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Environmental Pollution 120 (2002) 513–516

ENVIRONMENTAL POLLUTION

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New initiatives

Measurement of movement patterns of *Caenorhabditis elegans* (Nematoda) with the Multispecies Freshwater Biomonitor® (MFB)—a potential new method to study a behavioral toxicity parameter of nematodes in sediments

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Received 1 March 2002; accepted 11 May 2002

"Capsule": *The Multispecies Freshwater Biomonitor® (MFB) shows promise in monitoring nematodes in sediments.*

Abstract

The nematode *Caenorhabditis elegans* receives increasing attention in sediment ecotoxicology and new toxicity tests with sensitive test parameters are under development. In this study, the motility of *C. elegans* could be measured for the first time online in sediment, using the Multispecies Freshwater Biomonitor. Whereas single nematodes could not be recorded, groups of 10 nematodes gave typical locomotive signals in different media (water, agar, sediment) with comparable precision and accuracy. The results of this study encourage to develop a new rapid online whole-sediment toxicity test with behaviour as sensitive test parameter. © 2002 Published by Elsevier Science Ltd.

1. Introduction

In the last decade, the free living nematode *Caenorhabditis elegans*, a common model organism for genetic and developmental studies, has gained more and more attention in the field of ecotoxicology. Using various exposure substrate, such as aquatic media (Williams and Dusenbery, 1990a), soil (Donkin and Dusenbery, 1993), and freshwater sediments (Traunspurger et al., 1997; Höss et al., 1999), *C. elegans* served as test organism offering a variety of lethal and sublethal parameters (Anderson et al., 2001).

In various studies, the movement of *C. elegans* was shown to be a promising toxicity parameter for different toxicants, metals and organic compounds, using a computer tracking system (Williams and Dusenbery, 1990b; Anderson et al., 2001). The movement pattern was found to be at least as sensitive as other sublethal endpoints and the method showed to be less time consuming than measuring other toxicity endpoints, such as growth and reproduction (Dhawan et al., 1999; Anderson et al., 2001). However, the video-based computer tracking method does not offer the possibility of detecting movement in sediments or soils.

For measuring movement of organisms in sediments, a system is required that is independent of optical contact with the test organisms. The Multispecies Freshwater Biomonitor® (MFB) measures and analyses online different types of behavior of aquatic species based on the registration of changes in a high frequency alternating current, caused by the movements of the organisms in their test chambers (Gerhardt et al., 1994; Gerhardt, 1999). This method has already shown to be a valuable biomonitoring tool using crustaceans (daphnids, gammarids, shrimps), insect larvae (chironomids, mayflies), fish and tadpoles. The aim of this preliminary study was to test if the movement of nematodes can be detected with the MFB.

2. Materials and methods

The measurement principle of the MFB is based on the quadropole impedance conversion. Changes in an electrical field of high-frequency alternating current, that are caused by movements of organisms, can be automatically registered (record time: 4 min, interval: 6 min.) and analyzed with a Fast Fourier Transformation

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0269-7491/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0269-7491(02)00236-1

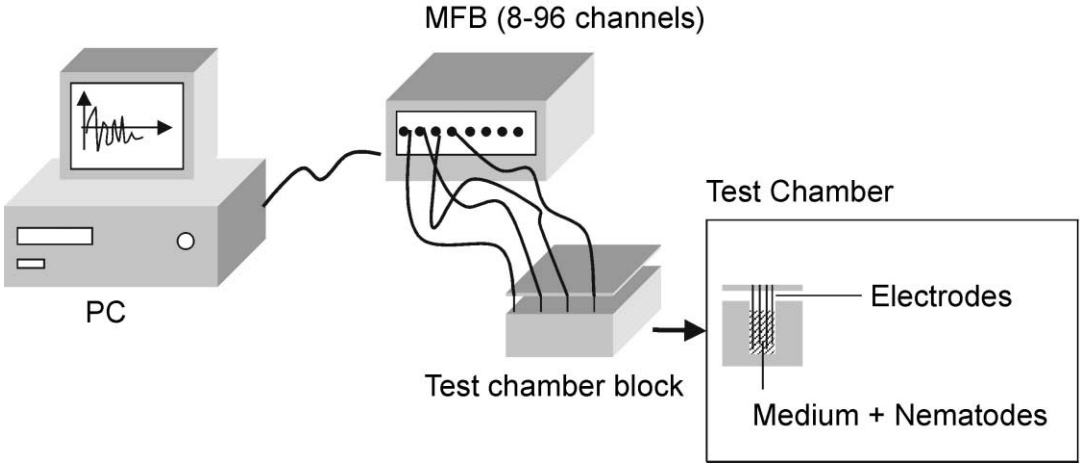

Fig. 1. Experimental setup with test chamber, Multispecies Freshwater Biomonitor (MFB), and analysis unit (PC).

