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Toxic Effects of Naphthalene-Spiked Sediment to Freshwater Oligochaete *Limnodrilus hoffmeisteri* from Chao Phraya Estuary

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ABSTRACT

The lethal and sublethal effects of naphthalene-contaminated sediment to *Limnodrilus hoffmeisteri* were determined using a 96 h, static-toxicity test. It was found that naphthalene was highly toxic to worms with 72 and 96 h LC_{50} values of 85.11 and 60.26 μg/g wet wt, respectively. The 96 h EC_{50} values for autotomy and sediment avoidance were 35.48 and 58.88 μg/g wet wt, respectively. The 96 h LOEC reworking activity value was 25 μg/g wet wt which was more than two times lower than the LC_{50}. The results suggested the usefulness of *L. hoffmeisteri* as a test organism to determine the toxicity of sediment polluted with naphthalene. Changes of morphological features and in behavioral responses of the worm can be used as criteria for sublethal toxicity testing.

Key words: oligochaete, autotomy, sediment avoidance, reworking activity, naphthalene

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds primarily derived from petroleum and coal. It is well recognized that PAHs have a high toxicity for most aquatic organisms. High toxicity of PAHs has been found in association with the water-soluble fractions of aromatic compounds dominated by 2-3 ringed aromatics such as naphthalene (Anderson *et al*., 1974). Naphthalene is a very common semivolatile PAH found in numerous petroleum products. Due to its low molecular weight (128.18), less sensitivity to photo-oxidation and its persistent nature in water, naphthalene is considered to be extremely toxic to exposed aquatic animals (Vijayavel and Balasubramanian, 2006). Several research studies on the toxic effects of naphthalene to aquatic biota have been reported, such as on the mysid shrimp *Mysidopsis bahia* (Barron *et al*., 1999), the amphipod *Diporeia* spp. (Landrum *et al*., 2003), and the mud crab *Scylla serrata* (Vijayavel and Balasubramanian, 2006). Most of these studies have demonstrated a water-borne toxicity, with a lack of published data related to the toxic effects of naphthalene in sediments.

Tubificids are aquatic oligochaetes which are widely distributed. They play an important role in freshwater sediment dynamics (Reynoldson, 1987). They are often found in abundance, especially in habitats where environmental conditions are adverse for other affected animals. Many representatives of the freshwater oligochaetes are commonly used as test organisms for the testing of chemicals as well as for the ecotoxicological assessment of sediment

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contamination (Phipps *et al*., 1993). Thus, they were considered to be a suitable test organism for toxicity testing.

The purpose of this study was to investigate the acute toxicity of naphthalene to tubificid oligochaetes using spiked sediment, following the test method developed by Meller *et al*. (1998). Both the lethal and sublethal effects of the toxicant on *Limnodrilus hoffmeisteri* were determined. The test organisms were collected from the Chao Phraya estuary where previous studies have reported that this species accounted for the vast majority of benthic species found in the lower Chao Phraya River (Kanchana-Aksorn and Petpiroon, 2006).

MATERIALS AND METHODS

Test animal and sediment

L. hoffmeisteri samples were manually collected from the Chao Phraya riverbank located adjacent to Tok road, Bangkorlaerm District, Bangkok, Thailand. Sediments were washed in a 500 μm mesh sieve with regional spring water and the worms were transported to the laboratory. They were classified according to Brinkhurst and Jamieson (1971). The identification was also confirmed by C. Erseus of the University of Gothenburg, Sweden (pers. comm.). The worms were acclimatized for seven days before the experiment. They were placed in a 15×20×7.5 cm^3 plastic box containing 2-3 cm depth of wet, sieved sediment with any particles greater than 500 μm removed. Spring water brought from the sampling site was used for rearing. The worms were fed with decomposing, ground, fish-food flake and maintained under the following conditions with aeration: lighting, 12 h light/12 h darkness; temperature, 27.03±1.21°C; pH, 7.49±0.31; and dissolved oxygen, 5.36±0.97 mg/l.

The test sediment was collected from the same location where the worms were collected. The sediment was washed through a 500 μm mesh sieve to remove extraneous materials and other organisms. The general constituents of the test sediment were: sand, 23%; silt, 27%; clay, 50%; and TOM, 3.52%. It was stored at 4°C prior to the experiment.

