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Changes in behavioural and physiological indices of medicinal leech exposed to crude oil

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Medicinal leeches (*Hirudo medicinalis*) were exposed for a 5-week period to the following concentrations of crude oil: 0.16, 0.33, 0.65, 1.3, 2.6, 5.2 and 10.4 g/l. Water and oil were renewed at 24-h intervals. Mobility and avoidance response were markedly increased in all test concentrations just after a new portion of oil had been added, while after 22 h increased mobility was recorded only in the 5.2–10.4 g/l concentrations and an increased avoidance response was observed only in the 1.3 g/l concentration. Changes in body shape occurred more frequently in groups of leech exposed to 0.33–10.4 g/l concentrations. A decrease of body weight was recorded after a 4-week exposure to 0.65–5.2 g/l concentrations. The possible reasons for the biphasic changes in body weight after exposure to the 10.4 g/l concentration are discussed. The amount of ingested blood was reduced after a 4-week exposure to the 0.33–10.4 g/l concentrations. No significant changes in excretion rate during the post-feeding period were observed. Thus, the most sensitive indices under study were avoidance response and mobility.

Key words: medicinal leech, crude oil, behaviour, physiological responses

INTRODUCTION

Toxicity of crude or refined oil and petroleum chemicals has been widely investigated using various marine organisms (Reish et al., 1997; Moles, 1998); however, their effects on freshwater organisms have largely been ignored. More recently, toxic effects of oil on freshwater invertebrates as well as terrestial species have been intensively studied (Jensen et al., 2002; Bhattacharyya et al., 2003) due to the increasing contamination of fresh-water, sediments and soils.

There are no available data on the influence of oil on the medicinal leech (*Hirudo medicinalis* L.). Investigation of this species is interesting from two points of view: first, the medicinal leech is an endangered species in various countries of Europe, second, it is very sensitive to heavy metal pollution (Флеров, 1989; Petrauskienë, 1999, 2001, 2003). Therefore, it seems promising to test the sensitivity of this animal to other toxicants including oil. The simplicity of keeping leeches under laboratory conditions as well as the simplicity of measuring various behavioural and physiological parameters makes the medicinal leech a good model animal for water pollution assessment. The advantages of using medicinal leeches in toxicity studies have been widely discussed in our previous papers (Petrauskienë, 2001, 2003). Behavioural and physiological indices of leeches (mobility, avoidance response, feeding activity, changes in body shape and weight, excretion rate) can be recorded comparatively easily.

The aim of the present study was to investigate changes in the behavioural and physiological responses of medicinal leeches during a long-term exposure to crude oil.

MATERIALS AND METHODS

Crude oil was sampled from the Girkaliai bore situated in Klaipëda region, Lithuania. The main characteristics of Girkaliai oil are as follows: density 815–827 kg/m^3; gas factor 49.1–52.3 m^3/t; boiling temperature 42 °C; sulphur content 0.07%; benzine fraction content 29.5–48%; benzine fraction consists of 71.06% of methane, 20% of naphthene and 8.9% of aromatic hydrocarbons.

