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Environmental impact statement

To reach a more realistic assessment of effects of dispersant application on nematodes and copepods, the present study used outdoor intertidal mesocosms. The application of chemical dispersants is an effective means of accelerating the dispersion of oil from the sea surface into the water column. Use of dispersant of third generation didn't provide protection of benthic organisms, even when oil and dispersants are mixed, no benefit was observed. It is not worthy to use this third generation dispersants for oil spill in nearshore areas.

Effect of crude oil exposure and dispersant application on meiofauna : an intertidal mesocosm experiment

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Abstract

Dispersant application is used as a response technique to minimize the environmental risk of an oil spill. In nearshore areas, dispersant application is a controversial countermeasure: environmental benefits are counteracted by the toxicity of dispersant use. The effects of the use of chemical dispersant on meiobenthic organisms and nematodes were investigated in a mesocosm experiment. A 20 days experiment was performed in four experimental sets of mesocosms. In three of them, sediments were contaminated, respectively by oil (500 mg Kg^{-1}), dispersed oil (oil + 5% dispersant), and dispersant alone, whereas in the last set sediments were kept undisturbed and used as reference (Re). Our results showed that meiobenthic response to oil contamination was rapid, for copepods and nematodes. One-way ANOVA showed a significant decrease of the abundance of copepods. In the case of nematods, univariate and multivariate analyses indicated a clear decrease of the abundance of the species after only 20 days of pollutant exposure and thus, reducing Shannon-Wiener diversity and Pielou's evenness. In contrast, *Sphaerolaimus gracilis* and *Sabateria sp.* became more frequent within disturbed assemblages and appeared to be resistant and/or opportunistic species at these kinds of toxicants. Moreover, responses of copepods and nematodes to the treatment seemed to be the same irrespective of whether only oil or oil + dispersant was perform. Main toxicities of dispersed oil come from not by "composition of newly formed oil and oil spill dispersant mixture" but by the "quantities of increased dispersed oil droplet".

Keywords: Crude oil; dispersant; sediment; mesocosm; nematodes; copepods.

1. Introduction

Oil spills cause serious environmental disasters, often leading to significant, short and long-term impacts on the environment and socioeconomic activity of impacted area. [1,2] Dispersants are designed to chemically disperse an oil slick so that the oil enters the water column, minimizing opportunities to strand on shorelines. [3] Dispersants may enhance oil bioavailability by creating more surface area in terms of multiple small oil droplets, allowing for increased biodegradation of the oil. [4,5]

Dispersants are known to be an appropriate solution for offshore spills. [3] At coastal areas, many studies have generated conflicting results related with the use of dispersants. [6-9] Many factors can contribute to these seemingly conflicting results; dosage and toxicity of dispersants differ, species within a community vary broadly in their response to contaminants, and the bioavailability of contaminants differs with sedimentary conditions. [10]

In European Atlantic coast, the minimum permitted water depth to spray dispersant is 10 m. [11] This restriction of minimum water depths was derived from studies on the dilution of dispersed oil in shallow water and took into consideration the ecological sensitivity of nearshore areas as they are nurseries for many aquatic species. However, a field study conducted by Baca *et al*. [12] suggests that in nearshore tropical ecosystems dispersant use minimizes the environmental damages arising from an oil spill.

The first step towards the assessment of advantages and potential risks of dispersed oil in these sensitive regions is to gain the knowledge of responses of susceptible benthic assemblages like meiofauna that serve as link between primary producers and higher trophic levels. This study aims to assess the toxicity of chemically dispersed oil at levels similar to those encountered in oil spill scenario. To simulate current oil dispersant application, our study uses a “third generation” dispersant, which is the most recent of formulation and is considered less toxic and more concentrated in tensio-active components than earlier ones.

