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ENQUÊTE MICROBIOLOGIQUE ET POSSIBILITÉ DE RÉUTILISATION DES EAUX USÉES POUR LA PRODUCTION VÉGÉTALE. UN CAS DE LA STATION D'EPURATION D'EL KOUWAER PENDANT LA PÉRIODE DE DYSFONCTIONNEMENT, ALGÉRIE

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Résumé

Description du sujet: La prolifération des bactéries filamenteuses et l'identification des microorganismes pathogènes, au niveau de l'eau épurée, influencent la valorisation des eaux usées dans l'irrigation en agriculture.

Objectifs: L'objectif de cette étude est d'évaluer la qualité microbiologique de la station d'épuration de la wilaya de Mascara pendant la période de dysfonctionnement afin de déterminer la possibilité de la réutilisation des eaux usées en agriculture.

Méthodes: 51 échantillons ont été prélevés pendant une période de quatre mois. Des examens microscopiques ont été réalisés sur les bacteries filamenteuse pour l'identification. Des bactéries pathogènes et une flore fongique ont été isolées, identifiées et dénombrées au niveau des eaux usées brutes et traitées.

Résultats: Les examens microscopiques montrent une diversité des bactéries filamenteuses: *Microthrix parvicella, Nostocoïda limicola* groupes (I, II et III), *Bogiotae* sp et *Sphaertilus natans*. Au niveau des eaux épurées vingt-deux (21) espèces pathogènes ont été isolées: *Enterobacteriaceae, Staphylocoquaceae, Pseudomonaceae, Aeromonas hydrophilae/caviae, Vibrio parahaemolyticus, Bacillus cereus et Streptococcus* sp., quatre (04) levures *Candida albicans, Candida glabrata, Trichosporon* sp et *Cryptococcus neofarmans* et cinque (05) espèces de champignons : *Aspergillus niger, Aspergillus flavus, Aspergilus ochraceus, Aspergillus fumigatus* et *Penicillium* sp. Les taux d'élimination des différentes flores est faible, sauf pour la flore totale qui est d'une valeur de 0,85.

Conclusion: La station d'El Kouwaer est caractérisée par une prolifération excessive de bactéries filamenteuses, un nombre important de bactéries pathogènes et de flore fongique, et une faible valeur des taux d'élimination. Selon les normes édictées par le journal officiel algérien, l'eau épurée peut être classée dans la catégorie B, elle peut être réutilisée pour l'irrigation des cultures telles que les céréales, les fourrages, les arbres fruitées.

Mots clés: Enquête microbiologique, eaux usées, période de dysfonctionnement, bulking, irrigation.

MICROBIOLOGICAL SURVEY AND WASTEWATER REUSE POSSIBILITY FOR CROP PRODUCTION. A CASE OF EL KOUWAER PLANT DURING DYSFUNCTION PERIOD, ALGERIA

Abstract

Description of the subject: The proliferation of filamentous bacteria and the identification of pathogenic microorganisms, at the level of treated water, influence the wastewater valorization in agricultural irrigation.

Objective: The objective of this study is to evaluate the microbiological quality of wastewater treatment plant in Mascara department during dysfunction period in order to determine the possibility of reutilization of effluent wastewater for crop irrigation.

Methods: 51 samples were collected over a four-month period. Microscopic examinations were performed on filamentous bacteria for identification. Pathogenic bacteria and fungal flora were isolated, identified and enumerated from raw and treated wastewater.

Results: Microscopic examinations show a diversity of filamentous bacteria: Microthrix parvicella, Nostocoïda limicola groups (I, II and III), Bogiotae sp and Sphaertilus natans. At the level of purified water twenty-two (21) pathogenic species were isolated: Enterobacteriaceae, Staphylocoquaceae, Pseudomonaceae, Aeromonas hydrophilae/caviae, Vibrio parahaemolyticus, Bacillus cereus and Streptococcus sp., four (04) yeasts Candida albicans, Candida glabrata, Trichosporon sp and Cryptococcus neofarmans and five (05) species of fungi: Aspergillus niger, Aspergillus flavus, Aspergilus ochraceus, Aspergillus fumigatus and Penicillium sp. The elimination rates of the different flora is low, except for the total flora which is a value of 0.85.

Conclusion: The El Kouwaer wastewater plant is characterized by an excessive proliferation of filamentous bacteria, an important number of pathogenic bacteria and fungal flora, and a low value of elimination rates. According to the norms issued by the Algerian official journal, the purified water can be classified in the category B, it can be reused for the irrigation of crops such as cereals, fodder, fruit trees.

Keywords: Microbiological survey, wastewater, dysfunction period, bulking, irrigation.

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INTRODUCTION

Algeria government works in order to preserve its water resources and to provide sustainable solution to water supply and management issues by carrying out a national water program [1]. The scarcity of water resources is causing serious problem at the moment that they are more and more limited. They are estimated at 19.2 billion m^3 of which 12.4 billion m^3 of surface water. By 2020, water needs will reach more than 8.3 billion m^3 of surface water per year [2]. Water reuse provides a promising alternative to face this crisis. Thus, the 1.702 operational WWTPs can double the currently mobilized volume. These infrastructures generate also nearly one billion cubic meters of treated water for agricultural irrigation making Algeria the leading African country in this field [3]. In recent years, attempts have been made to compensate irrigation water shortage through widespread wastewater application as a low-quality water resource for agriculture [4; 5]. The use of industrial or municipal wastewater in agriculture is a common practice in the world [6]. Wastewater reuse in irrigation would result in increasing agricultural production efficiency, surface water protection, reducing pressure on groundwater resources, and diminishing demand for chemical fertilizers, as well as decreasing wastewater treatment costs [7; 8]. As part of sustainable development, farmers will have to adopt alternative resources that balance the environment, economy and society. Wastewater reuse represents an opportunity for this challenge as it can alleviate the stress on scarce water resources and contribute to circular economies [9]. Thus, Wastewater treatment (WWT) is very important in modern life because it can affect various areas [10]. It aims mainly to remove biological pollutants from water and provides a major role in protecting the environment and public health [11], preserving the ecological balance [12], and reducing the high epidemic risk due to the presence of different pathogenic and multi-resistant microorganisms [13; 14]. Furthermore, other benefits of this treatment are the possibility of treated water reuses in various areas like agriculture manufacturing and human feed depending on bacteriological quality improvement of treated water [15; 16]. Several countries have adopted many waste water treatment technologies during the past years in order to increase reclamation efficiency and comply with discharge limits [17].

