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The impact of treated and untreated municipal wastewater on soil filamentous fungi.

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Dedication

I dedicate this work;

To my precious MOTHER, the source of tenderness
and Kind, who give me life, love, support and courage.
No dedication, no words, nothing can be enough to
express what you deserve for all the sacrifices you have
given me since my birth, thank you

To my precious FATHER, the support you have given me was a light
throughout my journey. Thank you for your love, your generosity, your
understanding, for everything you have done to me, no dedication, no words,
nothing can be enough to express the love and respect I have always for you.

Dear parents I wish that I make you proud of me, and find here
the result of long years of sacrifices and privations, may God keep my parents
and protect them.

To my dear BROTHERS and SISTER
Words are not enough to express my Thankful for your love and support and
encouragement during those years of study.

Special dedications to a SPECIALPERSON, who always support me, help
me; encourage me, and always being by my side.

To all my family a specially my GRANDFATHER, my GRANDMOTHER
and my AUNT. May God give them a long and joyful life.
And all FRIENDS I have known so far.
Thank you for your love and encouragement.
To my PROFESSORS and friends from the study.
To all those who contributed to the realization of this work
And finally, I dedicate this work to my SUPERVISOR
To thank him for all his efforts and help

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Abstract

The aim from this study is to investigate the effect of treated and untreated wastewater on the biodiversity of filamentous fungi in an agricultural soil planted with wheat. To evaluate the impact of wastewater on soil filamentous fungi communities, we isolated and identified species in treated and untreated municipal wastewater before it is used for irrigation to examine the possibility of input of exogenous filamentous fungi. Our experiment was carried out with 5 groups: Soil irrigated with mineral water (control); Soil irrigated with untreated municipal wastewater; Soil irrigated with treated municipal water, also we isolated and identified species in treated and untreated municipal wastewater.

Overall, our results showed that untreated municipal wastewater did not add any exogenous filamentous fungi to the soil. In the control group, where the soil was irrigated with distilled water, we identified five taxa: **Micor sp**, **Aspergillus niger**, **Alternaria sp**, **Pythium dissimile**, and **Geomyces sp**. The same taxa were found in soil irrigated with untreated municipal wastewater, but the genus **Geomyces sp** was not found. However, in sol irrigated with treated municipal wastewater, we identified six taxa; four of them were observed in control soil; **Micor sp**, **Aspergillus niger**, **Alternaria sp**, **Pythium sp**, and the other two: **Rhizopus nigricans** and **Absidia sp** were not.

Our results demonstrate that the irrigation with untreated wastewater has no impact on the diversity of filamentous fungi in agricultural soils in the region of Tébessa. The soil already seems poor in filamentous fungi and also poor in organic matter, indicating that these soils could not be fertile. Farmers must work hard to correct the structure and texture of these soils by adding organic matter and crop rotation and cultivating the soil with other crops besides wheat.

Keywords: Filamentous fungi, Soil, Treated wastewater, Untreated wastewater, Tebessa

Résumé

L'objectif de cette étude est d'étudier l'effet des eaux usées traitées et non traitées sur la biodiversité des champignons filamenteux dans un sol agricole planté de blé. Pour évaluer l'impact des eaux usées sur les communautés de champignons filamenteux du sol, nous avons isolé et identifié des espèces dans les eaux usées municipales traitées et non traitées avant qu'elles ne soient utilisées pour l'irrigation afin d'examiner la possibilité d'apport exogène de champignons filamenteux. Notre expérience a été réalisée avec 5 groupes : Sol irrigué avec de l'eau minérale (témoin) ; Sol irrigué avec des eaux usées municipales non traitées ; Sol irrigué avec de l'eau municipale traitée, Nous avons également isolé et identifié des espèces dans les eaux usées municipales traitées et non traitées.

Dans l'ensemble, nos résultats ont montré que les eaux usées municipales non traitées n'ont pas ajouté de champignons filamenteux exogènes au sol. Dans le groupe témoin, où le sol était irrigué avec de l'eau minéral, nous avons identifié cinq taxons : **Micor sp**, **Aspergillus niger**, **Alternaria sp**, **Pythium dissimile** et **Geomyces sp**. Les mêmes taxons ont été trouvés dans le group irrigués avec des eaux usées municipales non traitées, mais le genre **Geomyces sp** n'a pas été trouvé. Cependant, dans le groupe irrigué avec des eaux usées municipales traitées, nous avons identifié six taxons ; quatre d'entre eux ont été observés dans le sol témoin; **Micor sp**, **Aspergillus niger**, **Alternaria sp**, **Pythium sp** et les deux autres : **Rhizopus nigricans** et **Absidia sp** ne l'étaient pas.

Nos résultats démontrent que l'irrigation avec des eaux usées non traitées n'a pas d'impact sur la diversité des champignons filamenteux dans les sols agricoles de la région de Tébessa. Le sol semble déjà pauvre en champignons filamenteux et également pauvre en matière organique, indiquant que ces sols ne pourraient pas être fertiles. Les agriculteurs doivent corriger la structure et la texture de ces sols en ajoutant de la matière organique et rotation agricole et en cultivant le sol avec d'autres cultures que le blé.

Mots clés : Champignons filamenteux, Sol, Eaux usées traitées, Eaux usées non traitées, Tébessa

ملخص

تهدف هذه الدراسة إلى معرفة تأثير السقي بمياه الصرف الصحي المعالجة وغير المعالجة على التنوع البيولوجي للفطريات الخيطية في تربة زراعية مزروعة بالقمح. لتقييم تأثير مياه الصرف الصحي على المجتمعات الفطرية الخيطية في التربة قمنا بعزل والتعرف على الفطريات الخيطية الموجودة في مياه الصرف الصحي المعالجة والغير معالجة قبل استخدامها للري لفحص إمكانية إضافة فطريات خيطية غير موجودة في التربة. تم إجراء تجربتنا على 5 مجموعات: تربة مسقية بالمياه المعدنية) المجموعة الشاهدة(, تربة مسقية بمياه الصرف الصحي الغير معالجة؛ تربة مسقية بمياه الصرف الصحي المعالجة، كما قمنا بعزل والتعرف على الفطريات الخيطية الموجودة في مياه الصرف المعالجة وغير المعالجة.

بشكل عام، أظهرت نتائجنا أن مياه الصرف الصحي الغير معالجة لم تضيف فطريات خيطية خارجية إلى التربة في دراستنا. في المجموعة الشاهدة، حيث تم ري التربة بالماء المعدني، تم التعرف على خمسة أصناف **sp Micor:** و **Aspergillus niger** و **sp Alternaria** و **Dissimile Pythium** و. **sp Geomyces** تم التعرف على نفس هذه الأصناف في التربة المسقية بمياه الصرف الصحي الغير معالجة، ولكن لم يتم العثور على جنس **sp Geomyces** ومع ذلك في التربة المسقية بمياه الصرف الصحي المعالجة، لاحظنا تواجد ستة أصناف أربعة منهم تم التعرف عليهم في المجموعة الشاهدة **Micor sp** و **Aspergillus niger** و **Alternaria sp** و **Pythium sp** و الاثنان الآخران : **Rhizopus nigricans** و **sp Absidia** و لم يتم ملاحظة تواجدهم.

تظهر نتائجنا أن الري بمياه الصرف الصحي الغير المعالجة ليس له تأثير كبير على تنوع الفطريات الخيطية في التربة الزراعية في منطقة تبسة. تبدو التربة في منطقة الدراسة فقيرة فيما يخص التنوع البيولوجي للفطريات الخيطية كما أنها لا تحتوي على كمية ملائمة فيما يخص المواد العضوية، مما يشير إلى أن هذه التربة لا يمكن أن تكون خصبة. يجب على المزارعون تصحيح بنية هذه التربة عن طريق إضافة المواد العضوية و القيام بعملية المناوبة و تنويع المحاصيل وزراعة التربة بمحاصيل أخرى غير القمح .

الكلمات المفتاحية: الفطريات الخيطية، التربة، مياه الصرف الصحي المعالجة، مياه الصرف الصحي غير المعالجة ، تبسة

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List of abbreviations

FAO: Food and Agriculture Organization

WHO: World Health Organisation

EPA: US Environmental Protection Agency

pH: potential of hydrogen

CO_2: Carbon dioxide

C:N: The carbon-to-nitrogen ratio

NTU: Nephelometric Turbidity Units

SS: suspended solids

EC: electrical conductivity

SAR: sodium absorption rate

BOD: biological oxygen demand

COD: chemical oxygen demand

N: nitrogen

P: phosphate

K: potassium

Ca: calcium

Mg: magnesium

B: boron

Fe: iron

Mn: manganese

Zn: zinc

ANAT: Australian Network for Art and Technology

UAA: useful agricultural area

PS2': strategic proposal n 2

TSS: total suspended solids

PDA: potato dextrose agar

km^2: Square kilometer

%: percent

mm: millimeter

P (mm): precipitation per millimeter

Cm: centimeter

ms/cm: milli Siemens per centimeter

mg/L: milligramme per liter

°C: degree Celsius

w/v: weight per volume

h: hour

µL: microliter

Introduction

Introduction

Soils are very complex, having numerous constituents performing different functions mainly due to the activity of soil organisms **(Kostadinova et al., 2009)**. Soil microorganisms play a significant role in the ecosystem **(El-Enazi et al., 2018).** Soil quality is determined by the microbial composition and function changes during the decomposition of organic matter, recycling of nutrients and biological control **(Stefanis et al., 2013).**

Reusing treated wastewater, especially for irrigation, is becoming more common, governments and other official bodies worldwide are encouraging it **(Becerra-Castro, 2015).** Wastewater is produced as a result of multiple human activities, such as domestic, commercial, and industrial uses. The municipal wastewater composition depends on many factors: the number of inhabitants and the type of their activity, industrial development, location of point pollution sources, level of water consumption, day of the week and season, or even the design of the sewer and the treatment systems **(Becerra-Castro, 2015)**. Irrigation with wastewater may have implications at two different levels: alter the physicochemical and microbiological properties of the soil and/or introduce and contribute to the accumulation of chemical and biological contaminants in soil. The first may affect soil productivity and fertility; the second may pose serious risks to human and environmental health **(Becerra-Castro, 2015).**

Fungi are of fundamental importance and vital for the terrestrial environment's soil ecosystem; they play a key role in different essential processes, including elemental release by mineralization and organic matter decomposition **(Al-Enazi, 2018).** As well as elemental cycles and their activity contributes to the bio-deterioration and biodegradation of toxic substances in the soil. Fungi are often dominant in soil and comprise the largest pool of biomass (including other microorganisms and invertebrates). They also play a role in maintaining soil structure due to their filamentous growth habits and exopolymer production. Despite their important roles in the biosphere, fungi are frequently neglected within the broader environmental and microbiological spheres **(Geoffrey, 2007).**

Investigators of soil fungi encounter considerable problems in qualitative and quantitative studies because the fungi exist in the soil in a variety of morphological and physiological states (e.g., active, dormant, and even dead hyphae), various types of spores, and resting structures. It is important to assess the active hyphal fungi in soil or organic matter samples; but, despite some progress, this is still a challenging task **(Parkinson, 1983).**

In general, the success of fungi in reaching and colonizing a patch of soil is mainly due to their competitive saprophytic ability; expressed by fast mycelia growth, spore production, possession of an efficient and extensive system of powerful enzymes, and tolerance to antibiotics, salinity, heavy metals, soil pollution, fungicides, and temperature. The diversity of soil fungi depends on the soil's chemistry, texture, and water-holding capacity. Physical factors such as temperature, pH, organic matter, and water quality used for irrigation also greatly influence microbial life **(Azevedo, 2010).**

In this context, this study was carried out to investigate the effects of treated and untreated municipal wastewater on the biodiversity of filamentous fungi in agricultural soil planted with wheat.

