

Original paper

Effects of Drilling and Cementing Fluids on Indicator Species in Marine Coastal Benthic Systems

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Abstract

The paper studies toxicity of drilling and cementing fluids, used in offshore oil and gas operations, at concentrations of 10, 50, 100, 500 and 1000 mg/L for the mass species of benthic marine communities in the coastal part of the Black Sea: the eelgrass *Nanozostera noltii* and the amphipod *Chaetogammarus olivii*. The paper analyses effect of these toxic mixtures on the increase in biomass, leaves and roots of the eelgrass and on the survival of amphipods after 10, 20 and 30 days of exposure. Drilling fluid was shown to be more toxic than cementing fluid for the test organisms. Exposure to 10 mg/L of drilling fluid reduced the plant biomass growth by 49% after 10 days and by 62 and 78% after 20 and 30 days, respectively. With increase in the drilling fluid concentration to 50–100 mg/L, this indicator continued to decline rapidly to 60–80% relative to the control, and at a concentration of 500–1000 mg/L, the plants died. The roots of eelgrass were more sensitive to the toxicant than the leaves: the root growth showed a marked tendency to decrease by 48% relative to the control at toxicant concentrations of 50–100 mg/L after only 10 days. The harmful effect of the cementing fluid on the eelgrass was less pronounced than that of the drilling fluid. Exposed to the cementing fluid, the plants died at the highest concentration of the toxicant (1000 mg/L) after 30 days. No significant differences were found between the leaf growth in the test and control variants, but the root growth decreased significantly by 64 and 90% at 10 and 20 days at cementing fluid concentrations of 500 and 1000 mg/L, respectively. Throughout the experiment, the survival rate of the crustaceans exposed to over 10 mg/L drilling fluid was significantly lower than the control (30–85%). During exposure to the cementing fluid, however, significant differences were observed only at the highest concentration of 1000 mg/L. An ecotoxicological assessment of substances used in oil well drilling is necessary to determine their hazard when used in oil and gas production, as well as to select optimal components in their composition that contribute to reducing environmental damage to benthic marine communities.

Keywords: Black Sea, oil and gas complex, pollution, amphipods, *Zostera*, bioassay

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Влияние бурового раствора и тампонажной жидкости на виды-индикаторы морских прибрежных бентосных экосистем

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Аннотация

Исследовали токсичность используемых в морских нефтегазовых операциях бурового раствора и тампонажной жидкости в концентрации 10, 50, 100, 500 и 1000 мг/л для массовых видов бентосных морских сообществ прибрежной части Черного моря – зостеры *Nanozostera noltii* и амфипод *Chaetogammarus olivii*. Анализировали влияние этих токсичных смесей на прирост биомассы, листьев и корней зостеры и на выживаемость амфипод через 10, 20 и 30 сут. Показана бо́льшая токсичность бурового раствора по сравнению с тампонажной жидкостью для исследуемых организмов. Под воздействием бурового раствора в концентрации 10 мг/л прирост биомассы растения снизился на 49 % через 10 сут после начала эксперимента, а через 20 и 30 сут – на 62 и 78 % соответственно. При повышении концентрации бурового раствора до 50 и 100 мг/л этот показатель продолжал интенсивно снижаться до 60 и 80 % соответственно по отношению к контролю, а при концентрации 500 и 1000 мг/л растения погибли. Корни зостеры оказались более чувствительны к действию токсиканта, чем листья: прирост корней имел выраженную тенденцию к снижению на 48 % по отношению к контролю при концентрациях токсиканта 50 и 100 мг/л уже через 10 сут. Вредное влияние тампонажной жидкости на зостеру было выражено в меньшей степени, чем влияние бурового раствора. Под воздействием тампонажной жидкости в самой высокой концентрации (1000 мг/л) растения погибли через 30 сут инкубации. Достоверных различий между приростом листьев в опытных и контрольных вариантах не установлено, но прирост корней уже на 10 и 20 сутки достоверно уменьшился на 64 и 90 % при концентрациях тампонажной жидкости 500 и 1000 мг/л соответственно. На протяжении всего эксперимента выживаемость ракообразных, экспонированных в растворах с концентрацией бурового раствора более 10 мг/л, была достоверно ниже контроля на 30–85 %, тогда как при инкубации в растворах с тампонажной жидкостью достоверные различия отмечены только при самой высокой концентрации 1000 мг/л. Экотоксикологическая оценка токсичности веществ, применяемых при бурении нефтяных скважин, необходима для определения их опасности при нефте- и газодобыче, а также для выбора оптимальных компонентов в их составе, способствующих снижению экологического вреда для донных морских сообществ.

