# The effect of chemical dispersant of the third generation (Finasol OSR 62) on the microbial biodegradation process of Zarzaitine oil in water treatment

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ORIGINAL ARTICLE

# The effect of chemical dispersant of the third generation (Finasol OSR 62) on the microbial biodegradation process of Zarzaitine oil in water treatment

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**Abstract**
The application of chemical dispersants aims to stimulate microbial oil degradation by increasing the bioavailability of oil compounds. Overall, nine microcosms were prepared (three for each treatment) using treated sediment with (i) dispersant (*d*: 25 ppm), (ii) oil (500 ppm), and (iii) with oil + dispersant (500: 25 ppm), respectively. There are also three control microcosms containing only water and sediment without petroleum. Then, we analyzed bacterial abundance, total hydrocarbon, biological oxygen demand ($BOD_5$), and chemical oxygen demand (COD) in each microcosm. Bacterial response density was significantly affected after 40 days of exposure; it was higher in the control microcosm and *d* ($> 24.10^3$ cell/l) than in the other treatments. The index of total hydrocarbons was equal to 53 mg/kg dw in oil and 56 mg/kg dw in oil + dispersant. The higher $BOD_5$ found in oil and in oil + *d* shows the increased amount of oxygen consumed, which indicates enhanced bacterial activity. Microcosms treated with dispersant had higher COD than the others, but the dispersant did not stimulate microbial hydrocarbon degradation.

**Keywords** Bacterial density · Biodegradation · Biological oxygen demand · Chemical oxygen demand · Dispersant · Zarzaitine

## Introduction

The dispersants used to clean up oil spills are mixtures of surfactants in one or more organic solvents with specific formulations intended to facilitate the dispersion of hydrocarbons in the water column. Natural or induced movements of the water cause rapid diffusion of tiny droplets of hydrocarbons formed under the action of the dispersant, thus improving biodegradation. Since their introduction in the early 1960s, dispersants have become safer and increasingly efficient. Indeed, the so-called first generation of highly toxic products have now been replaced by third-generation compounds known as "concentrated" dispersants (Bælum et al. 2012) that are marketed as being harmless or having limited effects on the environment. However, evidence for the toxicity of dispersants in general, even those in the third generation, has been reported by several authors (Bertrand et al. 1983). This toxicity most often arises for the following: (1) the combined effects of dispersants and hydrocarbons can be additive, synergistic, or antagonistic (Fiocco et al. 1999); (2) dispersants can reduce the biodegradation of hydrocarbons, and increasing concentrations of dispersed hydrocarbons in the water column can have temporary toxic or inhibitory effects on natural bacterial populations (Fuller et al. 1999); and (3) dispersants can have toxic effects on marine populations such as coral, mussels, fish, and marine mammals (Ho et al. 1999).

Cleanup technologies for accidental oil spill treatment can be grouped into three general categories: chemical, physical

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and biological approaches (Chan 2011). Field studies carried out in oil contaminated marshes and beach sediments have demonstrated a wide range of biodegradation rates; oil biodegraded almost completely within a few weeks at some locations, while it persisted for tens of years at others (Short et al. 2006). Chemical oil dispersants are applied to spills at sea to reduce the risk of environmental impacts. The dispersant is applied as a spill mitigation technique to remove the oil slick from the water surface, thereby protecting birds or marine mammals from the suffocating effect of exposure to petroleum. Moreover, the dispersant drives the oil into the water column by creating tiny droplets that facilitate the biodegradation by oil-degrading microorganisms. Chemical remediation of a petroleum hydrocarbon contaminated site may be faster than bioremediation; however, this method is coarse, unsafe and has secondary effects (Chan 2011). In the USA, pre-approval for dispersant use in marine ecosystems is required only in specific circumstances of water depth and/or distance from the coast (Aurand and Coelho 2005). However, historical records suggest that the use of dispersant is more efficient closer to shore and in shallower water than in existing pre-approval areas. The accident of the Deepwater Horizon oil drilling rig in the Mississippi Canyon block, Gulf of Mexico, on April 20, 2010, resulted in the release of an estimated $5.3 \times 10^8$ kg of oil and $1.7 \times 10^8$ kg of gas from the Macondo well (MW) (Ryerson et al. 2012). It is the largest marine spill in US history (Atlas and Hazen 2011). The total volume of dispersants used in the entire Gulf spill was estimated at 1.84 million gallon (43,900 bbl). More than 1600 km of Gulf of Mexico shoreline were impacted by the DWH spill (Barron 2012). Although the slicks disappeared within weeks of capping the MW, and in spite of extensive cleanup activities along the affected beaches, oil-soaked sands have continued to wash ashore. Oil persistent in beaches are possibly re-dissolved or dispersed into the water column and may be potential threat to marine organisms especially the microbial community in sediments (Duran et al. 2015; Procópio 2020, 2021; Álvarez-Barragán et al. 2022). Dispersant may lead to dispersion of the oil to unaffected areas and to be transported deeper into the sediments. There is a need of assessing the impact of these events/procedures to protect vulnerable near-shore habitats. It is very important to know the influence of the dispersant on oil in the marine environment, the ability of bacteria to degrade oil and the derivatives resulting from chemical dispersion, as has already been published in the work of Cao et al. (2022a, b). It is also necessary to know the effects of oil, dispersant and products resulting from the chemical degradation of oil in the marine environment on the bacterial community, which is the subject of this work.

