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Bioaccumulation and subacute toxicity of mechanically and chemically dispersed heavy fuel oil in sea urchin (*Glyptocidaris crenulari*)

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Summary: Oil spills have a disastrous ecological impact on ecosystems but few data are available for the effects of dispersed oil on benthic marine organisms. In order to provide information for assessment, we analysed the hydrocarbon compositions of the mechanically dispersed water accommodated fraction (MDWAF) and the chemically dispersed water accommodated fraction (CDWAF) of No. 120 fuel oil, their bioaccumulation, and DNA damage related to oil exposure, using the sea urchin as a sentinel organism. The results show that the concentration of polycyclic aromatic hydrocarbon in the tissues of sea urchin exposed to the CDWAF is higher than that of those exposed to the MDWAF. The single cell gel electrophoresis assay results also indicated higher DNA damage from exposure to the CDWAF of oil. Thus, dispersants should be applied with caution in oil spill accidents.

Keywords: No. 120 fuel oil; dispersant; sea urchin; bioaccumulation; DNA damage; subacute toxicity.

Bioacumulación y toxicidad subaguda mecánica y químicamente dispersas de aceite combustible pesado de erizo de mar (*Glyptocidaris crenulari*)

Resumen: El derrame de petróleo tiene un desastroso impacto ecológico en los ecosistemas. Sin embargo, hay una falta de datos de los efectos de aceite disperso sobre los organismos marinos bentónicos. Con el fin de proporcionar información de evaluación de su efecto, hemos analizado la composición de hidrocarburos de fracción disuelta mecánicamente en el agua (MDWAF) y la fracción disuelta químicamente en el agua (CDWAF) No. 120 de aceite combustible, su bioacumulación y daños en el ADN relacionados con la exposición al petróleo, utilizando el erizo de mar como organismo centinela. Los resultados muestran que la concentración de hidrocarburos aromáticos policíclicos (PAH) en los tejidos de erizo de mar expuesta en CDWAF es mayor que en MDWAF. También que la concentración de hidrocarburos aromaticos policiclicos (HAP) en los tejidos de erizo de mar expuestos en CDWAF es mayor que en MDWAF. El resultado del ensayo de la célula individual por electroforesis en gel (SCGE) también indica el mayor daño en el ADN en CDWAF de aceite. Por lo tanto, la aplicación de dispersantes en accidentes de derrames de crudo ha de hacerse bajo control.

Palabras clave: No. 120 de aceite combustible; dispersante; erizo de mar; bioacumulación; daños en el ADN; toxicidad subaguda.

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INTRODUCTION

Oil spills have caused terrible environmental damage in the past few years. According to the ITOPF database, from 1973 to 2012 there were 1805 spills of 7 t and over. As one of the major sources of polycyclic aromatic hydrocarbon (PAH), oil spills have affected its presence and distribution in the marine environment. Marine organisms can be affected by long-term exposure to these persistent and bioaccumulative components of oil via several indirect processes mediated through the ecosystem.

It is therefore important to provide information on the bioaccumulation of PAH.

Several studies have evaluated the extent of marine oil pollution in some aquatic organisms by detecting bioaccumulation of PAH in tissues (Jensen et al. 2012, Alani et al. 2012), total hydrocarbon levels and biochemical responses (Oxidative stress, DNA damage and gene mutations) (Blanc et al. 2010, Cariello Delunardo et al. 2013, Weber et al. 2013). As one of the few model organisms considered by the European Agency, sea urchin embryos are widely used to evaluate oil pollution (Rial et al. 2013, 2014, Laramore et al. 2014), but few data on the possible bioaccumulation of PAH in sea urchins have been obtained.

As an important treatment method, from 1995 to 2005, oil dispersants were widely used to deal with 18% of oil spill accidents in the world (Wise et al. 2011). According to statistical data of the Chinese State Oceanic Administration, from 1973 to 2007 the total amount of oil spill accidents only in China's coastal areas was 2742. Dispersants were used to deal with those oil spills. Dispersants have many advantages in handling oil spills because the seawater surface oil is cleaned rapidly. Thus, dispersants can effectively protect plankton and offshore living organisms from contamination. At present, the third-generation dispersants have little toxic effects on aquatic organisms, dispersing the oil spill to form small oil droplets which are more soluble and degradable in the sea. However, the results of some studies have shown that dispersants have potential to increase the bioavailability of PAH to marine organisms (Almeda 2014, Lively et al. 2014, Goodbody-Gringley et al. 2013). Some reports show that dispersants do not increase oil toxicity (Dussauze et al. 2014, Hemmer et al. 2011, Long et al. 2002), but several others show that they make the effects of oil spills even worse (Milinkovitch et al. 2011, Mu et al. 2014, Rico-Martínez R et al. 2013).

