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# Microbial Solutions for Environmental Cleanup: *Sphingomonas paucimobilis* Role in Removing Heavy Metals and Aromatic Hydrocarbons

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**Abstract**

Bioremediation is considered a modern method for addressing pollution, with the objective of safeguarding the environment by eliminating heavy metals and aromatic hydrocarbon compounds from contaminated sites. Bacteria are heavily involved in these treatments because they are easily isolated, grow rapidly, and exhibit high adaptability to shifting environmental conditions. The main objective of this research was to isolate and identify *Sphingomonas paucimobilis* bacteria from soil contaminated with oil in southern Iraq. Additionally, the study aimed to evaluate their ability to remove heavy metals such as lead and cadmium as well as investigate their potential for degrading aromatic hydrocarbon compounds.

The results of the current study revealed the bacteria's ability to tolerate high levels of both lead and cadmium (200 and 50 ppm, respectively), as confirmed by the findings of the Minimum Inhibitory Concentration (MIC). The removal percentages for these metals were as follows: lead removal (58.78% and 27.95%) at concentrations of 25 and 50 ppm, and cadmium removal (48.21% and 22.37%) at concentrations of 25 and 50 ppm. The bacteria exhibited a high potential for effectively eliminating several aromatic hydrocarbon compounds, including naphthalene, 2-methylnaphthalene, pyrene, and benzo(b)fluoranthene + benzo(k)fluoranthene. The removal percentage was dependent on both concentration and incubation time, with higher removal observed at lower concentrations (2%) and longer incubation periods (28 days) (99.72%, 99.43%, 88.81%, 88.31%).

In conclusion, the present study provides important insights into the field of bioremediation by presenting *Sphingomonas paucimobilis* as a promising microbial agent capable of effectively addressing environmental pollution from heavy metals and aromatic hydrocarbons, thus underscoring its potential for real-world bioremediation applications.

**Keyswords**: -Bioremediation, environmental sustainability, pollution control, microbial, degradation

## 1. Introduction

The most prominent organic pollutants in the environment are those organic compounds contained in poly aromatic hydrocarbon compounds. The harmful effects of hydrocarbons on the environment result from multiple causes: 1. their toxicity and the damage/and or mutations they causes to the genetic components, as well as their carcinogenic nature; 2. their spreading in all environments and the difficulty of removing (Ghosal *et al.*, 2016); 3. their ability to congregate in various food chains, and thus is considered one of the substances that are dangerous to the general health of humans (Xue, 2005).

Several strategies have been developed to eliminate petroleum pollution, which has become a "global" environmental threat. Recently, Biodegradation is one of the most prominent of these strategies, which is based on the use of microorganisms as a tool to remove these pollutants. This is due to several reasons, the most prominent being environmentally friendly (Jaafar, 2019). Bacteria are currently considered the leading organism in the field of bioremediation (Jaafar, 2019). The entry of hydrocarbon pollutants into the environment has a negative impact on organisms in general, including bacteria. Truskewycz et al. (2019) stated that although soil contamination leads to a decrease in bacterial diversity, it increases their total activity. They attributed the reason for this to the adaptation of bacteria to such contaminated environment in addition to the enrichment of the indigenous soil microflora, which are capable of breaking down these pollutants occurring in the original soil. Therefore, the success of the hydrocarbon biodegradation process is subject to selecting the appropriate bacteria for specific hydrocarbons types and concentration, that the ability to biodegrade petroleum oil is associated with the concentration and composition of hydrocarbons (Xu *et al.*, 2018). The high levels of hydrocarbons lead to inhibiting the bacterial activity or even death the bacteria(Ma *et al.*, 2018), and the degradation ability varies with hydrocarbons types. The order of degradation is as follows: linear alkanes > branched alkanes > low molecular weight alkyl aromatics > monoaromatics > cyclic alkanes > polyaromatics > asphaltenes (Varjani &

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Upasani, 2017). Bacteria have proven effective ability in degradation of PAH (Fuchs *et al.*, 2011) .Previous studies have identified key bacteria that can act as eco-friendly agents in solving the problem of pollution caused by PAH compounds.

