



HAL
open science

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

Soumaya Elarbaoui, Latifa Smii, Zahrah Alhalili, Moêz Smiri

► To cite this version:

Soumaya Elarbaoui, Latifa Smii, Zahrah Alhalili, Moêz Smiri. The effect of chemical dispersant of the third generation (Finasol OSR 62) on the microbial biodegradation process of Zarzaitine oil in water treatment. Applied Water Science, 2022, 12, 10.1007/s13201-022-01803-3 . insu-03863332

HAL Id: insu-03863332

<https://insu.hal.science/insu-03863332>

Submitted on 21 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution| 4.0 International License



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

Soumaya Elarbaoui^{1,2} · Latifa Smii^{3,4} · Zahrah Alhalili¹ · Moêz Smiri^{1,2}

Received: 2 July 2022 / Accepted: 21 October 2022 / Published online: 2 November 2022
© The Author(s) 2022

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 (BOD₅), 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 BOD₅ 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

✉ Moêz Smiri
almoez@su.edu.sa

¹ College of Science and Arts – Sajir, Shaqra University, Riyadh, Saudi Arabia

² Laboratory of Water, Membranes and Environment Biotechnology (LEMBE), Technopole of Borj Cedria (CERTE), 2050 Hammam-Lif, Tunisia

³ Aix Marseille Université, CNRS, Université de Toulon, IRD, Mediterranean Institute of 12 Oceanography (MIO), UM 110, 13288 Marseille, France

⁴ Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), Carthage University, Tunis, Tunisia

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 5.3×10^8 kg of oil and 1.7×10^8 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% H₂O; 40 g) under alumina (grade 1 neutral; 1.5% H₂O; 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 N₂ gas. The extract was analyzed for alkyl homologous polycyclic aromatic hydrocarbons (PAHs) and other U.S. Environmental Protection Agency priority unsubstituted PAHs (Table 1).

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

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

| PAHs | <i>t_R</i> | 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 |

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) | <i>C</i> |
| Sediment treated with petroleum with a concentration of 500 ppm | <i>P</i> |
| Sediment treated with petroleum and dispersant | <i>P+D</i> |
| Sediment treated by dispersant | <i>D</i> |

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_2SO_4), 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 ($0.45 \mu m$), and the pellet was dried at $105^\circ C$ 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 $105^\circ C$. 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 NaSO₄ 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 (MWG-spa 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 | | | |
|------------------------------|-----------|-----------------------------|-------------|-------------|-------------|
| | | <i>C</i> | <i>P</i> | <i>P+D</i> | <i>D</i> |
| 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; Fraunhofer 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

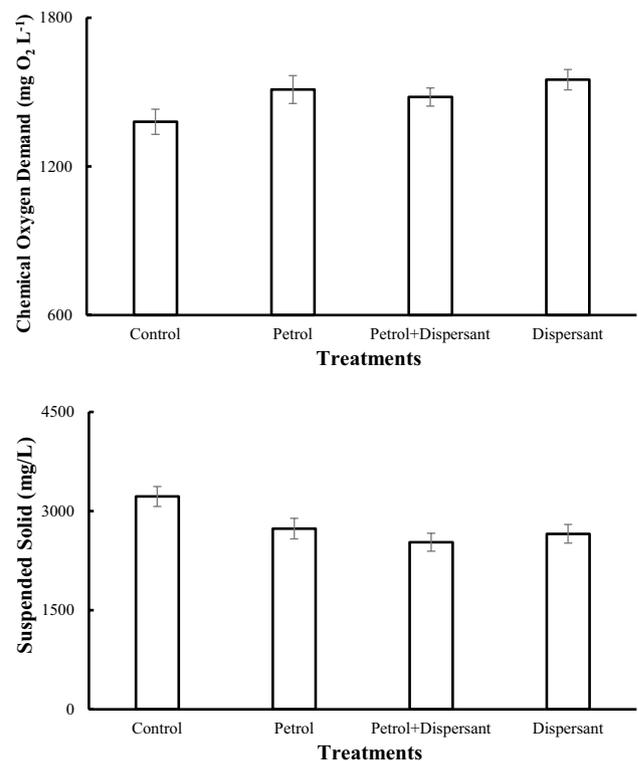


Fig. 2 Variation in COD (a) and SS (b) values after 40 days of incubation. COD is the amount of oxygen expressed in milligrams, consumed by existing materials in water and oxidizable under defined operating conditions. SS represent the solid particles responsible for the turbidity of water

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 | | | |
|--------------------------------------|--------|-----------------------------|---------------|---------------|---------------|
| | | <i>C</i> | <i>P</i> | <i>P+D</i> | <i>D</i> |
| 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 | | | |
|--------------------------------|------------------------------|-----------------------------|--------------------------------|------------------------------|-------------------------------|
| | | <i>C</i> | <i>P</i> | <i>P+D</i> | <i>D</i> |
| 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 CaCO₃ accumulation.

