

## **STUDY OF THE PHYSICO- CHEMICAL AND MYCOLOGICAL WASTEWATER OF AIN EL HAJAR CITY IN THE WILAYA OF SAIDA, ALGERIA**

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### **ABSTRACT**

The objective of this work is to study the physico-chemical and mycological wastewater of SAIDA city in Algeria; On the one hand, the aqueous effluent containing domestic waste and industrial waste; These waters have different fractions of toxic substances, they are often a significant hazard to the ecosystem. On the other hand the water is a seat of microbial life such as fungi, which are common contaminants of many nutrients.

The optimal conditions for the proliferation of certain fungal flora (the relationship between physicochemical parameters and mold growth) have been characterized, and new biological materials have been identified, they are likely to bio-accumulate or 'naturally absorb these pollutants in these effluents.

**Keywords:** fungal flora, mold, water effluents, biological materials.

### **1. INTRODUCTION**

In the purification step, the objective remains to eliminate the pollutants either according to their size or according to their eutrophisant character, the elimination of these components and the abatement of the micro-organisms can be assured by the biological processes.

The objective of our study is to determine the influence of the physicochemical characteristics on the presence and the development of the certain known fungic species for their purifying capacities of waste water. For that, we carried out physicochemical and mycologic analyzes upstream and with the downstream on the level of the treatment plant of AIN ELHADJAR city.

### **2. PRESENTATION OF THE PURIFICATION PLANT OF AIN EL HADJAR**

The treatment plant in Ain El Hajar works on the basis of activated sludge process with extended aeration with surface aerator at slow speed. Located in

Ain el Hajar (Saida) at the western entrance of the city. Waters admitted to the station are domestic and industrial water must pre-treated at the industrial units. The essential guarantees provided by the treatment plant are shown in Table 1:

**Table 1 Essential guarantees of treatment**

Parameters	Values
PH	Ranging between 6,5 and 8,5
Color	no perceptible
BOD 5	30 to 40 mg/1
COD	90 mg/1
Nitrogenize (N-NH <sub>4</sub> )	3-5 mg/1
Nitrogenize (N-NH <sub>3</sub> )	8-10 mg/1
Nitrification	70%
Greases and plant oils	20 mg/1
Mineral oils	05 mg/1

### 3. METHODS OF ANALYSIS

#### 3.1 Physico-chemical analysis (Rodier [1])

##### **Temperature:**

Temperature is a physical characteristic of thermal equilibrium. It governs the solubility of salts and gases; it plays a role in determining the pH.

##### **PH:**

The pH or potential hydrogen is an expression of the acidity or alkalinity of the medium.

#### 3.2 Chemical parameters

##### **Determination of the TA, TAC (alkalinity)**

The alkalinity of water is the presence of bicarbonates (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>-2</sup>) of hydroxide ions (OH<sup>-</sup>) and a more limits to silicate ions (HSiO<sub>3</sub><sup>-2</sup>) Phosphates (PO<sub>3</sub><sup>-4</sup>) or molecular species with weak acids.

### **Determination of hardness (total hardness TH)**

The hardness or total hardness of water is the sum of the concentrations of metal cations with the exception of those of alkali metals and hydrogen ion. In most cases, the hardness is mainly due to calcium ions and magnesium ions plus sometimes iron, aluminum, manganese and strontium.

### **Proportioning of chlorides (Method of Mohr)**

The chlorides exist in all water with variable concentrations. They can have several origins:

- Percolation through salted grounds;
- Water infiltration marinades in the ground water;
- Human activities and industrial.

### **Proportioning of dissolved oxygen (Method of Winkler)**

Oxygen; always present in water, is not a component, its solubility is function of the temperature, the partial pressure in the atmosphere and salinity; Its content is function of the origin of water.

### **The carbon dioxide**

Balances carbonic acids, bicarbonates, and carbonates are the basis of the properties and encrusting aggressive water, which depend on carbon gases in solution, alkalinity and pH.

### **Proportioning of sulfates**

Water practically always contains sulfates in very variable proportion.

### **Determination of nitrates**

Note that the nitrates participate in eutrophication, in periods of low oxygen, nitrate can serve as donors of O<sub>2</sub> and avoid anaerobic conditions.

