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ASSESSMENT OF STRESS-RELATED GENE EXPRESSION IN THE HEAVY METAL–EXPOSED NEMATODE *CAENORHABDITIS ELEGANS*: A POTENTIAL BIOMARKER FOR METAL-INDUCED TOXICITY MONITORING AND ENVIRONMENTAL RISK ASSESSMENT

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Abstract—The toxicity of cadmium, lead, chromium, and arsenite on *Caenorhabditis elegans* was investigated to identify sensitive biomarkers for environmental monitoring and risk assessment. Effects of these metals on stress-related gene expression, growth, reproduction, and mortality of *C. elegans* were investigated under laboratory conditions. The possibility of using *C. elegans* as a biosensor for environmental toxicity monitoring was also tested using a green fluorescent protein transgenic nematode. The 24-h median lethal concentrations of cadmium, lead, chromium, and arsenite in *C. elegans* were 846, 34, 115, and 92 mg/L, respectively. Cadmium exposure led to an increase in the expression of most of the genes tested. The degree of increase was more than threefold compared to control in heat shock protein 16.2, heat shock protein 70, metallothionein 2, cytochrome P450 family protein 35A2, glutathione-*S*-transferase 4, superoxide dismutase 1, catalase 2, *C. elegans* p53-like protein 1, and apoptosis enhancer 1 genes. The lead-, chromium-, and arsenite-exposed nematode, on the other hand, showed little change in gene expression. Alterations in growth and reproduction were observed in cadmium- and chromium-exposed worms. To consider a transgenic nematode as a biosensor for toxicity monitoring, the responses of stress-related gene promoters need to be tested with a variety of metals. The overall results suggest that cadmium exhibits a high level of tolerance compared to the other metals tested. Use of the responses of stress-related gene expression therefore has considerable potential as a sensitive biomarker for the diagnosis of cadmium contamination, and *C. elegans* seems to be a good biological model for this approach.

Keywords—*Caenorhabditis elegans* Stress-related gene expression Biomarker Metal toxicity monitoring Environmental risk assessment

INTRODUCTION

Caenorhabditis elegans, a free-living nematode that resides mainly in the liquid phase of soils, constitutes a useful bioindicator of environmental disturbances. Because of its abundance in soil ecosystems, its convenient handling in the laboratory, and its sensitivity to different kinds of stress, *C. elegans* frequently is used in ecotoxicological studies using various exposure media, including soil and water [1–3]. Because its genome has been completely sequenced, the functional relations of gene expression and phenotypic response have been widely investigated. Therefore, *C. elegans* is an attractive animal model for the study of the ecotoxicological relevance of chemical-induced, gene-level responses [4,5].

The current literature regarding *C. elegans* provides insight concerning the relative sensitivity of several of its lethal and sublethal endpoints [6–8]. In the present study, the toxicological effects of exposure to metals were assessed using *C. elegans*. Cadmium chloride (Cd), lead(II)nitrate (Pb), potassium dichromate (Cr), and sodium meta-arsenite (As) were chosen as test chemicals, both because of their abundance in the environment and because of the availability of toxicological data in the literature regarding nematodes and other organisms.

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

A lethal toxicity test was conducted to investigate the acute effects of heavy metals on *C. elegans*. Its responses on the molecular and physiological levels were subsequently investigated using sublethal exposure. As potential stress-related genes, heat shock protein (hsp-16.1, hsp-16.2, hsp-16.48, and hsp-70), metallothionein (mt-1 and mt-2), vitellogenin (vit-2 and vit-6), cytochrome P450 family protein 35A2 (cyp35a2), glutathione-*S*-transferase 4 (gst-4), superoxide dismutase 1(sod-1), catalase 2 (ctl-2), *C. elegans* p53-like protein (cep-1), and apoptosis enhancer (ape-1) were examined in a semiquantitative manner. Growth and reproduction were investigated as physiological descriptors of metal toxicity by measuring the body length and by counting the eggs of each worm, respectively. Finally, to test the potential of *C. elegans* as a biosensor for monitoring of metal toxicity, the metals-induced responses of GFP transgenic lines incorporating full-length hsp-16.2 and hsp-16.48 genes were investigated.

Table 1. Estimation of 24-h lethal concentrations in *Caenorhabditis elegans*[a]

		24 h LC (mg/L)	95% Confidence interval (mg/L)
Cadmium chloride	LC10	424	184 < LC10 < 582
	LC50	846	637 < LC50 < 1,064
	LC90	1,686	1,281 < LC90 < 3,353
Lead(II)nitrate	LC10	15	6 < LC10 < 23
	LC50	34	23 < LC50 < 46
	LC90	76	55 < LC90 < 143
Potassium dichromate	LC10	64	31 < LC10 < 86
	LC50	115	84 < LC50 < 151
	LC90	209	157 < LC90 < 32
Sodium metaarsenite	LC10	23	0.6 < LC10 < 50
	LC50	92	33 < LC50 < 151
	LC90	369	206 < LC90 < 3,830

[a] LC = lethal concentration; LC10 = 10% lethal concentration; LC50 = 50% lethal concentration; LC90 = 90% lethal concentration.

MATERIALS AND METHODS

Organisms

The wild-type *C. elegans* Bristol strain N2 was used in the present study. The organisms were maintained on plates containing nematode growth medium and seeded with *Escherichia coli* strain OP50 at 20°C using the standard method described previously by Brenner [9].

Metal exposure and sample preparation

Four types of endpoints (stress-related gene expression, growth, reproduction, and mortality) were assessed for exposure to Cd, Pb, Cr, and As. Nematodes were exposed to metals prepared in a K-medium (0.032 M KCl and 0.051 M NaCl) [10]. Young adults (age, 3 d) from an age-synchronized culture were used in all experiments. Worms were incubated at 20°C for 24 h without a food source and then subjected to each type of analysis. Three replicates for each metal concentration and a control were conducted for all test types. The metal concentrations were all nominal values.

Lethal toxicity tests

Each test consisted of five metal concentrations and a control. Using a dissecting microscope, 10 young *C. elegans* adults were transferred onto 24-well tissue culture plates containing 1 ml of the test solution per well. The worms were exposed for 24 h at 20°C. Following exposure, the numbers of live and dead worms were determined through visual inspection and by probing the worms with a platinum wire under a dissecting microscope.

