Toxic Effect of Benzene, Toluene and Formaldehyde to Response Behavior of Nematode (*Caenorhabditis elegans*) using Image Processing System

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Toxic, behavior, chemical, nematode and image processing system

ABSTRACT

Toxic Response behavior of nematode by exposure toxic chemicals is assessed using automatic recognition of line movement through image processing system under the microscope. The nematode *Caenorhabditis elegans* was exposed in different toxic chemicals to determine the toxic response. Toxicity of formaldehyde, benzene and toluene might affect on the movement behavior of nematodes. Toxic response behavior of nematode in three toxic chemicals have found to be similar. This study is identified some sequential line-movements of nematode that could be used as an alternative tool for the real-time monitoring of toxic substances in aquatic ecosystems in the future.

تقييم الاثر السمي للبنزين والتولوين والفورمالين على الاستجابة السلوكية للديدان الخيطية Nematodes (*Caenorhabditis elegans*) باستخدام نظام المعالجة التصويرية

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المُستلخص

تم تقييم سلوك الديدان الخيطية (Nematodes) عند تعريضها للسموم الكيميائية باستخدام الإدراك الذاتي للحركة الخطية عبر نظام المعالجة التصويرية المجهرية . تعرضت الديدان الخيطية (*Caenorhabditis elegans*) لأنواع مختلفة من المواد الكيميائية للتعرف على استجابتها للسموم. قد يتأثر سلوك حركة الديدان الخيطية بالمواد السامة الكيميائية عند تعريضها للبنزين والتولوين والفورمالين. أسفرت هذه الدراسة عن نتائج متشابهة في الاستجابة السلوكية للديدان الخيطية عند تعرضها للمواد الكيميائية الثلاثة (البنزين والتولوين والفورمالين). تمكنت هذه الدراسة من التعرف على بعض أنواع الحركة الخطية المتتابعة للديدان الخيطية والتي يمكن استخدامها كوسيلة بديلة لمراقبة المواد الكيميائية السامة في حينها عند دخولها إلى النظام البيئي المائي في المستقبل.

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الكلمات الدالة

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Introduction

The nematode *Caenorhabditis elegans* is widely used for studies of nervous system function and behavior. Considering that the *Caenorhabditis elegans* nervous system has a small number of neurons, all of which have been individually identified and characterized. Along with simple nervous system and feasibility in genetic manipulation (Huang and Schafer, 2007), behavior of the free living nematode *Caenorhabditis elegans*, has been a common model organism for genetic and developmental studies. The species garnered a special attention as an indicator species in the field of ecotoxicology (Gerhardt *et al.* 2002). Since 1990's response behaviors are regarded as sensitive to sublethal exposure to various toxic chemicals, (Lemly and Smith, 1986), (Dutta, *et al.,* 1992). Monitoring of the locomotor behavior has been an efficient means of evaluation of contaminated ecosystems with toxic chemicals (Teather *et al.,*2001). Effects of toxic chemicals on behaviors of organisms have been reported in various taxa, including crustaceans (Gerhardt *,et al.,*1998); (Abgrall, *et al.,*2000); (Roast, *et al.,*2000), snails (Ibrahim, *et al.,*1992), fish (Gray, *et al.,*1999); (Kwak, *et al.,*2002); (Oshima, *et al.,*2003); (Ji, *et al.,*2006) and insects (Chon, *et al.,*1998a); (Chon, *et al.,*1998b); (Ji, *et al.,*2007).

Continuous monitoring of movement behaviors requires automatic recognition of the line body shape for *Caenorhabditis elegans* Tracking of line-movement of *Caenorhabditis elegans* was reported by a group of researchers such as (Butcher, *et al.* 1999); (Hardaker, *et al.* 2001); (Baek, *et al,* 2002); (Weng, *et al.* 2003); (Geng *et al.* 2004); (Feng, *et al.* 2004); (Ajie, *et al.* 2005); (Stephens, *et al.* 2008). The reports stated above however, mainly concerned about introduction and establishment of tracking methods and pattern recognition of organisms. No extensive analysis of continuous line movements of *Caenorhabditis elegans* have been reported in response to toxic chemicals. Though lacking the full richness of a natural environment, this unconstrained motion allows for complex patterns of spontaneous motor behaviors (Croll, 1975), which are modulated in response to chemical, thermal and mechanical stimuli (Hedgecock and Russell, 1975). Here we explore the response behavior of the nematode, *Caenorhabditis elegans* in liquid media, based on continuous movement data, line tracking of test specimens was continuously conducted.