Ключевые слова: Черное море, нефтегазовый комплекс, амфиподы, зостера, биотестирование

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Introduction

The operation of offshore oil and gas facilities inevitably releases byproducts into the environment, and the rapid expansion of these activities significantly amplifies this impact. Coastal areas, characterized by high biological productivity, are extensively utilized for fishing, aquaculture, recreation, sports, shipping, and mineral extraction. These regions experience maximal and multifaceted anthropogenic stress, which adversely affects natural ecosystems, leading to their transformation and degradation, often resulting in the complete loss of resources or the impossibility of their use due to pollution and the mortality of aquatic organisms. To analyze such harmful processes and identify ways to prevent them, ecotoxicological methods are used. These methods, applied in both natural and laboratory settings, enable the assessment of the consequences of anthropogenic compounds entering the marine environment, evaluating their effects on natural systems, determining pollutant behavior, establishing permissible levels, and assessing their impact on marine biota.

Offshore oil and gas development releases not only oil and its derivatives but also drilling fluids (DFs) and other components used in well construction into the marine environment. DFs are complex mixtures comprising water, suspensions, emulsifiers, aerated liquids, organic solvents, heavy metals, and clay, used to flush wells during drilling [1, 2]. After use, DFs are classified as industrial waste and require proper disposal due to their toxicity, mutagenic and carcinogenic properties. In addition, cementing fluids (CFs) are used during drilling to cement wells. They also have a complex composition and contain hazardous and toxic substances [3, 4]. The volume of these components entering the marine environment is expected to increase, as the number of wells drilled rose from 39,000 in 2020 to 49,600 in 2022, with projections estimating up to 60,000 wells by 2026 [5].

Oil spills primarily affect surface waters and their inhabitants, whereas DF emissions impact benthic communities. Information on the pollution of the marine environment and aquatic organisms by DFs and other substances used in drilling operations is limited and inconsistent [6, 7]. The effects may vary significantly among representatives of different taxonomic groups [8, 9].

Macrophytes and higher aquatic vegetation, mainly represented by eelgrass in coastal benthic biocenoses, are highly vulnerable to drilling operations [10]. These

plants form a unique group, widely distributed in seas and oceans. Their communities play a critical role in the structure and functioning of coastal ecosystems, serving as food and habitat for numerous marine organisms, including commercially important species. Eelgrass exhibits high productivity [11, 12] and, alongside other marine macrophytes, contributes to the utilization of biogenic elements, such as carbon, which is essential for biosphere exchange and biogeochemical cycles. Seaweed and seagrass ecosystems support grazing, detrital, and food webs, stabilize sedimentary deposits, and play an important role in global carbon and nutrient cycles. These ecosystems host diverse flora and fauna, forming complex food chains [12]. The annual ecological value of one acre of seabed covered with algae and seagrasses is estimated to range from \$9,000 to \$28,000. Aquatic vegetation biocenoses perform many ecosystem functions, including storm protection, provision of food for commercial fish and invertebrates, and nutrient and carbon cycling, which are crucial for understanding the current state of nutrient cycles in the biosphere [13, 14]. However, eelgrass communities face intense anthropogenic impacts, particularly from the oil and gas industry, due to pollution from oil, dispersants, DFs, and heavy metals [15–18]. These plants absorb and accumulate components of these substances, making eelgrass an effective phytoremediator and indicator of polluted waters. Eelgrass is widely used in ecotoxicological studies to assess the accumulation and toxicity of various pollutants, necessitating comprehensive data on its responses to develop criteria for maximum permissible doses [16].

Intense anthropogenic activity has an extremely negative effect on eelgrass [19]. The plant's capacity to actively accumulate pollutants enables its use as a bioindicator for assessing the ecological state of coastal marine zones and in developing test systems for analyzing the toxicity of harmful substances. However, the concentrations of toxicants must be considered, as they can produce variable effects, as demonstrated in the case of oil pollution [20].