Sediment spiking

Naphthalene-spiked sediment was obtained by adding 1.0 g naphthalene (99% purity, Ajax Finechem, Australia) dissolved in 20 ml ethanol into 500 g of sediment. The spiked sediment was gently stirred for 1 h at room temperature prior to being serially diluted with unspiked sediment to produce the required concentrations. An unaltered sediment control (negative control) and a solvent control (positive control) were prepared for this experiment. Sediment for the solvent control was prepared by adding 10 ml ethanol to 250 g unspiked sediment and stirring the mixture for 1 h.

Since there was no available data on the toxicity level of naphthalene to *L. hoffmeisteri*, a range-finding test with test substance concentrations between 0.1 and 1,000 μg/g wet wt was performed to determine the concentrations for the definitive test in the exposure study.

Test setup

The test was conducted using a 96 h, static, short-term sediment toxicity test. There were five replicate test chambers per treatment. Each test chamber consisted of a 600 ml glass beaker with 2-3 cm sediment depth and 400 ml of overlying water. A hundred worms per test chamber were used as test organisms. The worms were not fed and no aeration was provided. The water layer was covered with a sheet of paraffin, to prevent the evaporation of naphthalene. The test was conducted at room temperature with a 12:12 h light:dark photoperiod under the following conditions: pH, 6.91±0.25; and dissolved oxygen, 2.51±0.41 mg/l.

Exposure study

A suitable range for the definitive test was determined from nominal concentrations of naphthalene (6.25, 12.5, 25, 50 and 100 μg/g wet wt). Diluted sediment was subsampled into each test chamber and the overlying water was gently poured over the sediment. The test chambers were placed for a 24 h equilibration period in the dark before the test organisms were added. Once the sediment had settled, test organisms were sampled from the culture by sieving the sediment through a 500 μm mesh to retain the worms. The animals were then transferred into a Petri dish. Only undamaged, actively creeping worms of uniform size (approx. length 2.0-2.5 cm) were randomly selected.

To assess the endpoint of the experiment, mortality was checked daily and dead worms were removed from the test chambers as soon as they were observed. Test worms were considered dead when there was complete immobilization and no response to pressing with a blunt grass rod. Worms were also checked visually every 24 h to monitor any sublethal effects causing morphological and behavioral changes including autotomy, sediment avoidance and reworking activity. The guidance and methods for evaluating the effects of naphthalene on *L. hoffmeisteri* in this study followed those of Meller *et al.* (1998). Morphological changes in the worms were determined using a binocular microscope.

Data analysis

The median lethal concentration (LC_{50}) and median effect concentration (EC_{50}) for each endpoint and their corresponding 95% confidence levels at each test concentration were estimated using log-probit analysis (Finney, 1971) after 24, 48, 72 and 96 h. Since the endpoint mortality, autotomy and sediment avoidance were small, a no-observed-effect concentration (NOEC) value for each endpoint was defined as the highest concentration exhibiting an effect <10%, with a lowest, observed-effect concentration (LOEC) value, as the lowest concentration with an effect ≥10%. To estimate the reworking activity, a qualitative comparison at the replicate level was performed. The reworking activity of all animals in one test chamber was defined as reduced when the visible number of traces left by the worms was distinctly lower than in the control chambers. The maximum effect of the endpoint of the reworking activity was met when all replicates of a specific concentration level indicated reduced reworking activity. The NOEC value of reworking activity was defined as the highest concentration with no replicate demonstrating reduced reworking activity and the LOEC value of reworking activity as the next highest concentration.

RESULTS

During the regular exposure phase of the definitive test, no effect on *L. hoffmeisteri* was observed with concentrations up to 6.25 μg/g wet wt, as well as with both the negative control and the solvent control. However, worms were observed to be stressed in the four highest exposure concentrations from 12.5-100 μg/g wet wt.

An overview of all endpoint values is given in Table 1. The LC_{50} values decreased with increasing exposure time from 48 to 96 h. This clearly revealed that the toxic effect of naphthalene was in relation to the exposure concentrations (Figure 1). Due to the lack of toxicity in the experiments with an exposure time of 24 h and 48 h, the LC_{50} values were greater than 100 μg/g wet wt. Signs of mortality were evident at 48 h of exposure with the LOEC value being 50 μg/g wet wt. The worms were observed protruding their tails above the surface of the sediment but they did not respond to a gentle mechanical stimulus to their posterior end.