Several types of biological dysfunctions may occur and constitute a permanent concern of WWTP especially those affecting the activated sludge treatment process [18]. The most frequent of these dysfunctions is the abundance of filamentous bacteria causing phenomenon designed by "Bulking". It largely promotes the continuing disruption and flocculation directly affecting purification performance [19]. The present study aims to survey de microbiological quality of El Kouwaer WWTP during dysfunction period. Mainly, analysis of water microbiological quality in aeration tanks that can interfere with dysfunction and impact purification performance including filamentous and pathogenic bacteria, and fungi.

MATERIALS ET METHODES

1. El Kouwaer plant description

"El Kouwaer" is one of the wastewater treatment plants located in northwest of Mascara department in Algeria (Fig. 1). It is geographically situated at coordinates (35°23'06.1"N 0°08'33.4"E) and 474 m of altitude. This plant belongs to the combined (unitary) sewerage system treating 13.000 m^3 DMF (Daily Medium Flow) of domestic wastewater for 100.000 Population Equivalent (PE). The plant installation includes three submersible pumps repressing a rate of 800 m^3/h. Two rectangular aeration tanks precept for each one sewage volume equivalent to 11.660 m^3. The oxygen supply is performed by 3 other turbines. El Kouwaer plant also comprises two cylindrical clarifiers representing an area of 1018 m^2 and 4 m deep. Sludge drying is carried out in 20 beds each one constitute an area of 250 m^2.

2. Sampling

All samples were prepared and stored according to the NF EN ISO 5667-2; 1991 requirements. A total of 51 samples were employed for bacteria isolation and enumeration including 17 samples of influent water, samples 17 of effluent water and 17 samples of aerations tanks. They, were taken during plant dysfunction period of four months, from March to June 2013. One sludge sample was used for tank's microscopic examination. Particularly, for bacteria enumeration samples of 200 ml were taken each week from influent water, effluent water and at the level of the two aeration tanks. Then, transferred in sterile flasks for microbiological analysis within 8h.

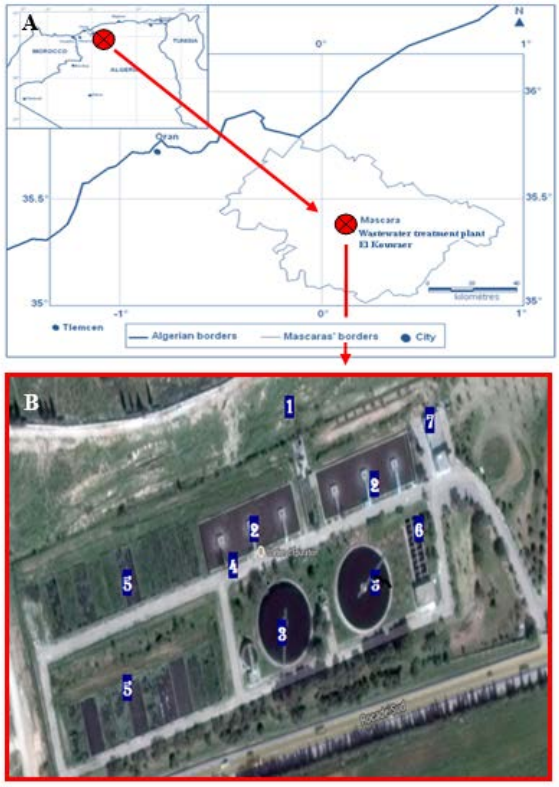

Figure 1: Geographical location of El Kouwaer sewage treatment plant (A: Geographic maps, B: general view of the station, 1: Effluent lift station; 2: aeration tanks; 3: clarifiers; 4: thickener; 5: sludge drying beds; 6: influent water tank; 7: administrative building).

3. Tank's microscopic examination

Tank's microscopic examinations aims to establish different morphological aspects of filamentous microorganisms. For dry state observation, 100 µl of sludge are placed on a microscope slide. Then, the reparation was fixed and observed using optical microscope at magnification of 100X [20]. For fresh state examination, 100 µl of the sludge sample was placed on a microscope slide. Then, microscopic observation was carried out using optical microscopy at a magnification of 100X [21; 22]. Gram staining technique was realized from 100 µl of the sludge sample that was taken and placed on microscope slide. The reparation was fixed and colored accordantly the basic technique steps, and observed at magnification of 100X [20].

4. Microbiological survey

4.1. Pathogenic bacteria evaluation

Pathogenic bacteria were isolated and identified from effluent wastewater samples using conventional techniques according to Delarras [23].

Isolation of *Pseudomonas aeruginosa* was carried out on Cetrimide agar, *Salmonella* and *Shigella* on Salmonella-Shigella medium, *Staphylococcus aureus* on Chapman medium, *Vibrio parahaemolyticus, Aeromonas hydrophilae/caviae* was performed on Alkaline Nutrient Agar. Characteristic colonies obtained were purified on the same selective medium used for isolation. Biochemical identification was carried out using API staph for Staphylococaceae, API 20E for Enterobacteriaceae and API 20NE for non-enteric Gram negative rods. All API systems were used according to the recommendations of the manufacturer with some modifications (Bio Merieux, France).

4.2. Fungal flora analysis

Mycoflora was isolated from effluent wastewater samples by suspension dilution technique using three media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Sabouraud medium [24]. Filamentous fungi were identified according to the corresponding determinative keys proposed by Kirk [25]. For yeast, identification was confirmed using API Candida galleries (Bio Merieux, France).

4.3. Filamentous bacteria determination

Filamentous bacteria were isolated from aeration tank samples based on the method described by Eikelboom [20] and some modifications made by Jenkins et *al.* [26] and Richard [27]. Their identification was established mainly by morphological criteria, most often associated with staining techniques. Briefly, microscopic examination was carried out one hour after sampling with or without preparation of serial decimal dilutions (10^{-1}, 10^{-2} and 10^{-3}). Dry and fresh states and Gram staining were performed according to usual bacteriological procedure. Images of microscopic observations were established using high resolution DP74 color camera connected to the WF10X/18mm Biological Microscope Wide Angle Hight Eyepiont Eyepiece Lens Field of View (18mm/0.8 in). Image analysis was realized with Image Tool software. Filamentous bacteria have were identified using morphological characters [26] and the classical approach for the identification of filamentous bacteria that is of Eikelboom [20], who studied more than 1100 activated sludge samples by phase contrast microscopy. Cell morphological features, like the presence or absence of a sheath or a slime layer, true or false branching, the length and shape of the filaments, and the diameter, were noted.