Several studies for biodegradation of petroleum oils have concentrated on bacteria, meiofauna and other benthic components. Microbial assemblages have been proposed as tools for monitoring the environment for their role in biogeochemical cycles. Understanding the fate of the spilled oil in the sediment is essential to assessing environmental impacts of oil spill. Dissolved or dispersed oil in sediment has a high bioavailability to marine organisms and has potentially toxic effects (de Almeida Couto et al. 2015; Maia et al. 2019). Effects of dispersant + oil on the microbial communities are not well known, even though they frequently end up in the marine environment. The general objective of work is to study in vivo the effects of hydrocarbons and/or dispersants on the physicochemical characteristics of sediments and water and on the density and diversity of bacteria. We also assess the degradation of total hydrocarbons in the presence of a third-generation dispersant. FINASOL OSR 62 is a third-generation dispersants contain a blend of two or more surfactants with glycol and light petroleum distillate solvents. The most common surfactants used are non-ionic and anionic. The concentration of surfactant with the solvent lies between 25 and 65% and tends to be higher than with first generation dispersants.

The present study was designed to test the following hypotheses: (1) The difference in bacterial responses with the surrounding medium, especially when mixing oil with dispersant, made it possible to assume a relationship between the process of oil dispersion and biodegradation. The biodegradation process of Zarzaitine oil was studied in the presence and absence of FINASOL OSR 62. (2) We examined the spread and proliferation of bacteria in the presence of oil to assume that Zarzaitine oil pollution caused bacteria inhibitory effects or induced proliferation. (3) Sometimes the process of chemical water purification entails the production of a new element that can be more hazardous, which calls for the assumption of a dispersion and oil effect on the distribution of microorganisms, which has called for the examination of oil-exposed and dispersed samples simultaneously. (4) To decide on the use of the dispersant in the case of oil contamination, we linked the biodegradation process, the distribution of microorganisms, and the water physicochemical properties to decide on the possibility of adopting the dispersant, especially FINASOL OSR 62 and deciding of its use as priority solution in case of oil spills.

## Materials and methods

### Coarse fractionation

The brut Tunisian light (Zarzaitine) selected for this study was obtained from The Tunisian Company for Refining Industries. The composition of the Zarzaitine oil was evaluated by a gravimetric method at the Spanish Council of Scientific Research. Open-column liquid–solid chromatography was used to fractionate the Zarzaitine oil. A glass column

packed with silica gel (60–100 mesh; 5% $H_2O$; 40 g) under alumina (grade 1 neutral; 1.5% $H_2O$; 20 g) was loaded with Zarzaitine (1 g) and the column eluted with hexane (2 column volumes), dichloromethane (2 column volumes), and methanol (2 column volumes) to provide aliphatic, aromatic, and polar fractions, respectively.

## Determination of PAHs in Zarzaitine

About 100 mg of oil was diluted with pentane (the pentane/oil ratio is 60:1 (w/w)) in 20 ml then sonicated for fifteen minutes to maximize the content of the pentane soluble fraction. The obtained mixture was filtered using an HVLP 4700 Millipore membrane filter. The equipment was then carefully cleaned with pentane to remove the amount of maltenic fraction sticking to the wall. Saturated and aromatic hydrocarbons were eluted with hexane (12 ml) and 50% DCM in hexane (15 ml, v/v). Fractionation was performed by adsorption chromatography in an open glass column packed with anhydrous sodium sulfate and an open glass column packed with 2 g neutral alumina (middle; activated at 400 °C, 5% water deactivated) and glass wool (bottom). The collected fractions were concentrated with a stream of $N_2$ gas. The extract was analyzed for alkyl homologous polycyclic aromatic hydrocarbons (PAHs) and other U.S. Environmental Protection Agency priority unsubstituted PAHs (Table 1).

