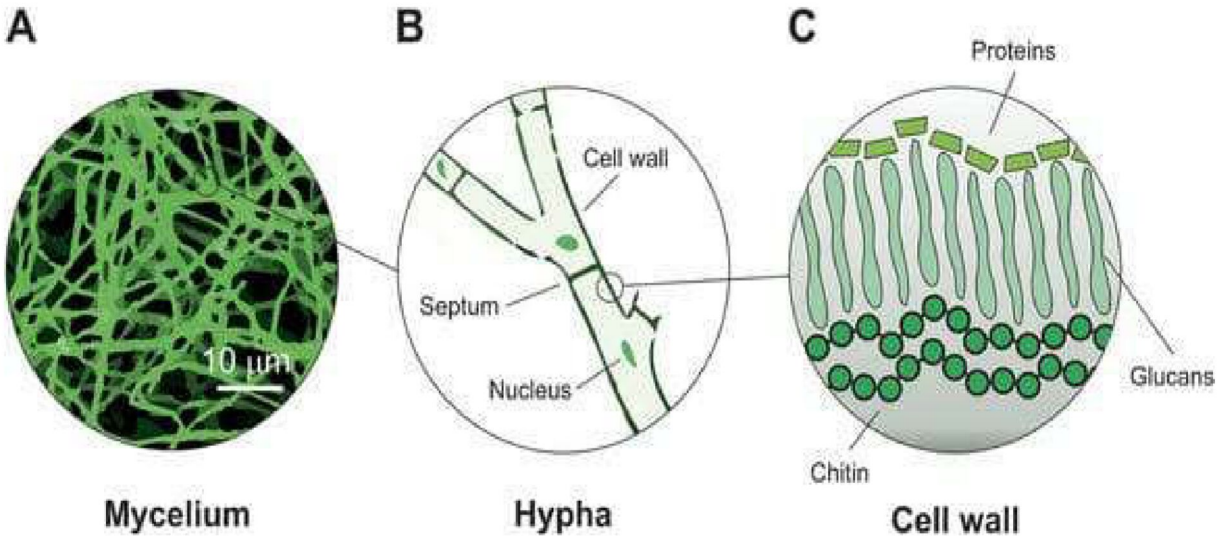

Figure .2. *Schematic representation of (A) Mycelium Fiber, (B) Hyphae and (C) The Cell Wall (**Haneef et al., 2017**).*

4.3. Types of keratinophilic fungi

The taxonomy of keratinophilic fungi is very complex since they embrace a diversity of families, including filamentous fungus that at most belong to Onygenales, saprophytic hyphomycetes fungi, and various other taxonomic groups. The nature of all dermatophytes is keratinolytic. However, we could divide them into three groups based on to the type of infection (**Kumar *et al.*, 2021**):

- Anthropophilic: infect humans.
- Aoophilic: infect animals.
- Geophilic: in soil.

4.4. Characteristics

We could summarize their characteristics as follows (**Summerbell, 2000**):

- All keratinophilic fungi are heterotrophs so they have to feed on preformed organic matter that they use as a source of energy and carbon.
- They have both sexual and asexual reproduction.
- They are eukaryotes, meaning they have a differentiated nucleus with a well-organized membrane.
- Conidial dimorphism.
- Anti-predation mechanisms against arthropods
- Independence of exogenous growth factors (vitamins and amino acids).
- Having high urease activity.
- Osmotolerance, which means resistance to sugars and salt, or only salt.
- They have a cell wall made up of polysaccharides, polypeptides and chitin; this wall is rigid, which is why they cannot engulf food particles but instead absorb simple and soluble nutrients that they obtain by disintegrating macromolecules (polymers) using depolymerizing enzymes.
- The fungal structure consists of a complex called thallus or mycelium, which is made up of multiple filaments or hyphae (hyphomycetes or molds) or, less often, by unicellular structures or yeasts.

4.5. Lifestyle

4.5.1. Keratinolysis

The fungal degradation of keratin consists of a mixed biochemical and mechanical process. The degradation of keratin substrates is due to the action of proteolytic enzymes known as keratinases. The secretion of these is induced by the presence of keratin in substrate (**Siesenop and Böhm, 1995**). This process is accompanied by different morphological adaptations of the fungus, which facilitate its mechanical action on said substrate. Many researches have studied the morphological appearance of keratinolysis in hair, differentiating two form of hair attack: superficial erosion and radial penetration. Superficial erosion consists of gradual thinning of the hair from the outside in. The hyphae that carry out this activity can maintain their normal appearance or dilate forming short branches, giving rise to finger-like structures. Radial penetration is an attack at any location on the hair by several

specialized hyphae that penetrate the hair at a right angle to the long axis of the hair . These latter structures are called perforating organs (**Guarro et *al.*, 1988; Cano et *al.*, 1991; Fusconi and Filipello Marchisio, 1991; Filipello Marchisio, 2000**).

4.5.2. Reproduction

To retain their ability to adapt, fungi must reproduce easily. The hyphae develop from a spore by emission from a germ tube; the simplest form occurs by apical growth of the hyphae; there is no intercalary growth, but nonterminal cells may branch (called holomorphs). Reproduction is carried out by means of spores and can be sexual (teleomorphic); which is a perfect reproduction produced by the union of two nuclei; or asexual (anamorphic); an imperfect reproduction (mitosporic fungi) occurs from a reproductive aerial mycelium without fusion of the nuclei (**Arenas, 2008**).

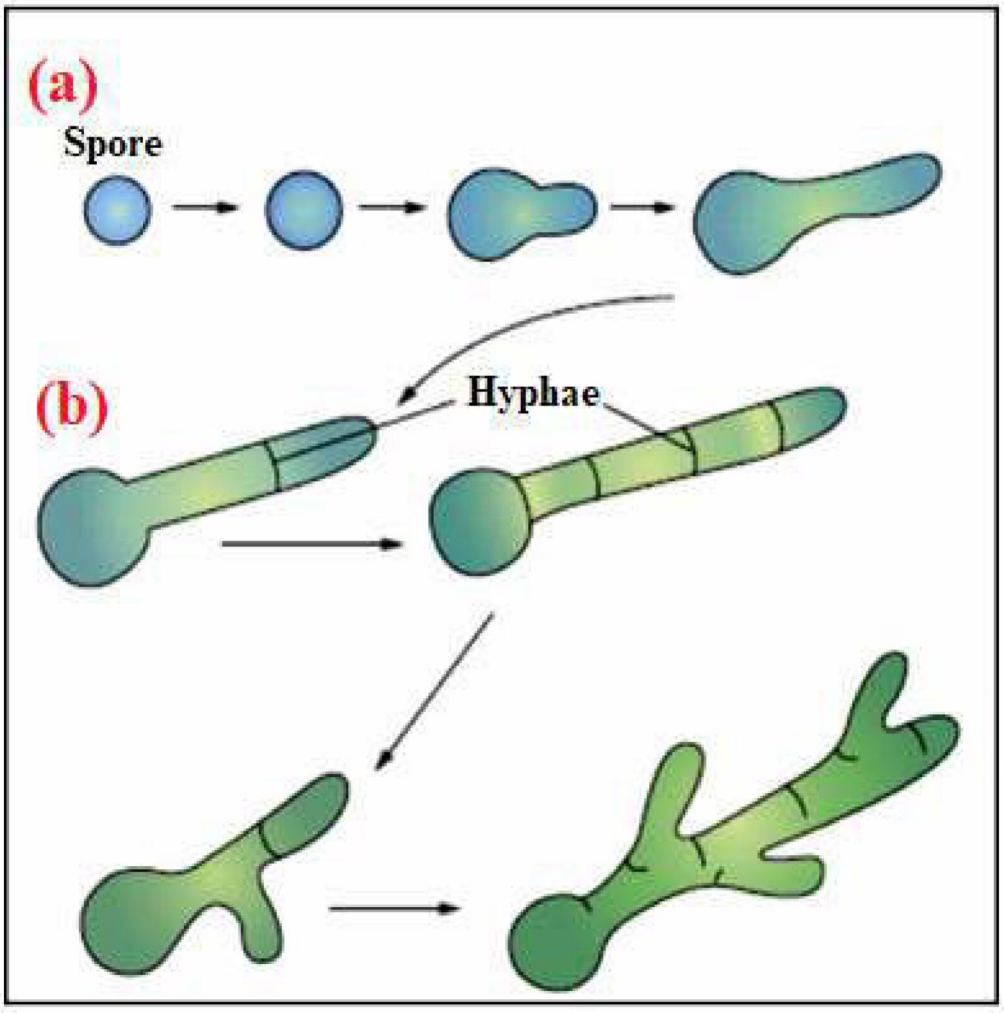

Figure .3. *Schema of fungi reproduction; (a) sexual and (b) asexual (**Córdoba et al., 2021**).*

4.5.2.1. Sexual reproduction

The sexual reproduction involves the union of nuclei. According to the involvement of the thallus in sexual reproduction with respect to the formation of the structure or sexual organs, two types of fungi are distinguished (**Alexopoulos, 1979; Schimek *et al.*, 2003**):

• *Holocarpic:* The entire thallus becomes a reproductive structure (organ). The somatic and reproductive phases do not coexist.

• *Eucarpic:* The reproductive organs arise only from a portion of the thallus, the rest continue their normal somatic activities. Sexual reproduction involves the union of two compatible nuclei and is separated into the following phases:

1. *Plasmogamy:* union of the two protoplasts and the reunion of the two nuclei in a cell
2. *Karyogamy:* union of the two nuclei, after plasmogamy.
3. *Meiosis:* Passage to the haploid state.

Plasmogamy and Karyogamy are almost simultaneous in primitive fungi, but they are separated in time and space in more complex fungi, so that the cells have two genetically distinct nuclei (dikaryotic and heterokaryotic). Dikaryotic hyphae can grow by duplicating their nuclei and maintaining heterokaryosis (**Alexopoulos, 1979**).

