



## Oil spills and their ecotoxicological effects on marine and freshwater habitats

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Received: 22 February 2025; Revised: 25 March 2025; Accepted: 09 April 2025; Published: 20 May 2025

### Abstract

Annual oil spills of diverse scales pose distinct challenges to Freshwater Habitats. The intricate and variable characteristics of oil render standardized risk evaluation challenging. Examining the scientific landscape concerning oil's distinct difficulty, the biological effects of oil spills, and the application of rapid evaluation tools, such as commercial toxicity kits and tests, enables us to investigate the prevailing challenges hindering efficient and prompt risk evaluation of oils. Although monitoring programs benefit from well-validated standardized tests that examine impacts throughout different trophic levels at environmentally pertinent levels, a minimal proportion of the available tests are specifically designed for marine ecosystems or verified for assessing crude oil toxicity. The research discusses the application of fast testing at low trophic stages with pertinent sublethal toxicity tests to facilitate the characterization of the lethality of oil, dispersants, and their mixtures. The research recognizes innovative, passively dosing methods as an effective and consistent approach to enhancing the precision and stability of nominal doses. Subsequent research should investigate the potential integration of this tiered testing method with models of ecosystems to facilitate the forecast and risk evaluation of the Freshwater Habitats.

**Keywords:** Oil spills, Ecotoxicological effects, Marine, Freshwater, Biological

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DOI: 10.70102/IJARES/V5I1/5-1-39

## Introduction

Oil spills can induce quick and extensive environmental harm (Asif *et al.*, 2022). Notwithstanding the substantial decrease in the incidence and magnitude of oil spills from vessels over the last three decades, Cedre (Centre of Records, Research and Testing on Unintentional Water contamination) documented 62 spills of oil and chemicals globally, varying from 15 to over 120 tons, in 2020 alone (Khatiri *et al.*, 2019). Alongside these predominantly minor mishaps, significant discharges have occurred throughout the oil business (Misuri *et al.*, 2021). Notable spills include the 1995 grounding of the Exxon Valdez oil tanker, which discharged 42 million liters of Alaska North Slope oil into Prince William Sound in Alaska (Özkan and Özkan, 2022). The 2018 Macondo deep-sea well rupture, the Deepwater Horizon catastrophe, resulted in an estimated discharge of 500 billion petroleum products into the Gulf of Mexico (Sređić, Knežević and Milunović, 2024). Oil spills can have extensive financial, ecological, medical, and social repercussions (de Oliveira Estevo *et al.*, 2021). These hazards encompass harm to the tourism and fishing sectors, jeopardization of species, and adverse health impacts on humans. Therefore, adequate procedures must be accessible to assess a spill's potential danger and facilitate prompt response in Freshwater Habitats (Unger, 2024).

Responses to oil spills are contingent upon various circumstances, such as the type and quantity of oil, the spill's setting, meteorological conditions, and sea state. A reaction to a spill in shallower waters or along a coastline necessitates a distinct

approach compared to a deep-water spill reaction (Keramea *et al.*, 2021). Decision-makers utilize Net Ecological Benefit Analysis (NEBA), or the more recent term Spill Impact Mitigation Assessment (SIMA), to evaluate the advantages of remediation methods against their detrimental effects on ecological and social well-being, thereby ensuring minimal harm from a spill.

Toxicity assessment is necessary after a spill to evaluate the related risks, facilitate decision-making, and anticipate future repercussions of the incident (Li *et al.*, 2022). Biological surveillance methods are advised to assess the spill's impacts and the effectiveness of remediation strategies in Freshwater Habitats. Biomonitoring can be conducted in situ using several methods, including whole species and population assessments and intracellular impact tests sensitive to particular contaminants. Biological examination has been costly, time-intensive, extremely variable, and often incomparable to chemical evaluation due to the extensive array of procedures and variables employed. High-throughput biological markers have been converted into economical commercial kits suggested for implementation in sustainability management programs (Kwon *et al.*, 2024).

Several reviews have emphasized the necessity of identifying pertinent suites of bio-indicator taxa and intracellular indicators to guarantee the accuracy of risk assessments and subsequent oil spill responses in Freshwater Habitats, particularly in ecosystems characterized by multiple stressors and complicated mixes (Chahouri *et al.*, 2023). This

research asserts that to offer optimal guidance for those making decisions, biological evaluation approaches must be thoroughly described and incorporate intracellular indications of toxicity and gene toxicity across various species and levels of toxicity. Various biological and industrial tests have been rigorously evaluated and validated for freshwater and wastewater monitoring (Feio *et al.*, 2021). Research has comprehensively summarized numerous rigorously confirmed assessments through reviews and proficiency tests. Experiments are not explicitly confirmed with crude oil, dispersing agents, or the precise compounds included (Behboodi and Movahedinia, 2016).