**Table 1** Concentration of 16 PAH (alkylated and parents) in the Brut Tunisian Light oil (Zarzaitine)

| PAHs | $t_R$ | ppm (µg/g) |
|---|---|---|
| Naphthalene | 6.68 | 510 |
| C1naphthalenes | 7.84 | 750 |
| C2 naphthalenes | 9.06 | 4675 |
| C3naphthalenes | 10.06 | 899 |
| C4naphthalenes | 11.25 | 664 |
| Acenaphthylene | 9.27 | 16 |
| Acenaphthene | 9.63 | 12 |
| Fluorene | 10.53 | 78 |
| Phenanthrene | 12.23 | 188 |
| Anthracene | 12.30 | 0 |
| C1 phenanthrenes-anthracenes | 13.57 | 1118 |
| C2 phenanthrenes-anthracenes | 14.31 | 1011 |
| C3phenanthrenes-anthracenes | 15.76 | 594 |
| C4phenanthrenes-anthracenes | 17.25 | 197 |
| Fluoranthene | 14.83 | 6 |
| Pyrene | 15.41 | 8 |
| 2-MPy, 4-MPy, 1-MPy | 16.93 | 123 |
| Benzo(a) anthracene | 18.67 | 7 |
| Chrysene | 18.78 | 77 |
| Benzo(b) fluoranthene | 21.73 | 6 |
| Benzo(k) fluoranthene | 21.80 | 5 |
| Benzo(e) pyrene | 22.43 | 18 |
| Benzo(a) pyrene | 22.56 | 4 |
| Indeno(1,2,3-c,d) pyrene | 25.31 | 0 |
| Dibenzo(a,h) anthracene | 25.36 | 3 |
| Benzo(g,h,i) perylene | 25.82 | 3 |

## Sampling site

To evaluate in microcosms the effect of the third-generation dispersant and the response of aquatic microorganisms to contamination of sediments by petroleum, we took water and sediment samples from the Sidi-Jehmi beach (north latitude: 36.72072349483175 and east longitude: 10.438728332519531), located in Borj Cedria, Governorate of Nabeul, northern Tunisia (Fig. 1). Samples were taken manually from the beach in sterile bottles.

## Zarzaitine contamination experience

The contaminant used during this study is Zarzaitine from the STIR's Bizerte refinery; it originates from the El-Borma deposit located in southwestern Tunisia and is marketed under the name of Zarzaitine mixture (it features low sulfur content and has a density of 0.8039). The third-generation dispersant used during experiments is characterized by a clear yellow coloration, a density of 0.79, very good storage stability, and a flashpoint above 28 °C. During contamination experiments, the sediment samples were first dried in an oven for 4 days at 60 °C, then distributed in microcosms in the order of 300 g of sediment per microcosm (Table 2). Several study parameters must be taken into account: (i) three control microcosms containing only water and sediment without petroleum (*C*); (ii) three microcosms treated with a concentration of petroleum c1 equal to 500 ppm (*P*); (iii) three microcosms containing only the dispersant (*D*); and (iv) three microcosms treated with petroleum concentration at 500 ppm and a third-generation dispersant applied by 1:20 assay ($P+D$). Incubation in each microcosm was carried out under oxygen pump aeration for 40 days.

## Characteristics of the water samples

### Physicochemical parameters

For the characterization of samples, we analyzed and evaluated the following parameters: pH was measured using pH meter (type 3510 pH-Meter). The salinity was determined using a conductivity meter. Biochemical oxygen demand (BOD) is the amount of oxygen necessary to oxidize organic matter by biological means. The measurement of the BOD was taken according to the nanometric method based on the principle of the WARBURG respirometer. Chemical oxygen demand (COD) is the amount of oxygen expressed


**Fig. 1** sampling site. Water and sediment samples from the Sidi-Jehmi beach (north latitude: 36.72072349483175 and East Longitude: 10.438728332519531), located in Borj Cedria, Government of Nabeul, northern Tunisia

**Table 2** Code used to identify the different microsomes

| Treatments | Code |
|---|---|
| Natural sediment (Control) | *C* |
| Sediment treated with petroleum with a concentration of 500 ppm | *P* |
| Sediment treated with petroleum and dispersant | *P* + *D* |
| Sediment treated by dispersant | *D* |

in milligrams consumed by existing materials in water and oxidizable. The principle of the determination was based on the oxidation of organic materials by an excess of potassium dichromate in an acid medium ($H_2SO4$), in the presence of silver sulfate as a catalyst and mercury sulfate as a complexing agent. We evaluated the quantity of oxygen (mg/L), used by the oxidation reactions, from the measurement of the residue of the reagents after 2 h of oxidation in the presence of excess of oxidant. COD was measured using a CR2200 calorimeter. The turbidity was measured using a TU-2016 turbidity meter. Suspended matter (MES) was determined by filtration of one liter of water on Whatman Millipore filters (0.45 µm), and the pellet was dried at 105 °C for 2 h (Ansari et al. 2010). The full alkalimetric title or TAC corresponds to the content of free alkalis, carbonates and bicarbonates in the water. These determinations are based on the neutralization of a volume of water with a dilute mineral acid in the presence of a colored indicator.

