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Evaluation the quality of the oil waste to AI-Nasiriya refinery and possibility of treatment by some filamentous fungi

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ABSTRACT

Nine filamentous fungi isolated from the soil contaminated with petroleum hydrocarbon and wastewaters from refinery located in the South part of AI-Nasiriya city by using dilution methods,(1kg) from soil and (1L) from wastewaters were collected in and outside the refinery randomly.Also soil samples were kept in sterilized plastic sac,and stored in refrigerator until analysis. Wastewater samples were also collected with (250 ml) cleaned and sterilized winkler bottle, and also stored in refrigerator until analysis.In present investigation was study the ability of isolated fungi to biodegradation hydrocarbons in wastewaters and determined total numbers of spores in all stage of fungi growth by using haemocytometer method. The results showed that *Aspergillus niger* were more dominant with 94% and *A.flavus* was 85%, the percent of *Penicillium sp.* reached to 72% while the percent of *A.fumigatus* reached to 69%. The remaining 5 fungal species isolated with a very low frequency and the percent reached to 11%. The results showed that *Penicillium sp.* Was the best fugus to decreased Total Dissolved Solid (TDS) from 700mg/I to 290mg/I in fourth week with 30c^o,pH 6,1.0gm / I Nacl,and the removal percent reached to 57%,in the same time the results showed that the temperature 20 c^o,30 c^o,pH 4,6 and 0.5, 1.0 of salt concentration were the optimum condition to biodegradation and decreased to TDS and TPH by axenic and mixed fungi. The result refer that the highest of total numbers of spores production in wastewater treatment were recorded with mixed fungi (*Aspergillus niger*, *A.fumigatus*, *A.flavus*, *Penicillium sp.*), in 20 c^o, pH 4, 0.5 gm / I NaCI. The results showed that the removal percent of Total Petroleum hydrocarbons(TPH) were reached to 99.99%, and the statistical methods obtained a significantly with a temperature in wastewater treatment with mixed fungi.

KEYWORDS: Keywords:*filamentous fungi; Refinery; Soil; Spores ;TDS; Wastewater.*

INTRODUCTION

high amount of water are consumed by industrial activities and consequently, significant amounts of wastewater are generated. Different levels of pollutants are discharged into the environment directly. The pollutants discharged from industries were effluent into the aquatic environments as well as the petroleum oil refineries with a partial treatment or without treatment. As not all refineries have the same processes, the effluents that are produced will have different chemical compositions depending on the type of treatment they received [1], [2]. Wastewater released by oil refineries contain large amount of toxic derivatives such as oil and grease, Phenols, Sulphides, Cyanides, Suspended solids, nitrogen compounds as well as heavy metals such as iron, nickel, copper, selenium, zink, molybdenum, etc [3]. Due to the ineffectiveness of purification systems, wastewater from the refineries may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem [4]. Thus the discharge of these effluents containing persistant chemicals into a receiving water body may result in the long-term effects to aquatic organisms [5].

The toxicity of oil refinery effluents to aquatic organisms have being reported in many literatures. Toxicity of petroleum refinery depends on a number of factors, such as quantity, volume and variability of discharge. The different components of the effluents may have varying effects and toxicity [6].[7]showed that the petroleum did not persist for long periods in the most soils even when relatively large quantities of petroleum have spilled, This is probably due to large part to the initial degradation by the action of sun light (Photodecomposition), so as by microbial attack when the oil sink. Fungi and other microorganisms have the ability to degrade several pollutants, including crude oil in the aquatic ecosystem and consumed them as a nutrient and energy source [8,9,10,11,12,13,14].They may also metabolize such as pollutants to substrates with low harmful effect on the environment [15],[16], [17].The efficiency of fungi isolated from soil and wastewater to treatment of effluent petroleum wastewater from AI-Nasiriya refinery has not been investigated, and the present study investigated the quality of the refinery effluent discharge.

MATERIAL AND METHODS

Characterization of Petrolum oil refinery wastewater:

Non-treated Petroleum oil refinery wastewater were collected from inside and outside refinery by using 5 litres polyethylene containers. The containers were rinsed several times with the effluent sample at the point of collection.The sample were bring to the laboratory within 1h of collection, TDS,TPH, Cations (Cd,Pb,Ni,Zn,Fe and Cu)in wastewater samples were determined according to standard methods [18].

Fungi strains and cultures conditions:

The fungi strains used in this study was isolated from soil and wastewaters samples collected from effluent discharged of refinery in AI-Nasiriya city. Only Potato Dextrose Agar (PDA) media were used to isolated fungi from soil and wastewaters, the media was supplemented with 250 mg /I Chloramphenicol to suppress bacterial growth. Plates and media were incubated at 25 c^o in the dark.Single colonies were picked from the plates under a dissecting microscope and transferred to appropriate media to allow fungal development. Stock cultures were maintained on the Potato Dextrose Agar slant, subcultured periodically and stored at 4 c^o.Fungal specimens were examined under light microscope after preparations and identified using morphological characters and taxonomical keys provided in the mycological keys [19].