Observation of the worms exposed to sediment-associated naphthalene revealed that naphthalene caused autotomy. This was initially

observed at 96 h of exposure with a concentration of 25 μg/g wet wt. The posterior region of the worms initially became constricted, then isolated from their bodies. The isolated part then degenerated and the tail was lost. In contrast, the control worms appeared to have no morphological alterations. The percentage of worms undergoing this process of autotomy increased proportionally with the duration of the experiment (Figure 1) and the naphthalene concentration (Table 1). The 48, 72 and 96 h EC_{50} were found to be 83.17, 46.77 and 35.48 μg/g wet wt, respectively.

Table 1 Lethal and sublethal effects of naphthalene associated with sediment on *L. hoffmeisteri*.

Endpoint	Data analysis	Exposure time			
		24 h	48 h	72 h	96 h
Mortality	LC_{50}	>100	>100	85.11	60.26
	95% CI			83.92-86.30	58.83-61.69
	NOEC	50	25	25	25
	LOEC	100	50	50	50
Autotomy	EC_{50}	>100	83.17	46.77	35.48
	95% CI		82.00-84.34	45.14-48.40	33.51-37.45
	NOEC	25	25	25	12.5
	LOEC	50	50	50	25
Sediment avoidance	EC_{50}	>100	>100	97.72	58.88
	95% CI			96.74-98.70	57.37-60.39
	NOEC	50	25	25	25
	LOEC	100	50	50	50
Reworking activity	NOEC	50	25	25	12.5
	LOEC	100	50	50	25

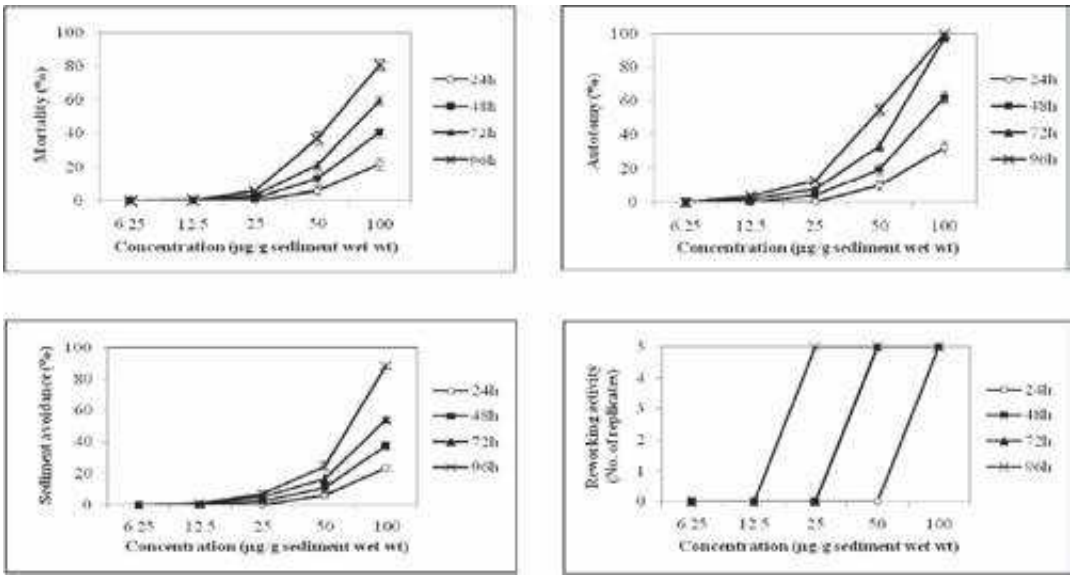

Figure 1 Effects of naphthalene on mortality, sediment avoidance, autotomy and reworking activity of *L. hoffmeisteri* after 24, 48, 72 and 96 h of test exposure in sediment.