## Characteristics of sediment samples

To determine the pH of sediment, we added 25 ml of distilled water to 10 g of dry sediment, followed by stirring for one hour followed by decantation for 15 min and pH was measured using a pH meter. Calcium carbonate level ($CaCO_3$) in sediment samples was determined using the Bernard calcimeter, which allows measurement of the volume of $CO_2$ released under the action of hydrochloric acid (HCl) on calcium carbonate ($CaCO_3$) present in each sediment sample. To determine the total surface (external and internal) of particles present in each sample of sediments, compared to the unit of mass, we measured the adsorption capacity of methylene blue. It is expressed in $m^2/g$. The organic matter (OM) was determined by the incineration method (loss on ignition or loss on calcination), which is not suitable for calcareous oils. The measurement of loss on ignition (PF) indicates the organic matter content and organic carbon content (Corg) of the oil. To determine the OM level, the sediment samples were burned at 105 °C. The organic matter is destroyed and released in the form of carbon dioxide ($CO_2$) and water vapor. After combustion, only the mineral fraction of the oil remained in the container. The count of the microbial load of samples was determined according to the probable number (NPP) technique. This technique takes place in three main stages: dilution, inoculation, and reading.

### Determination of hydrocarbons

To determine the level of hydrocarbons present in samples, we followed the steps of (i) elimination of the polar substances by purification with Florisil, (ii) analysis of an aliquot purified by capillary chromatography, using a non-polar column and a flame ionization detector (FID), and (iii) measurement of the total area of peaks between n-decane and the two specified mineral oils. Dry the sediment samples at 40° for 2 days, then crushed and sieved with sieves < 4 mm. Place 25 g of the sample and 25 g of sodium sulfate $NaSO_4$ in 250 ml glass bottles, 50 ml of hexane solvent was added, and the mixture was stirred for 30 min, and then filtered through glass flasks. The extraction is carried out using a rotary evaporator equipped with a sea bath, a condenser and a vacuum pump to concentrate the solvent, and add the hexane and then injection under GC-FID chromatography.

### Analysis of bacterial diversity using fingerprinting technique: ARISA

One microliter of DNA (corresponding to 1–5 ng) was amplified using universal bacterial primers 16S-1392F (5′-GYACACACCGCCCGT-3′) and 23S-125R (5′-GGGTTBCCCCATTCRG-3′), which amplify the ITS1 region in the rRNA operon plus ca. 282 bases of the 16S and 23S rRNA (Hewson and Fuhrman 2004). The primer 23S-125R was fluorescently labeled with the fluorochrome HEX (MWGspa BIOTECH). All PCRs were performed in a volume of 50 µl in a thermal cycler (Biometra, Germany) using the MasterTaq® kit (Eppendorf AG, Germany), which reduces the effects of PCR-inhibiting contaminants. Thirty PCR-cycles were used, consisting of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, preceded by 3 min of denaturation at 94 °C and followed by a final extension of 10 min at 72 °C. To check for eventual contamination of the PCR reagents, negative controls containing the PCR-reaction mixture but without the DNA template were run during each amplification. Positive controls, containing genomic DNA of *Escherichia coli* also were used. PCR products were checked on agarose-TBE gel (1%), containing ethidium bromide for DNA staining and visualization. Four different reactions were run for each sample and then combined to form two duplicate PCR reactions (Qiu et al. 2001), which were subsequently utilized for separate ARISA analyses. The two resulting PCR combined products were purified using the Wizard PCR cleanup system (Promega, Madison, WI), resuspended in 50 µl of Milli-Q water supplied with the cleanup system and then quantified. About 5 ng of amplicons were mixed with 14-µl of internal size standard (GS2500-ROX; Applied Biosystems, Foster City, CA) in deionized formamide, then denatured at 94 °C for 2 min and immediately chilled in ice. Automated detection of ARISA fragments was carried out using an ABI Prism 3100 genetic analyzer (Applied Biosystems). ARISA fragments in the range 390–1400 bp were determined using Genescan analytical software 2.02 (ABI), and the results analyzed adopting the procedure described by Danovaro et al. (2006), which included standardization of fluorescence among samples, elimination of "shoulder" and non-replicated peaks, and cut-off criterion. According to available literature (Danovaro et al. 2006; Hewson and Fuhrman 2006), we have set the accuracy in sizing at ± 3 bp for ARISA fragments lesser than 700 bp, ± 5 bp from 700 to 1000 bp and ± 10 bp for greater than 1000 bp.