RNA preparation and semiquantitative reverse transcription–polymerase chain reaction

Following 24-h incubation with exposure to sublethal concentrations of metals, nematodes were harvested for the preparation of RNA. Standard procedures were followed using Trizol reagent for total RNA isolation. The two-step reverse transcription–polymerase chain reaction (RT-PCR) method was used with RT Premix (Bioneer, Seoul, Korea) and PCR Premix kits (Bioneer) using a PTC-100 thermal cycler (MJ Research, Lincoln, MA, USA). The primers were designed on the basis of the sequences retrieved from the *C. elegans* database (http://www.wormbase.org). Actin mRNA was used for expression level normalization of the studied genes. The PCR products were sep-

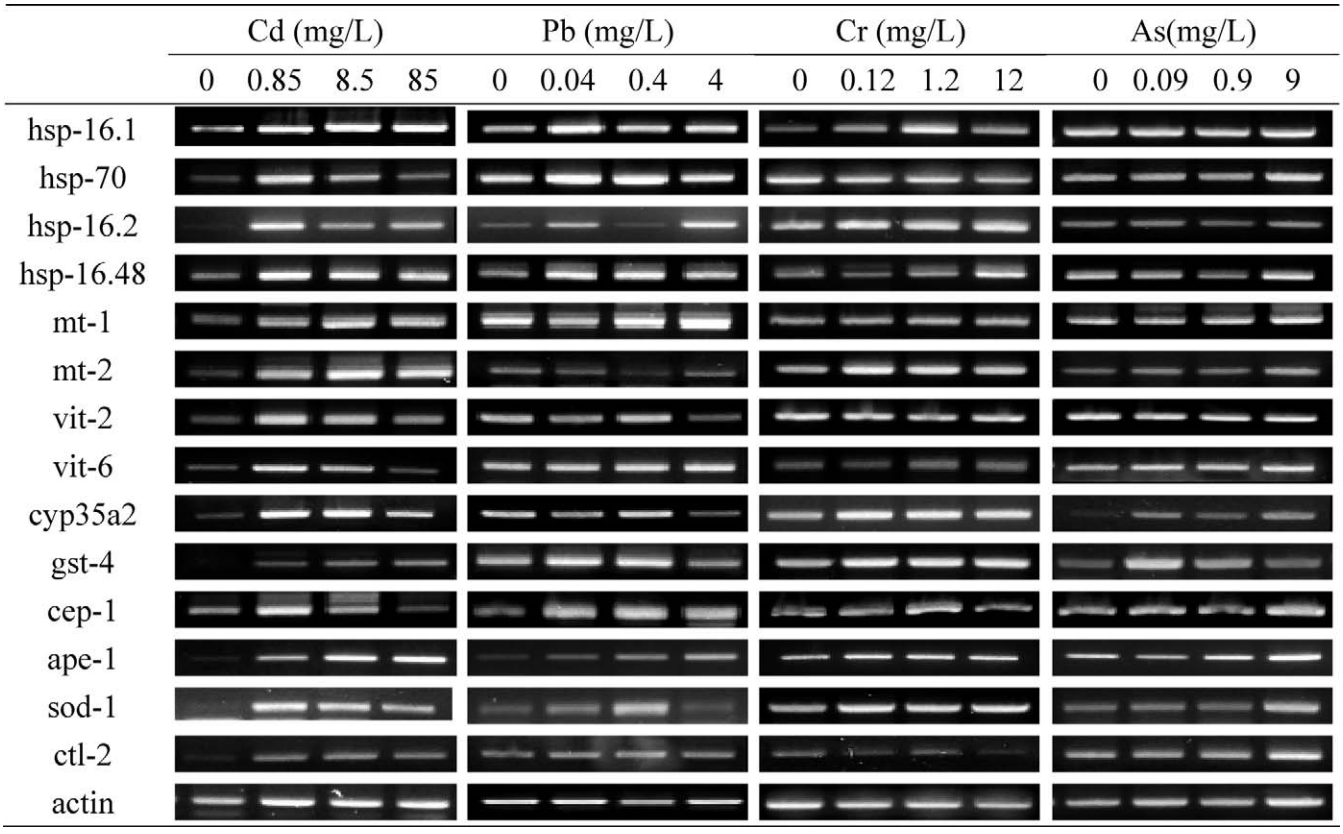


Fig. 1. Stress-related gene expression profiling in the young adult of *Caenorhabditis elegans* exposed to Cd, Pb, Cr, and As for 24 h.

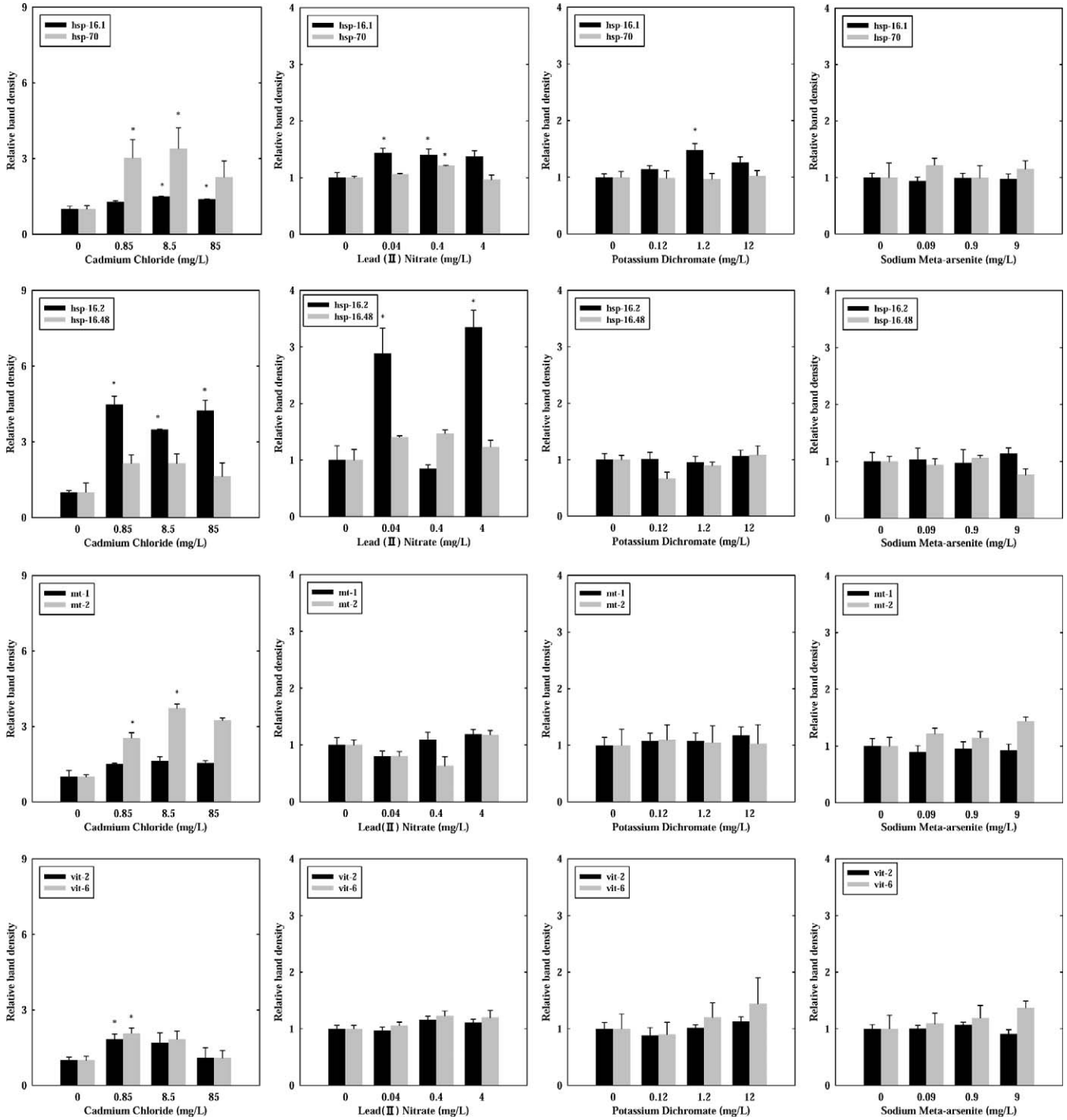


Fig. 2. Densitometric values of stress-related gene expression normalized using actin mRNA. Data are presented in arbitrary units compared to control (control = 1, $n = 3$, mean ± standard error of the mean). *$p < 0.05$.