Preparation of standardised inoculums:

Spores suspensions were prepared by adding 15 mL of sterile distilled water to mature (4-5 days) fungal colonies on PDA plates to dislodge the spores from the mycelium.The spores were counted using a haemocytometer (Neubauer,Germany) to obtain a spore concentration of a bout 10^5 spores / mI. These suspensions were then used to inculate 100 mL of wastewaters after sterilized in 500 mL Erlenmeyer flasks [20].The cultures were incubated at 25 c^o in an incubator and shaking three time in day.This resulting culture was then used as standard inoculum for further experiments.

Determination of biodegradation potential fungi:

To determine the biodegradation of oil degrading fungi. Add 500 mI of wastewaters from AI-Nasiriya refinery after sterilized by autoclave to Erlenmeyer flasks and incubated with 10 mI from axenic and mixed cultures of fungi spores (10^5 spore / mI), All flasks were coverd with non cotton wool absorbent and incubated at (10,20,30,40 c^o) with pH (2,4,6,8) by using HCL or NaOH and add (0.0,0.5,1.0,2.0 gm / I) Nacl. Control flasks were left without fungi and these experiments were duplicate. The flasks were shaken manually at regular intervals to allow adequate mixing and homogeneity of the contents.1 mI of liquid culture was examined with Haemocytometer under light microscope and culculated spores.

A nalysis of the fungal biodegradation activity was made using a computerized capillary gas chromatography with flame ionized GC- FID (Backard 438 A) and flame ionization detector set at 325 c^o. The carrier gas was Helium at flow rate of 30 mL / min. The column length 3m, internal diameter 1/ 8 mm. The temperature was programmed to increase from 100 to 300 c^o at 10 c^o / min. Individual compounds present in the fractions were determined by matching the retention time with authentic standards.

Statistical analysis:

The present study conducted on Anova (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, a significance difference.

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RESULTS AND DISCUSSION

The results of the chemical characterization of the oil refinery wastewater sample are shown in Table 1. The wastewaters sample have elevated levels of organic compounds as indicate by the concentrations of TDS, TPH.

Table 1. showed a reduction in the values of Zn,Cd,Pb,Ni,Fe in outside the refinery but Cu was increased.

Table 2. Showed the fungal strain isolated from soil and wastewaters of AI-Nasiriya refinery. *A.niger* was more dominant with 94% and *A.flavus* was 85%, the percent of *Penicillium sp.* reached to 72% while the percent of *A.fumigatus* reached to 69%. The remaning 5 fungal species isolated with a very low frequency while the percent reached to 11%. These results were similar to the findings of [21], which showed that increase in the fungal population when presence of oil in the soil. In the same time the similar results were record by [12], in their study obtained that *A.niger* were more frequency with 100% in all samples, but *A.fumigatus* and *Penicillium funiculosum* were 83%. Similar results reports have indicate the increase of microbial population in the presence of oil contaminated soil [22],in their study about the effect of oil spill on the composition of microbes in a soil, they found that the soil was dominant by a diversity of oil degrading fungi including *Penicillium sp.,Rhizopus sp., Thamanidium sp.,Cunninghamella* and *Candida.* The hydrocarbons may inhibited or caused by a death of certain microorganisms.On the other hand, there will also be increasing in numbers of certain microorganisms especially those capable of depending the hydrocarbons [23], The same result was obtained by [24],in this study showen that the numbers of oil degrading fungi were more than the numbers of oil degrading bacteria in the soil and changes in the flora of soil fungi following oil waste application and the study of [25] on the biodegradation potential of hydrocarbon assimilating tropical fungi. The statistical methods obtained significantly with temperature. This result was similar to findings of [26] which show that the growth of *Trichoderma virens* UKMP-IM was highest under acidic conditions and was able to grow in a relatively wide range of pH from 5 to 7 and suggesting that this isolate could degrade oil under not only acidic but also neutral conditions. [27] refer that temperature was affect on biomass production. [26] refer that heterotrophic organisms can degrading the oil and increasing in number.The increase in numbers of fungi in present study in 0.5gm Nacl due to the adaptation of osmosis and because to produce Polyols and glycerol compounds and these compounds work to osmosis bufferering and transport positive sodium ion systems [28], [29].

In the same time the high concentrations of salt produced increasing of spores production in some fungi [30].