Types of sexual reproduction

1. Copulation of planogamentos, flagellated gametes.
2. Gametangial contact or gametangia, the gametangia come into contact but without fusion, the male nucleus migrates through a pore or fertilization tube to the female gametangium.
3. Gametangial copulation or gametangiogamy, the gametangia or their protoplasts fuse, giving rise to a resistance zygote or spore.
4. Spermatization, plasmogamy produced by the union of a spermatium (uninucleated immobile gamete) with a receptor structure.
5. Somatogamy, fusion of somatic cells during plasmogamy (**Alexopoulos, 1979**).

4.5.2.2. Asexual reproduction

It occurs without fusion of gametes and corresponds mainly to the dispersal of asexual spores produced by specialized structures developed from the mycelium (**Bouchet *et al.*,**

2000). **Alexopoulos, (1979)** mentions that the types of asexual reproduction of fungi are divided into:

1. Fragmentation of the soma, each fragment becomes a new individual, they can be irregular or regular, regular fragmentation gives rise to two very important types:

• *Arthrospores:* The hyphae break down into the cells that form them and behave like spores.

• *Chlamydospores:* The hyphae fragment into the cells and are covered with a wall.

2. Fission or excision of somatic cells, to give two daughter cells by constriction and formation of a cell wall.
3. Budding of somatic cells, formation of a small evagination (yolk), to which a daughter nucleus migrates, each bud grows and separates, producing a new individual.
4. Sporulation, production of spores that germinate, causing a germ tube that will develop the mycelium. They are highly variable in shape, color, size, and number of cells. Some fungi produce only one type of spores, others produce up to four different types. They may be:

• *Sporangiospores:* spores produced in sporangia, sporangia are saciform structures whose content is converted entirely by segmentation into one or more spores that are surrounded by a spore wall. They can be of two types:

• *Zoospores:* mobile, uniflagellate or biflagellate, with smooth or barbulate flagella (with mastigonemas).

• *Aplanospores:* They do not present mobility.

5. Conidia, (conidiospores) spores produced on the apex or sides of hyphae.

4.5.3. Factors that affect keratinophilic fungi growth

When certain keratinophilic fungi that survive in the soil come into contact with a susceptible host, under adequate environmental and physiological conditions, they can proliferate on the host, giving an affectation generically called ringworm (**Garg *et al.*, 1985**). Many factors affect the occurrence, distribution and growth of keratinophilic fungi:

- *pH:* Most keratinophilic fungi grow at a particular pH, around 6.5 to 9 (neutral to weakly-alkaline); this could be explained by low enzyme activity in high acidic environments (**Jain and Sharma, 2011**).

- *Humidity:* It mainly depends on the type of keratinophilic fungi, some of them prefers high humidity while the others grow in soils with low humidity (**Iram *et al.*, 2013**).
- *Temperature:* The optimal temperature range for the majority of keratinophilic fungi is 25-27°C and when they are cultivated at temperatures above 40°C, they do not show growth. Therefore, it can be said that keratinophilic fungi, in general, are mesophilic; however, some strains can be thermotolerant and can even adapt themselves to survive in different temperatures (**Garg *et al.*, 1985**).
- *Biological factors:* The existence of keratinous substrates and microorganisms, which may counter the growth and occurrence of fungi. The nitrogen content in keratin varies in the different keratinized substrates, which may affect the colonization of keratinophilic fungi. Moisture availability for soil-dwelling fungi varies with habitats (**Kumar *et al.*, 2021**).
- Soil: The association of keratinophilic fungi with soil tends to correlate with the ability to develop heterothallic teleomorphic states, which means the perfect reproduction states of fungus (**Maruyama *et al.*, 2003**).
- The light: UV light is fungicidal, as it inhibits spore germination and eventually hyphal growth. It has been observed that in the summer months, the frequency of these fungi decreases, which could be partly due to the effect of radiation that inhibits their germination and growth. The intensity of said inhibition depends on the daily exposure time (**Garg *et al.*, 1985**).

4.6. Biodiversity

4.6.1. Biodiversity of keratinophilic fungi in aquatic ecosystem

Unlike underground water, superficial water is always exposed to air, making the occurrence of keratinophilic fungi happens frequently or accidentally (**Ulfig, 1991; Ulfig *et al.*, 1997**). These fungi grow on keratinous substrates in the superficial layer of bottom sediments and in overlying water under specific circumstances, such as the presence of oxygen and carbon dioxide along with nutrients, rainfall, water flow velocity, depth of the water body, aquatic vegetation, and other relevant parameters (**Tulasi *et al.*, 2021**). This would explain why different water bodies have different keratinophilic fungus species. The algae most commonly found in aquatic areas are Chrysosporium evoleeanui, C.

keratinophilum, C. tropicum, Tricho- phyton terrestre, and Microsporum gypseum (**Mangiarotti and Caretta, 1984**).

4.6.2. Biodiversity of keratinophilic fungi in surface sediments

Keratinophilic fungi are distributed all over the world with variable distribution patterns that depend on various factors: the temperature of the water, pH levels, dissolved elements such as oxygen, phosphates, ammonium, nitrates, waste microbial contamination, BOD, and excessive solar radiation related to a shortage of water (**Ulfig, 1996**). Furthermore, their presence and distribution in soil depend largely on the amount of keratinic material available (**Filipello Marchisio, 2000**). The ideal environments for the growth of keratinophilic fungi are woods, park soils, farmyards, and sediments of waterways and oceans enriched in moisture and organic compounds.(**Jamous *et al.*, 2000**) found that keratinophilic fungi tend to be most frequent in soils used for agriculture or livestock areas at which typical keratin resources appear.

4.7. Role of keratinophilic fungi

Keratin is an insoluble protein that has a fibrous helical structure and a large number of disulfide bonds that stabilize its quaternary structure, providing it with resistance to numerous proteases such as trypsin, pepsin, and papain, which is why it could be considered poorly biodegradable (**Grant *et al.*, 1986; Kunert, 2000; Soomro *et al.*, 2007**). It constitutes the main component of hair and feathers, whose degradation is carried out by specific proteolytic enzymes for keratin called keratinases (**Friedrich *et al.*, 1999; Riffel *et al.*, 2003**); and that they are potential hair and feather removal enzymes in industries that work with this material (**Takami *et al.*, 1992**). Keratinase enzymes have a broad substrate specificity since they can not only hydrolyze insoluble protein substrates such as keratin in feathers, wool, hair, nails, etc., but can also soluble protein substrates, including casein, gelatin, bovine serum albumin and hemoglobin (**Gupta *et al.*, 2006**). The potential use of these enzymes is distributed in different applications such as the detergent, textile, waste bioconversion, medicine and cosmetics industries (**Gradisar *et al.*, 2005**).

4.7.1. Role of keratinophilic fungi in aquatic ecosystem

The most important role of keratinophilic fungi is the self-purification of water in natural reservoirs and in various types of treatment plants , they contribute signifcantly the mineralization of insoluble in water protein substrates (for exemple brid feathers and

mammalian hair) by Keratinase enzymes which break down keratin into smaller compounds by cleaving the peptide bonds that hold the amino acids together in the keratin protein. This process results in the formation of smaller peptides and amino acids that can be utilized by other organisms. , this process releases nitrogen and other essential nutrients back into the ecosystem, which means participates in the cycling of essential nutrients, thereby maintaining the balance of micro- and macronutrients in lacustrine systems (**Bandh *et al.*, 2022 ; Jambholkar and yadav,2023**).

4.7.2. Role of keratinophilic fungi in surface sediments

The primary role is the natural degradation of keratinized residues especially α-keratins Which contains most common amino acids, cysteine residues and disulfide bridges, which makes it solid and difficult to degrade by other microorganisms.(**Filipello Marchisio, 2000**). It also contributes significantly to the conversion of energy and the cycling of minerals in the soil by the elimination of waste and producing food for animals, nutrients, adhesives, and rare amino acids that come from tannery and farm poultry wastes (**Filipello Marchisio, 2000 ;Kushwaha *et al.*, 2000**).

Chapter II

II. Materials and Methods

1. Geographical situation

The wetland of Sebkhet El Mahmel constitutes by its distinctive typology that it is the only natural body of water in the whole territory of the Wilaya of Khenchela, located in the southern steppe part of the Wilaya. The Sebkha sector belongs to the complex of continental wetlands of the high plateaus of southern Constantine, which covers the wilayas of Batna, Oum El Bouaghi, Khenchela and Tebessa. **(Bouakkaz, 2016).**

These wet areas are located between 750m and 1200m altitude; they constitute a long corridor bordered in the North by the Tell Atlas and the Saharan Atlas in the South. Their drainage is very compartmentalized, and they are crossed in scarps by small limestone ranges elongated Southwest and Northeast, which generally correspond to faulted or asymmetrical anticlines. This topography combines with the aridity to impede drainage. In the North, there is an exoreic drainage made up of small streams that meander through the High Plains before crossing the Tell. The center and the South are the domain of the endorheic drainage towards the Sebkhas. However, despite the national and international ecological importance that represents this wetland, it has never been subject to a study of classification as a wetland of importance. (**Bouakkaz, 2016)**

The sector of Sebkhet El Mahmel is located between the municipality of El Mahmel, which encompasses more than 80% of the area, and the remaining portion is situated within the municipality of Ain Touila. It is positioned in the eastern and northeastern extension of the Aures-Nemamcha Mountains, at the eastern boundary of the Wilaya of Khenchela, within the geographical area defined by the following coordinates

— Longitude: (7° 15' 33.88" and 7° 22' 47.28") East

— Latitude: (35° 20' 26.63" and 35° 24' 24.97") North

— Average altitude : 1070m

Area of the plan is about 612 ha in high water divided into three compartments:

- Chott ouled Bouali-Lakhlefna 75 ha
- Chott Oled Amara 370 ha
- Chott Ouled Mbarek 182 ha

While the total area of the Sebkha wetland is about 2800 ha. **(Bouakkaza, 2016)**

Photo.4. *General View of Sebkhet Ouled M'Barak, El-Mahmel, Khanchela (author's photo)*

2. Pedology

The majority of the saline soils are located in the Sebkha region, where they grow on sodium-rich rocks. It may be rocks naturally rich in sodium or rocks secondarily enriched in sodium coming from a salty aquifer of continental origin. Poor farming techniques cause salty upwelling that sterilize the soil, which leads to secondary enrichment. These salt rises have a variety of sources, one of the most commonly cited explanations is irrigation paired with high evapotranspiration. Saline soils have a straightforward profile with a single, comparatively thick horizon of mineral and organic matter covered with precipitated salt deposits.