Materials and Methods

(1) Nematodes

Caenorhabditis elegans wild type, strain N_2 was collected from the Molecular Ecotoxicology Laboratory for Environmental Biomarker Research, University of Seoul, South Korea. The specimens were cultured in agar medium with *E. coli* (strain Op_{50}) as food on crystal-grade polystyrene Petri dish (60 mm x 15 mm) under dark condition at 20°C ± 3°C. To obtain synchronized cultures for the experiments, larval stage-4 was transferred to a new agar plate, allowed to develop up to young adult stage for 12 hours, and finally young adult individuals were selected for the experiments.

(2) Toxic Chemicals and Concentrations

K-media (2.38g potassium chloride and 3g sodium chloride salt dissolved in 1 liter DDW) was used as the control to accommodate the individuals in the arena. For treatment, benzene and toluene were dissolved in K-media to make 1 mg/l benzene or 1 mg/l toluene. For formaldehyde solution, 37% formalin was diluted in K-media to make 1mg/l formaldehyde

(3) Observation System

The movement of *Caenorhabditis elegans* was observed and recorded under an observation system consisting of an observation arena, a camera and a desktop computer with software for image recognition. Experimental animal was transferred to the observation arena (1.5 mm in depth, 4 mm in diameters) filled with K-media (control) or 1 mg/l formaldehyde/ benzene/ toluene (treatment). Each experimental animal was allowed to acclimate for 5 minutes before tracking. After the individual was transferred to the arena, cover glass was placed on top of the arena carefully to prevent the air bubbles formed within the arena. By this method noise (i.e. reflection from liquid surface) was eliminated for recognition of line movement through the image recognition system. The movement behaviors of the specimens were similar with and without the cover glass according to preliminary observation. The amount of oxygen was sufficient in the arena during the observation period and hypoxia of specimens was not observed. The observation arena was placed on a Petri-dish (9 cm in diameter) filled with water used as a cooling plate for the arena. The observation arena was surrounded by a channel which was also filled with same liquid as the observation arena to prevent evaporation of the formaldehyde solution (figure 1).

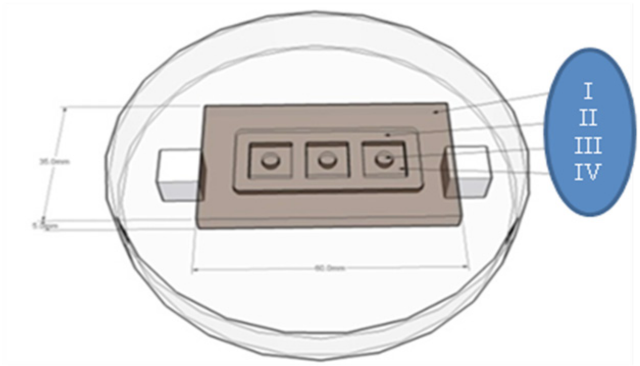

Figure 1: Petridis with Observation Plate (I. *Cover Glass*, II. *Channel*, III. *Observation Arena,* IV. *Observation Stage*)

(4) Detection of Line Movement and Analysis

The movement of the specimens was tracked for 3 hours from top view at every 0.25 second interval using a (Kukjae Electronics Co. Ltd.; IVC-841, CCTV) camera both for the control and treatment animals. The back light condition (back light; 0.2 Watt green diodes) was provided underneath the observation cage to enhance visual recognition of the specimens.

Considering the complexity in calculating the line movement of long animals (Baek, *et al.* 2002); (Geng, *et al.* 2003); (Feng, *et al.* 2004); (Ajie, *et al.* 2005); (Stephens, *et al.* 2008), we intended to demonstrate the structural changes were originated from segments (figure 2).