Fig 1,2,3,4 showed decreased of Total Dissolved Solid (TDS) in wastewater treatment with axenic and mixed culture of fungi. The highest decreased were recorded with axenic culture of *Penicillium sp.* in 30 c^o, pH 6, 1.0 gm / I Nacl and the value reached to 290 mg / I as well as the removal percentage reached to 57% Fig. 3 in fourth week when compared with control, this result due to that this fungus can degradation of dissolved solids substrates, the statistical methods obtained significantly with temperature 10 c^o and 30 c^o and in the same time no significant differences were observed in *Penicillium sp.* with different temperature.The results showed that *Penicillium sp.* Was the best fugus to decreased Total Dissolved Solid (TDS) from 700mg/I to 290mg/I in fourth week with 30c^o,pH 6,1.0 gm / I Nacl. It means that the fungal strains were able to degrade crude oil and consumption of its components. Crude oil consists of saturated and aromatic hydrocarbons and asphaltic compounds of varing molecular weight, complexity, and degree of susceptibility to microbial oxidation [31]. Mycellial organisms can penetrate insoluble substances such as crude oil and this increase the surface are available for microbial attach [8].

This result refer to that the greater capacity to remove crude oil due to the adaptation of this fungi to the pollutant composition as well as to the enzymatic systems of the fungi [32]. It means that the fungal strains were able to degrade crude oil and consumption of its components.Also [14] refer that the biodegradation process depend on the type of hydrocarbon,the genus, species, and may be the strain of the fungus, as well as on nutritional and fermentation conditions.In the same time [9] recorded that *A.niger* showed the largest colony diameter on medium with 20% kerosene amongst *A.terreus, Rhizopus sp.* and *Penicillium sp.* [33] refer that temperature had a significant role in biodegradation of hydrocarbons with direct effecting on the chemistry of the pollutants, and effecting the diversity of the microbial flora. Temperature also effected on the solubility of hydrocarbons and the activity of the bio- enzyme. The biodegradation constants of removal PAHS and TPH reduction manily reduced with decreasing temperature. The highest biodegradation rates that happenes in the range 15- 20 c^o in marine environment. At 20 c^o temperature the viscosity of the oil decreased, the volatility of the low molecular weight hydrocarbons were increased, bio- enzymatic oxidation were increased, and the accelerating the onset of biodegradation.

Fig 5,6,7,8 showed increased of total numbers of spores production in wastewater treatment with different fungi with increased of period incubation and in the same time the results refer that the highest of total numbers of spores production in wastewater treatment were recorded with mixed fungi (*Aspergillus niger*, *A.fumigatus, A.flavus*, *Penicillium sp.*), in 20 $c^{o,}$ pH 4, 0.5 gm / I NaCI Fig. 6.Fig.9 showed that the reduction of TPH was increased by an increase in the reaction's duration and increased temperature, pH and Nacl concentration in wastewaters treatment with mixed fungi cultures from 1 week to 4 week and the percentage removal of TPH reached to 99.99% when compared with control. These figures showed decreased concentration and appear many Peaks when compared with control. This result was similar to findings of [33],which show that the TPH value was reached to 99.98% when compared with initial concentration of 770 mg 1-1. [8] obtained that the mycelial organisms can penetrate insoluble substances such as crude oil and this increase the surface are available for microbial attack. [32] found that *Penicillium funiculosum* and *A.sydowii* were loss TPHS concentration to 86, 81 % respectively, and study of [34] on the biodegradation potential of hydrocarbon, they had been showed that *A.fumigatus* can removed of PHAS with 80 % after 120 days of exposure, and the same result was obtained by [35] in their study reported that *A.terres* and *Fusarium sp.* were the percent degradation to aliphatic compounds reached to 100 %.

Studies of [36] and [37] it is clear that very low salt concentrations reduce hydrocarbonoclastic activity and the optimum biodegradation results are reached whitin moderate salinity ranges.

First of all, it is necessary to consider that hydrocarbons are less bioavailable in hypersaline environments that in non saline ones [38].

Table 1: Chemical characterization of the oil refinery wastewaters.

Element	Inside Refinery	Outside Refinery
Zn	0.885*	0.460
Cd	0.137	0.083
Pb	0.121	0.093
Ni	0.283	0.062
Fe	2.299	1.160
Cu	0.128	0.164

* PPm

Table 2: Fungal strain isolated from soil and wastewaters from AI-Nasiriya refinery.

