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Note

Movement Responses of Sludge Worm *Tubifex tubifex* (Annelida, Oligochaeta) in Three Different Copper Concentrations

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Abstract Monitoring and assessing aquatic ecosystems using the behavior of organisms is essential for sustainable ecosystem management. Oligochaetes, which inhabit various freshwater ecosystems, are frequently used to evaluate the environmental conditions of freshwater ecosystems. *Tubifex tubifex* (Müller, 1774) (Oligochaeta, Tubificidae) is tolerant to organic pollution and has been used to evaluate the toxicity of toxicants, including heavy metals. We studied the behavioral responses of *T. tubifex* to three different copper concentrations (0.1, 0.5, and 1.0 mg L^{-1}). The specimens were exposed to copper in an observation cage containing 150 mL of dechlorinated water. Movement behavior (diameter, speed, acceleration, meander, and turning rate) was continuously observed for two hours before and after the copper treatments. After the treatments, the diameter shrank and showed rapid twisting movement under all the copper conditions. The turning rate had a positive correlation with meander and acceleration both before and after treatment at all three concentrations, whereas speed and meander had a negative correlation. Length and turning rate also showed a negative correlation. The correlation coefficient between speed and acceleration in the highest copper concentration changed from positive before treatment ($r=0.64$) to negative ($r=-0.52$) after treatment. Our results present the possibility of using behavioral parameters to detect copper contamination in freshwater ecosystems.

Key words: movement behavior, quantitative behavior, toxicity monitoring, oligochaetes, heavy metals

INTRODUCTION

Heavy metals are important environmental pollutants because of their toxicity, persistence in the environment, and bioaccumulative nature (Ali *et al.*, 2019). Although copper is an essential trace element in living organisms (Kumar and Shin, 2017), it exerts toxic effects on organisms following long-term exposure to high concentrations.

Aquatic oligochaetes are frequently used as indicators of organic pollution in freshwater (Kang *et al.*, 2017a) and in toxicity assessments because of their strong resistance to environmental pollution (Kang *et al.*, 2016; Kang *et al.*, 2017b). Toxicants affect various biological events in aquatic oligochaetes such as their survival, death, growth, development, reproduction, and appearance (Johnson *et al.*, 1993; Reynoldson *et al.*, 1996; Rodriguez and Reynoldson, 2011). Of these, *Tubifex tubifex* (Müller, 1774) has been frequently

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used to evaluate the toxicity of toxicants (Kang *et al.*, 2016). *T. tubifex* (also called sludge worms or sewage worms) is widely distributed in freshwater, lives in the water/sediment interface (Bouché *et al.*, 2000) and plays an important role in purifying toxic substances (Lucan-Bouché *et al.*, 1999). *T. tubifex* is relatively resistant to heavy metals (Milani *et al.*, 2003), showing various responses, including the inhibition of growth and reproduction and bioconcentration for copper exposure (Thit *et al.*, 2021). Reynoldson *et al.* (1996) reported that 48 h LC50 of copper was 0.18 and 0.26 mg L^{-1} in two populations of *T. tubifex* from the North American Great Lakes and Northern Spain, respectively. Maestre *et al.* (2009) revealed the 48 h LC50 of copper to *T. tubifex* was 0.1 mg L^{-1} in laboratory cultures.

Behavioral responses indicate the responses of organisms to environmental and physiological changes. Thus, measuring the behavioral responses of *T. tubifex* is important for determining changes in their biological traits under various environmental conditions (Bae *et al.*, 2021). The behavioral responses of *T. tubifex* were more sensitive than lethal effects for Cu, Cd, and Imidacloprid (Gerhardt, 2009). In addition, *T. tubifex* shows rapid twisting behavior in the early stages of exposure to high concentrations of copper, followed by a decrease in movement, decolorization, and collapse (Rathore and Khangarot, 2003). Meanwhile, the helical swimming behavior is more sensitively affected by exposure to copper than body reversal behavior in another tubificid red worm, *Limnodrilus hoffmeisteri* (O'Gara *et al.*, 2004). However, the differences in the behavior of *T. tubifex* as well as the median lethal concentration (LC50) of various toxicants depend on the origin of the tested organisms in Korea. Therefore, this study was aimed at evaluating the effects of copper on the behavioral responses of *T. tubifex*.