4.4. Calculation of microorganism frequencies

The Microbial Flora Frequency (MFF) of each flora (pathogenic bacteria and mycoflora) was calculated using the equation (1).

$$MFF = \frac{\text{Total Number isolate(s)of the species in all sampling unist (S)}}{\text{Total number of all sampling units (Q)}} * 100 \quad (1)$$

4.5. Bacterial flora enumeration

Bacterial flora enumeration was performed from influent and effluent wastewater samples. Total flora (TF) was enumerated using PCA medium according to the NF EN ISO 4833: 2003. Counts of total coliforms (TC) and fecal coliforms (FC) were carried out according to the standard methods described by NF EN ISO 4831: 2006. Enumeration of Fecal Streptococci (FS) was established as described by Delarras [23]. Enumeration of colony-forming unit was expressed using logarithmic notation, where the value shown is the base 10 logarithm (Log 10 CFU/ml) of the concentration.

Removal Allowance (RA) of different flora was estimated for each month using the following formula (2):

$$RA = -\text{Log } 10 \left(1 - \frac{P}{100}\right) \quad (2)$$

Where: the percentage of performance (P) was calculating using the formula (3):

$$P = \frac{\text{Log } 10 \text{ ewm} - \text{Log } 10 \text{ iwm}}{\text{Log } 10 \text{ ewm}} \quad (3)$$

Where: ewm: effluent wastewater per month; iwm: influent wastewater per month.

5. Statistical analysis

Bacterial enumeration data were analyzed by Principal Component Analysis (PCA) using Excel XLSTAT software version 2010.6.017.5.2.

RESULTS

1. Tank's microscopic examination

Dry and fresh state examinations showed excessive proliferation of filamentous microorganisms in sludge implicated in bulking phenomenon (Fig. 2A) and (Fig. 2B). Gram staining technique showed preliminary dominance of Gram positive filamentous bacteria compared Gram negative (Fig. 2C).

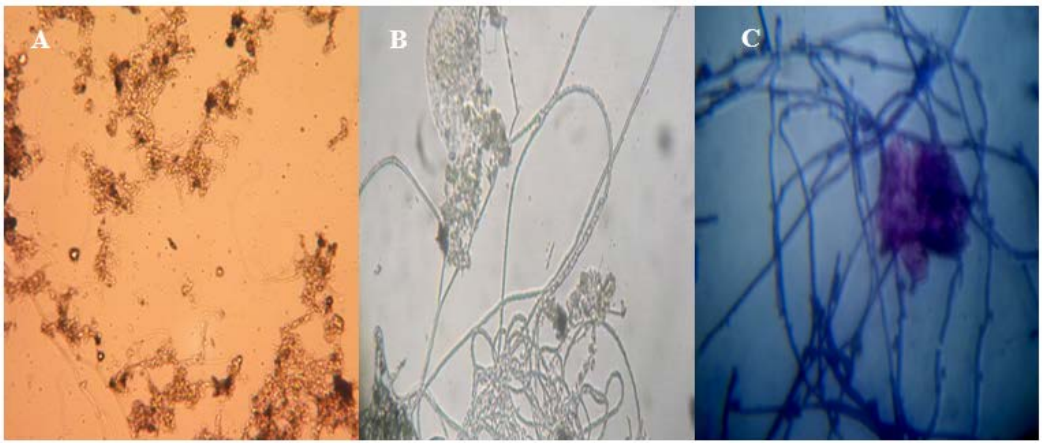

Figure 2: Microscopic observations of activated sludge (A: dry state; B: fresh state; C: Gram staining)

2. Microbiological survey

2.1. Pathogenic bacteria evaluation

Twenty-two (21) different pathogenic species were isolated from the in effluent wastewater samples (Table 1). The results indicated that the species belonging mostly to the *Enterobacteriaceae* family, which represents a Microbial Flora Frequency (MFF) of 38.00 % (06/17).

Followed by *Staphylocoquaceae* and *Pseudomonaceae* representing 23.80 % (04/17) and 19.04 % (03/17), respectively. Other pathogenic species represented 20.00 % (01/17) regrouping *Aeromonas hydrophilae/caviae, Vibrio parahaemolyticus, Bacillus cereus* and *Streptococcus* sp.

Table 1: Isolation, identification and microbial flora Frequencies (MFF) of pathogenic bacterial isolates.

Family	Q/S	MFF (%)	Isolate code	Genera and species (ID)
Enterobacteriaceae	06/17	38.00	EN1	*Enterobacter aerogenes*
			EN2	*Enterobacter sakazakii*
			EN3	*Escherichia coli*
			EN4	*Klebsiella oxytoca*
			EN5	*Klebsiella pneumoniae*
			EN6	*Salmonella enteritidis*
			EN7	*Shigella flexnerii*
Pseudomonaceae	03/17	19.04	PS1	*Pseudomonas aeroginosa*
			PS2	*Pseudomonas fluorescens*
			PS3	*Pseudomonas luteola*
			PS4	*Pseudomonas putida*
Staphylococcaceae	04/17	23.80	ST1	*Staphylococcus aureus*
			ST2	*Staphylococcus haemolyticus*
			ST3	*Staphylococcus lygdunensis*
			ST4	*Staphylococcus warneri*
			ST5	*Staphylococcus xylosus*
			ST6	*Staphylococcus epidermidis*
Other	04/17	20.00	AER1	*Aeromonas hydrophilae/caviae*
			BC1	*Bacillus cereus*
			SR1	*Streptococcus* sp
			VBC1	*Vibrio parahaemolyticus*

Q: Total number of all sampling units; S: Total Number isolate(s)of the species in all sampling unist; MFF: Microbial Flora Frequency, ID: Identification

2.2. Fungal flora analysis

Fungal flora analysis by determining of Microbial Flora Frequencies (MFF) revealed that the dominant yeast species in effluent wastewater samples were *Candida albicans* 60.00% (10/17) and *Candida glabrata* 30.00% (05/17) (Table 2). Other yeasts, like *Trichosporon* sp and *Cryptococcus neofarmans* represented 10.00% (02/17) for each one. In addition, among filamentous fungi, *Aspergillus niger* was dominant 70.60% (12/17). Followed, by *Aspergillus flavus* and *Penicillium* repreenting 18.00% (03/17). *Aspergillus fumigatus* and *Aspergilus ochraceus* were the lowest fungi isolated, as the MFF observed was 11.40% (02/17), for each filamentous fungus.