### **Determination of Nitrite**

Depending on the origin of the water, the nitrite content is quite variable in an acid solution, nitrite ions formed with sulfanilic acid diazonium salt, which reacts with n-(naphthyl-1)-ethylenediamine to give dihydrochloure a red dye purple dye that is determined by spectrophotometer.

### **Determination of total Phosphate and Orthophosphate**

Phosphorus is one of the essential components of the living matter; the phosphorated compounds have two origins, the human metabolism and the detergents.

## **Determination of Ammonium:**

The ammonium nitrogen (NH<sub>4</sub>-N) is presented in part as ammonium ions or in the form of ammonia, there is a pH-dependent equilibrium between these two forms. In a strongly alkaline solution containing practically as ammonia, it reacts with the hypochlorite ions to give it form monochloramine with a substituted phenol derivative indophenol blue which is determined by photometry; (the pH must be included 4 to 13).

## **3.3 Organic parameters**

### **COD**

COD reflects the amount of O<sub>2</sub> required to oxidize mg per liter Chemically the organic matter in the effluent.

### **BOD5**

BOD5 is defined as the concentration of oxygen consumed to achieve the destruction of non-nitrogenous compounds in the test conditions: incubation at 20 ° C and darkness and during a time of 05 days.

## **3.4 Mycological analysis**

### **3.4.1 Fungi Enumeration by the method of dilution**

It is to determine the mycoflora of the waste water and purified water (Ibrahim et al. [2]).

1 ml of the 10<sup>-1</sup> dilution is inoculated on the surface of the following media:

- C.D.A (Czapek Dox Agar)
- P.D.A (potatos Dextrose Agar)
- D.R.B.C

In addition 1 ml of stock solution is seeded on the surface of these environments. Before sowing, these environments are acidified by acid AClick except the DRBC. The incubation period is 5-7 days at 25 ° C.

### **3.4.2 Purification and identification**

Before its identification, a stock must be purified on culture medium (P.D.A).

For the thorough study of a stock, the technique of microculture described by Harris in 1969 gave us details more pushed on the morphological character of the various fungi.

#### **a) Method of microculture**

The technique consists in placing a sterile blade in a wet chamber on which is deposited a small square of medium P.D.A solid and sown on its edges by fungic spores, then to put a plate above.

After an incubation of 3-4 days, the plate is withdrawn and posed on a new blade containing a drop of lactophénol.

The identification of the stocks was carried out by optical microscopy while being based on the keys of identification illustrated in the guide of BARNETT, 1972.

#### **b) Method of tape:**

A small piece of Scotch tape is applied by its face sticking on the colony using a grip, then deposited on a drop of blue cotton on a blade slide. A second drop (more reduced) is then deposited on the higher face of the tape which is then covered with a plate cover-glass. It is advisable to eliminate excess from dye around the plate with a sheet of paper blotter.

### **3.4.3 Identification of Aspergillus species**

The identification of species of Aspergillus and Penicillium is performed by the method of Pitt (1973) and Ramirez (1982). This method is called Single spore, based on the relationship between the activity of water of the culture medium and temperature of incubation.

It involves the inoculation of spores of a few youth culture in hemolysis tubes containing a semisolid suspension containing 0.2% agar and 0.05 of Tween80. It is done in three different environments that are:

- Malt Extract Agar (AIM) at 25 ° C;
- Glycerol Nitrate Agar (G25N) at 25 ° C;
- Czapek Yeast Aar (CYA) at two different temperatures: 5 ° C and 37 ° C.

Confirmation of suspected strains Aspergillus flavus, Aspergillus is made by inoculation of the medium at 25 ° C. AFAP The latter gives a side of orange culture characteristic of this group.

## **4. RESULTS AND DISCUSSIONS**

### **4.1 Physico-chemical analysis**

The physical aspect of the waste water upstream of the station has a dark brown color and an unpleasant odor due to the presence of household waste and high levels of ammonia. On leaving the station, the water has a light yellow color with no odor due to the removal of mineral and organic matter in different treatment processes (table 2).

the temperature is an ecological factor, the results presented in the table show a slight difference between the air temperature (15-20 ° C), the upstream water (18 ° C) and downstream (16.7 ° c) of the station. This difference is the result of several factors, climate and water velocity in the pipes.

pH is an important element in defining the aggressiveness of a water or encrusting. The results (Table 2) are consistent with the prescribed standards (6.5 <pH <8.5).