Photo.5. *traces of salts on the outskirts of the sabkha (Author's photo)*

3. Climatology

The region of Khenchela is characterized by a continental climate, hot and dry in summer, cold and rainy in winter. The average rainfall is between 400 and 600 mm/year. The mountainous regions are covered with snow in winter. To study the climate of our area of study, we have exploited data collected at the meteorological station of El Hamma managed by the national office of meteorology, observed in the period from 2010 to 2021 **(Khenchela Meteorological station, 2023)**.

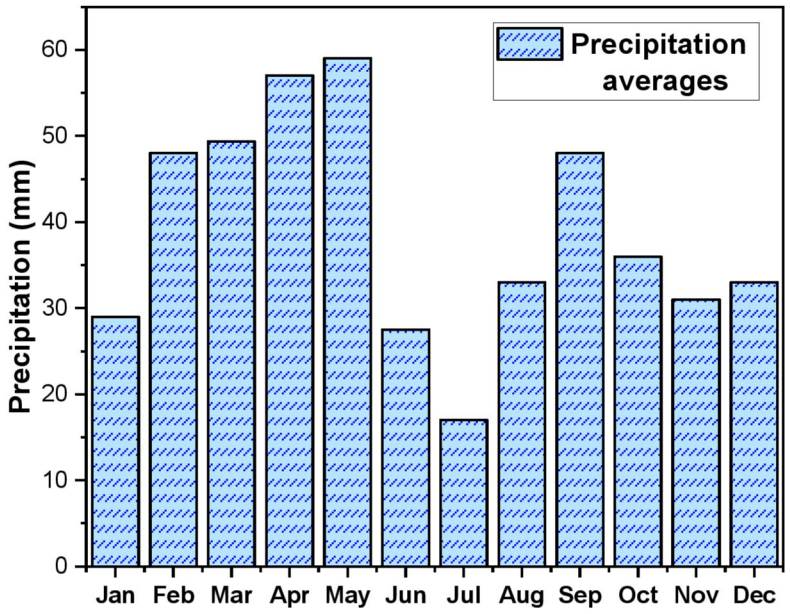

Fig.6. *Diagram of monthly average precipitation for the period 2010-2021*

According to the data in **Figure 6** and **Figure 7**, the average monthly rainfall during the years 2010–2021 is 447.48 mm. The rainiest month is May with an average of 58.30mm. On the other hand, the driest month is July with a monthly average of 15.93mm. We can see that July is the hottest month, with an average maximum temperature of 34.80°C, while December is the coldest month with an average minimum temperature of 3.5°C.

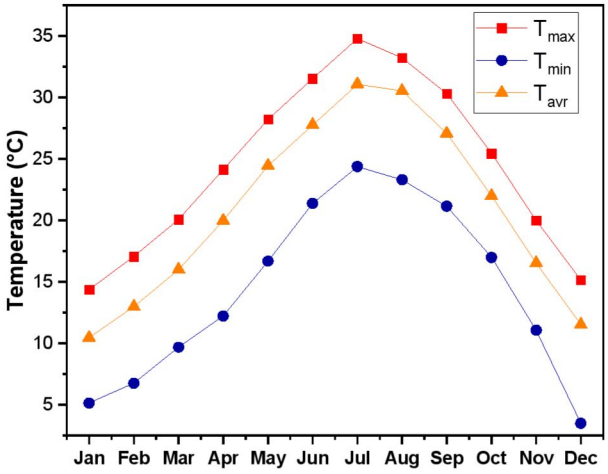

Fig.7. *Diagram of average monthly temperatures for the period 2010-2021*

4. Samples collection

On March 6, 2023, a total of six sediment samples were taken from various locations in Sebkhet El Mahmel. Using a spatula, samples were collected from the superficial layer of the soil, which had a depth of no more than 5 to 10 cm. As a result, 200 to 300g of sediment was gathered in sterilized polyethylene bags and taken to the lab for additional. The location of our samples are shown in **Fig.8**.

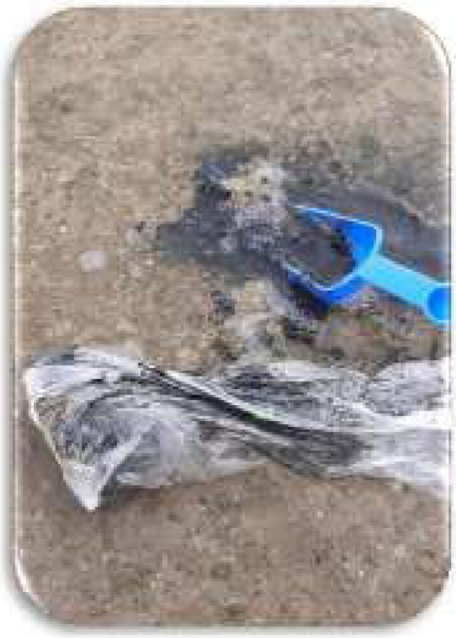

Photo.8. *Sample collecting*

Fig.9. *Localization of sample collection on arc*

5. determination of the characteristics of Sabkhet El Mahmel sediment

5.1. pH determinations

A total of 40g from each sediment sample, and it was thoroughly dissolved in 100ml of distilled water. After allowing the mixture to settle and decant, the pH of the supernatant after decantation was measured using a pH-meter.

5.2. Determination of electrical conductivity

Each sediment sample is tested for electrical conductivity by adding 20g of dried sediment to 100ml of distilled water. We used a conductometer to determine the conductivity after vigorous agitation for 30 minutes to an hour and 30 minutes of settling.

5.3. Determination of the rate of total organic matter

The organic matter content of the sediment can be determined using the sequential loss on ignition (LOI) technique, This technique is based on the principle that organic matter begins to burn at approximately 150°C and fully decomposes at 450°C, while the carbonates decompose at higher temperature **(Santisteban *et al.*, 2004)**. For each sample, 50 g of sediment was heated in an oven at 105°C for 48h. After the initial drying period, the sample were weighed (referred to as wt. at 105°C) subsequently, the sample were placed in muffle furnace at 450°C for 16h. Finally, the samples were weighed again (referred to as wt. at 450°C) to obtain the final weight of dried sediment. The LOI was calculated using the following formula:

$$LOI = (\text{wt. at } 105\ ^\circ C) - (\text{wt. at } 450\ ^\circ C) \times 100 / (\text{wt. at } 105^\circ C)$$

The results are presented up in Table 2.

Photo.10. *(a) The oven; (b) Muffle furnace*

6.1. Sample processing

To recover keratinophilic fungi, we used **Vanbreuseghem (1952)** hair baiting technique. Each 2 sediments samples of: Zone 01, Zone 02 , Zone 3 was thoroughly mixed, and smaller to 2 duplicate samples, approximately 50-70 g each, were placed into empty 90 mm sterile Petri dishes. Then, several pieces of sterile hair and feathers (10-15g) fragments were dispersed over the surface. Each Petri dish was moistened with 5-10 ml of sterile distilled water containing antibiotic (Gentamicine antibiotic 80 mg 2 ml).

6.2. Culture conditions

All baited samples were incubated at 25 -28 in the dark and moistened at intervals of 3-4 days to prevent the sediment from drying. The pallets were inspected daily for up to 40 days before being discarded.

Photo.11. *The autoclave*

7. Media and plates preparation

Keratinophilic fungus cultures were conducted on potato dextrose agar (PDA) media. The media than was autoclaved at 120°C for 15 minutes. We filled petri dishes with the media and left it to solidify for 24 hours. Two milliliters of la gentamicine were added to stop and prevent bacterial growth. After the solidification of agar, we placed the plates in a refrigerator set between 2 and 8 degrees Celsius**.**

Photo.12. *The hair baiting method*

Photo.13. *Incubator outside and inside*

8. Identification of the keratinophilic fungi

8.1. Preparation of lactophenol bleu

We put 10ml of Lactic acid with 10g of Phenol crystal, 0.5g of Methylene blue, 20ml of Glycerol and 10 ml of distilled water in a beaker, then we agitated it to homogenize the mixture. After that, we pored the solution in a flask until the use.

Photo.14. *Chemical products we use to prepare lactophnol bleu*

8.2. Identification of the keratinophilic fungi

The descriptions provided in the identification key used in the conventional taxonomy by **Barnett and Hunter (1999)** were utilized to identify the keratinophilic fungi. Slides from fungal isolates grown on PDA plates (obtained by direct inoculation of microfungi on cube media) were made, and they were then stained with Lactophenol Blue for morphological characterization and viewed under a microscope (40X and 60X), to evaluate the appearance of mycelium, the location of columella, as well as the spore's shape. Macroscopic (colony morphology, color, texture, form, colony appearance) and microscopic (septa in the mycelium, presence of certain reproductive structures, shape and structure of conidia) characteristics were used to identify fungal isolates.