Experiments were carried out on medicinal leech (*Hirudo medicinalis* L.) bred under laboratory conditions. Animals under study were 2 years old with the body weight 5–7.5 g. They were not fed 4 months before the initiation of experiments. Leeches were exposed to the following concentrations of crude oil: 0.16, 0.33, 0.65, 1.3, 2.6, 5.2 and 10.4 g/l. A semi-static experiment was performed; *e.g.*, water and oil were renewed at 24-h intervals. Animals were placed in standard 3-l glasses filled with 2 l of water; 5 specimens were placed in each glass. Twenty individuals were investigated for each test concentration of crude oil and 30 for control. Water specifications were as follows: t° 16–17 °C, pH 8.3–8.4, hardness 270–290 mg/l as $CaCO_3$, dissolved oxygen 7–9 mg/l (water was constantly aerated). Leeches were exposed for 5 weeks (wk) to crude oil; after 4 weeks they were fed and exposed for another 1 week period. The following behavioural responses were investigated: mobility (number of moving individuals within certain periods) and avoidance response (number of individuals escaping the test water). Behavioural responses were recorded twice a day in two observation sessions: one just after the water had been changed and a new portion of oil added, and the other 22 h following the change. The duration of sessions was 2 h. Multiple momentary recordings (every 10 min) of the number of animals that were moving in the test water or avoided the test water were performed. The number of animals with changes in body shape (that occur due to the permanent contractions of certain body muscles) was recorded every day during the observation sessions and during the water changing procedure. The strength of the attachment reflex and the weakness of the trunk muscles was evaluated every day during the water-changing procedure. The enhanced attachment reflex was considered in cases an animal was difficult to remove from the net or hand. The impaired strength of the trunk muscles was considered when an individual was feeble and soft. Leeches were weighed every week and an increase or a decrease of their body weight was evaluated. Changes of body weight were expressed in % considering that the initial body weight of animals was equal to 100%. After a 4-wk exposure to crude oil, leeches were fed fresh bovine blood that was pored into the pig intestine to which leeches can attach and pump out blood. Leeches were weighed before and after feeding trials. The size of blood meal was calculated as a difference between the post-feeding and pre-feeding weight and was expressed as a percentage from the pre-feeding weight. The rate of excretion followed 1 week after feeding was measured as a percentage of the weight lost during the period, *i.e.* as a difference between the post-feeding weight and the weight recorded 1 week after feeding expressed as a percentage of the post-feeding weight. A comparison of the mean value of mobility and avoidance response between control and treated groups was performed using the Mann–Whitney U test; changes in body shape, in the amount of ingested blood and in excretion rate were compared using chi-square citeria. Changes in body weight were considered significant when the difference between the control and the test value was more than 30%.

RESULTS AND DISCUSSION

We did not determine LC_{50} of crude oil for medicinal leeches, although some concentrations used in the present study caused lethal effects in the leech: 10% of animals died after 8 days of exposure to 10.4 g/l concentration of oil, 5% after a 5-d exposure to 0.65 g/l, and 5% after a 28-d exposure to 0.33 g/l. The mortality of larvae of rainbow trout exposed to the same crude oil used in the present study was observed in concentrations 7–14 g/l (Kazlauskienë, personal communication). Determination of LC_{50} requires a very large number of medicinal leeches. Our previous investigations of lethal effects of the other toxicants (heavy metals) showed that the 10-d LC_{50} of heavy metals for medicinal leech was very high, while sublethal effects were recorded at comparatively low concentrations (Petrauskienë, 2004). Therefore, it is more reasonable to investigate the long-term sublethal effects of toxicants on leeches. The priorities of investigation of long-term consequences of crude oil on invertebrates is discussed by Moles as well (2001).

Both behavioural responses (mobility and avoidance response) were markedly increased in all tested concentrations just after a new portion of oil had been added. There was no relation between the strength of concentration and the number of animals that were moving or escaping the test water (Figs. 1a and 2a). The mean value of these indices was much lower before oil changing, *i.e.* 22–24 h following oil changing (Figs. 1b and 2b). In the latter case, a significant increase in mobility was observed only in leech exposed to 5.2 and 10.4 g/l concentrations, while a significant increase of avoidance response was recorded only in leech exposed to 1.3 g/l. Most probably, a less pronounced avoidance response in 2.6–10.4 g/l concentrations of oil was due to the comparatively thick layer of oil that leeches had to cross performing avoidance behaviour. A marked increase of both indices just after a new portion of oil had been added indicates that the volatile fractions of crude oil caused a marked enhancement of these indices. The Girkaliai oil is composed mostly of the leight-weight hydrocarbons that evaporate rapidly. It is known that for crude oils the amount of the spill lost to evaporation can range from 20 to 60% within 24 h (Payne et al., 1983).

