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Toxicity evaluation in a paper recycling mill effluent by coupling bioindicator of aging with the toxicity identification evaluation method in nematode *Caenorhabditis elegans*

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Abstract

Toxicity identification evaluation (TIE) can be used to determine the specific toxicant(s) in industrial effluents. In the current study, the authors have attempted to combine the advantages of the model organism, *Caenorhabditis elegans*, with the virtues of the TIE technique, to evaluate and identify the toxicity on aging from a paper recycling mill effluent. The results indicate that only the toxicities from mixed cellulose (MC) filtration and EDTA treatment are similar to the baseline aging toxicity, suggesting that the suspect toxicants inducing aging toxicity may largely be the heavy metal substances in this industrial effluent. Examination of the accumulation of intestinal autofluorescence in adult animals further confirms that the short lifespans are actually due to accelerated aging. In addition, exposure to fractions of EDTA manipulations cannot result in severe defects of reproduction and locomotion behaviors in *C. elegans*. Moreover, high levels of Ca, Al, and Fe in the effluent may account for the severe toxicity on aging of exposed nematodes, by TIE assay. The study here provides a new method for evaluating environmental risk and identifying toxicant(s) from the industrial effluent using *C. elegans*.

Key words: metals; aging; toxicity identification evaluation (TIE); *Caenorhabditis elegans*

Introduction

As it may be difficult to determine which chemicals to analyze and also to predict the possible effects of the measured chemicals on the organism by chemical analysis, bioassay is used, as it has a better advantage of showing the total impact of the pollutant or pollutants and of providing the consequences of contaminants released into the environment. However, without chemical analysis, it will be difficult to measure specific chemicals in the assayed samples (Fjällborg *et al*., 2006). Of late, bioassay and chemical analysis have been combined to identify and determine the degrees of pollution in natural waters and soils (Kwok *et al*., 2005).

One of the approaches is to use toxicity identification evaluation (TIE), a bioassay-directed fractionation protocol. The USEPA (1996) first developed the TIE procedure, which makes it possible to determine what fractions of chemicals cause the observed effects on the bioassay and it allows the biologically active compounds in a complex mixture to be isolated prior to chemical analysis. TIE methods have proved to be an effective tool for characterizing and identifying toxicants in samples of freshwater and marine effluents, receiving waters, interstitial waters, sediment pore waters, whole sediments, metals in soil leachates, and leachates from products such as car tires (Ankley *et al*., 1992; Mount and Norberg-King, 1993; Nelson *et al*., 1994; Burgess *et al*., 2000; Ho *et al*., 2002; Kwok *et al*., 2005; Fjällborg *et al*., 2006). With the aid of TIE methods, the characterization of toxic effluent components are allowed to be evaluated and suspected, and the authors can comprehend the particular class of chemicals that the toxicant comes from, in phase I of the USEPA procedure. In addition, a benthic *in situ* TIE method was developed recently for separating key chemical classes of stressors in streams to help distinguish between point and nonpoint sources of pollution (Burton and Nordstrom, 2004; Custer *et al*., 2006). Based on the study on these procedures, guidelines for TIEs in marine receiving waters recommended a range of test organisms including macroalgae, echinoderms, bivalves, a gastropod, crustaceans, and fish (USEPA, 1996).

Nematode *Caenorhabditis elegans* is a free-living soil nematode that has been extensively used as a model organism, because it has properties of a short life cycle, simple cell lineage, low cost, and ease of maintenance (Riddle *et al*., 1997). In addition, *C. elegans* is sensitive to the testing toxicants, easy to manage in the laboratory, and

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available throughout the year, which helps it to satisfy the criteria for bioindicators (Wang and Yang, 2007; Wang *et al*., 2007a). By virtue of these properties, nematodes have been already developed for ecological risk assessment in soil (Peredney and Williams, 2000) and water (Ura *et al*., 2002), and a standardized method for conducting laboratory soil toxicity tests using *C. elegans* has been published in the America Society for Testing and Materials Guide E2172-01 (ASTM, 2002). Moreover, several endpoints, such as mortality, reproduction, behavior, and body length, have been used for acute toxicity testing with *C. elegans*, and the toxic effects have been evaluated in relatively short-term treatments (2–72 h), which provide a good benchmark for the assessment of biomarker responses in nematodes (Power and de Pomerai, 1999; Wang *et al*., 2007b). Nevertheless, no reports of similar experiments with terrestrial nematode *C. elegans* have appeared in TIE assays to date.