The ITOPF database shows that, from 1995 to 2005, a large proportion (66.8%) of the oil spill accidents were fuel oil spills. Because dispersants are less effective on heavier oils, heavy fuel oil can remain in the environment for longer time and has a more serious impact on the marine organisms. The purpose of this study is to analyse the mechanism of subacute toxicity of No. 120 fuel oil and oil dispersant on sea urchin by determining the indicators of bioaccumulation of petroleum hydrocarbon and DNA damage within 21 d. The paper may provide theoretical data for near-shore oil spill response and a comprehensive framework for the establishment of dispersant utilization policies in offshore areas.

MATERIALS AND METHODS

Experimental animals

One hundred sea urchins (*Glyptocidaris crenulari*) were collected from Haibao Farming Industry in Dalian, China. Then the sea urchins (3.5±0.5 cm) were selected and cultured at room temperature (18±0.5°C) for one week. Within the holding period, no mortality was observed and the sea urchins were fed with kelp once every 2 days under 12 h dark: 12 h light. Feeding was suspended for 24 h before the sea urchins were transferred to the testing solution. Normal healthy individuals were grouped according to the experiments.

Experimental system

The experimental system (Fig. 1) consisted of two devices (A and B). As a mixing device of mechanical dispersion (hereafter MD for short) and chemical dispersion (CD), Device A, adapted from Milinkovitch et al. (2011), was composed of six experimental tanks

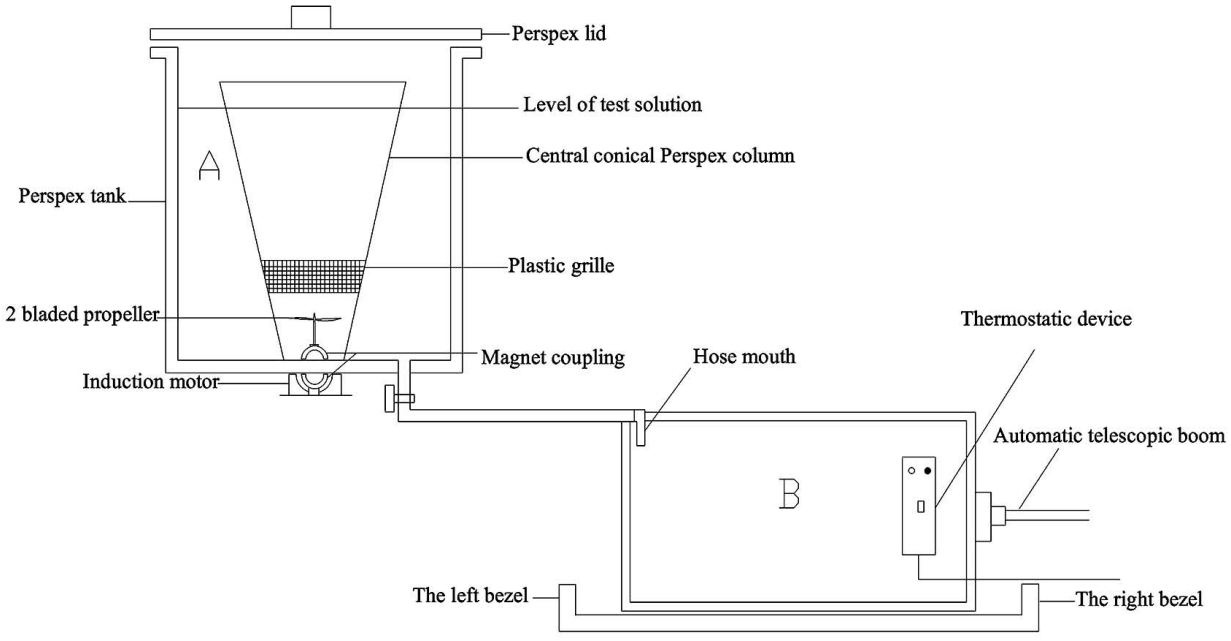

Fig. 1. – Experimental system.