Short-term changes in body shape were recorded just after a new portion of oil had been added (within 2 h). Part of leeches exposed to 5.2 and 10.4 g/l responded with a strong contraction of the whole body (the other part began to move). After 20–30 min quite the opposite response appeared – elongation of the whole body which lasted 10–20 min. The strong contraction disappeared after a 1-week exposure to oil, while elongation response was recorded during the whole experimental period. Leeches exposed to 0.16–2.6 g/l concentrations showed only body elongation, which appeared 20–30 min following oil changing and gradually disappeared after 1–1.5 h. Contraction of the whole body is often observed un-

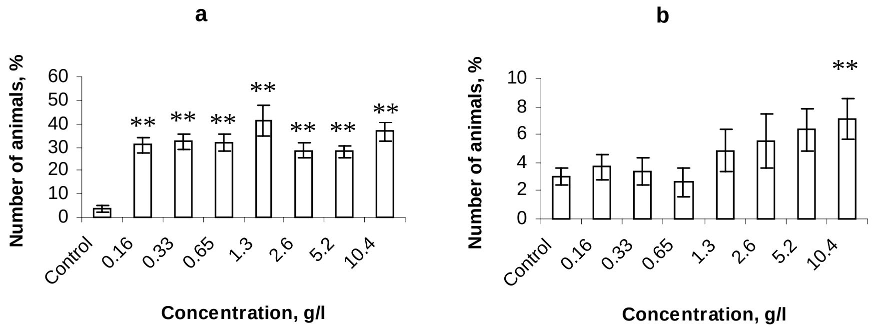

Fig. 1. Mobility of medicinal leech (number of animals (%) that performed various movements) just after oil was added (*a*) and after 22–24 h (*b*). The index significantly differs from the control value: * $p \le 0.05$, ** – $p \le 0.001$, *** – $p \le 0.0001$

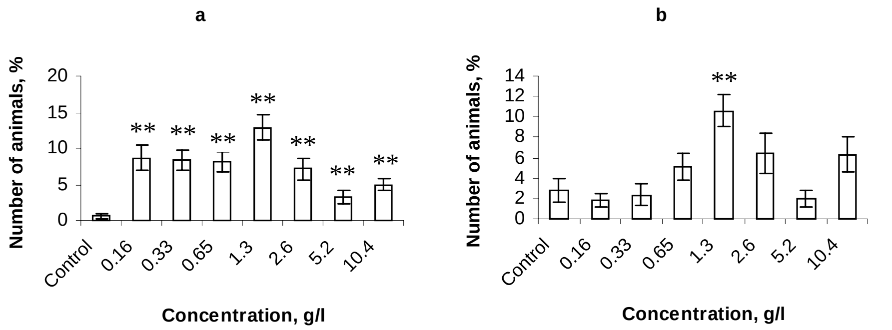

Fig. 2. Avoidance response (number of animals (%) that avoided test water) of medicinal leech just after oil was added (*a*) and after 22–24 h (*b*)
** and *** as in Fig. 1.

Table 1. **Long-term changes in body shape of medicinal leeches (number of animals with body distortions, %) during a 5-week exposure to various concentrations of crude oil**

Concentration, g/l	Exposure, wk				
	1	2	3	4	5
	Number of animals, %				
0 (control)	0	0	0	0	3.33
0.16	10	20	0	0	10
0.33	10	10	20	20	30*
0.65	10	20	10	30	40*
1.3	20	20	10	10	20*
2.6	10	10	30	10	30*
5.2	10	20	30	30	40*
10.4	30	30	20	20	40*

* *Significantly differs from the control value, p = 0.001.*

der natural conditions (the so-called whole body shortening reflex) when a mechanical stimulus is applied to the anterior part of the body. The shortening reflex is a rapid, almost synchronous contraction of all longitudinal muscles, for which the so-called fast-conducting neural system is responsible (Shaw, Kristan, 1995). Body elongation is observed in nature also as the so-called alert or resting postures (Sawyer, 1986). Leeches usually remain motionless holding these postures. Elongation of the body in our experiments was not a typical alert or resting posture, since leeches performed searching movements. Body elongation may occur due to a tonic contraction of the circular muscles all along the body.

