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Inventory of the Isolated and Identified Mycoflora from Water Polluted by Hydrocarbons of Sonatrach Skikda and Hassi Messaoud

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Abstract: Algeria has huge oil reservoir, which was used in the early years of independence as an essential element for its development by the national oil company SONATRACH. Organic pollutants among which include the group of hydrocarbons involved in environmental concerns a special place. This interest is due to their persistence in the environment and their toxicity. Their elimination of different ecosystems is a priority and requires the intervention of various biotic and abiotic factors. Discussing this study, we aim at evaluating the physicochemical characteristics of water samples and to make an inventory of the mycoflora from industrial effluents of two sites Sonatrach Skikda and Hassi Messaoud, in comparison with control sites.The total fungal mycroflora isolated is composed of different species belonging to the genera Aspergillus, Chaetomium, Cladosporium, Trichoderma, Penicillium and Rhizopus. As a result, we found that the tolerance of these strains from highly polluted environment indicates their good adaptation to harsh conditions: this illustrates their degradative capacity and so therefore their possible use in bioremediation process.

Key words: Hydrocarbons · Mycoflora · Tolerance · Bioremediation

INTRODUCTION

The development of human activities has become a threat to the environment which suffers from decades of harmful attacks. Algeria is the fourth largest oil producer in Africa and the reservoir are operated by SONATRACH.

The devastating effects of oil industrialization are assessed on the environmental, healthy and economical plans. Indeed, many real damage has been incurred in accidents (oil leak... etc), releases or deliberate spills, which can cause irreversible ecological catastrophe [1].

The assessment of oil pollution using quantitative and qualitative analysis is very expensive although it is essential in providing physicochemical data quantified. However, these measures show up the degree of pollution and measures the concentrations of pollutants and also measuring its effects [2]. For restoring polluted by petroleum products environments, bioremediation is the most effective and requested solution because it's more controlled and less expensive [3]. There are two main reasons that hydrocarbons persist in nature. First, the conditions necessary for their biodegradation are not present. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site [4]. If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavourable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation. However, there were some microorganisms that can survive in hydrocarbons contaminated site. Hydrocarbon may affect water microbial populations, stimulating growth of certain microorganisms and exerting toxic effects and inhibiting growth of others. So, identification and characterization of these microbial species is important to study about its potential candidate used in bioremediation. Metabolic processes of these organisms are capable of using

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chemical contaminants as an energy source rendering the contaminants harmless or less toxic products in most cases.

Therefore, in present investigation an interest to different releases of hydrocarbons and the status of their pollution in waters level. Another attempt has made to isolate and characterize fungal strains from the different sites. As well as an inventory was made.

MATERIALS AND METHODS

The quantitative and qualitative evaluation of the mycoflora was done during June, 2011 and made on water samples heavily rich with hydrocarbons: the first sample is taken from Sonatrach Skikda (North-East of Algeria) and the second one from Hassi Messaoud (South-East of Algeria).

Isolated and identified mycoflora populations were compared to those from control non-contaminated sites: the first one is from Collo (North-East of Algeria) and the second from Ouargla (South-East of Algeria).

Fungal strains were isolated in Petri dishes by these culture media: MEA (Malt Extract Agar: Malt Extract: 20 g; Agar-agar:15 g; chloramphenicol: 0,05g; distilled water: 1000 ml; pH: 5,6), PDA (Potato Dextrose Agar: potato extract: 4g; Agar-agar: 5 g; dextrose, 20g; water: 1000 ml; pH: 5,6) and Czapeck-Dox Agar: $NaNO_3$: 3 g: K_2HPO_4: 1 g; KCl: 0,5 g; $MgSO_4.7H_2O$: 0,5 g; $FeSO_4.7H_2O$:0,01g; Saccharose: 30 g; Agar-agar: 20 g; water: 1000 ml. The pH is adjusted at 4,5. They also were incubated at 37°C±2°C for five days. The different culture media were prepared according to the manufacturer's instructions.

The isolated fungi were identified conventionally according to their macromorphological and micromorphological characteristics, according to Domsch *et al.* [4] and Botton *et al.* [5]. The following morphological characteristics viz colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production …etc: were recorded. In some cases they were stained by cotton blue in Lactophenol. After determination of their genera, they were transferred to the media recommended by the authors of selected genus monographs for species identification. The process of identification usually involves specialized keys [4,5].

A physicochemical study of water samples was also performed. The analyses focused on the pH, temperature, biological oxygen demand (DBO_5), chemical oxygen demand (DCO) and the rate of hydrocarbons. Methods of analysis of the various physicochemical parameters are made according to Rodier's [6].