Amphipods are widely distributed in coastal zones and dominate benthic communities, including eelgrass beds. They are used in ecotoxicological studies due to their adaptability to laboratory conditions and sensitivity pollutants. As they lack larval stages, both juvenile and sexually mature adult crustaceans serve as test organisms [21, 22].

The study aims to investigate the toxicity of water-based DFs and CFs used in well cementing on dominant benthic species in the coastal Black Sea, specifically the seagrass *Nanozostera noltii* (Hornemann) and amphipods inhabiting its beds, *Chaetogammarus olivii* (H. Milne Edwards, 1830). The research evaluates the effects of stress induced by DFs and CFs from oil and gas production on the survival, growth, and development of these aquatic organisms, comparing the toxicity of

these substances based on the responses of test organisms from the seagrass and crustacean communities.

Material and methodology

DF is a viscous, light brown liquid with an ammonia-like odor, a pH of 10–11, a boiling point above 100°C, and a relative density at 20°C of 1.1–2.0. It is miscible with water and contains sodium chloride, silica and other additives. The substance is stable under normal conditions. CF, used for well cementing, primary consists of a dry cement mixture, defoamers, propylene glycol, calcium compounds, and other additives.

Eelgrass and amphipods were collected from the coastal area of Kazachya Bay (Sevastopol region) and immediately transported to the laboratory. The plants were washed to remove dirt and epiphytic flora and acclimated to experimental conditions in seawater for 3 days at $(22 \pm 2)^{\circ}\text{C}$. Toxicological experiments were conducted in accordance with established recommendations ¹⁾.

One-year-old vegetative eelgrass shoots, with an average weight of 328.5 ± 56 mg, were placed in 1.5 L glass containers (1 L working volume), with three plants per container, maintained at $(22 \pm 2)^{\circ}\text{C}$ under constant artificial lighting of 1500 lux. Sexually mature adult amphipods were placed in 500 mL aquariums, with 10 individuals per aquarium, and acclimated to experimental conditions for one week under natural light (12-hour daylight duration) at $(22 \pm 2)^{\circ}\text{C}$. Experiments were carried out in triplicate over 30 days.

Solutions of the test substances at concentrations of 10, 50, 100, 500 and 1000 mg/L were added directly to the water. Natural seawater with a salinity of 18‰, without added substances, served as the control. The water was replaced every 5–7 days. Plant biomass, including leaves and roots, was measured every 10 days. The effect of toxicants on crustaceans was assessed by mortality every 10 days over 30 days. During the experiment, amphipods were fed crushed brown algae and dried daphnia.

The results were statistically processed, and the mean value M and standard error of the mean m were calculated. Comparisons were performed using Student's t -test at a significance level of $p < 0.05$. The correlation between toxicant concentrations and amphipod survival rates was assessed using the CURVEFIT software (version 2.10–L).

¹⁾ Federal Agency for Fishery, 2009. *On the Approval of Methodological Guidelines for the Development of Water Quality Standards for Water Bodies of Fishery Significance, Including Standards for Maximum Permissible Concentrations of Harmful Substances in Waters of Water Bodies of Fishery Significance*: Order of the Federal Agency for Fishery of the Russian Federation dated August 04, 2009, No. 695. Moscow: Federal Agency for Fishery.

Results

The results revealed patterns and characteristics of the effects of toxicants on the indicator species of benthic systems. Variable effects of DFs and CFs on eelgrass growth and development were observed at different concentrations (Figs. 1–3). As shown in Fig. 1, 10 days after the experiment began, a significant ($p < 0.05$) reduction in plant biomass growth was observed at DF concentrations of 50 mg/L and higher compared to the control. A similar effect was noted after 20 days at a lower DF concentration of 10 mg/L, while at concentrations of 500 and 1000 mg/L, the plants died. After 30 days, the trend of reduced plant biomass growth relative to the control persisted, but the differences were not statistically significant. No correlation was found between DF concentrations and eelgrass biomass growth.