*Sphingomonas paucimobilis* is a notable bacterium known for its ability to degrade PAH compounds. Studies have demonstrated that this species can effectively break down a variety of PAHs, including naphthalene, phenanthrene, and pyrene. The bacterium initiates the degradation process using dioxygenase enzymes, which leads to the cleavage of aromatic rings and subsequent mineralization (Haritash & Kaushik, 2009). The *Pseudomonas* genus, particularly *Pseudomonas aeruginosa* and *Pseudomonas putida*, is extensively researched for its ability to degrade PAHs. These bacteria feature a versatile metabolic system that enables them to degrade a wide range of PAHs, such as pyrene, anthracene, and benzo[a]pyrene. The catabolic genes involved in PAH degradation in *Pseudomonas* are generally organized in clusters, which supports efficient degradation across diverse environmental conditions(Medić & Karadžić, 2022)

## 2. Materials And Methods

### *2.1. Collection of Samples*

In January 2022, thirty soil samples were gathered from two distinct stations within Basrah's southern city, North Rumaila oil field. These samples, taken from a depth of up to 20 cm, were meticulously labeled and stored in plastic bags. To get the samples ready for more analysis, each one was dried in the air, ground with a porcelain pestle and mortar, and then sifted through a 2 mm sieve. The fine soil parts that resulted were gathered in different bags and kept in a dry spot for later analysis.

### *2.2. Isolating and determining the identity of bacteria*

A scientific approach has been employed to isolate bacteria from soil samples, which involves adding varying concentrations of crude petroleum obtained from Al - Shua'aba Refinery (2% and 5%), to Mineral Salts Medium (MSM), with the composition: - KCl, $K_2HPO_4$, $KH_2PO4$, $FeSO_4.7H_2O$, NaCl, $MnSO_4.7H_2O$, $CaCl_2$, and distilled water. The pH of the medium is adjusted within a range of 7.0 - 7.8 to create optimal conditions for bacterial proliferation. 100 mL conical flask with a medium containing a specific concentration of crude oil and 1 gram of soil were prepared. The culture was then placed in a shaking incubator (120 rpm) at a temperature of 30°C for seven days. Nutrient agar from Hi Media (India) was employed to isolate *Sphingomonas paucimobilis.* The bacteria's identification involved morphological and biochemical assessments. Additionally, to improve precision in identification, the Vitek II automated device from Biomerieux (USA) was employed for bacterial identification (Jaafar, 2019).

### *2.3. Assays to assess tolerance to heavy metal exposure*

#### *2.3.1. Preparation of Heavy Metal Concentrations for Tolerance Assays.*

In readiness for tolerance assessments on heavy metal concentrations, we created stock solutions (1000 mg/L) of Lead nitrate and Cadmium nitrate salts. These salts were dissolved in sterile deionized water to form the stock solutions. The working standard solutions were made by diluting 100 ml of the stock standard solution of the chosen ion to a final volume of one liter with distilled water, from which working concentrations of metal salts were prepared following the protocol detailed in the cited study (Jaafar, 2019), (Etorki *et al.*, 2014).

#### *2.3.2. Assessment of Heavy Metal Tolerance*

To determine the tolerance of the isolated bacteria to heavy metals, a test was conducted. The bacteria were grown in heavy metal-free nutrient broth (NB, Hi media) at 30°C for 24 hours. Afterward, 0.1ml from the bacterial culture was aseptically transferred onto nutrient agar plates containing various concentrations of Cd and Pb (25, 50, 100, 250, 500, 1000, 1500, 1800 and 2000 ppm). These plates were then incubated at 30°C for 24 hours. The lowest concentrations of Cd and Pb that inhibited bacterial growth were identified as the tolerance levels. To ensure accuracy, this procedure was repeated three times, including a control experiment without heavy metals. This testing method was adapted from a study conducted by Andrea *et al.* (2017).