Our manuscript is organized as follows:

Chapter I present a synthesis of knowledge on filamentous fungi and the impact of wastewater on soil microbial communities.

-Chapter II: Materials and Methods

-Chapter III: Results and discussion

- Conclusion and perspectives

Chapter I:

Literature Review

1. Filamentous fungi

Fungi are a group of eukaryotic organisms that can be unicellular or multicellular, they include microorganisms like molds, yeasts, and mushrooms. They live on dead or living plants or animals' tissue; they are the primary decomposers of substances in the ecological system. Fungi are tremendous decomposers of organic waste material and most readily attack cellulose, lignin, gums, and other organic complex substances. Those organisms can also act under a wide range of soil reactions, from acidic to alkaline soil reactions **(Yuvaraj and Ramasamy, 2020).**

The term "filamentous fungi' is used for species producing filament like hyphae. It includes almost the entire fungal kingdom and is used in contradistinction to "yeasts," which are essentially unicellular fungi with vegetative cells capable of repeated budding **(Hawksworth et *al.*, 1988).**

2. Characteristics of filamentous fungi

Fungal cells are characterized by an extraordinary ability to secrete large amounts of proteins, metabolites, and organic acids into their growth medium. The fact that fungi are capable of surviving and, indeed thriving in extreme environments is an indication of their potential to withstand stresses imposed by perturbations in the environment **(Dighton, 2003).**

Filamentous fungi characterized by:

- **Hyphae:** Is a filamentous assemblage of tubular cells in which continuity is maintained between adjacent cells by the absence of cross cell walls (septa) or a septum perforated by a pore. The hyphae thus develop as a coenocytic structure, consisting of continual cytoplasmic connectivity between adjacent cells. Hyphae average 5– 6 mm in diameter and grow by wall extension at the tip (Fig 1) **(Hunsley, 1970).**

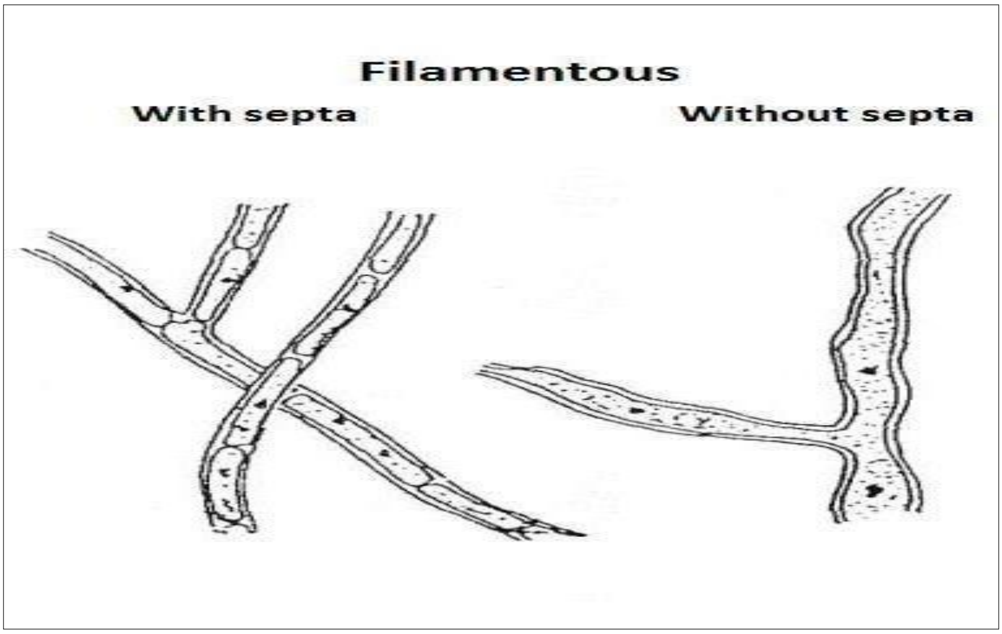

Fig 1. Filamentous fungi **(Kendrick, 1985)**

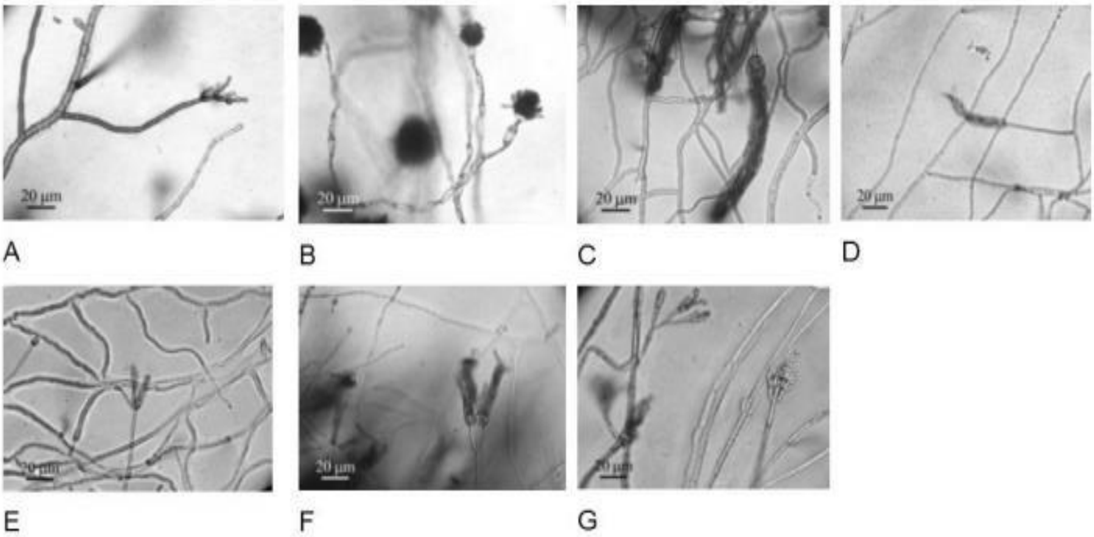

Fig. 2. Filamentous fungus *(A:Cladosporiumsp.; B: Aspergillus fumigatus; C to G :Penicilliumsp.)***(Pinto et *al.*, 2012).**

- **The fungal cell wall:** Although the chemical composition of cell walls can vary considerably between and within different groups of fungi, it consists of a structural scaffold of fibres which are cross linked, and a matrix of gel-like or crystalline material **(Hunsley, 1970).** It plays a significant role in development and integrity of the fundamental architecture required for survival and proliferation of the filamentous fungus. Also, the cell wall protects the fungus from abrasion, screens out poisons, and, by restricting inflation of the cytoplasm, allows the cell to become highly pressurized. It also serves as a scaffold for proteins that protect the inner polysaccharide layers and provide a dynamic interface with the surroundings **(Webster, 2007).**

- **Spores of fungi:** The reproduction by means of small spores is a cornerstone in the ecology of fungi. Spores may be organs of sexual or asexual reproduction, and they are involved in dispersal and survival. Many spores are dispersed passively by the action of gravity, air or water currents, rain splash, or by animals, especially insects. Dispersal may also occur by human traffic **(Webster, 2007).**
- **Nuclei**: Interphase nuclei in filamentous fungi are structurally similar to those in other eukaryotic organisms. They have a nuclear envelope perforated by numerous nuclear pore complexes enclosing a ribosome-free nucleoplasm packed with chromatin of varying densities and containing one or two nucleoli **(Borkovich, 2010).**
- **Mitochondria:** Like that of nuclei, the structure of fungal mitochondria is similar to that of other eukaryotic organisms. They are bound by a double-unit membrane with the internal membrane having a much increased surface area that is manifested in elaborate invaginations (i.e., cristae) that extend into the mitochondrial matrix. Fungal mitochondria differ from other eukaryotes in that their shape is more elongate, they are larger organelles, and their plate-like cristae are arranged as lamellae that are parallel to the organelle’s long axis. Mitochondria occur throughout the hyphal tip cytoplasm **(Borkovich, 2010).**
- **Vacuole:** is a dynamic organelle, enclosed by a single membrane, with a variety of structural forms: small vesicles, tubules, and large vesicles. It is an acidic compartment as a consequence of the activity of the vacuolar H-translocating ATPase (VATPase) in the membrane. It contains high concentrations of basic amino acids, polyphosphate, hydrolytic enzymes, and divalent cations **(Borkovich, 2010).**
- Photosynthetic pigments absent **(Borkovich, 2010).**
- Fungi associated with a range of plant and animal species. They occur in a variety of environments **(Wainwright, et al., 1997)**. Fungal pathogens of animals are capable of directly influencing faunal populations by causing the death of individuals or by reducing or increasing their fecundity **(Dighton, 2003)**.

3. Fungi lifestyle

The majority of fungi have a filamentous lifestyle. The evolution of the hypha has been pivotal to the success of filamentous fungi and in determining the uniqueness of their lifestyle. It has also had important consequences in determining the modes of morphogenesis of filamentous fungi, and how they operate as non-motile, heterotrophic organisms **(Read, 1994)**. Filamentous fungi in nature take the form of hyphae spreading upon or penetrating a substratum. If extension of such hyphae occurred far behind the apex then friction between hyphae and substratum would make advance difficult and lead to buckling, distortion and damage to the hypha. Such problems do not occur, as extension is confined to the apical region, ceasing when the hypha attains its full width. An understanding of hyphal growth hence requires a consideration of the form and ultrastructure of the extension zone and of events occurring in the apical region **(Michael et *al.*, 2001)**.

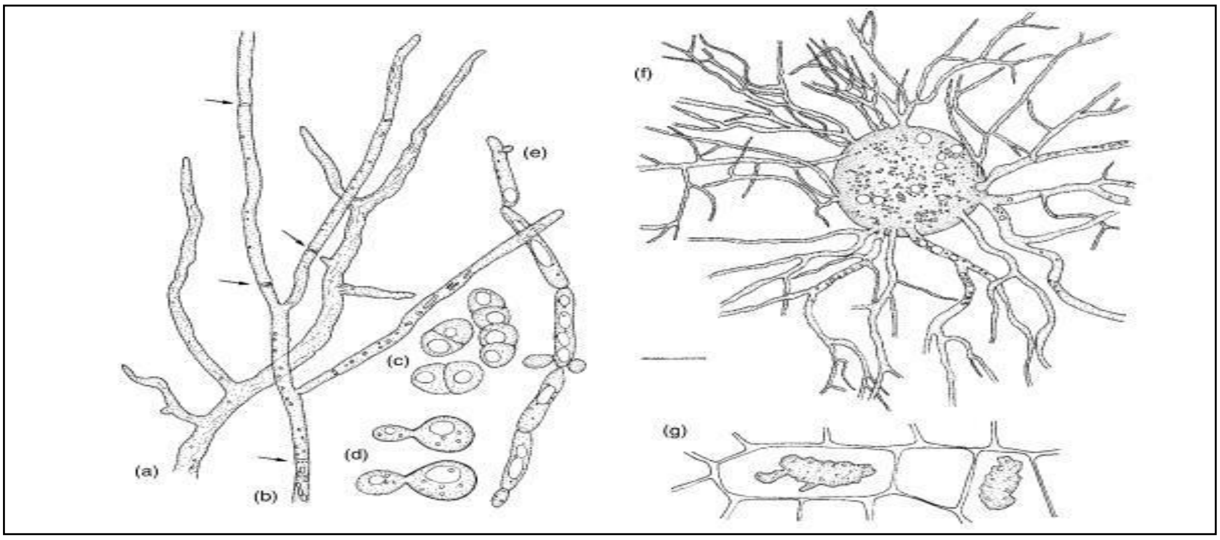

Fig.3. various growth forms of fungi (Webster 2007)

(a) Aseptate hypha of *Mucormucedo*(Zygomycota)
(b) Septate branched hypha of *Trichoderma viride* (Ascomycota).
(c) Yeast cells of *Schizosaccharomycespombe* (Ascomycota) dividing by binary fission.
(d) Yeast cells of *Dioszegiatakashimae* (Basidiomycota) dividing by budding.
(e) Pseudohypha of *Candida parapsilosis* (Ascomycota),
(f) Thallus of *Rhizophlyctisrosea* (Chytridiomycota)
(g) Plasmodia of *Plasmodiophorabrassicae* (Plasmodiophoromycota).

