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Time-dependent Toxic Effects of Cadmium Chloride on the Stress-related Gene Expression, Growth and Reproduction of the Soil Nematode *Caenorhabditis elegans*

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토양선충 *Caenorhabditis elegans*의 스트레스 관련 유전자 발현을 이용한 시간에 따른 카드뮴의 독성영향

노지연, 이정경, 권혁두, 최진희※

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요 약

카드뮴은 환경과 인체 위해도에 큰 영향을 미치는 중요한 환경오염물질로 잘 알려져 있다. 본 연구에서는 토양선충인 *Caenorhabditis elegans*에 카드뮴을 12시간과 48시간으로 나누어 처리하여 시간에 따른 장, 단기적 독성영향을 알아보고자 하였다. 이때 생리학적 수준으로 성장 및 생식을 조사하고, 분자수준에서 스트레스 관련 유전자들의 시간에 따른 발현 정도를 관찰하였다. 생식에서는 단기노출(12시간) 시 그 영향이 대조군에 비해 크게 나타났으며, mtl-2의 스트레스 관련 유전자가 증가하였다. 장기 노출(48시간) 시에는 cyp35a2, ape-1, sod-1, ctl-2 유전자가 대조군에 비해 약 2~4배 가량의 발현 증가 결과를 조사할 수 있었다. 본 연구결과들을 통해 스트레스 관련 유전자의 발현을 조사하는 것이 중요하고 민감한 생체지표가 된다는 것과 토양선충 *C. elegans*는 환경중 오염물질에 대한 장기, 단기적 영향을 평가하기 위한 좋은 생물학적 모델이 된다는 것을 알 수 있었다.

Key words : *Caenorhabditis elegans*, cadmium chloride, stress-related gene expression, growth, reproduction

INTRODUCTION

Cadmium is found in low abundance in the earth crust and most surface soil (Waalkes *et al.*, 1992), Cd and its compound have been implemented in a wide range of technological application, such batteries, dyes, paints and plastics (Martelli *et al.*, 2006). It currently ranks 7th on the United States Environmental Protection Agency's priority list of hazardous substance (ATSDR, 2003) and The Intenational Agency

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for Research on Cancer has classified Cd as human carcinogen (Waalkes, 2000). The accumulation and persistence of Cd in the environment constitute a threat to biological life, as witnessed by the chronic and acute poisoning of organisms (Lock *et al.*, 2001; Aruoja *et al.*, 2004; Guan *et al.*, 2004; Roh *et al.*, 2006). In this study, the time-dependent toxic effects of Cd exposure was assessed on stress related gene expression, growth and reproduction in *Caenorhabditis elegans*, especially.

C. elegans, a free-living nematode that lives mainly in the liquid phase of soils, is the first multi cellular organism to have its genome completely sequenced. The genome showed an unexpectedly high level of conservation with the vertebrate genome, which makes *C. elegans* an ideal system for biological studies, such as those in genetics, molecular biology and development biology (Jones *et al.*, 2001; Cui *et al.*, 2007). Due to its abundance in soil ecosystems, convenient handling in the laboratory and sensitivity to different kinds of stress, *C. elegans* is also a good animal model for the study of ecotoxicology, and thus, frequently used in ecotoxicological studies utilizing various exposure media, including soil and water (Williams and Dusenbery, 1990; Donkin and Williams, 1995; Boyd and Williams, 2003).

The aims of the current study were, fitst, to evaluate ecotoxicity of the Cd exposure in the soil nematode *C. elegans*, secondly, to investigate the timedependent effects of Cd, *C. elegans* was exposed for 12 and 48 h to Cd.

The aims of the current study were, first, to evaluate the acute toxicity of metals on *C. elegans*, secondly, to identify the sensitive genes expressed as part of the metal-activated stress responses of *C. elegans*, thirdly, to validate the ecotoxicological relevance of stress-related gene expression by investigating the physiological-level responses of *C. elegans*, and finally, to test the possibility of *C. elegans* being a biosensor for environmental toxicity monitoring, using the developing green fluorescent protein (GFP) transgenic nematode.

MATERIALS AND METHODS

1. Organisms

The wild-type *Caenorhabditis elegans* Bristol strain N2 was used in this study. *Caenorhabditis elegans* were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50, at 20°C, using the standard method previously described by Brenner (1974).