arated through electrophoresis on 1.5% agarose gel (Promega, Madison, WI, USA) and were visualized with ethidium bromide (Bioneer). All tests were replicated at least three times, and the relative densities of each band were determined with use of a Kodak EDAS 290 image analyzer (Kodak, Rochester, NY, USA) and a TFX-20.M ultraviolet transilluminator (Vilber Lourmat, Marne la Vallee, France).

Measurement of growth and reproduction

Following 24-h incubation with exposure to sublethal concentrations of metals, growth and reproduction were assessed. Growth was assessed by measuring worms that had been killed by the heat using microscopy with a scaled lens in each metal concentration and a control. The average length of the unexposed control worm was in the range of 0.10 to 0.12 mm. Preliminary assessment of reproduction was conducted by counting the eggs of each worm using microscopic inspection of the transparent *C. elegans* body in each metal concentration and the control. This procedure differs from more commonly used reproduction tests of offspring counting from an age-synchronized single worm, but this simple detection method seems to be appropriate for rapid screening of the effect on

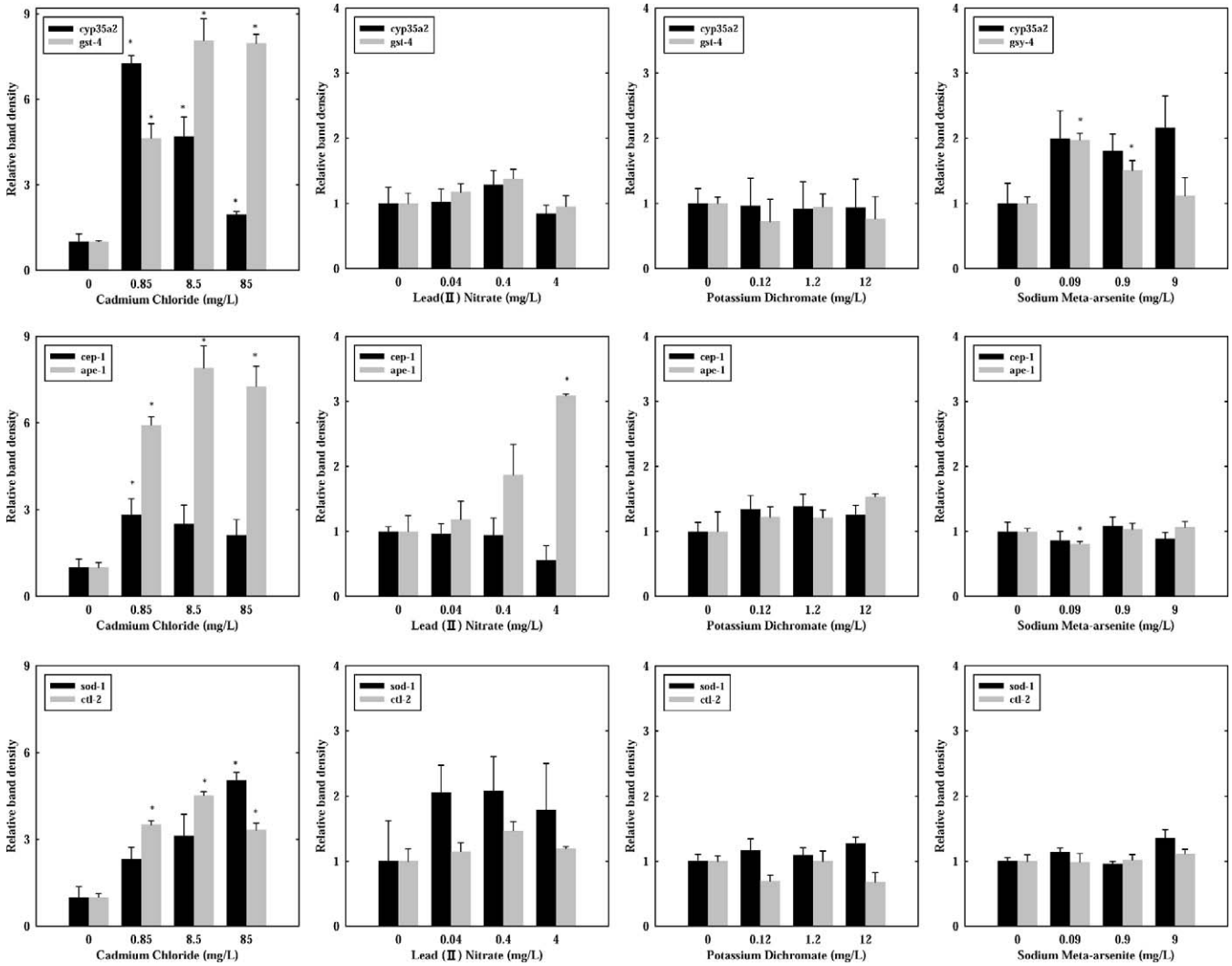


Fig. 2. Continued.

reproduction. The average number of eggs per worm in the unexposed controls was in the range of 20 to 40. One-hundred worms were examined per treatment for growth and reproduction experiments.

Detection of GFP transgenic C. elegans

The transgenic strains (hsp-16.2::gfp and hsp-16.48::gfp) of *C. elegans* were developed as described previously by Hong et al. [11]. Transgenic *C. elegans* were incubated for 24 h with sublethal concentrations of metals, and the fluorescence signal was examined from 50 independent transgenic worms per treatment. Fluorescence was observed using a Leica DM IRB microscope (Leica, Wetzlar, Germany), and the image was taken using a Leica DC 300FX camera (Leica). Levamisole (2 mM; Sigma-Aldrich Chemical, St. Louis, MO, USA) treatment was used to take pictures of the live worms. Fluorescence was quantified using a GENios microplate reader (TECAN, Maennedorf, Switzerland).

Chemicals

Analytical-grade metals were used. Cadmium chloride, Pb, and Cr were purchased from Sigma-Aldrich Chemical. Sodium meta-arsenite was purchased from Wako (Osaka, Japan).

