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Biodegradation of Some PAHs by a Locally-Isolated Strain of Actinomycetes

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Abstract

Samples from the River Nile, the Mediterranean, and the Red Sea were chemically and microbiologically evaluated. The chemical analysis proved that cations and anions concentrations were of higher values in seawater samples in comparison with those of the Nile water. The microbiological analysis showed that Cairo University Nile Bus Station samples (GCB-19) recorded the increased total viable microbial counts ($1.6x10^4$ CFU/ml). Total coliforms highest counts were analyzed in GCB-19 samples (110 cells/ml), whereas fecal coliforms highest counts (15 cells/ml) were recorded in Cairo University Nile Bus Station samples (GCB-17) and (GCB-19). PAHs were determined in the collected samples using GC/MS. Seawater samples had increased concentrations of PAHs in comparison with River Nile water ones. Fluorene was the major PAH in all water samples, for it ranged from 25 to 50 ppt. An actinomycetes isolate from the collected samples underwent molecular identification through the pair-wise alignment of its 16S rDNA sequence with sequences in the database. It was indicated that it was identical to *Streptomyces macrosporeus* with a 98.65% homology sequence. *Streptomyces macrosporeus* was examined for degradation efficiency of 0.1, 0.05, 0.025, and 0.0125 ppm of PAHs standard mix and 200 ppm of naphthalene, fluorene, and anthracene, individually for 5 days/37°C. It degraded all the detectable concentrations of the chemicals in the PAHs standard mix except for some non-degradable traces of naphthalene. Furthermore, it degraded naphthalene, fluorene and anthracene individually with a percentage of 99.99%, 94.88%, and 96.33%, respectively. Thus, the results of the current study reflected the biodegradation capability of *Streptomyces macrosporeus* for different PAHs.

Keywords: River Nile; Mediterranean Sea; Red Sea; chemical and microbiological analysis; *Streptomyces macrosporeus*; molecular identification; PAHs.

1. Introduction

With the increased consumption of petroleum fuels for various purposes, there has been an alarming rise in environmental pollution levels. This caused an increased accumulation of CO_2, unburnt hydrocarbons, particulate matter, oxides of sulfur and nitrogen, and other persistent organic pollutants. Yuewen and Adzigbli [1] indicated that oil pollutants in the marine environment have diverse effects on marine organisms. One of the most widespread organic environmental pollutants is polycyclic aromatic hydrocarbons (PAHs) which pose a potential risk to human health via marine biota [2]. PAHs have been identified as general causes of the deterioration of aquatic ecosystems in recent decades [3]. PAHs are environmentally persistent organic compounds which are a widespread group of several hundred chemically-related compounds with various structures and varied toxicity [4]. PAHs are well-known environmental pollutants and are included in the priority pollutant list of the European Union and US Environmental Protection Agency (EPA) due to their mutagenic and carcinogenic properties [5]. Abdel-Shafy and Mansour [6] stated that PAHs can be formed as products of incomplete combustion from either natural combustion sources (forest and brush fires) or man-made combustion sources (automobile emissions and cigarette smoke) and some are manufactured in the industry. So, PAHs are commonly detected in air, soil, and water. The mechanism of toxicity is considered to be interference with the function of cellular membranes and with enzyme systems that are associated with the membranes [6]. PAHs effects on immune system development, humoral immunity, and host resistance

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have been documented [7]. PAHs are rapidly adsorbed to the organic matter in sediments and due to their high hydrophobicity and lipophilicity, they remain in the environment for years [8]. Naphthalen, fluorene and anthracene are among PAH compounds. Bharti *et al*. [9] stated that fluorene is an organic compound which is high in hydrophobicity and toxicity. They are accumulating in the environment at an increasing concentration, posing a serious threat to living organisms. Microorganisms are capable of heterogeneous contaminant degradation and this is considered as an alternative tool to chemical and physical techniques for cleaning up polluted environments, as they are economically viable and less harmful to the environment; they can degrade or detoxify hazardous wastes into harmless substances such as carbon dioxide, water, and cell biomass [10]. Previous studies done revealed that bacterial strains isolated from PAHs contaminated sites had the ability to degrade PAHs to some extent when the polyaromatic compound was present at a very low concentration of 50-100 µg/l [11]. Fulekar[12] indicated that a consortium of indigenous microorganisms was found to be an effective source for the degradation of organic PAHs. The author indicated that this will be applicable to the toxic compounds to be degraded to clean up the environment. In this regard, Alsberi *et al.* [13] concluded that the usage of actinomycetes as alternative sources to be used in hydrocarbon degradation in the petroleum industry is potential. Actinobacteria are a group of diverse bacteria, having the ability to degrade a wide range of organic compounds particularly hydrophobic compounds such as PAHs [14]. In addition, Briceño *et al.* [15] stated that the use of microorganisms belonging to the *Streptomyces* genus is a promising bio-technological tool for the biodegradation of pesticides contaminated matrices.