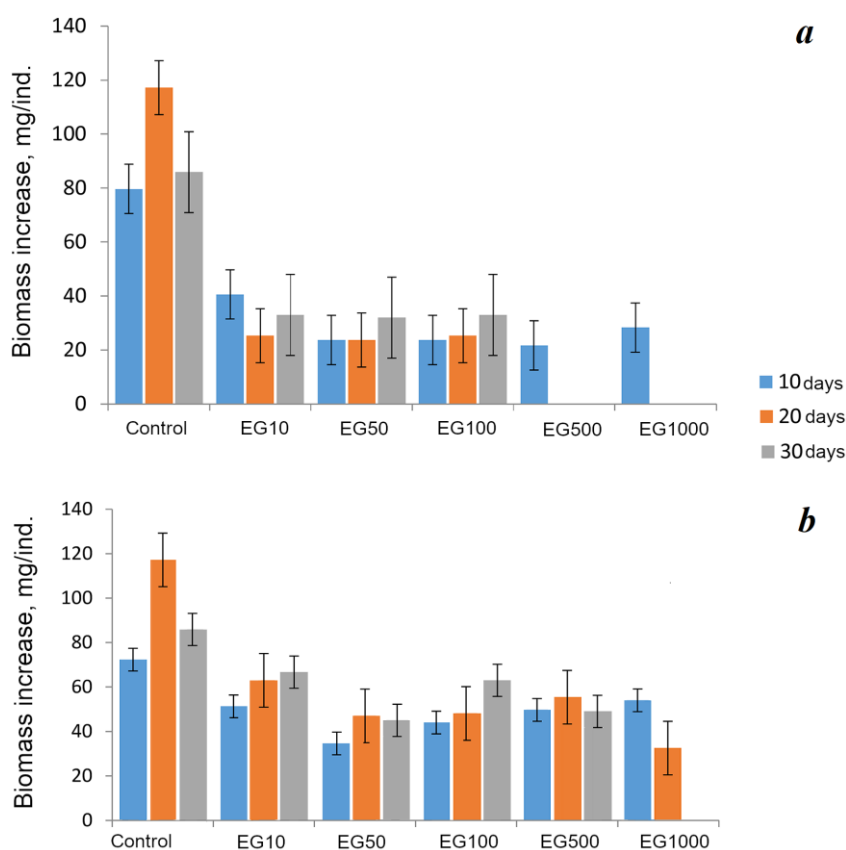


Fig. 1. Increase in seagrass *N. noltii* biomass (mg/specimen, Mean $\pm$ SEM) exposed to drilling fluid (a) and cementing fluid (b) in concentrations of 10 (EG10), 50 (EG50), 100 (EG100), 500 (EG500) and 1000 (EG1000) mg/L

When incubating eelgrass in solutions of toxicants at the studied concentrations for 10 days, no differences were observed compared to the control, although a trend toward reduced biomass growth was noted. After 20 days, a significant ($p < 0.05$) reduction in plant growth was observed at all tested concentrations. After 30 days, plants exposed to 1000 mg/L died, while no significant differences were found in the remaining experimental groups compared to the control. No correlation was found between toxicant concentrations and eelgrass biomass growth.

Changes in eelgrass leaf growth of under the influence of toxicants are shown in Fig. 2. DFs at the studied concentrations did not significantly affect leaf growth throughout the experiment, although a trend toward reduced growth was observed at high concentrations (500 and 1000 mg/L). When incubating eelgrass in a medium