Data analysis

Median lethal concentrations (LC50s) were derived through Probit analysis. The statistical differences between the control and treated worms were determined with the aid of the parametric *t* test. The Pearson test was conducted for the correlation study. All statistical analyses were conducted using SPSS 12.0.1 (SPSS, Chicago, IL, USA).

RESULTS

Acute toxicity was studied using LC50s derived through Probit analysis (Table 1). The 24-h LC50s of Cd, Pb, Cr, and As in *C. elegans* were 846, 34, 115, and 92 mg/L (4.6, 0.12, 0.4, and 0.7 mM), respectively. Based on the results of the acute toxicity test, three concentrations—corresponding to 1/1,000, 1/100, and 1/10 of the 24-h LC50—were selected for the sublethal exposure conditions. These concentrations were as follows: Cd, 0.85, 8.5, and 85 mg/L, respectively; Pb, 0.04, 0.4, and 4 mg/L, respectively; Cr, 0.12, 1.2, and 12 mg/L, respectively; and As, 0.09, 0.9, and 9 mg/L, respectively.

Figure 1 shows the stress-related gene expression profile measured in the young adults of *C. elegans* exposed to Cd, Pb, Cr, and As for 24 h. Cadmium exposure led to increases in the expression of most of the genes tested except for the

hsp-16.48 and mt-1 genes, for which no significant change was observed. The degree of increase was greater in hsp-16.2 (4.5-fold compared to control), mt-2 (3.7-fold), cyp35a2 (7.3-fold), gst-4 (8.1-fold), ape-1 (7.9-fold), sod-1 (4.5-fold), and ctl-2 (5-fold) (Fig. 2). In particular, the induction of the cyp35a2 gene expression was inversely concentration-dependent (7.3-, 4.7-, and 1.9-fold for 0.85, 8.5, and 85 mg/L, respectively). The responses of the stress-related genes to Pb, Cr, and As exposure were not as intense as their responses to Cd exposure. Lead exposure induced hsp-16.1, hsp-16.2, hsp-70, and ape-1 gene expression, and among these, the increase in the hsp-16.2 expression was most remarkable (~3.3-fold compared to control). Only hsp-16.1 gene expression was increased by Cr exposure, whereas As exposure induced hsp-16.1 and gst-4 gene expression. The increase in hsp-16.1 gene expression was observed in all worms that had been exposed to the four metals. The degree of increase, however, was less than 1.5-fold compared to control.

As a growth indicator, the changes in worm body lengths after metal exposure are presented in Figure 3. The worms, which had been exposed to Cd and Cr, showed a decrease in their body lengths. The most significant decrease was observed at the lowest concentrations of Cd (0.85 mg/L) and Cr (0.12 mg/L), which were 20 and 12.7% lower, respectively, compared to control. On the contrary, the body length rates were slightly increased by Pb and As exposure.

The effects of metals on the reproduction of nematodes were estimated by counting the number of eggs per worm in control versus metal-treated worms (Fig. 4). The number of eggs per worm was not changed by Pb or As exposure, whereas Cd and Cr exposure caused a serious decrease in egg formation. The most significant decrease was observed at the lowest concentrations of Cd (0.85 mg/L) and Cr (0.12 mg/L), which were 57.1 and 38.2% lower, respectively, compared to the control.

To identify any correlation between stress-related gene expressions and physiological effects, Pearson correlation tests were conducted on the 16 parameters studied, including the nominal concentration of metals (Table 2). For Cd exposure, 27 statistically significant correlations were observed, including that between body length and egg number per worm. For Pb, Cr, and As exposure, the nine, five, and eight correlations, respectively, were proven to be statistically significant.

Lastly, the possibility of transgenic *C. elegans* being a biosensor for environmental toxicity monitoring was tested using transgenic lines of the nematode. As shown in Figure 5, the hsp-16.2 and hsp-16.48 gene expression levels were semi-quantitatively assayed using GFP-based reporter transgenic nematodes. Transgenic nematodes were exposed to concentrations corresponding to 1/10 of the 24-h LC50 of each metal (Cd, 85 mg/L; Pb, 40 mg/L; Cr, 12 mg/L; and As, 9 mg/L), and the effects of two lower concentrations were additionally assessed for Cd exposure (0.85 and 8.5 mg/L). The fluorescence signals from both GFP transgenic lines increased after Cd exposure. The response of hsp-16.48::gfp, however, was greater than that of hsp-16.2::gfp. The increase in the fluorescence signal did not occur in a concentration-dependent manner, because it was observed only at the highest level of Cd exposure (85 mg/L). Lead, Cr, and As did not seem to affect these stress genes, because no change in fluorescence signal was observed.

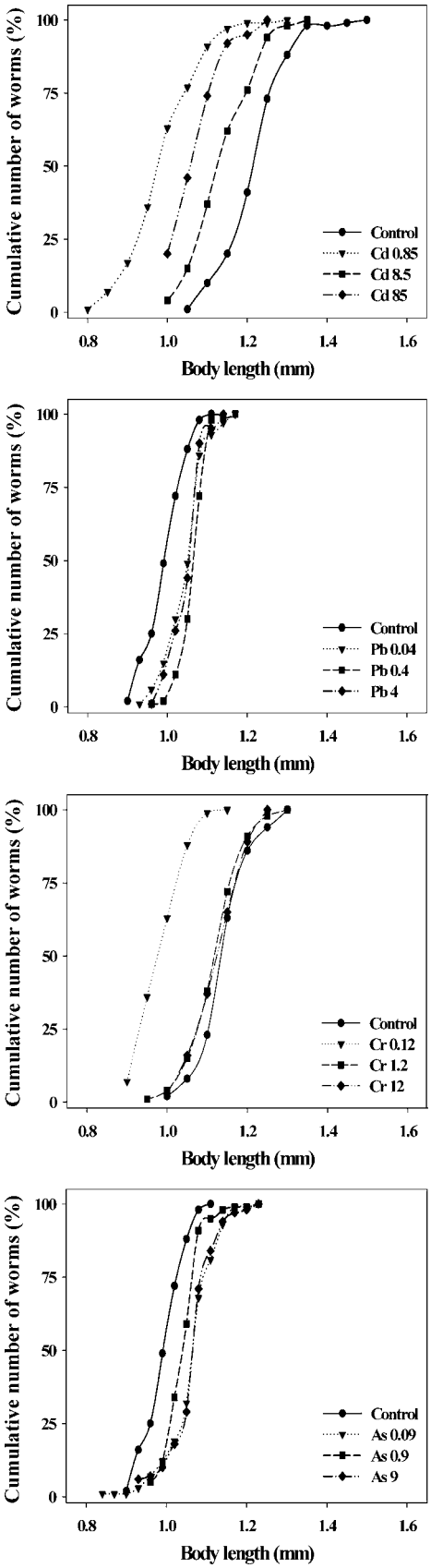


Fig. 3. Body length measured in young adult *Caenorhabditis elegans* exposed to Cd, Pb, Cr, and As for 24 h.