Fungal species	Numbers of fungal species appear	Frequancy %
Aspergillus niger Tighem	9	94
A.fumigatus Fresenius	6	69
A. flavus Link	8	85
Trichocladium opacum Harz	1	11
Alternaria alternate Keissler	1	11
Fusarium solani Link	1	11
Penicillum sp. Thom.	6	72
Trichoderma lignorum (Tode)Harz	1	11
Rhizoctonia solani Kuhn	1	11

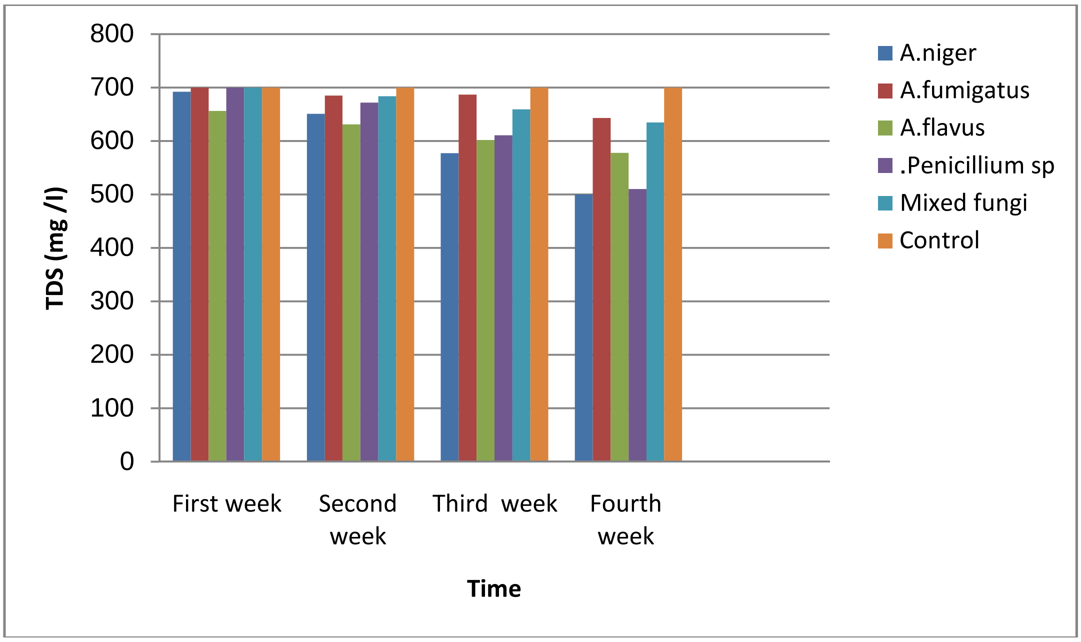

Fig. 1: Values of Total Dissolved Solid (TDS) in wastewater treatment with 10 c^{o}, pH 2 and 0.0 gm / I NaCI.

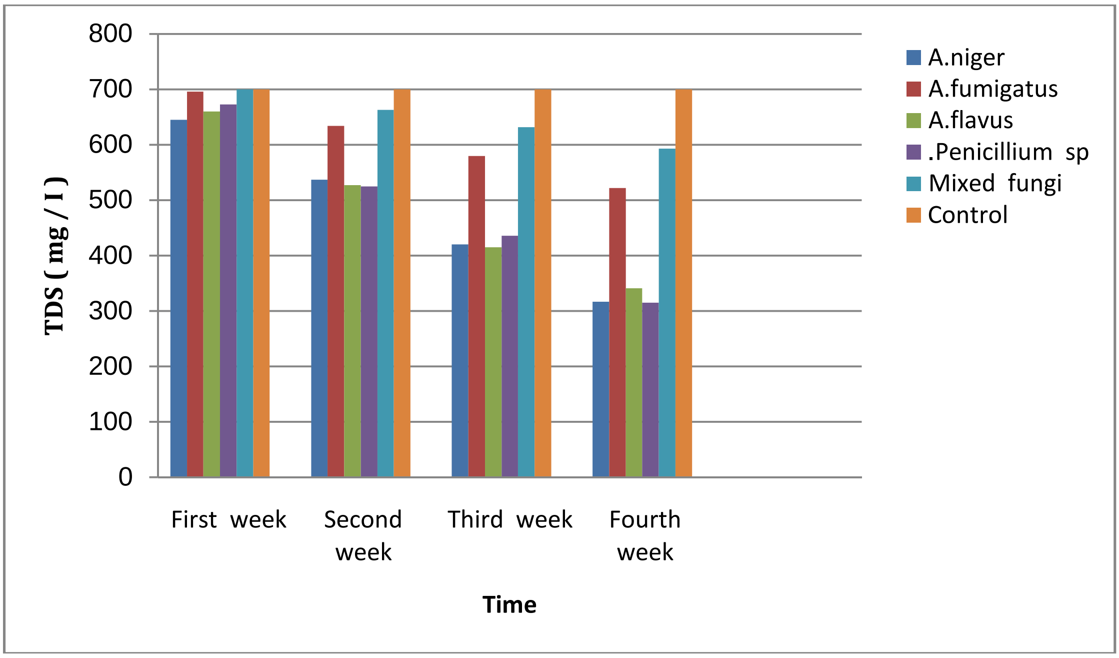

Fig. 2: Values of Total Dissolved Solid (TDS) in wastewater treatment with 20 c^{o}, pH 4 and 0.5 gm / I NaCI.

