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TOXICITY OF WATER SOLUBLE FRACTION OF CRUDE OIL ON MORPHOLOGY AND BEHAVIOR OF SOLDIER CRABS *Dotilla wichmanni* de Man, 1892

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ABSTRACT: Static bioassay test was conducted to assess the effect of Water Soluble Fraction (WSF) of crude oil on soldier crabs and to determine the median lethal concentration (LC_{50}) of 20% WSF at $\alpha = 0.05$. The results showed that the mortality rate of soldier crab varies depending on the TPH concentration and exposure time. The first toxicity effect could be observed at a level of 5.96 µg/l TPH in 48 hours, with a LC_{50} of 20 WSF of crude oil at 10.04 µg/l total petroleum hydrocarbons (TPH) after 96 hours. During exposure, morphological changes as the swelling of the walking legs, maxillipeds and gills could be observed. Behavioral records showed that the soldier crabs appeared to lose their balance and began to move rapidly in circles before finally dying.

Key words: Water Soluble Fraction of crude oil, morphology and behavior, Soldier crabs, median lethal concentration

INTRODUCTION

Crude oil is an important energy source for the agriculture and industry sectors, and its demand has increased every year especially in Thailand, which imports crude oil and petroleum products from abroad. Transportation of crude oil and petroleum products takes place in particular through sea routes to save costs and transport higher tonnage. However, its transports also cause leakage of oil and petroleum products during the process of unloading, and accidental or illegal dumping into the sea. ITOPF (2004) reported that oil leak around the world had increased by approximately 10,466 times in 1974–2003, resulting in the loss of more than 1.14 million tons of oil and petroleum products.

From 1997 to 2010, about 85 incidences of oil spills from tankers are documented in Thailand (Pollution Control Department, 2015). Considering that the main element in crude oil is hydrocarbon, oil spill affects the environment and causes massive problems in the marine environment (Pollution Control Department, 2015), especially in coastal areas such as beaches, mangroves and sea grass beds. In addition, oil leakage also affects coastal aquaculture, which is a very important economic sector in Thailand.

Crude oil is considered toxic and causes two main kinds of injury: physical and biochemical. The physical effects of freshly spilled crude oil are all too obvious, *e.g.*, birds and other animals coated in crude oil, struggling to survive. Crude oil not only destroys the insulating properties of animal fur and bird feathers, which can lead to hypothermia, but it also impairs animals' abilities to fly and swim, sometimes causing oiled animals to drown.

Sandy beaches are often affected by crude oil when oil spills. Beach areas are diverse marine habitats that support various aquatic animals that include soldier crabs *Dotilla wichmanni*, which are organic filter feeders and burrow in the sand (Hartnoll, 1973). *Dotilla wichmanni* is common species on sandy beach in Thailand, then play important role in sandy beach ecosystem.

As oil spills affect such habitats and have direct effects on soldier crabs, the present study focused on the effects of the toxicity of Water Soluble Fraction (WSF) of crude oil on the morphology and behavior of the soldier crabs. Understanding the effect of oil spills on the morphology and behavior of the soldier crabs may be an important key to gaining insight into the effect of oil spill on other marine organism in the field.

MATERIALS AND METHODS

This research assessed the toxicity of Water Soluble Fraction (WSF) of crude oil on the morphology and behavior of adult stage of soldier crabs *Dotilla wichmanni* de Man, 1892. Biological analysis through static bioassay was conducted. According to Anderson *et al*. (1974), Water Soluble Fraction of crude oil derived from Oman crude oil was prepared by mixing 1 part of crude oil with 9 parts sea water in 600 ml beakers. A magnetic stirrer was used to solubilize the mixture for 20 hours; during this time the beaker were covered with black plastic to prevent evaporation and oxidisation by light. Subsequently, the solution was kept static at 27°C and with a salinity of 30 psu for 6 hours to enable a complete solution separation. The lower clear phase, known as the Water Soluble Fraction of crude oil (100%) was diluted with sea water to produce a 20% WSF, which was used in experiments to determine its median lethal concentration (LC_{50}). Total Petroleum Hydrocarbon contraction was analyzed from WSF of crude oil and Qualitative and Quantitative Analyses of WSF by UV Fluorescence Spectroscopy (UVF) following the manual of IOC (1984).

A total of 180 specimens of mature stage soldier crabs with body weights 0.0–0.12 g and carapace widths of 0.7–1.0 cm were used in the experiment, mainly because at this size the crabs are mature and thereby suitable for the planned observations.