Third generation dispersants are the most commonly used nowadays. While most experimental studies assess the toxicity of the dispersant itself or the dispersed oil Water-Accommodated Fraction (WAF), [5,13,14] for pelagic organisms, our experimental approach give more consideration to benthic organisms. These organisms are ubiquitous in the marine environment and comprise an important link in the food chain, feeding on microalgae and bacteria and in turn being preyed upon by macrobenthic predators such as polychaetes, crabs and fishes. [15,16] They will be expected to be highly susceptible to sediment-associated pollutants because they live and feed in the sediments. Effects of contaminants on them are likely to be transmitted via the food chain and moreover bioaccumulation in them could be passed to higher trophic levels. [17]

Benthic nematodes are also well suited to bioassays because they are small, abundant, easily maintained, sediment-bound throughout their life history, quick to reproduce (in the order of weeks, [15,16,18] and sensitive to various toxicants. [19-21]

To reach a more realistic assessment of effects of dispersant application on nematodes and copepods, the present study used outdoor intertidal mesocosms which simulate as closely as possible, real-life conditions. We suppose that the use of dispersants didn't provide protection of benthic organisms. Findings of this study are interesting as they could help to establish the third-generation dispersant use policies in near shore areas.

2. Methods

2.1. Implementation of the intertidal mesocosm approach

Sediments were collected on the 9th June 2010 during low tide from an intertidal mudflat (46°15'20.23"N 1°08'33.78"W: Esnandes, France) and transferred to the experimental station of the FREDD (Fédération en Environnement pour le Développement Durable: CNRS, Université de La Rochelle, IFREMER) at the Marais de Lauzières (46°12'13.65"N 1°11'42.97"W, France).

Sediments were mixed for 30 minutes with a cement mixer (BETOMIX 160 L) before being introduced in PVC trays (H, W, L: 5 x 40 x 60 cm). These trays were randomly deployed into twelve benthoscosm devices. The latter were composed of two tanks (H, W, L: 40 x 60 x 80 cm), two evacuation tubes, a hose (16 mm Ø), a pump (Eheim compact 1000 L h^{-1}) and a mechanical timer (IDK PMTF 16A) allowing mimicking the tidal cycle (6 hours of low tide, 6 hours of high tide, two tides per day) (Figure 1). Intertidal mesocosms were filled with natural seawater from a pond of the experimental station.

2.2. Experimental design

Experimental design was composed of four treatments and was performed in triplicate. The levels of treatment were randomly assigned to intertidal mesocosm devices. Thus, three of the twelve intertidal mesocosm devices were qualified as references (Re). The latter ones contained contaminated sediment with dispersant (d), with oil (Oil) or with oil + dispersant (Oil + d).

2.3. Contamination proceeding

The concentrations used in the experiment simulated a range of sediment oil pollution comparable to the one of Amoco Cadiz oil spill (1978). [22] Four treatments were added progressively in a cement mixer and mixed during 30 minutes to ensure uniform mixing in this order: references (only 50 kg of sediment), dispersant (1.25 g of dispersant + 50 kg of sediment), oil (25 g of oil + 50 kg of sediment) then oil + dispersant (25 g of oil + 1.25 g of dispersant + 50 kg of sediment). The sediment (50 kg) of each treatment was divided into three equal portions for three replicates per treatment. After each treatment, the cement mixer was cleaned properly. Three PVC trays prior to random introduction to the intertidal mesocosm devices.

Brut Arabian Light (BAL) oil was selected for this study. Its composition was evaluated by the CEDRE (CEntre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux), certified according to ISO 9001 and ISO 14001. The oil was composed of 54% saturated hydrocarbons, 36% aromatic hydrocarbons, and 10% polar compounds. Details of oil PAHs composition is as described by Milinkovitch *et al.* [23]. The oil dispersant used in the current study was a third generation commercial formulation (Finasol; TOTAL Fluides, Paris, France). Its efficiency and acute toxicity was assessed by CEDRE using standard testing and approval procedures (NF.T.90-345 and NF.T.90-349, respectively).

2.4. Sampling

Temperature and salinity were measured starting from the three reference intertidal mesocosm with mean values of 19 ± 1.2°C and 31.01 ± 2.05 psu, respectively. Next, temperature was controlled every four days in each of the twelve intertidal mesocosms. No difference was observed between treatments within sampling dates. Temperature varied in relation to time with mean of 18.9 ± 2.5°C.