### Statistical analyses

Differences in the microbial community variables among control and treatments were tested using parametric one-way ANOVA tests. A posteriori paired multiple comparisons were made using Tukey HSD test when significant differences were $p < 0.05$.

## Results and discussion

### Determination of PAHs in Zarzaitine

The percentage of each fraction in Zarzaitine was determined. The brut Tunisian crude Zarzaitine is composed of 55.5% saturated hydrocarbons, 20% aromatic hydrocarbons, 3.3% polar compounds, and 21.16% asphaltenes, and the density of the Zarzaitine at 15 °C was 0.8039 (Table 1).

### Physicochemical characterization of water samples

The comparison of pH values measured at $t_0$ and after 40 days of incubation of the different types of treatment showed a slight variation in pH only in microcosms treated with the petroleum and dispersant mixture (see Table 3). This variation is characterized by a decrease in the pH value from 8.35 to 8.07. Decrease of pH can be explained by the degradation of petroleum whose rupture of these carbon chains releases the ions $H^+$ in water which tends to lower the pH of the medium. With respect to salinity, we recorded an increase in salinity in microcosms treated with the dispersant, about 185.55 ± 13.78 ms (Table 3), which was due to the composition of the dispersant. The significant increase in salinity was observed only when the dispersant was added individually. Salinity increased by 13% compared to dispersal-free water, while salinity decreased by 8% in presence of oil and dispersal together. To study the effect of mixing oil with dispersion, we conducted an experiment to compare the different results of the studied samples that enabled to conclude the hypothesis of a strong relationship between the process of oil dispersion and high water salinity. To examine

**Table 3** Impact of petroleum dispersion on the physicochemical properties of seawater

| Properties | Time 0 | After 40 days of incubation | | | |
|---|---|---|---|---|---|
| | | *C* | *P* | *P* + *D* | *D* |
| pH (T/20.8 °C) | 8.35 | 8.35 ± 0,02 | 8.35 ± 0.04 | 8.07 ± 0.3 | 8.38 ± 0.2 |
| Conductivity (ms) | 164 ± 1.9 | 164 ± 1.9 | 165 ± 13 | 151 ± 1 | 185 ± 13 |
| Turbidity (ntu) | ND | 33.93 | 0.77 | 0.52 | 0.20 |
| Potential (Mv) | ND | − 20 | − 20 | − 20 | − 20 |
| $DBO_{5\,(}$mg/L) | ND | 0 ± 0.00 | 20 ± 0 | 5 ± 7.07 | 0 ± 0.00 |
| TA ($10^{-3}$méq/L) | ND | 2.65 ± 0.91 | 1.35 ± 0.21 | 2.3 ± 0.42 | 1.35 ± 0.15 |
| TAC ($10^{-3}$méq/L) | ND | 1.9 ± 0.14 | 4.2 ± 0.28 | 2.65 ± 0.21 | 4.94 ± 0.06 |

possible mechanisms of action that have improved the productivity of dispersed oil disposal, the hypothesis has been adopted of a close correlation between water salinity and the chemical composition and functioning of the dispersant. Salinity affects the composition of oils. The interaction of NaCl with chemical bonds in organic compounds induced the decomposition process and thus improved the process of chemical dispersion (Ossipov et al. 2021). It is believed that many opinions largely agree on the role of high salinity in driving the process of chemical dispersion of oils. Salinity played an important role in dispersant effectiveness for almost all the oil–dispersant combinations (Cao et al. 2020; 2022a,b). Higher salinity levels will increase oil dispersion effectiveness by improving dispersant availability. When a dispersant is applied to an oil slick, its effectiveness in dispersing the spilled oil depends on factors such as oil properties, wave-mixing energy, temperature, and salinity of the water. Dispersion efficiency increased with increase in salinity for most oil–dispersant combinations (Chandrasekar et al. 2006).

The turbidity values obtained showed a significant drop in the microcosms treated compared to the control. From Table 3, Results show a significant decrease in turbidity exceeds 97–99% in water contaminated with dispersant and oil or both. We interpret these results of water clean by the role of these substances in the withdrawal of small impurities in the water, lowering them down or creating a floating layer on the surface, thus acting oil and dispersant as absorbents. The importance of oil in adsorption is highlighted in many researches (Wang et al. 2014; Frauenhofer et al. 2019), and dispersal in adsorption has been used in many cases (Kikkawa et al. 1988; Tomlinson et al. 2000; Palmqvist and Holmberg 2008; Haselhuhn 2013; Chen et al. 2018; Wang et al. 2020).