Thus, the aim of this study is to isolate, identify and use microorganisms (*Streptomyces* sp.), which are naturally occurring in the collected water samples, to degrade PAH compounds by using them as a sole carbon source.

2. Experimental

2.1.Sampling

Water samples were collected from different locations during the period from January 2016 to February 2019 (Table.1).

Samples of a 1 liter volume from each location were transported from the sampling site in ice boxes to the laboratory and were subjected immediately to chemical and microbiological analyses.

2.2. Chemical analysis of water samples

The pH value and electrical conductivity (EC) of water samples were determined according to APHA (1992). Cations (Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}) and anions (CO_3^{-2}, HCO_3^{-2}, Cl^{-}, SO_4^{-2}) were determined according to the Official Method of Analysis of A.O.A.C. International [16] at the water analysis lab., RCFF, ARC, Egypt.

2.3. PAHs determination in water samples

Using hexane, samples were extracted and were subjected to the GC/MS/MS analysis. The analysis was carried out using GC (Agilent Technologies 7890A) interfaced with a mass selective detector (MSD, Agilent 7000) that was equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m x 0.25 mm i.d. and 0.25 µm film thickness). Helium was the carrier gas with a linear velocity of 1ml/min. The injector and detector temperatures were 200 °C and 250 °C, respectively. The injected volume of the sample was 1 µl. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250 °C. The analytical technique used for quantification was MRM. A standard EPA 525 PAH Mix B was used in the quantitative determination of PAHs in the collected water samples. Calibration standards were prepared from the authentic compounds of the

Table.1. :Sampling sites.

Site	Date	Sample code	Location
Naama Bay-Sharm El-Sheikh	January 2016	NBS-16	27°54'32.0"N 34°19'39.0"E
Tiran Island Sharm El-Sheikh	April 2016	TIS-16	27°57'33.0"N 34°28'07"E
Montazah-Alexandria	August 2016	MoA-16	31°17'25.0"N 30°01'14.0"E
Giza (Cairo University Nile Bus Station)	August 2017	GCB-17	30°01'45.0"N 31°13'07.0"E
Cairo (Maspero Nile Bus Station)	May 2017	CMB-17	30°03'05"N 31°13'49.0"E
Giza (Cairo University Nile Bus Station)	February 2019	GCB-19	30°01'45.0"N 31°13'07.0"E
Cairo (Maspero Nile Bus Station)	February 2019	CMB-19	30°03'05"N 31°13'49.0"E

standard mix containing naphthalene, fluorene, phenanthrene, anthracene, Benzo(b)fluoranthene, Benzo(k)-fluoranthene as well as Benzo(a)pyrene. The identification of components was based on a comparison of their mass spectra, retention time and peak area with those of the above mentioned authentic compounds.

2.4. Microbiological analysis of water samples

Total viable microbial counts were determined according to APHA [17] on a nutrient agar medium [18]. The most probable number (MPN) technique was used according to APHA [19] for counting total and fecal coliforms in MacConkey liquid medium [20].

2.5. Microbial screening and isolation

Minimal salt medium (MM) was used for the screening of microorganisms are able to use the PAHs standard mixed solution as a sole carbon source for their growth. The medium contained (per liter): 2.13 g Na_2HPO_4, 1.3 g KH_2PO4, 0.5 g NH_4Cl, 0.2 g $MgSO_4$. $7H_2O$, and trace elements solution (1 ml per liter) [21]. The PAHs standard mix solution was added in different concentrations (0.1, 0.05, 0.025, 0.0125 ppm) to sterile melted MM-agar flasks under aseptic conditions. Each melted solid MM flask was separately inoculated with 0.1 ml of each of the collected water samples separately. The inoculated medium was then poured into the plates allowing solidification. Plates were incubated at37^{o}C till the observation of a growth.

The microbes which were capable of growing on and using the added PAHs standard mix solution, as a sole carbon source, were the only ones grown on the MM, and accordingly the colonies displaying different morphologies were isolated and underwent further purification on nutrient agar plates.

2.6. Microbial identification

2.6.1. Morphological identification

The isolated microorganisms underwent Gram staining for examining bacterial isolates and lactophenol staining for examining fungi and were examined under a light microscope to characterize general morphological features of the isolates.

