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Lethality Toxicities Induced by Metal Exposure During Development in Nematode *Caenorhabditis Elegans*

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Abstract Lethality changes were investigated during development in 4 h metal exposed *Caenorhabditis elegans*. Exposure to examined metals caused severe lethality toxicities in L1- and L2-larvae, in L3-larvae exposed to examined metals at concentrations of 50 and 100 μM and to Pb, Hg, and Cr at the concentration of 2.5 μM, in L4-larvae exposed to examined metals at concentrations of 50 and 100 μM, and in adults exposed to Pb, Hg, and Cr at the concentration of 100 μM. Moreover, the lethality toxicities induced by Pb and Hg in L1 larvae for 4 h could be largely comparable to those in young adults for 24 h.

Keywords Lethality · Metal exposure · Developmental stage · *Caenorhabditis elegans*

Nematode *Caenorhabditis elegans*, a free-living soil nonparasitic animal inhabiting the aquatic component of the soil environment, or interstitial water, has been used in a variety of ecological risk assessment in soil (Donkin and Dusenbery 1993; Peredney and Williams 2000; Boyd and Williams 2003; Graves et al. 2005), water (Mutwakil et al. 1997; Hitchcock et al. 1997; Ura et al. 2002; Wang et al. 2008), and sediments (Traunspurger et al. 1997). The detailed knowledge of its biology, including movement, feeding, development, and reproduction is available (Riddle et al. 1997), which provides the essential feature for the development of *C. elegans* as a biomonitor. *C. elegans* has a thin, translucent body, and a short life cycle. *C. elegans* can tolerate wide pH, salinity, and water hardness ranges, and is easy to obtain the age-synchronized nematode populations (Hitchcock et al. 1997). Considering the fact that the potential for genetic variability in nematode populations can be minimized by the hermaphroditic nature of the nematode (Brenner 1974), *C. elegans* can serve as an ideal test organism with the approximately same size, age, health, and reproduction status. The concentration-response relationships have been established based on the assessment of nematode endpoints after exposure to toxic metals and organic pollutants (Anderson et al. 2001; Roh et al. 2007; Sochová et al. 2007). A standardized method for conducting laboratory soil toxicity tests using *C. elegans* was published in the America Society for Testing and Materials (ASTM) Guide E2172-01 in 2002. It has been further suggested that *C. elegans* could substitute for vertebrate organisms in predicting mammalian acute lethality from metals (Williams and Dusenbery 1988). Moreover, the toxicity evaluation in a paper recycling mill effluent by coupling bioindicator of aging with the toxicity identification evaluation method has been performed in *C. elegans*, and the authors' data suggest that the suspected toxicants for aging toxicity might be mainly the heavy metal substances in industrial effluent from the paper recycling mill (Wang et al. 2008).

Caenorhabditis elegans has been shown to be effective in the detection of environmental contamination by monitoring the adverse effects on lethality (Williams and Dusenbery 1988, 1990; Peredney and Williams 2000; Ura et al. 2002), lifespan (Wang et al. 2007a, b), reproduction (Anderson et al. 2001; Wang and Wang 2008b), development (Wang and Yang 2007; Wang and Wang 2008b), feeding (Jones and Candido 1999; Anderson et al. 2001), behavior (Wang and Xing 2008), and behavioral plasticity (Wang et al. 2007b; Wang and Wang 2008a). Among the examined endpoints, the lethality is the most widely accepted one to evaluate the

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toxicity from environmental contamination or exposure in nematodes. This endpoint has been used to assess toxic constitutes within the pharmaceutical mixture (Dengg and van Meel 2004), and explored for the assessment of synergistic toxicity of multiple heavy metals and the improvement of heavy metal stress and toxicity assays by coupling a transgenic reporter in a mutant nematode strain (Chu and Chow 2002; Chu et al. 2005). Furthermore, the endpoint of lethality was used to examine the possible heavy metal resistance in *daf-2* and *age-1* long-lived mutants of *C. elegans* (Barsyte et al. 2001).

The choice of an optimal endpoint for testing environmental toxicants depends on several factors, including sensitivity, and the sensitive endpoints can permit detection of lower levels of toxicants and allow the use of shorter testing intervals (Anderson et al. 2001). Recently, in order to improve the sensitivity of endpoints, reporter transgenes consisting of a fragment of the promoter from the *C. elegans* heat-shock proteins of *hsp-16* and *hsp-70* that control the transcription of a β-galactosidase reporter (*LacZ*) or a green fluorescent protein (*gfp*) have been generated and successfully used for assessment of toxicological response to metals or other toxicants (Mutwakil et al. 1997; Chu and Chow 2002; Chu et al. 2005; Dengg and van Meel 2004; Wang et al. 2007b; Wang and Wang 2008a). Moreover, with the aid of the endpoint of feeding, it has been reported that when young adult adults were examined, L1 *C. elegans* larvae may represent one of the most sensitive invertebrate so far adopted for metal toxicity assay (Jones and Candido, 1999; Chu and Chow 2002). Nevertheless, previous studies have not excluded the possible effects of other developmental stages on the lethality toxicity from metal exposure at the L1-larval stage. Thus, in this study, we selected four metals (Pb, Hg, Cd, and Cr) and performed a 4 h toxicity assay to investigate the changes of lethality endpoint at different developmental stages (L1, L2, L3, L4, and young adult) in metal exposed *C. elegans*.