The objective of this study was to attempt to combine the advantages of the model organism, *C. elegans*, with the virtues of the TIE technique when evaluating the environmental risk and determining the true toxicant(s). In the present study, the authors explored the TIE method to evaluate the toxicity on aging from a paper recycling mill effluent using *C. elegans*. Their study will provide a new method for evaluating environmental risk and identifying individual toxicant(s) from polluted water.

1 Materials and methods

1.1 Sample collection

Water samples were collected from the paper recycling mill in Nanjing, China. The paper recycling mill was near the Yangtze River, and most of the industrial effluent from this mill had been released into the Yangtze River. On obtaining the water sample, the sample was stored at 4°C until measurement. All reagents used in this study were of analytical grade.

1.2 Preparation of nematode cultures

All strains used in the current study were originally obtained from the Caenorhabditis Genetics Center (Minneapolis, MN, USA). They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 22°C, as described by Brenner (1974). Gravid animals were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (0.45 mol/L NaOH, 2% HOCl). Age synchronous populations of nematodes (L4-stage larva) were obtained by collection, as described in published work (Donkin and Williams, 1995). The L4-stage larvae were washed with double-distilled water twice, followed by washing with modified K medium once (50 mmol/L NaCl, 30 mmol/L KCl, 10 mmol/L NaOAc, pH 5.5). Exposures were performed in 12-well sterile tissue culture plates. Approximately 100 animals were transferred in 50 μl of each exposure solution using a micropipettor. All exposures were 2 d long and were carried out in 22°C incubator, in the absence of food.

1.3 Life span assay

The method was performed as described by Swain *et al*. (2004). The exposed and control animals were picked and placed on the assay plates, and the time was recorded as $t = 0$. About 20 to 35 worms were placed on a single plate, and adult nematodes were transferred every two days to fresh plates during the brooding period. The numbers of survivors were scored daily. Nematodes that failed to respond to repeated touch stimulation were considered as dead. The data were representative of at least three trials. The data was statistically analyzed using a two-tailed, two sample *t*-test (Minitab Ltd., Coventry, UK).

1.4 Brood size and generation time assay

The methods were performed as described by Swain *et al*. (2004). Briefly, brood size was assayed by placing the exposed or control animals onto individual wells of tissue culture plates. The P0 (the parental generation) nematodes were transferred to a new well every one and half days. Progeny were counted on the day following the transfer. The generation time referred to the time from P0 egg to the first F1 (the first filial generation) egg. For both the brood size and the generation time test, at least 10 replicates were performed for statistical purposes.

1.5 Thrashing and body bends assay

The methods were performed following the published work (Tsalik and Hobert, 2003). To assay the head thrashes, exposed and control animals were washed with double-distilled water, followed by washing with modified K medium. Every nematode was transferred into a microtiter well containing 60 μl of modified K medium on the top of agar. After a 1-min recovery period, the head thrashes were counted for 1 min. A thrash was defined as a change in the direction of bending at the mid body. To assay the body bend frequency, exposed and control animals were picked onto a second plate, and scored for the number of body bends in an interval of 20 s. A body bend was counted as a change in the direction of the part of the nematode corresponding to the posterior bulb of the pharynx along the *Y*-axis, assuming that the nematode was traveling along the *X*-axis. Fifteen animals were examined for each assay.

1.6 Assay of intestinal autofluorescence

The method was performed as described by Boehm and Slack (2005). The images were collected for endogenous gut fluorescence using a 525-nm bandpass filter, without automatic gain control, to preserve the relative intensity of a different animal's fluorescence. Day 4, day 8, and day 12 adults were photographed and examined on the same day to avoid effects of light source variance on fluorescence intensity.

1.7 Toxicity identification evaluation (TIE)

Caenorhabditis elegans were exposed to aliquots of industrial effluent that had been subjected to one of the physical TIE manipulations (filtration, pH adjustment,

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and C18 solid-phase extraction). The TIE approach was performed according to the USEPA guidelines for marine TIE studies (USEPA, 1991; Mount and Norberg-King, 1993), as also drafts of industrial effluent and sediment TIE procedures, with some minor modifications, according to the researchers (Mount and Hockett, 2000; Kwok *et al*., 2005).