DISCUSSION

Nematodes are becoming popular bioindicators of pollution stress because of their ecological significance, short life cycle,

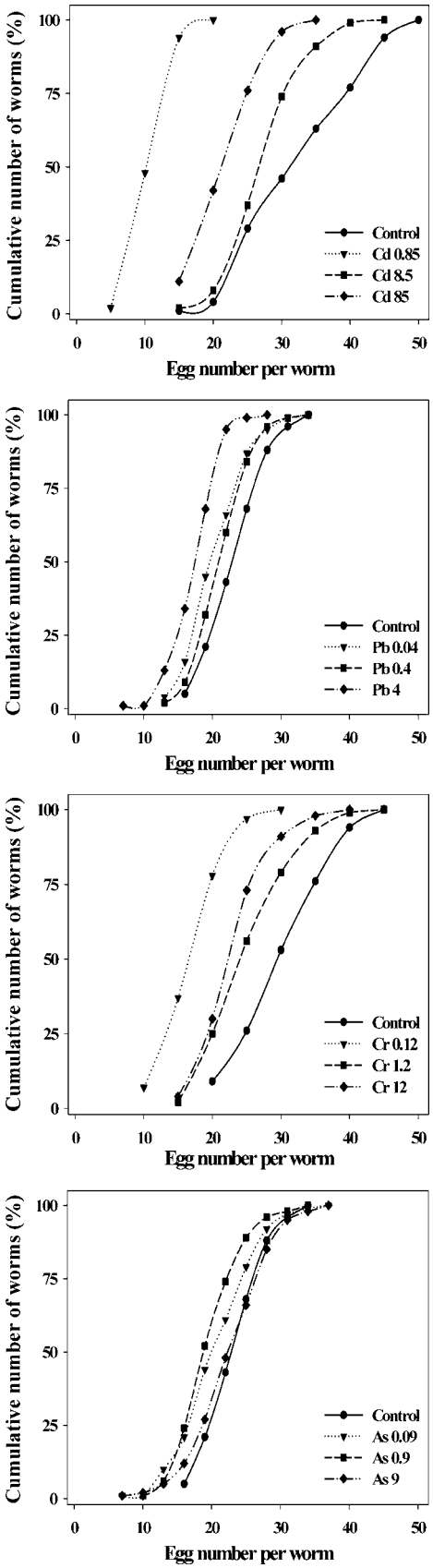


Fig. 4. Egg number per worm measured in young adult *Caenorhabditis elegans* exposed to Cd, Pb, Cr, and As for 24 h.

and convenience of culturing and maintaining large numbers of worms in the laboratory [3,7,10]. Table 3 covers most of the metal toxicity tests performed with *C. elegans*, including results from the present study. As shown in Table 3, the current metal toxicity study with *C. elegans* focuses on organism-level endpoints, such as mortality, behavior, growth, or reproduction. Only a few experiments, however, have ventured into a direct comparison of multiple endpoints under a common set of experimental conditions. In the present study, sensitive genes expressed as part of metal-activated stress responses were identified in *C. elegans* using four important metals (Cd, Pb, Cr, and As), and an attempt was made to validate their ecotoxicological relevance by investigating their physiological-level responses, such as growth and reproduction, and by comparing these with the stress-related gene expression profile.

Acute toxicity tests using *C. elegans* have been performed by many investigators [3,10,12–14]. The LC50s of Cd, Cr, and As obtained in the present study are in accordance with the *C. elegans* aquatic toxicity data reported previously by Williams and Dusenbery [10] (Table 3). The order of acute toxicity (LC50s) observed for *C. elegans* after 24 h of exposure was as follows: Pb > As > Cr > Cd. In the present study, *C. elegans* showed a high level of tolerance of, or resistance to, Cd exposure. When comparing the acute toxicity from one metal to another using LC50 in molar units for concentrations, Cd was approximately 5- to 10-fold more toxic than Cr or As and was almost 40-fold more than Pb. That Cd exhibits such a high level of tolerance compared to the other metals tested may imply that these organisms possess efficient defense mechanisms preventing Cd-related damage (i.e., the existence of a specific protein that protects against Cd toxicity in *C. elegans*). The Cd-responsive gene cdr-1 and its family recently have been identified and characterized in *C. elegans* [15,16]. This protein may contribute to this animal's high tolerance level toward Cd exposure, as observed in the present study. Different from mt or hsp, cdr-1 was reported to have been induced only by Cd and not by other metals, organic chemicals, or physical stresses. An acute toxicity study showed that *C. elegans* survived at a high level of Cd exposure. Such tolerance suggests an efficient molecular/biochemical or physiological level of protection against Cd toxicity. Different from the Pb, Cr, and As responses, for which little gene expression change was observed, Cd exposure led to increases in the expression of most of the stress-related genes tested, including hsp-16.2, mt-2, cyp35a2, gst-4, ape-1, sod-1, and ctl-2. In particular, it has been reported that almost all cyp35 forms in *C. elegans* are moderately or strongly inducible by different xenobiotics in a cyp450 gene expression–screening experiment [4,17]. A sensitive response at the molecular level may contribute to organism-level resistance, which may be translated into high LC50s. Physiological-level alterations, such as growth, reproduction, feeding, movement, or behavior, have been used as endpoints for chemical-induced toxicity testing in *C. elegans* [7,12,18–20] (Table 3). The effects of xenobiotics on the growth and reproduction of the test organisms are broadly accepted test parameters and are much more sensitive indicators of toxicity than lethality, as shown in the present study (Figs. 2 and 3). The decreases in body length and egg number per worm observed after Cd and Cr exposure may induce alteration in the growth and reproduction of the nematode population in the long term. In particular, for Cd exposure, an increase in many stress-related gene expressions occurred concomitantly with this deterioration on the physiological level.