**Table 2 Physical characteristics**

Parameter	Waste water	Purified water	units
Color	Dark chestnut	Yellow clear	/
Odor	Unpleasant	Without odor	/
T°	18	16.7	°C
pH	7.78 (with 25°C°)	7.82 (with 25°C°)	/

#### 4.2 Chemical parameters (Table 3)

For our samples, TA = 0, which usually occurs in water with a pH <8.3. TAC values indicate that the waste water is slightly alkaline against the alkalinity decreases during treatment, because in absence of CO<sub>2</sub>, ions (HCO<sub>3</sub><sup>-</sup>) are removed after precipitation with sludge.

TH = 3.8 meq / l of purified water is a normal value of hardness, for against the wastewater has a high hardness TH =19.9 meq / l that is to say that water is extremely hard.

#### Chlorides

These values indicate that the waste water is slightly alkaline on the other hand this alkalinity decreases during treatment because in absence of CO<sub>2</sub>, ions (HCO<sub>3</sub><sup>-</sup>) are evacuated after precipitation with muds.

It is noted that our samples have very high Cl<sup>-</sup> values about 1533.6mg/l and 1079.2 mg/l with the upstream and the downstream respectively.

#### Oxygen dissolves

From table 3, we note an increase in the O<sub>2</sub> content in the water treated (2.794 mg/l) compared to the waste water (0.79 mg/l), this saturation is due to the

process treatment to realize in the form of the surface aerators on the level of the basin of ventilation and under the effect of the reduction in the micro-organisms which consume O<sub>2</sub>.

### **The carbon dioxide**

We noted that there was an opposite relation between the concentration of oxygen dissolves and the carbon dioxide.

### **Sulfate**

The results clearly show an important rate of ammonium in wastewater 113 mg / l, this concentration is reduced during treatment to 18.1 mg / l under the action of specific bacteria (nitrification).

### **Nitrate**

The values obtained are very low but and are consistent with the standards. In addition, the results show a difference between the NO<sub>3</sub><sup>-</sup> concentration of the waste water and purified water. This decrease due to the consumption of nitrogen by microorganisms.

### **Nitrites**

It was found that their use as a corrosion inhibitor in water treatment plants can lead to misinterpretation. Nitrites can appear as by-products during chlorination of water in the microbial decontamination of water.

### **Phosphates and orthophosphates**

The phosphates escape in major part from the treatment of the traditional biological purification plant and so are found in the rejections. The physicochemical treatments of precipitation can eliminate from 80 with 85% of phosphates according to the reagent used and the pH.

**Table 3 Chemical parameters**

Parameters	Waste water	Purified water	Units
TAC	15.2	2.2	meq/l
MT	0	0	meq/l
TH	19.9	3.8	meq/l
Chloride	1533.6	1079.2	mg/l
oxygen dissolves	0.79	2.794	mg/l
the carbon dioxide	1.263	0	meq
Sulfate	0.184	0.096	mg/l
Nitrates	0.16	0.03	mg/l
Nitrite	0.59	0.067	mg/l
Phosphates	71	5.3	mg/l
Orthophosphate	123	4.3	mg/l
Ammonium	113	18.1	mg/l

### 4.3 Organic parameters

#### COD

The sample taken at the upstream has a high COD value decreases against the result of precipitation of organic and mineral particles during the treatment processes. The value of the COD of the treated water is of the order of 59 mg / l (Table 4). The values found are consistent with normal values (<90mg / l).

#### BOD5

BOD5 of the wastewater is very high (160 mg / l) due to the presence of microorganisms and the organic biodegradable load by BOD5 against purified water (25mg / l) was acceptable according to standards (from 30 to 40 mg / l), this value is related to the decrease in the concentration of micro-organisms after biological treatment (Table 4).



**Table 4 the organic parameters**

Parameter	Upstream	Downstream	Standards
DCO (mg/l)	126	59	90
DBO5 (mg/l)	160	15	< 40

#### 4.4 Mycologic analysis

The analysis of the results obtained by the method of dilution allows the average appreciation of the degree of pollution (Rate of contamination), while giving an indication of the contaminant flora of all our samples. The expressions of these results are concretized in the figures which follow (Figures 1, 2, 3 ).