Materials and Methods

The metal concentrations used in this study were referred to our previous description (Wang et al. 2007a, b; Wang and Yang 2007; Shen et al. 2009; Xing et al. 2009). Three concentrations of $CdCl_2$, $CrCl_2$, $HgCl_2$, and $Pb(NO_3)_2$ solutions were used in the current work, and they were 2.5, 50, and 100 μM, respectively. Metal concentrations of exposed solutions were analyzed by atomic absorption spectrophotometry (AAS; Pye-Unicam model SP9, Cambridge, UK). All the chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Nematodes used in the present study were wild-type N2, originally obtained from the *Caenorhabditis* Genetics Center (funded by the NIH National Center for Research Resource, USA). They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20°C as described (Brenner 1974). Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (0.45 N NaOH, 2% HOCl). Age synchronous populations of larval (L1, L2, L3, or L4-stage) or young adult animals were obtained by the collection as described (Donkin and Williams 1995). The collected nematodes were washed with double-distilled water twice, followed by washing with modified K medium once (50 mM NaCl, 30 mM KCl, 10 mM NaOAc, pH 5.5) (Williams and Dusenbery 1990). Exposures were performed in 12-well sterile tissue culture plates. The exposures were 4 h long at the L1- through L4-larval stages, and the exposures were 4 h or 24 h long at the young adult stage. All exposures were carried out in 20°C incubator in the absence of food.

For the lethality assay, approximately 500 larvae or young adult animals treated by metal exposure were counted under a dissecting microscopy, where the inactive ones were scored. All assays were replicated three times.

All data in this article were expressed as mean ± SE. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA). One-way analysis of variance (ANOVA) followed by a Dunnett's *t*-test was used to determine the significance of the differences between the groups. The probability levels of 0.05 and 0.01 were considered statistically significant.

Results and Discussion

We first examined the possible changes of lethality toxicities induced by metal (Pb, Hg, Cr, and Cd) exposure at concentrations of 2.5, 50, and 100 μM at the younger larval (L1 through L3) stages in wild-type N2 nematodes. Under our experimental conditions, we seldom detected the appearance of lethal animals in wild-type nematode population without metal treatment at the L1- though L3-larval stages. In contrast to this, exposure to metals of Pb, Hg, Cr, and Cd at different concentrations for 4 h all caused the severe ($p < 0.01$) lethality toxicities at the L1-larval stage compared to control (Fig. 1). Similarly, exposure to metals of Pb, Hg, Cr, and Cd at all examined concentrations for 4 h resulted in the severe ($p < 0.01$) lethality toxicities at the L2-larval stage compared to control (Fig. 2). Exposure to all examined metals at concentrations of 50 and 100 μM, respectively, for 4 h also induced the formation of severe ($p < 0.01$) lethality toxicities at the L3-larval stage compared to control (Fig. 3). The significant lethality toxicities could be further observed in nematodes exposed to Pb ($p < 0.01$), Hg ($p < 0.01$), and Cr ($p < 0.05$) at the