Microbiological characterization

The study of the bacterial load in different treatment present high density (24.10³ cells/L) at *t*₀. It decreased after 40 days of incubation to reach 9 × 10³ cells/L in the control microcosm (*C*), 1,3 × 10³ cells/L in (*P+D*) microcosm, and 1,4 × 10³ 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 | | | |
|--------------------------------|--------|-----------------------------|----------|------------|----------|
| | | <i>C</i> | <i>P</i> | <i>P+D</i> | <i>D</i> |
| 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 (Sommerfield 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 | <i>S</i> | <i>D</i> | <i>J'</i> | <i>H'</i> |
|-----------|----------|----------|-----------|-----------|
| 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 Alpha-proteobacteria (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 CaCO₃ 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

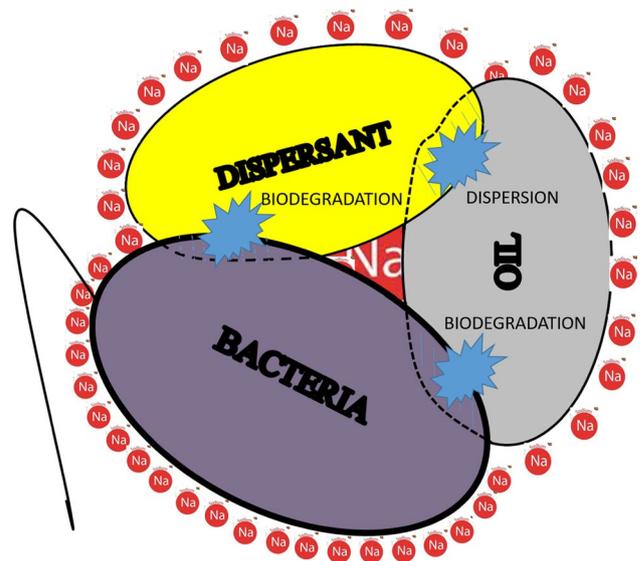


Fig. 3 Role of bacteria in oil/dispersant biodegradation under saline water conditions

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 microcosms. The bacterial density decreased from $> 24 \times 10^3$ cells/L in the control microcosms to $1,4 \times 10^3$ 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.

Acknowledgements Author would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

Funding The author(s) received no specific funding for this work.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