#### *2.3.3. Assessment of the Bacterium's Heavy Metal Removal Capacity*

To examine the bacterium's capacity for heavy metal removal, the following steps were undertaken. First, the bacteria were cultured in 10 ml of NB broth at 30°C for one day. Then, 2 ml of the bacterial suspension was introduced into conical flask containing 100 ml NB broth with the concentration 25 and 50 pp of Pb and Cd. The mixture was then left to incubate at 30°C for 24 hours.

Following the incubation period, the culture was centrifuged at 3000 rpm for 20 minutes. The supernatant was then collected and subjected to analysis to determine the removal of Pb and Cd using a flame atomic absorption spectrophotometer (AAS 6300, Shimadzu, Japan). This process was repeated three times to ensure the accuracy and reliability of the results.

The following equation was utilized to compute the percentage of removal:

% elimination = (reduction in heavy metal concentration ÷ Initial heavy metal concentration) × 100 (Andrea *et al.*, 2017)

#### *2.3.4. Assessing the bacterium's capacity for oil degradation*

To evaluate the bacterium's ability to degrade oil, an experimental setup was utilized. A 50 mL Erlenmeyer flask containing Mineral Salt Medium (MSM) was prepared with crude oil concentrations of 2% and 5%. To this medium, 1 mL of an overnight bacterial culture was added. The flasks were then incubated in an orbital shaker set at 25°C for durations of 7 and 28 days, with a shaker speed of 120 rpm. After the incubation period, the remaining crude oil was quantified. The experiment was replicated to ensure the accuracy and reliability of the results (Jaafar, 2019).

#### *2.3.5. Recovery of residual crude oil components*

The method described by Emiliana (Pandolfo E., 2023) was employed to extract residual crude oil from the MSM medium. This extraction procedure involved the addition

of 50 ml of carbon tetrachloride (CCl4) solvent to the bacterial culture. CCl4 is a commonly utilized solvent for extracting hydrocarbons while concurrently inhibiting bacterial growth and activity. The addition was conducted under continuous agitation of the mixture. Subsequently, the culture was transferred to a separating funnel and allowed to undergo settling. The separation procedure involved employing a separation column with dimensions of 25 cm in length and 3 cm in diameter, as outlined by (Farid, 2006). This column was packed with 8 grams of silica gel and a small amount of glass wool. The residual oil, dissolved in 25 ml of benzene, was cautiously poured into the separation column. The aromatic fraction was collected in a 50 ml beaker. Similar extraction techniques were conducted for the control flasks. Gas chromatography was used to estimate the hydrocarbons present in the aromatic fraction, the suspension was then decanted, and the remaining oil underwent drying in an oven set at 40°C to eliminate the CCl4 solvent.

## 3. Results And Discussions

### *3.1. Bacterial Identification*

The bacteria isolated were characterized based on their morphology and biochemical features. To optimize the identification process, an automated bacterial identification tool (Vitek II, C8300 Biomerieux USA) was utilized. The resulting outcome achieved a confidence level of 95%.

**Table 1**. The cellular morphology and biochemical properties of *S. paucimobilis*.

| Phenotypic characteristic | |
|---|---|
| Appearance based on morphology. | Results |
| Configuration | Rode |
| Mobility | + |
| Color | Yellowish |
| Biochemical reactions | |
| Formation $H_2S$ | - |
| Nitrite reduction | - |
| Urease | + |
| Oxidase | + |
| Simon citrate | + |
| Sugar(Glucose, Sucrose) | - |