Table 2. Pearson coefficient of correlations between measured parameters in *Caenorhabditis elegans*[a]

	hsp-16.1	hsp-16.2	hsp-16.48	hsp-70	mt-1	mt-2	vit-2	vit-6	cyp35a2	gst-4	cep-1	ape-1	sod-1	ctl-2	Body length	Egg number
Cadmium chloride																
hsp-16.1	0.359	0.417	−0.071	0.013	0.361	0.420	−0.450	−0.475	−0.401	0.579	0.049	0.438	0.298	0.182	−0.229	−0.074
hsp-16.2		0.767	0.822	0.895	0.968*	0.998**	0.541	0.509	0.445	0.968*	0.788	0.983*	0.959*	0.972*	−0.452	−0.289
hsp-16.48			0.828	0.823	0.900	0.778	0.609	0.592	0.664	0.756	0.917	0.866	0.903	0.814	−0.912	−0.813
hsp-70				0.989*	0.893	0.796	0.914	0.899	0.876	0.682	0.978*	0.838	0.923	0.932	−0.710	−0.645
mt-1					0.937	0.872	0.857	0.837	0.796	0.772	0.957*	0.897	0.958*	0.975*	−0.648	−0.556
mt-2						0.968*	0.634	0.606	0.587	0.931	0.900	0.993**	0.997**	0.977**	−0.660	−0.517
vit-2							0.497	0.463	0.407	0.982*	0.773	0.988*	0.954*	0.959*	−0.459	−0.290
vit-6								0.999**	0.974*	0.334	0.869	0.545	0.687	0.723	−0.621	−0.637
cyp35a2									0.978*	0.298	0.856	0.514	0.661	0.697	−0.619	−0.643
gst-4										0.255	0.874	0.487	0.642	0.642	−0.748	−0.783
cep-1											0.679	0.968*	0.904	0.890	−0.748	−0.783
ape-1												0.841	0.926	0.898	−0.838	−0.773
sod-1													0.982*	0.964*	−0.590	−0.431
ctl-2														0.984*	−0.679	−0.547
Body length															−0.563	−0.434
																0.979*
Lead(II)nitrate																
hsp-16.1	0.274	0.657	−0.072	−0.505	0.718	0.720	0.460	0.549	−0.641	−0.528	−0.998**	0.951*	0.134	0.054	0.261	−0.886
hsp-16.2		0.514	0.914	0.409	−0.051	−0.343	0.351	0.655	0.173	0.485	−0.321	0.442	0.985*	0.665	0.951*	−0.650
hsp-16.48			0.137	−0.568	−0.050	0.550	−0.190	0.108	−0.748	−0.481	−0.697	0.527	0.362	−0.222	0.283	−0.854
hsp-70				0.737	−0.153	−0.690	0.391	0.619	0.553	0.797	0.031	0.172	0.971*	0.820	0.940	−0.305
mt-1					−0.089	−0.948	0.457	0.444	0.969*	0.990**	0.498	−0.213	0.560	0.834	0.589	0.328
mt-2						0.398	0.824	0.680	−0.116	−0.198	−0.681	0.792	−0.091	0.341	0.151	−0.407
vit-2							−0.149	−0.164	−0.941	−0.968*	−0.705	0.478	−0.499	−0.640	−0.452	−0.477
vit-6								0.933	0.370	0.374	−0.441	0.691	0.380	0.813	0.598	−0.347
cyp35a2									0.278	0.405	−0.552	0.781	0.657	0.863	0.823	−0.588
gst-4										0.942	0.645	−0.375	0.341	0.707	0.373	0.541
cep-1											0.513	−0.246	0.630	0.811	0.628	0.292
ape-1												−0.950	−0.179	−0.062	−0.295	0.913
sod-1													0.339	0.360	0.497	−0.877
ctl-2														0.748	0.968*	−0.517
Body length															0.859	−0.197
																−0.561
Potassium dichromate																
hsp-16.1	0.211	0.801	0.647	0.812	0.873	−0.223	0.831	0.892	−0.402	−0.442	0.088	0.906	0.808	−0.572	0.333	−0.106
hsp-16.2		−0.358	0.013	−0.395	0.471	0.229	0.283	0.525	−0.967*	−0.022	0.792	0.430	0.309	0.103	0.194	−0.158
hsp-16.48			0.389	0.943	0.643	−0.086	0.464	0.453	0.124	−0.627	−0.197	0.684	0.720	−0.787	−0.046	−0.267
hsp-70				0.653	0.236	−0.882	0.934	0.753	−0.031	0.377	−0.498	0.295	0.075	0.194	0.902	0.690
mt-1					0.511	−0.411	0.650	0.547	0.207	−0.337	−0.430	0.569	0.533	−0.540	0.264	0.066
mt-2						0.251	0.546	0.767	−0.676	−0.712	0.545	0.997**	0.970*	−0.738	−0.305	−0.515
vit-2							−0.663	−0.374	−0.308	−0.715	0.768	0.190	0.393	−0.543	−0.910	0.935
vit-6								0.938	−0.350	0.128	−0.170	0.591	0.376	−0.020	0.800	0.434
cyp35a2									−0.625	−0.109	0.174	0.792	0.604	−0.202	0.613	0.145
gst-4										0.249	−0.842	−0.637	−0.541	0.144	−0.106	0.318
cep-1											−0.522	−0.692	−0.858	0.974*	0.697	0.917
ape-1												0.483	0.530	−0.346	−0.436	−0.715
sod-1													0.965*	−0.735	0.009	−0.470
ctl-2														−0.880	−0.247	−0.668
Body length															0.575	0.804
																0.867

Table 2. Continued.

	hsp-16.1	hsp-16.2	hsp-16.48	hsp-70	mt-1	mt-2	vit-2	vit-6	cyp35a2	gst-4	cep-1	ape-1	sod-1	ctl-2	Body length	Egg number
Sodium meta-arsenite																
hsp-16.1	0.046	0.914	−0.891	0.318	−0.299	0.876	−0.849	0.906	0.575	−0.417	−0.408	0.552	0.874	0.987*	0.393	0.361
hsp-16.2		−0.243	0.276	−0.913	0.907	−0.405	0.117	−0.043	−0.620	−0.842	0.751	0.834	−0.387	0.192	−0.797	0.389
hsp-16.48			−0.999**	0.611	−0.466	0.876	−0.966*	0.735	0.567	−0.257	−0.733	0.216	0.987*	0.841	0.471	0.468
hsp-70				−0.641	0.480	−0.863	0.970*	−0.701	−0.556	0.234	0.767	−0.170	−0.989*	−0.810	−0.473	−0.479
mt-1					−0.903	0.658	−0.512	0.288	0.687	0.558	−0.940	−0.615	0.719	0.164	0.802	−0.067
mt-2						−0.719	0.273	−0.452	−0.893	−0.729	0.712	0.531	−0.599	−0.178	−0.975*	0.447
vit-2							−0.721	0.908	0.884	0.073	−0.612	0.159	0.915	0.812	0.786	−0.007
vit-6								−0.574	−0.337	0.420	0.714	−0.241	−0.925	−0.780	−0.248	−0.679
cyp35a2									0.783	−0.148	−0.233	0.515	0.734	0.911	0.601	−0.059
gst-4										0.486	−0.489	−0.106	0.665	0.507	0.966*	−0.462
cep-1											−0.276	−0.819	−0.101	−0.505	0.671	−0.787
ape-1												0.497	−0.798	−0.256	−0.583	−0.278
sod-1													0.090	0.677	−0.359	0.329
ctl-2														0.784	0.596	0.352
Body length															0.298	0.351
																−0.524