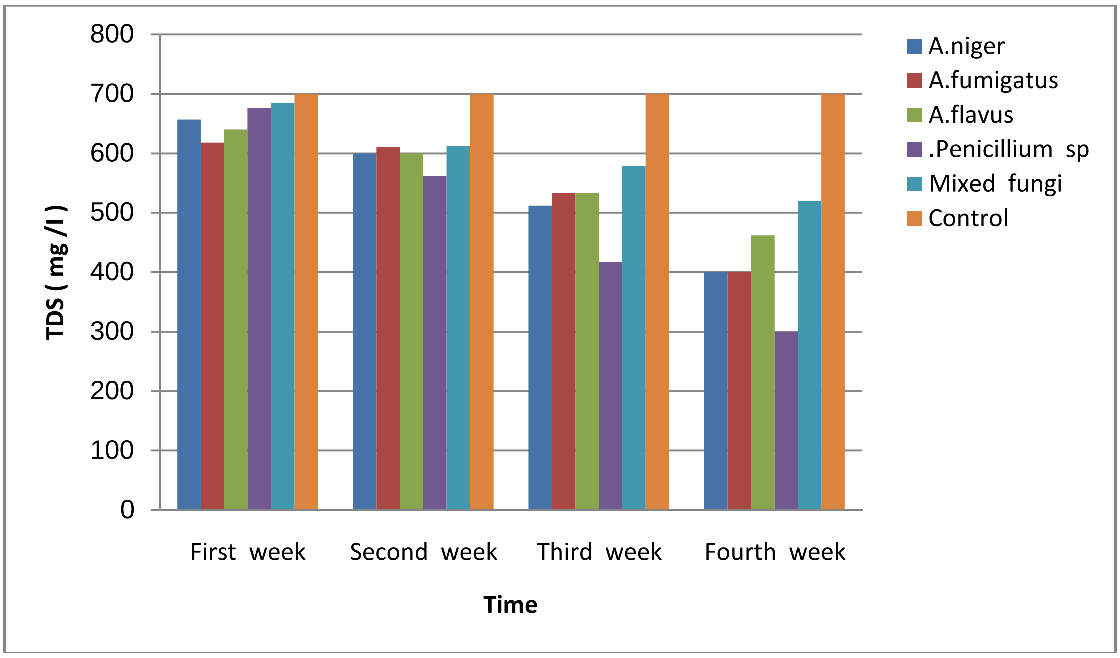

Fig. 3: Values of Total Dissolved Solid (TDS) in wastewater treatment with 30 c^{o}, pH 6 and 1.0 gm / I NaCI.

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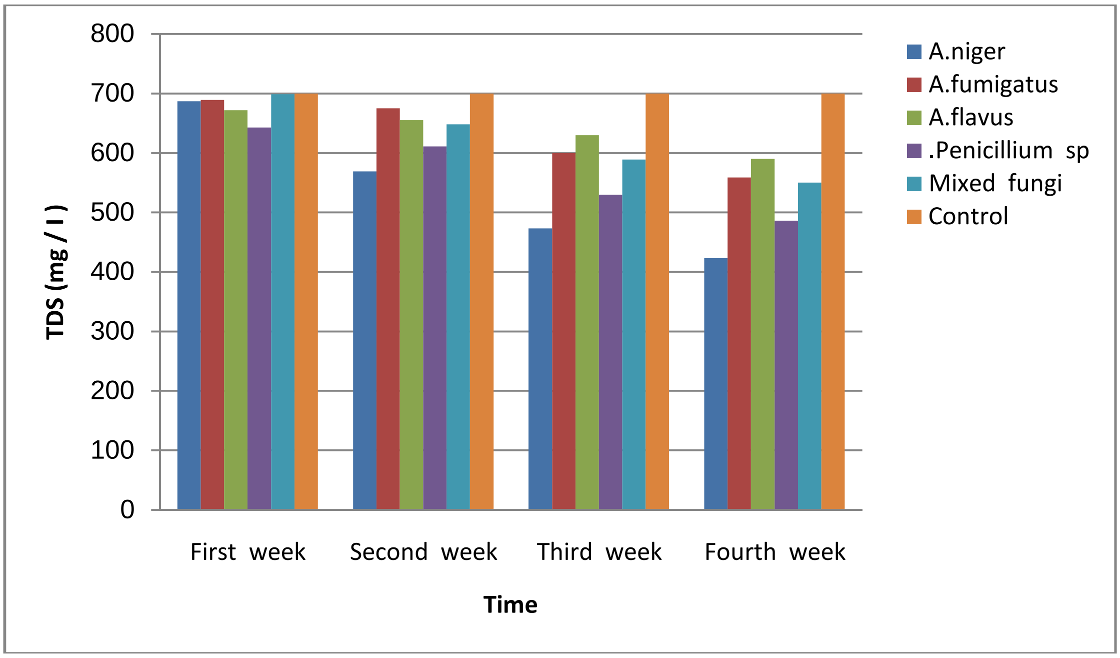

Fig. 4: Values of Total Dissolved Solid (TDS) in wastewater treatment with 40 c^{o}, pH 8 and 2 gm / I NaCI.