Table 1. – Concentration of 19 PAHs (alkylated and parents) in the MDWAF (5:1000) and CDWAF (5:1000) of No. 120 fuel oil. The 19 PAHs represents the 16 US-EPA PAHs and three supplementary PAHs (retene, benzo(e)pyrene and perylene).

	Molecular weight (g mol^{-1})	MDWAF (ng ml^{-1})	CDWAF (ng ml^{-1})
Naphtalene	128.2	18.42	22.78
C1-Naphthalene	142.3	43.21	70.33
C2-Naphthalene	158.2	39.42	95.47
C3-Naphthalen	173.2	32.40	38.13
C4-Naphthalen	188.2	14.93	34.64
Acenaphthylene	152.2	0.70	2.00
Acenaphthene	154.2	2.91	5.86
Fluorene	166.2	5.52	9.35
C1-Fluorene	181.2	0.11	0.04
C2-Fluorene	196.2	0.22	0.15
C3-Fluorene	211.2	0.07	0.07
Phenanthrene	178.2	5.96	17.35
Anthracene	178.2	1.54	1.96
C1-phenanthrenes/anthracenes	193.2	0.40	0.29
C2-phenanthrenes/anthracenes	208.2	0.48	0.40
C3-phenanthrenes/anthracenes	223.2	0.22	0.33
C4-phenanthrenes/anthracenes	238.2	0.63	0.55
Fluoranthen	202.3	0.77	2.47
Pyrene	202.3	4.49	7.93
Retene	234.3	0.33	6.11
Benzo(a)anthrancene	228.3	0.15	1.27
Chrysene	228.3	0.11	0.44
C1-Chrysene	243.3	0.07	0.04
C2-Chrysene	258.3	0.04	0.04
C3-Chrysene	273.3	0.04	0.07
Benzo(b)Fluoranthene	252.3	0.29	10.11
Benzo(k)Fluoranthene	252.3	0.26	0.36
Benzo(e)Pyrene	252.3	0.44	8.77
Benzo(a)Pyrene	252.3	0.48	1.93
Perylene	252.3	0.15	0.18
Indeno(123-c,d)Pyrene	276.3	0.18	0.25
Benzo(g,h,i)Perylene	276.3	0.04	0.11
Dibenzo(a,h)anthracene	278.4	0.04	0.22

(units) covered by a lid. Magnetic stirring was changed to the bottom of Device A to prevent oil from adhering to a two-bladed propeller. In Device B, sea urchins were exposed to the treatment solution. The thermostatic device was used to control water temperature at 18°C. In order to simulate waves, an automatic telescopic boom was installed and the boom stopped when Device B touched the right/left bezel. The switching frequency of the pushing and pulling was 0.03 Hz. The entire system was stored in a temperature-controlled room (18°C).

Chemicals

No. 120 fuel oil was provided by the Liaoning Maritime Safety Administration and the composition of the oil was determined by the IJRCPTS (International Joint Research Centre for Persistent Toxic Substances), an ISO 14001–certified laboratory. Composition and concentration of PAHs (including the 16 priority PAHs listed by US-EPA) in the mechanically dispersed water accommodated fraction (MDWAF) and the chemically dispersed water accommodated fraction (CDWAF) are presented in Table 1.

Dispersant: The '919 Oil Dispersant' manufactured by Zhenjiang Braun Ship Supplies is in accordance with oil spill dispersant application criteria (GB 18188.2-2000) and approved by Maritime Affairs. It was evaluated by IJRCPTS and was considered effective enough for use in marine environments and non-toxic at the concentrations recommended by the manufacturer.

Exposure methods

Seawater was treated and supplied by Dalian Crest Fluid Equipment Co., Ltd. (China). Oil and water were mixed in accordance with the volume ratios (0.32:1000, 0.63:1000, 1.25:1000, 2.5:1000 and 5.0:1000). The stock solution of MD and CD was mixed by Device A for 24 h and then the solution was settled for 4 h to allow the largest oil droplets to resurface. After one week of acclimation, sea urchins were exposed to different concentrations of CDWAF and MDWAF for 21 d. Ninety sea urchins were exposed in Device B (10 L) with continuous aeration under the exposure cycle of 12 h dark:12 h light. During the exposure period, the mixing system (Device A) was devised to stop mixing of oil dispersant droplets and the lip was removed to simulate natural weathering. The solution of the exposure system (Device B) was replaced every two days and supplemented by Device A. The control group (seawater) was also individually exposed to similar conditions. Physicochemical parameters were measured during exposure (Table 2).