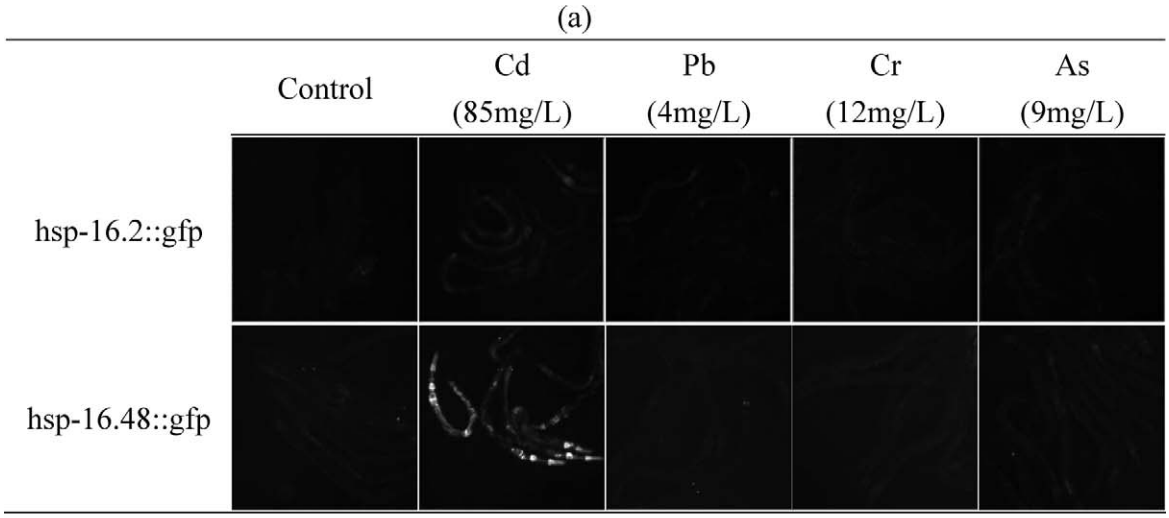
[a] See text for definitions of abbreviations. Asterisks indicate significant correlations (* $p < 0.05$; ** $p < 0.01$).

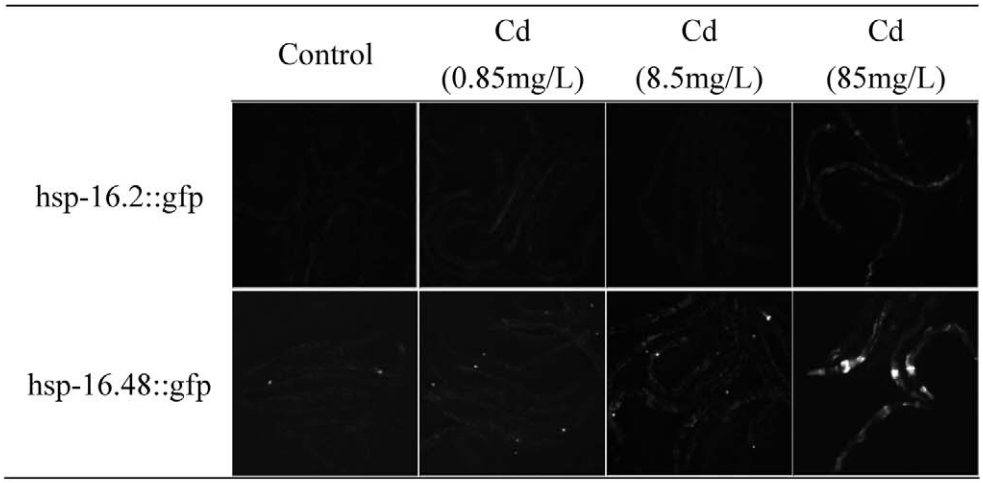
Lead and As seem to have little impact on the growth and reproduction of *C. elegans*, because only a slight stimulatory effect on worm growth was observed.

As mentioned previously, one of the aims of the present work was to reveal a link or correlation between a validated toxicity endpoint (i.e., growth and reproduction) and upstream-induced gene expression. This is interesting with reservations, particularly for ecotoxicological purposes. The statistical tests revealed 27 significant correlations in Cd exposure. Their biological/ecological meanings, however, should be interpreted, because a correlation study alone cannot provide any causal relationship between these parameters. Direct experimental demonstrations of the wider relationships between molecular/biochemical-level effects and their subsequent consequences at higher levels of biological organization are needed to establish causal relationships. The characterization of the causal relationships between the biomarker responses and the effects at higher biological levels will help to define the sublethal hazards of chemicals in this animal.

The use of transgenic animals is not a new approach in environmental monitoring. Recently, a fish transgenic model was developed. It has received much attention [21–24], and its promising capability was demonstrated. Nonetheless, most of the protocols require skills-based, long, and costly experiments, which make them difficult to adapt for the rapid routine assessment of field samples. *Caenorhabditis elegans* allows the preparation of a large number of staged and genetically homogeneous animals in the laboratory in a short time. Moreover, the advantage of a rich collection of gene engineering approaches and well-established transgenetic approaches also presents a short cut to the development of a sensitive biosensor that other organism models cannot surpass. Indeed, different promoters (e.g., hsp and mt) and alternative reporters (e.g., GFP, β-galactosidase, and luciferase) have been tested in different transgenic designs [25–27]. In the present study, because hsps are thought to play roles in various stress conditions, it was hypothesized that hsp-16.2 and hsp-16.48 are general stress-response proteins. Moreover, the response level of hsp16-lacZ transgenic *C. elegans* toward $CdCl_2$, $Pb(NO_3)_2$, and $NaAsO_2$ exposure was reported previously as 100, 30, and 50 μM, respectively [25]. However, unexpectedly, the responses of gene expression levels, semiquantitatively assayed using the gfp-based reporter hsp-16.2 and hsp-16.48 transgenic nematodes, were not very sensitive toward the four metals tested. To identify the transgenic nematode as a biosensor for toxicity monitoring, the responses of a broad range of stress-related gene promoters to various classes of chemicals should be screened.

Xenobiotically induced gene expression is considered to be a highly promising tool in biomonitoring for the early detection of environmental contaminants [4,28,29]. Gene expression endpoints are not only sensitive and useful in estimating the effects of toxicants on expected populations but also may provide insight regarding the mechanisms underlying these effects. Their field application is still limited, however, because these systems are not capable of completely integrating the physiological status of a living organism and, thus, have low ecological relevance. Whereas the parameters from higher levels of biological organizations, such as growth and reproduction, are accepted as valid and standardized endpoints, their responses are, nonetheless, not very sensitive or specific. The main advantage of the gene expression test compared to the growth or reproduction test is the increased sensitivity and





(b)