At the end of 20 days, three samples of sediment were collected per intertidal mesocosm device (n = 9), meiofauna were collecting using a 10 cm^2 hand-cores to a depth of 5 cm. Sediment was preserved with 4% formalin.

2.5. Sample processing

Meiofaunal taxa, defined here as metazoans that pass through a 1 mm mesh sieve and are retained on a 40 µm mesh, [24] were sieved following the resuspension–decantation methodology, [25] and stained with Rose-Bengal (0.2 g 1^{-1}) for easy counting under a stereo dissecting microscope. During this step,–nematodes (one hundred individuals per replicate) were randomly picked out and placed in 21% glycerol, evaporated to anhydrous glycerol, and then mounted on slides, [26] for microscopic identification. Nematodes were identified at species level using the pictorial keys of Platt and Warwick, [27,28] and the NeMys online identification key. [29]

2.6. Data analyses

A majority of the data analysis followed standard community analysis methods described by Clarke and Warwick, [30] using the PRIMER 5.0 software package. For nematodes of every intertidal mesocosm, five univariate indices were considered: nematode abundance (I), number of species (S), diversity (Shannon-Wiener index, H'), species richness (Margalef's, d) and evenness (Pielou's, J'). One-way ANOVAs were used to test for overall differences between these indices and the abundance of copepods and the Tukey HSD multiple comparisons test was used in pairwise comparisons of treatments. When required, data were transformed (log (x + 1)) to achieve homogeneity of variances and normality of residuals. An alpha level of 0.05 was assumed.

Three multivariate analyses were also applied. First, pairwise analysis of similarities (ANOSIM) was carried out to determine if there were significant differences between nematode assemblages from different intertidal mesocosms. Second, non-parametric Multi-Dimensional Scaling (MDS) ordination was performed based on measures of Bray-Curtis similarity in order to visualize the variability in species composition between treatments. Third, SIMPER (similarity percentages) was used to determine the contribution of every species towards dissimilarity between treatments.

3. Results

3.1. Univariate indices

One-way ANOVA showed a significant decrease in abundances of copepods (Fig. 2A) and nematodes (Fig. 2B) in contaminated intertidal mesocosms comparing to reference mesocosm. No significant difference between oil and oil + dispersant treatment was recorded.

A total of 13 nematode species were recorded in all the intertidal mesocosms (Table 3). Copepod abundance and nematode univariate measures relative to the 4 different treatments are shown respectively in Figure 2 and Figure 3. ANOVA showed significant differences between the treatments. According to multiple comparisons tests, contaminated mesocosms presented significant lower values than reference ones; univariate measures didn't present significant differences between oil and oil + dispersant treatments with the exception of H' and J' which vary significantly between all treatments.

3.4. Multivariate analyses

The highest number of genera and species was observed in reference treatment (Table 3). ANOSIM results (Table 1) revealed a significant effect of d, Oil and Oil + d on structure of nematode assemblages. All treatments were significantly different from the reference and from each other but there was no difference between Oil and Oil + d. Based on this analysis, three groups were discernibly distinguished: Re, d and oil (with and without dispersant). This lack of significant difference between oil and oil + dispersant is also visible in the MDS analysis (Fig. 5) which showed a clear separation between contaminated and reference mesocosms, suggesting the influence of the contaminant treatments on nematode community structure.

The species contributing to about 70% of the dissimilarity between reference and contaminated intertidal mesocosms indicated by SIMPER are given in Table 4. The reference community was dominated by *Daptonema oxycerca*, *Chromadora macrolaima*, *Sphaerolaimus gracilis* and *Sabateria* sp. (Table 4). The treatment d was dominated by *S. gracilis*, *D. oxycerca* and *Sabateria* sp. (Table 4). Further in the dispersed oil intertidal mesocosms (Oil and Oil + d), *S. gracilis* and *Sabatieria* sp. have constitute the most frequent species (Table 4). Increasing numbers of *S. gracilis*, *Sabatieria* sp. and *D. oxycerca* and decreasing number of *C. macrolaima* and *A. paraspinosus* were responsible for significant difference between Re and d (40.43%). Mechanical and chemical dispersion of the crude oil led to an increase in the abundances of *S. gracilis* and *Sabatieria* sp., and a decrease in the abundance of *D. oxycerca* and *C. macrolaima* causing significant differences between reference and the treatments Oil (59.53%) and Oil + d (56.85%).