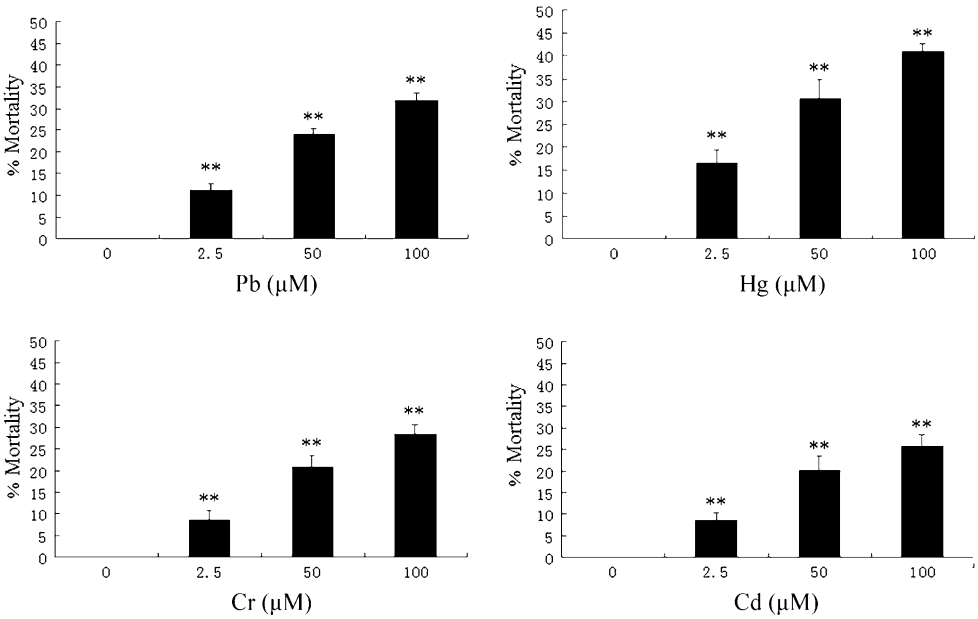

Fig. 1 Lethality toxicities induced by metal exposure at the L1-larval stage in wild-type N2 nematodes. Bars represent mean ± SE. $^{**}p < 0.01$ vs. 0 μM

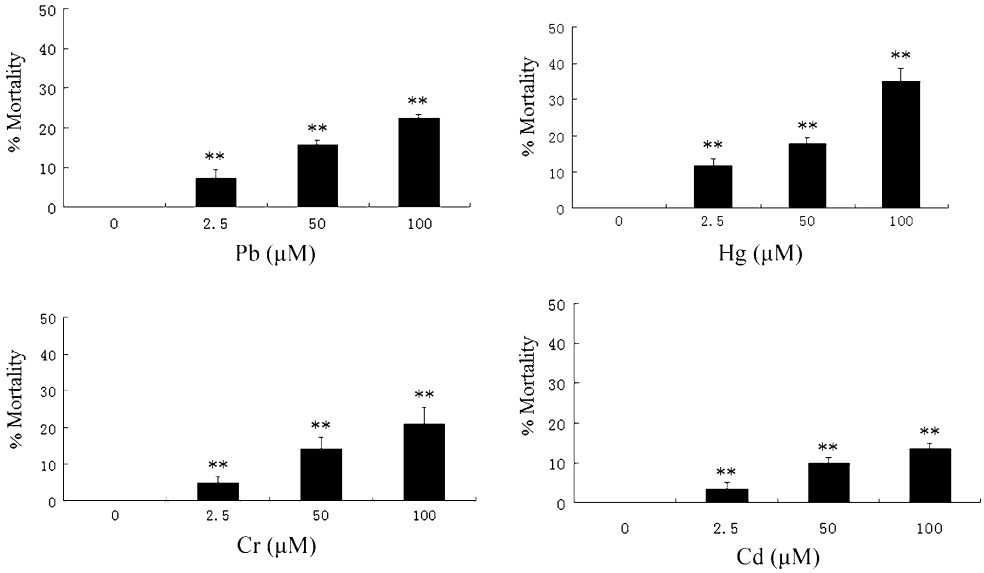

Fig. 2 Lethality toxicities induced by metal exposure at the L2-larval stage in wild-type N2 nematodes. Bars represent mean ± SE. $^{**}p < 0.01$ vs. 0 μM

concentration of 2.5 μM at the L3-larval stage, whereas exposure to 2.5 μM of Cd would not cause the formation of severe lethality toxicities at the L3-larval stage (Fig. 3).

Previous study on the 48 h toxicity assay using Cd as a reference metal suggests that nematodes in the late larval stages might have similar sensitivities as those of adults (Chu and Chow 2002). We next examined the lethality changes in nematodes exposed to metals at different concentrations for 4 h at the L4-larval and young adult stages. Exposure to examined metals at concentrations of 50 and 100 μM for 4 h induced significantly ($p < 0.01$) increased lethality toxicities, whereas exposure to examined metals at the concentration of 2.5 μM for 4 h could not resulted in the formation of severe lethality toxicities at the L4-larval stage compared to control (Fig. 4). Furthermore, only exposure to metals of Pb ($p < 0.01$), Hg ($p < 0.01$), and Cr ($p < 0.05$) at the concentration of 100 μM for 4 h could cause the noticeably elevated lethality toxicities, whereas exposure to Cd at the concentration of 100 μM for 4 h would not induce the lethality toxicities at the young adult stage compared to control (Fig. 5). In addition, exposure to examined metals at concentrations of 2.5 and 50 μM did not result in the severe lethality toxicity at the young adult stage (Fig. 5). Therefore, the L4 larvae and young adults can only be used to detect the limited lethality toxicity from metal exposure for 4 h.