In nutrient-poor environments, fungal hyphae adopt a searching strategy, forming fast effuse growth with a low hyphal density. On substrates with high nutrient availability the same fungus adopts a slow, dense pattern of hyphal growth as the hyphae utilize the resources availablewith photosynthetic pigments being absent; fungi have a heterotrophic mode of nutrition. Fungi obtain their nutrients by extracellular digestion due to the activity of secreted enzymes, followed by absorption of the solubilized breakdown products (Fig.02), which together make up the mycelium **(Webster, 2007)**.

4. Biodiversity of filamentous fungi in soil

About 80 000 to 120 000 species of fungi have been described to date, although the total number of species is estimated at around 1.5 million **(Kirk, 2001)**.This would render fungi one of the least-explored biodiversity resources of our planet. Fungi exist as a variety of functional groups, and are associated with a range of plant and animal species **(Webster, 2007)**. Fungi constitute an important component of the ecosystem. Fungi have been found in all the major ecosystems of the world and have been seen to play a large variety of roles **(Dighton, 2003)**.Soil is a primary source of fungal growth, and is associated with the roots of all plant species. Fungi produce a wide range of bioactive metabolites, which can improve plant growth **(Muthuraman and Murugaragavan, 2020).**

5. Role of filamentous fungi in soil and environment

The oldest established uses of filamentous fungi are those concerned with food for man. Remarkably, not more than 20 species are currently exploited commercially (e.g. *Agaricusbrunnescens*, 'mushrooms'; *Lentinula edodes*, 'shii-take'; *Tricholomamatsutake*, 'matsu-take'; *Volvariellavolvacea*, 'padi straw mushroom **(Hawksworth, 1988).**

5.1. Role of filamentous fungi in soil

- **Fungi in Rock Breakdown:** Fungi alone produce organic acids that are capable of breaking down rock. Yeasts and filamentous fungi, such as *Aspergillus niger*, alone are involved in rock solubilization, releasing cations from amphibolite, biotite, and orthoclase. **(Ascaso and Wierzchos, 1995)**.*Penicillium* and yeasts were also found to be able to dissolve calcium-rich rocks, such as limestone, marble, and calcium phosphate **(Chang andLi, 1998)**.
- **Fungi in symbiotic association:** with plant roots, play a role in the dissolution of parent rock material in more established soils. Sometimes fungi alone are capable of this activity, but often it is an evolved partnership between the fungi and bacteria that work in a consortium. **(Dighton, 2003).**
- **Keeping Soils Together:** The role of fungi in these communities of bacteria, algae in lichen symbioses, is to physically hold the mineral soil particles together. The fungal hyphae penetrate between the soils mineral particles and act as a web to physically retain soil particles. The role of fungi and bacteria in the formation and stability of soil aggregates is of fundamental importance to both the fertility of soil and carbon storage and sequestration within soils **(Dighton, 2003)**.

- **Fertility:** Dead plant parts (above- and below-ground) are returned to the soil, where the activities of bacteria, saprotrophic fungi, and soil fauna degrade the complex organic components. They utilize the carbon skeletons for energy, these organisms rely on extracellular enzymes to degrade the complex organic molecules contained in the litters. **(Dighton, 2003)**.

- **Immobilization and movement of nutrients:** During the course of decomposition, the element is not in a soluble form in the soil solution, but is immobilized in microbial tissue. The amount of accumulation within the fungal component varies among ecosystems, depending on the chemical composition of the plant parts available for decomposition and the main fungal groups involved in the process. Evidence for elevated nutrient concentrations in fungal tissue above that of the underlying substrate. Their measures of nutrient content of fungal fruit bodies the duration of immobilization of those nutrients into fungal hyphae before translocation to the fruit body, however, could be an important aspect of the control fungi have on the rates and timing of release of pulses of nutrients within the ecosystem **(Dighton, 2003)**.

5.2. Role of Fungi in the environment

The importance of fungi contribution to primary production in forested ecosystems contributes only some 1% of total ecosystem biomass, the percentage of net primary production represented by fungi was 14 – 15% (or 45% in young forest stands and 75% in mature stands) when combined with the fine root biomass supporting the fungal tissue**(Vogt et *al.*, 1982).**

- **Fungi and climate change:** As a result of industrialization, increased use of automobiles, consumption of fossil fuels, atmospheric carbon dioxide concentrations are continuing to increase. The effect of increased availability of CO_2 could increase the C:N ratio of plant litter, making the role of fungi as saprotrophs more important. Fungi diversity thus will be maintained and favor those species capable of producing enzymes for the acquisition of major nutrients from organic sources **(Dighton, 2003)**.
- **Fungi and nitrogen in the environment:** Fungal biomass correlated negatively to nitrogen leaching, but this is not necessarily a causal relationship. Increased fungal biomass is not only a consequence of reduced fertilization, but also a cause of reduced nitrogen losses to the environment **(Franciska, 2009)**

- **Table 01:** Ecosystem services provided by fungi **(Dighton, 2003)**.

Ecosystem service		**Fungifunctional group**
Soil formation	Rock dissolution	Lichens, Saprotrophs, Mycorrhizae
Providing fertilityfor primary production	Particle binding Decomposition or organic residues Nutrient mineralization Soil stability (aggregates)	Saprotrophs, Mycorrhizae Saprotrophs, (Ericoid and ectomycorrhizae) Saprotrophs,(Ericoid and ectomycorrhizae) Saprotrophs, (Arbuscular mycorrhizae)
Primary production	Direct production Nutrient accessibility Plant yield Defenseagainstpathogens	Lichens Mycorrhizae Mycorrhizae, pathogens Mycorrhizae, Endophytes, Saprotrophs
Plant community Structure Secondary production	Defense against herbivory Plant – plant interactions As a food source Population/biomass regulation	Endophytes Mycorrhizae, pathogens Saprotrophs, Mycorrhizae Pathogens
Modification of pollutants Carbonsequestration and storage		Saprotrophs, Mycorrhizae Mycorrhizae, (Saprotrophs)

5.2.The use of fungi in sludge treatment

Certain Filamentous Fungi species are reported to have several notable advantages in the sludge treatment. The effect of fungal potentiality can increase the degradability, settleability and dewater ability of wastewater sludge and its contributions accelerate the sludge management strategy for future research and application. However, the role of Filamentous Fungi in sludge treatment for improving sludge settling, dewatering and degradation is not well established **(Moret *al.*, 2010).**

6. The use of wastewater in irrigation

In general, standards are based on the evaluation of physicochemical and microbiological parameters. Physicochemical characterization of wastewater includes the evaluation of several properties such as turbidity [Nephelometric Turbidity Units (NTU) or suspended solids (SS)], acidity (pH), salinity [electrical conductivity (EC),sodium absorption rate (SAR)],organic load [biologi-cal (BOD) or chemical oxygen demand (COD)], and nutrients[total N and/or NO_3^- and P in form of PO_4^{3-} phosphate]. The microbiological characterization of wastewater is mainly focused on the presence of potential human pathogens and parasites, and is generally based on the enumeration of faecal indicators and nematode eggs. Irrigation with wastewater may have implications at two different levels: alter the physicochemical and microbiological properties of the soil and/or introduce and contribute to the accumulation of chemical and biological contaminants in soil. The first may affect soil productivity and fertility; the second may pose serious risks to the human and environmental health. The impact of wastewater irrigation on soil ecosystem services, which relies on an adequate equilibrium of diversity and activity of soil microbiota, crucial for soil health, may be not properly covered by the available guidelines (**Becerra-Castro, 2015)**.

7. The impact of wastewater on soil microbial communities

Soil microbial communities are established on basis of a complex net- work of interrelations between abiotic (physical and chemical soil properties) and biotic factors (macro- and microbiological soil components). The impact of wastewater on soil microbial communities is supposed to depend on direct inputs of exogenous microbiota, which, in an improbable worst case scenario, would lead to the elimination of autochthonous microorganisms; the indirect effects produced by wastewater, which may contribute to change the physicochemical soil properties and, therefore, induce microbial community disturbances(**Becerra-Castro, 2015)**.

7.1. Physicochemical soil properties

According to **Becerra-Castro (2015)** the physicochemical soil parameter (PH, Organic matter,Nitrogen, phosphorus, Salinity)seems to be an important determinant of the richness (number of different species) and diversity (variety of organisms) of soil bacterial communities.

- **pH:** Variations on pH can influence the solubility of different soil components, such as metals. The increase of free metals in soil irrigated with wastewater was related to a decrease of soil pH. In turn, the concentration and availability of metals have the potential to affect the microbial communities. This parameter seems to be an important determinant of the richness (number of different species) and diversity (variety of organisms) of soil bacterial communities. **(Becerra-Castro, 2015).**

- **Organic matter:** Soil organic matter is essential as nutrient reservoir and in soil Structure. The organic matter-related pools are amongst the soil properties most affected by wastewater irrigation. Effects on soil aggregate stability and water retention capacity; modify soil microhabitats and, hence, influence the soil microbial communities. **(Becerra-Castro, 2015).**
- **Nitrogen, phosphorus and other plant nutrients:** The availability of plant nutrients such as N, P or K is essential for plant growth. One of the advantages of wastewater irrigation is the supplying of nutrients; wastewater can also be a source of other macro and micronutrients such as Ca, Mg, B, Mg, Fe, Mn or Zn. Both N and P contents may impact soil microbial communities, in particular the activity associated with the cycling of these elements. The excessive provision of nutrients in soil such as available P and nitrate may be leached into the surface and groundwater, causing eutrophication or toxicity in other habitats. The excess of nutrients can also disturb the autochthonous soil microbial communities. **(Becerra-Castro, 2015).**
- **Salinity:** wastewater irrigation may promote soil Stalinization (increase of soluble salts concentration) or sodification (increase of sodium ions relative to other cations). In turn, Stalinization and sodification are also associated with the increase of electrical conductivity. These are the most commonly reported negative effects on microbial communities caused by wastewater irrigation (reduce fungal and bacterial counts). **(Becerra-Castro, 2015).**
- **Contaminants:** Wastewater transports different types of contaminants (e.g., metals, organic micropollutants), which through irrigation may accumulate in soil. These contaminants may diffuse or propagate to the surrounding environment and in soil may hinder its fertility and/or disturb the microbial communities; and consequent effects on plant growth and/or contamination, metals may also disturb the autochthonous microbial communities (e.g. reduction of microbial bio- mass or alteration of the community structure **(Becerra-Castro, 2015).**