Experiment was set up for 4 different TPH concentrations, *i.e*., 0, 5.96, 10.04, and 12.54. For each concentration, 45 specimens of soldier crabs were used for the three replicates, one crab per container. The soldier crabs were exposed for 24, 48, 72 or 96 hours to different TPH concentrations. All specimens of soldier crabs were incubated in 200 ml of the respective TPH concentration without changing the solution during the 96 hours exposure period. Their behavior and morphological changes were documented such as color, appendages (legs, gills *etc*.) and movement. External and internal organs of soldier crab were observed under stereo microscope such as maxillipeds, gills and walking legs.

Wilk's Lambda statistic was used to investigate the effect of TPH concentration to the mortality rate. According to McMahon and Doyle (1997), analysis of Variance (ANOVA) at $\alpha = 0.05$ was used to investigate the effect of TPH concentration on the mortality rate at each time period and the Least Significant Difference technique (LSD) at $\alpha = 0.05$ was calculated to compare the results from each TPH concentration and the mortality rates of the soldier crab at each time period. NOAEL (no-observed-adverse-effect level), which are used to derive threshold safety exposure dose to animals such as derived no-effect level, occupational exposure limit and acceptable daily intake. (Kinnberg *et al*., 2000; Jumel *et al*., 2002; Hutchinson *et al*., 2006; Hutchinson *et al*., 2009; Lammer *et al*., 2009), was reported in the present study.

RESULTS AND DISCUSSION

The toxicity of Water Soluble Fraction of crude oil on soldier crabs *Dotilla wichmanni* is shown in Table 1. The Total Petroleum Hydrocarbon of crude oil of WSF was estimated at the concentrations at 0.00, 5.96, 10.04 and 12.54 µg/l. The soldier crabs were exposed to the WSF of crude oil for 24 to 96 hours and their behaviors were documented. The first concentration which affected the soldier crabs was at 5.96 µg/l TPH. Although the soldier crabs survived for 24 hrs, mortality could be observed after 48 hrs to 13.33% of the tested animals. With increasing exposure (72 and 96 hrs), the mortality rates reached a stable plateau of 26.67%. Higher THP exposure (10.04 µg/l TPH) resulted after 24 hrs with a mortality rate of 13.33%, which increased to 50.33% at 96 hrs exposure time. At the highest concentration 12.54 µg/l TPH a mortality of 16.67% could be observed after 24 hrs and 23.33% (after 48 hrs), 33.33% (after 72 hrs), and 53.33% after 96 hrs. From these results it was calculated that the LC_{50} of Water Solution Fraction of crude oil was at 10.04 µg/l TPH in 96 hrs.

The comparison between each time period and each TPH concentration showed firstly, that the mortality rate during the first 24 hours was not significantly different ($\alpha = 0.05$) between 0 and 5.96 µg/l TPH as well as, 10.04 and 12.54 µg/l TPH. Secondly, the mortality rate at 48 hours was not significantly different ($\alpha = 0.05$) between TPH concentration at 10.04 and 12.54 µg/l. Thirdly, the mortality rate at 72 hours was not significantly different ($\alpha = 0.05$) between 5.96 and 12.54 as well as, 10.04 and 12.54 µg/l. Finally, the mortality rate at 96 hours was not significantly different ($\alpha = 0.05$) between 10.04 and 12.54 µg/l TPH.

Table 1. Mortality of soldier crabs *Dotilla wichmanni* in Total Petroleum Hydrocarbon of crude oil of WSF from 24 to 96 hours.

Total Petroleum Hydrocarbon (µg/l TPH)	Soldier crab (specimens)	Percentage mortality (%) (average ±SD)			
		24 hrs	48 hrs	72 hrs	96 hrs
0.00	45	0	0	0	0
5.96	45	0.00±0.00	13.33±6.02	26.67±2.30	26.67±2.30
10.04	45	13.33±6.02	28.33±4.50	42.33±9.81	50.33±2.51
12.54	45	16.67±7.76	23.33±5.13	33.33±2.88	53.33±5.50
10.04 µg/l TPH		96 hrs LC_{50} of crude oil			