The median values of the total mycoflora, as well as the various fungal stocks appeared by the method of dilution on medium PDA for the samples of water, testify to a rate of contamination by a high fungic flora for the two types of water with analyzed (waste water and purified). This rate spreads out between  $(7,47 - 11,09) \times 10^2$  UFC /l for waste water (E) and between  $(5,49 - 8,11) \times 10^2$  UFC/l for that purified water (S).

The predominance of *Aspergillus* on these samples is definitely visible. Noting the dominating intervention of *Aspergillus flavus* (figure 5), *Aspergillus fumigatus* (figure 6) with this potential of pollution on the two types of water but with an increase on waste water. More precisely on E3 and E1.

The result also makes it possible to count certain species belonging to the flora of grounds such (*Cladosporium* sp, *Rhizopus* (+++)...).

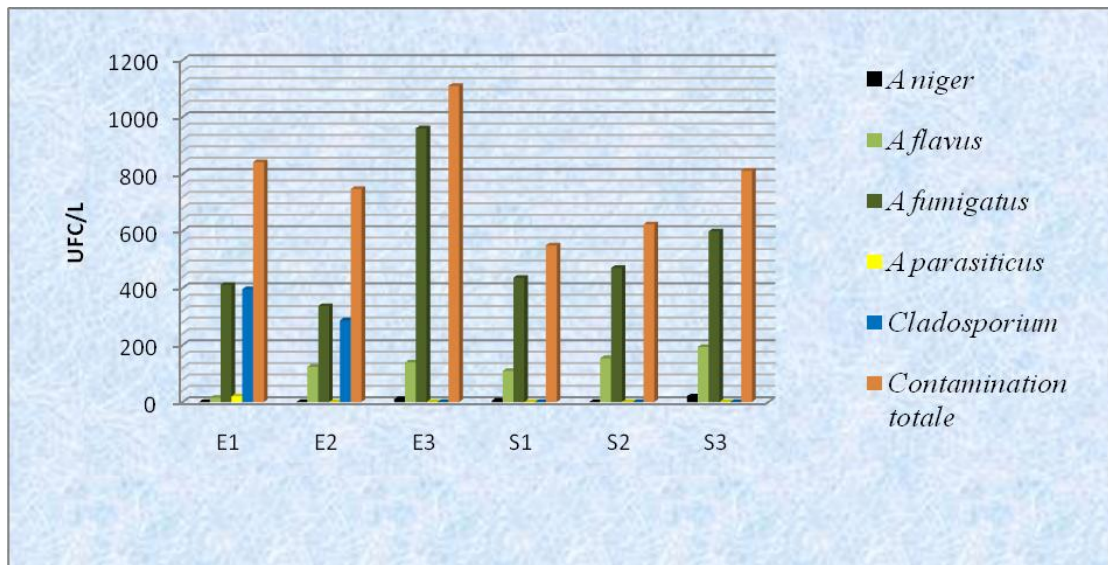
On the medium CDA, the Figure 2 confirms the degree of pollution of our samples, the rate of contamination is lower compared to that on the PDA  $(8,46 - 12,92) \times 10^2$  UFC/l than the upstream of the treatment plant (E) and  $(6,33 - 10,49) \times 10^2$  UFC/l with the downstream (S).

With regard to the DRBC, the samples show a very great contamination compared to the other mediums (PDA, CDA) figure 3 the rate of contamination of waste water and between  $(13,21 - 17,27) \times 10^2$ , these value decrease until  $(10,89-15,49) \times 10^2$  for purified water.