Chemical analysis

Polycyclic aromatic hydrocarbon seawater concentrations

PAH concentrations were assessed in each tank, at T=0 d and following sea urchin exposure (T=7 d, 14 d, and 21 d), using the mean of three replicated

Table 2. – Sea urchin weight (values represent mean±SD; n=9 per treatment) and physicochemical parameters measured during organism exposure (values represent mean±SD of 11 tank measurements at T=0 d).

Parameters	Control	Mechanical dispersion	Chemical dispersion
Temperature (°C)	18.0±0.5	18.0±0.5	18.0±0.5
pH	8.12±0.04	8.13±0.03	8.15±0.02
Salinity (‰)	31.4±0.1	31.4±0.1	31.3±0.1
Dissolved oxygen (mg l^{-1})	7.0±0.5	7.1±0.5	7.0±0.5
Sea urchin weight (g)	11.4±0.4	11.4±0.5	11.6±0.6

measurements for each time point. After sampling, a 24-h settling phase was used to separate oil droplets and particulate matter from the seawater. Then, 150 µL of a solution of 5 perdeuterated internal standards (Naphthalene d_8 (210 µg ml^{-1}), Bihenyl d_{10} (110 µg ml^{-1}), Phenanthrene d_{10} (210 µg ml^{-1}), Chrysene d_{12} (40 µg ml^{-1}), and Benzo[a]pyrene d_{12} (40 µg ml^{-1})) was diluted in 10 ml of absolute methanol, and this volume of methanol was added to the liquid phase of the samples. PAHs were extracted from the seawater and analysed by thermal desorption coupled to a capillary gas chromatography-mass spectrometer (GC-MS). A total of 19 PAHs (alkylated and parents) were quantified. Based on the detection limits of this method, accurate results at concentrations of 1 ng ml^{-1} were possible.

PAH sea urchin intestinal tissue concentrations

The concentrations of 19 PAHs (alkylated and parents) in the sea urchin intestinal tissue were assessed. At different time points PAHs were extracted from the tissue, using the mean of three replicated measurements for each time point. Prior to extraction, 150 µl of a solution of 5 perdeuterated internal standards and 50 ml of an ethanolic solution of potassium hydroxide (2 mol l^{-1}) were added to sea urchin tissue in 250-ml flasks and placed for 3 h in a drying cupboard at 60°C. After alkaline digestion, 3 ml of demineralized water was added and samples were extracted with 2×2 ml of ethyl acetate/n-hexane 40/60. The resulting extract was then concentrated using a rotary evaporator to 1 ml, purified on a silica gel column (5.5 g of silica, hydrocarbon were eluted with 50 ml of n-hexane/dichloromethane 60/40) and concentrated to 200 µl for analysis. Aromatic compounds were analysed by GC-MS, with an approximate quantification limit of 5 µg kg^{-1} of dry weight.

The single cell gel electrophoresis (SCGE) assay

The SCGE was performed according to the procedure described by Andrade et al. (2004) and Cariello Delunardo et al. (2013) with some adaptations. 0.8% (w/v) normal melting point agarose was pre-coated on a microscope slide. The cells of tissue and gonad were dispersed to produce individual cells and suspended in molten low-melting-point agarose at 37°C. This mono-suspension (5 µl of tissue cells and 95 µl of low-melting-point agarose) was spread on pre-coated normal melting point agarose on a microscope slide and topped with normal melting point agarose. After encapsulation, the slides were then immersed into a fresh lysis solution of precooling to lyse the cells in the dark for 2 h at 4°C. Subsequently, the slides were washed with distilled water to remove all salts and immersed in an alkaline buffer solution (300 mM NaOH and 1 mM EDTA, pH 13) for 15 min for DNA helicase. The slides were settled for 10 minutes for electrophoresis in the same buffer solution. The electrophoresis was carried out at 4°C to prevent cellular DNA loss caused by the high-temperature electrophoresis liquid. After electrophoresis the slides were washed with 0.4 M Tris (pH 7.5) and immersed in Tris for 15 min. The slides were stained for 20 min with 50 µl 20 µg ml^{-1} ethidium bromides. Finally, the slides were submerged into the stop solution and cleaned for observation under a fluorescence microscope (excitation filter of 510-560 nm and barrier filter of 590 nm). In order to calculate injury rates, 100 cells were randomly chosen and analysed (Cook et al. 1976).