The results presented in Fig. 2 show a significant increase in chemical oxygen demand (COD) of (*P*), (*P* + *D*) and (*D*) samples compared to the control (*C*). The low chemical oxygen demand could be explained by the small content of organic matter in the control sample. Contamination of samples with petroleum and/or dispersant imposed high level of COD. On the other hand, there is a decrease in biochemical

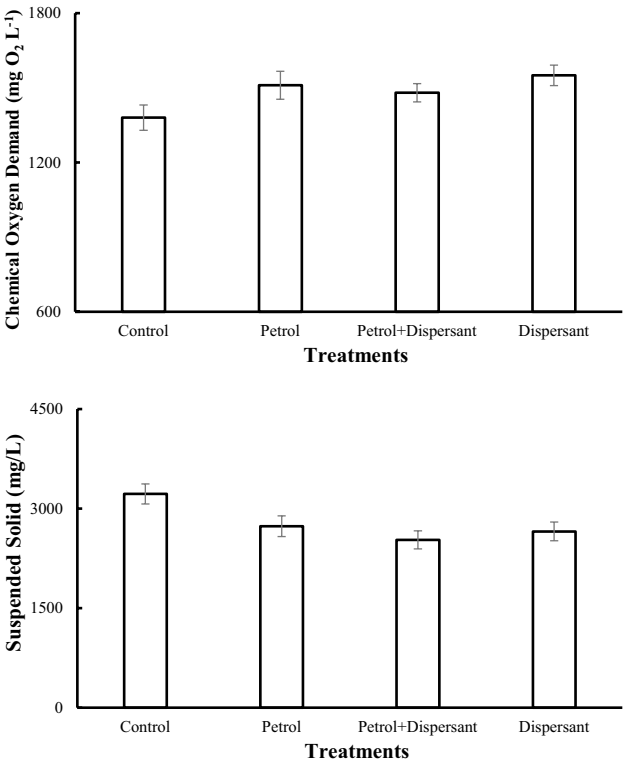


**Fig. 2** Variation in COD (**a**) and SS (**b**) values after 40 days of incubation. COD is the amount of oxygen expressed in milligrams, consumed by existing materials in water and oxidizable under defined operating conditions. SS represent the solid particles responsible for the turbidity of water

oxygen demand (BOD) in microcosm (*P* + *D*). From these results, it can be concluded that the dispersant plays an important role in the chemical degradation of petroleum more than microorganisms. According to Fig. 2, the contamination by petroleum and/or dispersant caused a reduction in the suspended matter (MES) is noted after. Decrease becomes more significant after the contact of petroleum and dispersant, which can be explained by the fixation of fine particles in suspension by petroleum and the dispersant. The decrease in the suspended matter in microcosms *P*, *P* + *D*, and *D* is accompanied by a decrease in turbidity,

**Table 4** Impact of petroleum dispersion on the physicochemical properties of the sediment

| Properties | Time 0 | After 40 days of incubation | | | |
|---|---|---|---|---|---|
| | | *C* | *P* | *P*+*D* | *D* |
| PH | 16.8 | 9.06 ± 0.03 | 8.95 ± 0.22 | 8.94 ± 0.1 | 9.22 ± 0.38 |
| Conductivity (ms) | 16.42 | 16.8 ± 0.14 | 9.87 ± 0.60 | 11.52 ± 0.31 | 9.9 ± 0.85 |
| Specific surface ($m^2$/g) | ND | 26.16 ± 7.39 | 31.39 ± 14.79 | 52.31 ± 14.77 | 52.32 ± 14.79 |
| $CaCO_3$ (%) | ND | 9.15 ± 0.65 | 17.97 ± 0.40 | 15.4 ± 1.32 | 5.63 ± 0.18 |

**Table 5** Result of microbial load count

| Properties | Time 0 | After 40 days of incubation | | | |
|---|---|---|---|---|---|
| | | *C* | *P* | *P*+*D* | *D* |
| Bacterial density (NPP) | $24_{*}10^3$ cells/L | $9_{*}10^3$ cells/L | $12.6_{*}10^3$ cells/L | $1.3_{*}10^3$ cell/L | $1.4_{*}10^3$ cells/L |
| OM | ND | 18 ± 0.01 | 14 ± 0 | 12 ± 0.03 | 4 ± 0.02 |
| Hydrocarbon index (Mg/Kg (MS)) | 150 | < 11 | 53 | 56 | < 11 |

which becomes more significant in microcosm treated by the dispersant (*D*) compared to the (*P*+*D*)- and *P*-treated microcosms. The results could be explained by the presence of small hydrocarbon molecules released in suspension under the degradable action of the dispersant and microorganisms.