2.6.2. Molecular identification

Bacterial DNA extraction

A nutrient broth medium was used for growing the bacterial isolate for 24 hours at 37^{o}C, and then it was centrifuged at 12000 g for 5 min. for harvesting. The bacterial pellet was washed three times using a 0.85% NaCl saline solution. GeneJET Genomic DNA purification Kit (Thermo Scientific, Lithuania) was used to extract the genomic DNA. A Nanodrop spectrophotometer and agarose gel electrophoresis were used for checking the DNA yields and purity.

Identification of the bacterial isolate by 16S rRNA gene sequencing

The universal primers F-27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1494 (5′-CTACGGYTACCTTGTTACGAC-3′) were used for amplifying the 16S rRNA gene fragment of the bacterial isolate using a PCR machine (Bio-rad T100 thermal cycler). The checking of the PCR products was performed using agarose gel electrophoresis. The purification of the PCR products was implemented using a gel extraction kit and they were sequenced by Macrogen Korea.

Phylogenetic analysis of the bacterial isolate

The Neighbor-Joining method was used for inferring evolutionary history. The computation of the tree was performed using the Maximum Composite Likelihood method. The analysis involved 28 nucleotide sequences; 6 sequences of 16S rRNA gene were amplified from the bacterial isolate of the current study, while 22 sequences representing the most similar hits were obtained from the NCBI Gene Bank Database. An evolutionary analysis was conducted in MEGA5 software.

2.7. Evaluation of the degradation capability of microbial isolates on PAHs

2.7.1. Evaluation of the degradation capability of actinomycetes for the PAHs standard mix

Actinomycetes were grown on a 50 ml broth MM in 100 ml-flasks containing 0.1, 0.05, 0.025, 0.0125 ppm of the PAHs standard mix for 5 days at 37^{o}C. After 5 days of incubation, the samples were taken from the cultures to examine the efficiency of degrading the PAHs using GC-MS and the efficiency percentage was calculated according to the following equation: (C1-C2/C1) X 100, where C1 is the initial concentration while C2 is the remaining concentration of the PAHs after the microbial degradation.

2.7.2. Evaluation of the degradation capability of actinomycetes for individual naphthalene, fluorene and anthracene

The MM was used to evaluate the degradation capability of the actinomycetes for 200 ppm of each of naphthalene, anthracene, and fluorene individually. The mentioned PAHs were used as the sole carbon source in the medium for the growth of the actinomycetes. One thousand ppm of naphthalene, phenanthrene, and fluorene were separately dissolved

in acetone to prepare the stock solution. The effect of each PAHs compound (200 ppm) on the growth of actinomycetes was determined by adding 10 ml of stock solution from each of naphthalene, fluorene, and anthracene to 40 ml sterilized MM in 250 ml flasks which were inoculated with the actinomycetes. The inoculated flasks were then incubated at 37ºC for 5 days. Total bacterial counts were determined before and after the incubation to determine the effect of PAHs on the counts of the bacteria. The broth cultures were collected and centrifuged at 10000 rpm/10 mins. to harvest the microbial cells. The harvested microbial cells were then subjected to overnight drying using the oven at a temperature of 60 °C and dry weights were determined. GC-MS was used to determine the degradation efficiency of the bacteria.

3. Results and discussion

3.1. Chemical analysis of water samples

The pH and EC were determined in the representative water samples. The data in Table (2) revealed that pH slightly changed according to the sampling site difference and ranged from 7.1 to 7.3 in the Nile water samples, and from 7.8 to 8 in seawater samples. These results are consistent with the World Health Organization standard guidelines [22] which stated that the range of the pH values for aquatic life is 6.5–9.0, for drinking water from 6.5 to 8.5, and irrigation from water 6.5 to 8.4. Accordingly, pH outside the standard guidelines range may cause a nutritional imbalance or may contain a toxic ion. In our study, it was investigated that the change in pH was accompanied by changes in Electrical conductivity. Results revealed that EC ranged from 0.33 to 0.35 mS/cm in the Nile water samples, while in seawater samples they ranged from 58.77 to 63.61 mS/cm. This is in accordance with the study results of the study by El-Sayed and Salem [23] who studied the EC of surface water of the River Nile and found that it ranged from 0.427 to 0.505 mS/cm. It was concluded that these higher EC values in seawater samples have resulted from the concentrations of soluble salts, cations, and anions [24].