1.7.1 Filtration

For vacuum filtration, 0.45-μm Millipore HA mixed cellulose (MC) filters (cellulose acetate/cellulose nitrate) were used with a glass Millipore vacuum filtering system (Millipore, Billerica, USA). The filters were prerinsed with 300 ml of both 10% nitric acid and Milli-Q water before filtering the industrial effluent.

1.7.2 pH adjustment

The filtered aliquots (50 ml) of effluent were adjusted to pH3 (the pH value of the effluent was adjusted with HCl to 3.0), pHi (the effluent without pH adjustment) or pH10 (the pH value of effluent was adjusted with NaOH to 10.0) in silanised 100 ml Duran Schott bottles. Either HCl or NaOH was added to the industrial effluent blanks, and the pH was measured to ascertain that the changes in pH were the same as those in the effluent. Adjusted solutions were left for 3–4 h before being adjusted back to the initial pH value.

1.7.3 Carbon 18 solid phase extraction (C18 SPE) and methanol elution

Alltech Extract-Clean 1,000 mg/6 ml columns were preconditioned with methanol. The filtered samples were then pumped through the conditioned C18 SPE column with a flow rate of 1 ml/min. The methanol was concentrated and mixed with the manipulated samples. The aliquot corresponding to the first bed volume was allowed to go to waste, to avoid collecting the conditioning water in the void volume of the column. The post-column water was collected and readjusted to pH 7 for toxicity testing. The test concentrations chosen for $Na_2S_2O_3$ were 220, 440, and 880 mg/L, respectively. An aliquot of a fresh stock solution of $Na_2S_2O_3$ was added to each test vial. The vials were left for at least one hour prior to toxicity assay with *C. elegans*. The test concentrations chosen for EDTA were 27.5, 55, and 110 mg/L, respectively. The EDTA-treated vials were left for 3 h, to allow for EDTA complexation of metals in the effluent prior to the toxicity assay with *C. elegans*.

1.8 Determination of metal content

The samples and standards were made up to 6 ml with 8.8% HNO_3. The metals were analyzed by atomic absorption spectrophotometry (Pye-Unicam model SP9, Cambridge, UK).

1.9 Statistical analysis

All the data in this article were analyzed using one-way ANOVA followed by a one-tailed Dunnett's test. Paired-sample *t*-tests were performed. A probability level of 0.05 was considered statistically significant.

2 Results

2.1 Toxicity characterization from the paper recycling mill effluent in the TIE assay

Life span was a very important endpoint to assess toxicity from toxins in *C. elegans*. The endpoint of aging was mainly selected in the current study to evaluate the toxicity from the paper recycling mill effluent. Maximum and median life spans were explored to evaluate the aging toxicity. The minor modified TIE manipulations were performed as shown in Fig.1 and Table 1. The baseline toxicity of the original effluent (collected on 24 April, 2006) was very toxic as both mean lifespan and maximum lifespan of the baseline were significantly ($P < 0.01$) decreased. Compared to the control, all manipulations could cause remarkable ($P < 0.01$) mean lifespan defects, except the pH3 filtration. Again, compared to the baseline toxicity of the original effluent, the pH3 ($P < 0.01$) and pH10 ($P < 0.05$) filtration, C18 SPE tests at pH3 ($P < 0.05$), pHi ($P < 0.05$), and pH10 ($P < 0.05$), and $Na_2S_2O_3$ treatments ($P < 0.05$) could result in relatively reduced