MATERIALS AND METHODS

1. Test organisms

The sludge worm *Tubifex tubifex* (Müller, 1774) was used to test the treatment of copper ($CuSO_4$) at three different concentrations (0.1, 0.5, and 1.0 mg L^{-1}). The test organisms were obtained from a farm supplying sludge worms for fish food in Korea (http://www.bizidduk.com). Before experimenting, we identified the test organisms using mitochondrial cytochrome oxidase subunit 1 (CO1) of test organisms in the GenBank of NCBI (https://www.ncbi.nlm.nih.gov/). A stock population was maintained in an aquarium with dechlorinated tap water (water temperature: 22±1°C; L16 : D8) and a 10 cm layer of sand at the bottom, and food (Tetra Bits, Tetra®) was supplied twice a week (Wilt and Wessells, 1967). The test organisms were acclimated under the same experimental conditions for at least one week before the experiments.

2. Observation of movement behaviors

The test organisms were randomly chosen from the stock population. Glass observation cages (10×10×5 cm^3, horizontal×vertical×height) were installed inside a constant-temperature water bath (DWB-22) at 22°C to observe the movement behaviors of the specimens. The observation cages were filled with 150 mL of dechlorinated tap water. The movement behavior of the specimens was observed before and after treatment with copper ($CuSO_4$) in the observation cages. A single worm was gently placed at the center of the observation cage. We observed the behaviors for two hours (1 h before copper treatment and 1 h after copper treatment) using a webcam (Logitech webcam C905). The observations were repeated for 10 different individuals at three different concentrations (0.1, 0.5, and 1.0 mg L^{-1}).

3. Measurement of parameters in the movement behaviors

Based on our experience with test organisms' behaviors and suggestions in previous studies (Park *et al.*, 2005; Ji and Park, 2012), we can classify the behavior of test organisms into five behavioral parameters (i.e., diameter, speed, acceleration, turning rate, and meander). The movement track (i.e., the position of the test organism in the observation cage) of each individual was recorded every 10 s from the recorded video images using an image-processing tool (i-Solution version 2001, iMTechnology). Then, the behaviors were extracted from the movement tracks using an image-processing program in MATLAB (version 7.9.0) (The Mathworks, 2001). The parameters are defined as follows:

- Diameter (mm): diameter of the smallest circle covering the entire body of the specimen.
- Speed (mm s^{-1}): Average movement speed.
- Acceleration (mm s^{-2}): changing speed ratio
- Turning rate (rad s^{-1}): the sum of angular changes in radi-

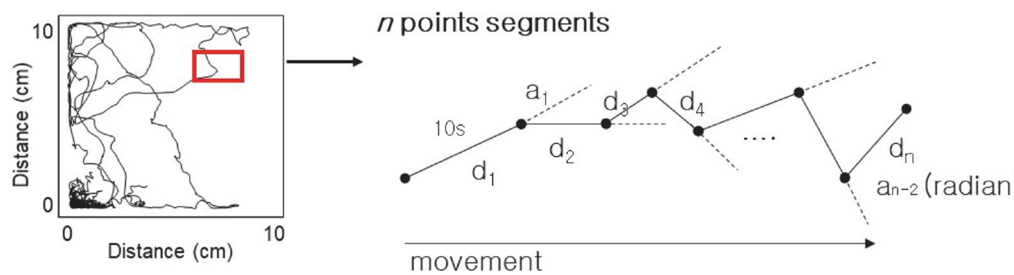

Fig. 1. Schematic diagram to measure behavioral parameters in the segment of movement track.