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

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Álvarez-Barragán J, Cravo-Laureau C, Duran R (2022) Fungal-bacterial network in PAH-contaminated coastal marine sediment. *Environ Sci Pollut Res Int*. <https://doi.org/10.1007/s11356-022-21012-4>
- Ansari ZA, Parveen F, Shahin B (2010) Response of meiofauna to petroleum hydrocarbon of three fuel oils. *Proc Nat Acad Sci* 80:138–143
- Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. History. *Environ Sci Technol* 45:6709–6715
- Aurand D, Coelho G (2005) Using laboratory, mesocosm, and field data in ecological risk assessments for near-shore dispersant use (Conference Paper). In: International oil spill conference, IOSC 2005; Miami Beach, pp 8912–8916
- Bælum J, Borglin S, Chakraborty R, Fortney JL, Lamendella R, Mason OU, Auer M, Zemla M, Bill M, Conrad ME, Malfatti SA, Tringe SG, Holman H, Hazen TC, Jansson JK (2012) Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environ Microbiol* 14:2405–2416
- Barron MG (2012) Ecological impacts of the Deepwater Horizon oil spill: implications for immunotoxicity. *Toxicol Pathol* 40:315–320
- Ben Said O, Cravo-Laureau C, Armougom F, Cipullo S, Ben Khelil M, Ben Haj Yahya M, Douihech A, Beyrem H, Coulon F, Duran R (2021) Enhanced pilot bioremediation of oily sludge from petroleum refinery disposal under hot-summer Mediterranean climate. *Environ Technol Innov* 24:102037
- Bertrand JC, Rambeloarisoa JF, Rontani JF, Giusti G, Mattei G (1983) Microbial degradation of crude oil in sea water in continuous culture. *Biotechnol Lett* 5:567–572
- Cao Y, Zhang B, Zhu Z, Song X, Cai Q, Chen B, Dong G, Ye X (2020) Microbial eco-physiological strategies for salinity-mediated crude oil biodegradation. *Sci Total Environ* 727:138723. <https://doi.org/10.1016/j.scitotenv.2020.1387>
- Cao Y, Zhang B, Greer CW, Lee K, Cai Q, Song X, Tremblay J, Zhu Z, Dong G, Chen B (2022a) Metagenomic and metatranscriptomic responses of chemical dispersant application during a marine dil-bit spill. *Appl Environ Microbiol* 88(5):e0215121. <https://doi.org/10.1128/aem.02151-21>
- Cao Y, Kang Q, Zhang B, Zhu Z, Dong G, Cai Q, Lee K, Chen B (2022b) Machine learning-aided causal inference for unraveling chemical dispersant and salinity effects on crude oil biodegradation. *Bioresour Technol* 345:126468. <https://doi.org/10.1016/j.biortech.2021.126468>
- Chan H (2011) Biodegradation of petroleum oil achieved by bacteria and nematodes in contaminated water. *Sep Purif Technol* 80:459–466. <https://doi.org/10.1016/j.seppur.2011.05.028>
- Chandrasekar S, Sorial G, Weaver J (2006) Dispersant effectiveness on oil spills—impact of salinity. *ICES J Mar Sci* 63:1418–1430. <https://doi.org/10.1016/j.icesjms.2006.04.019>
- Chen J, Eraghi Kazzaz A, AlipoorMazandarani N, Hosseinpour Feizi Z, Fatehi P (2018) Production of flocculants, adsorbents, and dispersants from lignin. *Molecules* 23(4):868. <https://doi.org/10.3390/molecules23040868>
- Danovaro R, Luna GM, Dell'anno A, Pietrangeli B (2006) Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. *Appl Environ Microbiol* 72:5982–5989
- de Almeida Couto CR, Alvarez VM, Marques JM, de Azevedo JD, Seldin L (2015) Exploiting the aerobic endospore-forming bacterial diversity in saline and hypersaline environments for biosurfactant production. *BMC Microbiol* 28:240. <https://doi.org/10.1186/s12866-015-0575-5>
- Duran R, Cuny P, Bonin P, Cravo-Laureau C (2015) Microbial ecology of hydrocarbon-polluted coastal sediments. *Environ Sci Pollut Res Int* 22:15195–15209. <https://doi.org/10.1007/s11356-015-5373-y>
- Elarbaoui S, Richard M, Boufahja F, Mahmoudi E, Thomas-Guyon H (2015) Effect of crude oil exposure and dispersant application on meiofauna: an intertidal mesocosm experiment. *Environ Sci Process Impacts* 17:997–1004. <https://doi.org/10.1039/c5em00051c>
- Fiocco R, Daling P, DeMarco G, Lessard R, Canevari G (1999) Chemical dispersibility study of heavy bunker fuel oil. In: Proceedings 22nd arctic and marine oil spill program technical seminar, pp 173–186

