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Development and application of a sediment toxicity test using the benthic cladoceran *Chydorus sphaericus*

T. Dekker[a], G.D. Greve[a, b], T.L. Ter Laak[a, c], M.E. Boivin[a, b], B. Veuger[a, d], G. Gortzak[a], S. Dumfries[a], S.M.G. Lücker[a], M.H.S. Kraak[a], W. Admiraal[a] and H.G. van der Geest[a,*]

[a]Department of Aquatic Ecology & Ecotoxicology, IBED, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands
[b]Expert Centre for Substances, RIVM, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[c]IRAS, Utrecht University, Yalelaan 2, 3584 CL Utrecht, The Netherlands
[d]NIOO-KNAW, Centre for Estuarine and Marine Ecology, P.O. Box 140, 4400 AC Yerseke, The Netherlands

* Corresponding author. Tel.: +31 20 525 7721; fax: +31 20 525 7716.

Abstract

This study reports on the development and application of a whole sediment toxicity test using a benthic cladoceran *Chydorus sphaericus*, as an alternative for the use of pelagic daphnids. A *C. sphaericus* laboratory culture was started and its performance under control conditions was optimised. The test was firstly validated by determining dose–response relationships for aqueous cadmium and copper and ammonia, showing a sensitivity of *C. sphaericus* (96 h LC_{50} values of 594 μg Cd/L, 191 μg Cu/L and 46 mg ammonia/L at pH 8) similar to that of daphnids. Next, sediment was introduced into the test system and a series of contaminated sediments from polluted locations were tested. A significant negative correlation between survival and toxicant concentrations was observed. It is concluded that the test developed in the present study using the benthic cladoceran *C. sphaericus* is suitable for routine laboratory sediment toxicity testing.

1. Introduction

During the past few decades, water quality has generally improved (Admiraal et al., 1993), but sediments still contain high concentrations of xenobiotic compounds (Koelmans and Moermond, 2000). Therefore, sediments may act not only as a sink, but also as a source of a wide range of chemical substances, such as nutrients, metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), that have been deposited previously in high concentrations (Beurskens et al., 1993). Determining the effects of sediment bound toxicants on benthic biota is therefore a necessary step in ecological risk assessment. However, sediment toxicity is often tested by exposing *Daphnia magna* to pore water (Côté et al., 1998, Reinhold-Dudok van Heel and Den Besten, 1999 and Lahr et al., 2003). *D. magna* is not a benthic organism and isolated pore water may not reflect toxicant exposure in situ and thus may not provide reliable information on the impact of pollutants in sediments. Moreover, benthic animals are exposed to sediment bound toxicants in a different way than pelagic animals, due to e.g. ingestion and direct contact with the contaminated soil (Rönnpagel et al., 1998 and Sibly et al., 1999). Therefore we considered the use of benthic

cladocerans (Chydoridae) which, unlike daphnids, can be tested in their natural micro-habitat, i.e. the sediment. Although their distribution is well studied (Duigan and Kovach, 1991, Whiteside et al., 1978 and Hann and Zrum, 1997), only few papers focus on aspects of the life history of chydorids (Keen, 1979, Robertson, 1988 and Dekker et al., 2002). The available information indicates that chydorids may be useful and sensitive test species (Koivisto et al., 1992, Dekker et al., 2002 and Bossuyt and Janssen, 2005).

One of the most common benthic cladoceran species is *Chydorus sphaericus*, a sediment dwelling species occurring in a variety of habitats (Duigan and Kovach, 1991, Fryer, 1995 and Van de Bund and Spaas, 1996), feeding mainly on detritus (Fig. 1). The abundance of chydorids in littoral regions of freshwater lakes makes them an important component of the aquatic ecosystem (Williams, 1982). They hold a key position in the food web by converting organic material into their own body mass that becomes in turn available for predators such as juvenile fish. This way their presence influences the transfer of chemicals in sediments and in the food chain. Hence, determining the effect of sediment bound toxicants on chydorids may be a good estimate for the potential effect of such contaminants on the benthic community. Being cladocerans, chydorids have the same advantages as daphnids for use in experiments, such as ease of handling and parthenogenetic reproduction. Therefore *C. sphaericus* may be a suitable species for standardized laboratory toxicity testing.