Statistical analysis

Statistical analysis was carried out using SPSS13.0 data analysis software. One-way ANOVA was performed in order to assess the effects of different exposure conditions. If $p<0.05$, the difference is significant; if $p<0.01$, the difference is extremely significant.

RESULTS

Component analysis of PAH

PAHs in the MDWAF and the CDWAF of No. 120 fuel oil were detected and are presented in Table 1. It was found that the water accommodated fraction of No. 120 fuel oil included 19 PAHs, and the concentration of PAHs in CDWAF (5:1000) was higher than in MDWAF (5:1000).

Toxicity of MDWAF and CDWAF

In the subacute toxicity experiment, the solution concentrations of MD and CD oil were from 0.32:1000 to 5:1000 (V_{oil}/V_{water}). Throughout the experiment, no mortality was found in control groups. Mortality was not observed by exposure to either the MDWAF or the CDWAF of oil during 21 d. At T=14 d, adhesion and activities of sea urchins were normal in all test groups. However, body weight of sea urchins in the MDWAF and the CDWAF showed different degrees of reduction (Table 2 and 3) after 21 d, while activities of sea urchins in the MDWAF (5:1000) and the CDWAF (1.25:1000, 2.5:1000 and 5:1000) declined. At T=21 d, in the MDWAF, abnormal sea urchins were not ob-

Table 3. – Sea urchin body weight in MDWAF, CDWAF and control group at T=21 d.

Concentration (V_o/V_w)	Control	MDWAF	CDWAF
	11.4±0.2	-	-
0.32:1000	-	11.4±0.3	11.5±0.3
0.63:1000	-	11.3±0.3	11.4±0.2
1.25:1000	-	11.2±0.3	11.2±0.3
2.5:1000	-	11.0±0.3	10.9±0.4
5:1000	-	10.8±0.4	10.6±0.4

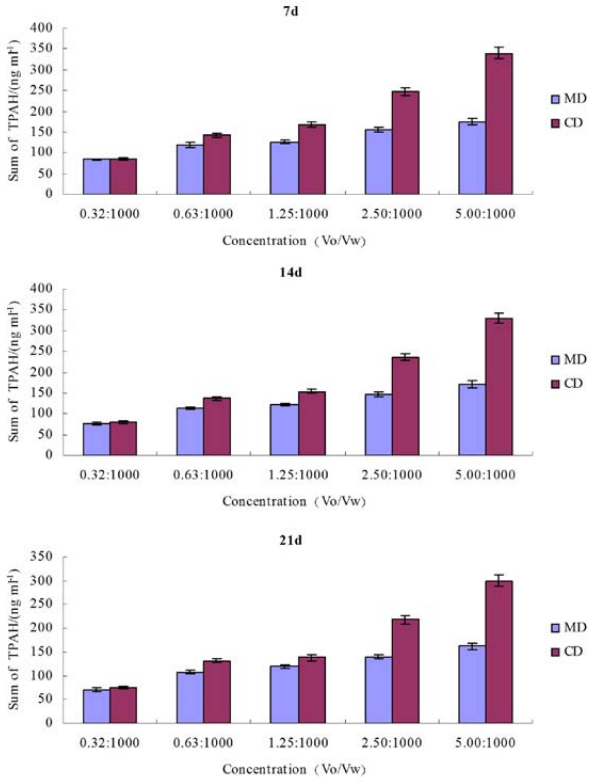

Fig. 2. – Concentration of total PAHs in chemically and mechanically dispersed water accommodated fraction of oil within different exposure times (7, 14 and 21 d).