## Characterization of sediment samples

The results presented in Table 4 showed pH variations between treatments. The specific surface area increased considerably in the presence of dispersant and in (*P*+*D*) microcosm, which represents a risk of $CaCO_3$ accumulation.

## Microbiological characterization

The study of the bacterial load in different treatment present high density ($24.10^3$ cells/L) at $t_0$. It decreased after 40 days of incubation to reach 9 $10^3$ cells/L in the control microcosm (*C*), 1,3 $10^3$ cells/L in (*P*+*D*) microcosm, and 1,4 $10^3$ cells/L in (*D*) microcosm (Table 5). The reduction in bacterial load in control microcosm could be explained by the lysis of bacterial cells due to the depletion of the substrate and the accumulation and toxicity of metabolic waste. The significant decrease in bacterial load in treated microcosms could be explained by the toxicity of bacterial metabolism products and by the inhibitory effect of dispersed hydrocarbon molecules following the degradation of oil (Gerlach 1978). Adding a new bacterial substrate (the dispersant) can attract microorganisms more than hydrocarbons. The dispersant was not toxic to endogenous bacteria. Bacteria isolated from the aftermath of the Louisiana oil accident were capable of degrading the various compounds of the dispersant Corexit 9500, such as hydrocarbons, glycols, and dioctyl sulfosuccinate.

**Table 6** Effects of petroleum dispersion on the biodegradation of hydrocarbons

| Treatments | Time 0 | After 40 days of incubation | | | |
|---|---|---|---|---|---|
| | | *C* | *P* | *P*+*D* | *D* |
| PF | | 18 ± 0.01 | 14 ± 0 | 12 ± 0.03 | 4 ± 0.02 |
| Hydrocarbon index (Mg/Kg (MS)) | 150 | < 11 | 53 | 56 | < 11 |

## Effects of petroleum dispersion on hydrocarbon biodegradation

The total hydrocarbon index did not show a significant change between the microcosms treated with both the mixture (petroleum and dispersant) or with petroleum alone (Table 6). In contrast, many studies (in mesocosms or microcosms) have shown an increase in the biodegradation of hydrocarbons when dispersants were used (Somerfield et al. 1994; Rodriguez et al. 2007). The chemical dispersion of Zarzaitine increased the penetration of hydrocarbons in sediments and facilitated the complexation of oil with suspended particles. The dispersant accelerated the adsorption of petroleum into the sediment (Chandrasekar et al. 2006). The biodegradability of samples enabled us to conclude that the dispersed or non-dispersed Zarzaitine was biodegradable (29.65%) under these experimental conditions. Arabian light crude oil was degraded more than 65%, while fuel Zarzaitine rises to 11% (Fleeger and Chandler 1982; Elarbaoui et al. 2015). The variation in the rate of biodegradation can be attributed, at least in part, due to the nature of the Zarzaitine, which is composed of several types of hydrocarbons made up of different families that are difficult to assimilate by microorganisms.

**Table 7** Mean of univariate indices values for bacterial assemblages observed in microcosms, exposed to different treatments (*C*: control, *d*: dispersant, oil: Oil, oil + dispersant: Oil + *d*). *S* = number of species; Margalef's *D* = species richness; *H'* = Shannon–Wiener index; Pielou's *J'* = evenness

| Microcosm | *S* | *D* | *J'* | *H'* |
|---|---|---|---|---|
| *C* | 49 | 10.42 | 0.786 | 3.0585 |
| *d* | 72 | 15.42 | 0.79515 | 3.4005 |
| Oil + *d* | 54 | 11.51 | 0.8296 | 3.3095 |
| Oil | 39 | 8.252 | 0.84215 | 3.085 |