4. Discussion

The impact of oil, oil + dispersant and dispersant on meiofauna using simulation systems of intertidal mudflat was investigated. After 20 days of exposure, copepod abundances resulted reduced by half in all the mesocosms treated with contaminants, with no differences between mixture of sediment and oil and chemical dispersion of the crude oil (ie. Oil and Oil + d). The nematode abundance decreased upon the addition of dispersant, oil and oil + dispersant to 71.95%, 32.54 %and 31.28%, respectively. These results are supported by several previous studies, [19,29,31-33] which proved that among aquatic invertebrates, crustaceans are sensitive to crude oil. As well, this is consistent with the findings of Jung *et al.* [34], who recorded that the abundance of copepods decreased rapidly upon the addition of crude oil at concentrations over than 1000 ppm in 10 L outdoor microcosms which were manipulated over an exposure period of 8 days. These authors found that contaminated sediments reduced significantly the abundance of many groups of crustaceans such as gammarids, ostracods, tanaids and copepods. Among meiobenthic fauna, harpacticoid copepods are particularly sensitive to hydrocarbons. [19,35-40]

In the current mudflat experiment, the abundance of copepods exposed to only dispersant decreased. However, despite the possible toxicity of dispersant alone on copepods, their combination with oil didn't enhance the negative effects of oil on their abundance.

In comparison with reference mesocosm, the one treated with dispersant alone presented increased abundances of the predator/omnivore *S. gracilis* and the two non-selective deposit feeders *D. oxycerca* and *Sabateria sp.* The mesocosms treated with oil and oil + dispersant were characterised by increased abundances of *S. gracilis* and *Sabateria sp.* as well, but also by the decrease of *D. oxycerca. S. gracilis* is a predator which forages exclusively on other nematodes in non stressed conditions. However, when dead nematodes become more available for the high amounts of pollutants in the sediments, it could change its preferences by feeding as a scavenger. [41] Genera such as *Daptonema* and *Sabatieria* are often considered as very tolerant to various kinds of toxicants. [41,42] The enhance of opportunistic non-selective deposit feeder species may be also a consequence of the increase in bacteria abundance in sediments, [43-45] an event which generally occurs after oil spills, [46] or seepages.[47] In treatments oil and oil + dispersant, the presence of *D. oxycerca* became less remarkable than its equivalent belonging to the same feeding group, *Sabatieria sp.*

The results of this study demonstrated that chemically dispersed oil did not have more negative toxic effect after 20 days than the mixture of sediment and oil on nematode assemblages. No bibliographic data were accessible for free-living marine nematodes. However, analogous facts were observed for diatoms. [9] Indeed, they concluded that the diatoms were found to be much more sensitive to dispersants than to the water accommodated fraction (WAF), and more sensitive to the chemically enhanced WAF (CEWAF) than to either the WAF itself or the dispersants. They observed that the exposure to dispersants and CEWAF caused membrane damage, while exposure to WAF did not. The observed toxicity bore no relationship to PAH concentrations in the water column or to total petroleum hydrocarbon (TPH), suggesting that an undescribed component of the oil was causing toxicity. [9] Rico Martinez *et al*. [48] observed that Corexit 9500A and oil are similar in their toxicity.

The copepod and nematode results suggest independent rather than synergistic interaction toxicity between oil and dispersants (Fig. 2). Main toxicities of dispersed oil come from not by “composition of newly formed oil and oil spill dispersant mixture” but by the “quantities of increased dispersed oil droplet”. In contrast, Rico Martinez *et al*. [48] showed that when Corexit 9500A and oil are mixed, toxicity to *B. manjavacas* increases up to 52-fold.