Table 1 Mean life spans of various TIE manipulations

Phase extraction	Mean lifespan (d)	R_{mo}	R_{mc}	Maximum lifespan (d)	N	P_o	P_c
Control	14.8 ± 0.8			26, 32	24		
Original effluent	10.7 ± 0.2		0.72	19, 18	29		< 0.01
MC filter	10.5 ± 1.5	0.98	0.71	17, 18	31	NS	< 0.01
MC filter pH3	12.5 ± 1.5	1.17	0.85	19, 20	27	< 0.01	NS
MC filter pH10	11.8 ± 0.6	1.10	0.80	21, 22	24	< 0.05	< 0.01
C18 pH3	11.7 ± 0.4	1.09	0.79	21, 21	19	< 0.05	< 0.01
C18 pHi	12.1 ± 1.1	1.13	0.82	18, 20	21	< 0.05	< 0.05
C18 pH10	11.5 ± 0.3	1.06	0.78	18, 19	24	< 0.05	< 0.01
$Na_2S_2O_3$ 220 mg/L	11.5 ± 0.4	1.07	0.77	18, 19	19	< 0.05	< 0.01
$Na_2S_2O_3$ 440 mg/L	11.9 ± 0.4	1.11	0.80	19, 21	24	< 0.05	< 0.01
$Na_2S_2O_3$ 880 mg/L	11.8 ± 0.6	1.10	0.80	20, 20	20	< 0.05	< 0.01
EDTA 110 mg/L	9.9 ± 1.2	0.93	0.67	16, 17	19	NS	< 0.01
EDTA 55 mg/L	9.7 ± 1.1	0.91	0.66	15, 15	21	NS	< 0.01
EDTA 27.5 mg/L	9.5 ± 1.2	0.89	0.64	13, 14	24	NS	< 0.01

Results of one typical experiment are shown. pH3: the pH value of the effluent was adjusted with HCl to 3.0; pHi: pH value was the effluent without pH adjustment; pH10: the pH value of the effluent was adjusted with NaOH to 10.0. R_{mo}: ratio of the mean life span divided by that of the original effluent; R_{mc}: ratio of mean life span divided by control. N: number of animals examined; P_o: probability of survival being different from that of the original effluent. P_c: probability of survival being different from control. NS: no significant differences. Data are expressed as mean ± SD.

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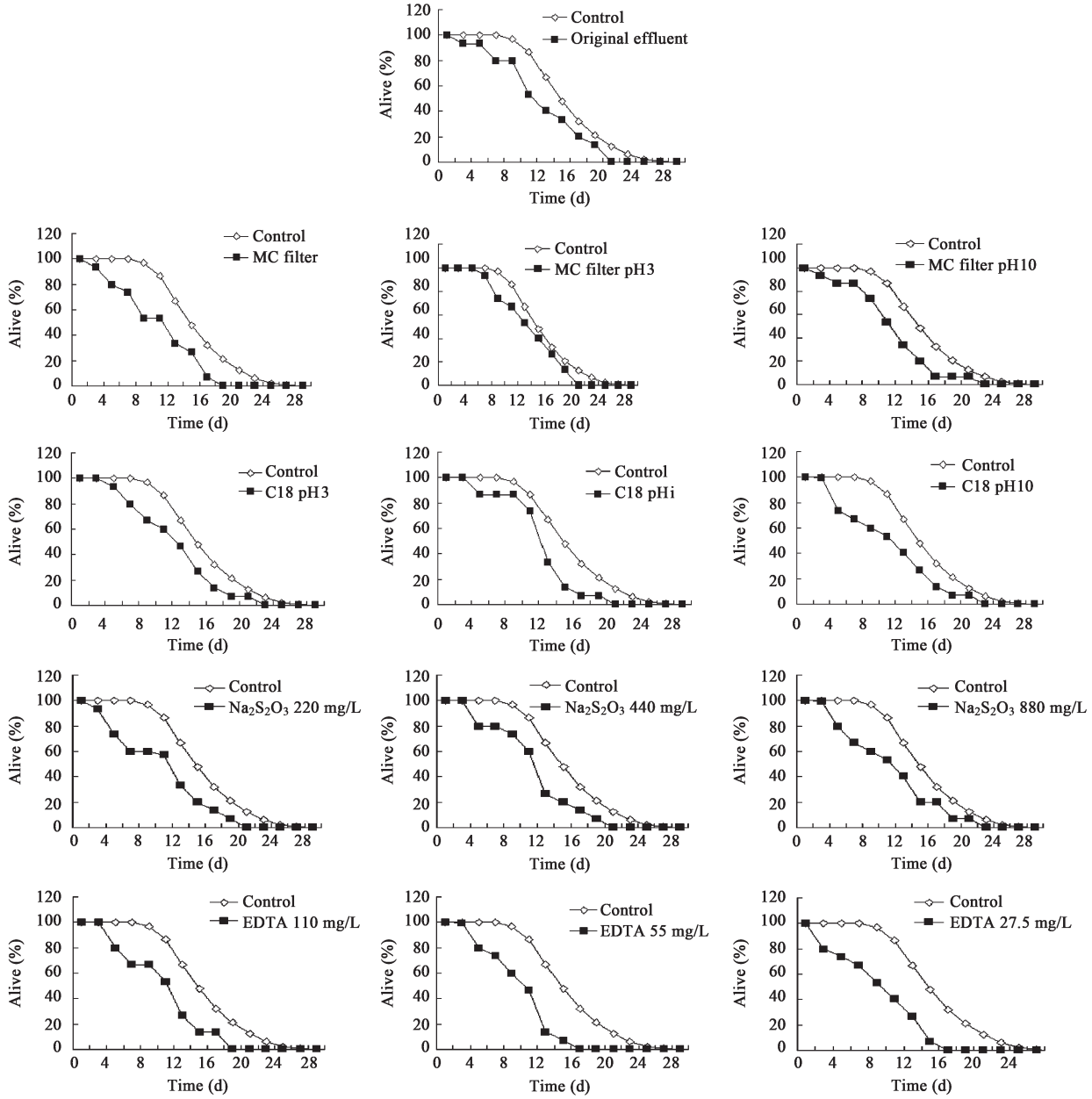