Table 2: Isolation, identification and Microbial Flora Frequencies (MFF) of mycofloral isolates

Mycoflora	Q/S	MFF (%)	Isolate code	Genera and species (ID)
Yeast	02/17	10.00	Y1	*Cryptococcus neofarmans*
	02/17	10.00	Y2	*Trycosporon* sp
	10/17	60.00	Y3	*Candida albicans*
	02/17	30.00	Y4	*Candida glabra*
Fungi	12/17	70.60	ASP1	*Aspergilus niger*
	03/17	18.00	ASP2	*Aspergilus flavus*
	02/17	11.40	ASP3	*Aspergilus ochrasus*
	02/17	11.40	ASP4	*Aspergillus fumigatus*
	03/17	18.00	PNC1	*Penicillium* sp

Q: Total number of all sampling units; S: Total Number isolate(s)of the species in all sampling unist; MFF: Microbial Flora Frequency, ID: Identification

2.3. Filamentous bacteria determination

Various filamentous bacteria were identified from El kouwaer plant during dysfunction period that are major bulking agents (Fig. 3). They include three *Nostocoïda* (*Nostocoïda limicola* group I*; Nostocoïda limicola* group II and *Nostocoïda limicola* group III)*; Microthrix parvicella; Beggiatoa* sp*;* and *Sphaerotilus natans. Nostocoïda limicola* group I (Fig. 3A1) and *Nostocoïda limicola* II (Fig. 3A2) belong to Gram positive bacteria. *Nostocoïda limicola* III (Fig. 3A3) is filamentous bacteria characterized by loop shape, established dented wall and variable *Microthrix parvicella* is a Gram positive bacterium which is characterized by formation of spaghetti morphology (Fig. 3B). *Beggiatoa* sp is a Gram positive bacterium endowed with flexible undulating form (Fig. 3C). *Sphaerotilus natans* is characterized by long filament lengths and false branches with a bumpy wall (Fig. 3D).

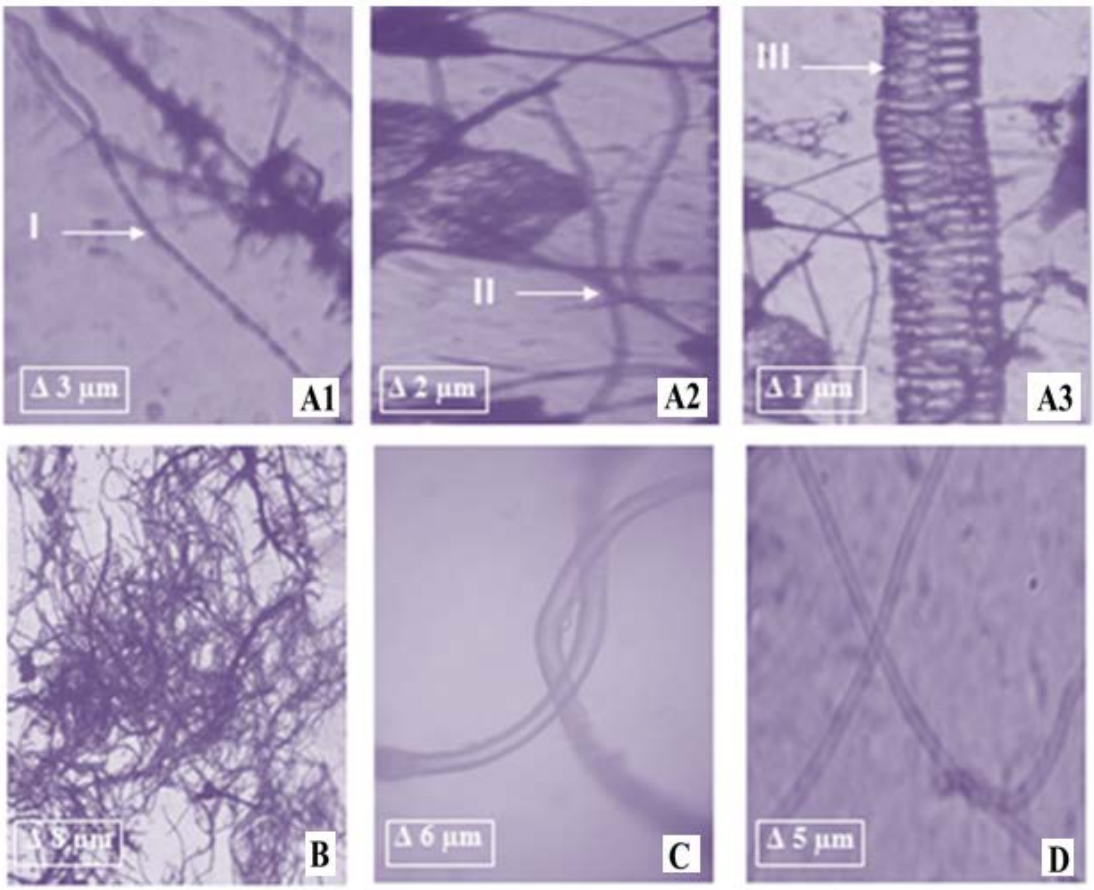

Figure 3: Gram staining observations of filamentous bacteria (A1: *Nostocoïda limicola* group I: A2: *Nostocoïda limicola* group II, A3: *Nostocoïda limicola* group III; B: *Microthrix parvicella*; C: *Beggiatoa sp.*; D: *Sphaerotilus natans.*

Evaluation of Microbial Flora Frequencies (MFD) showed the dominance filamentous bacteria group *Nostocoïda limicola* (group I, group II, and group III) representing and MFD of 82.35% (14/17) from all the 17 aeration tank samples (Table 3). Followed by, *Microthrix parvicella* and *Beggiatoa* sp. representing MVD values 58.82% (10/17) and 29.41% (5/17), respectively. *Sphaerotilus natans* was not dominant, as it was isolated only in 17.64% (05/17) of the aeration tank samples.