Photo.15. *Laminar flow hood*

Chapter III

III. Results and Discussion

1. Results

According to the descriptions found in the identification key used in **Barnett and Hunter's (1999)** traditional taxonomy, the keratinophilic fungi were identified based on:

- Macroscopic characteristics of the colonies: color, texture, shape and appearance.
- Microscopic characteristics of the mycelium or spores: septation in mycelium, presence of specific reproductive structures, shape and structure.

1.1. Sampling point 1

1.1.1. Hair as a substrate

The six fungus strains that were found in the soil of sample 1 might be categorized as follows based on their morphology:

- Strain 1 belongs to the species *Chrysosporium* (**Fig.16**)
- Strain 2 belongs to the species *Penicillium SP1* (**Fig.18**)
- Strain 3 belongs to the species *Aspergillus SP1* (**Fig.20**)
- Strain 4 belongs to the species *Aspergillus SP2* (**Fig.22**)
- Strain 5 belongs to the species *Alternaria SP* (**Fig.24**)
- Strain 6 belongs to the species *Aspergillus SP3* (**Fig.26**)

Chrysosporium SSP (≈ C. keratinophilum): White flaky and powdery colonies with light-brown backside. The mycelium produces ovoid or ampulliform aleuria that are formed laterally, intercalary barrel-shaped aleuria, and terminal or lateral conidia (aleuria).

Penicillium SP1 (≈ P. thomi)*:* Spherical smooth, ovoid conidia, a greenish gray colony color. Septate mycelium, biverticillate conidiophore followed by several branches of Metulae that carry the Phialides arranged in tight brushes and at the end the Sporocysts, which release the conidia.

Aspergillus SP1 (≈A. stelliformis, A. sydowii, A. flavus)*:* It appears as irregularly shaped, furrowed buff-yellowish colonies with off-white spores. The mycelium is transparent and not septate, unbranched conidiophores ending in vesicles bearing Metulae and Phialides (biseriate conidial head), spherical and smooth conidia.

Aspergillus SP2 (≈ A. sclerotiorum): Brown color. These colonies with thin margins and relative fructification develop quickly. Mycelium is transparent, septate, conidiophores are long with a smooth wall, terminate in spherical vesicles bearing metulae and phialides all around the vesicle, spherical conidia with a yellow to orange-brown color.

***Alternaria SP*:** Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

***Aspergillus SP3*:** Beige to hazelnut brown tint, smooth conidiophore, phialides borne by metula inserted mainly on the upper part of the vesicle, conidia smooth, globose to slightly elliptical, and columnar aspergillus heads.