Anderson *et al*. (1974) found that a LC_{50} mortality rate was 19.8 µg/l TPH for 96 hrs exposure to the post-larva of the South Louisiana shrimp *Palaemonetes pugio* (referred to as *Palaemonetes pugio*) and *Penaeus aztecus*, while juvenile stages of the shrimp *Litopenaeus setiferus* show the same effect at a TPH concentrations of 6.49 µg/l of crude Kuwait oil (Bo, 2003). Larval stages of the Venezuelan lobster *Homarus americanus* (Anderson *et al*., 1974) and shrimp *Pandalus hypsinotus* (Wells and Spraque, 1976) reacts even more sensitively to crude oil (for *Homarus americanus* concentrations from 0.86 to 4.90 µg/l TPH, for *Pandalus* values around 2.7 µg/l TPH). Moreover the lobster *Homarus* show a loss of balance (Anderson *et al*., 1974) as observed in this study. Rice *et al*. (1979) revealed that sensitivity to crude oil generally increases from invertebrates to fish, but it is actually better correlated with their habitats. In the case of sensitivity of various species to crude oil using different systems, Vanderhorst *et al*. (1976) report the bioassay of a No.2 fuel oil dispersion at 96 hrs LC_{50} of coon stripe shrimp is 0.8 µg/l. However, crude oil is an extremely complex mixture of different compounds ranging from simple low molecular weight to very complex polynuclear aromatic hydrocarbons containing numerous isomers (Duffus, 1982).

In this context, soldier crabs may be able to tolerant higher concentrations as adult specimens were used in this study and the animals are benthic organism that usually borrow in the sand. However, the crude oil clearly had an observable effect on the soldier crabs *Dotilla wichmanni* morphology. Already at low TPH concentration of 5.96 µg/l the external and internal organs (maxillipeds, gills and walking legs) of soldier crabs were swollen and their cuticle began to turn gray (Fig. 1).

Figure 1. Soldier crabs after being exposed to the crude oil showing morphological changes: a gray color change in the cuticle, and swollen maxilliped and walking legs.

The soldier crabs may be able to tolerate higher TPH concentrations by increasing their metabolism and thereby facilitating oil detoxification. Cappuzzo and Lancaster (1982) reported that swelling of the liver is a result of plasma protein synthesis, *i.e.* balance of blood plasma, aneurysm of the aorta, and lose of lamellae. Kornberg (1981) found that hydrocarbons affect the metabolism of organisms and functions of cells. The hydrocarbons infiltrate the fat layer of the cell membranes, which in consequence hinder the metabolic exchange between the inner and outer cells compartment. Nicolas (1999) reported that TPH has been demonstrated to be mutagenic and carcinogenic precursors, as well as to impair various physiological functions in fish and other organisms. Alterations of endocrine systems have been frequently reported, sometimes resulting in impairments of reproductive processes or physiological responses such as changes in plasma glucose, osmotic and ionic regulation, blood oxygen, hematocrit and hemoglobin concentration.

Al-Kindi *et al*. (2000) reported a variety of blood–borne parameters recognized as reliable tools in determining the relative severity of stress in fish. The petroleum hydrocarbons affected endocrine responses including changes in plasma catecholamines, corticosteroids, and thyroid hormones. Physiological responses included changes in plasma glucose, osmotic and ionic regulation, blood oxygen, hematocrit and hemoglobin concentration.

Gusmão *et al*. (2012) reported results from chronic toxicity test conducted with newly hatched fish larvae exposed for 21 days to sublethal concentrations of WSF (2.5, 5, 10, and 20% of WSF), plus one control. Survival and growth were significantly lower in the highest concentration. Several histopathological changes were found in the gills (*e.g*., hyperplasia, aneurysms, edema, and necrosis), kidney (*e.g*., nuclear alterations, decrease in the hematopoietic cells), and liver (*e.g*., hypertrophy, karyorrhexis, and karyopyknosis). An index of branchial lesion was proposed to standardize gill lesions to different pollutants.

Franco *et al*. (2017) reported a crude oil toxicity to fiddler crabs (*Uca longisignalis* and *Uca panacea*) from the Northern Gulf of Mexico: impacts on bioturbation, oxidative stress and histology of the hepatopancreas. Fiddler crabs were exposed for periods of 5 or 10 days to oil concentrations up to 55 mg/cm2 on the sediment surface. Their burrowing was delayed, small hole and they transported less sediment in the presence of oil. The hepatopancreas had elevated levels of oxidative stress and a higher abundance of blister cells, which play a role in secretory processes.

In general, for the experiment, the soldier crabs show an unusual rapid escape behavior when exposed for 24 to 48 hrs to crude oil and try to evade by swimming to the water surface (Fig. 2A). Affected animals are not able to stay in the water column and exhibit normal behaviour. With increased exposure 72 to 96 hrs the specimens instead sink back to the tank bottom, show a walking behavior with loss of balance (observed by its up-side down positions) and they subsequently died (Fig. 2B and Table. 2).