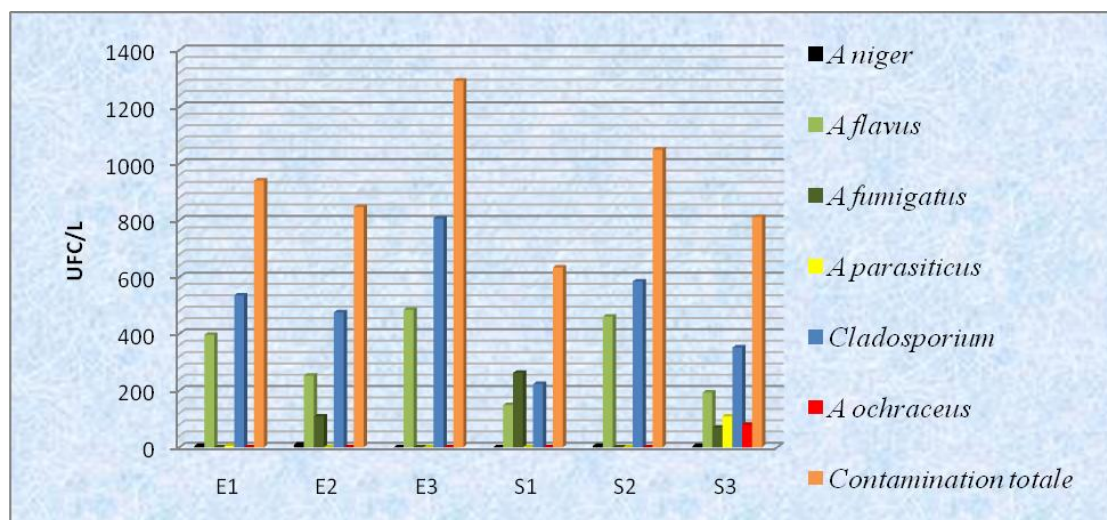
The pH of all samples reveals that our samples have a neutral pH (7.78 to 7.82). Note here that the fungi develop normally at pH values between 3 and 8, and have optimum growth at pH values between 5 and 6. The course summarizes the results of mycological analysis shows a strong and diversified presence of important fungal species in all samples. The preferred mold for the organic medium (PDA) is well above the mineral

(CDA). This is consistent with the work of Moussaoui [3], Benmansour-Brixi [4] and Belyagoubi [5].

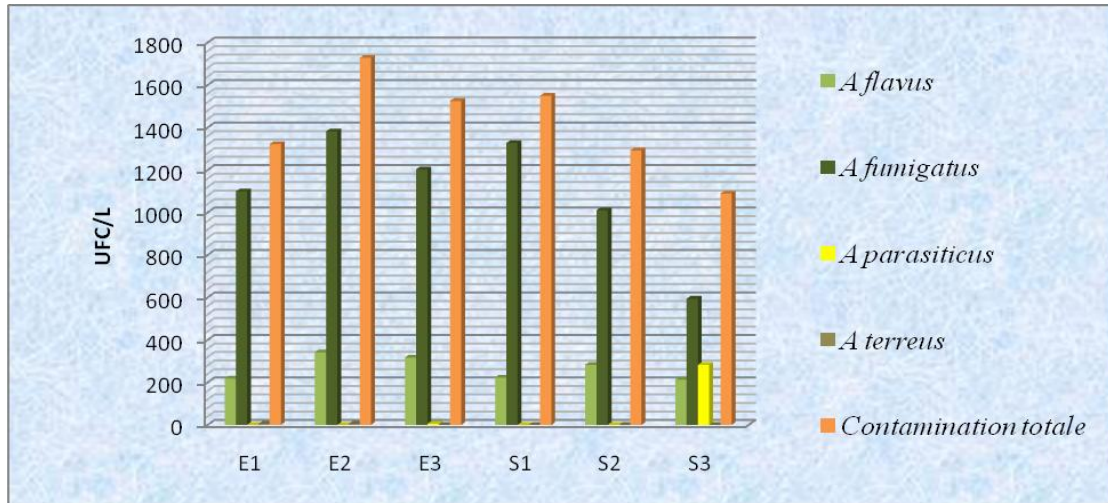
This analysis also allowed the identification of different genera representing the flora of soil types: *Cladosporium* (figure 9) and the strong presence of Mucorales (*Rhizopus*) (figure 7) that may play an important role in water treatment (fixation of heavy metals such column 3 grams of *Rhizopus* can purify 600 ml of zinc at 50 mg/l to a level below 0.1 mg / l) (Fourest [6]). Note the use of *Aspergillus Niger* (figure 4) in the biological treatment of polluted water (degradation of organic pollutants: eg phenol).