Table 3: Isolation, identification and Microbial Flora Frequencies (MFF) of filamentous bacteria isolates

Filamentous bacteria (ID)	Q/S	MFF (%)
Nostocoïda limicola (groups I, II and III)	14/17	82.35
Microthrix parvicella	oct-17	58.82
Beggiatoa sp	mai-17	29.41
Sphaerotilus natans	mars-17	17.64

Q: Total number of all sampling units; S: Total Number isolate(s) of the species in all sampling unist; MFF: Microbial Flora Frequency, ID: Identification

2.4. Bacterial flora enumeration

Enumeration results of various bacterial floras in effluent and influent wastewater samples during dysfunction period (four months) showed low reduction of most of all microorganisms enumerated, except for total coliforms (Fig. 4). The enumeration of the colonies-formed expressed using logarithmic notation showed that for Total Flora (TF) (Fig. 4A) in influent wastewater samples, a minimum of 3.4 Log CFU/100ml value during (April, May and June), and a maximum of 3.5 Log CFU/100ml corresponding to March. In addition, their number in effluent wastewater samples ranged between a minimum of 2.6 Log CFU/100ml recorded in March and maximum of 2.8 Log CFU/100ml corresponding to June.

For total coliforms (TF) numeration, a maximum of 3.14 Log CFU/100ml was recorded during most of all the dysfunction period (four months) in influent wastewater samples, however, in effluent wastewater a minimum of 3.13 Log CFU/100ml we recorded during (April, May and June), and a maximum of 3.14 CFU/100 ml in March (Fig. 4B). For Fecal Coliforms (FC), their number ranged between a minimum of 2.7 Log CFU/100 unregistered in March and a maximum of 2.9 Log CFU/100 ml recorded in (April, May and June) for influent wastewater samples. For effluent wastewater samples, their enumeration values ranged among a minimum of 2.1 Log CFU/100 ml unregistered during (March, April and March) to a maximum of 2.3 Log CFU/100 ml recorded in June (Fig. 4C). Results of Fecal Streptococci (FS) enumeration varied between a minimum of 2.6 Log CFU/100 ml in March and a maximum of 2.8 Log CFU/100 ml recorded during (April and June) for influent wastewater samples. For effluent wastewater samples their values varied between a minimum of 2.1 Log CFU/100 ml in May and a maximum of 2.2 Log CFU/100 ml recorded during (March, April and June) (Fig. 4D).

Removal Allowance (RA) of different flora was estimated for each month. The results of Total Flora (TF) enumeration (Fig. 4A) revealed a minimum removal allowance of 0.59 recorded in June and a maximum of 0.85 corresponding to March. For Total Coliforms (TF), a maximum removal allowance of 0.008 was recorded in March (Fig. 4B). Reduction of Fecal Coliforms (FC) ranged between a minimum of 0.52 unregistered in March to a maximum of 0.8 recorded in April (Fig. 4C). Results of Fecal Streptococci (FS) removal allowance revealed that it varied between a minimum of 0.43 in March and a maximum of 0.58 Log recorded in April (Fig. 4D).

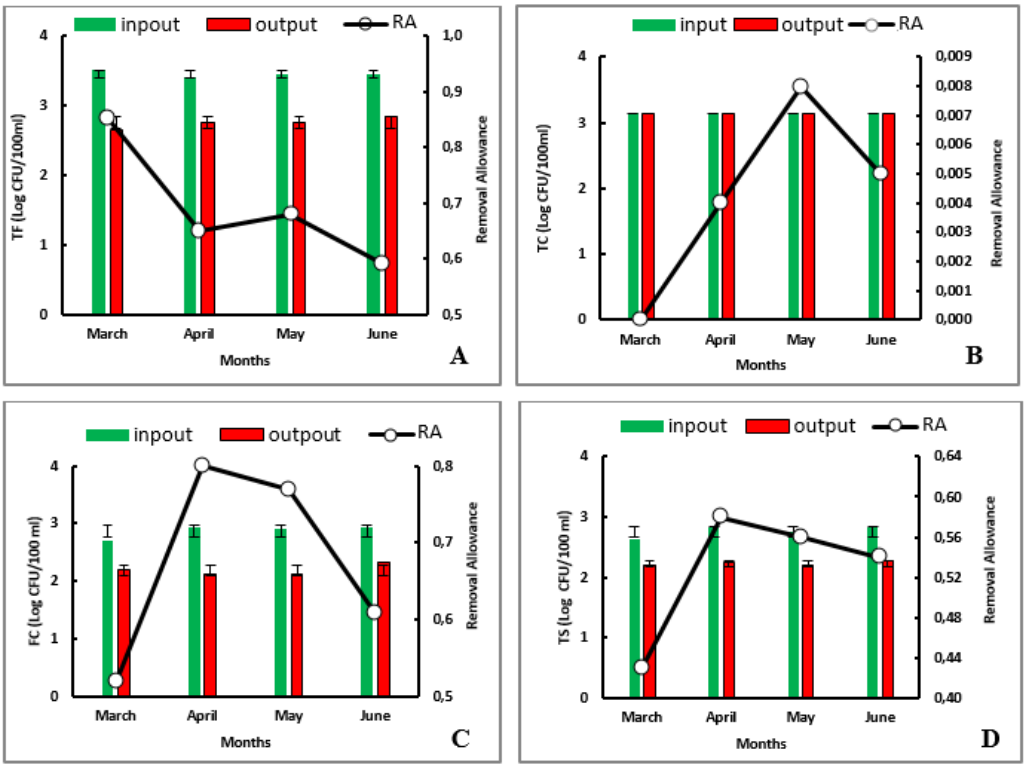

Figure 4: Bacterial flora enumeration (RA: Removal Allowance; A: Total Flora (TF); B: Total Coliforms (TC); C: Fecal Coliforms (FC); D: Fecal Streptococci (FS).

Graphical representation of Principal Component Analysis showed that bacterial variable space of the factorial plane F1-F2 expressed 93.64% of the variance (Fig. 5). First axis F1, presented variance equal to 70.34 %, expressed to its positive pole by fecal streptococci and total flora of effluent water. Total flora (influent water) and total coliforms (effluent water) were negatively correlated. This axis provided information on most of the parameters that determine the degree of mineral and organic pollution. Second axis F2, contributory component 23.30% of the total variance which was positively correlated to the fecal coliforms (effluent water) and negatively correlated to the fecal streptococci and total coliforms (influent water).