RESULTS AND DISCUSSION

Physicochemical Water Analysis

pH: Each microorganism requires a determined pH between 4 and 8, bacteria grows better at neutral to basic pH, on the contrary, fungi prefers an acidic pH; there are many exceptions [7].

Knowledge of pH means understanding the type of environment in which microorganisms grow. The recorded values of pH (Fig. 1) indicate that it's alkaline; the values are between 9.1 and 7.8; in contrast to control sites which are close to neutral. These records are different from the maximum water of industrial installations where the pH value is typically between 5.5 and 8.5 [8].

Temperature: Each microorganism requires a determined temperature for its growth [7]. We note that samples temperature from different sites varied from 27.1°C to 35.8°C, value higher compared to control sites are between 33°C and 25°C, although sampling values have taken place in June (Fig. 2). It's noted that the temperature values of Collo and Skikda's samples exceed standards of industrial effluents (30° C) [8].

Chemical Oxygen Demand (COD): According to the results, the values of COD are varied. The sample of Hassi Messaoud shows an increase compared to other sites (628 mg / l), followed by that of Ouargla with 125 mg / l. These values exceed the limit of COD of industrial effluents which are fixed at 120 mg / l [8].

The lowest value was recorded from the sample of Skikda (4.18 mg / l).

Biological Oxygen Demand (BOD): It shows that the recordes of Hassi Messaoud are very high (314 mg / l), followed by those calculated at Skikda (100 mg / l). They clearly exceed the acceptable standards of industrial effluents limited to 40 mg / l [8].

BOD of control sites are between 10 mg / l at Collo and 40 mg / l at Ouargla. (Fig. 4).

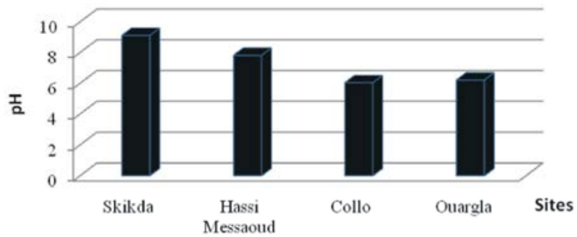

Fig. 1: pH values recorded of water samples

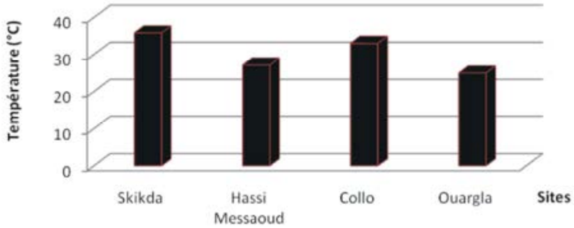

Fig. 2: Temperature values recorded of water samples

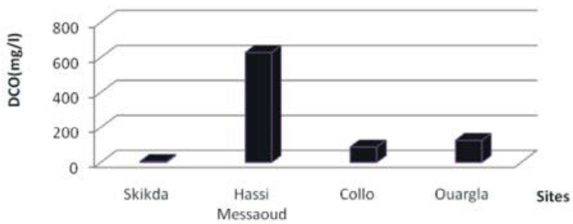

Fig. 3: COD values recorded of water samples.

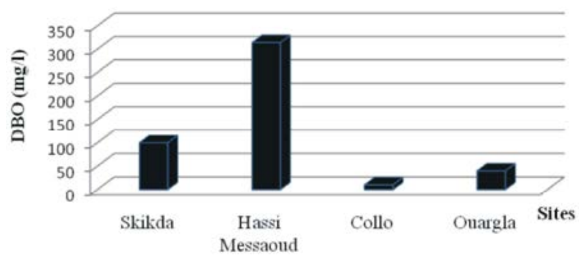

Fig. 4: BOD values recorded of water samples

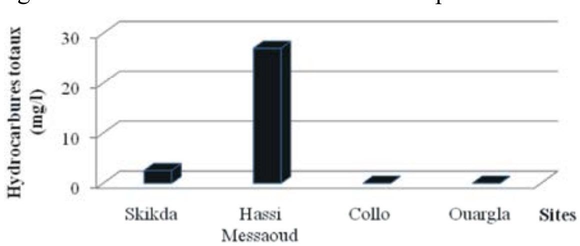

Fig. 5: Values of total hydrocarbons recorded from water samples

Total Hydrocarbons: Organic substances in the waste water are primarily hydrocarbons. The results show that hydrocarbons are present in almost all sampling sites with the value of 27 mg / l at Hassi Messaoud and 2.5 mg / l recorded at Skikda. In the control sites, the value of total hydrocarbons is zero. (Fig. 5).