The application of chemical dispersants can be an effective means of accelerating the dispersion of oil from the sea surface into the water column. [11] This in turn helps to accelerate dilution and biodegradation of the oil, [49] and can reduce the environmental and economic impact of spilled oil in offshore.

The dispersant showed toxic effects both for copepods and nematodes therefore results support the first hypothesis. Use of dispersants didn’t provide protection of benthic organisms. Even when oil and dispersants are mixed, no benefit was observed. The experience clearly showed that dispersant is toxic for benthic organisms in nearshore areas. It is not worthy to use this third generation dispersants for oil spill.

An experimental approach taking into account other kinds of crude oil and other components of the benthic ecosystem would provide supplementary information. From this, further intertidal mesocosm experimentations are needed to evaluate the bioremediation potential vs. dispersants and petroleum compounds at the so-called “small food web” (bacteria, protists, and meiofauna).

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Table 1 ANOSIM results (R statistic and significance level with $p < 0.05$) of pairwise tests for pairwise differences between treatments and control using square-root-transformed nematode abundance data.

Groups	**Re, Oil**	**Re, Oil + d**	**Re, d**	**Oil, Oil + d**	**Oil, d**	**Oil + d, d**
R statistic	0.968	0.991	0.716	0.561	0.614	0.907
Significance level	0.100	0.100	0.100	0.100	0.100	0.100

Data are the mean of two independent experiments. Significance level of sample statistic: 0.1%
Re: reference, d: dispersant, Oil: oil, Oil + dispersant: Oil + d.

Table 2 Average dissimilarity between treatments.

Average dissimilarity (%)	**Re**	**d**	**Oil + d**	**Oil**
Re	-	-	-	-
d	40.43	-	-	-
Oil + d	56.85	34.07	-	-
Oil	59.53	43.52	24.81	-

Re: reference, d: dispersant, Oil: oil, Oil + dispersant: Oil + d.

Table 3 Proportion (%) ± standard deviation of nematode species identified in reference (Re) and contaminated benthocosms (d: dispersant, Oil + dispersant: oil + dispersant, oil: Oil). Species accounting for ~ 70% of overall dissimilarity between treatment groups are ranked in order of importance of their contribution to this dissimilarity.

Average dissimilarity (%)	**Re**	**d**	**Oil + d**	**Oil**
Anoplostoma viviparum	1.56 ± 3.06	0	0.22 ± 0.63	0
***Anticoma* sp.**	0.22 ± 0.63	0	0	0
Axonola mus paraspinosus	7.33 ± 4.67	2.22 ± 1.81	7.44 ± 0.83	4.44 ± 4.19
Chromadora macrolaima	15.22 ± 8.64	2.78 ± 2.20	4.33 ± 2.16	0.78 ± 1.13
Daptonema hirsutum	8.78 ± 4.73	6.22 ± 1.69	3.11 ± 0.99	3.44 ± 4.03
Daptonema oxycerca	23.33 ± 7.06	25.22 ± 7.33	3 ± 1.05	7.33 ± 5.21
***Gamphionema* sp.**	2.78 ± 2.70	1.78 ± 1.69	6.89 ± 2.08	2.11 ± 1.85
Paramonohystera sp.	3 ± 2.11	0	0.22 ± 0.42	0.44 ± 1.26
Praeacanthonchus punctatus	3.22 ± 1.81	1.22 ± 0.92	0.33 ± 0.67	1.67 ± 1.63
Ptycholaimellus jacobi	5.89 ± 3.96	3.78 ± 1.87	1 ± 1.15	0.67 ± 0.82
Sabatieria sp.	10.22 ± 5.79	20.89 ± 4.48	26.56 ± 4.03	18.44 ± 1.95
Sphaerolaimus gracilis	11.33 ±3.20	31.78 ± 7.48	44.67 ± 8.25	57.67 ± 4.76
Terschellingia longicaudata	7.11 ± 4.41	4.11 ± 3.51	2.22 ± 2.30	5.90 ± 3.20

Re: reference, d: dispersant, Oil: oil, Oil + dispersant: Oil + d.