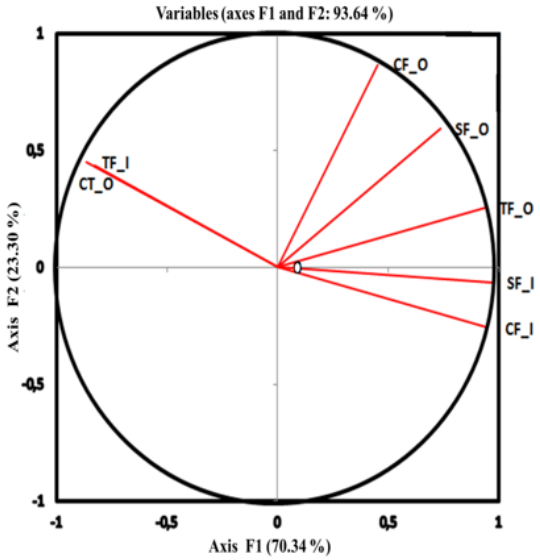

Figure 5: Graphical Principal Component Analysis (PCA) representation (CF-O: Fecal Coliforms- Output; SF-O: Fecal Streptococci-Output; TF-O: Total flora-Output; SF-I: Fecal Streptococci-Input; CF-I: Fecal Coliforms-Input; CT-O: Total Coliforms-Output; TF-I: Total Flora-Input).

DISCUSSION

The evaluation of the microbiological quality of WWTPs and wastewater reuse in agriculture for irrigation are indissociables parameters for safe and sustainable agriculture. In this current investigation, tank's microscopic examination revealed excessive proliferation of filamentous microorganisms. This could promote the continuing disruption and fluctuation directly affecting purifying performances and limite the clarifier hydraulic capacities [18]. The majority of plants during dysfunction period, revealed excessive proliferation of filamentous microorganisms [28; 29]. Proliferation of these microbes at high levels, responsible of phenomenon designed by “sludge bulking” or “bulking”, is a major cause of sludge process deficiency. Sludge bulking occurs when the sludge fails to separate out in the sedimentation tanks. It arises from an overgrowth of filamentous organisms inducing an important loss of biomass, that considerably reduce the removal efficiency of Suspended Solids (SS), Chemical Oxygen Demand (COD) and nutrients. Preventing sludge bulking is a challenge residing in the characterization of the large diversity and the specific ecology of the causing-filamentous microogganisms [30]. It has been reported also that, during functioning period, most of the filamentous bacteria found in activated sludge are Gram negative, only few stains are Gram positive bacteria [18].

In this study pathogenic bacteria analysis showed dominance of *Enterobacteriaceae*, *Staphylocoquaceae* and *Pseudomonaceae*. Wheares, a number of investigations like reported by Hamaidi-Chergui et *al.* [31] who mentioned the absence of certain pathogens such as Salmonella and Vibrio in wastewater samples during plant dysfunction period. The potential transmission of pathogenic microorganisms, essentially enteric pathogens, can cause various redoubtable diseases such as typhoid fever, dysentery, diarrhea and vomiting [32]. Experiments of detection of pathogens have demonstrated that the detection of pathogenic microorganism in treated water is influenced by $WWTP_S$ operational parameters as hydraulic system, residence time, seasonality and the composition of effluent water [33]. The nature and concentration of pathogens in sewage depend also on the health of source populations [34]. In this current study, the presence of filamentous fungi (*Aspergillus* and *Penicillium*), and yeasts (*Candida*, *Trichosporon* sp and *Cryptococcus*) at high frequencies may be explained by the load of organic matter in the raw water during dysfunction period. All these fungal species representing hight Microbial Flora Frequences (MFF) can be attributed to their metabolic potentiality preserving their distribution and survival under unfavorable conditions. Similar results were reported by Hamaidi-Chergui et *al.* [31] mentioning the presence of several species of the genus *Candida* especially *Candida albicans*, and fungi like *Aspergillus* sp and *Penicillium* sp in WWTP during analysis of raw and treated water. The presence of those fungi and yeasts can cause serious health risks [35]. Their origin can come either from feces or from the environment.

In this current work, various filamentous bacteria were identified from El Kouwaer plant during dysfunction including *Nostocoïda; Microthrix parvicella; Beggiatoa* sp*;* and *Sphaerotilus natans***.** Identifying which filaments bacteria dominating will help to understand the condition in the treatment system so that corrective changes can be made [36]. Different filamentous bacteria will dominate in wastewater depending on nutrients [37]. Accurate of filamentous bacteria in activated sludge systems is, therefore, an essential step in the characterization of the bulking phenomenon [38]. Their identification is made primarily on the basis of morphological features and microscopic characterization [20; 39].

Determination keys have been developed based essentially on the observations of their morphological criteria [26]. Particularly, *Nostocoïda limicola* and its various morphotypes (groups I, II and III) is a typical domestic filament [40]. Several studies showed that *Nostocoïda limicola* was among the most morphotypes observed in the sludge plants affected by bulking phenomenon [41]. *Microthrix parvicella*, belonging to the group IV, recover its carbon source requirements mainly from short chain fatty acids at the level of effluent water [20]. It can dominate in the municipal treatment plants causing operational difficulties, such as flocculation defects and foaming [42]. The study performed by Milobedzka & Amuszynsk [43] showed also that *Beggiatoa* sp. is one of the most abundant species featured bulking phenomenon. *Sphaerotilus natans* is another major filamentous bulking agent implicated in plant dysfunction treatments. This filamentous bacterium can be present in activated sludge during normal function and dysfunction state. Recent studies have shown that excessive proliferation of *Sphaerotilus natans* is the key factor of bulking phenomenon [44].

In this study, bacterial flora enumeration results (total flora; fecal coliforms and fecal streptococci) showed that influent water samples were charged with those pathogenic microorganisms compared to their bacterial number in effluent water. While, stabilization of total coliforms number was observed. Bourouache et *al*. [45] reported persistence of fecal streptococci compared to fecal coliforms during plant dysfunction period. For any given wastewater treatment system, there are essentially two pathogen removal factors: how long the pathogen stays in the system and how quickly it dies. The former is governed by the hydraulic flow regime and the latter depends on the ecology of the reactor [46]. The evaluation of microbiological quality of wastewater is based on the concept of these germs often called indicators. Their presence means the existence of a contamination by materials, and their abundance is an indication of the risk level of the pathogenic microorganism presence. This investigation has demonstrated particularly, the persistence of Fecal Streptococci (FS) compared to Fecal Coliforms (FC) during dysfunction period as established by their variations in the reduction of their corresponding Removal Allowance (RA) values.