The data also indicated that the highest concentrations of anions and cations were detected for seawater samples in Naama bay - Sharm El-Sheikh. The prevalent cation was Na^+ where its concentrations ranged from 400 to 469.57 mg/l in seawater samples and from 1.03 to 2.13 mg/l in the Nile water samples. Generally, cation and anion concentrations were of higher values in seawater samples and were of lower values in the Nile water samples. Besides, results showed that CO_3^- was not detected in all of the collected water samples. In this respect, El-Sayed and Salem [23] investigated the concentrations of Na^+ in surface Nile water samples and they ranged from 38.6 mg/l to 45.66 mg/l. The authors mentioned that the sodium ion represents one of the main ions dissolved in natural waters and when reacting with chloride ions, forms rock salt which is NaCl. Thus, as expected pH, EC, anions, and cations values of seawater samples exceed those of the Nile water.

3.2. PAH determination in different collected water samples

PAHs were determined in the collected water samples (Table.3) and the results revealed that seawater samples reported the highest concentrations of PAHs in comparison with the Nile water samples. It was recorded that fluorene was the major PAH in all water samples as it ranged from 25 to 50 ppt. The maximum concentration of fluorene was scored in NBS-16 water samples (50 ppt), while its low concentration (25 ppt) was scored in CMB-19 water samples. Data also indicated that naphthalene, phenanthrene, and Benzo(b)fluoranthene were reported only in NBS-16 water samples. Also, it can be indicated that naphthalene scored the maximum concentration (240 ppt) among all PAHs in all samples. The present study suggests that total PAHs of 50-342.86 ppt is to some extent safe and will have a weak, but not harmful, effect on marine organisms. This is in accordance with the conclusion derived from Mazmanidi *et al.* [25] who stated that the harmful effect of hydrocarbons on aquatic organisms in seawater was at a total concentration of about 50000 ppt. In this concern, Awad [26] stated that PAHs recorded on the KSA coasts ranged from 1880 to 412000 ppt. In this respect, El-Agroudy *et al.* [27] studied the distribution of different PAHs in coastal waters and some marine organisms of the Gulf of Suez considering anthropogenic sources. The results indicated that total PAHs were ranged from 20.95 to 99.24 ppt. The authors mentioned that these values were lower than those recorded by Awad [26] in the Arabian Gulf water; they were 300-455000 ppt. Kavouras *et al.* [28] stated that the high contribution of pyrolytic sources resulted in the general distribution of PAHs in water samples, due to the predominance of parent PAHs over the alkylated derivatives. In general, petroleum transportation, offshore exploitation, and/or natural seeps are the most important PAHs sources in seawater samples. In this regard, El Nemr *et al.* [29] stated that the detection of PAHs fractions may be due to the incomplete combustion process (pyrolytic origin). Petroleum often contains more phenanthrene relative to anthracene as phenanthrene is the most thermodynamically stable tricyclic aromatic isomer [27].

Table.2. Chemical characteristics of water samples

Sample	pH	EC (µS/cm)
NBS-16	8	63.71
TIS-16	7.8	58.77
MoA-16	8	60.61
GCB-17	7.2	0.35
CMB-17	7.1	0.39
GCB-19	7.1	0.35
CMB-19	7.3	0.33

	Cations (mg/l)				Anions (mg/l)			
	Ca+2	Mg+2	Na+	K+	CO3-	HCO3-	Cl-	SO4-2
NBS-16	4.23	191.88	469.57	10.85	ND*	2.83	5.37	668.31
TIS-16	28.17	157.55	400.00	8.19	ND*	2.36	491.53	100.02
MoA-16	26.76	166.75	434.78	9.96	ND*	2.36	516.95	118.94
GCB-17	1.14	0.93	1.31	0.15	ND*	1.51	1.36	0.94
CMB-17	1.97	0.89	1.03	0.16	ND*	1.60	0.85	1.60
GCB-19	1.57	0.97	2.13	27.67	ND*	ND**	1.52	45.3
CMB-19	1.68	0.92	1.54	0.92	ND*	ND**	0.87	1.39

*Not determined, **Not detected.

. The PAHs in fuels are not destroyed during the combustion process, due to the survival mechanism, and they exist in the stack flue gas or engine exhaust [30]. A direct correlation existed between the PAH emission and formation from the exhaust streams and PAH content in the fuel [31]. Another mechanism of the PAH emission is the formation as products of incomplete combustion (PICs) of both biomass and fossil fuels that contain carbon and hydrogen [32] during incineration, industrial production and transportation activities. In general, PAHs usually exist as a complex mixture of various individual compounds [33]. PAHs are also the most significant contaminants in the marine environment and they have been reached the pristine areas like the Arctic and Antarctica via long-range transport [32]. It was reported that PAH concentrations vary depending on the emission sources. Cheruiyot *et al*. [30] mentioned that the mobile sources of PAHs include emissions from ships which are equipped with either compression or spark-ignition internal combustion engines. Marr *et al*. [34] mentioned that several studies have identified these vehicles as major sources of PAH emissions in the environment. These aromatic compounds' emissions depend on several factors, including the engine type, fuel composition, load and age, mileage, PAH content in the lubricant oil, lubricant oil combustion, driving mode, and emission control devices such as air filters [35].