Long-term changes in body shape were observed during 1–5 weeks of exposure. The body was distorted by narrowing or broadening some parts of the trunk. The leeches could have one or several narrowings of the trunk. The narrowing of the trunk appeared sometimes in control leeches as well (in our case it appeared in 1 specimen of 30), however, untreated leeches never had several narrowings of the trunk. The number of animals that had body distortions are represented in Table 1. One can see that the number of affected leeches varied during the experiment in different concentrations of oil. The majority of treated leeches had the highest number of distortions after 5 weeks of exposure to crude oil. A significant increase of the number of animals that had body distortions was observed in groups of leeches exposed to 0.33 g/l and higher concentrations. However, there was not a distinct relation between the strength of concentration and a number of animals with distortions. Changes in body shape occur due to permanent contractions of body muscles in certain segments that evoke a narrowing or broadening of some parts of the trunk. The distortion of body was observed after exposure to trichlorphon, phenol and heavy metals as well (Лапкина, Флеров, 1980; Petrauskienë, 1999, 2003). Changes in body shape of the leech have been shown to correlate with suppressed activity of the enzyme acetylcholinesterase (Лапкина и др., 1988). The reduced inactivation of acetilcholine leads to the permanent depolarization of muscles, therefore, a muscle is constantly contracted. The simple response – changes of the body shape – reflects disturbances in the enzyme activity that occur in the central nervous system of the medicinal leech. Other responses under study (mobility, contraction or elongation of the whole body) reflect the irritation or suppression effects of oil chemicals on the leech nervous system.

When manipulating leeches during water and oil changing procedures, it was possible to notice that the trunk of leeches became feeble after a 1-week exposure to 0.33–10.4 g/l concentrations of oil. The feeble leeches usually had an enhanced attachment reflex of the posterior sucker, as it was rather difficult to remove them from the net or hand. The weakness of body trunk and enhanced attachment reflex disappeared after 2 weeks of exposure to 0.33–0.65 g/l concentrations, while in stronger concentrations it was recorded during a 3-week exposure. Later it was recorded only in 10% of animals exposed to a 5.2 g/l concentration.

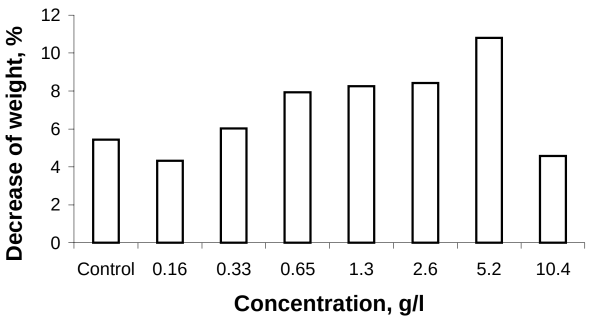

Fig. 3. Changes in body weight (a decrease of weight (%) considering the initial weight as 100%) of starving medicinal leech exposed for 4 weeks to crude oil

Changes in body weight of leeches after a 4-week exposure to crude oil are presented in Fig. 3. Oil concentrations 0.65–5.2 g/l evoked a significant decrease of body weight (differences from the control value were more than 45%). However, after a 4-week exposure to the highest concentration of oil (10.4 g/l) a decrease of weight was lower than in the control group. Certainly it does not mean that the 10.4 g/l concentration is less toxic to leeches. A more detailed analysis (comparison of changes every week) showed that leeches exposed to a 10.4 g/l concentration were losing their weight during the first two weeks of exposure, while during the third and fourth weeks their weight was increasing. Losing body weight may indicate the increased energetic demands needed for the detoxication of the organism. The increasing body weight in animals deprived of food indicates disturbances of osmoregulation processes. It might occur by several ways. The body fluids of leeches are hyperosmotic to the surrounding water, which is constantly penetrating the relatively thin semipermeable integument. Elimination of excess water is constantly performed by nephridia under normal conditions (Boroffka, 1968). The toxicity of crude oil may reduce excretion processes through nephridia or enhance the permeability of the integument, consequently the body weight may increase due to the increased water content in the leech. Some nephridiopores may be obstructed with oil particles reducing excretion as well. It is also known that leeches may ingest water and greatly increase their body weight (up to 60–100%) under the influence of lethal concentrations of phenol, trichlorphon and some other pesticides (Виноградов, Лапкина, 1976; Лапкина, Флеров, 1980). We did not observe leeches ingesting water, and an increase of body weight reached only 2.85% in our experiments.