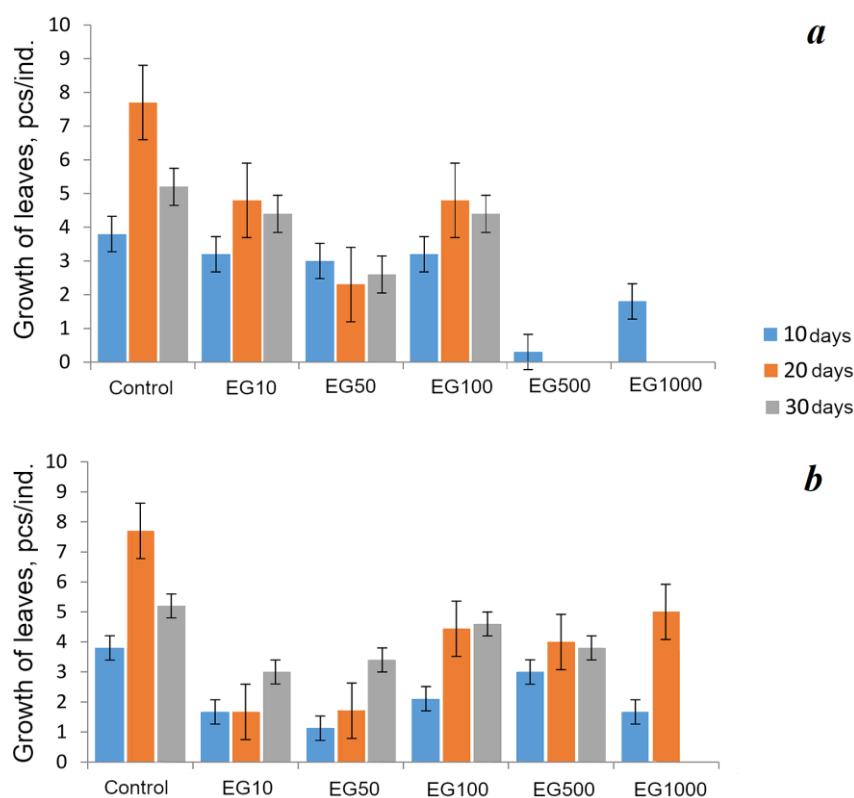


Fig. 2. Growth of seagrass *N. noltii* leaves (pcs./specimen, Mean $\pm$ SEM) exposed to drilling fluid (a) and cementing fluid (b) in concentrations of 10–1000 mg/L. Notation: See Fig. 1

with CFs, no significant differences from the control were observed after 10 days. After 20 days, a significant ($p < 0.05$) reduction in leaf growth was detected at low concentrations (10 and 50 mg/L), but not at higher concentrations. After 30 days, no differences were found between the control and experimental groups at any concentration, except at 1000 mg/L, where the plants died. No correlation was found between leaf growth and the concentrations of either toxicant.

Fig. 3 shows data on the effects of the tested toxicants on eelgrass root growth. A significant ($p < 0.01$) reduction in root growth was observed for plants incubated in DFs at a concentration of 500–1000 mg/L after 10 days. After 20–30 days,