Fig. 1 Effects of Toxicity identification evaluation (TIE) physical manipulations on the life span of *C. elegans*.

lifespan toxicity among different manipulations, and their toxicities were significantly distinguished from those of the baseline toxicity. In contrast, the toxicity from MC filtration and EDTA treatment was similar to the baseline toxicity. Therefore, the suspect toxicants inducing aging toxicity might mainly be the heavy metal substances in this industrial effluent.

To determine whether the short lifespan from different manipulations is due to accelerated aging or to an unrelated cause, the authors further investigated the accumulation of intestinal autofluorescence in adult animals. Intestinal autofluorescence, an established maker for aging, is caused by lysosomal deposits of lipofuscin, which accumulates over time in aging animals (Boehm and Slack, 2005). As shown in Fig.2, all manipulations cause more rapid accumulation of intestinal autofluorescence in animals on day 4, after the L4-larva stage, than that in the control. Manipulations with fractions of original effluent, MC filtration and EDTA treatments (110, 55, and 27.5 mg/L) resulted in more rapid accumulation of intestinal autofluorescence in animals on day 8 and day 12 after the L4-larva stage, than that in control. In particular, only the accumulation rates of intestinal autofluorescence in animals exposed to fractions of MC filtration and EDTA treatment were similar to those in animals exposed to baseline toxicity on day 4, day 8, and day 12, after the L4-larva stage. These results were largely consistent with the life span analysis given earlier, suggesting that the evaluated toxicities from different manipulations were actually due to accelerated aging.

Usually, heavy metal exposure could cause multi-biological toxicities (Swain *et al*., 2004; Wang and Yang, 2007). Next, the authors examined the effects from EDTA manipulations on reproduction and locomotion behaviors

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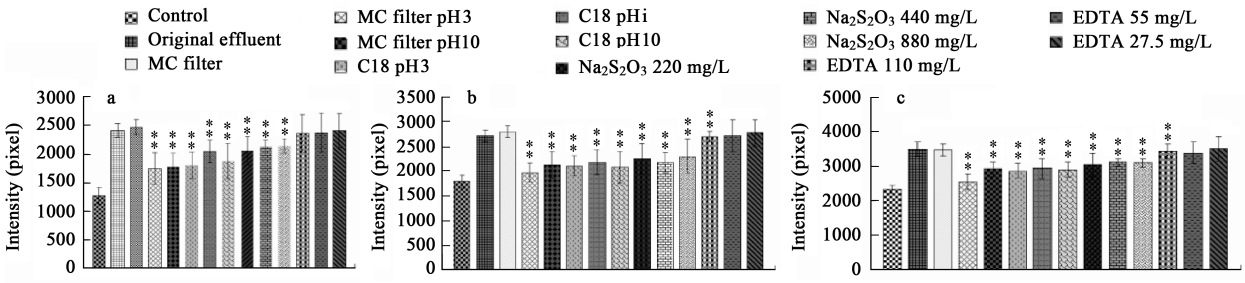

Fig. 2 Effects of TIE physical manipulations on the gut lipofuscin autofluorescence in animals on day 4 (a), day 8 (b), and day 12 (c) after the larva-to-adult transition. Bars represent means ± SD. $^{**}P < 0.01$.