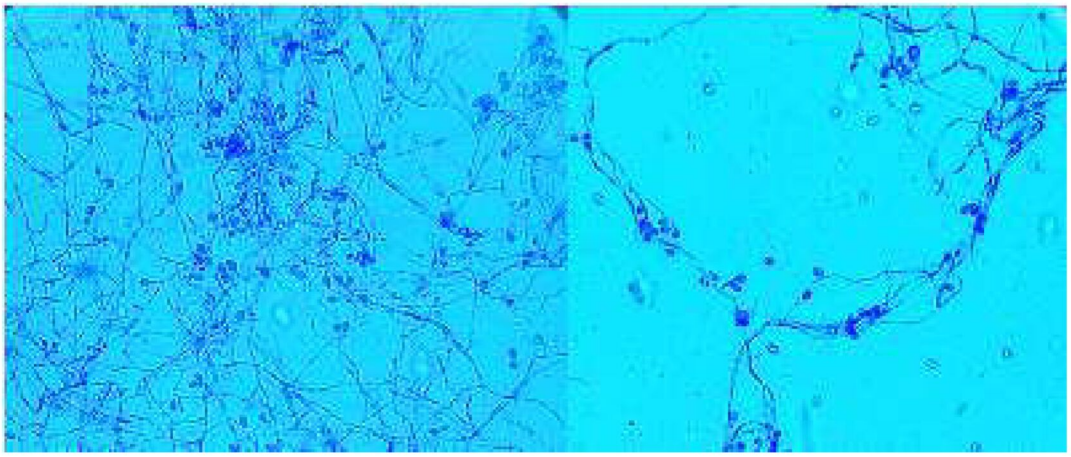

Fig.16. *Microscopic observation of Chrysosporium SP X40*

Fig.17. *Petri dish surface side of Chrysosporium SP*

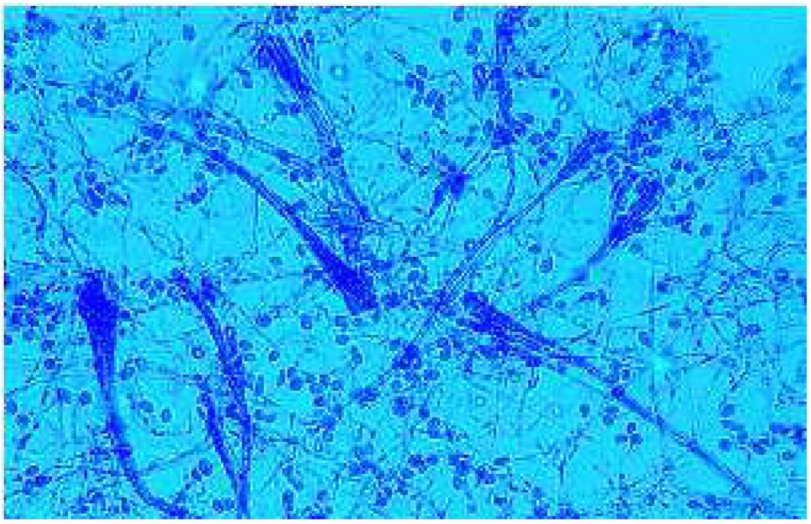

Fig.18. *Microscopic observation of Penicillium SP1 X40*

Fig.19. *Petri dish reverse side of Penicillium SP1*

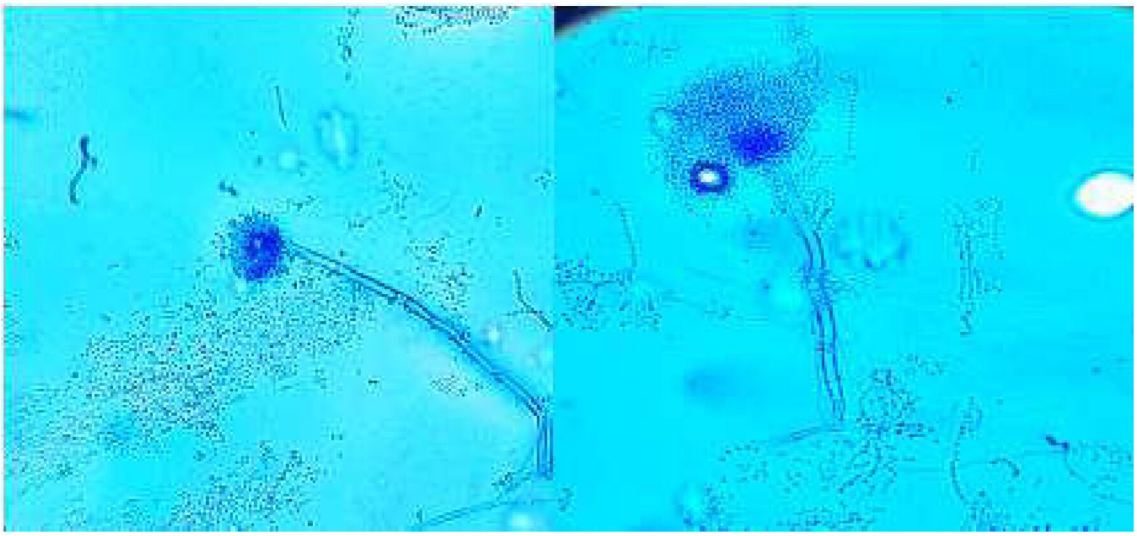

Fig.20. *Microscopic observation of Aspergillus SP1 X40*

Fig.21. *Petri dish surface reverse of Aspergillus SP1*

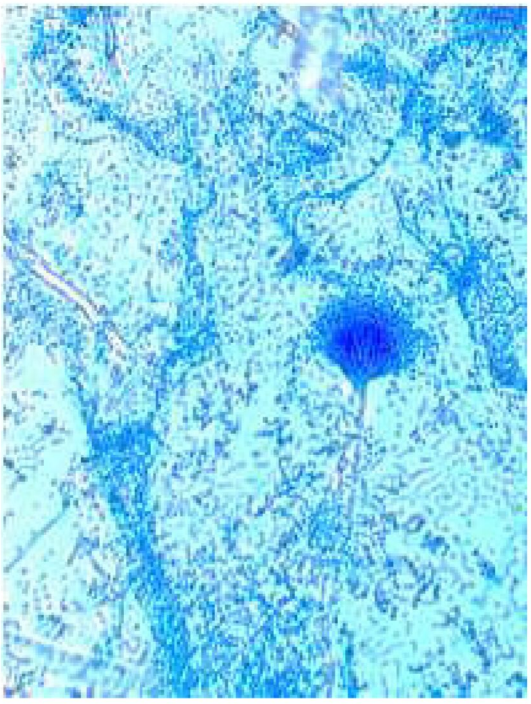

Fig.22. *Microscopic observation of Aspergillus SP2 X40*

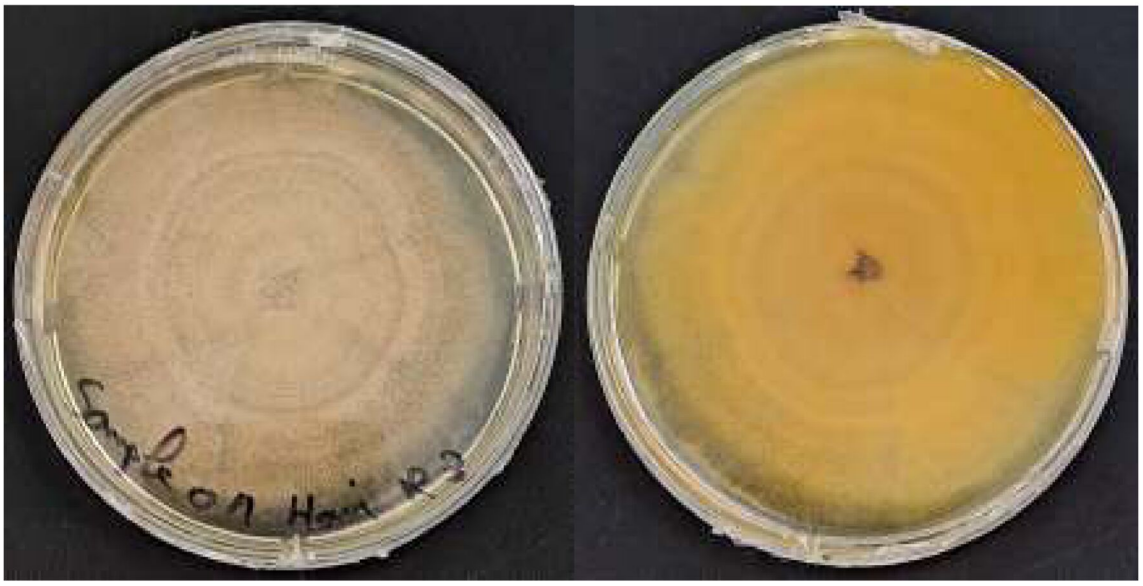

Fig.23. *Petri dish surface and reverse sides of Aspergillus SP2*

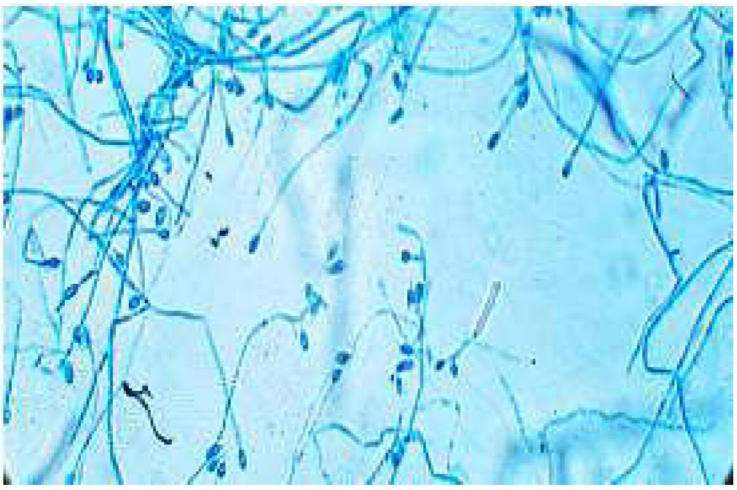

Fig.24. *Microscopic observation of Alternaria SP X40*

Fig.25. *Petri dish surface and reverse sides of Alternaria SP*

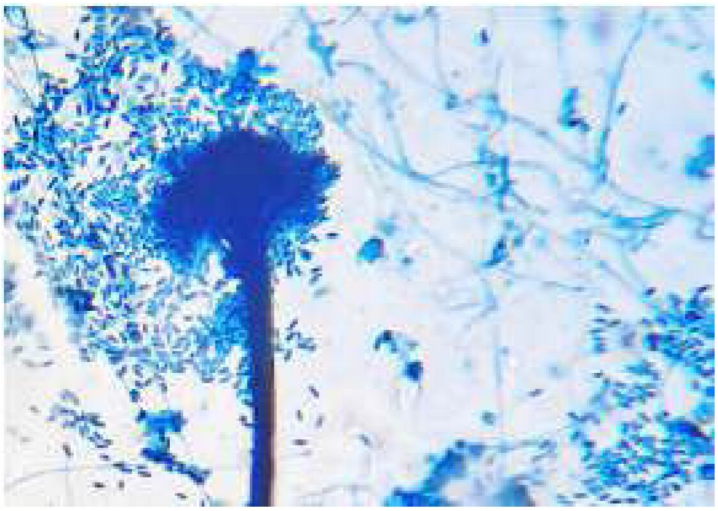

Fig.26. *Microscopic observation of Aspergillus SP3X40*

1.1.2. Feathers as a substrate

We found three fungus strains in the soil of sample 1 that could be categorized as follows based on their morphology:

- Strain 1 belongs to the species *Penicillium SP1* (**Fig.27**)
- Strain 2 belongs to the species *Alternaria SP* (**Fig.28**)
- Strain 3 belongs to the species *unidentified* (**Fig.29**)

Penicillium SP1 (≈ P. thomi)*:* Spherical smooth, ovoid conidia, a greenish gray colony color. Septate mycelium, biverticillate conidiophore followed by several branches of Metulae that carry the Phialides arranged in tight brushes and at the end the Sporocysts, which release the conidia.

Alternaria SP: Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

Unidentified species

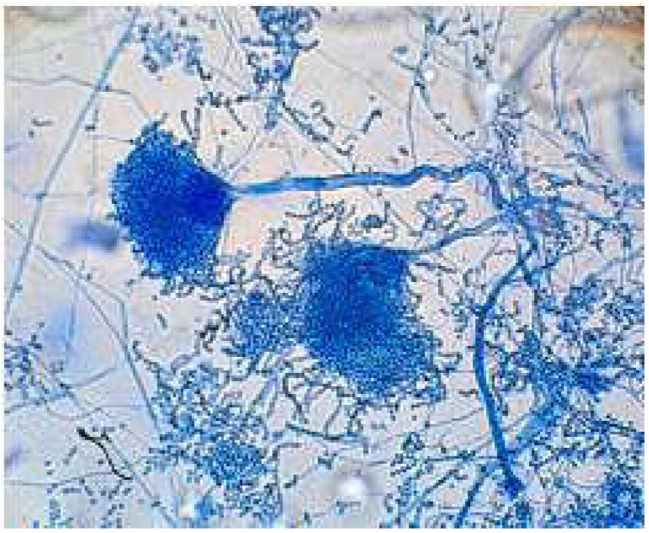

Fig.27. *Microscopic observation of Penicillium SP1 X40*

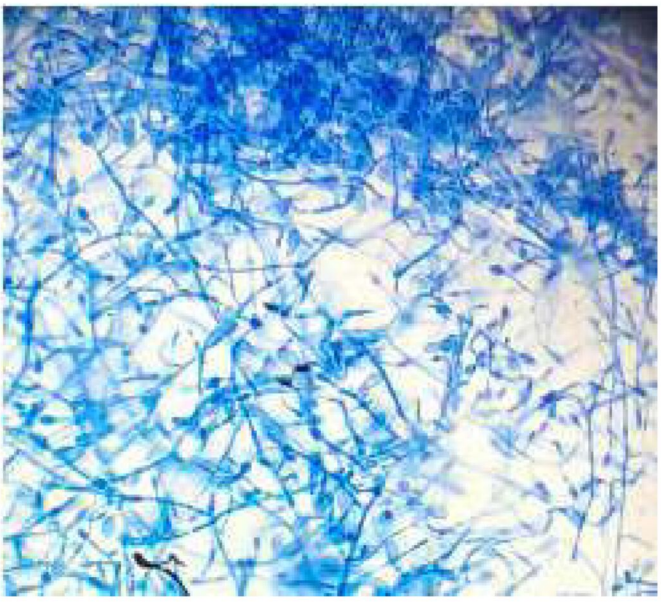

Fig.28. *Microscopic observation of Alternaria SP X40*

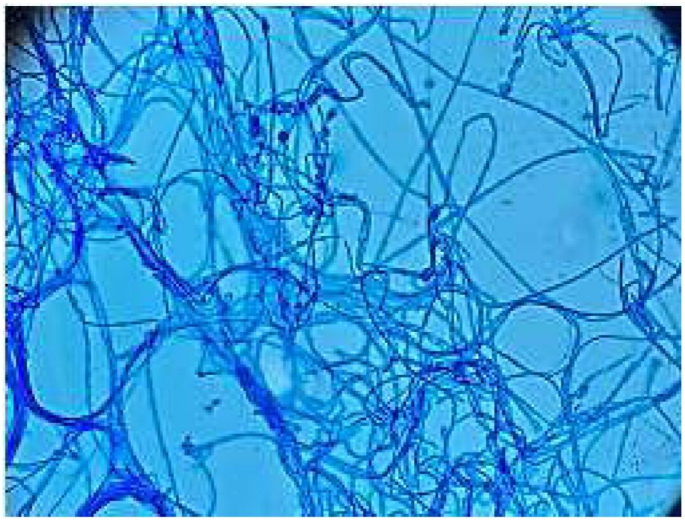

Fig.29. *Microscopic observation of the unidentified species X40*

Fig.30. *Petri dish surface and reverse of sample 1 feather*

1.2. Sampling point 2

1.2.1. Hair as a substrate

The four fungus strains that were found in the soil of sample 2 could be categorized as follows based on their morphology:

- Strain 1 belongs to the species *Chrysosporium SP (***Fig.31**)
- Strain 2 belongs to the species *Aspergillus SP1* (**Fig.32**)
- Strain 3 belongs to the species *Alternaria SP* (**Fig.33**)

- Strain 4 are *Unidentified species* (**Fig.34**)

***Chrysosporium SP (≈ C. keratinophilum*):** White flaky and powdery colonies with light-brown backside. The mycelium produces ovoid or ampulliform aleuria that are formed laterally, intercalary barrel-shaped aleuria, and terminal or lateral conidia (aleuria).