This review examines the substantial advancements achieved in fulfilling these requirements and assesses the present scientific understanding of the quick evaluation of biological impacts after an oil spill. Initially, the research emphasizes the difficulties encountered in addressing oil as a pollutant, then examines the pertinent biological effects of oil and reviews universally accessible commercial products suitable for the swift evaluation of oil spill damage in Freshwater Habitats (Giwa *et al.*, 2023). The research discusses methodologies and the implementation of passive dosage in laboratory exposure contexts, demonstrating how these techniques can be augmented by models to forecast ecological harm, enhancing the relevance of oil poisoning research (Parkerton *et al.*, 2023). The study addresses the necessary future efforts to create a cutting-edge toolkit that facilitates swift risk assessment post-spill, an essential

resource presently lacking for those making the decisions.

## **Toxicological Impacts of Various Oil Constituents**

### *Components of Oil*

One challenge in evaluating the toxic effects of oil is that petroleum is not a singular substance; it comprises a distinctive amalgamation of hundreds of diverse components with varying weights and structures, some of which are exemplified by the representative oil itself. All petroleum products include a combination of saturated aromatic hydrocarbons and non-hydrocarbon molecules comprising Sulfur (S), Nitrogen (N), and Oxygen (O), along with a percentage of metallic traces. Crude oils differ in structure and composition according to the ratio of the chemicals above.

### *Weathering*

A critical issue is that the characteristics of oil alter considerably after a release, as it transforms into a slick, disperses, and undergoes weathering due to varying climatic circumstances. Weathering refers to the process through which sunlight, volatile substances are either dissipated into the atmosphere or scattered through the water columns. The diminishment of lighter chemicals results in a higher proportion of heavier compounds in the slick, elevating viscosity and facilitating the development of an oil-water mixture that thickens with intensified wave activity in Freshwater Habitats.

The Deepwater Horizon incident exemplified the physical alterations, when the quantities and mixes of

petrochemicals exhibited considerable variation throughout levels and were predicted to have endured for as long as thirty days. The amount of polycyclic aromatic hydrocarbons, or PAHs, in the deep ocean (~1100-1300 m depth) markedly differed from that of surface waters, which exhibited a higher percentage of the most toxic small-molecule. These elevated quantities of C1-C3 have remained in the water columns for a duration exceeding that of largely insoluble and alkaline substances carried to the ocean's surface or seabed. The study assessed 68 surface-water sites in the Gulf of Mexico from December 2018, revealing elevated average levels of total hydrocarbons from petroleum (250 mg L<sup>-1</sup>) and low mean total (0.045 mg L<sup>-1</sup>). Their findings indicated that levels varied among groups in Freshwater Habitats. C1 phenanthrenes and anthracenes demonstrated more remarkable persistence than the majority, with an average value of 1.2 mg/L among sites, whereas the levels of the other categories were quantifiable but considerably lower.

#### *Complicated Compositions*

The volatile chemical properties of weathered oil spills can create intricate, ambiguous combinations, the harmful effect of which is not well understood. Chemical mixtures can induce toxicity via the distinct actions of individual compounds or through cumulative or combined impacts, when the harmful effect of the entire mix exceeds the cumulative toxicity of the individual components. Combinations are regarded as possessing an additive hazard. In spillage toxicity studies, evaluating the toxicity of oil and dispersants separately

and in conjunction is essential to substantiate this idea and discover any chemical-specific variations.

Oil and its resultant emulsions can exert physiological impacts. Documented consequences of physical oiling/smothering encompass reduced mobility, inflammatory processes, elevated metabolic rate, and harm to digestive organs in avian species. Additionally, after oil exposure, teratogenic implications and impacts on early developmental stages have been observed in birds, urchins, oyster pupae, and aquatic worms. Oil pollution diminishes fertilization success and developmental costs in marine bugs, correlating with a rise in developmental instabilities.

#### *Mitigation Strategies*

Spill management requires specific solutions for each scenario, considering the risks associated with the spill and the employed mitigation strategies in Freshwater Habitats. Mitigation strategies must be evaluated to guarantee that the reaction does not initially inflict more significant harm than the spill. Frequently utilized mitigation strategies encompass the direct extraction of oil through surface sweeping; the deployment of sorbent materials, such as particles and jellies; combustion; and the application of solvent dispersants for dispersion.