Significant changes in the community composition, increases in diversity, and high temporal variability are considered as a positive response of the microbial communities to chemical pollution [36].

In the same context, Fouad *et al.* [37] stated that biological augmentation technology using adding bacterial strains consortium for drainage wastewater

Table.3. PAH determination in different collected water samples

PAHs compounds	Samples						
	NBS-16	MoA-16	TIS-16	GCB-17	CMB-17	GCB-19	CMB-19
Naphthalene	240	-	-	-	-	-	-
Fluorene	50	33	40	35	28	30	25
Phenanthrene	7	-	-	-	3	-	-
Anthracene	27	-	10	-	-	-	-
Benzo(b)fluoranthene	0.86	-	-	-	-	-	-
Benzo(k)fluoranthene	15	67	-	-	-	-	-
Benzo(a)pyrene	3	9	-	-	-	-	-
Total concentration (ppt*).	342.86	109	50	35	31	30	25

*ppt: part per trillion.

in El-Rahawy drain in Egypt is active and cost-effective for the degradation of organic and inorganic pollutants.

3.3. Microbiological analysis of water samples

The total viable microbial counts as well as total and fecal coliforms were enumerated in the collected water samples (Table.4). The data revealed that the highest total viable bacterial counts were detected in GCB-19 water samples (1.6 x 10^4 CFU/ml). The highest counts of total coliforms scored 110 cells/ml in GCB-19 water samples and the highest count of fecal coliforms was recorded for both GCB-17 and GCB-19 water samples (15 cells/ml). In this concern, El-Shenawy *et al.* [38] stated that the sewage discharge, harbor activities, boats and vessels as well as heavy recreational activities are the main sources of bacterial contamination. In the same context, Ibrahim and El-Shenawy[39] investigated the microbiological profile of some sites along with the Red Sea and the results showed high counts of detected bacteria fluctuating between 1.1x10^2 and 2.4 x10^2/CFU100 ml for *Escherichia coli* at Marina Sharm and Naama Bay. The authors explained this by the increased recreational activities during the long summer season, leading to huge amounts of untreated waste discharged from the high numbers of vessels and boats in the coastal water, in addition to heavy marine sports. Recent studies showed that there is a higher bacterial and archaeal diversity in inland freshwater than in marine environments [40, 41].

3.4. Morphological identification of the isolated microorganisms

A total number of 19 microbial isolates were obtained from the collected water samples (Table.5). Through the use of a light microscope, the morphological identification reflected that 4 of these isolates were observed as gram-positive cocci, 6 were gram-positive bacilli, 7 were gram-negative bacilli, 1 was related to actinomycetes and 1 to fungus.

Furthermore, the actinomycetes isolate was selected to conduct further studies including morphological and molecular identification, as well as characterization for the degradation efficiency of different PAHs at varying concentrations.

It was reflected from the images taken from the light microscope that actinomycetes isolates were gram-positive and were branched into filaments with elongated and small sized-cells. Colonies in the Petri-plates were white in color, and rough and crumbly in texture.

3.5. Molecular identification of actinomycetes

For more precise and accurate identification of the studied bacterial isolate, since morphological attributes showed some variability, the molecular techniques of the DNA sequencing were applied. Wilmotte *et al.*[42], Nelissen *et al.* [43]; Garcia-Pichel *et al.* [44] and Nübel *et al.* [45] stated that the molecular approaches are useful, especially when learning that the 16S rDNA sequencing is an established method for identifying bacteria to overcome the problems of microscopic examination that does not conveniently provide enough information for accurate identification. The DNA was successfully amplified from the bacterial isolate using universal primers and separated on a gel for verification. The sequencing of 16S rDNA sequences was conducted using the same primers which were used for amplification. Accordingly, the phylogenetic relationship of the isolated strain was studied.

The obtained 16S rDNA sequence was
CTCCCTCCCACAAGGGGTTGGGCCACCGGCT
TCGGGTGTTACCGACTTTCGTGACGTGACGG
GCGGTGTGTCAAGGCCCGGGAACGTATTCAC
CGCAGCAATGCTGATCTGCGATTACTAGCGA
CTCCTACTTCATGGGGTCGAGTTGCAGACCC
CAATCCGAACTGAGACCGGTGCAGCCCAAGA
CATAAGGGGCATGATGACTTGACGTCGTCCC
CACCTTCCTCCGAGTTGACGCTTTTTGAGATT
CGCTCCACCTCGCGGTATCGCAGCTCATTGTA
CCGGCCATTGTAGCACGTCCCGGCGGTCTCC

Table.4.Total viable counts (TC), total and fecal coliforms counts in the water samples.