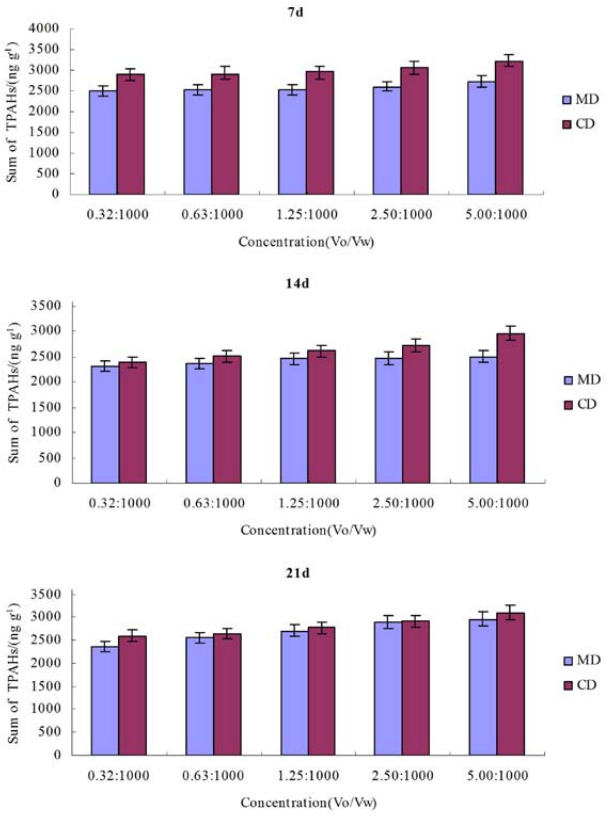

Fig. 3. – Concentration of total PAHs in the intestine of sea urchins exposed to CDWAF and MDWAF within different exposure times (7d, 14 d and 21 d).

served except for 5:1000 exposures (30% abnormal). In the CDWAF, 30%, 60% and 90% abnormal were observed for 1.25:1000, 2.5:1000 and 5:1000 exposures, respectively.

PAH in seawater and the intestinal tissues of sea urchins

No PAH was detected in the tissue of the sea urchin control group in any exposure medium at 0 d.

The total PAH concentration of different exposure solutions at 7, 14, and 21 d were shown in Figure 2. The results showed that total PAH concentration in the CDWAF was higher than that in the MDWAF in the same exposure time. For example, at T=7 d of MDWAF exposure, following exposure to five test solutions (0.32:1000, 0.64:1000, 1.25:1000, 2.5:1000 and 5:1000), the total PAH concentrations were 84, 118, 126, 157 and 175 ng ml^{-1}, respectively. For CDWAF exposure, following exposure to five test solutions the total PAH concentrations were 85, 142, 168, 248 and 340 ng ml^{-1}, respectively. However, there was no significant increase in PAH concentration between the CDWAF and the MDWAF at the low concentration ($P<0.05$).

It was found that the total PAH concentration in the intestines of sea urchins was slightly decreased at 14 d and increased at 21 d in the MDWAF and the CDWAF (Fig. 3). During the exposure, the concentration of total PAH in the CDWAF was higher than that in the MDWAF. Between the CDWAF and the MDWAF, the difference in total PAH concentration in sea urchin intestinal tissue gradually decreased with the prolongation of exposure time. Correlations were found between total PAH concentrations in sea urchin intestinal tissue (Fig. 3) and the concentrations of the exposure solution (Fig. 2). The PAH concentration was increasing gradually with growing concentrations of exposure solution.

Bioaccumulation factor

Table 4 presents the measured bioaccumulation factor (BAF= [total PAH] in sea urchin tissue/ [total PAH] in sea water) for the samples in the MDWAF and the CDWAF (Milinkovitch et al. 2011). The BAF

Table 4. – Bioaccumulation factor for MDWAF and CDWAF exposure.

BAF (V_o/V_w)	7 d		14 d		21 d	
	MDWAF	CDWAF	MDWAF	CDWAF	MDWAF	CDWAF
0.32:1000	29.7	35.3	27.5	29.3	28.1	31.7
0.63:1000	21.4	20.6	20.0	17.7	21.6	18.6
1.25:1000	20.2	17.6	19.6	15.6	21.5	16.5
2.50:1000	16.6	12.3	15.7	11.0	18.5	11.8
5.00:1000	15.5	9.5	14.3	8.7	16.9	9.1

Table 5. – DNA damages (TL, tail length; %TDNA, tail DNA%; TM, tail moment) of sea urchin intestinal cells within 7.14 and 21 d; * indicates statistically significant differences ($p<0.05$) with respect to the corresponding MDWAF treatment.