***Aspergillus SP1 (≈A. stelliformis, A. sydowii, A. flavus*)**: It appears as irregularly shaped, furrowed buff-yellowish colonies with off-white spores. The mycelium is transparent and not septate, unbranched conidiophores ending in vesicles bearing Metulae and Phialides (biseriate conidial head), spherical and smooth conidia.

***Alternaria SP*:** Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

Unidentified

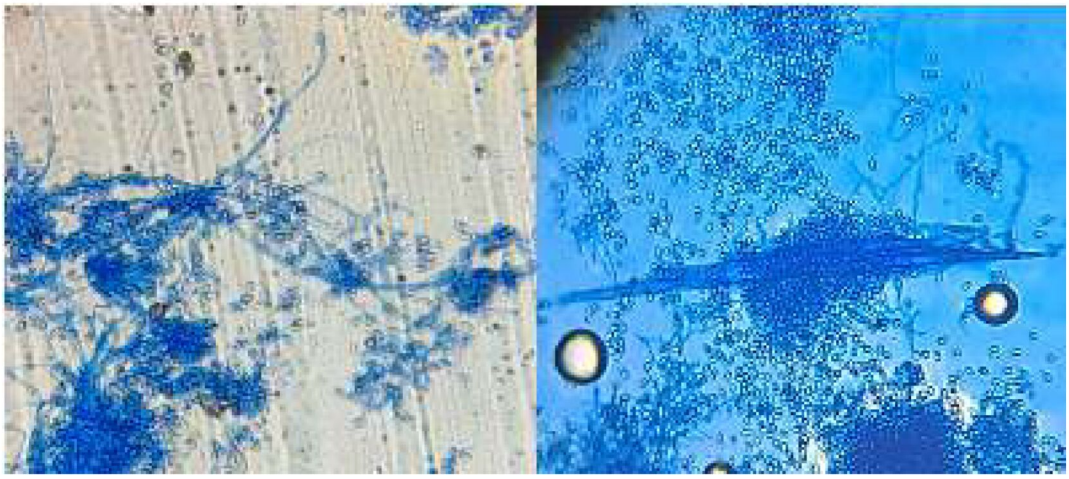

Fig.31. *Microscopic observation of Chrysosporium SP X40*

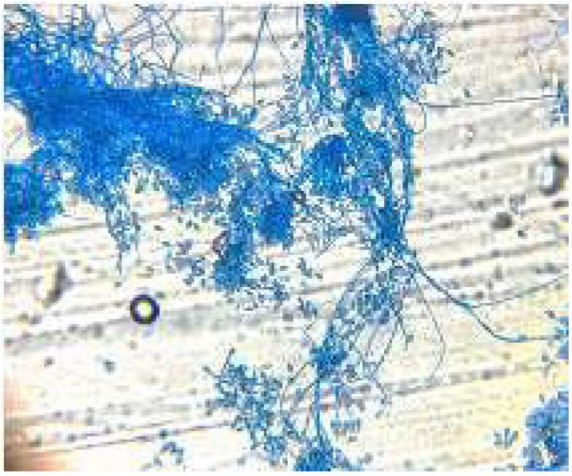

Fig.32. *Microscopic observation of Aspergillus SP1 X40*

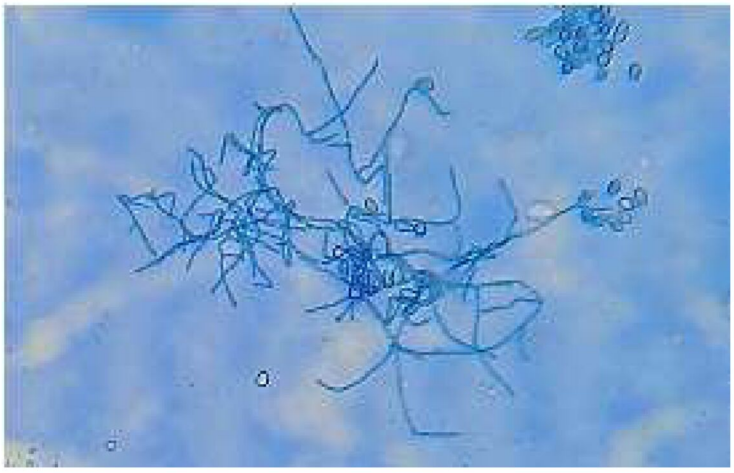

Fig.33. *Microscopic observation of Alternaria SP X40*

Fig.34. *Microscopic observation of the unidentified species X40*

1.2.2. Feathers as a substrate

We found three fungus strains in the soil of sample 1 that could be categorized as follows based on their morphology:

- Strain 1 belongs to the species *Alternaria SP* (**Fig.35**)
- Strain 2 belongs to the species *Chrysosporium SSP* (**Fig.36**)

***Alternaria SP*:** Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

Chrysosporium SSP (≈ C. keratinophilum): White flaky and powdery colonies with light-brown backside. The mycelium produces ovoid or ampulliform aleuria that are formed laterally, intercalary barrel-shaped aleuria, and terminal or lateral conidia (aleuria).

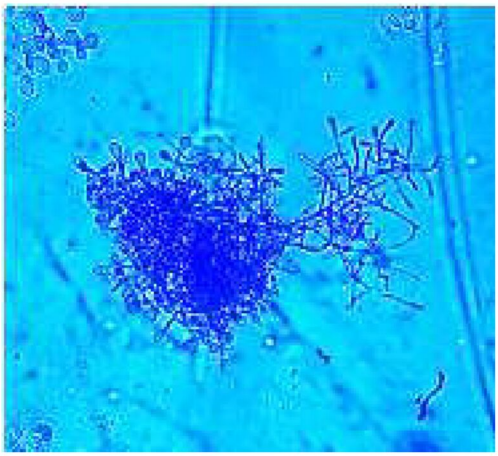

Fig.35. *Microscopic observation of Alternaria SP X40*

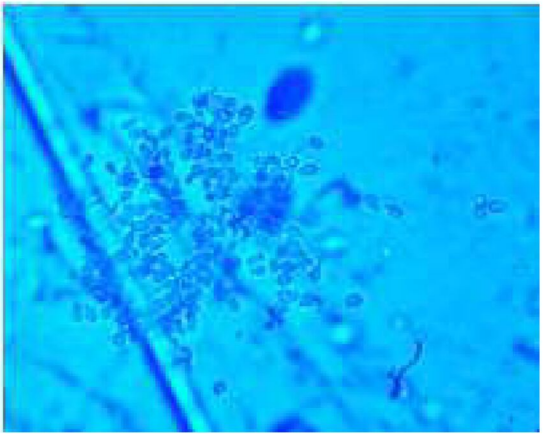

Fig.36. *Microscopic observation of Chrysosporium SP X40*

1.3. Sampling point 3

1.3.1. Hair as a substrate

The five fungus strains that were found in the soil of sample 1 might be categorized as follows based on their morphology:

- Strain 1 belongs to the species *Aspergillus SP1* (**Fig.37**)
- Strain 2 belongs to the species *Aspergillus SP2* (**Fig.38**)
- Strain 3 belongs to the species *Penicillium SP1* (**Fig.39**)
- Strain 4 belongs to the species *Mucor SP* (**Fig.40**)
- Strain 5 belongs to the species *Alternaria SP* (**Fig.41**)

Aspergillus SP1 (≈A. stelliformis, A. sydowii, A. flavus)*:* It appears as irregularly shaped, furrowed buff-yellowish colonies with off-white spores. The mycelium is transparent and not septate, unbranched conidiophores ending in vesicles bearing Metulae and Phialides (biseriate conidial head), spherical and smooth conidia.

***Aspergillus SP2 (≈ A. sclerotiorum*)**: Brown color. These colonies with thin margins and relative fructification develop quickly. Mycelium is transparent, septate, conidiophores are long with a smooth wall, terminate in spherical vesicles bearing metulae and phialides all around the vesicle, spherical conidia with a yellow to orange-brown color.

***Penicillium SP1 (≈ P. thomi*)**: Spherical smooth, ovoid conidia, a greenish gray colony color. Septate mycelium, biverticillate conidiophore followed by several branches of Metulae that carry the Phialides arranged in tight brushes and at the end the Sporocysts, which release the conidia.

***Mucor SP*:** The colonies have a woolly texture with a brown color on the surface. Filaments broad and not septate, Sporocystophore bearing a globose sporocyst, the presence of chlamydospores and the constriction of the sporocyst under the columella and round spores.