Samples	TC (CFU/ml)	Total coliforms (Cells/ml)	Fecal coliforms (Cells/ml)
NBS-16	3.5 x 10^2	0	0
TIS-16	2 x 10^2	0	0
MoA-16	5 x 10^2	0	0
GCB-17	1.5 x 10^3	46	15
CMB-17	1.7 x 10^3	0	0
GCB-19	1.6 x 10^4	110	15
CMB-19	5.4 x 10^3	24	9

Table.5. Microbial isolates in the collected water samples.

Samples	No. of microbial isolates/each sample	Morphological Shape
NBS-16	2	1 G^+ cocci, 1 G^+ bacilli
TIS-16	2	1 G^+ cooci, 1 G^+ bacilli
MoA-16	2	1 G^+ cocci, 1 G^+ bacilli
GCB-17	3	1 G^- actinomycetes, 1 fungus, 1 G^+ bacilli
CMB-17	3	3 G^- bacilli
GCB-19	3	1 G^+ bacilli, 1 G^- bacilli , 1 G^+ cocci,
CMB-19	4	3 G^- bacilli, 1 G^+ bacilli

CGTGAGTCCCCAGCACCACAAGGGCCTGGTG GCAACACGGGACAAGGGTTGCGCTCGTTGCG GGAATTAACCCAACATCTCACGACACGAACT GACCACCGCCATGCACCACCTGGACACCGAC CACAAGGG. This sequence was aligned with sequences of other bacteria obtained from the Genbank and a phylogenetic tree was designated to determine the relationships between species. The pair-wise alignment of the obtained sequence with sequences in the database showed that the obtained sequence was identical to *Streptomyces macrosporeus* (Accession # HQ607420) with a homology sequence of about 98.65%. The phylogenetic tree presented in Figure (1) corresponds to the partial 16S rDNA gene phylogeny of the order bacteria.

3.6. Degradation of PAHs by actinomycetes

After growing *Streptomyces macrosporeus* in the medium containing different concentrations of the mixed PAHs, the data showed that *Streptomyces macrosporeus* degraded all detectable concentrations of the chemicals in the mixed standard (Table.6) except for naphthalene as some traces were not degraded by that bacterial strain. These results are in accordance with the results of Balachandran *et al.* [46] who studied the degradation efficiency of the *Streptomyces* isolate ERI-CPDA-1 which was recovered from an oil-contaminated soil in Chennai, India. The results showed that the isolate degraded naphthalene with an efficiency of 99.14% and phenanthrene with an efficiency of 17.5% in 5 days at 30 °C.

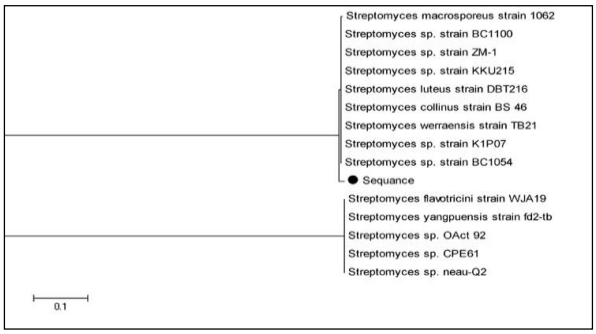

Fig.1. Phylogenetic tree of *Streptomyces macrosporeus.*

Add to this, Wei *et al.* [47] stated that *Streptomyces* sp. Hlh1 was the only strain that showed pyrene degradation, although PAHs are being persistent in the environment due to their increased molecular size and their slow degradation by few microbes. Thus, actinomycetes have peculiar characteristics *i.e.* resistance to drought, survival, and growth in alkaline pH and the production of extracellular enzymes which make them suitable candidates for biodegradation [48]. In this regard, Ferradji *et al.* [49] stated that *Streptomyces* spp. AB1, AH4 and AM2 are good PAH degraders and could be used for the biodegradation of PAH contaminated soils.