7.2. The effect of wastewater on soil fungi

Wastewater possesses different biological, physical and chemical effects on the environment and especially on fungi, the soil irrigated with wastewater continent many of elements including heavy metals are required by living organisms for their normal function, these elements considered one of the most harmful industrial pollutants in the environment also at high concentrations, they become toxic **(Mlitan, 2015)**. The fungal population has the ability to resist higher concentrations of metals **(Iram et *al.*, 2013)**.Which means that some fungi are able to grow in the presence of heavy metals **(Zafar, et *al.*, 2006).** Fungi diversity is low in the soil irrigated with wastewater and this lower take to a diminution of enzymes; decomposition of the complex organic components; immobilization and movement of nutrients; the fertility of soil; and keeping soils together (aggregate), that's means reduction on the role of fungi in the soil.Wastewater without treatment may have nitrogen and is mostly present in the form of NH4. Nitrogen is an essential nutrient for biological growth. It is one of the main components in all living organisms such as plants and microorganisms such as fungi **(Mlitan et al., 2015).**

Chapter II:

Materials and methods

1. Geographical Situation

Tebessa, a border town with Tunisia, is located in in the northeast of Algeria **(Fig.3)**. The plant cover of the Tebessa generally consists of forests, scrubland and reforestation; the rest of the surface is divided between agricultural land and land unproductive. The development of agriculture is strongly compromised by the characteristics of the soil, flows, and the intensity of human action. The forests are mainly populated by Aleppo pine, a species that best adapts to climatic conditions of the region, and does not require much water (200 mm/year) but requires limestone soil **(Mihi et *al.*, 2019)**.

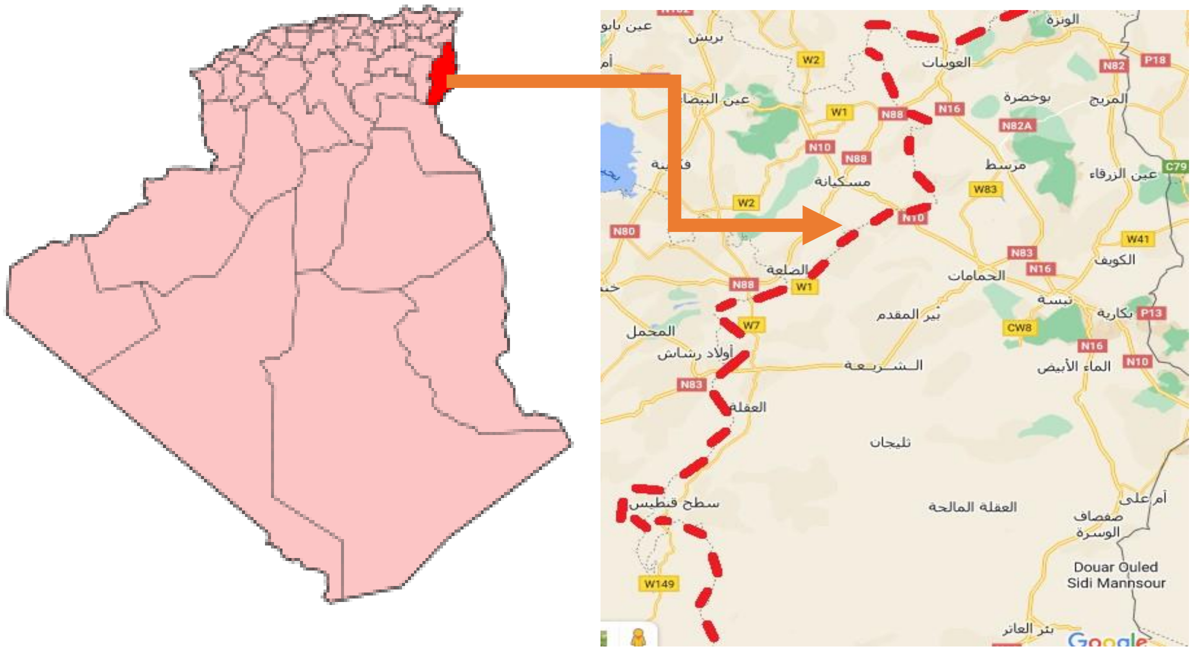

Fig.04: Geographical Situation of the wilaya of Tebessa.

2. Climate and rainfall

Tebessa climate is semi-arid Mediterranean with hot and dry summers from October to May and mild winters with low rain from June to September. According to long-term meteorological data of Tebessa climatic station (1984–2013), the average annual temperature is 15.84 °C. The mean annual rainfall is 379.77 mm. The wettest month is September (43.41 mm), while the driest month is July (14.37 mm) **(Djellab et *al.*, 2019).**

The Wilaya of Tebessa is characterized by a mild semi-arid climate in its northern part and a Saharan climate in its southern part. Geo-climatic heterogeneity divides the territory schematically into three zones: the northern mountainous zone with a mild semi-arid climate, the middle steppic zone with a semi-arid climate and the southern Saharan zone. This zoning, carried out by the ANAT, is essential to take into account when developing development strategies because each area has its own wealth and specific needs. The aridity of the climate, the frequency of periods of drought makes the water network sparse and the quantity of water available remains insufficient **(Boubir, 2017).**

Table 02. Summary of surface area, percentage, and description of precipitation in the Wilaya of Tebessa **(Boubir, 2017).**

Factor	Classes	Description	Area (km^2)	(%)
Precipitation (mm/year)	[104–229]	Slight	3113.73	23.48
	[230 354]	Moderate	3510.24	26.47
	[355 478]	High	6121.38	46.16
	[479–604]	Very high	515.86	3.89

Monthly average precipitation: Based on data collected at the Tébessa station for twenty-one years from 1997-2018, the monthly average precipitation values are represented in the following table:

Table 03: Monthly average precipitation in (mm) of Tebessa over the 21-year period (1997/1998-2017/2018**) (Boubir, 2017).**

Month	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
P (mm)	46,4	38,64	33,75	33,49	32,94	22,28	31,75	37,68	47,36	26,15	15,94	28,56	394.98

The rainfall variations of the months (September – August) of the station of Tebessa; indicate that the maximum value is marked in the month of May with an average of 47.36 and a minimum value for the month of July with an average of 15.94**(Chenatlia and Hamaili, 2020).**

3. Agriculture

The territory of Tebessa was classified in zone V: «Zone of the steppe and the Saharan Atlas», it was characterized by the weakness of the densities of settlement, the modest crop agricultural production, the absence of industrial activity, fairly basic road infrastructure, low urbanization and the scarcity of urban areas, some mining operations are its main assets. The total agricultural area of the country is estimated at 134,9713 hectares, but only 27% is the useful agricultural area (UAA). Cereals account for more than 48% of the UAA with a yield of 10 quintals/hectare. Fodder crops account for only 16% of the UAA and their production has reached a yield rate of 8Quintaux/hectare despite the effects of the cereal producer price policy which has prompted the farmer to move towards more lucrative wheat production to the detriment of other more strategic speculations. The importance of fodder crops is linked to the development of sheep rearing and livestock, which are the main source of wealth for Tebessa. Vegetable crops occupy only 1% of the UAA and are dominated by potato production with a yield of 340 quintals/hectare. The development of agriculture is hampered by the low mobilization of irrigation potential and agricultural companions are often affected during periods of drought **(Boubir, 2017).**

The most important economic activity of the area is agriculture. Large amounts of synthetic nitrogen fertilizers, such as urea 46%, ammonium nitrate 33.5%, liquid fertilizer N-32%, or commercial complex fertilizers with different proportions (%) of nitrogen, phosphorous, and potassium [(N=15, P_2O_5=15, K_2O= 15), (N=8, P_2O_5=15, K_2O=15), and (N=9, P_2O_5=18, K_2O=28)] are applied during farming season (summer and autumn) **(Rouabhia et *al.*, 2009).**

Table 04: Summary of surface area, percentage, and description of land use in the Wilaya of Tebessa **(Rouabhia et *al.*, 2009)**.

Factor	Classes	Description	Area (km^2)	(%)
Land use	Forest and maquis	Dense vegetation	1963.99	14.81
	Pastures and steppes	Sparse vegetation	7405.07	55.84
	Agriculture and arboriculture	Farmland	3815.25	28.77
	Urban zone and bare soil	Bare land	76.92	0.58

4. Samples collection

Surface soil samples not exceeding 10 cm depth, used in this study were collected from agriculture areas in the department of TEBESSA **(35°24'18.82"N N and 8° 3'58.11"E)** in the North-East of Algeria **Fig 04.** Three areas of one 1 M^2 were irrigated separately at 50% of field capacity for 45 a days (ones every 7days) by treated, untreated municipal wastewater, and mineral water as a control group. The sampling took place on 27 February 2022. Three samples were collected at different locations (A, B and C) **Fig 05** and **Fig 06.** The soil was collected with a pre-sterilized trowel. The samples were stored in sterile zipper polythene bags at 4 ˚C until used. According to the farmer who owns the agricultural land, the farmland sol is irrigated with only with rain water planted by wheat, no pesticides or herbicide were used in the field during the last 6 year of exploitation. Nitrogen fertilizer (urea or ammonium sulfate) were used after wheat cultivation. Treated municipal wastewater used for this study consisted of tertiary treated municipal wastewater.

Fig.05: localization of agricultural land

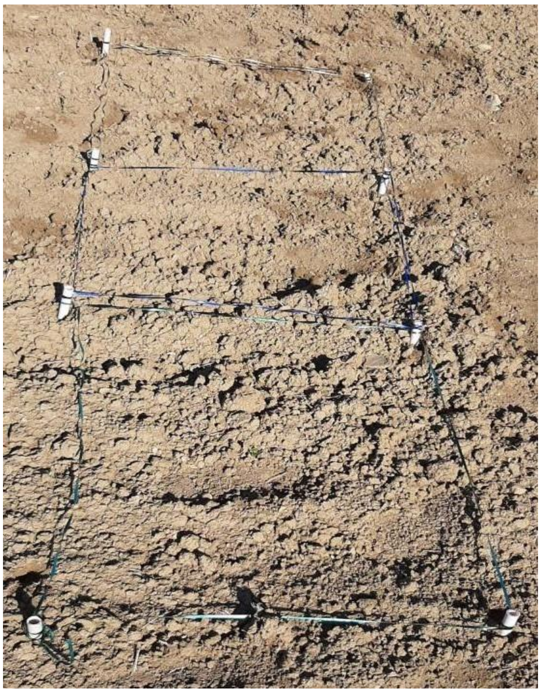

Fig. 06: Three areas of one 1 M^2 were irrigated for 45 a days (ones every 7days)

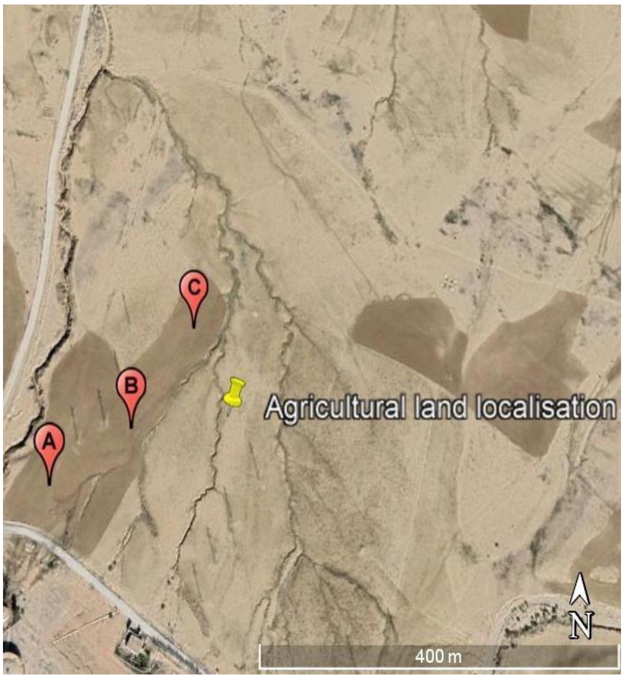

Fig 07: locations of the collected samples (A, B and C)

5. Physico-chemical parameters of treated and untreated wastewater

The physico-chemical parameters of treated and untreated wastewater were provided by the national sanitation Office (ONA), Annaba Zone.