(FFT); (Gerhardt et al., 1994; Gerhardt, 2001). Measurement unit is a test chamber that can vary in size, type of construction (e.g. flow-through, static), electrode-arrangement and material according to the test species, its ecological requirements and the parameters to be measured. In this study, vertical, cylindrical test chambers were used (Fig. 1). The chambers are slots in a block made of polyacrylate with an inner diameter of 0.6 cm and a depth of 1.5 cm that were equipped with four vertically arranged platinum electrodes ($\varnothing = 1$ mm, length = 1.5 cm).

Measurements were performed at room temperature (20 °C) in three different types of medium: (1) Aqueous medium: Volvic water (300 μl), (2) natural freshwater sediment (Lake Ammersee, Germany) that was dried for 16 h at 105 °C and again water saturated with Volvic water, and (3) NG agar (Brenner, 1974) with overlying Volvic water. Nematode movement was measured in six replicates and five successive measurements over a period of 1 h (30 measurements). As control, signals were also measured in an empty chamber, without nematodes.

Caenorhabditis elegans var. Bristol, strain N2, was maintained in stocks of dauer larvae on NG agar according to standard procedures (Sulston and Hodgkin, 1988). Pieces of agar plates with dauer larvae were transferred to agar plates with a fresh bacterial lawn (*Escherichia coli* OP50). After 5 days all developmental stages were available. For measurements on agar, pieces of an agar plate with *C. elegans* of all developmental stages (ca. 10 individuals) were placed in the test chamber. The nematodes were delivered and kept as dauer larvae on NG agar at room temperatures with a 12 h/12 h photoperiod.

3. Results and discussion

Single nematodes could not be registered in the present test chambers in either medium, since the test chambers were too large and the amplification of the MFB too low. Groups of nematodes (10 and 50 individuals) generated motility signals in all types of media. Fig. 2 clearly shows the difference between an empty chamber and the chambers containing nematodes and aqueous medium, sediment, or agar. Motility consisted of irregular signals of low signal amplitudes (up to 250 mV) and low signal frequencies (< 2.5 Hz), higher frequencies occurred to much lesser extent, thus showing a decreasing function in the FFT-diagram (Fig. 2). Concerning the original signals as well as the FFTs, no difference in the signals of *C. elegans* could be found between the different media. However, the reproducibility of the signals varied with the type of medium. While in aqueous medium and on agar the motility signals could be reproduced in only 27 and 21% of the total measurements ($n = 30$), we found a reproducibility of 47% in sediment ($n = 30$). The higher percentage of registered movements in the sediment might be due to a better registration because of the possibility for the nematodes to move in a three-dimensional way, in contrast to aqueous medium and agar, where they just move on the bottom of the test chamber and the agar surface, respectively. Also indirect effects, such as the amplification of the movements by moving sediment particles might have improved the reproducibility of movement signals. The average amplitudes of the signals, however, were the same in all media (Fig. 2). The measurements in aqueous medium and sediment showed no differences in signals produced by 10 compared to 50 nematodes which indicates that the movement signals are not dependent on the number of nematodes per chamber, once a threshold is exceeded.

The results of the present study show that the MFB might be used with nematodes as test organism in toxicity assessment and online biomonitoring of aqueous phases such as waste water or ground water, as well as of solid phases such as sediments and soils. As other studies have shown, motility of *C. elegans* is as sensitive