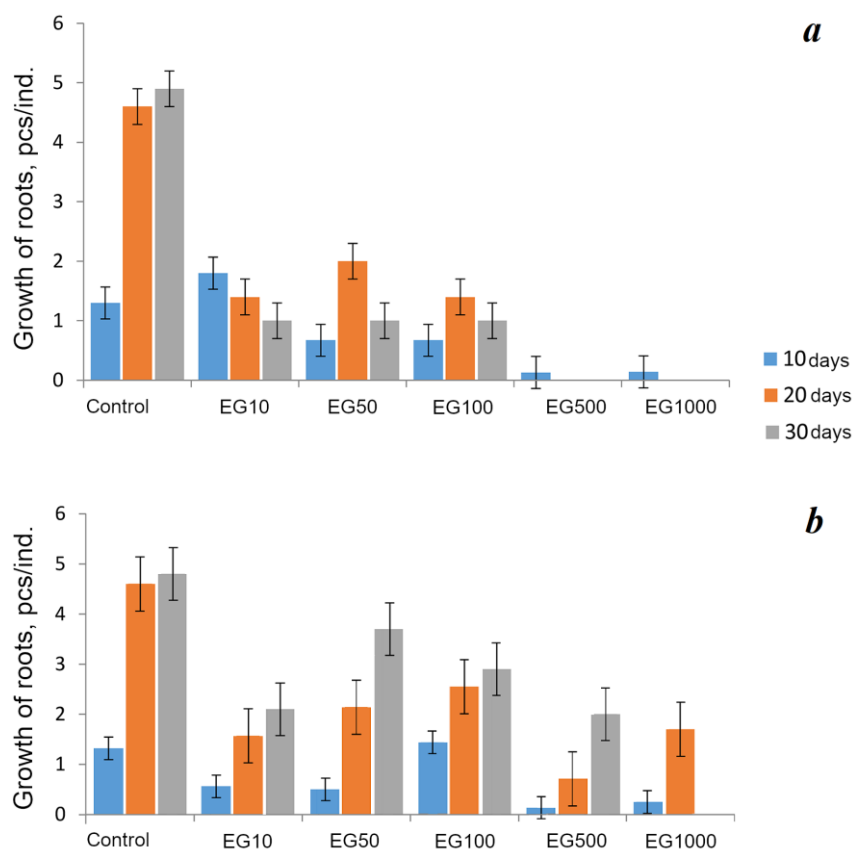


Fig. 3. Growth of seagrass *N. noltii* roots (pcs./specimen, Mean $\pm$ SEM) exposed to drilling fluid (a) and cementing fluid (b) in concentrations of 10–1000 mg/L. Notation: See Fig. 1

the same effect was observed at lower DF concentrations of 10 and 50 mg/L. After 10 days, a moderate correlation was identified between root growth and toxicant concentration ($Y = 610 - 420.9X$, $r = 0.490$, $R^2 = 0.37$).

When incubating eelgrass in media with varying toxicant concentrations, a significant ($p < 0.01$) reduction in root growth was observed after 10 days at concentrations of 500 and 1000 mg/L. This effect persisted after 20 and 30 days, but at 1000 mg/L after 30 days, the plant died. A moderate correlation was identified between root growth and toxicant concentration after 10 days ($Y = 0.94 - X$, $r = 0.39$, $R^2 = 0.23$).

Fig. 4 presents data on the survival of amphipods exposed to DFs and CFs. A significant ($p < 0.05$) reduction in amphipod survival was observed after 10 days at a DF concentration of 50 mg/L. After 20 days of exposure to media with DFs, a significant ($p < 0.05$) reduction in amphipod survival was detected at concentrations of 100 mg/L and higher. A strong correlation was identified between amphipod survival and DF concentration ($Y = 54.4 - 0.05X$, $r = 0.87$, $R^2 = 0.89$).

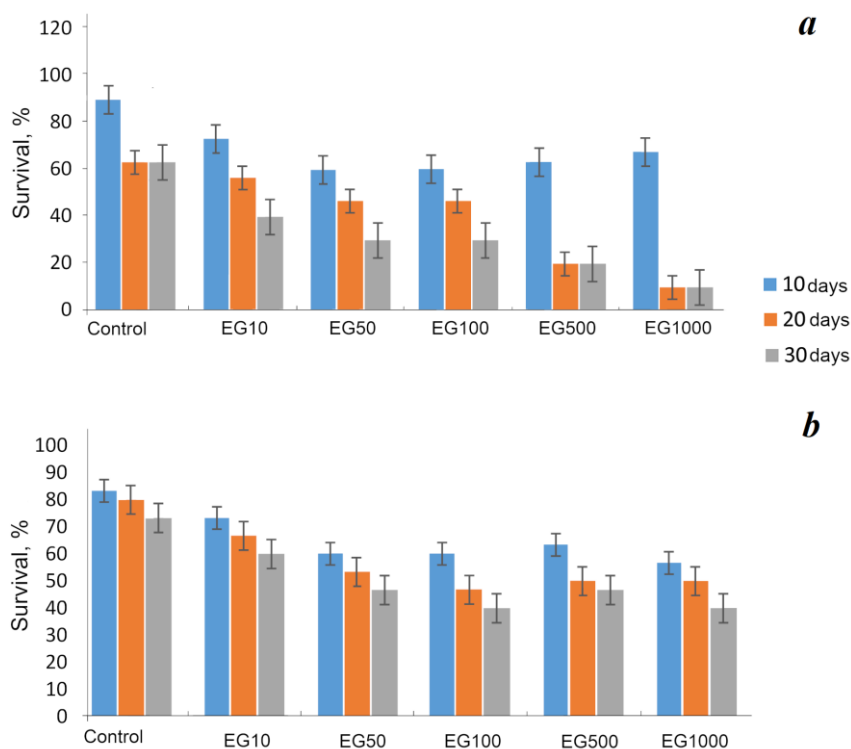


Fig. 4. Survival of amphipods (% Mean $\pm$ SEM) exposed to drilling fluid (a) and cementing fluid (b) in concentrations of 10–1000 mg/L. Notation: See Fig. 1

After 30 days, in the experimental groups with DFs, the trend persisted, but the correlation was weaker ($Y = 41.9 - 0.04X$, $r = 0.48$, $R^2 = 0.59$). When amphipods were maintained in solutions with varying concentrations of toxic substances, a significant reduction in survival was observed at a concentration of 1000 mg/L across all study periods, but no correlation was found between survival and toxicant concentration.