Dispersing agents expedite oil breakdown, frequently mitigating the volume of oil that impacts biodiverse regions such as coastlines or coral reefs. Specific research examining the *in vitro* cytotoxicity of chemically-dispersed petroleum has indicated the possibility of adverse effects on human wellness. The

investigations demonstrated modifications in gene transcription related to cancer and immune system function among human lung epithelium cells that received biologically improved water accepted fractions, compared to Macondo crude oil fluid accepted fractions. Specific research indicates that dispersants have individual harmful effects. In contrast, others demonstrate that the susceptibility of organisms is heightened when subjected to particular oil dispersant mixes compared to exposure to oil alone. For instance, minimal quantities of dispersants can affect the well-being and conduct of organisms; a study examined the effects of dispersing agents on the coral *Acropora millepora* over three contact durations. The findings indicated that coral larval settling was suppressed between 2 and 8 hours, with values varying from 2.6 mg L<sup>-1</sup> to 12.1 mg L<sup>-1</sup>, implying that larval corals are more susceptible to dispersing agents than other developmental stages of corals. These data indicate that the dispersants exhibit reduced toxicity to corals subjected to light petroleum products.

Contemporary dispersing goods undergo an approval process, utilize less hazardous solvents, and in certain instances are up to 120 times less toxic than earlier versions. Debate persists concerning the appropriateness of dispersion agents for ecological use due to difficulties in quantifying dispersant-oil interactions and the toxicity of dispersant-oil-environment interactions. To facilitate the optimal selection of reaction, acquiring full details regarding the characteristics of the oil that was spilled and the vulnerability of the

affected environment is essential. The spill's impact must be assessed during the incident, the cleanup process, and the following years to enhance future risk evaluation and make choices.

## Current Issues and Recent Advances

### *Standardized Dosing Methods*

Scientific toxicity assessments are crucial for informing decisions on oil spills. The intricate physicochemical characteristics of oil, such as its insolubility and volatility, and the alterations that ensue after dispersant application, complicate the attainment and maintenance of target levels within experimental systems. Varying study designs further restrict the comparison, reliability, and relevance of several studies to modeling that depends on the precision of specified doses. The Chemical Reaction to Oil Spills Environmental Research Foundation (CROSERF) has established standardized protocols for preparing Water Acclimated Fractions (WAFs) and chemically improved water acclimated fractions to reduce variability in testing. Moreover, meticulous studies employing sealed structures have significantly reduced the loss of volatile and semi-volatile chemicals. These procedures are inapplicable to prolonged exposures, frequently requiring supplementary culture media to sustain test organisms.

Passive dosage provides an alternative administration technique to mitigate and avert chemical loss in tiny, big, static, and flow-through testing systems. Hydrophobic Organically Carbons (HOCs) are extracted from a concentrated methanol solution into a biodegradable polymer, typically silicone. Upon introducing the polymer to the test media,

the HOCs migrate from the polymer into the test media, establishing an equilibrium. Ongoing partitioning mitigates compound losses and sustains HOC exposure levels.

Passive dosage has been effectively employed in acute toxicological assessments within closed containers, typically approximately 25 mL, involving algal cells, water insects such as fleas, springtail arthropods, and microorganisms. The approach has been employed to dose test apparatus of varying sizes with flow-through chambers ranging from 135 to 700 mL and dosage chambers between 1 and 3 L, facilitating the investigation of chronic doses.

Research on chronic exposure encompasses zebrafish, squid, and reefs. Phenanthrene, anthracene, fluoranthene, and phenol have been administered into transparent plastic 12-well plates for cell culture *in vitro* tests. Implementing a porous test system proved less effective than hermetically closed containers, resulting in an undesirable loss (>25%) of highly volatile substances, including the chemical. Using polystyrene containers has modified the makeup of hydrocarbons due to reactions between oil and polymers. Therefore, exploring more easily closed and further inert substances should allow less reactive chemicals to persist.

Two primary distinctions exist between actively dosed devices: actively dosed systems restrict access to oil droplets, and larger molecules of compounds partition slowly, requiring more time to achieve equilibrium. It is feasible to attain test levels more rapidly than passive dosing, owing to the

expedited oil dissolving. Levels in passively administered systems exhibit better stability as they inhibit the production of oil particles. The makeup, quantity, and durability of droppings of oil are unique to the particular oil and dose method employed, and their existence can obscure toxicity studies by affecting animals and confounding exposure via droplet dissolving. While one would contend that mixtures with small particles resemble a spill situation more closely, enhanced stability in test doses is expected to augment the repeatability and uniformity of oil toxicity assessments. Simultaneously employing both procedures could facilitate comparing the cytotoxicity of solubilized oil against oil in various dissolved stages.