Fig. 1. Four developmental stages of *C. sphaericus*. Stage 1: neonate, stage 2: second instar with swollen ovaria, stage 3: third instar with eggs, stage 4: fourth instar with newly born neonates and swollen ovaria.

This study reports on the development and application of a sediment toxicity test using the benthic cladoceran *C. sphaericus*. In order to optimize the *C. sphaericus* laboratory culture and the control performance in the toxicity test, the influence of food type and temperature was analysed. The developed test, so far testing water exposure only, was validated by determining dose–response relationships for cadmium and copper that allowed comparison with *Daphnia* species which are often tested for these metals (Kluttgen and Ratte, 1994, Mark and Solbé, 1998 and Nebeker et al., 1986). In addition, the sensitivity of *C. sphaericus* to ammonia was determined, since ammonia toxicity is one of the most frequently occurring confounding factors in sediment toxicity tests (Lahr et al., 2003). Finally, sediment was introduced into the test system and this step was validated by subjecting the test to a series of contaminated sediments from polluted locations.

2. Materials and methods

2.1. *C. sphaericus* laboratory culture

The clone of *C. sphaericus* used in this study was reared from one gravid female collected in the summer of 1998 in the Drontermeer, a eutrophic, sandy lake in The Netherlands.

The animals were kept in plastic containers (l × w × h = 13 × 8 × 6 cm) filled with 300 ml of M7 medium (Elendt, 1990) and about 1 g of pre-combusted (3 h at 550 °C) quartz sand (Sibelco M32, Antwerp, Belgium; grain size 100 μm–400 μm). Three times a week, the animals were fed 2 ml of a food suspension consisting of dried, ground nettle powder (*Urtica dioica*) (0.5% w/v) and 30×10^6 μm^3 *Nitzschia perminuta*/ml medium. Every week, around 70% of the culture medium was renewed, by decanting most of the medium from the container. Along with the medium, some of the animals in the culture were also removed. This partial removal prevented crowding which assured that the females continued to reproduce parthenogenetically and did not form ephippia. Every month each container was replaced by a new one that was inoculated by decanting part of the contents of an old container into it. The temperature in the culture room was maintained at 20 °C ± 1 °C and a light:dark regime of 16:7 h with twice half hour twilight in between was applied.

2.2. *C. sphaericus* test

One day before the start of the experiment, adult females containing parthenogenetic eggs were taken from the culture and transferred into glass jars containing 75 ml of medium and 5 drops of food suspension (see above). The jars were placed overnight in a climate room under the same culture conditions (see above). The next day, newborn neonates (0–24 h) were collected and used for the experiments.

All tests were performed at least in triplicate. The tests were carried out in small, round glass dishes (3 ml) with a diameter of 3 cm and a depth of 7 mm with rounded edges to which 2 ml of medium, 20 neonates and 1 drop of food suspension (see above) were added. The containers were loosely covered with a lid to prevent evaporation and the tests were performed under the same conditions as the culture (see above). After 96 h the animals were collected under a dissecting microscope and survival, growth and developmental stage were determined. Growth was calculated by subtracting the average initial length (n = 20) from the individual final length. Body length was measured with an image analyzer from the edge of the carapace above the nauplius eye to the posterior margin of the carapace where it splits. Developmental stage was determined by classifying the animals into the four stages as shown in Fig. 1, after which the mean developmental stage was calculated. In the sediment toxicity test 0.3 g of sediment was introduced into the test system and pre-combusted (3 h at 550 °C) quartz sand (Sibelco M32, Antwerp, Belgium; grain size 100 μm–400 μm) was used as control sediment.