***Alternaria SP*:** Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

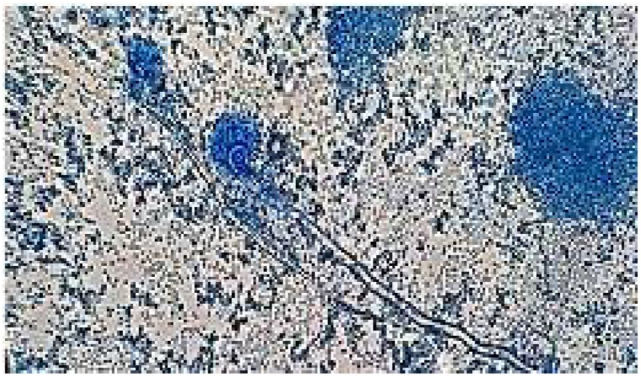

Fig.37. *Microscopic observation of Aspergillus SP1 X40*

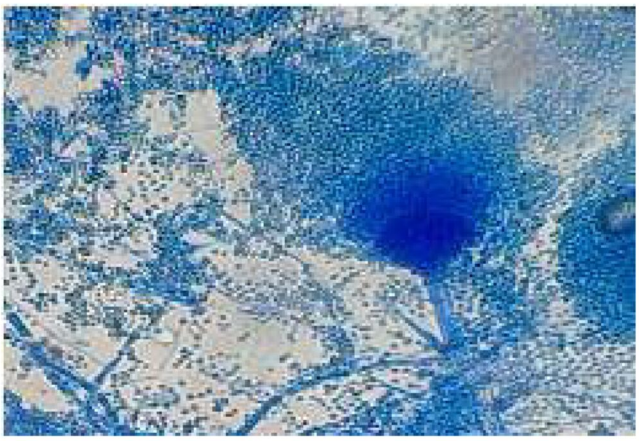

Fig.38. *Microscopic observation of Aspergillus SP2 X40*

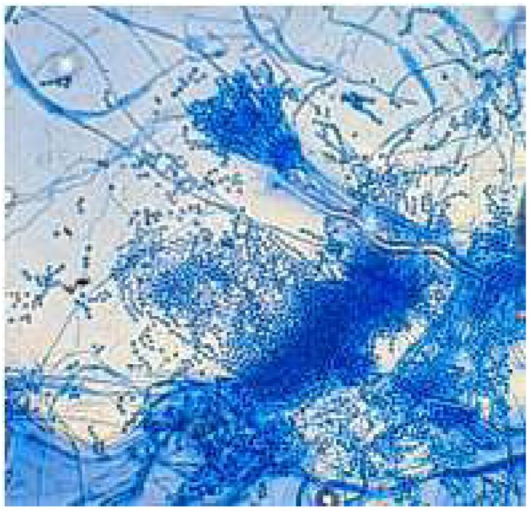

Fig.39. *Microscopic observation of Penicillium SP1 X40*

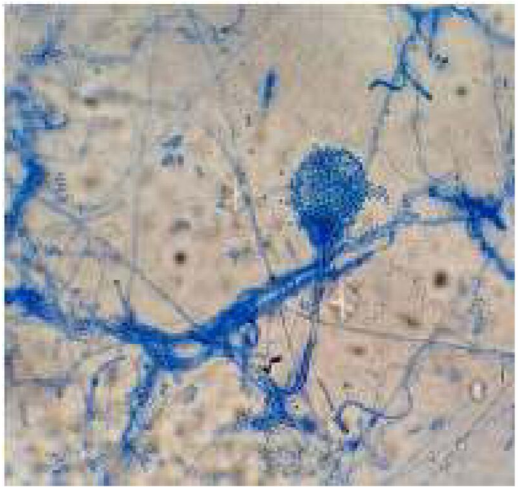

Fig.40. *Microscopic observation of Mucor SP1 X40*

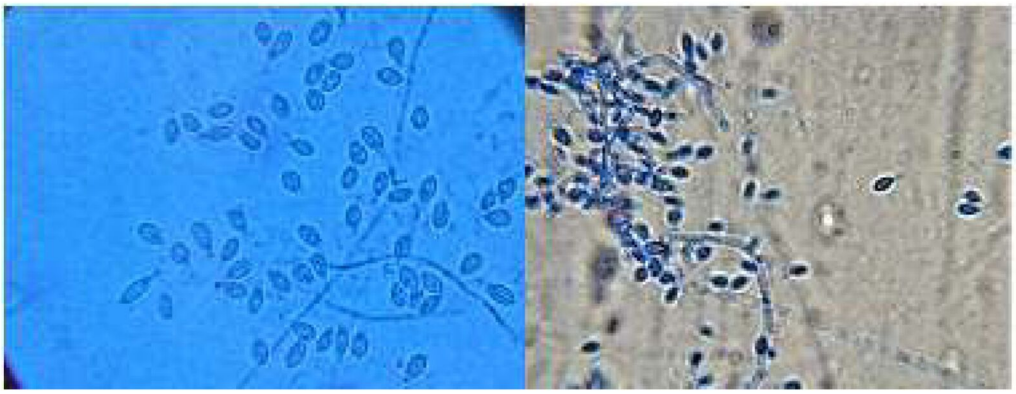

Fig.41. *Microscopic observation of Alternaria SP X40*

1.3.2. Feathers as a substrate

We observed one only fungi strain in the soil of sample 3 that could be identified based on its morphology as the species Alternaria SP (**Fig.42**).

Alternaria SP: Black to greyish color. These include two to three clearly distinct circular white spots of mycelium and green rings of conidia. Additionally, we observed a quick development in the culture medium and the emergence of conidia with a green-yellow hue.

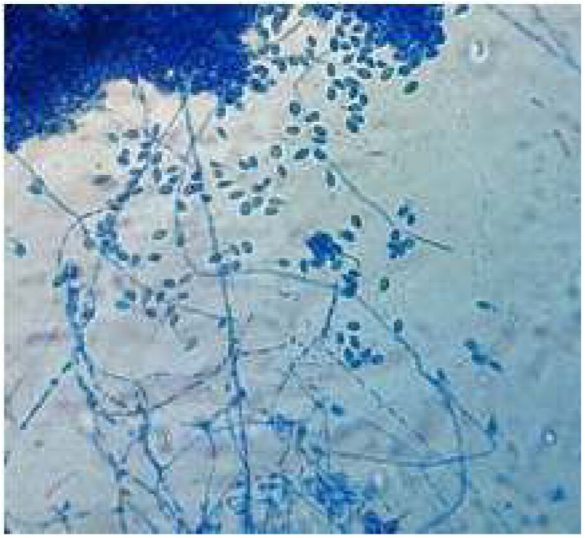

Fig.42. *Microscopic observation of Alternaria SP X40*

1.4. Characteristics of Sabkhet El Mahmel water

The physical parameters of the water were measured using a multiparameter YSI ProDss instrument on 06-03-2023. The obtained results are presented in **table 1**.

Table.1. *Characteristics of Sabkhet El Mahmel water, pH, electrical conductivity, dissolved oxygen and total dissolved solids*

	pH	EC µs/cm	Do mg/l	TDs mg/l
S1	7.98	17736	5.98	8369.170
S2	8.69	17919	7.11	7189.315
S3	8.83	18052	7.83	7244.588

1.5 Characteristics of soil samples

Table.2. *Characteristics of the soil samples*

	pH	EC µs/cm	Organic matter %
S1	6.76	13.9	54.01
S2	7.83	6.6	40.09
S3	7.50	8	25.72

2. Discussion

Fungi are eukaryotic microorganisms that perform fundamental ecological functions as saprophytes, mutualists, or pathogens of plants and animals. Fungi are widely distributed in all terrestrial ecosystems, but the distribution of species has been poorly documented (**Tedersoo *et al.*, 2014**). Various roles are played within ecological processes, such as the nitrogen and carbon cycle and soil formation (**Van Der Heijden *et al.*, 2008**). The kingdom of fungi is one of the most diverse groups of organisms on earth, comprising approximately 100,000 described species, but it is estimated that the real scope of global fungal diversity corresponds to 0.8 to 5.1 million species (**Tedersoo *et al.*, 2014**).

Knowing the diversity, not only of fungi, but also of all species is a reference that can be used to measure the impact of humanity on ecosystems. Recently, the distribution of keratinophilic fungi has shown worldwide interest, since every time new pathogenic microorganisms that were considered as saprophytes emerge (**Dehghan *et al.*, 2019**). Since the soil and sediment represents an important reservoir of these microorganisms, it is interesting to know their distribution in this type of habitat (**Anane *et al.*, 2015**).

The hair-baiting technique allows the isolation of microorganisms with a certain affinity for keratin. The presence of this type of fungus in the sediment depends on various factors, such as physical and chemical parameters, the climate and the presence of plants, animals and humans, pollution as well as the region or area factors. In our study, we investigated the biodiversity of keratinophilic fungi in "Sebkhat El Mahmal" an aquatic ecosystem that has been polluted for years by untreated wastewater.

Three different zones in the sebkha were chosen based on their proximity to the point of entry of the wastewater:

- Sampling point or zone 1: at the level of the sewage inlet.
- Sampling point or zone 2: in the middle of Sebkhat el Mahmal.
- Sampling point or zone 3: at the end of the Sebkhat el Mahmal, farthest from the sewage inlet.

Two sets of criteria were used to evaluate the isolated fungi in our study: macroscopic criteria based on the characteristics of colonies and microscopic criteria based on fungal morphology. Our findings indicated the existence of eight species, which are listed in the table below (**Table. 3**).

Table.3. The isolated species of keratinophilic fungi in sediment (+ presence / - absence)

Zone / Species	Hair			Feathers		
	Zone 1	**Zone 2**	**Zone 3**	**Zone 1**	**Zone 2**	**Zone 3**
Chrysosporuim sp	+	+	-	-	+	-
Penicillium sp	+	-	+	+	-	-
Aspergillus sp1	+	+	+	-	-	-
Aspergillus sp2	+	-	+	-	-	-
Alternaria sp	+	+	+	+	+	+
Aspergilluis sp3	+	-	-	-	-	-
Mucors sp	-	-	+	-	-	-
Unidentified species	-	+	-	+	-	-

Analysis of our results of isolation indicates the efficiency of our culture technique in the isolation of keratinophilic fungi from the sediment. All isolates' colonies were obtained in hair while only 50% of them were found in feathers. This could be explained by the fact that feathers are constructed of beta-keratin, a tougher variety and hard to decompose compared to alpha-keratins that forms hair (**Sharma *et al.*, 2016**).

For hair as substrate results, zone 1 was more abundant and diverse as it contains almost all species except *Mucors sp* and the unidentified colonies. While area 2 was the less diverse one containing: *Chrysosporium sp, Aspergillus sp1, Alternaria sp* and the unidentified species. As for the zone 3, it was similar to the zone 1, except the absence of *Chrysosporium sp* and *Aspergillus sp3* and the appearance of *Murco sp*, which was not found in both other zones. As for feather results, we find a convergence in the number of species. We recorded three species in zone 1: *Penicillium sp, Alternaria sp* and unidentified species. Moreover, there are two species in zone 2: *Chrysosporium sp*, *Alternaria sp*. As for zone 3, only one species was found: *Alternaria sp.*

- *Chrysosporium sp:* was present in Zone 1, Zone 2 and absent in Zone 3. This suggests that *Chrysosporium sp* tends to thrive in the proximity of the wastewater entry (Zone 1 and Zone 2), but its presence diminishes as you move away from the source (Zone 3).