Exposure time	V_o/V_w	MDWAF			CDWAF		
		TL (μm)	%TDNA	TM (μm)	TL (μm)	%TDNA	TM (μm)
7d	0:1000	0.18±0.08	0.054±0.04	0.19±0.09	0.16±0.03	0.052±0.04	0.18±0.06
	0.32:1000	0.52±0.12	0.43±0.13	0.49±0.17	0.79±0.28	0.85±0.32	0.72±0.26
	0.63:1000	0.97±0.32	1.06±0.25	0.98±0.27	1.24±0.45	1.08±0.39	1.17±0.25
	1.25:1000	1.32±0.45	1.28±0.36	1.29±0.24	1.49±0.39	1.54±0.29*	1.42±0.37
	2.50:1000	1.77±0.54	1.82±0.28	1.69±0.37	2.14±0.44*	2.08±0.35*	1.97±0.31*
	5.00:1000	1.97±0.24	1.86±0.32	1.95±0.27	2.54±0.43*	2.48±0.31*	2.38±0.33*
14d	0:1000	0.15±0.06	0.038±0.05	0.22±0.07	0.17±0.02	0.043±0.04	0.20±0.05
	0.32:1000	0.42±0.12	0.48±0.16	0.49±0.14	0.53±0.28	0.48±0.34	0.52±0.34
	0.63:1000	0.87±0.24	0.76±0.34	0.85±0.27	1.19±0.45	1.26±0.27*	1.17±0.31*
	1.25:1000	1.25±0.17	1.34±0.26	1.29±0.24	1.43±0.28	1.38±0.35	1.41±0.24
	2.50:1000	1.67±0.39	1.46±0.41	1.53±0.37	2.09±0.46*	1.98±0.42*	2.11±0.31*
	5.00:1000	1.82±0.24	1.66±0.34	1.73±0.28	2.36±0.52	2.42±0.44*	2.36±0.42*
21d	0:1000	0.16±0.04	0.042±0.03	0.21±0.08	0.16±0.03	0.038±0.04	0.22±0.07
	0.32:1000	0.72±0.12	0.78±0.16	0.59±0.14	2.29±0.28*	2.07±0.34*	2.72±0.34*
	0.63:1000	2.97±0.24	2.06±0.34	2.35±0.27	3.14±0.46	2.68±0.39*	3.47±0.29
	1.25:1000	2.68±0.28	2.25±0.24	2.83±0.35	3.56±0.35	3.15±0.37*	3.81±0.26
	2.50:1000	3.13±0.37	3.26±0.31	4.87±0.42	15.38±0.68*	8.54±0.57*	5.19±0.62
	5.00:1000	4.35±0.31	5.49±0.38	6.34±0.33	20.16±0.86*	17.28±0.77*	13.53±0.92*

showed a downward trend with the increase in testing solution concentration for MD and CD exposure. The BAF was found to be significantly higher (except for the lowest concentration) in the following exposure to MD fuel oil than to CD oil. The BAF in 14 d was the lowest both in the MDWAF and the CDWAF of oil.

DNA damage in sea urchin exposed to the MDWAF and the CDWAF

The SCGE assay results were given by three parameters: tail length (TL), DNA fragments in the comet tails (%TDNA) and tail moment (TM). Table 5 presents DNA migration data (TL, %TDNA, TM) of intestinal cells in sea urchin exposed to the MDWAF and the CDWAF. Compared to the controls, it was found that the MDWAF and the CDWAF both induced DNA damage to intestinal cells at all the tested concentrations. DNA damage was always more accentuated for those exposed to the higher concentration of the MDWAF and the CDWAF. The SCGE assay results showed different degrees of increase of TL, %TDNA and TM with increasing concentrations of the MDWAF and the CDWAF during the same exposure time. At the end of exposure, the levels of DNA damages (TL, %TDNA and TM) were higher in CDWAF than in MDWAF, except for the lowest concentration (0.32:1000). DNA damage in high concentration of CDWAF was higher than that in the respective MDWAF. The Pearson's correlation coefficients for the relationships between concentration of test solution and DNA damage of sea urchin intestinal cells were as follows: $r=0.832$ for TL ($P=0.040$), $r=0.968$ for %TDNA ($P=0.001$) and $r=0.948$ for TM ($P=0.004$) in MDWAF at 21 d; $r=0.961$ for TL ($P=0.002$), $r=0.994$ for %TDNA ($P=0.001$) and $r=0.971$ for TM ($P=0.001$) in CDWAF at T= 21 d.