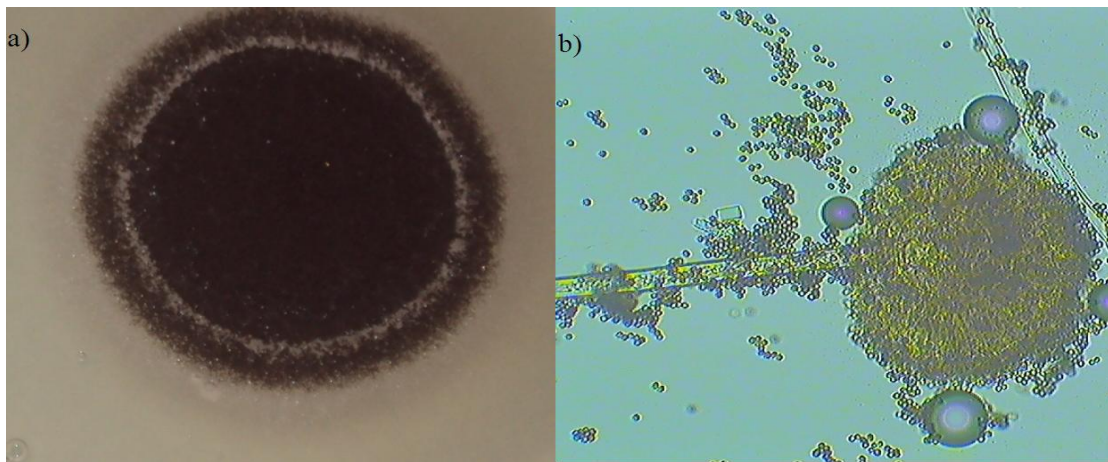
**Fig.1 Median values of the Total mycoflora and the various fungic stocks revealed by the method of dilution in the samples of waste water (E) and the water purified (S) on the PDA. (UFC/g)**



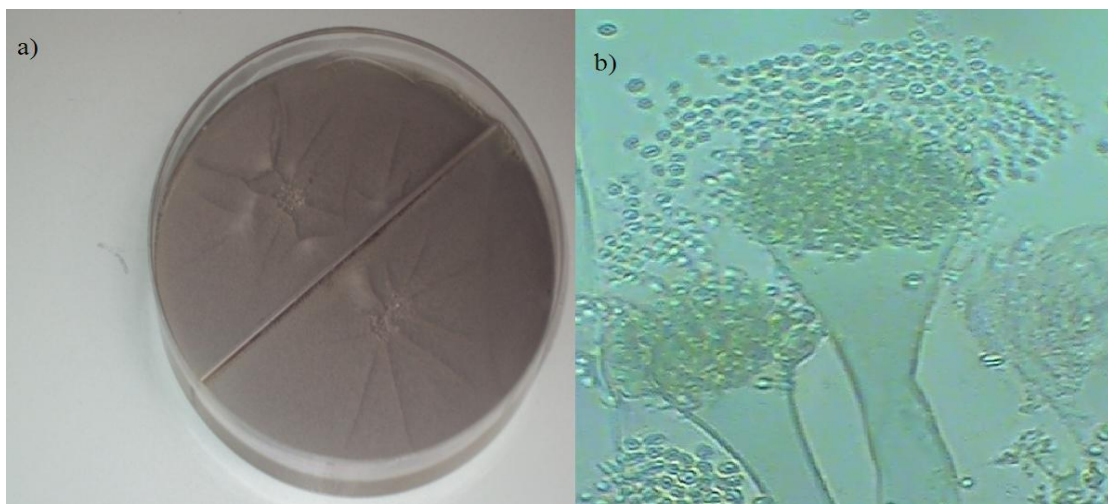
**Fig.2 Median values of the Total mycoflora and the various fungic stocks revealed by the method of dilution in the samples of waste water (E) and the water purified (S) on the CDA. (UFC/g)**



**Fig.3 Median values of the Total mycoflora and the various fungic stocks revealed by the method of dilution in the samples of waste water(E) and the water purified (S) on the DRBC. (UFC/g)**