Table 4 Species responsible for differences between control and treated microcosms based on similarity percentages (SIMPER) analysis of square-root transformed data. (+): more abundant; (-): less abundant. Species accounting for ~ 70% of overall dissimilarity between treatment groups are ranked in order of importance of their contribution to this dissimilarity.

Average dissimilarity (%)	**Re**	**d**	**Oil + d**	**Oil**
Axonolaimus paraspinosus	8.78	-	+	-
Chromadora macrolaima	15.22	-	-	-
Daptonema oxycerca	23.33	+	-	+
Ptycholaimellus jacobi	5.89	-	-	-
Sabatieria sp	10.22	+	+	+
Sphaerolaimus gracilis	11.33	+	+	+
Terschellingia longicaudata	7.11	-	-	-

Re: reference, d: dispersant, Oil: oil, Oil + dispersant: Oil + d.

List of figures:

Fig. 1 Experimental tray deployed in intertidal mesocosm device equipped with tidal cycle system.

Fig. 2 Mean (± SE) of a) copepod, b) nematode abundances (ind. 10 cm^{-2}) observed in benthocosms, exposed to different treatments (C: reference, d: dispersant, oil: Oil, oil + dispersant: Oil + d) $^{a, b, c, d}$: Different letter showed significant differences ($p < 0.05$).

Fig. 3 Mean (± SE) of univariate indices values for nematode assemblages observed in benthocosms, exposed to different treatments (C: reference, d: dispersant, oil: Oil, oil + dispersant : Oil + d). a) S = number of species, b) Margalef's d = species richness, c) H' = Shannon-Wiener index, d) Pielou's J' = evenness, $^{a, b, c, d}$: Different letter showed significant differences ($p < 0.05$).

Fig. 4 Non-metric MDS ordination of square-root transformed nematode species abundance from reference (C) and contaminated benthocoms (d: dispersant, oil: Oil, oil + dispersant: oil + d).

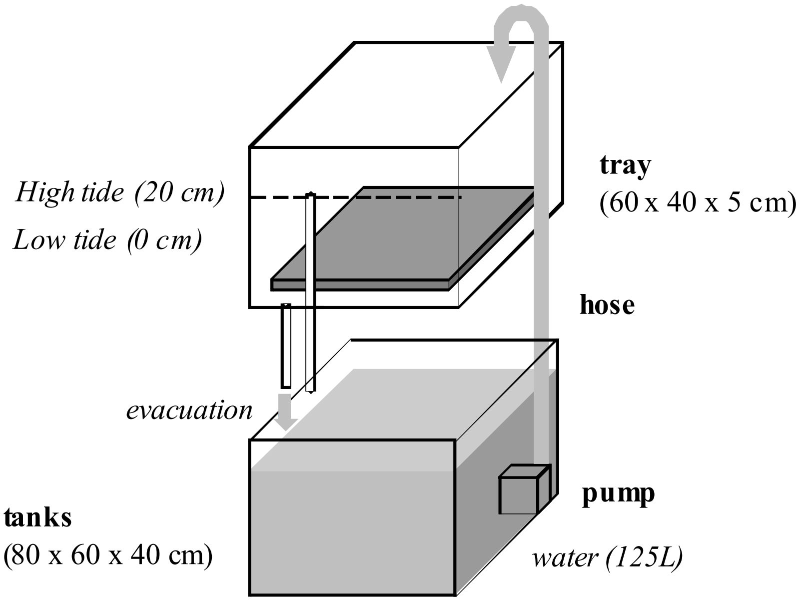
tray
(60 x 40 x 5 cm)
High tide (20 cm)
Low tide (0 cm)
hose
evacuation
pump
tanks
(80 x 60 x 40 cm)
water (125L)