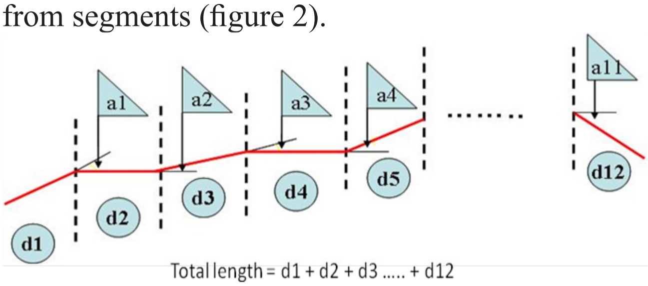

Figure 2: Schematic Diagram of *C. elegans* Body is Divided into Twelve Sub-segments and Showing the Segments Length (*e.g.,* d1, d2, *etc*) and the Angles (*e.g.,* a1, a2, *etc*).

The parameters such as speed, acceleration, stop duration, stop number, turning rate and meander were measured for each point by using MATLAB (version 7.0.1.). The lines presenting the body of the specimens were extracted based on subtraction from the background image and skeletonizing. The head and tail of the line body were recognized according to the activity and movement sequences identified (Baek, *et al.,* 2002); (Geng, *et al.,* 2003); and (Stephens, *et al.,* 2008). After extraction of the line body shape, the line was evenly divided into twelve segments and, numbers were assigned to points from 1 to 13 in order from head to tail (see, figure 2). The length of each segment and the curvatures between the neighboring segments were recorded in each time interval.

Results and Discussion

(1) Results

To monitor the sequential line-movements of *Caenorhabditis elegans*, six parameters on each 13 points were measured. The average values of speed, acceleration, stop time, stop duration, turning rate and meander were compared with control and treatment at 1 mg/l of formaldehyde, benzene and toluene (figure 3).