- *Penicillium sp* shows a different distribution pattern. It is present near the wastewater entry (Zone 1) and at the farthest point (Zone 3), but it is absent in Zone 2.

- *Aspergillus sp1* is consistently present across all zones, indicating that it can tolerate a range of water quality conditions influenced by wastewater effluent.

- Similar to *Penicillium sp*, *Aspergillus sp2* is present near the wastewater entry (Zone 1) and at the farthest point (Zone 3), but it is absent in Zone 2.

- *Alternaria sp* is found in all zones, suggesting that it can survive and thrive across different areas affected by wastewater effluent.

- *Aspergillus sp3* is only present near the wastewater entry (Zone 1) and absent in Zone 2 and Zone 3.

- *Mucor sp* is only present at the farthest point from the wastewater entry (Zone 3) and absent in Zone 1 and Zone 2.

Overall, our results suggest that different species of fungi exhibit varying patterns of presence and absence in relation to the quality of water affected by wastewater effluent. Some species are more prevalent near the wastewater entry point, while others show a more dispersed distribution. These findings provide insights into how the fungi populations are influenced by the changing water quality resulting from the wastewater discharge.

In our study, the water quality and the sediment parameters, including pH, Electrical conductivity (EC), and total dissolved solids (TDS), for three different zones (Zone 1, Zone 2, and Zone 3) in "Sebkhat El Mahmal" seemed to be influenced by wastewater effluents.

The pH values gradually increase from Zone 1 to Zone 3. The EC shows a slight increase from Zone 1 to Zone 3, suggesting an increase in the concentration of dissolved ions in the water. Which impact sediment pH and Ec. The total dissolved solids exhibit some variation between zones, with Zone 1 having the highest TDS and Zone 2 and Zone 3 showing lower values. The pH and EC increase, and the decrease of TDS from Zone 1 to Zone 3 indicate the dilution of alkaline water in "Sebkhat El Mahmal by the wastewater effluents.

The pH is one of the most common and important measurements in routine chemical soil analysis, since it controls chemical and biological reactions in it. It can be affected by several factors such as the type and quantity of organic and inorganic constituents that contribute to the acidity of the sediment, the concentration of salts, etc... (**Böhme and Ziegler, 1969**).

The pH values are in the range of 6.5-9, a neutral to slightly alkaline pH that is ideal for

the growth and activity of keratinophilic fungi according to **Jain and Sharma (2011)**, and similar to the results found by **Da Silva *et al.*, (2009)** and **Pakshir *et al.*, (2013)** that proves the presence of keratinophilic fungi in sediment samples corresponding to pH values 7 to 9. This preference on the part of keratinophilic microorganisms for a slightly alkaline pH may be due to the fact that keratinolytic enzymes are produced at pH values from 6 to 9 and particularly extracellular keratinase is active at pH 9. Various works mention that keratinophilic fungi are not found in sediment with acid pH from 3 to 4.5; Some authors even report that sediment and soil with a pH of 5.9 are free of keratinophilic fungi (**Malek *et al.*, 2013; Pakshir *et al.*, 2013**).

According to **Lal (2004)** and **Mlitan *et al.*, (2015)**, pollutants from wastewater affects soil and sediment characteristics, including aeration, pH, salinity and organic carbon storage. They also supply the soil and sediment with nutrients, like nitrogen, which is an essential element for all microorganisms, including fungi; this explains the high number of keratinophilic colonies in Area 1 because it is the closest one to the outlet of wastewater. We found that *Alternaria SP* are the most adaptable fungus to the physical and chemical conditions of soil (temperature, pH, nutrients…) and to the source of keratin (hair or feathers), being present in the three areas, same results have been reported by **Saber *et al.*, (2010)** and **Kumari *et al.*, (2020)**. The second most prevalent keratinophilic fungus belongs to the genus *Aspergillus* (SP 1, SP 2 and SP 3), which is widespread and can be found in any environment, like soil, water, food, plants, and many other apparently common areas (**Ward et al., 2006**). They are hyaline filamentous fungi, which have conidiophores to which the spores are attached. They can grow in a wide temperature range from 0 ºC to 45 ºC. Some species are related to human skin infections and food poisoning (**Gugnani, 2000; Bonifaz, 2010; Martínez *et al.*, 2013**). The isolates collected in this study are yellowish to brown, which agree with results found in the literature (**Klich, 2002; Varga *et al.*, 2004**).

Chrysosporuim sp and some of its species can cause skin infections and onychomycosis in humans. Colonies are very similar to those of dermatophytes, growing moderately at 25ºC and some species can grow at 37ºC. We observed that the hyphae present septa and produce micro conidia that are born along the hyphae, similar to the results found by other researchers (**Rajendra, 2000; Dongyou, 2011**). Another common fungus we found is *Penicillium*, it can grow in a diverse range of habitats such as soil, vegetation, air, and various food products. Some species are associated with infections in humans, especially in immunocompromised

patients (**Duong 1996; Walsh et *al.*, 1999**). The genus is characterized by the development of erect conidiophores, usually branched, smooth or rough, hyaline or pigmented. Metulae arise from the ramifications and from the end of these the phialides that contain conidia in chains (**Mc Ginnis, 1980; Visagie et *al.*, 2014**). There is a field of wheat near the area three of the Sebkha, this explains why Mucors sp occurs only there, these species being known for a long time for their ability to grow on straw (**Sadasivan, 1993**).

Another crucial factor that affects the growth of these microorganisms is the salinity; it could be identified by the measure of electrical conductivity, which varies with the variation of the concentration of dissolved salts available in soil, a high conductivity indicates a high value of salinity. According to studies conducted by **Lozupone et *al.* (2007) and Auguet et *al.* (2010)**, soil salinity has been proven to be the most significant factor influencing the global distribution of soil microorganisms. However, there is a lack of information in literature on the effect of saline soils on the keratinophilic fungi growth and function. In our study, the values of electrical conductivity of the three areas are 13.9 $\mu s.cm^{-1}$, 6.6 $\mu s.cm^{-1}$ and 8 $\mu s.cm^{-1}$, respectively. These values are quite low, and it is hard to investigate its effects on fungal growth.

According to **(Simanjuntak *et al.*, 2020),** sediment organic matter content consists of 5 classes, namely: > 35% (very high organic matter content); 17-35% (high organic matter content); 7-17% (medium organic matter content); 3.5-7% (low organic matter content).

The presence and abundance of keratinophilic fungi in sediment can be influenced by the availability and quality of organic matter **(Ogórek *et al.*, 2022).** In our study higher concentrations of organic matter were measured in zone one (54.01 %) than in zone two (40.09 %) and zone three (25.72 %). In zone one, which is the entrance of wastewater to Sebkhat el Mahmal, could be with high inputs of hair, feathers, or other keratinous materials, may provide a favorable environment for the growth and colonization of keratinophilic fungi. Conversely, in the zone two and three with lower organic matter inputs, the abundance of these keratinophilic fungi may be lower.

Overall, the relationship between keratinophilic fungi and organic matter in sediment is symbiotic, with the fungi relying on the organic matter as a food source, and their activity contributing to the decomposition and recycling of organic materials in the ecosystem.

Conclusion

The soil is a rich source and a fundamental habitat for fungi. For some species, it is the place where they complete their biological cycle, for others it is just a temporary environment where they remain until they manage to reach their definitive niche. Fungi perform various functions in the ecosystem, they can be beneficial, by establishing mutualistic relationships, or harmful and by infecting humans, animals, and plants with diseases. Keratinophilic fungi are first-line decomposers, essential for the biological cycle of keratin and its derivatives, giving them a huge importance for treating wastewater and sewage. They are present in the environment with a variable distribution pattern, which depends on different factors that determine their presence; physicochemical characteristics of the soil and the region, the latter includes the climate, plants, animals and people. The main objective of this work is to study the diversity of keratinophilic fungi in Sebkhat El Mahmal as zone being polluted by wastewater.

A total of nine keratinophilic microorganisms were isolated from three areas of the Sebkha. Since the pH range of the soil samples was almost the same (6.76 to 7.83), a relationship between this and the distribution of the isolated microorganisms was not observed. The climate of the Sebkha was ideal for the growth of keratinophilic fungi. Greater diversity of keratinophilic microorganisms were observed in the soil samples from area 1. We noticed the number of the isolated species decrease the farther we move away from the mouth of wastewater and sewage.

For the samples grown on hair, considering the exception of Mucors SSP and the unknown colonies, area 1 has nearly all species, making it greater in variety and abundant. Area 2 and area 3 were almost similar in the number of fungal colonies, Aspergillus SP1, Alternaria SP, Chrysosporium SSP, and unidentified species were present in area 2, while Penicillium SSP1, Aspergillus SP1, Aspergillus SP2, Alternaria SP and Mucors SSP were in the last area.

As for the ones grown on feathers, we observe a convergence in the number of species for feather results. In region 1, we found three different species: Penicillium SSP, Alternaria SP, and an unidentified species. Furthermore, Chrysosporium SSP and Alternaria SP were found in region 2. Only one species, Alternaria SP, was discovered in region 3.

To conclude, our results shows and confirms the impact of wastewater on the biodiversity of keratinophilic fungi on Sebkhat el Mahmal, as a crucial factor that could directly affect the physicochemical characteristics of soil; like salinity, pH, concentration of nutrients and minerals and the percentage of organic matter, which as a result control the biodiversity of keratinophilic fungi.

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