Sediment avoidance by the worms was observed within 48 h from a value of 50 μg/g wet wt upwards. The EC_{50} values were 97.72 and 58.88 μg/g wet wt for 72 and 96 h, respectively. To describe this behavioral change, in the negative control and the solvent control, the worms were vertically distributed throughout the whole sediment. In contrast, the animals in the treatments did not burrow immediately throughout the contaminated sediment. These worms aggregated on the surface of the contaminated sediment, and then burrowed into the surface. The observation also demonstrated that most animals initially burrowed into the spiked sediment and then some of them returned to the sediment surface during the following 96 h. The body part of these worms became white, through disintegration. The color of the worms was paler than those in clean sediment.

With regard to the reworking activity, the worms were found to entirely burrow into sediment within a few minutes with the anterior portion hidden in the substrate. They temporarily inhabited burrows not necessarily vertical and then left traces as footprints while digging through the sediment. Complicated and dense burrows of *L. hoffmeisteri* were observed in the sediment that was deeper than 3 cm. The reworking activity of all animals was defined as reduced when the visible number of traces left by the worms was distinctly lower than that in the control sediments. From the assumption above, the results of this study showed that naphthalene influenced the reworking activity at a sediment concentration of 50 μg/g wet wt within 48 and 72 h and at a sediment concentration of 25 μg/g wet wt within 96 h.

DISCUSSION

All statistical values for *L. hoffmeisteri* obtained in this study are expressed as micrograms naphthalene per gram wet sediment because this work was carried out using toxicity tests with naphthalene dissolved in ethanol and spiked into the sediments. In addition, as *L. hoffmeisteri* is a benthic species, the decision was made to evaluate naphthalene toxicity in the sediments. In contrast, most reports on toxicity tests of naphthalene have placed considerable attention on the water-column since the statistical values were reported in milligrams or micrograms per liter, such as the work of Barron *et al.* (1999), Landrum *et al.* (2003) and Vijayavel and Balasubramanian (2006).

The 72 and 96 h LC_{50} values from this study were 85.11 and 60.26 μg/g wet wt, respectively. Although aquatic oligochaetes are commonly used in several sediment quality assessments (e.g., Kukkonen and Landrum, 1994; Weinstein *et al.*, 2003), there is a lack of studies on toxicity testing that have been conducted using naphthalene-spiked sediment exposures with this organism. Due to this limitation, it is difficult to determine the toxicity of sediment-associated naphthalene on these animals and other benthic invertebrates. However, a few data revealed the toxicokinetics of naphthalene in sediment using some aquatic species such as zebra fish *Brachydamio rerio* (Djomo *et al.*, 1996) and mollusk *Corbicula fluminea* (Narbonne *et al.*, 1999). The results from the current study have provided the first information on the tolerance of *L. hoffmeisteri* to naphthalene using a sediment toxicity test. It is also suggested that the more study that is carried out on the toxicity of naphthalene on oligochaete worms, the better confirmation there will be that it is a suitable test organism for sediment toxicity testing.

The LC_{50} values of naphthalene obtained in this study may be compared with those of other PAHs. In the present study, *L. hoffmeisteri* had a 96 h LC_{50} of 60.26 μg/g wet wt. This was considerably lower than the LC_{50} values for other aquatic oligochaetes. Weinstein *et al.* (2003) reported no mortality in the estuarine water tubificid oligochaete *Monopylephorus rubroniveus*

to fluoranthene sediment concentrations greater than 191,765 μg/g_{oc} dry wt for 10 days of exposure. Similarly, the freshwater oligochaete *Lumbriculus variegatus* had a seven-day LC_{50} value greater than 226 μg/g wet wt to sediment-associated pyrene (Kukkonen and Landrum, 1994). These values indicated that naphthalene was more toxic than other PAHs. It has been presumed that naphthalene was acutely toxic to aquatic invertebrates due to a low molecular weight and high hydrophilicity (Vijayavel and Balasubramanian, 2006) and it is commonly available in water and easily taken up by organisms. Work by Anderson *et al*. (1974) supported that middle distillates were extremely toxic to exposed aquatic animals because of the higher concentrations of two- and three-ring aromatics.