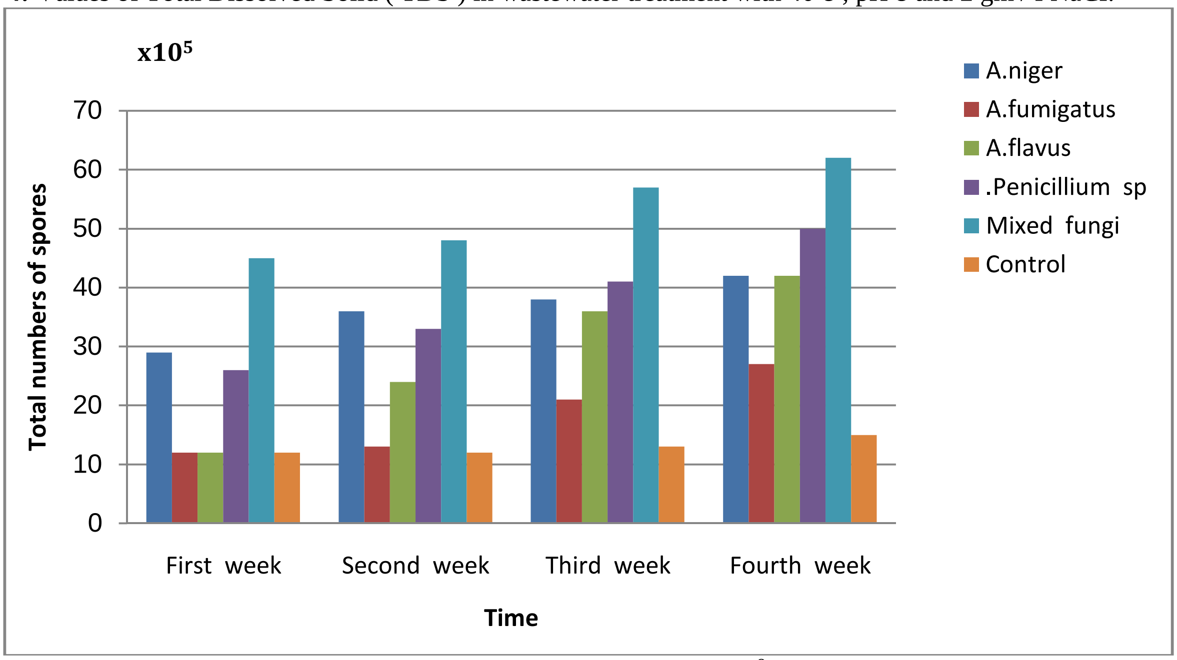

Fig. 5: Total numbers of spores production in wastewater treatment in 10 c^{o}, pH 2 and 0.0 gm / I NaCI.

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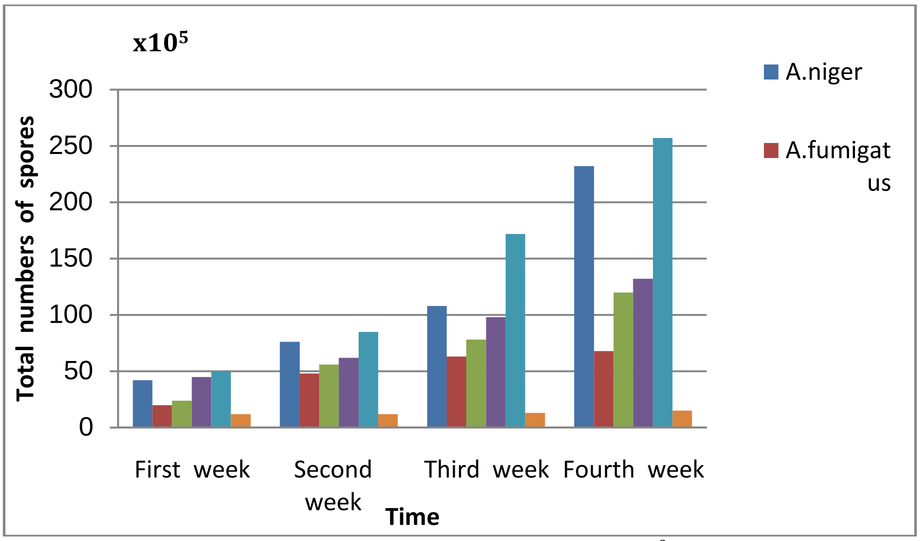

Fig. 6: Total numbers of spores production in wastewater treatment in 20 c^{o}, pH 4 and 0.5 gm / I NaCI.