### *3.2. Pattern of metals resistance by bacteria*

The minimum inhibitory concentration (MIC) denotes the smallest dosage of a substance needed to inhibit observable growth of a microorganism following an overnight period of incubation. MICs are predominantly employed in diagnostic settings to ascertain breakpoints for antimicrobial resistance and also serve as a research method for evaluating the in vitro efficacy of newly developed antimicrobial compounds (Chhetry *et al.*, 2022). The results of bacterial tolerance to heavy metals (Pb and Cd) are represented in the Table 2. Results indicated that cadmium (Cd) exhibited greater toxicity than lead (Pb), as reflected by their respective minimum inhibitory concentrations (MICs) ,(500 and 2000 ppm), respectively. Metals that demonstrated lower MIC values were found to be more toxic, whereas metals with higher MIC values were considered to be less toxic (Mishra & Mishra, 2018). Jaafar (2019) recorded high tolerance to the Pb than Cd by *S. paucimobilis* (Tangaromsuk *et al*., 2002) . This can be explained based on the concentrations present in the environment to which bacteria have adapted (Sodhi *et al.*, 2020a). Pollution stemming from a particular metal has the potential to boost the tolerance of bacterial communities towards that metal. Furthermore, a variety of physical and chemical factors play a substantial role in heightening bacteria's susceptibility to varying concentrations of metals (Campillo-Cora *et al.*, 2023), (Manikant Tripathi *et al*., 2024). The findings of the current study are also in line with those of previous research (Afzal *et al.*, 2017), (Jaafar, 2019), (Sodhi *et al.*, 2020b).

### *3.3. Removal of heavy metals by bacteria*

In the bioremediation study, two different concentrations of Pb (25 and 50 ppm) and Cd (25 and 50 ppm) were applied under consistent conditions, including a temperature of 25°C, pH of 7, vibration at 120 rpm, and an incubation period of 24 hours. The results of the study demonstrated that the bacteria used in the experiment were capable of removing both Pb and Cd. The removal percentages for Pb were 27.95% and 58.78% for the respective concentrations, while for Cd, the removal percentages were 22.37% and 48.21% (Table 2). These results indicate that the removal process followed a diffusion pattern, as the removal percentage increased with higher metal concentrations. Dong *et al.* (2023) indicated high percentage of heavy metals removal by SRB bacteria as increase the concentration, increasing the concentration of metals ions facilitated the binding of these metals with functional group in bacterial cell wall. Results also indicated higher remediation ability of bacteria in relation to Pb than Cd. This may be due to various mechanisms employed by organisms to eliminate heavy metals, as well as the heavy metal's capability to cause oxidative damage, thereby diminishing the organisms' remediation potential (Oziegbe *et al.*, 2021).

**Table 2.** The minimum inhibitory concentration (MIC) (ppm), and the elimination of heavy metals (%)

| Levels of heavy metal concentrations. | MIC (ppm) | Percentage (%) of removal during 24 hours. |
|---|---|---|
| Pb(50ppm) | 2000 | 58.78 |
| Pb(25ppm) | | 27.95 |
| Cd(50ppm) | 500 | 48.21 |
| Cd(25ppm) | | 22.37 |

### *3.4. Bacterial aromatic hydrocarbons degradation study*

The evaluation of degradation potentials exhibited by bacterial strains isolated from polluted sites is of utmost importance when it comes to designing and developing a durable bioremediation strategy (Azadi & Shojaei, 2020). Results of the present study indicated that the isolated bacteria identified as *S. Paucimobilis* was able to degrade *TPH* compound with the total removal capacity reach to (99.82% and 99.90%) for both concentrations (2% and 5%) during the incubation period of 7 days, while the removal capacity during the 28 days' incubation reach to (99.76% and 99.88%). Li *et al.* (2020) in their study indicated ability of *S. changbaiensis to degrade the TPH within the percentage* $39.2 \pm 1.9\%$ during 30 days of incubation. Srivastava & Kumar (2019) reported ability of

*S. Paucimobilis* to degrade PAH compound. Sphingomonas sp. has integrated a diverse set of metabolic pathways for aromatics into its system. These pathways include meta-cleavage of catechol, dihydroxylation, and subsequent meta-cleavage of aromatic rings mediated by dioxygenase and dehydrogenase enzymes, as well as ring cleavage via Baeyer-Villiger oxidation. These metabolic capabilities enable Sphingomonas sp. to efficiently break down and utilize a wide range of aromatic compounds (Zhou *et al.*, 2022).