3.6.1. Degradation efficiency of naphthalene, fluorene, and anthracene by Streptomyces macrosporeus

Streptomyces macrosporeus was examined for its degradation efficiency; it degraded each of naphthalene, fluorene and anthracene individually in a concentration of 200 ppm for each (Fig. 2). This was in order to compare the efficiency of degrading them individually and in the mixed form. The data showed that *Streptomyces macrosporeus* degraded naphthalene, fluorene and anthracene with a percentage of 99.99 %, 94.88%, and 96.33%, respectively. These results were in agreement with the study results of Chaudhary *et al.* [50], who screened *Streptomyces rochei* for its degradation capability to degrade anthracene, fluorene, phenanthene, and pyrene in the presence of 0.1% yeast extract as a co-substrate in a minimal medium. The authors reported that the isolate was found to degrade the four PAHs between 28% and 92% within 15 days at a 100 ppm level. Bourguignon *et al.* [51] reported that the use of indigenous actinomycete strains isolated from different contaminated environments could help remove naphthalene, phenanthrene, and pyrene when grown in minimal culture media. The authors recorded that the selected microorganisms showed an enhanced degradation of phenanthrene, and pyrene in the presence of glucose,

Table.6. *Streptomyces macrosporeus* degradation of PAHs

PAHs compounds	Conc. (ppm*)							
	0.0125	Degradation %	0.025	Degradation %	0.05	Degradation %	0.1	Degradation %
Naphthalene	0.0009	92.8	0.0008	96.8	0.0006	98.8	0.008	92
Fluorene	<1ppt**	100	<1ppt	100	<1ppt	100	<1ppt	100
Phenanthrene	<1ppt	100	<1ppt	100	<1ppt	100	<1ppt	100
Anthracene	<1ppt	100	<1ppt	100	<1ppt	100	<1ppt	100
Benzo(b)fluoranthene	<1ppt	100	<1ppt	100	<1ppt	100	<1ppt	100
Benzo(k)fluoranthene	<1ppt	100	<1ppt	100	<1ppt	100	<1ppt	100
Benzo(a)pyrene	<1ppt	100	<1ppt	100	<1ppt	100	<1ppt	100

*ppm: part per million, **ppt: part per trillion.

which acted as a co-substrate for growth. Naphthalene is the simplest, most volatile, and least toxic of the PAHs. In several research laboratories, naphthalene has been used as a model to develop catalysts and biological processes that have the potential to effectively destroy PAHs [52].

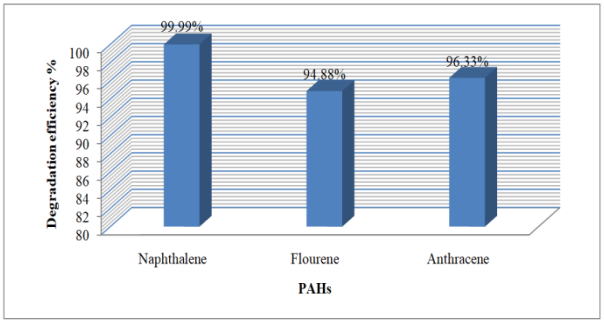

Fig.2. Degradation efficiency percentages of *Streptomyces macrosporeus* for naphthalene, fluorene and anthracene.

In this concern, Rojo [53] stated that hydrocarbon degradation occurs via terminal oxidation, subterminal oxidation, ω-oxidation, and β-oxidation.

Generally speaking, n-alkanes are oxidized to their corresponding alcohols by an electron carrier dependent monooxygenase system (alkane hydroxylases). It leads to the oxidation of the methyl group to form primary alcohol which is dehydrogenated to produce aldehyde and carboxylic acid. These undergo β-oxidation pathway, and finally enter the tricarboxylic acid cycle (TCA) with CO_2 and water as end products.

In addition, total viable bacterial counts of the *Streptomyces macrosporeus* strain were determined at zero time and at the end of the experiment (Table.7) to observe the effect of the used PAHs on the microbial growth. The data reflected that bacterial counts were decreased at the end of the experiment. It was observed that the total counts were decreased with the highest percentage when grown in the presence of anthracene (82.18%), compared with the other tested compounds. The fluorene-grown strain showed a lower percentage of decrease in the microbial counts (66.92%) in comparison with the other compounds. In this regard, Fulekar [12] indicated a decrease in the CFU and cease in the growth of microbial consortium isolated from a petrochemical contaminated soil for the biodegradation of naphthalene at varying concentrations. The author mentioned that microbes entered the pseudo-stationary phase as the compound was not enough to maintain the growth of the population. In the same context, wet and dry weights of *Streptomyces macrosporeus* grown on naphthalene, fluorene, and anthracene were determined (Fig.3). The best weights for *Streptomyces macrosporeus* were shown in anthracene followed by fluorene and naphthalene, respectively. Thus, the results of the current study indicated high percentages of the used PAHs with no additions for organic supplements as the PAHs acted as the sole carbon sources for the microbial growth and this may explain why the microbial counts decreased at the end of the experiment.