2. Sample preparation

Three types of endpoints (growth, reproduction and stress-related gene expression) were assessed to investigate the multi level effects of $CdCl_2$ on *C. elegans*. Pure analytical-grade $CdCl_2$ (Sigma Aldrich Chemical, St. Louis, MO, USA) was used and it was dissolved in water. Nematodes were exposed to Cd prepared in a K-medium (0.032 M KCl, 0.051 M NaCl) (Williams and Dusenbery, 1990). Three replicates for each metal concentration and a control were conducted for all the test types. The Cd concentrations were all nominal values.

3. Growth and reproduction tests

Following the 12 and 48 h incubation with exposure to sublethal concentrations of Cd, growth and reproduction were assessed, respectively. Growth was assessed by measuring the worms that had been killed by the heat through microscopy, with a scaled lens in each sample. Reproduction was preliminarily assessed by counting the eggs of each worm through the microscopic inspection of the transparent *C. elegans* body in each sample. Although this procedure differs from more commonly used reproduction tests of offspring counting from an age-synchronized single worm, this simple detection method seems appropriate for the rapid screening of the reproduction effect. One hundred worms were examined per treatment for growth and reproduction experiments.

4. Semi-quantitative reverse transcription polymerase chain reaction

Following the 12 and 48 h incubation with exposure to sublethal concentrations of Cd, nematodes were harvested for the analysis of stress response gene expression using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR), as described previously (Roh *et al.*, 2006). Briefly, the two-step RT-PCR method was used with RT-PCR Premix (Bioneer, Seoul, Korea), using a PTC-100 thermal cycler (MJ Research, Lincoln, MA, USA). The primers were designed based on the sequences retrieved from the *C. elegans* database (www.wormbase.org).

5. Data analysis

The statistical differences between the control and treated worms were determined with the aid of the parametric *t* test.