2.3. Food and temperature

The following food items were tested: ground *Urtica* powder, the fish foods Tetraphyll and Tetramin, a green alga (*Selenastrum capricornutum*), a diatom (*N. perminuta*) and *N. perminuta* combined with *U. dioica* powder. The optimal quantities of these single food items had been determined in pilot experiments. The *U. dioica* powder, Tetraphyll and Tetramin were suspended in medium (1%

w/v) of which 2 drops were added to the test jars. The green alga was fed in a volume of 1×10^6 μm^3/ml and the diatom in a volume of 60×10^6 μm^3/ml. These volumes were determined by using a Coulter Counter. The combination of *U. dioica* powder and *N. perminuta* was fed in quantities of 0.5% w/v *U. dioica* suspension and a *N. perminuta* volume of 30×10^6 μm^3/ml medium, respectively, being half the amounts fed in the single food item treatments. The following temperatures were tested: 5, 10, 15, 20, and 25 °C. One-way analysis of variance (ANOVA) tests followed by Scheffe's post hoc test were conducted to test for significant differences between treatments and controls (Sokal and Rohlf, 1995).

2.4. Cu, Cd and ammonia toxicity

Cadmium and copper were both added as chlorides, using a 1 g/l stock solution. To measure the actual total metal concentrations in the water, two replicate samples (0.5 ml) were taken at the start and at the end of the experiment. Since the test volume should be 2 ml (see above), the experiments were initiated with 3 ml. The water samples were acidified with 20 μl 70% nitric acid, centrifuged for 10 min at 3000 rpm and transferred into new eppendorf vials. The samples were analysed by air–acetylene Flame Atomic Absorption Spectrometry (Perkin–Elmer 1100B, Norwalk, CT, USA) or by Furnace Atomic Absorption Spectrometry (Perkin–Elmer 5100PC/HGA600/AS60 equipped with Zeeman background correction, Norwalk, CT, USA). Quality of the metal analyses was ascertained by analyzing blanks and reference material (NIST:SRM 1643, National Institute of Standards and Technology, Gaithersburg, MD, USA). Survival, growth and developmental stage were plotted against the actual total metal concentration in the water. From these dose–response relationships, EC_{50} values and their corresponding 95% confidence limits were calculated using the log–logistic curve fitting procedure of Haanstra et al. (1985).

The ammonia toxicity test was performed in glass vials of 50 ml to allow pH measurements with a relatively large pH electrode. One day prior to the experiments 20 ml of aerated M7 Elendt medium was added to each vial. The pH of each vial was established at 8.0 by adding the 20 nM buffer POPSO (disodiumsalt, Sigma), NaOH (0.01 M) and HCl (0.01 M). Total ammonia concentrations were set by adding NH_4Cl from a stock solution of 150 mg/L NH_4Cl in M7 medium. The vials were placed in an incubator where the conditions were the same as during the metal toxicity experiments. Shortly before the start of the experiment, the pH was measured and adjusted if necessary. Before placing the test vials in the incubator and just before the experiment was terminated, 2 ml of medium was taken from each test vial for analysis of the actual total ammonia concentration in the water, by means of a discrete analyzer for total ammonia. By means of a speciation model, the concentration of NH_3 was determined for each treatment using the mean pH and total ammonia concentration. The model used for this purpose is described by Emmerson et al. (1975) and expresses the NH_3 concentration as a fraction (f) of the total ammonia concentration:

$$f = 1/10^{pK_a - pH} + 1$$

where pK_a = dissociation constant of NH_4^+, at 20 °C in freshwater (=9.407313106) and pH = mean pH during the experiment.

Survival was plotted against the actual total ammonia concentration and against the NH_3 concentration in the water. From these dose–response relationships, EC_{10} and EC_{50} values and their corresponding 95% confidence limits were calculated using the log–logistic curve fitting procedure of Haanstra et al. (1985).