Although these results showed that naphthalene was highly toxic to aquatic organisms in sediment exposure, the toxicity levels of this chemical in the other studies were lower compared to data presented on other PAHs. Geyer *et al*. (1981) reported that aromatic hydrocarbon compounds with low solubility were more toxic than compounds with high solubility. Karcher *et al*. (1988) indicated that naphthalene was the least toxic due to its low coefficient of volatilization and could be rapidly volatilized. Moreover, it was found that the low adsorption rate of naphthalene in sediment, compared with anthracene, phenanthrene, pyrene and benzo(a) pyrene, was a factor affecting the decrease of PAHs bioavailability for zebra fish *Brachydamio rerio* (Djomo *et al*., 1996) and for mollusk *Corbicula fluminea* (Narbonne *et al*., 1999). The results from the current study could not be compared with the results reported by these authors due to the specific emphasis on naphthalene. Certainly, comparative sediment toxicity studies among various PAHs including naphthalene on aquatic oligochaetes is an area worthy of further investigation.

The results from microscopic examination revealed a naphthalene induced autotomy of the caudal region of *L. hoffmeisteri* from a threshold level of 25 μg/g wet wt at the end of the exposure time. Autotomy is a morphological response to chemical exposure which is known to occur in oligochaetes as a means of protecting themselves against an increase in internal concentrations of toxicants (Roberts and Dorough, 1984; Lucan-Bouche *et al*., 1999). However, the loss of segments may lead to the death of a single individual after an extended exposure period. This phenomenon was also observed by Lucan-Bouche *et al*. (2000) who found segmentation and disintegration of the rear part of the body of *Tubifex tubifex* exposed to copper induced fragmentation. The data from the current study verified that autotomy of *L. hoffmeisteri* was a valid endpoint of sublethal toxicity induced by naphthalene.

From the investigations in this study, it is known that *L. hoffmeisteri* has the ability to avoid stress induced by naphthalene associated with sediment at a concentration of 50 μg/g wet wt. Keilty *et al*. (1998) found that the burrowing behavior of oligochaetes was an excellent response variable for assessing pollutant impact on benthic communities. Their work stated that a worm was not considered to be burrowing if an estimated 75% of its body was above the sediment surface. However, very little published work has been presented on freshwater oligochaete behavioral responses to toxicant-contaminated sediment. McMurtry (1984) indicated that sublethal doses of copper and zinc increased the movement of *T. tubifex* and *L. hoffmeisteri* from the treated sediment to surrounding uncontaminated sediment. Similarly work reported by Meller *et al*. (1998) observed that these two worm species initially burrowed into sediment contaminated with lindane and copper sulfate and later returned to the sediment surface during the following 24 h of exposure.

The experiment with naphthalene-

contaminated sediment described here demonstrated that the reworking activity of *L. hoffmeisteri* was reduced within 96 h at a concentration of 25 μg/g wet wt. This phenomenon was in agreement with Meller *et al.* (1998) who noticed a change in the reworking behavior of tubificids when exposed to sediment-associated lindane and copper sulfate. The decline in reworking activity was accompanied by a reduction of worm biomass which reflected decreased feeding rates. These events were confirmed by Keilty *et al.* (1988) who investigated subacute responses of *L. hoffmeisteri* exposed to endrin in sediment. Likewise, Lotufo and Fleeger (1996) demonstrated that sediment contaminated with pyrene and phenanthrene caused a reduction in the egestion rate and reproduction of *L. hoffmeisteri*. The current study, however, assessed neither worm biomass nor feeding rate. Further investigations should consider these activities to better explain any reduction in the reworking activity which affects the population dynamics of *L. hoffmeisteri*.

In the present study, lethal as well as sublethal effects demonstrated a dose-response relationship. It was found that the EC_{50} values of autotomy for *L. hoffmeisteri* were nearly two times lower than the LC_{50} values, while the EC_{50} values of sediment avoidance were similar to the LC_{50} values. This level of response suggested that naphthalene had a greater potential to change morphological features than to directly kill individuals. The observations also implied that, by its loss, the posterior end of the worms may be more sensitive to chemical pollutants.

CONCLUSION

The results obtained in the present study indicated that sediment-associated naphthalene poses a high risk to *L. hoffmeisteri*. The change in the morphology and the behavior of *L. hoffmeisteri* due to the concentration of naphthalene-spiked sediments could become a useful biosensor in the detection of overloading by this pollutant.

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