Discussion of research results

The results demonstrated the toxic effects of the tested substances on representative species of the benthic community, including eelgrass and amphipods. The observed effects varied with toxicant concentration and exposure duration, enabling assumptions about the mechanisms of toxicity and potential consequences for the studied benthic organisms.

Our studies demonstrate that eelgrass biomass growth decreased by 49% after 10 days of incubation in media containing DFs at a concentration of 10 mg/L. With continued exposure, the reduction in biomass growth at this DF concentration was 62–78%. At higher concentrations (50–100 mg/L), biomass growth continued to decline significantly (by 60–80% compared to the control), and at concentrations of 500–1000 mg/L, the plants died. Other researchers have also reported reduced productivity (in terms of carbon absorption and growth rate) in seagrass of the genus *Thalassia* after 10 days of exposure to DF concentrations of 200 and 1000 µL/L [17]. However, our studies found no differences in leaf growth between eelgrass exposed to DFs and the control, whereas root growth showed a marked reduction (by 48%) compared to the control at toxicant concentrations of 50–100 mg/L after just 10 days. In subsequent periods, this trend was observed across all tested concentrations. Notably, eelgrass root growth is influenced by many factors, including soil substrate, oxygen availability, biogenic elements, water mixing, and the presence of toxicants, as confirmed by our findings. Thus, different plant parts responded differently to the effects of DFs, with roots being more sensitive to adverse effects than leaves, consistent with findings from other researchers [23].

The toxic effect of CFs on eelgrass was less pronounced than that of DFs. At the highest CF concentration (1000 mg/L), plants died after 30 days of incubation. After 10 days, no differences in biomass or leaf growth were observed between the control and experimental samples across all tested concentrations, but root growth was significantly reduced at CF concentrations of 500–1000 mg/L. In subsequent experimental periods, leaf growth remained comparable to the control, whereas biomass growth was significantly reduced after 20 days at CF concentration of 50 mg/L and higher. Root growth inhibition persisted at these concentrations from 20 to 30 days, but biomass growth after 30 days showed no significant difference from the control. Thus, it is possible to note different effects of the toxicant

on different parts of the plant, which was established when studying the effect of DFs on eelgrass. Notably, roots were more sensitive to the effects of CFs than leaves, as similarly observed with DFs.

It has been demonstrated that various toxins, including nutrients and sulfides at high concentrations, can severely impair the growth and survival of the eelgrass population [19]. Different plant parts can react differently to toxicants, including biogenic elements, associated with a higher affinity of leaves to ammonium compared to roots. A negative effect on *Zostera noltii* biomass growth was observed at sulfide concentration below 200 $\mu\text{mol/L}$. Under natural conditions, eelgrass habitat expansion did not occur at sulfide concentrations exceeding 1000 $\mu\text{mol/L}$, which is associated with reduced root viability upon contact with sulfides. However, researchers have noted that the productivity of *T. testudinum* in laboratory and field conditions unaffected by DFs after 6- and 12-week exposure periods [24], confirming the need to investigate different effects of DFs on benthic flora species.

The impact of DFs on eelgrass can be both direct and indirect. Indirect effects of drilling and DFs arise from habitat degradation. During well drilling, numerous suspended particles are generated, significantly reducing water transparency and impeding sunlight penetration. This inhibits photosynthetic processes in plants and suppresses their growth, including that of epiphytic microflora [25]. When suspended particles settle, they form a layer that restricts nutrient supply to eelgrass roots, an effect particularly pronounced with CFs, as demonstrated in our studies. Consequently, nutrient exchange between the environment and the plant is disrupted. Additionally, the introduction of xenobiotics into water alters its physicochemical properties, further negatively affecting the survival and growth of aquatic organisms.