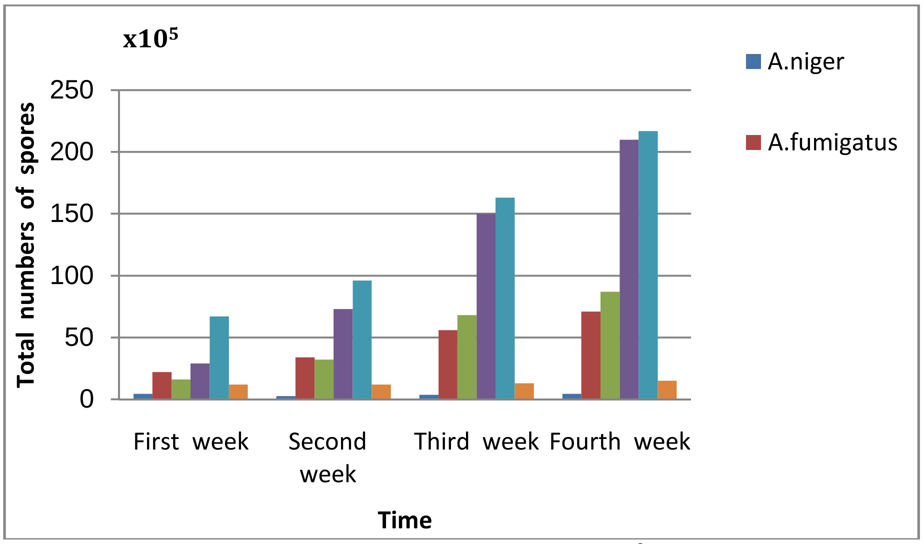

Fig. 7: Total numbers of spores production in wastewater treatment in 30 c^{o}, pH 6 and 1.0 gm / I NaCI.

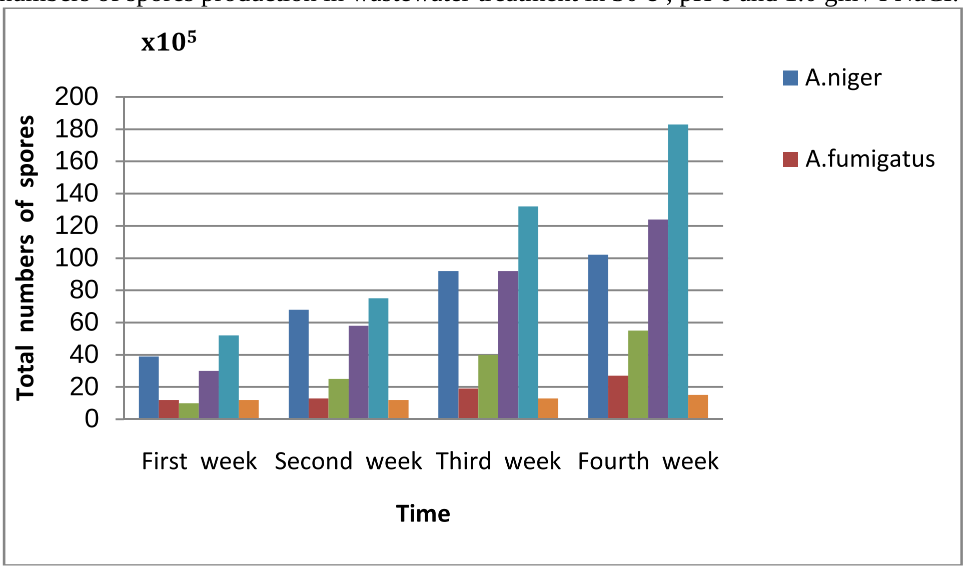

Fig. 8: Total numbers of spores production in wastewater treatment in 40 c^{o}, pH 8 and 2.0 gm / I NaCI.