Table 2. **Feeding activity (amount of ingested blood, in %) of medicinal leeches after 4-week exposure to various concentrations of crude oil**

Concentration, g/l	Amount of blood, %
0 (control)	45.11
0.16	42.96
0.33	8.75*
0.65	9.32*
1.3	11.11*
2.6	10.96*
5.2	10.16*
10.4	11.40*

* *See Table 1.*

The amount of blood sucked by leeches after a 4-week exposure to crude oil is shown in Table 2. Leeches exposed to a 0.16 g/l concentration ingested approximately the same amount of blood as did animals of control groups. Other treated leeches ingested a markedly smaller amount of blood: leeches exposed to 0.33–10.4 g/l concentrations ingested only 8.75–11.40% of their pre-feeding weights. There was no relation between the strength of concentration and a decrease of the ingested amount of blood. The impaired feeding activity of the medicinal leech under the influence of oil means that the impact of oil was rather dangerous to the leech. Feeding activity is very important for every species in general and for the medicinal leech in particular due to the infrequent feeding opportunities: adult leeches feed 1–3 times a year.

No significant changes in excretion rate during the post-feeding period were observed. A decrease of body weight (which reflects the intensity of excretion rate after feeding) during one week following feeding was 7–16% in different groups. This index varied greatly in different groups of the treated leech and in control groups as well.

CONCLUSION

The present study was the first step in evaluating the impact of oil on the medicinal leech. Short-term and long-term disturbances in leech behaviour and physiological functions were determined. A comparison of behavioural and physiological responses under study showed that mobility and avoidance response were the most sensitive indices: changes of these indices were recorded in animals exposed to the lowest investigated concentration (0.16 g/l) of crude oil. Impaired feeding activity, changes in body shape, weakness of body trunk muscle and enhanced attachment reflex were observed in animals exposed to 0.33 g/l and higher concentrations. A significant decrease of body weight was recorded under the influence of 0.65 g/l and higher concentrations.

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ÞALIA NAFTA PAVEIKTØ MEDICININIØ DËLIØ ELGESIO IR FIZIOLOGINIØ RODIKLIØ POKYÈIAI

Santrauka

Medicininës dëlës (*Hirudo medicinalis*) buvo veikiamos 5 savaites ðiø koncentracijø þalia nafta: 0,16, 0,33, 0,65, 1,3, 2,6, 5,2, 10,4 g/l. Nafta ir vanduo buvo keièiami kas 24 val. Judrumas ir vengimo reakcija stipriai padidëdavo tuoj po visø tirtø koncentracijø naftos pakeitimo; praëjus 22 val. padidëjæs judrumas iðlikdavo tik esant 5,2–10,4 g/l koncentracijos, o padidëjusi vengimo reakcija – tik esant 1,3 g/l koncentracijos naftai. Kûno formos pokyèiai daþniau atsirasdavo grupëse dëliø, paveiktø 0,33–10,4 g/l koncentracijos nafta. Kûno svorio sumaþëjimas registruotas po 4 sav. poveikio 0,65–5,2 g/l koncentracijos nafta. Aptartos dvifaziø svorio pokyèiø, atsiradusiø veikiant 10,4 g/l koncentracijos nafta, prieþastys. Prisiurbto kraujo kiekis buvo sumaþëjæs po 4 sav. poveikio 0,33–10,4 g/l koncentracijos nafta. Neaptikta patikimø ðalinimo procesø greièio pokyèiø pomitybos laikotarpiu. Taigi jautriausi ið tirtø rodikliø buvo vengimo reakcija ir judrumas.

Raktaþodþiai: medicininë dëlë, þalia nafta, elgsena, fiziologiniai pokyèiai