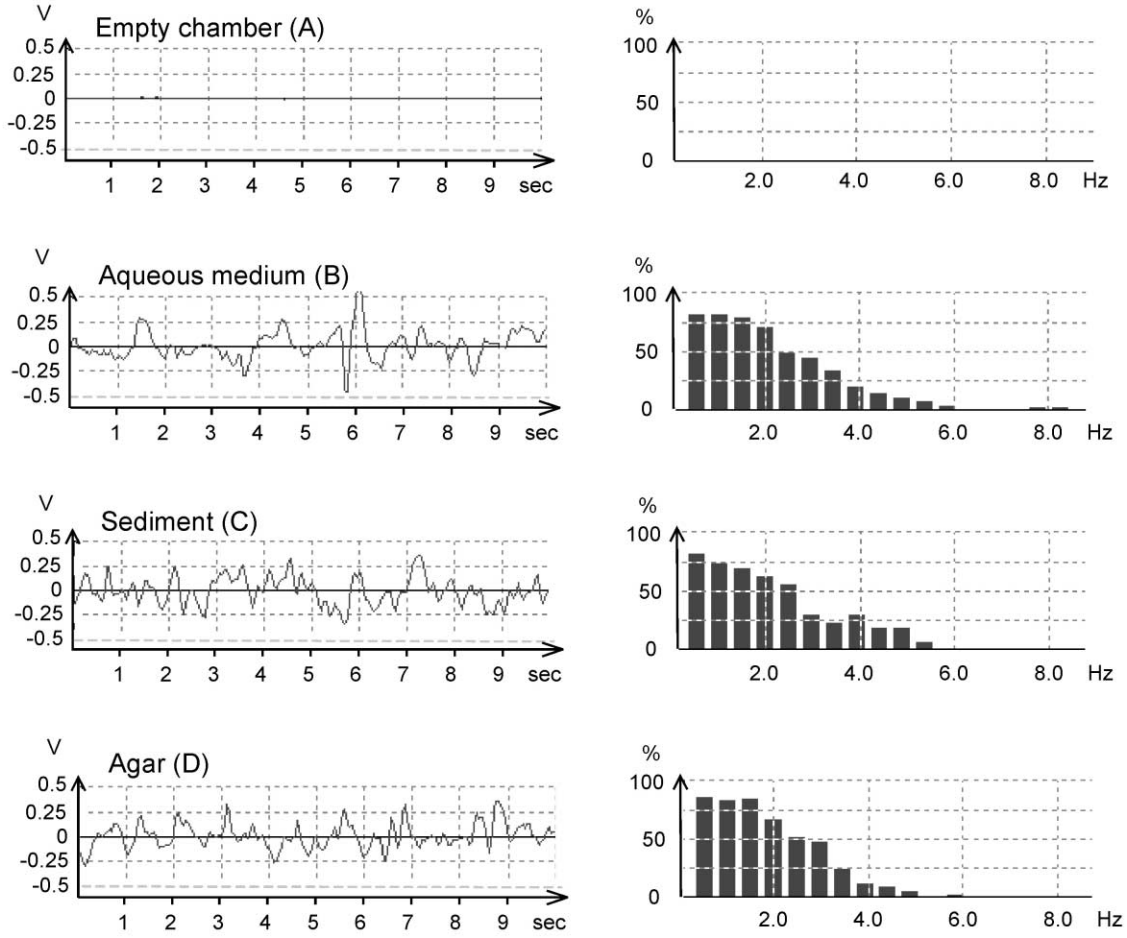

Fig. 2. Motility signals (Volt; left) and Fast Fourier Transformation (FFT) histogramms (%; right) of measurements (zoomed: 10 s out of 240 s) in test chambers containing no organisms (A), aqueous medium + 10 nematodes (B), freshwater sediment + 10 nematodes (C), and agar + 10 nematodes (D).

as other sublethal parameters such as reproduction and, additionally, might be an indicator for neurobehavioral toxicity (Williams and Dusenbery, 1990b; Anderson et al., 2001). Moreover, changes of motility can be detected within several hours of exposure which offers the possibility of a more rapid toxicity screening than growth and reproduction of the tested organisms.

An important advantage of the MFB over the video tracking system would be the possibility of toxicity assessment in sediment samples, as only exposure in whole sediment can estimate the real bioavailability of contaminants. *C. elegans* feeds and moves in the interstitial water of soils and sediments (Schiemer, 1975) and thus is exposed to contaminants via porewater, particle contact, and feeding. Generally, the use of nematodes for the MFB would be of high ecological relevance, as nematodes are the individual and species richest metazoan organism group in sediment and soils and represent an important component of benthic food webs (e.g. Traunspurger, 1996). However, further research and standardization of this innovative bioassay is needed in order to evaluate its complete spectrum of application and utility.

Acknowledgements

The gifts of *Caenorhabditis elegans* and *Escherichia coli* from the Caenorhabditis Genetic Center (Theresa Stiernagle) are gratefully acknowledged.

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