- Fleeger JW, Chandler GT (1982) Meiofauna responses to experimental introduction of crude-oil in a salt-marsh. *Am Zool* 22:858–858
- Frauenhofer E, Cho J, Yu J, Al-Saigh Z, Kim J (2019) Adsorption of hydrocarbons commonly found in gasoline residues on household materials studied by inverse gas chromatography. *J Chromatogr A* 1594:149–159. <https://doi.org/10.1016/j.chroma.2019.01.076>
- Fuller C, Bonner J, Mc Donald T, Page C, Bragin G, Clark J, Aurand D, Hernandez A, Ernest A (1999) Comparative toxicity simulated beach sediments impact with both whole and chemical dispersions of weathered Arabian crude oil. In: Proceeding of the 22 arctic and marine oil spill program (AMOP) technical seminar, Calgary, Alberta, p 659
- Gerlach SA (1978) Food chain relationships in subtidal silty marine sediments and the role of meiofauna in stimulating bacterial productivity. *Oecologia* 33:55–69
- Haselhuhn HJ (2013) Dispersant adsorption and effects on settling behavior of iron ore. *Min Metall Explor* 30(3):188–189. <https://doi.org/10.1007/bf03402269>
- Hewson I, Fuhrman JA (2004) Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Appl Environ Microbiol* 70:3425–3433
- Hewson I, Fuhrman JA (2006) Improved strategy for comparing microbial assemblage fingerprints. *Microbiol Ecol* 51:147–153
- Ho KAY, Patton L, Latimer JS, Pruell RJ, Pelletier M, McKinney R, Jayaraman S (1999) The chemistry and toxicity of sediment affected by oil from the North cape spilled into Rhode Island sound. *Mar Pollut Bull* 38:314–323
- Kikkawa H, Takezaki H, Otani Y, Shoji K, Ogawa J, Kanamori S (1988) Effect of adsorption characteristics of dispersant on flow and storage properties of coal–water mixtures. *Powder Technol* 55(4):277–284. [https://doi.org/10.1016/0032-5910\(88\)80038-x](https://doi.org/10.1016/0032-5910(88)80038-x)
- Maia M, Capão A, Procópio L (2019) Biosurfactant produced by oil-degrading *Pseudomonas putida* AM-b1 strain with potential for microbial enhanced oil recovery. *Bioremediat J* 23:302–310. <https://doi.org/10.1080/10889868.2019.1669527>
- Ortmann AC, Lu Y (2015) Initial community and environment determine the response of bacterial communities to dispersant and oil contamination. *Mar Pollut Bull* 15:106–114. <https://doi.org/10.1016/j.marpolbul.2014.11.013>
- Ossipov K, Panyukova DI, Mokochunina TV, Trukhina MV, Maryutina TA (2021) Effect of water salt composition on oil spill dispersant efficiency determined in laboratory conditions. *Ind Lab Diagn Mater* 87:5–11. <https://doi.org/10.26896/1028-6861-2021-87-9-5-11>
- Overholt WA, Marks KP, Romero IC, Hollander DJ, Snell TW, Kostka JE (2015) Hydrocarbon-degrading bacteria exhibit a species-specific response to dispersed oil while moderating ecotoxicity. *Appl Environ Microbiol* 6:518–527. <https://doi.org/10.1128/AEM.02379-15>
- Palmqvist L, Holmberg K (2008) Dispersant adsorption and viscoelasticity of alumina suspensions measured by quartz crystal microbalance with dissipation monitoring and in situ dynamic rheology. *Langmuir* 24(18):9989–9996. <https://doi.org/10.1021/la800719u>
- Procópio L (2020) Changes in microbial community in the presence of oil and chemical dispersant and their effects on the corrosion of API 5L steel coupons in a marine-simulated microcosm. *Appl Microbiol Biotechnol* 104(14):6397–6411. <https://doi.org/10.1007/s00253-020-10688-8>
- Procópio L (2021) The oil spill and the use of chemical surfactant reduce microbial on corrosion API 5L steel buried in saline soil. *Environ Sci Pollut Res Int* 28(21):26975–26989. <https://doi.org/10.1007/s11356-021-12544-2>
- Qiu X, Wu L, Huang H, McDonel PE, Palumbo AV, Tiedje JM, Zhou J (2001) Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl Environ Microbiol* 67:880–887
- Rodriguez JG, Monica I, Rosario DLH, López J, Lastra M (2007) Polycyclic aromatic hydrocarbons (PAHs), organic matter quality and meiofauna in Galician sandy beaches, 6 months after the Prestige oil-spill. *Mar Pollut Bull* 54:1046–1052
- Ryerson TB, Camilli R, Kessler JD, Kujawinski EB, Reddy CM, Valentine DL, Atlas E, Blake DR, de Gouw J, Meinardi S, Parrish DD, Peischl J, Seewald JS, Warneke C (2012) Chemical data quantify Deepwater Horizon hydrocarbon flow rate and environmental distribution. *Proc Natl Acad Sci USA*. <https://doi.org/10.1073/pnas.1110564109>
- Short JW, Maselko JM, Lindeberg MR, Harris PM, Rice SD (2006) Vertical distribution and probability of encountering intertidal Exxon Valdez oil on shorelines of three embayments within Prince William Sound, Alaska. *Environ Sci Technol* 40:3723–3729
- Somerfield PJ, Gee JM, Warwick RM (1994) Soft sediment meiofaunal community structure in relation to a long-term heavy metal gradient in the Fal estuary system. *Mar Ecol Prog Ser* 105:79–88
- Tomlinson A, Scherer B, Karakosta E, Oakey M, Danks TN, Heyes DM, Taylor SE (2000) Adsorption properties of succinimide dispersants on carbonaceous substrates. *Carbon* 38(1):13–28. [https://doi.org/10.1016/s0008-6223\(99\)00087-1](https://doi.org/10.1016/s0008-6223(99)00087-1)
- Wang J, Chen Z, Chen B (2014) Adsorption of polycyclic aromatic hydrocarbons by graphene and graphene oxide nanosheets. *Environ Sci Technol* 48:4817–4825. <https://doi.org/10.1021/es405227u>
- Wang L, Gao C, Feng J, Xu Y, Li D, Zhang L (2020) Adsorption properties of comb-shaped polycarboxylate dispersant onto different crystal pyraclostrobin particle surfaces. *Molecules* 25(23):5637. <https://doi.org/10.3390/molecules25235637>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.