RESULTS AND DISCUSSION

Nematodes are becoming popular bioindicators of

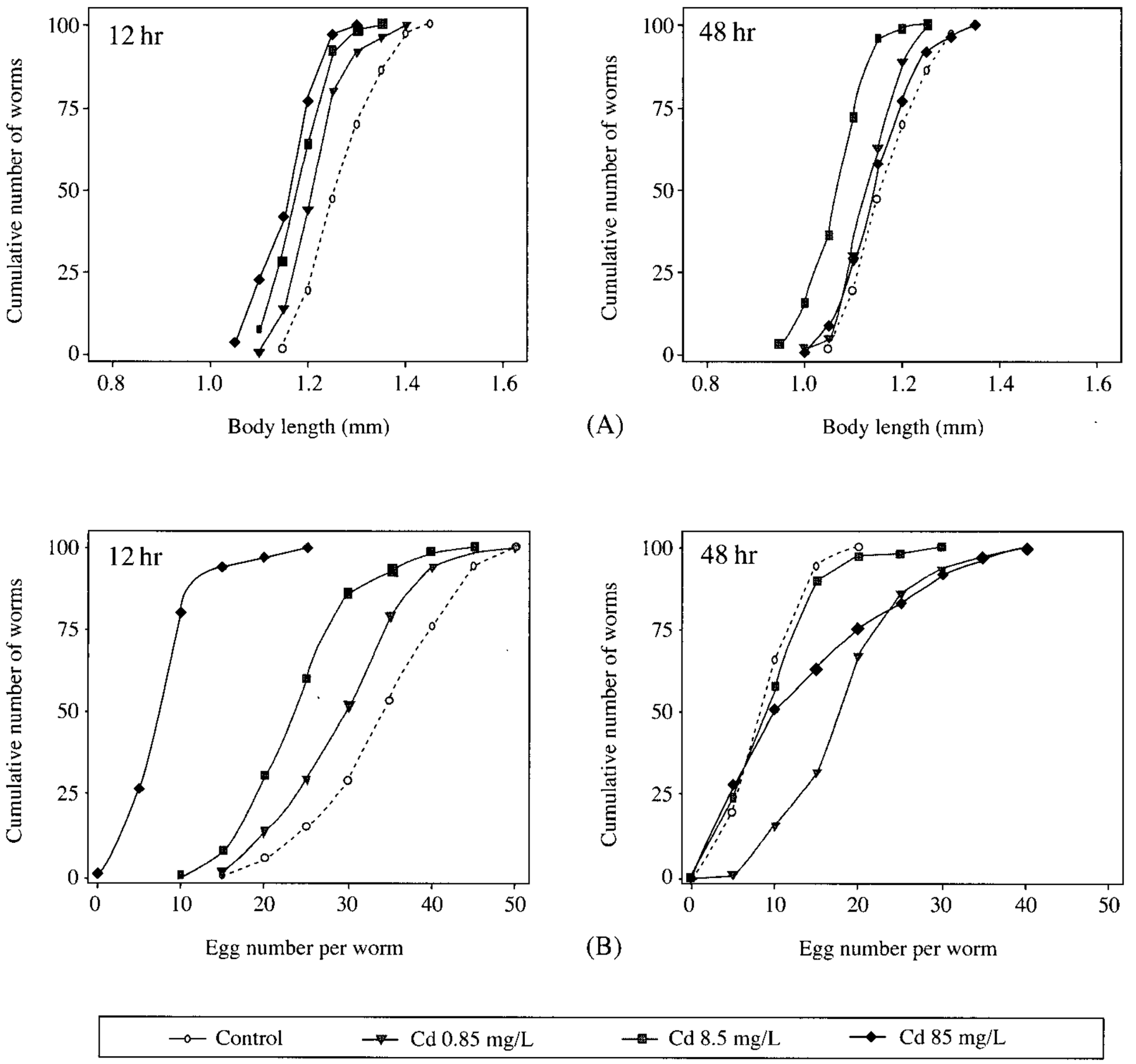

Fig. 1. Growth (A) and reproduction (B) indicators examined in the young adult of *Caenorhabditis elegans* exposed to $CdCl_2$ for 12 and 48 h.