We further examined the possible similarities or differences for the lethality toxicity between metal exposure for

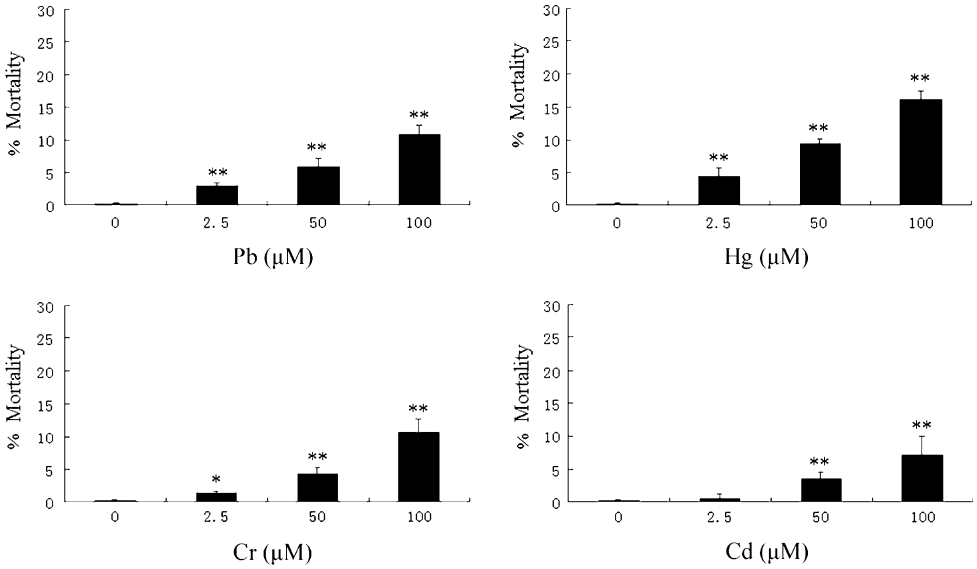

Fig. 3 Lethality toxicities induced by metal exposure at the L3-larval stage in wild-type N2 nematodes. Bars represent mean ± SE. *$p < 0.05$ vs. 0 μM; **$p < 0.01$ vs. 0 μM

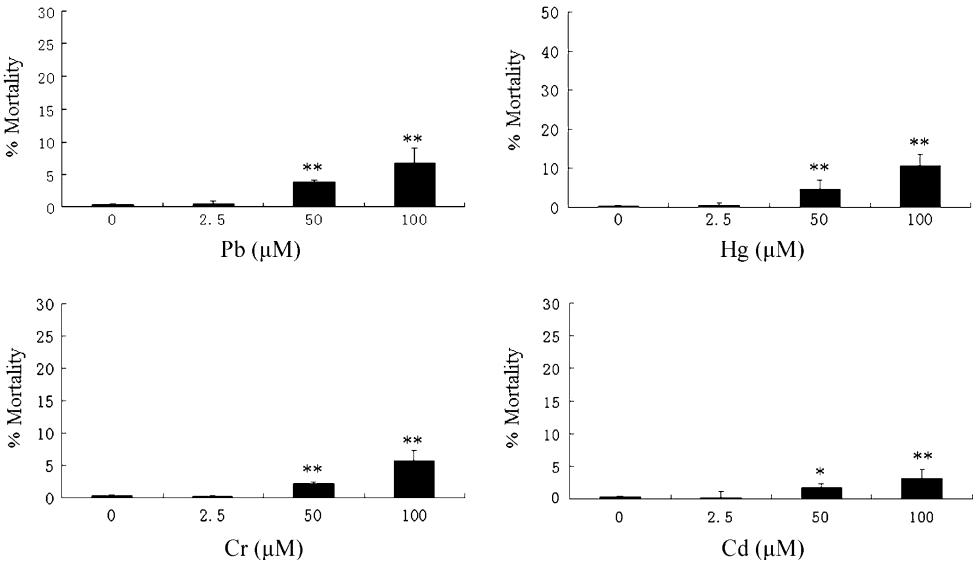

Fig. 4 Lethality toxicities induced by metal exposure at the L4-larval stage in wild-type N2 nematodes. Bars represent mean ± SE. *$p < 0.05$ vs. 0 μM; **$p < 0.01$ vs. 0 μM

4 h at the L1-larval stage and metal exposure for 24 h at the young adult stage. Nematodes exposed to Pb and Hg at all examined concentrations for 4 h at the L1-larval stage showed similar lethality toxicities from those observed in nematodes exposed to Pb and Hg for 24 h at the young adult stage (Fig. 6). Nevertheless, nematodes exposed to Cr and Cd for 4 h at the L1-larval stage still exhibited less lethality toxicities than those observed in nematodes exposed to Cr and Cd for 24 h at the young adult stage (data not shown). These data suggest that the lethality toxicities induced by some specific metals at the L1-larval stage for 4 h can be largely comparable to those at the young adult stage for 24 h in wild-type N2 nematodes.