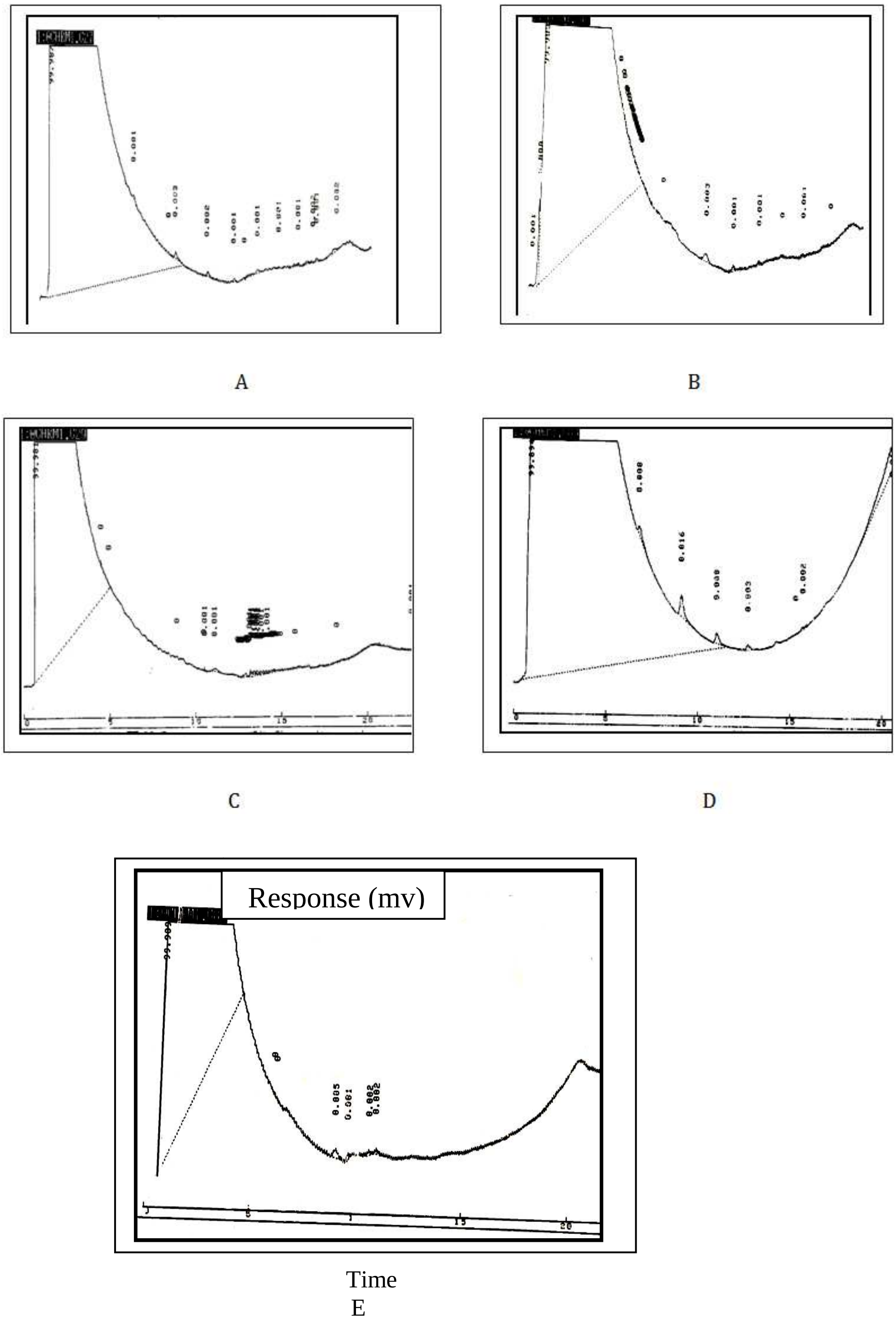

Fig. 9: GC- Chromatogram of removal Total Petroleum Hydrocarbon (TPH) in wastewaters in treatment with mixed fungi (A: First week, B:Second week, C:Third week,D:Fourth week, E:control).

Conclusion:

The oil degrading fungi isolated from the soil and wastewaters in the present study included *A.niger*, *A.flavus*, *A.fumigatus* and *Penicillium sp.*, and the density of these fungi were dominant than other fungi. These fungi were well adapted to degrade and consumed the crude oil. The mixed culture of these fungi were beter than the axenic fungi to removel of TPH.

The reduction of TDS and TPH enhanced with increasing reaction's duration time, for given experimental conditions. The pH was effect on biodegradation of TDS, and TPH reduction, also the highest reduction obtained at pH 6.

The 0.5 and 1.0 gm / I salt concentration (NaCI) a chieved the highest efficiency in biodegradation of TDS and TPH reduction. The temperature 20 c^o, 30 c^o was a chieved the highest efficiency in biodegradation of TDS and TPH.

Rehabilitation of oil contaminated soil and wastewaters by the culture of mixed fungi (*A.niger, A.flavus, A.fumigatus* and *Penicillium sp.*) were promising as it can reduce the oil pollution to acceptable levels for reuse of land and water within a short period.

The data obtained in the present investigation was advanced our knowlege of petroleum hydrocarbons and behavior of fungi in polluted soils in different location, and how these fungi to breakdown or biodegradation petroleum hydrocarbons in environment,as well as can used these organisms to removel pollution now and also in future.

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