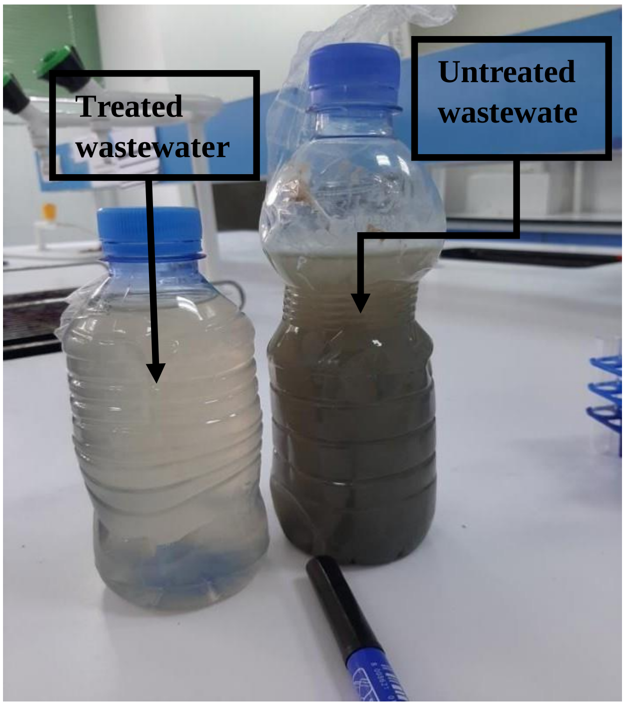

Fig.08: Treated and untreated wastewater samples

Table 05: Characteristics of treated and untreated wastewater (ONA, 2021)

Parameters	Waste water	Treated waste water
EC [ms/cm]	3,20	2,98
pH	7,46	7,50
TSS [mg/L]	117.67±9,37	42.8±2,51
BOD_5 [mg/L]	161.24±11,2	27.9±1,9
COD [mg/L]	279.5±18,4	68.30±4,6
Total Phosphorus [mg/L]	20.9±3,1	8.65±0,9
Total Nitrogen [mg/L]	52.3±1,38	18.7±0,33

6. Physico-chemical Characteristics of agriculture soil

7. 6.1- pH determinations

40g of soil from each sample is dissolved in 100ml of distilled water by stirring. After decantation, the pH of the supernatant is determined using a pH meter.

6.2-Determination of electrical conductivity

To determine the electrical conductivity of each soil sample, 20g of soil sieved (2mm), air-dried was added to 100ml of distilled water. After vigorous stirring for 1 hour followed by settling for 30 minutes, the conductivity is measured using a conductivity meter.

6.3-Determination of the rate of total organic matter

We used the Weight Loss-on-Ignition (LOI 360^{o}) method. This procedure is used for the estimation of soil organic matter by the loss of weight in a sample heated at a temperature high enough to burn organic matter but not so high as to decompose carbonates.The rate of total organic matter represents the difference between the dry weight (50g) and the weight of the ashes found after the incineration of already dried soil (48 hours at 105C°) for 16 hours at 450C° in a muffle furnace **(Combs and Nathan, 1998**).

LOI= (wt. at 105°C) – (wt. at 360° C) x 100 / Wt. at 105° C

First we weight 50g of soil and put it in the oven to dry it for 48 hours at 105^{0}C; after 48h we get out the soil from the oven and weight it (the soil weight was 43g). After that we put the dried soil in a muffle furnace for 16 hours at 450^{0}C; after 16h we weight it (the soil weight was 41,67g). In the end with using the equation we determinate the rate of total organic matter **(table 6)**.

Fig.09: The oven

Fig.10 : muffle furnace

Table 06: Characteristics of agriculture sol

Parameters	**Sol**
EC [ms/cm]	0,13
pH	8,2
Organic matter [%]	3,09±0,02

8. Sample processing and culture conditions

7.1. Sample processing

Isolation of fungi was performed by standard spread plate technique (technique for qualitative assessment).The method consist in the incubation of a substratum sample in a sterile nutrient medium where usually, numerous colonies will be produced. These colonies can be transferred to new culture media to obtain pure cultures **(Bills *et al.*, 2004).**

9. Media and plates preparation

Potato dextrose agar (PDA) media was used for filamentous fungi cultures. The media was autoclaved for 30 minutes at 121°C. The media was poured in Petri-dishes and allowed to solidify for 24 hours. To suppress and inhibit bacterial growth, 30 mg/l of la gentamicine was added. Once the agar was solidified we then put plates in an inverted position for 24 hours at room temperature **(Suhail et *al.*, 2007)**.

Fig.11 : the autoclave

8.1. Culture conditions

The experiment was carried out with 5 groups and 3 replicate for each:

- **Control group:** sol irrigated with mineral water

- **Group 2:** Sol irrigated with untreated municipal wastewater

- **Group 3:** Sol irrigated with treated municipal water

-**Group 4:** Treated municipal wastewater

-**Group 5:** Untreated municipal wastewater

One (1) gram of each soil sample was weighed and then added to a conical centrifugal tube containing 9 mL of sterile saline (0.9% w/v). The tubes were then agitated over a tube agitator (VelpScientifica 2x^3) for 10 minutes. The samples were then decanted for fifteen minutes.

Fig. 12: tube agitator (VELP SCIENTIFICA 2x^3)

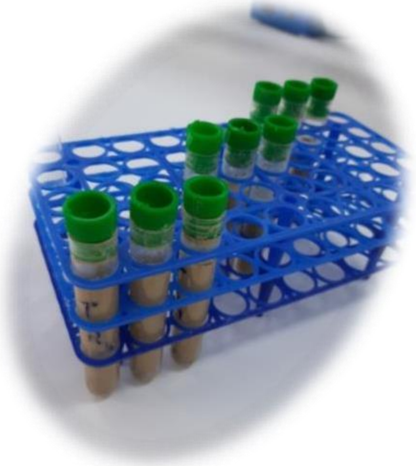

Fig. 13 : conical centrifugal tubes

The supernatants (500 µL) were seeded by spreading method, using a sterile Drigalsky loop, on the surface of Petri dishes (90x15mm).

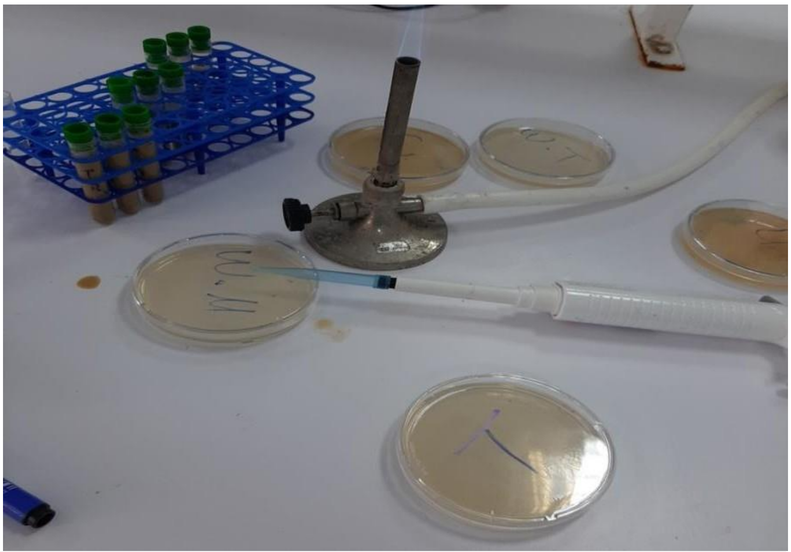

Fig.14 : Spreading method

All plates were incubated at 28°C for five to seven days and examined for the presence of filamentous fungi every 24 h. the prominent colonies were picked for identification. Concerning the qualitative assessment of filamentous fungi in treated and untreated municipal wastewater, 500 µL of water was also spread on PDA plates and incubated at 28 C **(Pont and *al.*, 2013).**

Fig.15 : Incubator outside and inside

8.2. Identification of filamentous fungi

8.2.1. Preparation of lactophenol bleu

We put 10ml of Lactic acid with 10g of Phenol crstal; and 0,5g of Methylene blue and 20ml of Glycerol with 500ml of distillated water in a beaker agitated it until it mixed; then we put the solution in flask until the use.

8.2.2 Identification of filamentous fungi

The filamentous fungi were identified according to the descriptions obtained in the identification key used in the classical taxonomy Barnett and Hunter (1999). Slides were prepared from fungal isolates, grown on PDA plates (obtained by inoculating microfungi directly on a small square of medium), Lactophenol bleufor morphological characterization and were examined under a microscope (at 40x and 60x) to ascertain mycelia appearance, sporangiophore position, columella and spore shape. The fungal isolates were identified on the basis of macroscopic (colony morphology, color, texture, shape, appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia). The isolated fungi were identified to the genus and the species level if possible on the basis of their morphological characters and microscopic analysis by using suitable media, slide cultures and the most taxonomic guides and standard procedures.

Chapter III:

Results and Discussion

III .Results and discussion

1. Results

The filamentous fungi were identified according to the descriptions obtained in the identification key used in the classical taxonomy of **Barnett and Hunter (1999).**

The identification of these genera is based essentially on the macroscopic characters of the colonies (color, texture, shape, appearance of colony.) and on the microscopic characters of the mycelium or spores (septation in mycelium, presence of specific reproductive structures, shape and structure).