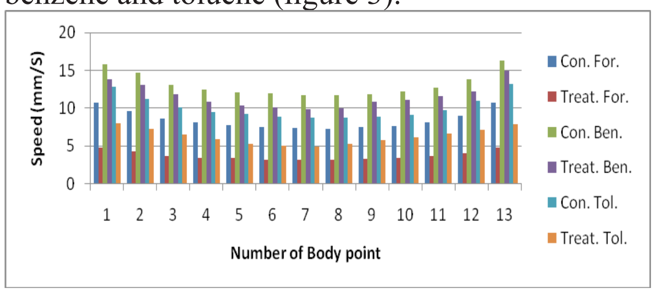

Figure 3A: Speed

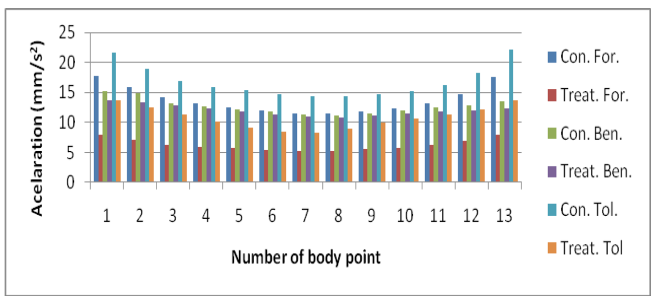

Figure 3B: Acceleration

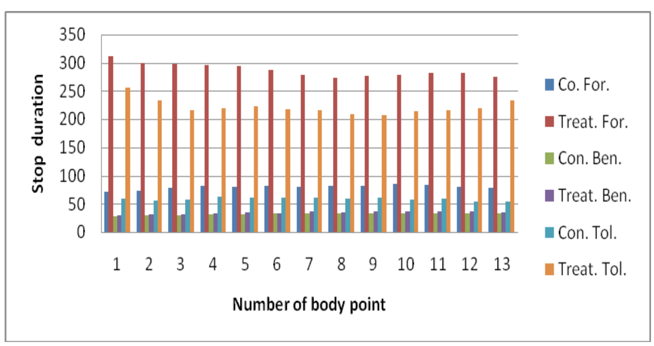

Figure 3C: Stop Duration

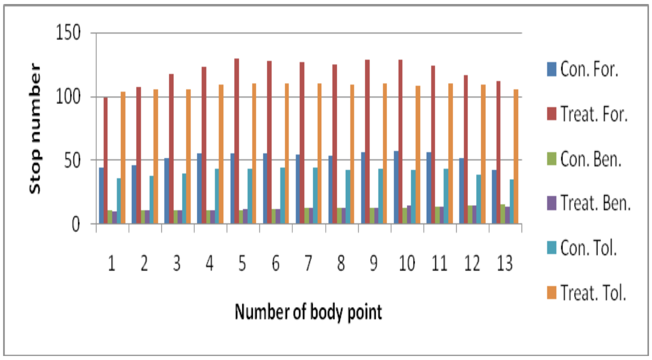

Figure 3D: Stop Number

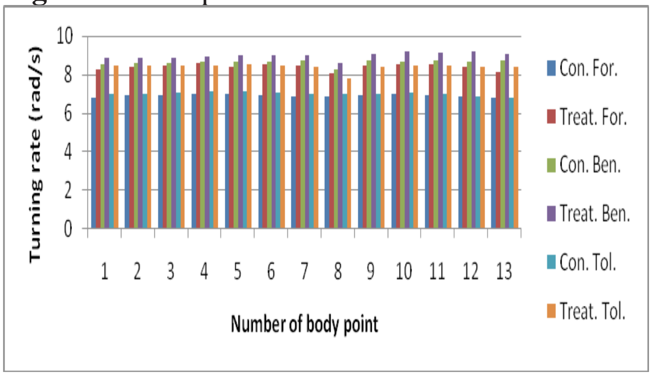

Figure 3E: Turning Rate

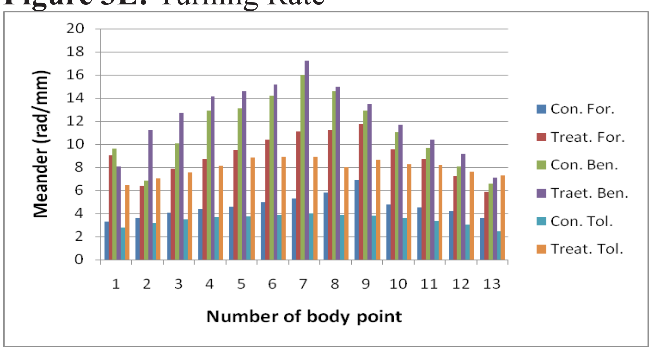

Figure 3F: Meander

Figure 3(A-F): Comparison of Line Tracking Parameters of *Caenorhabditis elegans* Treated with 1 mg/l of Formaldehyde, Benzene and Toluene. ((3A) *Speed*, (3B) *Acceleration*, (3C) *Stop Duration*, (3D) *Stop Number* (3E) *Turning Rate*, (3F) *Meander*. *(Con.) *means Control,* (Treat.) *means treatment,* (For.) *Indicates Formaldehyde,* (Ben.) *Indicates Benzene, and* (Tol.) *Indicates Toluene*).

The results show that the average values of speed and acceleration were lower than that of control. Among the three chemical, formaldehyde has shown maximum reduction of movement behavior followed by toluene and benzene. However the average values of speed, acceleration, stop duration stop number and meander shown more differed at all points along the body before and after treatment with 1 mg/l formaldehyde, benzene and toluene (figure 3).

Speed and acceleration are increased in a higher degree at end of the body after the treatments, while stop number and meander increased at the points located in the center of the body. This indicated that body movement was changed after the chemical treatments, more curvature occurred in the center along with higher speed at the end.

Angle values for control and treatments were almost smaller at all points (figure 4) and anglesa1~a11, were matched with figure 2, (see, figure 2). But after treatment with 1 mg/l formaldehyde, benzene and toluene, the values of angles were relatively increased in the center position. The angle values at 1 mg/l formaldehyde as well as benzene and toluene were higher than control at all points, the discrepancies between the experiments were relatively higher in the center positions. This indicated that the body of the treated nematode tended to contract and fold strongly after the treatments with 1 mg/l formaldehyde, benzene and toluene.