**Table 3.** Total percentage (%) of PAH compound degradation by *S. Paucimobilis* in different concentration of crude oil and incubation period

| 24 h incubation period | | 28 incubation period | |
|---|---|---|---|
| Total percentage (%) of PAH compound degradation | | Total percentage (%) of PAH compound degradation | |
| Concentration 2% | Concentration 5% | Concentration 2% | Concentration 5% |
| 99.82% | 99.90% | 99.76% | 99.88% |

### *3.5. Special degradation ability of different aromatic compounds*

Present results related to the degradation ability of *S. Paucimobilis* for the flowing compound (Naphthalene,2-methylnaphthalene, pyrene, and Benzo(b) fluoranthene+Benzo(k)fluoranthene during 24h incubation period were (99.80, 96.69, 71.13, and 56.11%) and (83.71, 87.89, 49.74, and 50.535%) for concentration 2% and 5% respectively (Fig.1, 2). Results related to the degradation the same compounds but during 28 days' incubation were as follow (99.72, 99.43, 88.81, and 88.31%) and (98.84, 16.32, 18.32, and 16.08%) for concentration 2% and 5% respectively (Fig.3, 4). The results demonstrated the strong capability of *S. Paucimobilis* to degrade the PAH compound, with a predicted concentration-dependent ability. At higher initial concentrations of mixed PAHs, it was found that increased toxicity levels and competitive inhibition occurred. Fu *et al.* (2014) indicated ability of Sphingomonas sp. to degrade phenanthrene, anthracene, fluoranthene and pyrene, with the degradation percentage reaching to (99 ± 0.4%, 67 ± 2%, 97 ± 3%, 72 ± 8%, and 6 ± 2%) each at the level 100 mg $L^{-1}$ and benzo[a]pyrene at 10 mg $L^{-1}$, respectively. Li *et al.* (2023) predicated that S. *multivorum* was more efficient in degrading fluorine and phenanthrene with the efficiency of 74.6% and 76.4% in 10 days, respectively. Zhang *et al.* (2022) reported in their study The ability of *Sphingobium* sp. to degrade naphthalene reached 100%. Additionally, it demonstrated partial degradation of pyrene, chrysene, and indole within 6 hours, with degradation rates of 39.0%, 78.0%, and 55.3%, respectively. (Zhou *et al.*, 2016), demonstrated ability of six strain of sphingomonas to degrade fluorine with different removal percentage (87.2 ± 3.8 85.4 ± 4.5 74.9 ± 4.7 83.0 ± 5.4 68.7 ± 3.1 63.2 ± 1.9, N.G.). The Sphingomonas bacteria are classified as bacteria that reside in soil that has been polluted with hydrocarbon compounds. Their notable capacity to degrade numerous aromatic hydrocarbon compounds into smaller components can be attributed to their high proficiency in this process (Kertesz *et al.*, 2018). This susceptibility can be interpreted as follows: 1-The reason behind this capability can be attributed to the existence of a diverse collection of genes that code for ring-hydroxylating dioxygenases, enzymes that play a crucial role in the degradation of aromatic hydrocarbon compounds; 2-The occurrence of multiple insertion elements and transposons, in conjunction with the arrangement of degradative genes in various dispersed clusters, offers the possibility of forming more efficient combinations of existing genes. This, in turn, can lead to the development of novel degradation pathways with enhanced capabilities (Kertesz *et al.*, 2018).