2.5. Sediment toxicity

The sediment toxicity test using *C. sphaericus* was applied to a series of eight contaminated sediments and two controls, one with and one without sand. In addition, the following possible confounding factors were measured in a comparable separate setup: pH and the concentrations of NO_2^-, NO_3^- and NH_4^+. The sediments originated from a polluted brook in the centre of The Netherlands, De Wenumse Beek, contaminated by former copper mills, paper mills and other industries. Cu, Pb, Cd, Zn and ∑PAK [EPA 16] concentrations are given in Table 1. The sediments were homogenized and frozen at −20 °C in order to kill the autochthonous organisms. One day prior to the experiments the test vials were filled with 0.3 g sediment and 2 ml medium. After 96 h the animals were hand-sorted from the sediments under a dissecting microscope and survival and growth were determined. A *t*-test was applied to test for significant differences between treatments and controls. To reduce the number of sediment variables, a principal component analysis was performed with the different concentrations of toxicants (Pb, Cd, Cu, Zn and PAH). The scores of the sediments on the first principal component served as a new variable for toxicant concentration (factor tox). It was tested if survival correlated with the factor tox using the Pearson correlation test.

Table 1.

Concentrations of Cu, Pb, Cd, Zn and ∑PAK [EPA 16] in sediments from the Wenumse Beek in mg/kg dw

Sample	Cu	Pb	Cd	Zn	PAH	Factor tox
Control	0	0	0	0	0	
Sand Control	0	0	0	0	0	
A	30	47	0.52	50	1.20	−0.48
B	28	14	<0.4	0.43	0.32	−0.73
C	190	32	<0.4	37	3.10	−0.55
D	42	20	<0.4	18	0.78	−0.68
E	240	140	0.63	170	4.6	0.09
F	2400	400	1.3	360	30	2.14
G	67	33	<0.4	75	0.39	−0.55
H	5000	210	<0.4	160	17	0.76

Factor tox are the scores of the sediments on the first principal component in a PCA using the contaminant concentrations (Cd, Cu, Zn, Pb, ∑PAHs), see Section 2.

3. Results

3.1. Food

A high survival was observed for animals feeding on *U. dioica* powder, green alga, *N. perminuta* and the combination of *N. perminuta* and *Urtica* (Fig. 2). Only Tetraphyll caused a significantly ($p < 0.05$) lower survival. Growth in the presence of *N. perminuta* and the combination of *N. perminuta* and *U. dioica* was significantly ($p < 0.05$) higher than for the other food sources, the latter sustaining the best growth. The combination of a *N. perminuta* and *U. dioica* was therefore chosen as food source for both cultures and experiments.