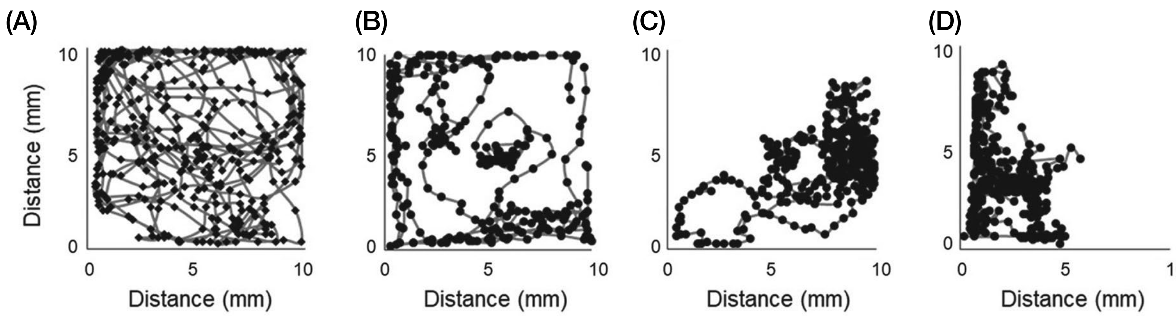

Fig. 2. Examples of movement tracking of *T. Tubifex* by different Cu concentrations (10 × 10 × 5 cm, glass cage). (A) without treatment, (B) 0.1 mg L^{-1}, (C) 0.5 mg L^{-1}, (D) 1.0 mg L^{-1}.

ans in absolute values divided by the cumulative duration of the movement.

− Meander (rad mm^{-1}): angle change per movement distance

Behavioral parameters of the tested organisms, such as body length (mm), speed (mm s^{-1}), acceleration (mm s^{-2}), turning rate (radian s^{-1}), and meander (radian mm^{-1}), were measured based on the position of the head of the specimen. Fig. 1 shows a schematic diagram for measuring the behavioral parameters. Details of the measurements are reported in Ji and Park (2012).

4. Statistical analysis

To determine the behavior of *T. tubifex* at different copper concentrations, we analyzed the behavioral parameters in three steps. First, a paired *t*-test was conducted to evaluate the differences before and after copper treatment. Second, correlation coefficients between behavioral parameters were calculated at three different concentrations of copper to determine the relationship between the parameters. Third, classification and regression trees (CART) were constructed to differentiate between movement behaviors before and after treatment. All analyses were conducted using R (R Core Team, 2020). A paired *t*-test and correlation coefficients were calculated using the package 'stats' (R Core Team, 2020) and CART was conducted using the package 'rpart' (Therneau and Atkinson, 2019) package in R.

RESULTS AND DISCUSSION

Before copper treatment, the test organisms moved widely in the observation cage, whereas the organisms with copper treatments moved in a relatively narrow area (Fig. 2). Similarly, as the copper treatment concentration increased, the movement tracks were limited to certain areas of the observation cages. Individuals exposed to 0.1 mg L^{-1} copper seemed to move on a larger area than those exposed to 1.0 mg L^{-1}. Behavioral parameters were significantly different before and after copper treatment at high concentrations (i.e. 1.0 and 0.5 mg L^{-1}) (paired *t*-test, $p < 0.05$) (Fig. 3). Diameter (mm) significantly decreased at all tested concentrations (paired *t*-test, $p < 0.05$), whereas acceleration (mm s^{-2}), turning rate (radian s^{-1}), and meander (radian mm^{-1}) increased significantly (paired *t*-test, $p < 0.05$). However,