Moreover, we observed that exposure to Cr and Cd at all examined concentrations for 4 h caused less lethality toxicities than those from exposure to Pb at L1- through L3-larval stages (data not shown). In contrast, exposure to Hg at the concentration of 100 μM for 4 h resulted in moderately higher lethality toxicities than those from exposure to Pb at L1- through L3-larval stages (data not shown). In addition, exposure to 50 μM of Hg also induced higher lethality toxicity than that from Pb exposure at the L1-larval stage (data not shown). Therefore, the lethality toxicity manner for examined metals is Hg > Pb > Cr > Cd based on 4 h assay under our experimental conditions.

In the present study, we investigated the lethality toxicities induced by metals at different developmental stages in model organism of *C. elegans*. To investigate the sensitivity of larvae nematodes to lethality toxicity from metal exposure, we exposed the nematodes at different developmental

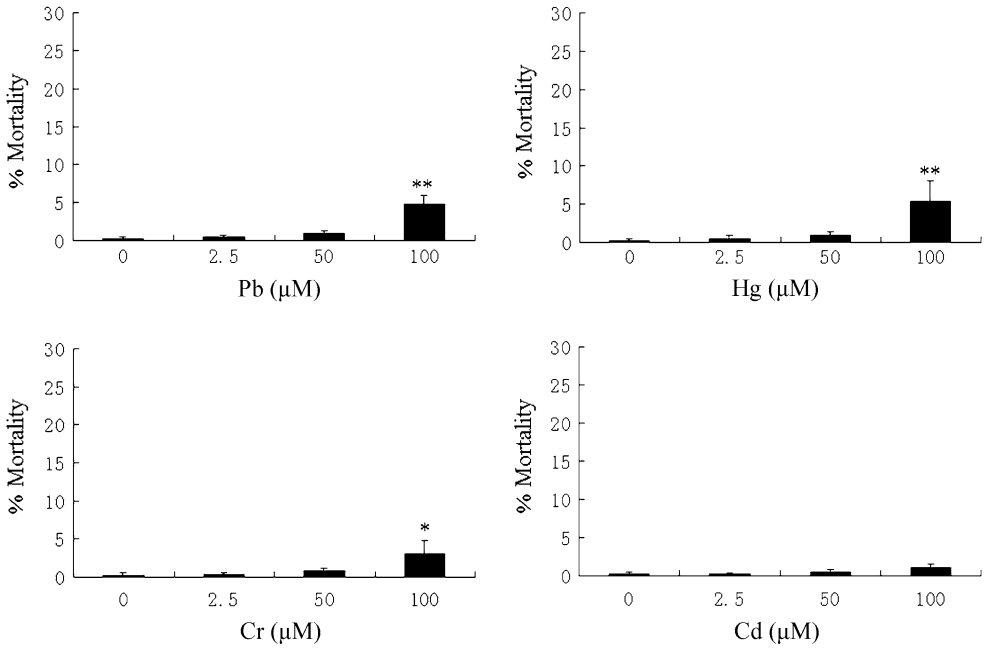

Fig. 5 Lethality toxicities induced by metal exposure at the young adult stage in wild-type N2 nematodes. Bars represent mean ± SE. $*p < 0.05$ vs. 0 μM; $**p < 0.01$ vs. 0 μM

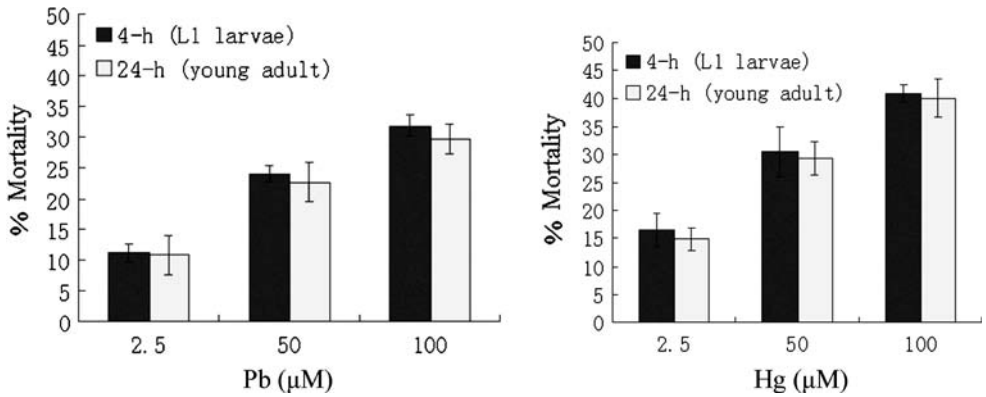

Fig. 6 The lethality toxicities induced by metal of Pb or Hg at L1-larval stage for 4 h are largely comparable to those at the young adult stage for 24 h in wild-type N2 nematodes. *Bars* represent mean ± SE