Fig. 1

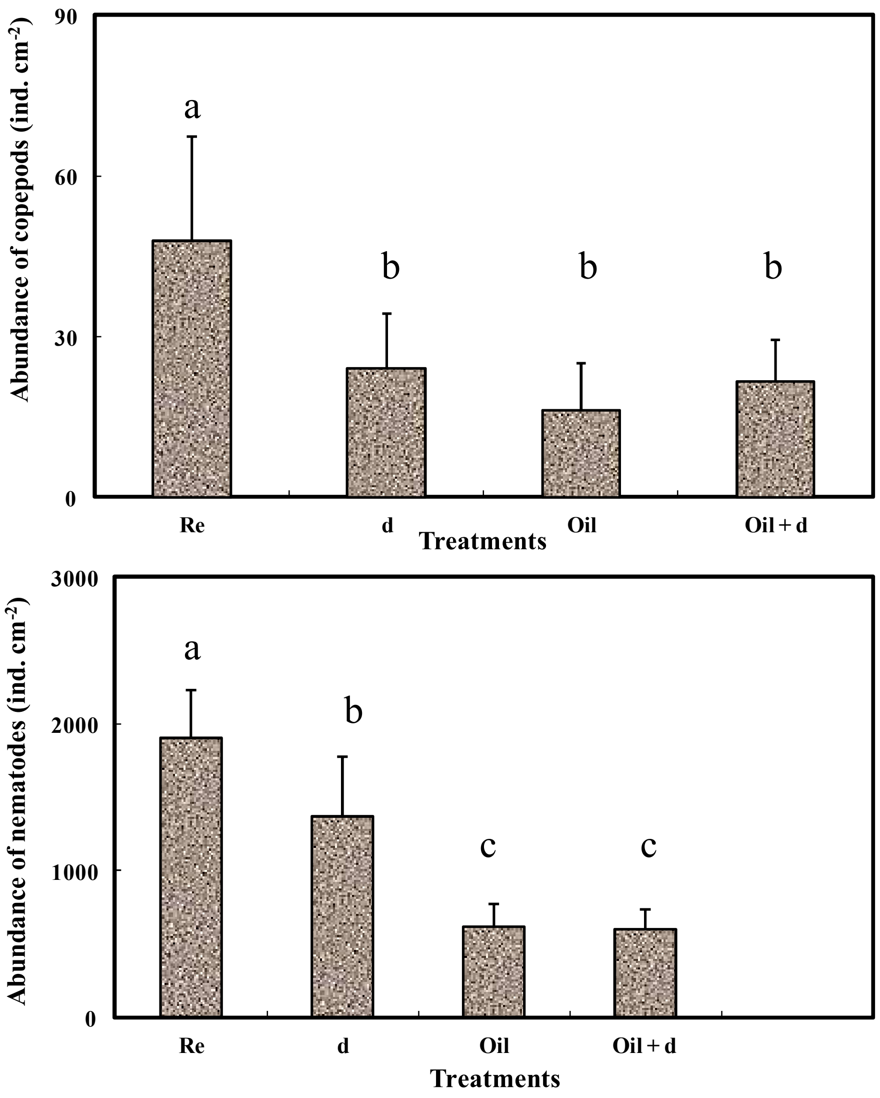
Abundance of copepods (ind. cm^{-2})
90
60
30
0
a
b
b
b
Re
d
Oil
Oil + d
Treatments
Abundance of nematodes (ind. cm^{-2})
3000
2000
1000
0
a
b
c
c
Re
d
Oil
Oil + d
Treatments