Fig.16: Isolation of filamentous fungi

1.1. Control Soil

Based on morphology, fungal strains obtained from soil irrigated by non-polluted water (distilled water), 5 strain found can be classified as follows:

Strain 1 belongs to the species ***Micor SP*** (Fig 19)
Strain 2 belongs to the species ***Aspergillus niger*** (Fig 20)
Strain 3 belongs to the species ***AlternariaSp*** (Fig 21)
Strain 4 belongs to the species ***Geomyces SP*** (Fig 22)
Strain 5 belongs to the specie ***Pythium dissimile*** (Fig 23)

Strain 1: Sporangiophores erect, pale reddish yellow, simple or branched, bearing sporangia terminally. Sporangia globose, yellow, smooth, columellate on dehiscence: collumellae globose. Sporangiospores long ellipsoidal, various in size, guttulate. **Watanabe, (2002)**

Strain 2: Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriatephialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. **Watanabe, (2002)**

Strain 3: Conidiophores pale brown, simple or branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia catenulate, mostly up to 9 in a chain, often branched. Conidia porosporous, acropetally developed, (dark) brown, cylindrical or spindle-shaped, often with cylindrical beaks, muriform composed of 3–4 (-8) transverse walls and 1–2 longitudinal walls. **Watanabe, (2002)**

Strain 4: The colonies are small in size, Gray-brown in color, they are fluffy, short, flat with a central elevation. The contours are rounded. The conidiophores give rise to one or more chains of arthroconidia (barrel-shaped). **Watanabe, (2002)**

Strain 5: Sporangia lobate, terminal or intercalary, rarely discharging zoospores, mostly functioned as conidia germinated by germ tubes. Oogonia mainly terminal, bearing no antheridia. Antheridia, possibly hypogynous, but not recognizable. Oospores formed parthenogenetically, plerotic, with thick oogonium wall, containing single oil globules. **Watanabe, (2002)**

Fig.17: *Petri dish Surface side of control soil*

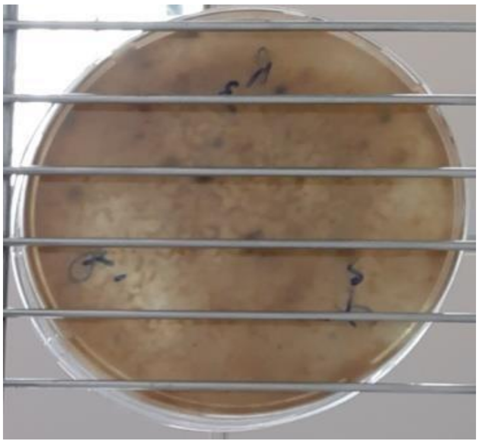

Fig.18: *Petri dish reverse side of control soil*

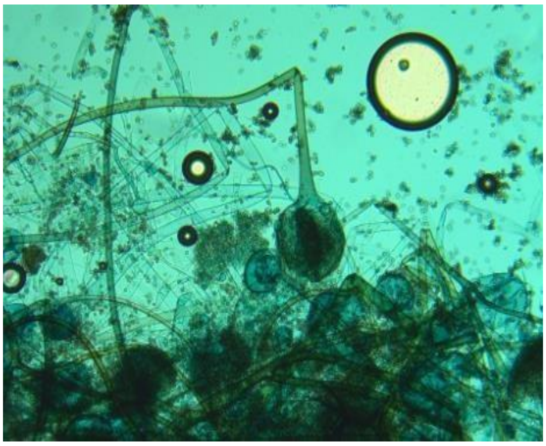

Fig.19: *Microscopic observation of**MicorSp** X40*

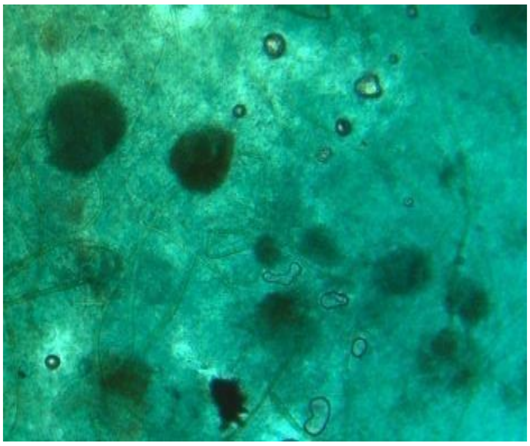

Fig.20: *Microscopic observation of **Aspergillus niger**X40*

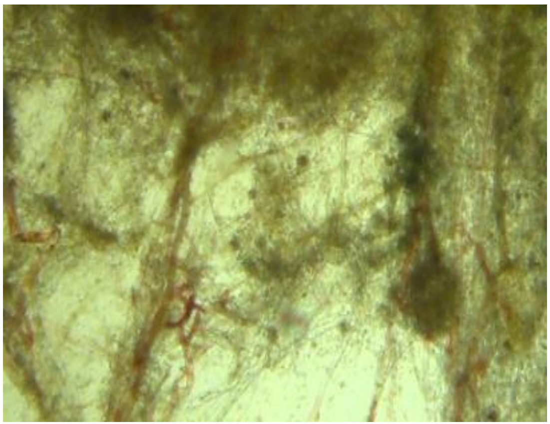

Fig.21: *Microscopic observation of **Alternaria Sp**X40*

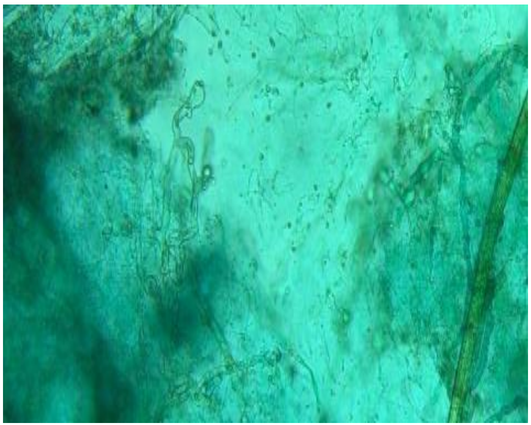

Fig.22: *Microscopic observation of **Geomyces SP**X10*

***Fig.23:** Microscopic observation of **Pythium dissimile**X40*

1.2. Soil with untreated wastewater

Based on morphology, fungal strains obtained from soil irrigated by untreated wastewater, 4 strain found can be classified as follows:

Strain 1 belongs to the species ***Micor SP*** (Fig 26)

Strain 2 belongs to the species ***Aspergillus niger*** (Fig 27)

Strain 3 belongs to the species ***AlternariaSp*** (Fig 28)

Strain 4 belongs to the species ***Pythium SP*** (Fig 29)

strain 1: Sporangiophores erect, pale reddish yellow, simple or branched, bearing sporangia terminally. Sporangia globose, yellow, smooth, columellate on dehiscence: collumellae globose. Sporangiospores long ellipsoidal, various in size, guttulate. **Watanabe, (2002)**

Strain 2: Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriatephialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. **Watanabe, (2002)**

Strain 3: Conidiophores pale brown, simple or branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia catenulate, mostly up to 9 in a chain, often branched. Conidia porosporous, acropetally developed, (dark) brown, cylindrical or spindle-shaped, often with cylindrical beaks, muriform composed of 3–4 (-8) transverse walls and 1–2 longitudinal walls. **Watanabe, (2002)**

Strain 4: Sporangia lobate, terminal or intercalary, rarely discharging zoospores, mostly functioned as conidia germinated by germ tubes. Oogonia mainly terminal, bearing no antheridia. Antheridia, possibly hypogynous, but not recognizable. Oospores formed parthenogenetically, plerotic, with thick oogonium wall, containing single oil globules. **Watanabe, (2002)**

Fig.24 : *Petri dish Surface side of soil with untreated*

Fig.25: *Petri dish reverse side of soil with untreated wastewater*

Fig.26: *Microscopic observation of **MicorSp**X40*

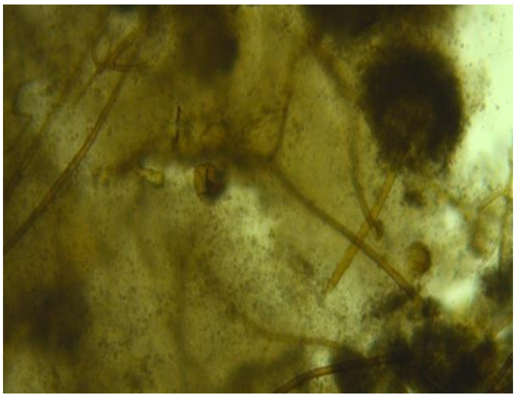

Fig.27: *Microscopic observation of **Aspergillus niger**X40*

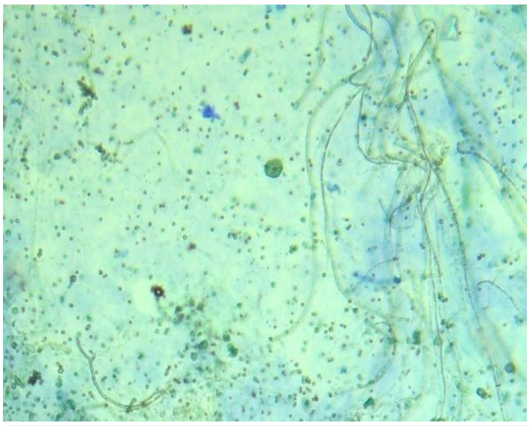

Fig.28: *Microscopic observation of **AlternariaSp**X40*

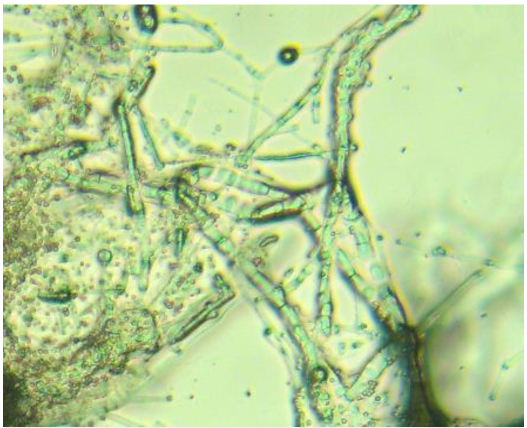

Fig.29: *Microscopic observation of **Pythium SP**X40*

1.3. Soil with treated wastewater

Based on morphology, fungal strains obtained from soil irrigated by treated wastewater, 4 strain found can be classified as follows:

Strain 1 belongs to the species .***Micor sp*** (Fig 32)
Strain 2 belongs to the species ***Rhizopus nigricans*** (Fig 33)
Strain 3 belongs to the species ***Absidia sp*** (Fig 34)
Strain 4 belongs to the species ***Aspergillus niger*** (Fig 35)
Strain 5 belongs to the species ***Alternaria Sp*** (Fig36)

Strain 6 belongs to the species ***Pythium SP*** (Fig 37)

Strain 1: Sporangiophores erect, pale reddish yellow, simple or branched, bearing sporangia terminally. Sporangia globose, yellow, smooth, columellate on dehiscence: collumellae globose. Sporangiospores long ellipsoidal, various in size, guttulate. **(Watanabe, 2002).**

Strain 2: Sporangiophores erect, simple or branched, yellowish to dark brown, rhizoidal, connected directly to sporangiophores, bearing sporangia terminally. Sporangia globose, dark brown to black, minutely spiny, apparently subglobose after maturity, columellate on dehiscence; columellae globose, brown. Sporangiosporessubglobose to subellipsoidal, pale brown, with bluish stripes (lines). **(Watanabe, 2002).**

Strain 3: Sporangiophores hyaline, erect, branched, often curved, bearing columellate and apophysate sporangia terminally. Sporangia pale brown; columellae hemispherical; apophyses flask-shaped; both columellae and apophysessubglobose or ellipsoidal. After deliquescence of sporangia, sporangial wall partially left. Sporangiosporessubhyaline, ellipsoidal or ovate, 1-celled. **(Watanabe, 2002).**

Strain 4: Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriatephialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. **(Watanabe, 2002).**