## Changes in the microbial communities

The number of OTUs, richness and diversity indexes in all microcosms (control and treatments) are reported in Table 7. Libraries from control, dispersant and Oil + *d* had a higher richness than Oil. Experiments reveal that the microbial community in term of abundance and diversity are affected by oil and dispersant. The widespread application of chemical dispersants to treat oil spills enabled the mitigation of biodegradation barriers caused by high salinity (Cao et al. 2022a, b). Authors concluded that there was a positive causal relation between the high rate of biodegradation of oil and the increasing numbers of microorganisms in the presence of dispersant. In the same way, results in Table 7 show increase in number of species (*S*) and species richness (*D*) in dispersant and mixed mediums (47% and 10%, respectively), but decreased in oil medium (21%). Similarly, Shannon–Wiener index (*H'*) increased in dispersant, mixed and oil mediums (11%, 8% and 1%, respectively). Moreover, evenness (*J'*) increased in oil, mixed and dispersant mediums (7%, 5% and 1%, respectively). Recently, Ben Said et al. (2021) were isolated and characterized oil-degrading bacteria in oily sludge from the Tunisian Company for Refining Industries (STIR). The major strains were affiliated to the Gammaproteobacteria, especially, the *Pseudomonas* genus and Beta- and Alphaproteobacteria (Firmicutes and Actinobacteria). Based on previous publications in this area, studies have shown a range of oil-analyzing bacteria in a different salinity that can range from 0 to 120 g/L (Cao et al. 2022a). The effectiveness of the degradation process is linked to the physicochemical properties of water, most notably salinity. We further note through the results that the biodegradation process is subject to a combination of factors affecting the organic compounds of the oils, the ability of microorganisms to withstand different ecological factors, including the environmental characteristics of their medium and different physiological interactions. Researchers in these studies have shown that dispersant significantly stimulates bacteria proliferation and diversity at high salinity, as opposed to oil causes in salinity. When mixing oil with dispersant, bacteria were not affected much at high salinity. These results show relationships between the biodegradation of oil, bacteria, dispersant, oil and salinity (Fig. 3). Bacteria have the potential to enhance the toxicity of crude oil by producing biosurfactants, and the same bacteria may reduce the toxicity associated with dispersed oil through degradation or sequestration. Ortmann and Lu (2015) underscore large variability in bacterial responses to hydrocarbon pollutants, implying that bioremediation success varies with starting biological and environmental conditions. Increases in the contributions of hydrocarbon-degrading taxa and decreases in common estuarine bacteria were observed in response to dispersant and/or oil in two mesocosms experiments. Overholt et al. (2015) showed that bacterial strains grow with Corexit as the sole carbon and energy source. Hydrocarbon-degrading bacterial species demonstrate a unique response to dispersed oil compared to their response to crude oil, with potentially opposing effects on toxicity.

## Conclusion

Change in the Zarzaitine and dispersant microcosm properties poses a risk of $CaCO_3$ accumulation. Moreover, Zarzaitine pollution caused an increase in BOD, pH, TAC, salinity, and a decrease in MES and turbidity. The addition of the third generation dispersant for decontamination is also the origin of the variation in the physicochemical parameters of marine water, in particular the COD, TAC, and salinity which became significant after the addition of the dispersant

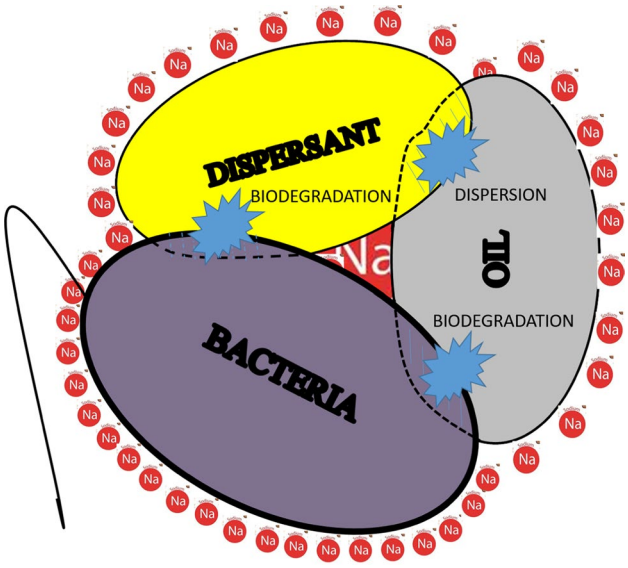


**Fig. 3** Role of bacteria in oil/dispersant biodegradation under saline water conditions

to microcosm not treated with petroleum. The specific surface area increased considerably in the presence of dispersant. The turbidity decreased compared to the oil-treated microsomes. The bacterial density decreased from > 24 $10^3$ cells/L in the control microcosms to 1,4 $10^3$ cells/L in microcosm containing the dispersant, while the microbial diversity increased. The total hydrocarbons index in the microcosms treated with the mixture (petroleum and dispersant) or with petroleum alone are the same. The chemical oxygen demand decreased in microcosm treated with dispersant and petroleum compared to the microcosm treated by petroleum, which could be explained by the degradation of organic matter and the consumption of dissolved oxygen. The decrease in the suspended matter (SS) implies that the dispersant attacks the finest particles. When there is an oil spill, there is a change in the majority of the physicochemical parameters. It is important to use a mechanical elimination of petrol to minimize the quantity of dispersant, the intervention time and the diffusion of chemical substances.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.



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