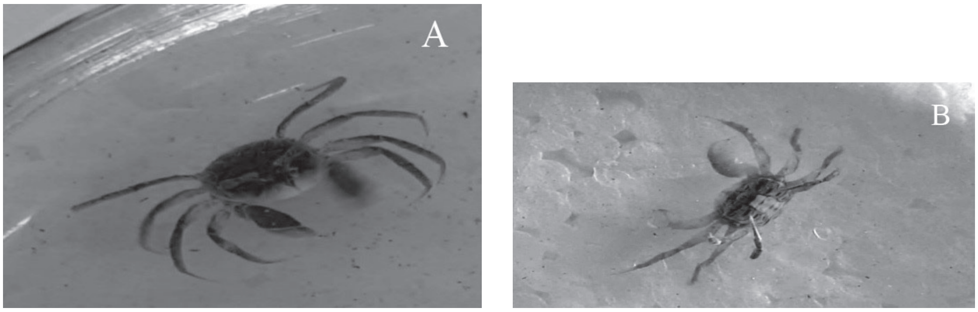

Figure 2. A. soldier crab in its normal walking position; B. soldier crab lost its balance and finally died after prolonged exposure.

Table 2. Behavior of soldier crabs in Water Soluble Fraction of crude oil in 24, 48, 72 and 96 hrs.

Time [hrs]	Total Petroleum Hydrocarbon [μg/l TPH]	Observation of the soldier crabs behavior
24	0.00	Soldier crab in its normal walking position at the bottom of tank.
	5.96	Soldier crabs unusually walk fast.
	10.04	Soldier crabs unusually walk fast, then die.
	12.54	
48	0.00	Soldier crab in its normal walking position at the bottom of tank.
	5.96 10.04 12.54	Soldier crabs are unusually walking much faster than in 24 h exposure, then die.
72	0.00	Soldier crab in its normal walking position at the bottom of tank.
	5.96	Soldier crabs are unusually walking slower than in 48 h exposure, then die.
	10.04 12.54	Soldier crabs are unusually walking slower than in 48 h exposure, while trying to find air near the surface of oil solution. Then, they lose the ability to walk and maxillipeds can no longer move, then die.
	0.00	Soldier crab in its normal walking position at the bottom of tank.

Time [hrs]	Total Petroleum Hydrocarbon [µg/l TPH]	Observation of the soldier crabs behavior
96	5.96	Soldier crabs are unusually walking slower than 72 h exposure, then die.
	10.04 12.54	Soldier crabs are unusually walking and moving slower than in 72 h exposure while trying to move up the surface of oil solution. Then, the maxillipeds can no longer move and they lose the ability to walk, then stop walking, and while gradually losing balance, their movements slow down, then they stop and die.

Duffus (1982) explained that groups of aromatic hydrocarbon oil with low boiling point are highly toxic and water soluble. They inhibit the formation of blood affecting the respiratory system with the gills of soldier crabs being affected by TPH, resulting in swelling and likely stimulation of central nervous animal hypoxia, and loss of balance. Saturated hydrocarbon compounds with low boiling point also make the exposed animals drowsy or put them in coma (observed from the paralysed soldier crabs) that eventually result in deaths. This effect could also be observed in the present study when the soldier crab become motionless and subsequently die.

The present study intended to estimate the maximum exposure concentration and period of crude oil on soldier crab survival. The present results indicate that the NOAEL of crude oil should be less than 5.96 µg/l TPH. This is consistent with the findings of Phukaokaew (2015) who stated that concentrations of petroleum hydrocarbons higher than 1 µg/g dry weight affect the survival of soldier crabs. Animals that inhabit contaminated areas as Samet Island in Rayong Province are not able to dig holes and show a decreased sand molding behavior. Normal crabs in non-affect area can mold sand at a rate of 15 pellets/min, while effected specimens show a decreased rate (6–10 pellets/min) (Sukhsangchan, 2014). Contaminated animals also stop feeding, however this does not appear theirs weight in the short-term (Phukaokaew, 2015).

CONCLUSION

Results of testing the toxicity of crude oil that show the effect on the morphology and behavior of the soldier crab *Dotilla wichmanni* as summarized as follows:

The median lethal concentration (LC_{50}) of Water Soluble Fraction (WSF) of crude oil on soldier crabs is given at a concentration 10.04 µg/l TPH during an exposure period of 96 hrs. Morphological change (*i.e.*, color changes of the cuticle and swelling of the maxillipeds, walking legs, and gills) are observable already at lower concentrations (5.96 µg/l TPH) and within shorter period (24 hrs). In parallel the behavior of soldier crabs change, the animals start to move faster than the control group. Affected soldier crabs try to move to the water surface, but sink back to the tank bottom and die finally. The no observed effect level (NOAEL) of Water Soluble Fraction of crude oil be less than 5.96 µg/l TPH to avoid a decline of soldier crab population.

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