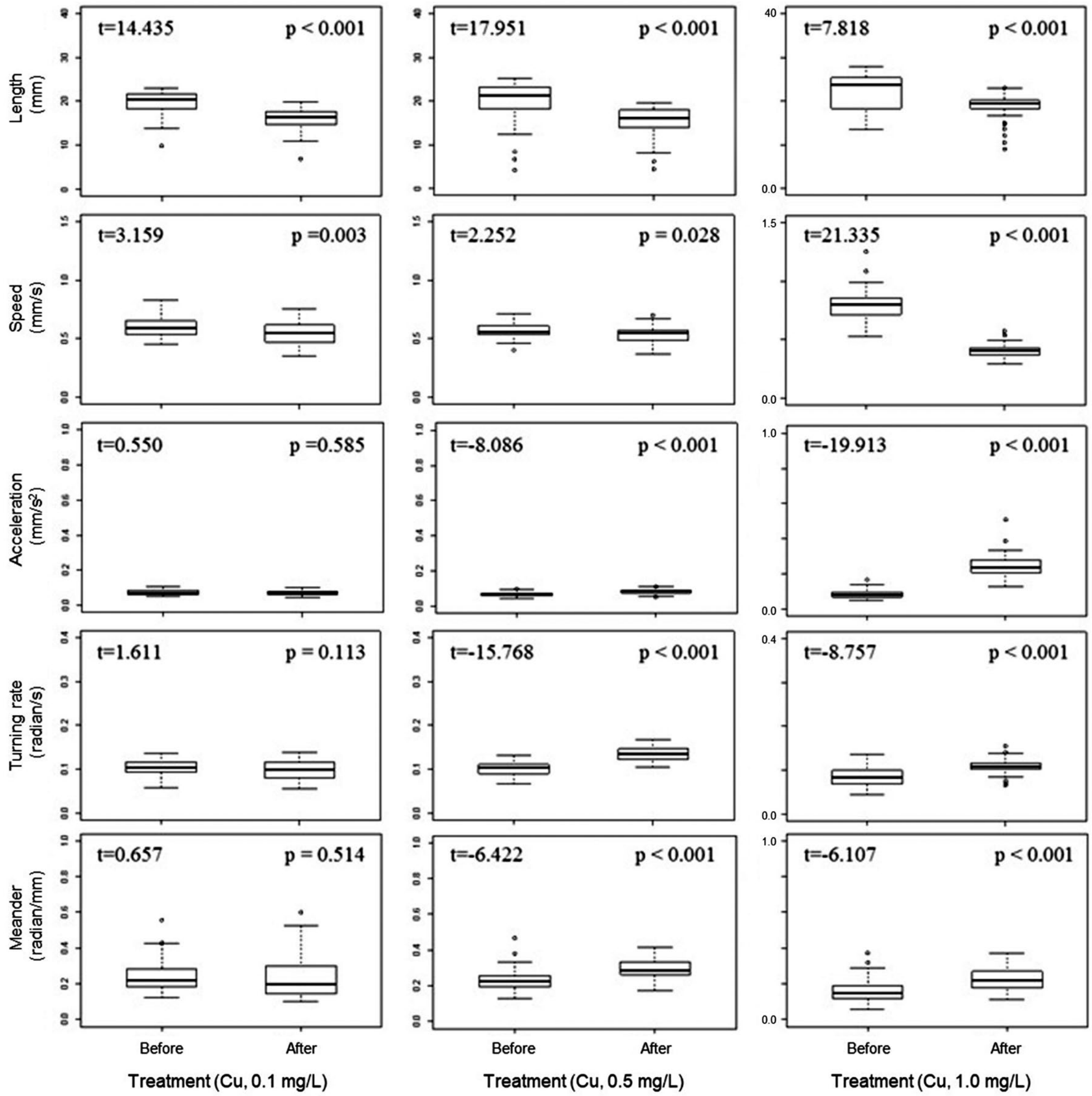

Fig. 3. Comparison of behavioral parameters between before and after treatments of copper at three different concentrations. Paired *t*-test was conducted.

behavioral parameters (length and speed) were significantly different only at low concentrations.