stages to metals at different concentrations for 4 h. One of the important reasons to perform the 4 h exposure is that the life cycle of *C. elegans* is very rapid, and its postembryonic development will last only for 36 h through four larval stages, L1–L4 to the adult (Riddle et al. 1997). Four hour exposure will allow us to determine the specific sensitivity of L1, L2, L3, or L4 larvae to the specific form of toxicity from metal exposure. Three concentrations (2.5, 50, and 100 μM) of examined metals were selected in this project. Based on our previous studies, exposure to 2.5 μM of metals for more than 24 h usually only be able to induce a trace toxicity in young adult nematodes, whereas exposure to 50 μM and 100 μM of metals for more than 24 h can often result in a severe toxicity in young adult nematodes (Wang et al. 2007a, b; Wang and Yang 2007; Shen et al. 2009; Wang and Xing 2008; Xing et al. 2009). Moreover, to exclude the possible effects of food, the metal exposure was performed in 12-well sterile tissue culture plates in 20°C incubator in the absence of food.

The previous studies have demonstrated that *C. elegans* is suitable for the evaluation of lethality toxicity from environmental stresses including metal exposure (Williams and Dusenbery 1988, 1990; Peredney and Williams 2000; Ura et al. 2002) and organic pollutant exposure (Roh et al. 2007; Sochová et al. 2007). Our data here suggest that younger larvae (L1, L2 and L3) nematodes showed more severe lethality toxicity than L4 larvae and young adult nematodes exposed to examined metals for 4 h, which are largely consistent with previous studies on the performance of younger animals in nematodes and other organisms. Young organisms are more sensitive to the toxic effects of pesticides, and preweanling rats were twice as sensitive as adults to aldicarb (Morser 1999). In addition, at least for some endpoints, young rats are more sensitive to a range of chlorpyrifos doses; however, the magnitude of age-related differences depends on the specific endpoint and time of assessment, as well as age and sex of the test subject (Morser 1999, 2000). The 7 day LD50 increased from

1.65 mg Cd/kg body weight in 7 day-old mice to 4.08 mg/kg in adult mice, and the interaction between Cd and this metal-binding protein may be also affected by age after exposure to this toxic metal (Thomas et al. 1987). Moreover, the detectable limit was an order of magnitude higher than that defined with L1 nematodes when young adults were used for toxicity evaluation (Williams and Dusenbery 1988, 1990; Jones and Candido 1999). In addition, after a 48 h toxicity assay, the mortality curve on the L1 population had a sharp slope between 0 and 1,500 μM, but a LC50 value of 5,520 μM was obtained with adult nematodes, suggesting that the L1 animals were more sensitive than adult animals (Chu and Chow 2002). Our results further suggest that such a conclusion can also be obtained from a 4 h toxicity assay at specific developmental stages. Especially, the lethality toxicities induced by some specific metals (Pb and Hg) at the L1-larval stage for 4 h could be largely comparable to those at the young adult stage for 24 h in wild-type N2 nematodes (Fig. 6). Previous observations on the sensitivity of L1-larvae nematodes to metal toxicity might be the combination effects of different developmental stages, and the L1-larval stage may play a predominant role in inducing the final lethality toxicity in metal exposed nematodes.

Moreover, based on the 48 h toxicity assay using Cd as a reference metal from Chu and Chow (2002), nematodes in the late larval stages had similar sensitivities as those of adults. In this study, our data demonstrated that, under our experimental conditions, exposure to examined metals at concentrations of 50 and 100 μM for 4 h could still induce significantly increased lethality toxicities at the L4-larval stage (Fig. 4). In contrast, only exposure to metals of Pb, Hg, and Cr at the concentration of 100 μM for 4 h could cause the noticeably elevated lethality toxicities at the young adult stage (Fig. 5). Therefore, although the L4 larvae and young adults can only detect the limited lethality toxicities from metal exposure for 4 h, the L4-larval nematodes are still somewhat more sensitive than adult nematodes for the lethality toxicity evaluation. This can be at least partially explained by the differences of our assay from that performed by Chu and Chow (2002). The 48 h toxicity assay from Chu and Chow (2002) could not exclude the possible effects of other developmental stages on the lethality toxicity at the L1-larval stage. Even for the assessment of lethality toxicity at the young adult stage, a 48 h toxicity assay will not allow us discriminate the possible reason(s) of finally observed toxicity. It is mostly possible that the finally observed toxicity for the 48 h assay is the combined effects of lethality toxicity on the young adult and that on adult nematodes. The 4 h toxicity assay at a specific developmental stage performed in the current work can effectively exclude the possible effects from other developmental stages. Moreover, such a 4 h toxicity assay at the L1-larval stage with lethality as the ecologically relevant endpoint will be more convenient for the assessment from environmental contamination.