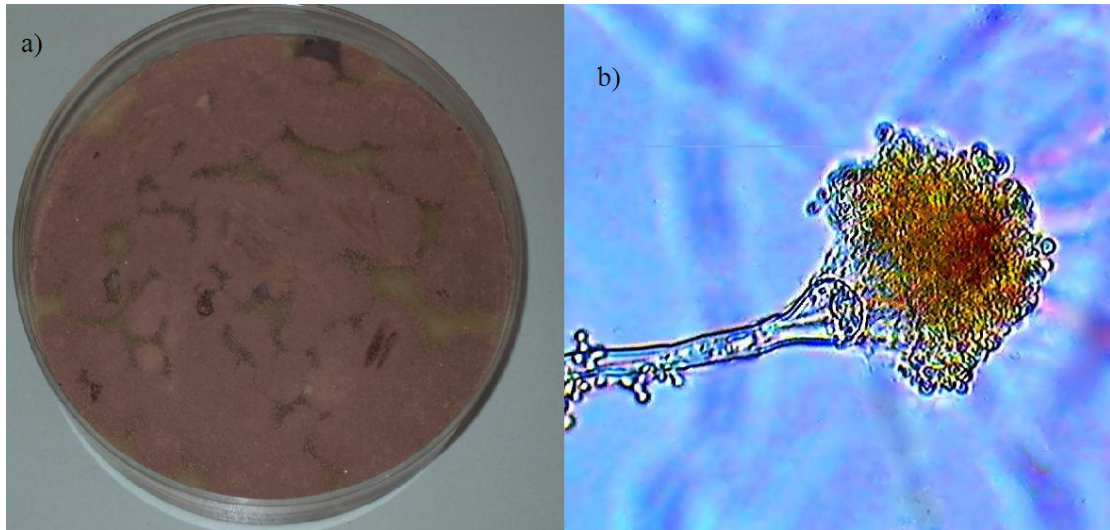


**Fig.4 *Aspergillus Niger* (a Macroscopic aspect ; b microscopic Aspect Gx100).**



**Fig.5 *Aspergillus Fimugatus* (a Macroscopic aspect ; b microscopic Aspect Gx100).**

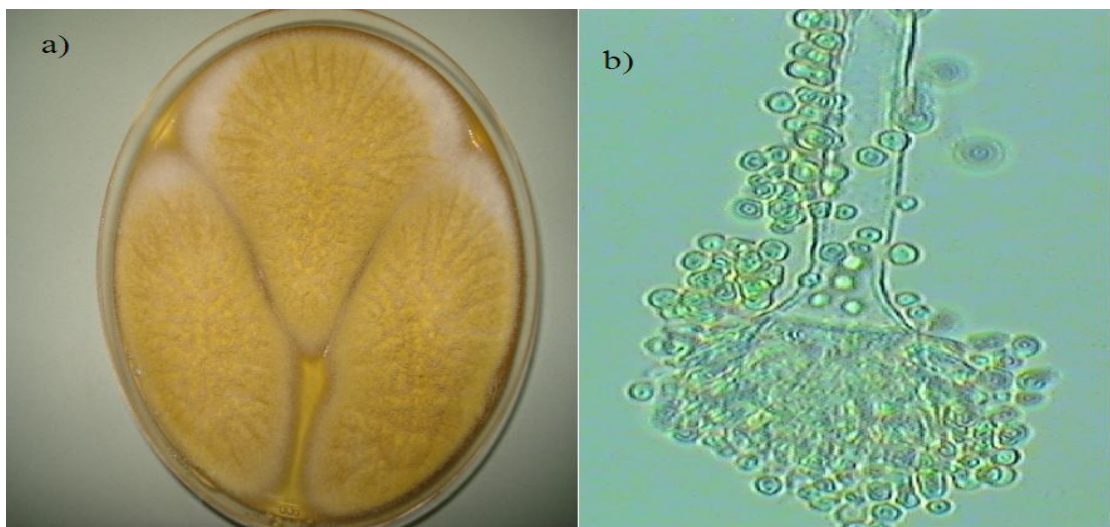




**Fig.6 *Aspergillus terreus* (a Macroscopic aspect ; b microscopic Aspect Gx100).**



**Fig.7 *Mucorales (Rhizopus sp)* (a Macroscopic aspect ; b microscopic aspect Gx100).**



**Fig.8 *Aspergillus Flavus* by method "Individual Spore" (a Macroscopic aspect ; b Microscopic aspect Gx100).**



**Fig.9 Macroscopic aspect *Cladosporium sp***

## **5. CONCLUSION**

We did some analysis of physico-chemical and mycological urban wastewater upstream and downstream of the city of AIN EL HADJAR. The results indicate a continued presence of indicators of fecal contamination by mold and a high rate of physico-chemical, upstream from this station with a decrease in the rates below. This decrease shows the effectiveness of different treatments that down at this station. On the other hand, we have shown the influence of some physicochemical parameter (pH, temperature, oxygen, ...) that can be responsible for the growth of mycological species. The latter can be valued in the wastewater treatment (fixing metal, each microorganism is chosen appropriately according to their affinity for each metal, degradation of organic pollutants such as phenol).

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