This can be explained by the difference in their resistance degrees as described many authors [47; 48]. In addition, this is can suggest a dysfunction notably at the level of the tertiary treatment system of "El Kouwaer" wastewater treatment plant. The comparison between the bacteriological accounts of effluent wastewater and the regulation standards given by the Algerian inter-ministerial decree of 2 January 2012, setting the specifications of purified wastewater that can be used for irrigation purposes, allows to categorizing of El Kaouwer effluent wastewater in category B because the none elimination and high account of Total Coliforms (TF) used as indicator of fecal contamination. This wastewater can be reused only for irrigation of cereals, fodder, fruit trees and industrial crops [49].

CONCLUSION

Analysis of El Kouwaer wastewater treatment plant during dysfunction period revealed microbiological poore quality affecting treatment performances. Thus, allowed to raise a serious sanitary and environmental problem. At the level of treated water, the presence of pathogenic bacteria, fungal floras and filamentous bacteria responsible for "bulking phenomenon" indicated major dysfunctions. The water effluent can be classified in category B which can be used effectively in agriculture. A number of provision techniques, such as chlorination, may be taken for the disinfection and the amelioration of the microbiological quality of treated wastewater after its reuse in farming for irrigation of a restricted number of crops.

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Suplemetary material

Table 1 : Biochemical characters of the isolated bacteria « *Enterobacteriaceae* »

Test	Isolates							
	EN1	EN2	EN3	EN4	EN5	EN6	EN7	VBC1
ONPG	+	+	-	+	+	-	-	-
ADH	-	+	-	-	-	+	-	-
LDC	+	+	+	+	+	+	+	+
ODC	-	+	+	-	-	+	+	+/-
CIT	+	+	-	+	+	+	-	-
H_2S	-	-	-	-	-	+	-	+
URE	-	+	-	+	+	-	-	+/-
TDA	-	+	-	+	-	-	-	-
IND	-	+	+	+	-	-	+	+
VP	-	+	-	-	+	-	+	-
GEL	+	+	-	-	-	-	+	+
GLU	-	+	+	+	+	-	-	+
MAN	+	+	+	+	+	+	-	+
INO	+	-	-	+	+	-	-	-
SOR	+	+	+	+	+	+	-	?
RHA	+	+	+	+	+	+	-	?
SAC	+	+	+	+	-	-	-	-
MEL	+	+	+	+	+	-	+	?
AMY	+	+	-	+	-	-	+	?
ARA	+	+	+	+	+	-	-	+

EN1:*Enterobacteraerogenes.* **EN2:***Enterobactersakazakii.* **EN3:** *Escherichia coli.***EN4:***Klebsiellaoxytoca*.**EN5:***Klebsiellapneumonia*.**EN6:** *Salmonella enteritidis.***EN7:***Shigellaflexnerii.* **VBC1:***Vibrioparahaemolyticus.* **ONPG:**β-galactosidase (Ortho nitrophenyl-galactoside degradation). **ADH:** Arginine dihydrolase. **LDC:** Lysine decarboxylase. **ODC:**Ornithinedecarboxylase. **CIT:** Citrate assimilation. **H_2S:** H_2S production. **URE:** Urease. **TDA:**Tryptophanedesaminase. **IND:**Tryptophane assimilation (indole production). **VP:VogesProskauer test (Acetoin production)**. **GEL:** Gelatin degradation. **GLU:** Glucose assimilation. **MAN:**Mannitol assimilation. **INO:** Inositol assimilation. **SOR:** Sorbitol assimilation. **RHA:**Rhamnose assimilation. **SAC:**Saccharose assimilation. **MEL:**Melibiose assimilation. **AMY:** Amygdaline assimilation. **ARA:** Arabinose assimilation.

Table 2: Biochemical characters of the isolated bacteria*"Staphylococcaceae"*

Test	Isolates					
	ST1	ST2	ST3	ST4	ST5	ST6
GLU	+	+	+	-	-	+
FRU	+	+	+	-	-	+
MNE	+	+	+	-	+	+
MAL	+	+	+	+	+	+
LAC	+	+	-	-	+	+
TRE	+	+	+	-	+	-
MAN	+	+	-	-	-	-
XLT	-	-	-	-	-	-
MEL	-	+	+	-	+	-
NIT	+	+	+	+	+	+
PAL	+	-	-	-	-	+
VP	-	+	+	-	-	+
RAF	-	-	-	-	+	-
XYL	-	+	+	-	+	-
SAC	+	+	+	-	+	+
MDG	-	-	-	-	-	-
NAG	+	+	+	-	-	-
ADH	+	+	+	+	+	+
URE	-	+	+	+	+	+

ST1: *Staphylococcus aureus*.**ST2:***Staphylococcus haemolyticus*. **ST3:***Staphylococcus lugdunensis*. **ST4:** *Staphylococcus warneri*.**ST5:** *Staphylococcus xylosus*.**ST6:***Staphylococcus epidermidis*.**GLU:** D-glucose assimilation. **FRU:** D-fructose assimilation. **MNE:** D-mannose assimilation. **MAL:** Maltose assimilation. **LAC:** Lactose assimilation. **TRE:** D-trehalose assimilation. **MAN:** D-mannitol assimilation. **XLT:**Xylitol assimilation. **MEL:** D-melibiose assimilation. **NIT:**Réduction of Nitrates to Nitrites .**PAL:** Alcaline phosphatase. **VP:**VogesProskauer test (Acetoin production). **RAF:** Raffinose assimilation. **XYL:** Xylose assimilation. **SAC:** Saccharose assimilation. **MDG:**Methyl-α-D-glucopyranoside assimilation. **NAG:** N-acetyl-glucosamine assimilation. **ADH:** Arginine dihydrolase. **URE:** Urease.