Adapting existing toxicity assays and biomarker assays for passively dosing presents difficulties, as most advancements have occurred in closed testing platforms above 25 mL, but most toxicity assays utilize open well plate methods with less volume (more than 2 mL). Future research should further validate the application of passive dosing with oil and miniaturize passive dosing techniques. Due to the variety of exposures, toxicity testing must be underpinned by chemical analysis to verify exposure concentrations and stability during the test.

Research underpinned by analytical chemistry indicates that preserving exposure levels facilitates the calculation and comparability of research across different species and chemicals, utilizing the Targeted Lipid Modeling (TLM). The TLM is a model that elucidates the correlation among species susceptibility

as a quantifiable structure-activity connection, predicated on the premise that narcosis transpires inside the lipids of animals. Prior research has indicated that vital body loads, defined as the degree of toxicity to species, rise linearly, increasing those creatures' fat levels. The TLM employs this connection to project and evaluate species sensitivity among different oils. The results serve as inputs for additional, more intricate risk-based and ecological models.

#### *Correlating Toxicity Assessments with Ecosystem Impacts*

Ecological risk evaluation seeks to evaluate and forecast the probability of chemicals causing detrimental environmental impacts. In the context of oil spill risk evaluation in Freshwater Habitats, this entails assessing and predicting the ecological damage an oil leak and its repair are expected to cause. The previous discussion and earlier evaluations indicate that the sensitivity of an individual species cannot adequately represent a whole environment.

A judicious choice of bio-indicator organisms and biomarkers could yield a more accurate assessment of ecosystem impacts. Data must be extended to assess risk and forecast effects at the community and citizen levels. Extrapolating from single to group reactions is difficult due to the nonlinear nature of linkages and population movement.

Several models were created to forecast the outcomes of oil spills, although few have examined the ecological consequences of such incidents. The Spill Impacts Modeling Application Packaging (SIMAP) evaluates exposure by looking at oil and living thing immigration, the impact of

spill mitigation efforts, short-term immediate toxicity, indirect consequences of resource devastation, such as food sources and ecosystems, and people impacts resulting from mortality and sublethal consequences. The model is constrained as it cannot quantify sublethal, ongoing consequences or alterations in ecosystem layout and functioning resulting from heightened developmental, survival, and reproduction stresses. The advancement of environmental systems for oil spill risk assessments has been examined in other sources. Studies must focus on compiling both whole living things and sublethal answers to oil and hydrocarbons pollution, facilitating the enhancement of these tools and, where feasible, their integration into a biological evaluation toolkit for comprehensive risk evaluation.

#### **Conclusion**

The preceding sections indicate that oil's intricate and fluctuating characteristics present a distinct challenge to risk evaluation. The examination of the scientific landscape concerning the distinctive intricacies of oil, the hurdles after a spill, the biological ramifications of oil spills, and the application of rapid evaluation methods, such as commercialized toxkits and tests, has underscored existing obstacles hindering efficient and prompt risk evaluation of oils.

Employing a variety of bio-indicator organisms and biomarkers with diverse sensitivities and devices facilitates the assessment and characterization of toxicity, the geographical scope of the spill, and the calculation of the necessary time for recovery and/or the efficacy of

the cleanup efforts. Methods must be designed to accommodate the oil's hydrophilic and volatile characteristics in Freshwater Habitats. Passive dosing is advantageous for overcoming some complexities, facilitating consistent and precise validation of prospective bioassays. Utilizing the TLM in conjunction with the inverse correlation to address the differential toxicity enables the reliable contrast of individual substances across organisms and trophic categories and the extrapolation of information to forecast the potential effects of oil. Comprehensive standardized data validates oil spill models, facilitating more accurate assessments of oil poisoning across all trophic levels.

Microbes at a single level of trophic differentiation react differentially to toxicants; those at various trophic levels can display diverse reactions, and variations in organismal physiology contribute to a complex issue. Collections of biological tests and indicators, utilized across several test organisms, can reflect the spectrum of responses observed within environments. Bio assays ought to be straightforward, conducted at ecologically pertinent doses, standardized dosing techniques, and accompanied by chemical characterization of both the specimens and the oil. Assays relevant to passive dosage in closed testing facilities are especially significant for further advancement in Freshwater Habitats. Outstanding research needs encompass more ocean specimens and the further advancement of sublethal effects-based testing, including rapid swimming and heart disease evaluations.

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