The direct impact of DFs has a toxic effect due to the presence of heavy metals and organic compounds, which accumulate in plants and impair their physiological functions. This results in the inhibition of overall plant growth and that of specific parts (leaves and roots), as demonstrated in our study, as well as the suppression of dispersal and reproduction. Furthermore, eelgrass beds may become unsuitable habitats for other marine organisms, such as amphipods, fish, and mollusks, that rely on them.

For example, under natural and experimental conditions, researchers have observed changes in the abundance of benthic invertebrates. Macrofauna exposed to DFs or clay used in well cementing exhibited significantly reduced abundance compared to the control group [26]. Our studies demonstrated that CFs were less toxic to amphipods than DFs. Throughout the experiment, the survival rate of amphipods exposed to DF solutions at concentrations above 10 mg/L was significantly lower (by 30–85%) than the control, whereas during incubation in CF solutions, significant differences were observed only at the highest concentration of 1000 mg/L,

when this indicator was 46% lower compared to the control group. Thus, the toxic effects on amphipods were specific to CFs and DFs.

Researchers have observed that, under natural conditions, some seagrasses were less sensitive to oil, dispersed oils and DFs than intertidal communities, including corals, sponges, echinoderms, mangroves, invertebrates and mollusks [27]. Notably, the number of benthic invertebrates was significantly reduced by DFs in laboratory settings, but these effects were not observed in natural environments. In field conditions, invertebrate density was comparable between control and DF-treated areas but significantly lower than in laboratory controls, while species diversity remained consistent between field and laboratory conditions [23].

The global trend of increasing oil and gas production in coastal marine areas poses a significant threat to benthic ecosystems and their inhabitants. Changes in the physicochemical properties of water, increased turbidity, and reduced light availability for aquatic organisms can trigger cascading effects across the marine ecosystem. Given the critical role of seagrasses and their associated invertebrates in coastal ecosystems, this study highlights the vulnerability of benthic communities to the introduction of DFs and their components, particularly under changing environmental conditions [28, 29].

Conclusion

Long-term exposure to DFs and their components disrupts eelgrass metabolic processes, causes leaf mortality, and induces tissue degradation, threatening the overall health and viability of seagrass. Our studies demonstrated that, at a DF concentration of 10 mg/L, plant biomass growth decreased by 49% after 10 days. After 20–30 days, the reduction in biomass growth at this concentration was 62–78%. At higher concentrations (50–100 mg/L), biomass growth declined significantly (by 60–80% relative to the control), and at 500–1000 mg/L, the plants died. Eelgrass roots were more sensitive to DFs than leaves: no differences in leaf growth were observed between DF-exposed eelgrass and the control, whereas root growth was significantly reduced by 48% relative to the control at toxicant concentrations of 50–100 mg/L after just 10 days. The toxic effect of CFs on eelgrass was less pronounced than that of DFs. At the highest toxicant concentration (1000 mg/L), plants died after 30 days of incubation. No significant differences in leaf growth were found between experimental and control groups, but root growth was significantly reduced by 64–90% at CF concentrations of 500–1000 mg/L after 10–20 days. Changes in the growth rate of seagrasses (eelgrass) and reductions in the number of benthic invertebrates, resulting from environmental disturbances and alterations in physicochemical properties, can lead to irreversible modifications of coastal benthic communities. CFs were less toxic to amphipods than DFs, as evidenced by a significant reduction (by 30–85%) in amphipod survival in DF solutions at concentrations above 10 mg/L compared to the control, whereas significant differences in CF solutions were observed only at the highest concentration of 1000 mg/L. Consequently, measures to preserve ecosystems and mitigate the impacts of offshore drilling are essential. Toxicity tests enable the evaluation of biological responses and determination of concentrations at which DF emissions and

drill cuttings discharges affect indicator species and test organisms. These impacts include changes in autotrophic and heterotrophic individuals/populations, community structure, and energy flow processes within seagrass ecosystems and their associated invertebrates. Ecotoxicological methods facilitate the assessment of environmental impacts throughout the drilling cycle. This integrated approach provides valuable insights into the consequences of drilling operations, supporting informed decision-making aligned with environmental safety principles and aiding in the development of environmental profiles and impact assessments for various waste management strategies.

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