Strain 5: Conidiophores pale brown, simple or branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia catenulate, mostly up to 9 in a chain, often branched. Conidia porosporous, acropetally developed, (dark) brown, cylindrical or spindle-shaped, often with cylindrical beaks, muriform composed of 3–4 (-8) transverse walls and 1–2 longitudinal walls **(Watanabe, 2002).**

Strain 6: Sporangia lobate, terminal or intercalary, rarely discharging zoospores, mostly functioned as conidia germinated by germ tubes. Oogonia mainly terminal, bearing no antheridia. Antheridia, possibly hypogynous, but not recognizable. Oospores formed parthenogenetically, plerotic, with thick oogonium wall, containing single oil globules **(Watanabe, 2002).**

Fig.30: *Petri dish Surface side of soil with treated wastewater*

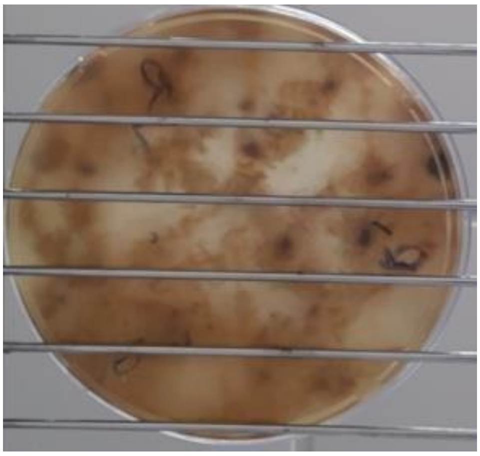

Fig.31: *Petri dish reverses side of soil with treated wastewater*

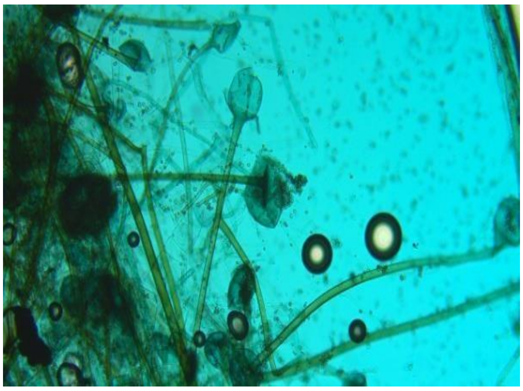

Fig.32: *Microscopic observation of* ***Micor SP****X10*

Fig.33: *Microscopic observation of* ***Rhizopusnigricans****X40*

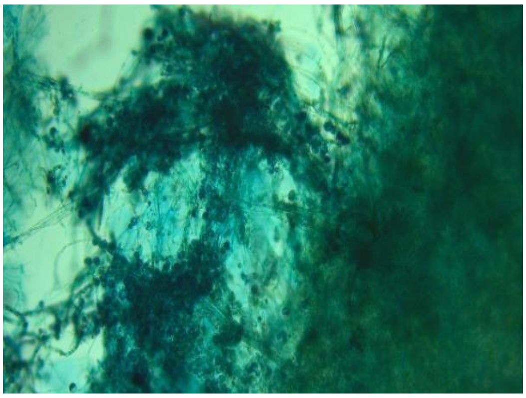

Fig.34: *Microscopic observation of* ***Absidiasp****X40*

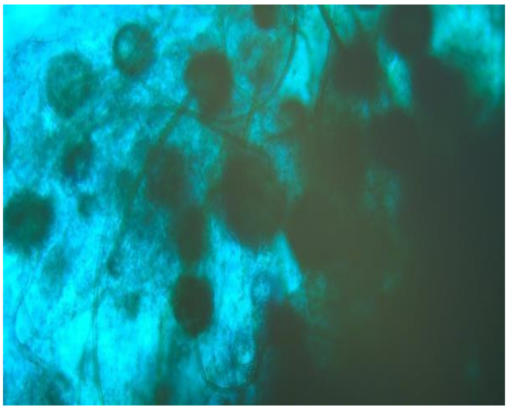

Fig.35: *Microscopic observation of* ***Aspergillus niger****X40*

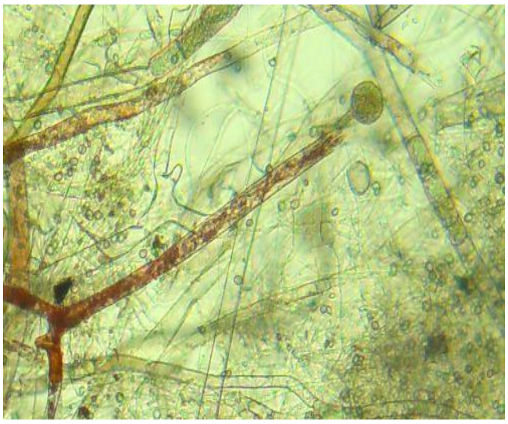

Fig.36: *Microscopic observation of* ***Alternaria Sp****X10*

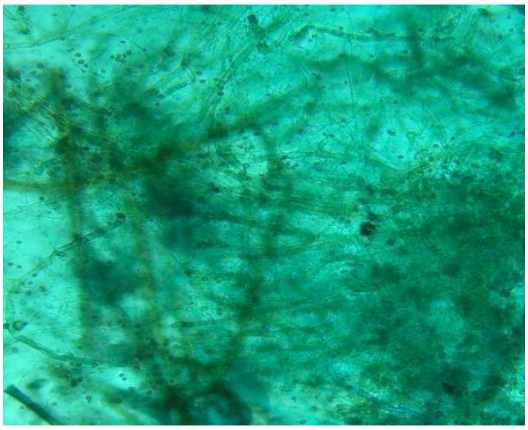

Fig.37: *Microscopic observation of* ***Pythium SP****X40*

1.4.Untreated wastewater isolates

Based on morphology, fungal strains obtained from untreated wastewater, 3 strains found can be classified as follows:

Strain 1 belongs to the species. ***Aspergillus niger*** (Fig 40)
Strain 2 belongs to the species ***Pythium sp*** (Fig 41)
Strain 3 belongs to the species ***Penicilluim sp*** (Fig 42)

Strain 1: Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriatephialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate **(Watanabe, 2002)**

Strain 2: Sporangia lobate, terminal or intercalary, rarely discharging zoospores, mostly functioned as conidia germinated by germ tubes. Oogonia mainly terminal, bearing no antheridia. Antheridia, possibly hypogynous, but not recognizable. Oospores formed parthenogenetically, plerotic, with thick oogonium wall, containing single oil globules. **(Watanabe, 2002)**

Strain 3: Conidiophores hyaline, erect, slightly rough, developed from aerial hyphae, branched penicillately at the apexes, with primary or secondary metula, verticillatephialides and catenulate conidia at each phialide, forming cylindrical columnar grayish green conidial heads: phialides pen-pointed with abruptly sharpened tips. Conidia phialosporous, pale green, globose or subglobose, 1-celled, verrucose or minutely echinulate on the surface. **(Watanabe, 2002)**

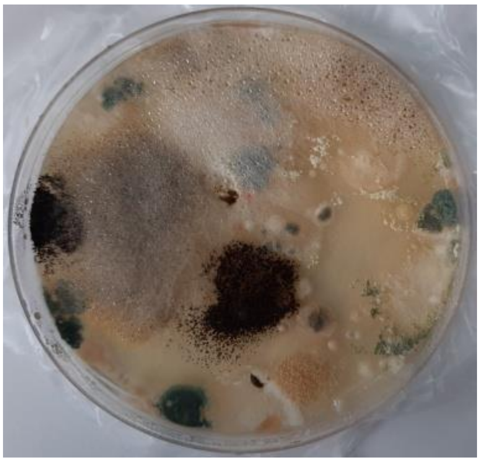

Fig.38: *Petri dish surface side of untreated wastewater*

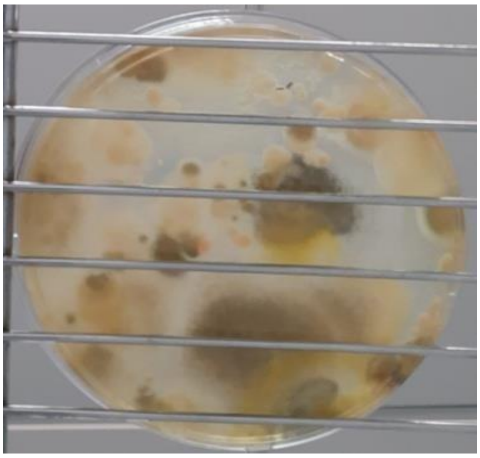

Fig.39: *Petri dish reverse side of untreated wastewater*

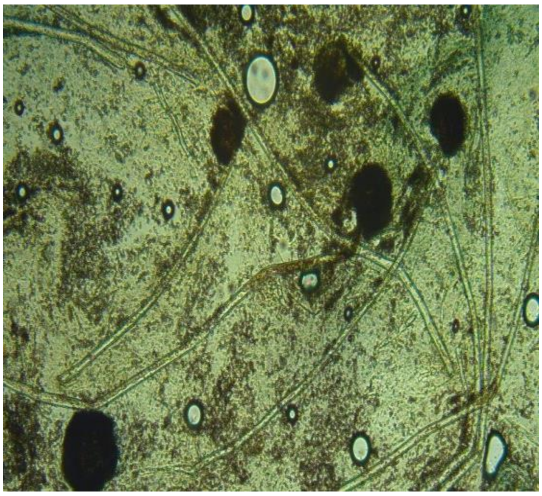

Fig.40: *Microscopic observation of* ***Aspergillus niger****X40*

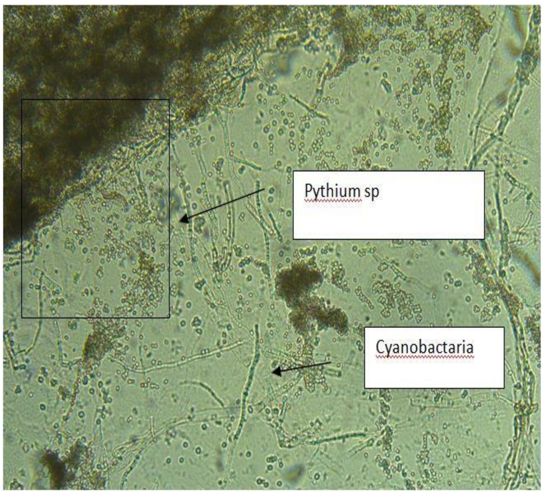

Fig.41: *Microscopic observation of* ***Pythium sp****X10*

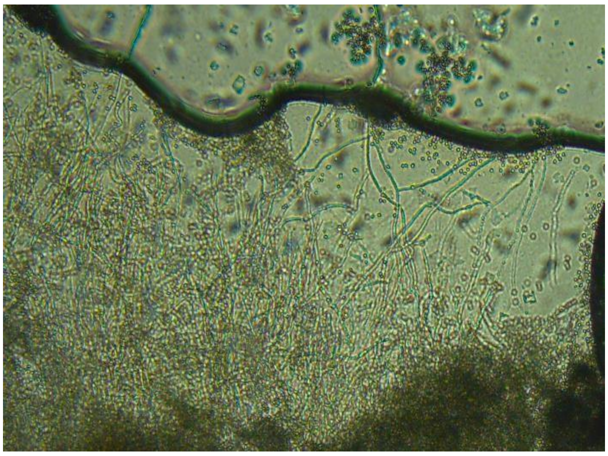

Fig.42: Microscopic observation of ***Penicilluimsp****X10*

According to the *Petri dish reverse side I suspect the* presence of ***Penicilluimsp*** (septate hyphae)

1.5.Treated wastewater isolates

Based on morphology, fungal strains obtained from treated wastewater, 2 strains found can be classified as follows:

Strain 1 belongs to the species. ***Cyanobacteria (anabena or nostoc) no fungi*** (Fig. 45)
Strain 2 belongs to the species ***Pythium SP*** (Fig. 46).