Correlation coefficients between behavioral parameters changed at the three different concentrations of copper (Table 1). The coefficients mostly showed similar patterns before and after copper treatment. For example, the overall turning rate was positively correlated with meander and acceleration both before and after treatment at all three concentrations ($p < 0.001$), whereas speed and meander were negatively correlated ($p < 0.001$). Length and turning rate also showed a negative correlation ($p < 0.001$). Speed and acceleration had strong positive correlation at both before and after treatments of 0.1 and 0.5 mg L^{-1} ($r > 0.6$, $p < 0.001$). However, the correlation coefficient between speed and acceleration was opposite before ($r = 0.64$, $p < 0.001$) and after the treatment ($r = -0.52$, $p < 0.001$) at 1.0 mg L^{-1}.

Movement patterns differed before and after the treatment based on the differences in five behavioral parameters through CART (Fig. 4). The movement patterns were more precisely classified at higher concentration treatment (Prediction of accuracy (ACC) = 0.83 at 1.0 mg L^{-1}) than at the low concentration (ACC = 0.71 at 0.1 mg L^{-1}). Speed and length

Table 1. Correlation coefficients between behavioral parameters at three different concentration of copper treatments.

Concentration (mg L^{-1})	Treatment	Parameter	Diameter	Speed	Acceleration	Meander
Cu 0.1	Before	Speed	0.16			
		Acceleration	−0.17	0.63		
		Meander	−0.18	−0.52	−0.30	
		Turning rate	−0.35	−0.26	0.32	0.61
	After	Speed	0.24			
		Acceleration	0.03	0.65		
		Meander	−0.34	−0.43	−0.30	
		Turning rate	−0.45	−0.06	0.37	0.56
Cu 0.5	Before	Speed	0.21			
		Acceleration	−0.03	0.69		
		Meander	−0.16	−0.67	−0.28	
		Turning rate	−0.30	−0.24	0.38	0.64
	After	Speed	−0.09			
		Acceleration	−0.23	0.86		
		Meander	−0.02	−0.64	−0.37	
		Turning rate	−0.26	0.19	0.56	0.41
Cu 1.0	Before	Speed	0.30			
		Acceleration	−0.05	0.64		
		Meander	−0.38	−0.57	−0.22	
		Turning rate	−0.48	−0.32	0.29	0.65
	After	Speed	0.05			
		Acceleration	−0.14	−0.52		
		Meander	−0.22	−0.32	−0.14	
		Turning rate	−0.41	−0.08	0.14	0.61

Statistical significance levels: $r > |0.13|$, $p < 0.05$

were included as important parameters influencing the behavior of the test organisms at all concentrations. At the 1.0 mg L^{-1} treatment, speed was the first parameter to differentiate between the patterns, followed by acceleration and length (Fig. 3A). At the 0.5 mg L^{-1} treatment, meander was the first parameter, and speed and length were selected as major parameters influencing the behavior of test organisms (Fig. 3B). At 0.1 mg L^{-1} concentration, length was the most important, followed by the turning rate and speed (Fig. 3A).

In this study, the behavioral parameters of *T. tubifex* in response to copper treatment were examined. Copper is widely used in fertilizers and pesticides (Ji *et al.*, 2020). However, if high concentrations of Cu flow into freshwater ecosystems, freshwater biodiversity can be disrupted. We found that different copper concentrations resulted in differences in the behavior of *T. tubifex*. *T. tubifex* reduced diameter by contracting or twisting the body when exposed to copper. Acceleration, turning rate, and meander increased with copper treatment, indicating that the movement direction of *T. tubifex* frequently changes when exposed to copper. Therefore, it can be interpreted that *T. tubifex* turns its body as a result of the stimulation. The decrease in speed indicates that copper stimulation resulted in a decrease in the distance traveled by *T. tubifex*. This also shows a negative correlation between speed and acceleration at high a concentration (1.0 mg L^{-1}). These changes in motility are thought to be useful for the early detection of contaminants such as copper. This might have caused the high tolerance of *T. tubifex* to pollutants. Therefore, *T. tubifex* could be useful for detecting high concentrations of copper in polluted areas. Although our results show that the behavior of *T. tubifex* could differ according to the gradient of anthropogenic disturbances, such as different