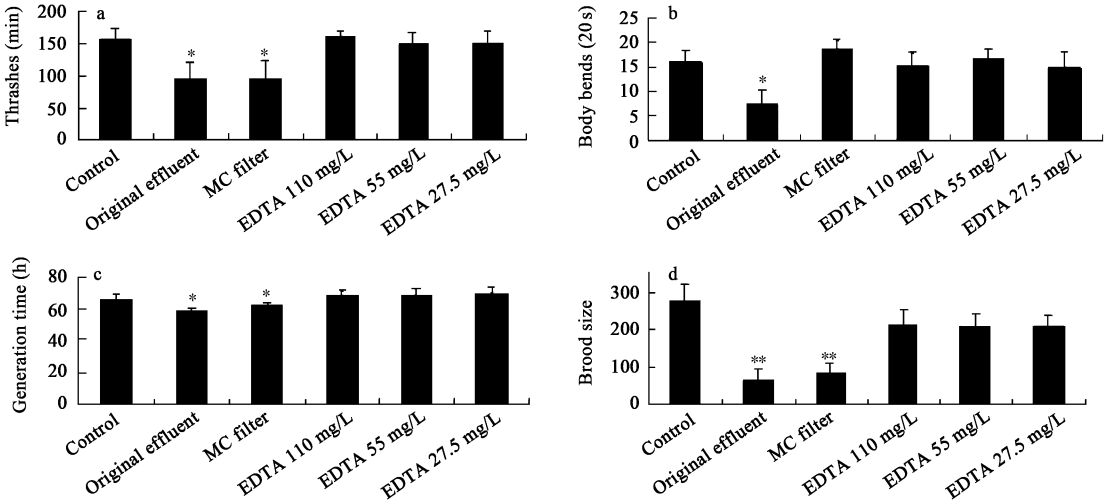

Fig. 3 Effects of TIE physical manipulations on the reproduction and locomotion behaviors of *C. elegans*. Bars represent means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

in animals. Generation time and brood size were selected for the reproduction assay. The reproductive speed was evaluated by generation time, whereas, the brood size could reflect the reproductive capacity. As shown in Fig.3, no obvious toxic effects were found on generation time and brood sizes from EDTA manipulations, compared to the control, with severe toxic effects (generation time, $P < 0.05$; brood size, $P < 0.01$) from baseline toxicity and MC filtration manipulation. Furthermore, head thrash and body bend were selected for locomotion behavior assay. Similarly, no significantly toxic effects were found on thrashes and body bends from EDTA manipulations, compared to the control, with severely toxic effects ($P < 0.05$) from baseline toxicity and MC filtration manipulation. Therefore, the toxicity on the reproduction and locomotion behavior from the original effluent might not mainly be caused by metal toxicity, and could be from other fractions.

2.2 Toxicity identification from the paper recycling mill effluent in the TIE assay

The earlier results suggest that the toxic effects on aging might be mainly from metal toxicity. Again, the authors determined the metal contents in industrial effluent from the paper recycling mill by atomic absorption spectrophotometry. As shown in Fig.4, Si (92.8 μg/ml), Na (314 μg/ml), Al (33.4 μg/ml), Fe (43.1 μg/ml), Mg (37.2 μg/ml), Mn (0.5 μg/ml), and Zn (1.46 μg/ml) were detected in

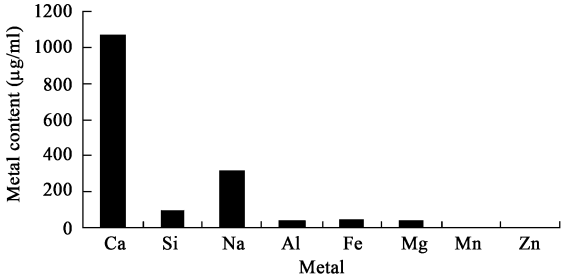

Fig. 4 Metal contents in industrial effluent.

the industrial effluent from the paper recycling mill. In particular, the authors found a high level of Ca (1,069 μg/ml) in the liquid effluent. In addition, trace amounts of Cd (< 0.001 μg/ml), Co (< 0.001 μg/ml), Cr (< 0.01 μg/ml), Ni (< 0.01 μg/ml), and Pb (< 0.001 μg/ml) were also detected.