Strain 1: there is no fungi in this strain only Cyanobacteria ***(anabena or nostoc)***

Strain 2: Sporangia lobate, terminal or intercalary, rarely discharging zoospores, mostly functioned as conidia germinated by germ tubes. Oogonia mainly terminal, bearing no antheridia. Antheridia, possibly hypogynous, but not recognizable. Oospores formed parthenogenetically, plerotic, with thick oogonium wall, containing single oil globules**. (Watanabe, 2002)**

Fig.43: *Petri dish surface side of treated wastewater*

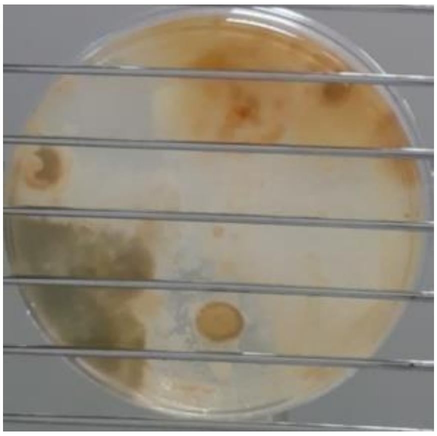

Fig.44: *Petri dish reverse side of treated wastewater*

Fig.45: *Microscopic observation of* ***Cyanobacteria (anabena or nostoc)*** *X40*

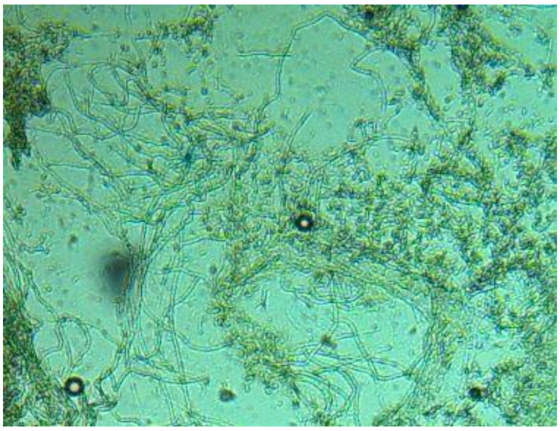

Fig.46: *Microscopic observation of* ***Pythium sp*** *X10*

2. Discussion

Population growth and water scarcity necessitate alternative agriculture practices, such as reusing wastewater for irrigation. Domestic wastewater has been used for irrigation for centuries in many historically low-income and arid countries and is becoming more widely used by high-income countries to augment water resources in an increasingly dry climate **(Slobodiuk, and *al.*, 2021).**Soil microbial communities are established on basis of a complex network of interrelations between abiotic (physical and chemical soil properties) and biotic factors (macro- andmicrobiological soil components). The impact of wastewater on soil microbial communities is supposed to depend on direct inputs of exogenous microbiota, which, in an improbable worst case scenario, would lead to the elimination of autochthonous microorganisms by competition **(Becerra-Castro, 2015)**.

In this context, in our study, we investigated the effects of treated and untreated municipal wastewater on the biodiversity of filamentous fungi in an agricultural soil planted with wheat. To evaluate the impact of wastewater on soil filamentous fungi communities, we isolated and identified species in treated and untreated municipal wastewater before it is used for irrigation to examine the possibility of input of exogenous filamentous fungi. Our experiment was carried out with 5 groups

- Control group: Sol irrigated with mineral water

- Group 2: Sol irrigated with untreated municipal wastewater

- Group 3: Sol irrigated with treated municipal water

-Group 4: Treated municipal wastewater

-Group 5: Untreated municipal wastewater

The isolated fungi in our study were examined using two categories of criteria: macroscopic based on colony characteristics and microscopic based on fungal morphology. The results are shown in the table below **Table 07**.

Overall, our results showed that untreated municipal wastewater did not add any exogenous filamentous fungi to the soil. In the control group, where the soil was irrigated with distilled water, we identified five taxa**: *Micorsp*, *Aspergillus niger*, *Alternariasp*, *Pythium dissimile***, *and* ***Geomyces sp***. The sametaxa were found in soil irrigated with untreated municipal wastewater, but the genus ***Geomycessp*** was not found. However, in sol irrigated with treated municipal wastewater, we identified six taxa; four of them were observed in control soil; ***Micorsp***, ***Aspergillus niger***, ***Alternariasp***, ***Pythium sp***, and the other two:***Rhizopusnigricans*** *and* ***Absidiasp***were not.

- **Table 07:**.The isolated filamentous fungi in soil (+ presence / **-**absence)

Species	Control soil	Soil with untreated wastewater	Soil with treated wastewater
Micor sp	+	+	+
Aspergillus niger	+	+	+
Alternariasp	+	+	+
Geomces sp	+	–	–
Pythum dissimile	+	+	+
Rhizopus nigricans	–	–	+
Absidia sp	–	–	+

In untreated wastewater, we observed the three taxa: ***Aspergillus niger***, ***Pythium sp***, and ***Penicillium sp;*** on the other hand, in treated wastewater, we found ***Penicilliumsp***, ***Pythium sp***, and a lot of **Cyanobacteria (Anabaena and Nostoc)**. According to **Azevedoet *al.*,(2010)** the biodiversity of soil fungi depends on the chemistry, texture and water holding capacity of the soil. Physical factors such as temperature, pH, organic matter, also have a great influence on the microbial life. Soil temperature affects all chemical reaction rates; therefore microorganisms have little control over how fast their own metabolic processes take place.

In our study, soil Ph was around pH = 8, 2 and EC= 0,13 ms. This alkalinity maybe explains the low biodiversity of the soil in this experiment (Control group). Effectively, filamentous fungi are known to prefer acid than alkalinesoil. According to **Kemmitt et *al.*, (2006),** one of the most influential factors affecting the microbial community in soil is pH, this factor strongly influences abiotic factors, such as carbon availability. Fungi and bacteria are the two groups that dominate the microbial decomposer community, and, crudely defined, they share the function of decomposing organic matter in soil, indicating that there is a strong potential for interaction **(Rousk et *al.*, 2009)**. The study of **Rousk et *al.*, (2009),** showed that neutral or slightly alkaline conditions favored bacterial growth. Conversely, an acid pH favored fungal growth. This resulted in an increase in the relative importance of fungi by a factor of 30 from pH 8.3 to pH 4.5.Filamentous fungi are well known for their ability to degrade lignocellulosic biomass and have a natural ability to convert certain products of biomass degradation, for example glucose, into various organic acids. Organic acids are suggested to give a competitive advantage to filamentous fungi over other organisms by decreasing the ambient pH. They also have an impact on the ecosystem by enhancing weathering and metal detoxification **(Liaud, 2014).** Soil electrical conductivity (EC) is a measure the salinity of soil. It is an indicator of nutrient availability and loss, soil texture.Soils that have an EC value of less than 1 dS/m (ms/cm) are a non saline soil **(Smith, and Doran, 1997).**

In our experiment, irrigation with no saline-treated and untreated wastewater EC = 3,20 ms and 2,98 ms, respectively, did not affect soil salinity or filamentous fungi's biodiversity. Even though we found two more taxa (***Rhizopus nigricans*** *and* ***Absidia sp***) in the soil irrigated with treated wastewater, the irrigation with treated and untreated water pH = 7,50 and 7,46 , respectively, did not influence or change the ph of the soil. These results could be due to eliminating other contaminants in the wastewater like heavy metals, pesticides, or herbicides harmful to sensible filamentous fungi.In our study untreated wastewater COD = 279,5 mg/l > 90 mg/l the permissible limits of Algerian standards for reuse in irrigation.

Concerning the total phosphorus and nitrogen of treated and untreated wastewater; it seems to be not high to contaminate the soil according tothe permissible limits of Algerian standards for reuse in irrigation 30mg/l **(OJRA ,2012).**However, the presence of those two essential elements could be crucial in soil for developing fungi. According to **Mlitan et *al.*, (2015).** Wastewater without treatment may have nitrogen and is mostly present in the form of NH4. Nitrogen is an essential nutrient for biological growth. It is one of the main components in all living organisms such as plants and microorganisms such as fungi.

Concerning the effect of organic matter of treated and untreated wastewater on soil filamentous fungi diversity, our results show that the high BOD=161,24 in untreated wastewater could positively affect fungi development due to the low rate of organic matter in soil 3,09 %. However, this seems not to be happening. We can explain this by the edaphic soil factors.Soil organic matter is essential as nutrient reservoir and in soil Structure. The organic matter-related pools are amongst the soil properties most affected by wastewater irrigation. Effects on soil aggregate stability and water retention capacity; modify soil microhabitats and, hence, influence the soil microbial communities **(Becerra-Castro, 2015).** In Tebessa region, according to the soil map of the region (Jaseix-Bellon, 1948), the study area is part of the steppe highlands, characterized by clay-limestone type with more or fewer pebbles **(Zereg, 2019**).The spatial organization of soils is crucially important in affecting belowground function, and the associated delivery of ecosystem services.The distribution of water within soils plays a crucial role in governing fungal development and activity, as does the spatial distribution of nutrient resources **(Ritz and Young, 2004).**

Our results demonstrate that irrigation with untreated wastewater has no impact on the diversity of filamentous fungi in agricultural soils in the region of Tébessa. The soil already seems poor in filamentous fungi and also poor in organic matter, indicating that these soils could not be fertile. Farmers must work hard to correct the structure and texture of these soils by adding organic matter and rotating and cultivating the soil with other crops besides wheat.

Conclusion

Conclusion

The biodiversity of soil fungi depends on soil chemistry, nature and physical factors. Therefore microorganisms have no control over their metabolic processes. Filamentous fungi are known to favor acid over alkaline soil and pH has a significant influence on the vitality factors of these communities.

The reuse of alternative wastewater for irrigation water in many low-income countries is due to water scarcity and demographic growth. Developed countries have also used it to increase water resources. Wastewater is supposed to affect biological communities in the soil, which in turn will eliminate microorganisms. So in our study we examined the effects of treated and untreated municipal wastewater on the biodiversity of filamentous fungi in wheat-grown agricultural soil. To assess the impact of these waters on biological communities in the soil.

In the experiment, irrigation with treated and untreated wastewater did not affect soil salinity or biodiversity, nor did pH affect soil. These results can be due to the elimination of pollutants in wastewater. The presence of some other elements can be critical in the soil for the development of these fungi.

Organic matter in soil is essential as nutrient stock and in soil structure. Material-related organic complexes are among the soil properties most affected by wastewater irrigation. Effects on soil stability and water retention capacity; Modify micro-housing in the soil, there by affecting the microbial communities of the soil.

Conclusively, our results show that untreated wastewater irrigation has no impact on the diversity of filamentous fungi in agricultural soil in the Tebessa area. Soil already appears poor in filamentous fungi and also poor in organic matter, suggesting that this soil cannot be fertile. So, Farmers should work hard to correct the structure and texture of this soil by adding organic materials, rotating the soil and growing them with other crops along with wheat.

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