Fig. 2

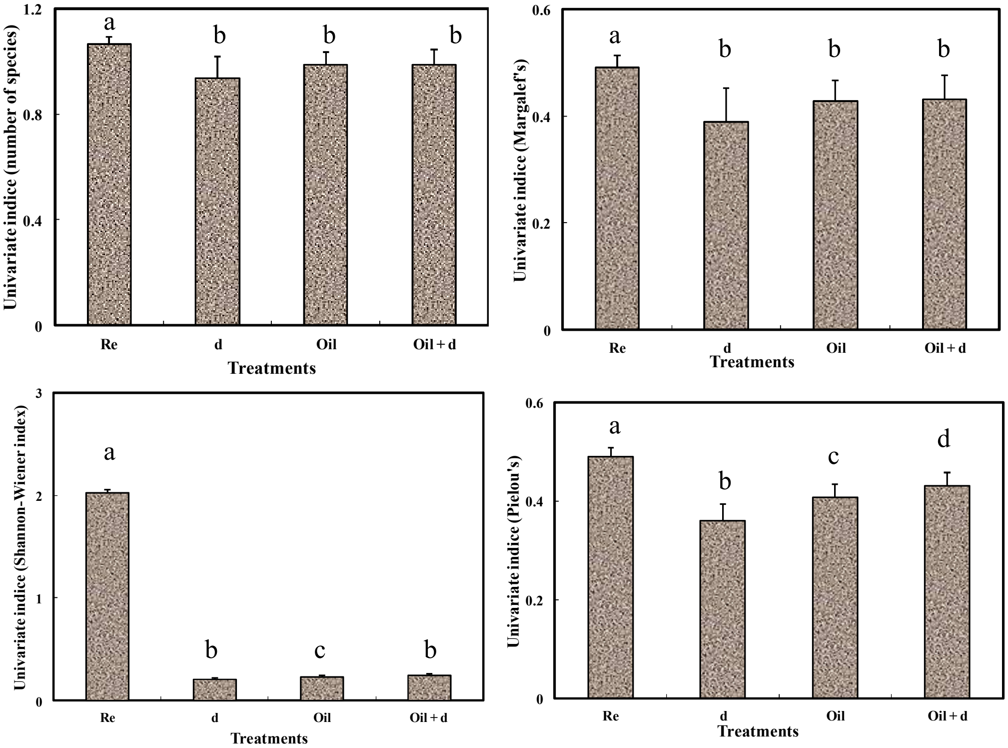
Univariate indice (number of species)
1.2
0.8
0.4
0
a
b
b
b
Re
d
Oil
Oil + d
Treatments
Univariate indice (Margalef's)
0.6
0.4
0.2
0
a
b
b
b
Re
d
Oil
Oil + d
Treatments
Univariate indice (Shannon-Wiener index)
3
2
1
0
a
b
c
b
Re
d
Oil
Oil + d
Treatments
Univariate indice (Pielou's)
0.6
0.4
0.2
0
a
b
c
d
Re
d
Oil
Oil + d
Treatments

Fig . 3

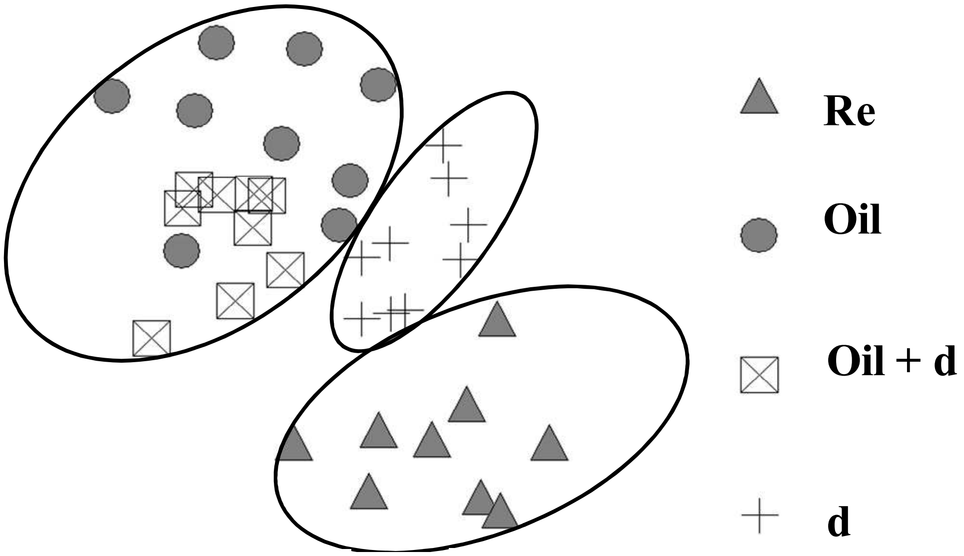

Fig. 4