2.3 Toxicity confirmation from the paper recycling mill effluent in the TIE assay

To confirm the possible toxicity from metals in the industrial effluent, the authors examined the toxicity from metal exposure on the *C. elegans* aging. The metal concentrations were prepared according to the detected contents in the original effluent. As shown in Fig.5 and Table 2, exposure to Mg, Mn, and Zn did not result in aging toxicity in exposed animals. In addition, because the contents of

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Table 2 Mean life spans of various metal toxicities

Phase extraction	Mean lifespan (d)	R_{mo}	R_{mc}	Maximum lifespan (d)	N	P_o	P_c
Control	14.8 ± 0.8	–	–	26, 32	27	–	–
Original effluent	10.7 ± 0.2	–	0.72	19, 18	31	–	<0.01
Al (33.4 μg/ml)	12.5 ± 1.4	1.17	0.85	22, 24	29	NS	<0.05
Ca (1,069 μg/ml)	10.9 ± 0.9	1.02	0.74	22, 22	31	NS	<0.05
Fe (43.1 μg/ml)	10.7 ± 2.7	1.00	0.72	22, 21	21	NS	<0.05
Na (314 μg/ml)	16.0 ± 2.1	1.50	1.08	27, 29	29	<0.01	NS
Mn (0.5 μg/ml)	16.0 ± 2.7	1.50	1.08	28, 28	31	<0.01	NS
Zn (1.46 μg/ml)	17.0 ± 3.1	1.59	1.15	27, 26	34	<0.01	NS
Mg (37.2 μg/ml)	17.0 ± 2.9	1.59	1.15	28, 30	29	<0.01	NS

Data are expressed as mean ± SD. R_{mo}, R_{mc}, N, P_o and P_c are the same as that in Table 1.

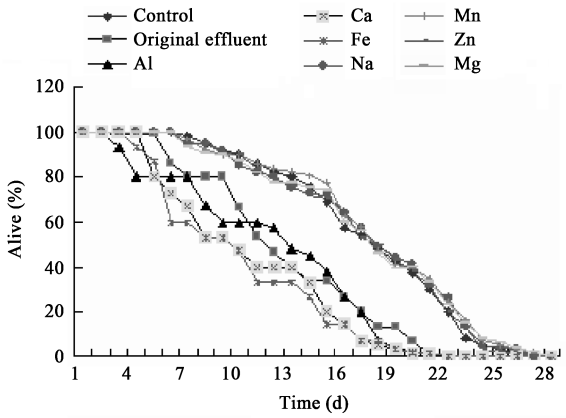

Fig. 5 Life spans in wild-type animals exposed to metals.

Cd, Co, Cr, Ni, and Pb were very low, these metals could not cause detectable aging toxicity (data not shown). In contrast, exposure to Ca, Al, and Fe resulted in significantly ($P < 0.05$) severe aging defects compared to the control. Ca, Fe, and Al could be taken up by nematodes according to the investigation using inductively coupled plasma mass spectrometry (data not shown). No prominent differences were observed between baseline toxicities and the aging toxicities from Ca, Al or Fe exposure.

3 Discussion

TIE can be used to determine the specific toxicant(s) in marine effluents and sediments (Burgess *et al*., 2000; Kwok *et al*., 2005). In the current study, the TIE technique was performed to characterize, determine, and confirm the major toxicants for aging toxicity from a paper recycling mill, using *C. elegans* as a bioindicator. The results and the studies from other laboratories show that a number of characteristics of *C. elegans* make this model organism suitable for use in marine TIE. These properties include the following: (1) the lifespan is very short (approximately three weeks under optimal conditions); (2) the short test duration (48 h) can minimize the chemical changes in the industrial effluent; (3) both acute and chronic aging toxicities can be examined; (4) *C. elegans* is sensitive to a wide range of contaminants or toxicants (Peredney and Williams, 2000; Ura *et al*., 2002); (5) it is easy for performing different TIE manipulations; (6) nematodes can live within interstitial water and soil, and can be present within the anticipated microenvironment once they are placed in direct contact with any contaminant (Dhawan *et al*., 1999); (7) *C. elegans* do not rapidly migrate from stressful conditions and are tolerant to high salinity and external pH (data not shown); (8) the small test volumes required permit multiple manipulations to be performed simultaneously.