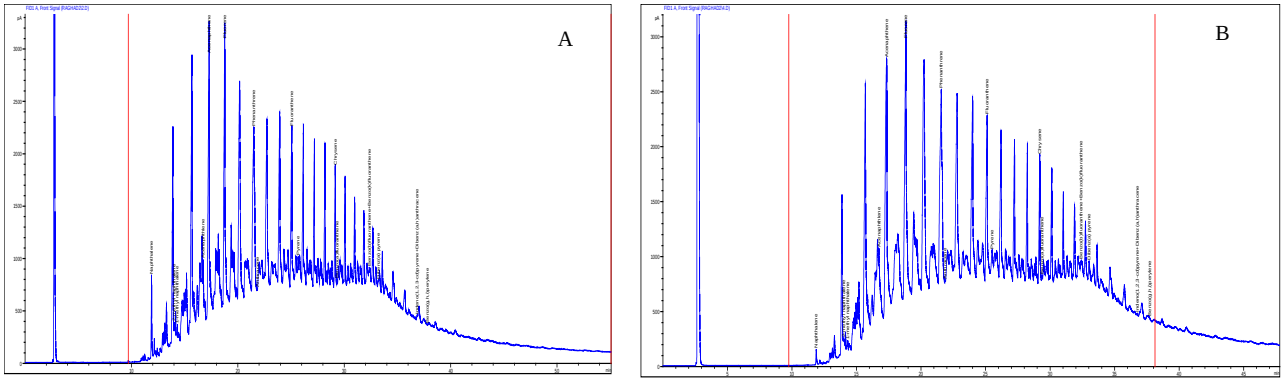


**Figure1.** Sample A consisted of Crude oil (Aromatic fraction, 5%) combined with *S. Paucimobilis*, whereas sample B contained Crude oil (Aromatic fraction 5%) without *S. Paucimobilis*. Both samples were subjected to a 24-hour incubation period.

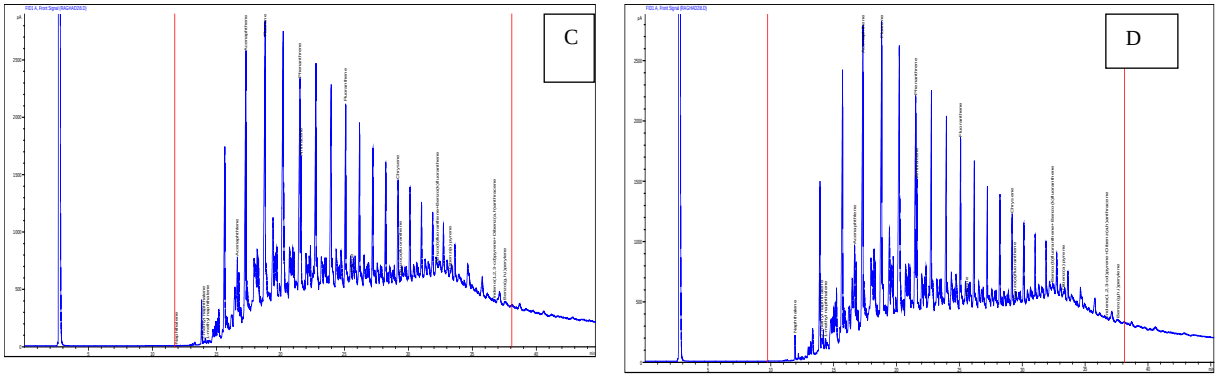


**Figure2**. Sample C contained Crude oil with an aromatic fraction of 2%, along with *S. Paucimobilis*, while sample D contained Crude oil with an aromatic fraction of 2% without *S. Paucimobilis*. Both samples underwent a 24-hour incubation period.