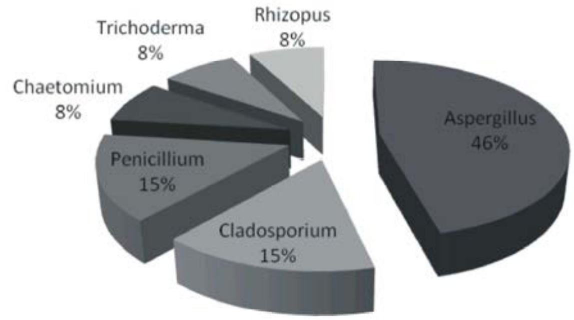

Fig. 6: Distribution of fungal genera

Microbiological Analyses: The quantitative evaluation of the total isolated mycoflora on different medium from water sampling reveals a significant number developed at 37°C. Those of control sites are very low (Table 1). The species are distributed in a heterogeneous manner in all analyzed samples. The filamentous fungi can grow on hydrocarbons, with Aspergillus and Penicillium species being the most frequently reported [9-10].

The total isolated mycoflora is estimated at 13 species, with a dominance of Aspergillus (Table.1.). This effective reflects a variety in species for contaminated sites by hydrocarbons. A rare presence of Chaetomium, Rhizopus and Trichoderma was noted in all samples.

Aspergillus fumigatus is the most powerful type of the isolated mycoflora species, which is saprophytic fungus, widespread, reported in several media by many authors. It was previously isolated in the waters and sediments in Eastern Algeria [11] and in soils of the North-East of Algeria [12].

Fungi have a potential of morphological development in response to specific environmental situations [13]. We can say that they are endowed with a remarkable ability to adapt to difficult conditions [12].

We noted an important presence of *Aspergillus fumigatus* in all studied sites; however someone occupy only control sites: the case of *Aspergillus clavatus* and *Penicillium citreonigrum* (Table 1).

Aspergillus occupy first place in the inventory with 6 species, followed by Cladosporium and Penicillium with 2 species, the rest is represented only by one species (Fig. 6). Elshafiea *et al.* [14] showed that Aspergillus and Penicillium species are more active than the others.

Cladosporium sphaerospermum occupies only sites of Collo and Skikda, *Penicillium chrysogerum* in its turn is isolated only from water sample of Hassi Messaoud and Ouargla.

Table 1: The list of species identified in each site

Souches	Skikda	Collo	Hassi Messaoud	Ouargla
Aspergillus fumigatus	*	*	*	*
Aspergillus flavus			*	
Aspergillus niger		*	*	*
Aspergillus clavatus		*		
Aspergillus parasiticus			*	
Aspergillus versicolor			*	
Chaetomium tetrasporum	*		*	
Cladosporium sphaerospermum	*	*		
Cladosporium cladosporioides	*			
Penicillium chrysogerum			*	*
Penicillium citreonigrum		*		
Rhizopus stolonifère			*	
Trichoderma viride	*			

Most of isolated and identified fungal strains from water samples of different sites are capable of adapting to the most difficult environmental conditions (polluted and unpolluted environment). Ecological conditions of wastewater have certainly imposed a selection of microorganisms and some environmental parameters permit a number relatively varied of species to live in such biotope [11].

However, among all the strains isolated and identified, only *Aspergillus fumigatus* is clearly the most adapted to hydrocarbons. Other strains have a preferential tolerance.

The vast majority of fungal species degrade hydrocarbons. Most researchers have focused their studies on fungi «white rot» and especially *Phanerochaete chrysosporium* [17-18].

They haven't the same degradation rate as bacteria [13] but it remains limited by the presence of nutrients [13-17].

Fungi don't use hydrocarbons as only source of carbon and energy, the environment must contain an additional one which helps them to break down and mineralize the aromatic hydrocarbon chain [19,24]. They grow very quickly and their cell wall presents a large fixation capacity and often shows high intracellular retention values. The cell wall performs the role of protection and exchange between the cell and the external environment [19].

CONCLUSION

The physicochemical results of the study sites have a significant impact on the composition of the mycoflora both quantitatively and qualitatively. However, the physical environmental factors (temperature, pH, moisture... etc.) are a necessary support to their life.

The pH is the most important influence on the biological activity of micro fungi. The majority develops in an area of 4.5 to 8.0 and the optimum range is between 5.5 to 7.5.

The results show that hydrocarbons are present in the samples of Hassi Messaoud and Skikda, however those of control sites are zero. Hydrocarbons in whatever form are generally the most common contaminant that requires remediation due to their widespread occurrence and the risks they pose, they are toxic to all forms of life.

Microorganisms are widely distributed in nature. They are important agents of degradation of all organic matter.

This study allowed us to obtain a fairly rich mycoflora inventory, composed of several fungal species which may survive in specific conditions of pollution by hydrocarbons.

That is how the fungal species can be used in bioremediation processes, because they have the characteristics of biological decontamination agents.

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