At the same time, some limitations were also raised for this TIE-coupled bioindicator assay. The nematode is not suitable for evaluation of the toxic effects on the structure and functions of some important organs, since nematodes do not have heart, liver, lung or kidneys. Similarly, the toxicities from the assayed industrial effluent induce some significant diseases, which cannot be investigated by establishing the corresponding disease model, in nematodes or *C. elegans*. In addition, some solvent solutions of identified compounds will rapidly result in severe lethal animals, which will not allow one to determine or confirm the exact toxicant(s) from the assayed samples.

In the present study, some metals were identified as possible major toxicants for the aging toxicity of the industrial effluent from the paper recycling mill, by TIE assay. Several evidences were raised to support this conclusion. First, in the TIE assay, only the toxicities on aging from MC filtration and EDTA treatment were similar to the baseline toxicity. Second, investigation on the accumulation of intestinal autofluorescence in adult animals indicated that the short life spans from manipulations of MC filtration and EDTA (110, 55, and 27.5 mg/L) treatments were actually due to accelerated aging. Third, a high level of metals (Ca, Fe, and Al) was detected in the assayed industrial effluent in the TIE assay. Internal Ca^{2+} concentration was closely related to the induction of cell death or cell senescence in animal cells (Demaurex and Distelhorst, 2003). An overload of Fe exposure could result in free radical-mediated damage by inducing reactive oxygen species (ROS) production, in response to oxidative stress (Rachmilewitz *et al*., 2005). Al overload may contribute to the induction of aging by at least partially affecting the mitochondrial DNA fragmentation (Swegert *et al*., 1999).

Moreover, the authors found that the identified high level of Ca appeared to have a strong synergistic effect in combination with the metals of Fe and Al at assayed concentrations, and the combination exposure of Ca, Fe, and Al exhibited more severe aging defects than that in animals exposed to the original effluent (data not shown). However, the identified high level of Ca had only a neutralizing effect on other trace metals in inducing aging

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toxicity in the assayed industrial effluent, such as, Mg, Mn, Zn, Cd, Co, Cr, Ni, and Pb (data not shown). Therefore, the synergistic effects on the aging toxicity from the exposure to the combination of Ca, Fe, and Al might at least partially be able to be counteracted by other organic compounds in the assayed industrial effluent. Nevertheless, the possibility could not be excluded that the organic compounds in the assayed industrial effluent were also involved in the induction of aging toxicity. Manipulations with MC filters (pH10, $P < 0.01$), C18 pH3 ($P < 0.01$), C18 pHi ($P < 0.05$), C18 pH10 ($P < 0.01$), and $Na_2S_2O_3$ treatments (220, 440, and 880 mg/L, $P < 0.01$) could cause significantly shortened life spans compared to the control (Table 1, Fig.1). In addition, the toxic effects of some organic compounds on aging might be possibly counteracted by other organic compounds.

Furthermore, in the current study, only the aging toxicity in the TIE assay was focused on. The possible toxicant(s) was not examined for reproduction and locomotion behaviors, because the toxicity on the reproduction and locomotion behavior from the original effluent might not be caused mainly by metals. However, the data indicated that the tested industrial effluent could cause severe reproduction and locomotion behavior defects in *C. elegans*. Therefore, it suggested that the nematode, together with suitable endpoints, could also be used for characterizing, identifying, and confirming the toxicant(s) for other toxicities in the TIE assay. Or else, this model organism could be exploited to systematically identify the toxicants causing multi-biological defects, with the aid of the TIE technique.

In addition, the availability of recombinant technology could make it possible to improve the sensitivity of assays by transgenesis in *C. elegans*. Transgenic strains carrying stress-inducible *lacZ* reporter genes and heat shock promoter driven *gfp* reporter genes were already constructed (Mutwakil *et al*., 1997; Chu and Chow, 2002), which would be very valuable for assessing the toxicity from marine sediments and effluents.

4 Conclusions

Overall, the authors' data suggested that the suspected toxicants for aging toxicity might be mainly the heavy metal substances in the industrial effluent from a paper recycling mill, by TIE assay. High levels of Ca, Al, and Fe in the assayed effluent might account for a severe toxicity on the aging of exposed nematodes. The present study and studies from others suggested that the characteristics of *C. elegans* made this model organism suitable for use in marine TIE assays.

Acknowledgments

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