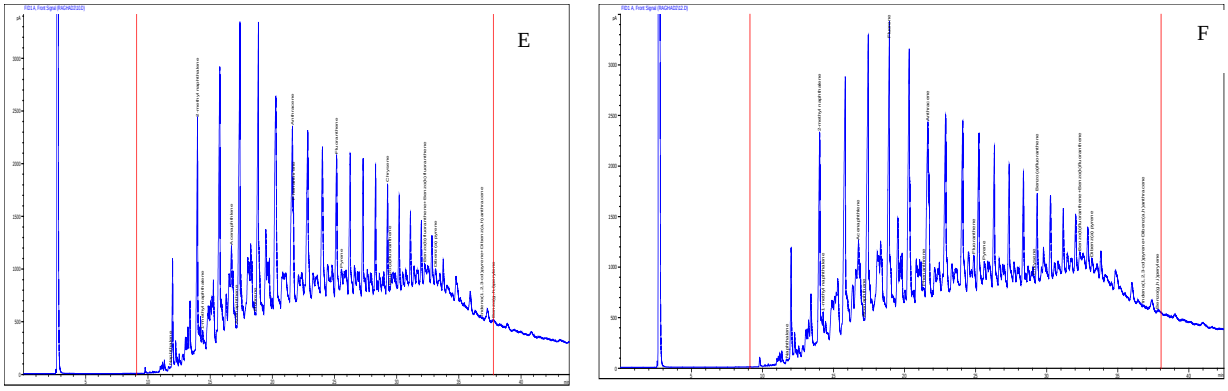


**Figure3**. E Crude oil (Aromatic fraction, 5%) with *S. Paucimobilis* and F: Crude oil (Aromatic fraction 5%) without *S. Paucimobilis.* 28 days' incubation period.

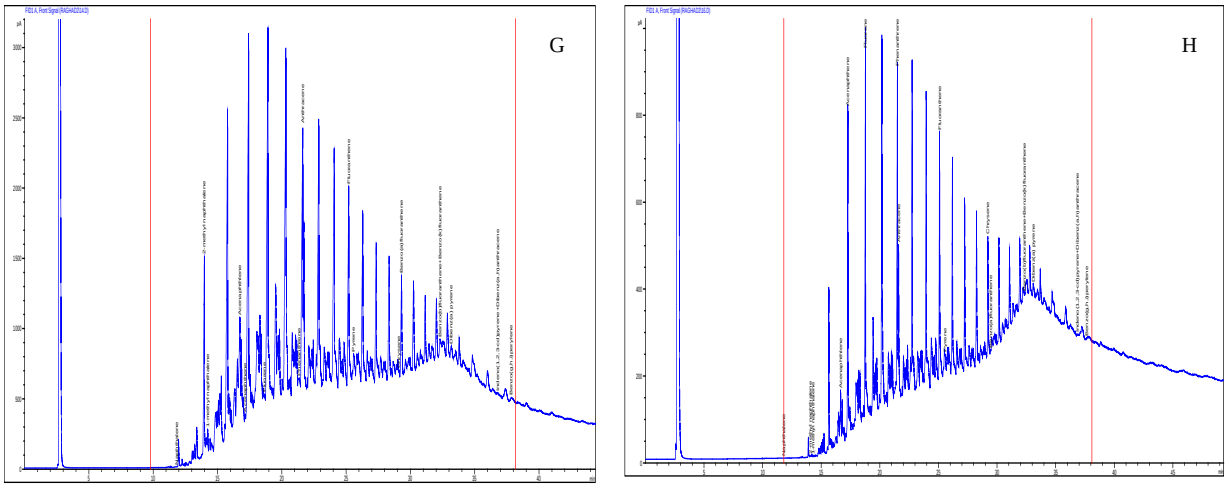


**Figure4**. E Crude oil (Aromatic fraction, 2%) with *S. Paucimobilis* and F: Crude oil (Aromatic fraction 2%) without *S. Paucimobilis.* 28 days' incubation period.

## 4. Conclusion

In conclusion, this research highlights the potential of Sphingomonas bacteria in bioremediation, especially for removing heavy metals and aromatic hydrocarbons from polluted environments. The bacteria exhibited strong tolerance to high levels of lead and cadmium, achieving significant removal rates. Additionally, Sphingomonas demonstrated the ability to degrade a variety of aromatic hydrocarbons, indicating its versatility in addressing different contaminants. These findings emphasize the value of using Sphingomonas as a sustainable solution for pollution cleanup. Further exploration and application of these bacteria could play a crucial role in enhancing bioremediation techniques and promoting environmental conservation.

## 5. Grant Details

The research was self-funded, as it was not conducted using any external support.

## 6. Conflicting Interests and Ethics

The authors assert that there are no conflicts of interest associated with the publication of this manuscript. Furthermore, they confirm strict adherence to ethical guidelines, encompassing plagiarism, informed consent, misconduct, data fabrication or falsification, double publication or submission, and redundancy.

## 7. Life Science Reporting

This research did not involve any experiments that posed a threat to life sciences.

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