Furthermore, based on our 4 h assay, we concluded that the lethality toxicity manner for examined metals was Hg > Pb > Cr > Cd under our experimental conditions. This lethality toxicity manner largely agrees to previous reports. Based on the toxicity assay at the young adult stage, the order of metal toxicity was Cu > Pb > Cd for each endpoint, including lethality and movement (Anderson et al. 2001). The data from Chu and Chow (2002) demonstrated that, among their examined metals, the more toxic class included mercury, copper, lead, and chromium with a steep slope of killing curve, and the less toxic group included nickel, cadmium, aluminium, cobalt, zinc, and manganese with a flat shallow killing curve according to the 48 h toxicity assay on L1-larval nematodes. Similarly, adults cultured from L1-larval stage appeared more sensitive than adults cultured from dauer larvae for the lethality toxicity from metal exposure (Donkin and Williams 1995). Our previous study on the locomotion behavioral toxicity induced by acute metal exposure also demonstrated that the neurotoxicity from Pb and Hg exposure was more severe that that from Cr and Cd (Wang and Xing 2008).

In addition, previous studies have also suggested that the metal toxicity can be further improved by the mutation of some specific genes in nematodes (Barsyte et al. 2001; Swain et al. 2004; Chu et al. 2005; Hughes and Stürzenbaum 2007). For example, the toxicity from cadmium exposure could be magnified by the knock-out or wild-type subjected to a knock down by RNAi of metallothionein genes in nematodes (Swain et al. 2004). Our recent data suggested that mutation of metallothionein genes (*mtl-1* and *mtl-2*) could result in more severe lethality toxicity from metal exposure in L1-larval nematodes compared to that in wild-type N2 nematodes (D. Wang, personal communication). Therefore, the combinational use of younger nematode larvae and mutation of a specific gene will greatly increase the sensitivity of nematodes in detecting the toxicity from environmental contamination.

Overall, younger larvae (L1, L2 and L3) nematodes exhibited more severe lethality toxicity than L4 larvae and young adult nematodes after 4 h of metal exposure. Moreover, the lethality toxicities induced by some specific metals (Pb and Hg) at the L1-larval stage for 4 h can be largely comparable to those at the young adult stage for 24 h. Such a 4 h toxicity assay at the L1-larval stage with lethality as the ecologically relevant endpoint will be more convenient for the assessment from metal exposure.

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References

America Society for Testing and Materials (ASTM) (2002) Standard guide for conducting laboratory soil toxicity tests with the nematode *C. elegans*. E 2172-01. In: Annual book of ASTM standard, vol 11.05. ASTM, West Conshohocken, PA, pp 1606–1616

Anderson GL, Boyd WA, Williams PL (2001) Assessment of sublethal endpoints for toxicity testing with the nematode *C. elegans*. Environ Toxicol Chem 20:833–838

Barsyte D, Lovejoy DA, Lithgow GJ (2001) Longevity and heavy metal resistance in *daf-2* and *age-1* long-lived mutants of *C. elegans*. FASEB J 15:627–634

Boyd WA, Williams PL (2003) Availability of metals to the nematode *C. elegans* toxicity based on total concentrations in soil and extracted fractions. Environ Toxicol Chem 22:1100–1106

Brenner S (1974) The genetics of *C. elegans*. Genetics 77:71–94

Chu KW, Chow KL (2002) Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. Aquat Toxicol 61:53–64. doi:10.1016/S0166-445X(02)00017-6

Chu KW, Chan SKW, Chow KL (2005) Improvement of heavy metal stress and toxicity assays by coupling a transgenic reporter in a mutant nematode strain. Aquat Toxicol 74:320–332. doi:10.1016/j.aquatox.2005.06.006

Dengg M, van Meel JCA (2004) *C. elegans* as model system for rapid toxicity assessment of pharmaceutical compounds. J Pharmacol Toxicol Methods 50:209–214. doi:10.1016/j.vascn.2004.04.002

Donkin SG, Dusenbery DB (1993) A soil toxicity test using the nematode *C. elegans* and an effective method of recovery. Arch Environ Contam Toxicol 25:145–151

Donkin SG, Williams PL (1995) Influence of developmental stage, salts and food presence on various end points using *C. elegans* for aquatic toxicity testing. Environ Toxicol Chem 14:2139–2147

Graves AL, Boyd WA, Williams PL (2005) Using transgenic *C. elegans* in soil toxicity testing. Arch Environ Contam Toxicol 48:490–494. doi:10.1007/s00244-004-0031-2

Hitchcock DR, Black MC, Williams PL (1997) Investigations into using the nematode *C. elegans* for municipal and industrial wastewater toxicity testing. Arch Environ Contam Toxicol 33:252–260

Hughes S, Stürzenbaum SR (2007) Single and double metallothionein knockout in the nematode *C.* elegans reveals cadmium dependent and independent toxic effects on life history traits. Environ Pollut 145:395–400. doi:10.1016/j.envpol.2006.06.003

Jones D, Candido EPM (1999) Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode *C. elegans* relationship to the cellular stress response. J Exp Zool 284:147–157