4. Conclusion

Due to the highly toxic effects of PAHs and their long-term accumulation in the environment, so much effort was expended including microbial biodegradation to get rid of their toxic effects. Therefore, this study concluded that a locally-isolated *Streptomyces macrosporeus* was capable of degrading various concentrations of different PAHs which in turn, contributes to ridding the environment of the harmful effects of these PAHs.

Table.7. Total viable bacterial counts (CFU/ml) of *Streptomyces macrosporeus* at zero time and after five days of incubation.

PAHs	Zero time	After five days	% of decrease in microbial counts after 5 days
Naphthalene	$66.5x10^4$	$22x10^4$	66.92 %
Fluorene	$60x10^3$	$24x10^3$	60 %
Anthracene	$50.5x10^4$	$9x10^4$	82.18

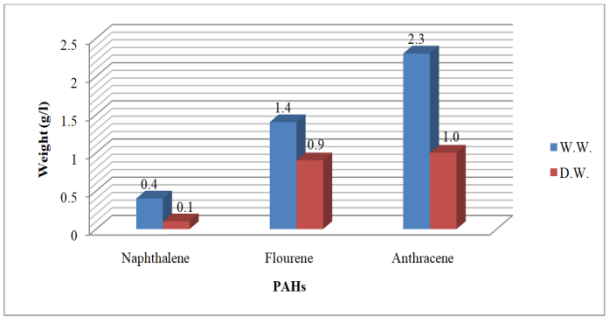

Fig.3. Wet and dry weights determined for *Streptomyces macrosporeus.*

5. Conflicts of Interest

The authors declare that there are no conflicts of interest.

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التكسير الحيوي لبعض من الـPAHs بإستخدام عزلة محلية من الأكتينوميسيتات

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المستخلص العربي

تم إجراء تقييم كيميائي وميكروبيولوجي لعينات تم أخذها من مياه نهر النيل والبحرين المتوسط والأحمر، وأوضحت نتائج التحاليل الكيميائية زيادة تركيزات الكاتيونات والأنيونات بعينات البحر عنها بعينات بمياه نهر النيل. وقد أظهرت نتائج التحاليل الميكروبيولوجية إرتفاع الأعداد الميكروبية (1.6 x104 CFU/ml) بعينات مرسى الأتوبيس النهري بجامعة القاهرة (GCB-19). وسجلت عينات GCB-19 زيادة في أعداد البكتريا القولونية الكلية total coliforms (110 cells/ml) بينما سجلت عينات مرسى الأتوبيس النهري بجامعة القاهرة (GCB-17) و(GCB-19) زيادة في أعداد البكتريا القولونية fecal coliforms (15 cells/ml). وتم تقدير نسبة PAHs بعينات المياه التي تم جمعها وذلك بإستخدام جهاز GC/MS. وأوضحت النتائج زيادة نسبة الـPAHs في عينات مياه البحر مقارنة بعينات مياه نهر النيل. وتبين أن وجود الـFluorene بجميع العينات التي تم جمعها بتركيزات تراوحت من 25 إلى 50 ppt. وتم عمل تعريف لعزلة من الأكتينوميسيتات وذلك بمقارنة تتابع الـ16S rDNA لها مع تتابعات الـDNA بقاعدة بيانات الـGenebank وتبين وجود تشابه مع سلالة *Streptomyces macrosporeus* بنسبة 98.65%. وتم اختبار هذه السلالة في التكسير الحيوي لتركيزات 0.1, 0.05, 0.025 and 0.0125 ppm من الـ PAHs standard mix بالإضافة إلى تركيز 200 ppm من كل من الـnaphthalene و الـ fluorene و الـanthracene وذلك بصورة منفصلة لكل مركب على حدة بالتحضين على درجة حرارة 37 درجة سيليزية لمدة 5 أيام. وقد تبين قدرة هذه السلالة على التكسير الحيوي لكل التركيزات التي يمكن تقديرها من الـ PAHs standard mix فيما عدا الـnaphthalene الذي تبقى منه نسبة قليلة جدا لم يتم تكسيرها. كما تم تكسير الـnaphthalene و الـfluorene والـanthracene كل على حدة بنسبة وصلت 99.99% و94.88% و 96.33% على التوالي مما يؤكد قدرة هذه السلالة البكتيرية على التكسير الحيوي للـPAHs.

الكلمات الدالة: نهر النيل، البحر المتوسط، البحر الأحمر، التحليل الكيميائي، التحليل الميكروبي، الأكتينوميسيتات، *Streptomyces macrosporeus*، التعريف الجزيئي، PAHs، naphthalene، fluorene ، anthracene ، التكسير الحيوي.