Table 3:Biochemical characters of the isolated bacteria *"Bacillus"*

Test	GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	MDG	NAG	AMY	ARB	ESC	SAL	MAL	LAC
BC1	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

BC1:*Bacillus subtilis*. Carbon assimilation profiles obtained: **GLY:** Glycerol. **ERY:**Erytrol. **DARA:** D-arabinose. **LARA:** L-arabinose. **RIB:** Ribose. **DXYL:** D-Xylose. **LXYL:** L-Xilose. **ADO:** D-Adonitol. **MDX:**Metil-β-D-xylopyranoside. **GAL:** Galactose. **GLU:** Glucose. **FRU:** Fructose. **MNE:**Manose. **SBE:**Sorbose. **RHA:**Rhamnose. **DUL:**Dulcito. **INO:**Inozitol. **MAN:**Manitol. **SOR:** Sorbitol. **MDM:**Metil-α-D-manopyranoside, **MDG:**Metil-D-glucopyranoside. **NAG:** N-acetylglucosamine. **AMY:** Amygdaline. **ARB:**Arbutine. **ESC:**Esculin. **SAL:**Salicin. **MAL:** D-celobiose. **LAC:** D-maltose.

Table 4:Biochemical characters of the isolated bacteria*"Streptococcaceae"*

Test	VP	HIP	ESC	PYR	αHE	βGAL	ARG	RIB	ARA	βHE	MEL	SOR	LAC	INU
SM1	+	-	+	-	+	-	-	-	-	-	-	-	+	+

SM1:*Streptococcus mutans*.**VP:** Sodium pyruvate. **HIP:**Hippuratehydrolysis.**ESC:** Esculine hydrolysis. **PYR:**Pyrrolidonylarylamidase (L-pyrrolidonyl-α-naphthylamidehydrolysis). **βGAL:** β-Galctosidase (P-nitrophenyl-β-D-galactopyranosidehydrolysis). **αHE:**α-Hemolysin.**βHE:**β-Hemolysin. **ARG:** Arginine dihydrolase. **RIB:** Ribose fermentation. **MEL:**Melibiose fermentation. **SOR:** Sorbitol fermentation. **LAC:**Lacose fermentation. **ARA:** Arabinose fermentation. **INU:**Inuline fermentation.

Table 5:Biochemical characters of the isolated yeasts

Test	Isolates			
	Y1	Y2	Y3	Y4
GLU	+	+	+	+
GAL	-	+	+	-
SAC	+	+	+	-
TRE	+	+	+	+
RAF	+	+	-	-
βMAL	-	-	+	-
αAMY	+	+	+	-
βXYL	+	+	+	-
βGUR	-	-	-	-
URE	+	-	-	-
βNAG	-	-	+	-
βGAL	-	+	+	-

Y1:*Cryptococcusneofarmans*. **Y2:***Trycosporon*sp. **Y3:***Candida albicans*. **Y4:***Candida glabra*. **GLU:** Glucosefermentation. **GAL:** Galactose fermentation. **SAC:** Saccharose fermentation. **TRE:**Trehalose fermentation. **RAF:**Rafinose fermentation. **βMAL:**β-Maltosidase. **αAMY:** α-Amylase. **βXYL:** β-Xylosidase. **βGUR:** β-Giucuronidase. **URE:**Urease. **βNAG:** N-acetyl-β-glucosaminidase. **βGAL:** β-Galactosidase.

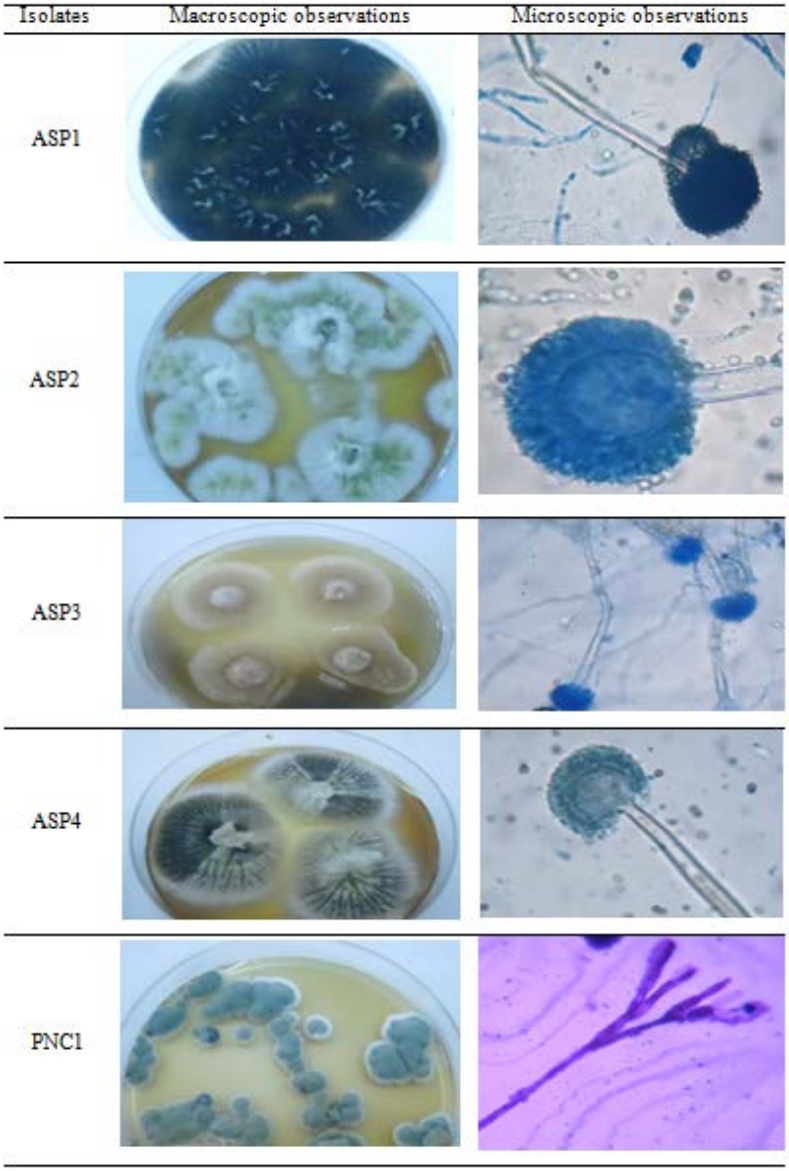

Figure 1: Macroscopic and microscopic characters of the isolated filamentous fungi (ASP1**:** *Aspergilus niger*, ASP2: *Aspergilus flavus*, ASP3: *Aspergilus ochrasus*, ASP4*: Aspergilus fumigatus*, PNC1: *Penicillium* sp).