Morser VC (1999) Comparison of aldicarb and methamidophos neurotoxicity at different ages in the rat behavioral and biochemical parameters. Toxicol Appl Pharmacol 157:94–106. doi:10.1006/taap.1999.8675

Morser VC (2000) Dose-response and time-course of neurobehavioral changes following oral chlorpyrifos in rats of different ages. Neurotoxicol Teratol 22:713–723. doi:10.1016/S0892-0362(00)00087-8

Mutwakil MHAZ, Reader JP, Holdich DM, Smithurst PR, Candido EPM, Jones D, Stringham EG, de Pomerai DI (1997) Use of stress-inducible transgenic nematodes as biomarkers of heavy metal pollution in water samples from an english river system. Arch Environ Contam Toxicol 32:146–153

Peredney CL, Williams PL (2000) Utility of *C. elegans* for assessing heavy metal contamination in artificial soil. Arch Environ Contam Toxicol 39:113–118. doi:10.1007/s002440010086

Riddle DL, Blumenthal T, Meyer BJ, Priess JR (1997) C. ELEGANS II. Cold Spring Harbor Laboratory Press, Plainview, New York

Roh J, Jung I, Lee J, Choi J (2007) Toxic effects of di(2-ethylhexyl) phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode *C. elegans*. Toxicology 237:126–133. doi:10.1016/j.tox.2007.05.008

Shen L-L, Xiao J, Ye H-Y, Wang D-Y (2009) Toxicity evaluation in nematode *C. elegans* after chronic metal exposure. Environ Toxicol Pharmacol 28:125–132. doi:10.1016/j.etap.2009.03.009

Sochová I, Hofman J, Holoubek I (2007) Effects of seven organic pollutants on soil nematode *C. elegans*. Environ Int 33:798–904. doi:10.1016/j.envint.2007.03.001

Swain SC, Keusekotten K, Baumeister R, Stürzenbaum SR (2004) C. *elegans* metallothioneins new insights into the phenotypic effects of cadmium toxicosis. J Mol Biol 341:951–959. doi:10.1016/j.jmb.2004.06.050

Thomas DJ, Winchurch RA, Huang PC (1987) Ontogenic variation in acute lethality of cadmium in C57BL/6 J mice. Toxicology 47:317–323

Traunspurger W, Haitzer M, Höss S, Beier S, Ahlf W, Steinberg C (1997) Ecotoxicological assessment of aquatic sediments with *C. elegans* (nematode)—a method testing liquid medium and whole-sediment samples. Environ Toxicol Chem 16:245–250

Ura K, Kai T, Sakata S, Iguchi T, Arizono K (2002) Aquatic acute toxicity testing using the nematode *C. elegans*. J Health Sci 48:583–586

Wang D-Y, Wang Y (2008a) Phenotypic and behavioral defects caused by barium exposure in nematode *C. elegans*. Arch Environ Contam Toxicol 54:447–453. doi:10.1007/s00244-007-9050-0

Wang D-Y, Wang Y (2008b) Nickel sulfate induces numerous defects in *C. elegans* that can also be transferred to progeny. Environ Pollut 151:585–592. doi:10.1016/j.envpol.2007.04.003

Wang D-Y, Xing X-J (2008) Assessment of locomotion behavioral defects induced by acute toxicity from heavy metal exposure in nematode *C. elegans*. J Environ Sci 20:1132–1137

Wang D-Y, Yang P (2007) The multi-biological defects caused by lead exposure exhibit transferable properties from exposed parents to their progeny in *C. elegans*. J Environ Sci 19:1367–1372

Wang D-Y, Shen L-L, Wang Y (2007a) The phenotypic and behavioral defects can be transferred from zinc exposed nematodes to their progeny. Environ Toxicol Pharmacol 24:223–230. doi:10.1016/j.etap.2007.05.009

Wang Y, Xie W, Wang D-Y (2007b) Transferable properties of multi-biological toxicity caused by cobalt exposure in *C. elegans*. Environ Toxicol Chem 26:2405–2412

Wang X-Y, Shen L-L, Yu H-X, Wang D-Y (2008) Toxicity evaluation in a paper recycling mill effluent by coupling bioindicator of aging with the toxicity identification evaluation method in nematode *C. elegans*. J Environ Sci 20:1373–1380

Williams PL, Dusenbery DB (1988) Using the nematode *C. elegans* to predict mammalian acute lethality to metallic salts. Toxicol Ind Health 4:469–478

Williams PL, Dusenbery DB (1990) Aquatic toxicity testing using the nematode *C. elegans*. Environ Toxicol Chem 9:1285–1290

Xing X-J, Du M, Xu X-M, Rui Q, Wang D-Y (2009) Exposure to metals induces morphological and functional alteration of AFD neurons in nematode *C. elegans*. Environ Toxicol Pharmacol 28:104–110. doi:10.1016/j.etap.2009.03.006