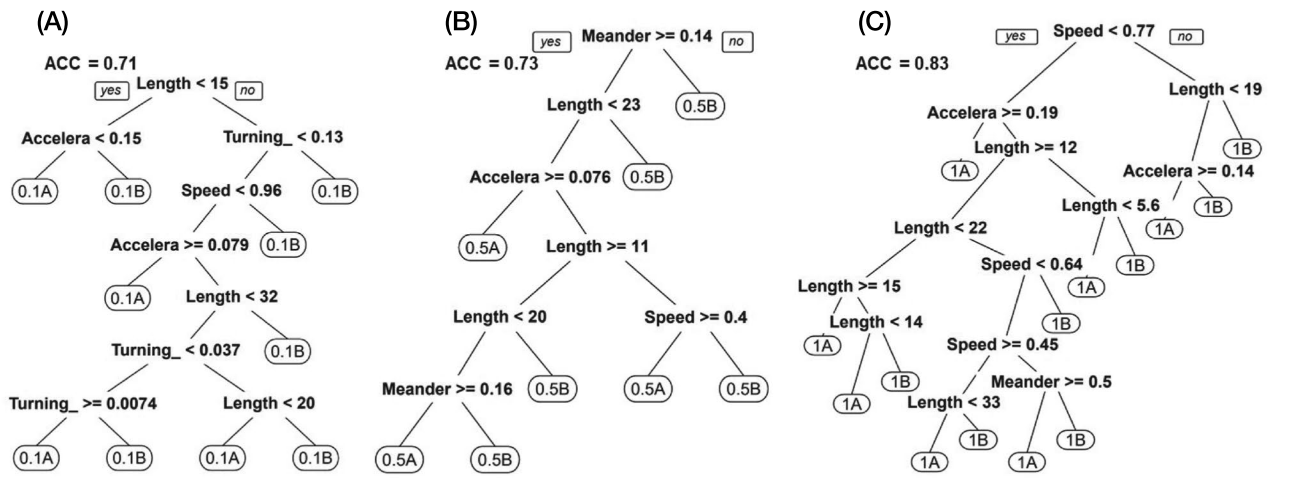

Fig. 4. Discrimination of behavioral patterns between before and after treatments of copper based on five behavioral parameters responding to three different concentrations of copper through CART. (A) 0.1 mg L^{-1}, (B) 0.5 mg L^{-1}, (C) 1.0 mg L^{-1}. The alphabet in the final nodes indicates before (B) or after (A). The number 0.1, 0.5 and 1 in the final nodes represent the concentration of Cu treatment.

copper conditions, our research has some limitations. When we conducted the behavioral experiments in the observation cages, we used dechlorinated tap water without sediments to analyze the full body shape of *T. tubifex*. *T. tubifex*, which is a burrower, inhabits both stream sediment and water (Mermillod-Blondin, 2011; Kornijów *et al.*, 2021) and feeds on organic particles from sediments (Kornijów *et al.*, 2021). Thus, the toxicity of sediment can be more valuable than that of water in determining the behavior of *T. tubifex*. Some researchers have tried to conduct experiments with spiked sediments using *T. tubifex*, however, they only considered EC50 (Meller *et al.*, 1998), LC50 (Maestre *et al.*, 2009), or lethal concentrations (Meller *et al.*, 1998), which are higher than the concentrations used in the study of behavior patterns. Further research into the behavioral differences, such as vertical movement, in *T. tubifex* in toxic sediments, is necessary to understand their behavioral changes in freshwater ecosystems.

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Author contribution Conceptualization: Y.-S. Park, M.-J. Bae, Experiment and data analyses: H. Kang, Writing: H. Kang, M.-J. Bae, Y.-S. Park, Editing: Y-S. Park, Review: all authors

Conflict of interest The authors declare no conflict of interest.

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