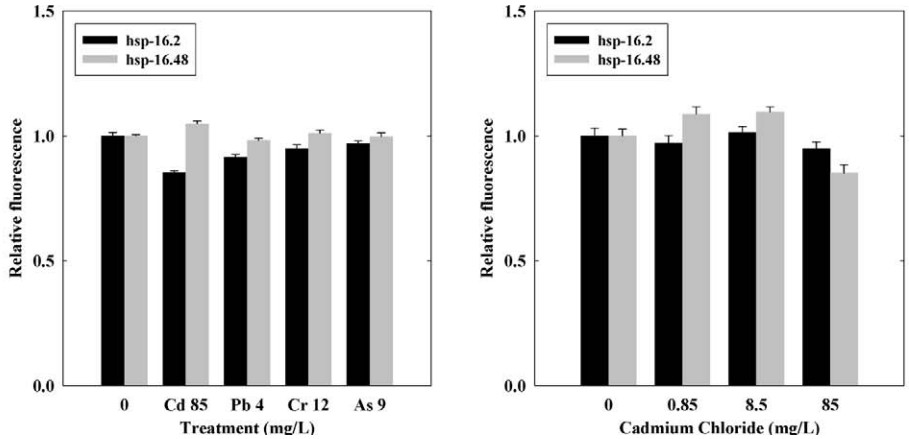


Fig. 5. Response of transgenic strains (hsp-16.2::gfp and hsp-16.48::gfp) of *Caenorhabditis elegans* exposed to Cd, Pb, Cr, and As for 24 h (**a**). Quantified fluorescence values are presented in arbitrary units compared to control (control = 1 [**b**], $n = 3$, mean ± standard error of the mean). gfp = green fluorescent protein; hsp = heat shock protein.

Table 3. Metal toxicity tests performed on *Caenorhabditis elegans*

Metals	Toxic endpoints[a]		Compound	Exposure condition	Concentration (mg/L)	Reference
Cd	Lethality	24-h LC50	$CdCl_2$	K-media	904	[10]
			$CdCl_2$	K-media	1,322	[12]
			$CdCl_2$	K-media	2,772.2	[13]
			$CdCl_2$	Agar plate	566	[10]
			$CdCl_2$	K-media	850	Table 1
		96-h LC50	$CdCl_2$	K-media	6.1×10^{-2}	[10]
	Behavior	24-h EC50	$CdCl_2$	K-media	25	[12]
	Feeding	24-h EC50	$CdCl_2$	K-media	23.4	[7]
	Movement	24-h EC50	$CdCl_2$	K-media	30.6	[7]
	Growth	24-h EC50	$CdCl_2$	K-media	26.9	[7]
	Reproduction	72-h LC50	$CdCl_2$	K-media	27.6	[7]
	Metallothionein expression	24-h LOEC	$CdCl_2$	K-media	18.3	[31]
		24-h LOEC	$CdCl_2$	K-media	457.5×10^{-3}	[32]
	CDR-1 gene expression	24-h LOEC	$CdCl_2$	K-media	183×10^{-3}	[15]
	Stress-related gene expression	24-h LOEC	$CdCl_2$	K-media	0.85	Figs. 1 and 2
Pb	Lethality	24-h LC50	$Pb(NO_3)$	K-media	129	[10]
			$PbCl_2$	K-media	202	[12]
			$Pb(NO_3)_2$	Agar plate	421	[10]
		96-h LC50	$Pb(NO_3)_2$	K-media	6.2×10^{-2}	[10]
		24-h LC50	$Pb(NO_3)_2$	K-media	40	Table 1
	Behavior	24-h EC50	$PbCl_2$	K-media	9	[12]
	Feeding	24-h EC50	$PbCl_2$	K-media	8.2	[7]
	Movement	24-h EC50	$PbCl_2$	K-media	8.6	[7]
	Growth	24-h EC50	$PbCl_2$	K-media	7.5	[7]
	Reproduction	24-h EC50	$PbCl_2$	K-media	5.5	[7]
	Stress-related gene expression	24-h LOEC	$Pb(NO_3)_2$	K-media		Figs. 1 and 2
Cr	Lethality	24-h LC50	$K_2Cr_2O_7$	K-media	156	[10]
		24-h LC50	$K_2Cr_2O_7$	K-media	120	Table 1
		96-h LC50	$K_2Cr_2O_7$	K-media	5.9×10^{-2}	[10]
	Stress-related gene expression	24-h LOEC	$K_2Cr_2O_7$	K-media	0.12	Figs. 1 and 2
As	Lethality	24-h LC50	$NaAsO_2$	K-media	182	[10]
		24-h LC50	$NaAsO_2$	K-media	90	Table 1
		96-h LC50	$NaAsO_2$	K-media	173	[10]
	Stress-related gene expression	24-h LOEC	$NaAsO_2$	K-media	0.09	Figs. 1 and 2
Cu	Lethality	24-h LC50	$CuCl_2 \cdot 2H_2O$	K-media	22	[10]
			$CuCl_2$	K-media	91	[12]
			$CuCl \cdot 2H_2O$	Agar plate	170	[10]
		96-h LC50	$CuCl \cdot 2H_2O$	K-media	0.256	[10]
	Behavior	24-h EC50	$CuCl_2$	K-media	3	[12]
	Feeding	24-h EC50	$CuCl_2$	K-media	9.0	[7]
	Movement	24-h EC50	$CuCl_2$	K-media	11.2	[7]
	Growth	24-h EC50	$CuCl_2$	K-media	10.2	[7]
	Reproduction	24-h EC50	$CuCl_2$	K-media	7.1	[7]
Al	Lethality	24-h LC50	$Al(NO_3)_3$	K-media	79	[10]
			$Al(NO_3)_3$	K-media	49	[12]
			$Al(NO_3)_3$	Agar plate	137	[10]
	Behavior	24-h EC50	$Al(NO_3)_3$	K-media	3	[12]
Zn	Lethality	24-h LC50	$ZnCl_2$	K-media	202	[10]
			$ZnCl_2$	K-media	257	[12]
			$ZnCl_2$	Agar plate	347	[10]
		96-h LC50	$ZnCl_2$	K-media	1.32	[10]
	Behavior	24-h EC50	$ZnCl_2$	K-media	18	[12]

[a] CPR = cadmium-responsive gene; EC50 = median effect concentration; LC50 = median lethal concentration; LOEC = lowest-observed-effect concentration.

specificity, as shown in Table 3. The parallel determination of a variety of stress-inducible genes (e.g., by using a DNA microarray) will promote implementation of this approach [5,30]. Depending on the selected marker genes, this approach has the potential of identifying substance class–specific effects. In this context, it is important to reveal the potential relationships between the observed toxicity and the induced gene expression. Reliable, sensitive, and specific test systems therefore are needed, particularly for risk assessment of a low-level mixture of xenobiotics in the environment, which affects both the wildlife and human health on a subcellular level. Using sensitive and reproducible detection methods, it is possible to establish significant pollution-induced changes on specific gene expressions and to generate new and accurate assays.

The overall results suggest that Cd exhibits a high level of tolerance compared to the other metals tested, which may imply that *C. elegans* possess efficient defense equipment that prevents Cd-related damage. Use of the responses of stress-related gene expression therefore has considerable potential as a sensitive biomarker for the diagnosis of Cd contamination, and *C. elegans* seems to be a good biological model for this approach.

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