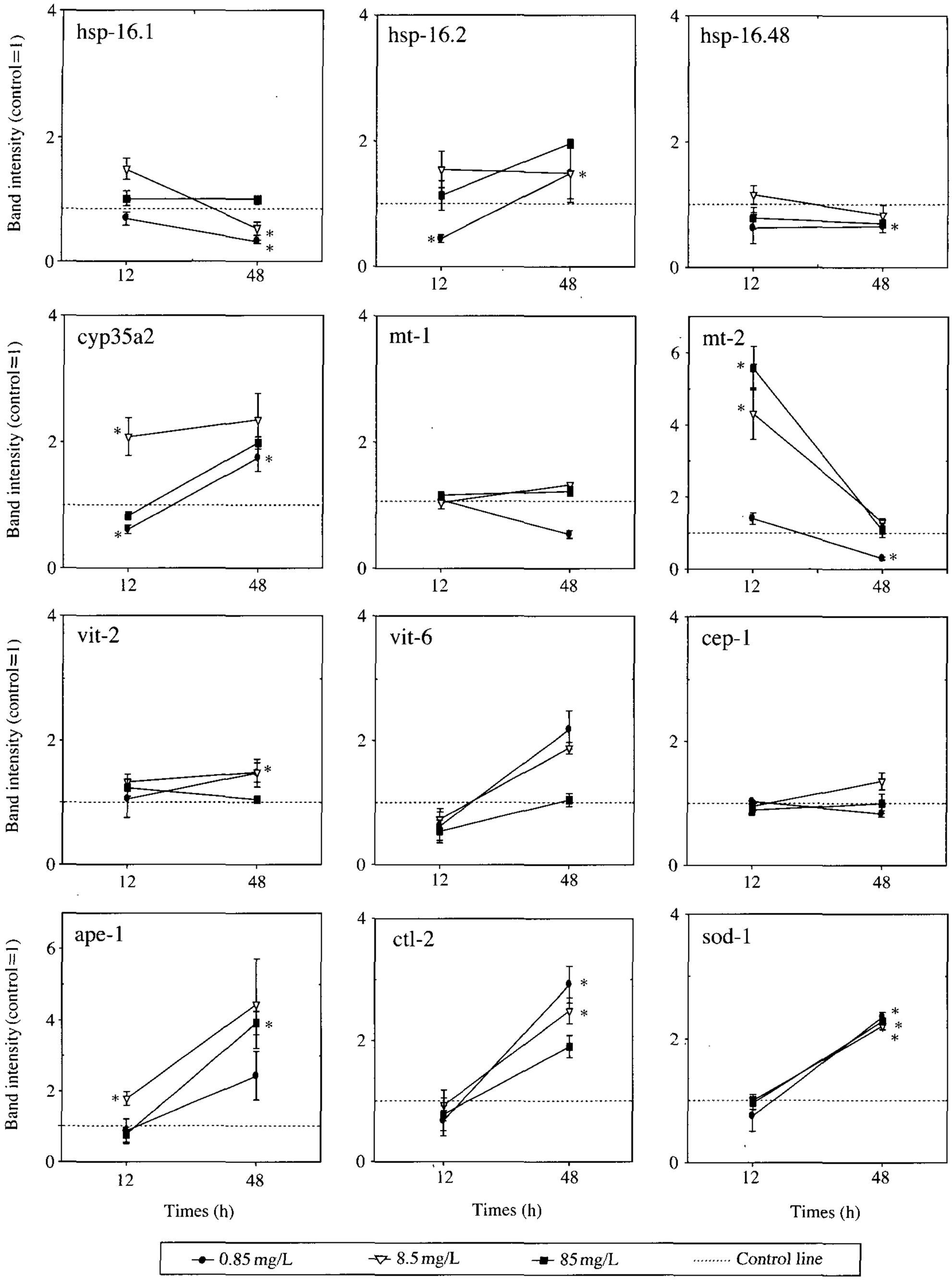

Fig. 2. Stress-related gene expression profiling in the young adult of *Caenorhabditis elegans* exposed to Cd for 12 and 48 h. Densitometric values were normalized using actin mRNA and are presented in arbitrary unit compared to control (control=1; n=3; mean±SEM; $*p<0.05$).

pollution stress because of their ecological significance, their short life cycle, and the convenience of forming cultures from them and of maintaining a large number of them in the laboratory (Anderson *et al.*, 2001; Kohra *et al.*, 2002; Boyd and Williams, 2003; Tominaga *et al.*, 2003). In this study, sensitive genes expressed as part of metal-activated stress responses were identified in *C. elegans* using Cd, and an attempt was made at validating their ecotoxicological relevance by investigating their physiological-level responses, such as growth and reproduction. The effects of xenobiotics on the growth and reproduction of the test organisms are broadly accepted test parameters, as shown in this study (Fig. 1). The decreases in egg number per worm observed after 12 h of Cd exposure may induce alteration in the reproduction of the nematode population in the short term.

As a growth indicator, the changes in the worms' body lengths after 12 h and 48 h of Cd exposure were presented in Fig. 1-A. The body length was not changed by exposure to Cd. The average length of the worms were in the range of 1.0 to 1.2 mm. However, the worms, which had been exposed to Cd for 12 h, showed a serious decrease in their egg formation, whereas that did not change after 48 h of exposure (Fig. 1-B). The most significant decrease was observed at the lowest concentrations of Cd (0.85 mg/L) for 12 h, which were 80.0% lower compared to the control.

The stress response gene expression profiles were evaluated on the young *C. elegans* adults that were exposed to Cd for 12 and 48 h (Fig. 2). As potential stress-response genes, heat-shock protein (*hsp-16.1*, *hsp-16.2* and *hsp-16.48*), metallothionein (*mtl-1* and *mtl-2*), vitellogenin (*vit-2* and *vit-6*), the cytochrome p450 family protein 35A2 (*cyp35a2*), superoxide dismutase-1 (*sod-1*), catalase-2 (*ctl-2*), the *C. elegans* p53-like protein (*cep-1*) and the apoptosis enhancer protein (*ape-1*) were selected based on our previous study (Roh *et al.*, 2006). No significant effect was observed in the nematode exposed to Cd exposure for 12 and 48 h on the expression of the heat shock protein genes, such as hsp-16.1, hsp-16.2, hsp-16.48, was observed. 48 h exposure led to increases in the expression of cytochrome P450, vitellogenin, apoptosis enhancer, catalase and superoxide dismutase genes. The degree of increase was greater in cyp35a 2 (2-fold compared to the control), vit-6 (2.1-fold), ape-1 (4.5- fold), ctl-2 (2.5-fold) and sod-1 (2.4-fold). However, at 12 h, only the mtl-2 gene expression was increased about 5.6-fold compared to the control. Moreover, the induction of mtl-2 gene expression occurred in an exposure concentration-dependent manner (1.4, 4.3 and 5.6 folds for 0.85, 8.5, and 85 mg/L, respectively).

Xenobiotically induced gene expression is considered a highly promising tool in biomonitoring for the early detection of environmental contaminants (Roesijadi, 1994; Yoshimi *et al.*, 2002; Menzel *et al.*, 2005). Gene expression endpoints are not only sensitive and useful in estimating the effects of toxicants on expected populations, but may also provide insight into the mechanisms underlying these effects.

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