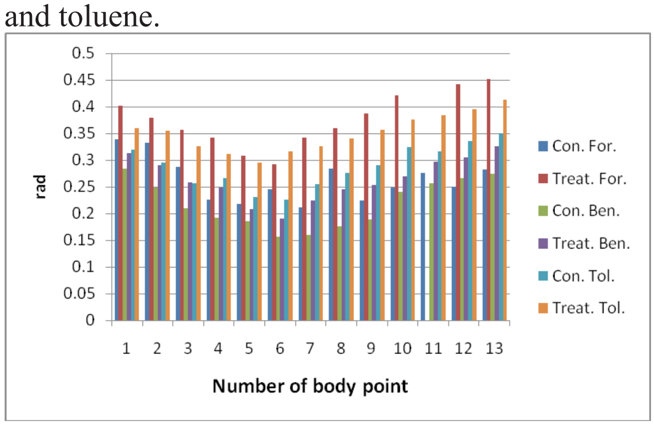

Figure 4: Comparison of Angles of the Sub-segment of *C. elegans* Treated with 1 mg/l Formaldehyde, Benzene, and Toluene before and after the Treatments.
*(Con.) *means Control,* (Treat.) *means treatment,* (For.) *Indicates Formaldehyde,* (Ben.) *Indicates Benzene, and* (Tol.) *Indicates Toluene*).

(2) Discussion

Sequential line-movements of *Caenorhabditis elegans* were used to detect toxic response behavior. Movement parameters and body shapes were useful for quantifying the response behaviors.

To compare the line-movement parameters, partial differentiations between the control and those treated with formaldehyde, benzene and toluene were increased little after treated with chemicals, except speed and acceleration. The speed and acceleration decreasing directly after treatment of formaldehyde, benzene and toluene, indicated a toxic effect on locomotor behavior. The values of speed and acceleration decreased due to the toxic effect of chemicals and thus the movement pattern of *Caenorhabditis elegans* shown greater toxic response to 1 mg/l formaldehyde, followed by 1 mg/l toluene and benzene. Behavioral data is difficult to analyze due to many factors (biotic and abiotic) involving in a complex manner (Chon, 2011). Behavioral response to toxic chemical indicates valuable information in establishing environmental assessment guidelines (Teather, *et al.* 2001).

This study demonstrated swimming movement behavior that are efficiently extracted through different parameters to illustrate outline of behavioral responses to chemical stressors. The comparison of pattern composition illustrated that the movement tracks were dominated by the active and wide ranging movement patterns before the treatments, while the tracks were mainly covered with non-active patterns with low speed, strong turning rate and meander after the treatments (see, figure 3). This indicated that *Caenorhabditis elegans* showed more abnormal behaviors under the chemical stressors. Same observation has been reported in *Daphnia magna* (Liu ,*et al.* 2010). The angles of the body segments showed more significant differences than movement parameters. The Angle values were comparatively increased after treatment of 1 mg/l formaldehyde, benzene and toluene, especially at a4, a5, a7, a9-a13. This indicated that the body of the treated nematode tended to contract and fold strongly after the treatments.

Caenorhabditis elegans has been shown to be a suitable test organism for ecotoxicological assessments of sediments and soils (Hoss, *et al.* 2001). Researchers reported that *Caenorhabditis elegans* has several locomotives behavior such as rapid withdrawal, crawling, body reversal and helical swimming. These are stereotyped behaviors that can be used for sub-lethal toxicology (Rogge and Drewes 1993); (Ding, *et al.* 2001). The change of movement patterns observed after treatment such as short turns and shaking and backward movements were not clearly presented with the movement patterns in short sequence. According to authors experience these patterns tended to be more effectively detected in some longer sequences. This study is identified some sequences of line-movements of the nematode that confirmed the toxicological effect on the nematode's behaviors.

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