DISCUSSION

PAH is a huge threat to the environment due to the carcinogenicity and mutagenicity of PAH. However, PAH exhibits subacute toxicity and bioaccumulation potential due to low removal efficiency in traditional biological detoxification processes (Herbes and Schwall 1978). After the dispersant treatment, in the CDWAF the concentrations of most single compounds of PAH were increased. The increased concentration of single compounds of PAH could increase the toxic effects to sea urchin, and PAH mixtures had a higher toxicity than expected. This was an important factor that led to the increase in subacute toxicity.

The toxicity of PAH

The sea urchin experiments showed that fuel oil pollution treated by dispersant led to a decrease in activity. A similar decrease in activity of other marine animals (shrimps and copepods) as a result of oil has been reported (Ekanem et al. 2011, Jiang et al. 2012). It has been suggested that the oil water accommodated fraction (WAF) is able to disturb the normal operation of physiological and biochemical systems (Jiang et al. 2012). Most oil WAF is highly lipophilic and may be easily ingested by marine organisms. Sea urchins have a huge surface area because water enters from the tube feet, the madreporite and other places throughout the body. The organic compounds (especially PAH) of oil WAF could easily accumulate in the animals via the feeding behaviour and surface contact (Carls et al. 2006, Jiang et al. 2012). Therefore, these toxic effects could be related to the toxicity of PAH.

Bioaccumulation of PAH in the tissues of sea urchins

In a comparison of the MDWAF and the CDWAF of oil, our results showed that chemical dispersants led to an increase in PAH concentrations in the water column. Moreover, the PAH concentrations of the tissues of sea urchins in the CDWAF were higher than those in the MDWAF. These results are consistent with those of previous studies that have revealed an increase in PAH uptake due to dispersant application (Mielbrecht et al. 2005, Wolfe et al. 2001). However, the BAF declined at the same exposure time in the MDWAF and the CDWAF. A similar phenomenon was also reported

by Li et al. (2008). Although the BAF declined, the higher PAH concentration in sea urchin tissues in the CDWAF suggested that chemically dispersed oil increased the biological availability.

DNA damage

As shown in Table 5, DNA damage levels in the intestinal tissues of sea urchins exposed to the CDWAF were higher than those in sea urchins exposed to the MDWAF. Changes in DNA damage levels showed a significant positive correlation with the exposure test solution concentration ($P<0.05$).

DNA damage could be caused by environmental factors such as UV radiation and oxidative stress (Binelli et al. 2009, Ciereszko et al. 2005). However, PAH can generate reactive oxygen species by redox and form DNA adducts with DNA. This process probably causes DNA single bond breakage and results in DNA damage. Some studies have found that PAH could induce DNA damage in marine organisms. Humphries (2006) showed increasing DNA damage in mussel cells related to increasing PAH concentrations. A sea urchin experiment found that levels of DNA damage were proportionally increased by exposure concentration and time(Woo et al. 2006), similar to a previous report. For this reason, the results could be explained by the fact that the higher concentration of PAH in the CDWAF increased the level of DNA damage (Teixeira et al. 2012).

CONCLUSIONS

By comparing exposure to the MDWAF and the CDWAF, our results demonstrate that the use of chemical dispersants increases contaminant uptake by the sea urchin, and leads to lower activity, higher bioavailability of the PAH of the WAF of fuel oil and more severe DNA damage to sea urchins. Similar results have been reported in other marine organisms (Milinkovitch et al. 2011, Ramachandran et al. 2004). The adverse effects of chemically dispersed fuel oil solutions are probably due to the presence of smaller oil droplets and an increase in the PAH concentration in the water.

This study aimed to obtain information about the subacute effects of dispersant application on benthos in offshore areas. However, the experiment was carried out in the laboratory to simulate the effects of dispersant application in real oil spill accidents. Though exposure conditions cannot be completely consistent with the real situation, the results provide information on potential ecological impacts to improve evaluation of dispersant application. The adverse effects reported should be taken into account before dispersants are applied in offshore areas.

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