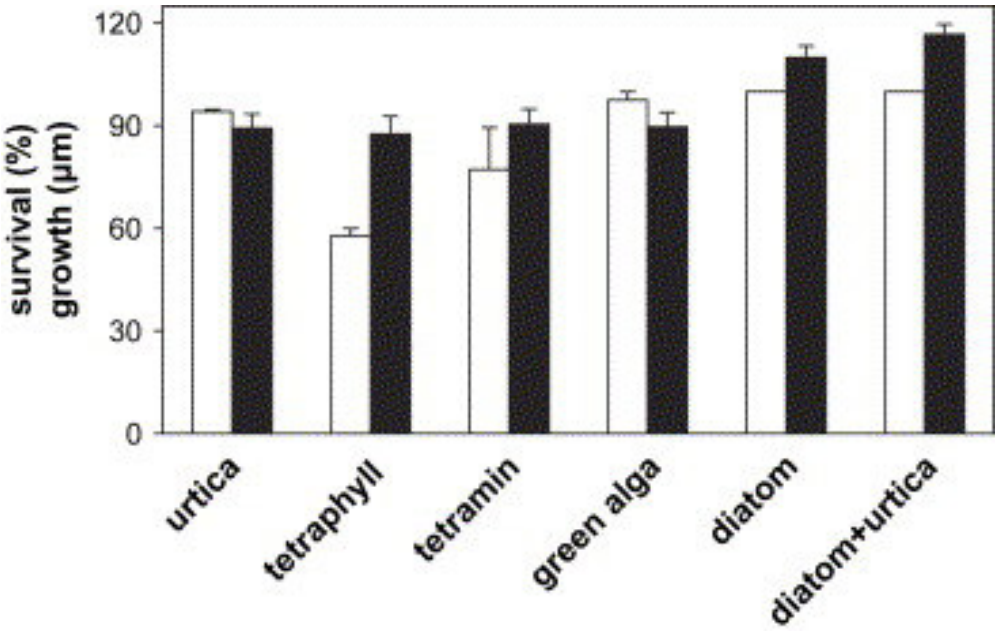

Fig. 2. Survival (white bars) and growth (black bars) of *C. sphaericus* after 96 h, feeding on different food sources. Error bars represent standard errors.

3.2. Temperature

No significant differences were found for survival at temperatures between 5 °C and 20 °C, but survival at 25 °C was much lower (Fig. 3). Developmental stage increased significantly ($p < 0.05$) with increasing temperature (Fig. 3) and therefore the most suitable temperature for chydorid toxicity test was considered to be 20 °C. Moreover, most standard toxicity tests are also performed at 20 °C which facilitate comparisons.

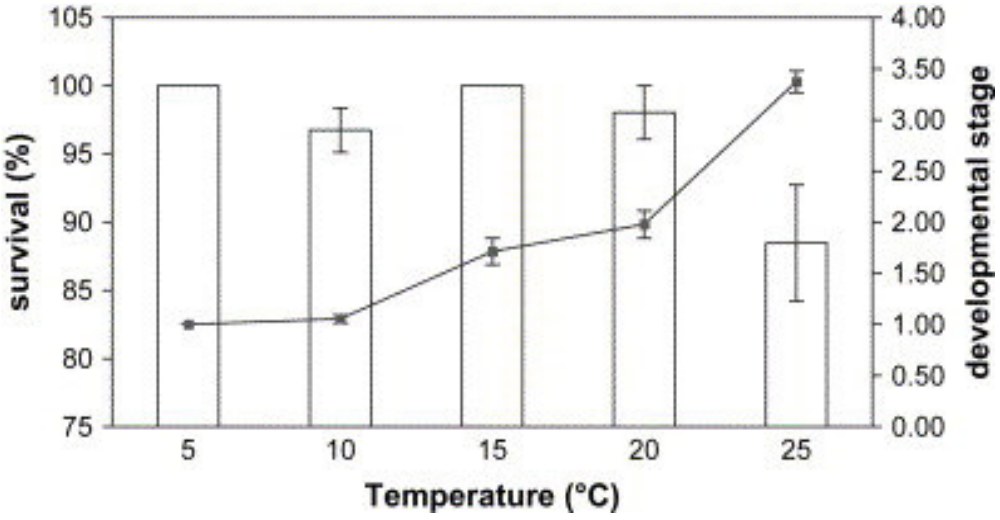

Fig. 3. Survival (bars) and developmental stage (line) of *C. sphaericus* after 96 h at five temperatures. Error bars represent standard errors.

3.3. Cu and Cd toxicity

Control survival was above 90%. Clear dose–response relationships were observed for survival, growth and development of chydorids exposed to copper or cadmium (Fig. 4). The corresponding LC_{50} and EC_{50} values are listed in Table 2. *C. sphaericus* responded differently to either metal. In the case of cadmium, the EC_{50} values for the sub-lethal endpoints growth and development were lower than the LC_{50}. In contrast, for Cu the LC_{50} was lower than the EC_{50}s, indicating that the some surviving animals were still able to develop.

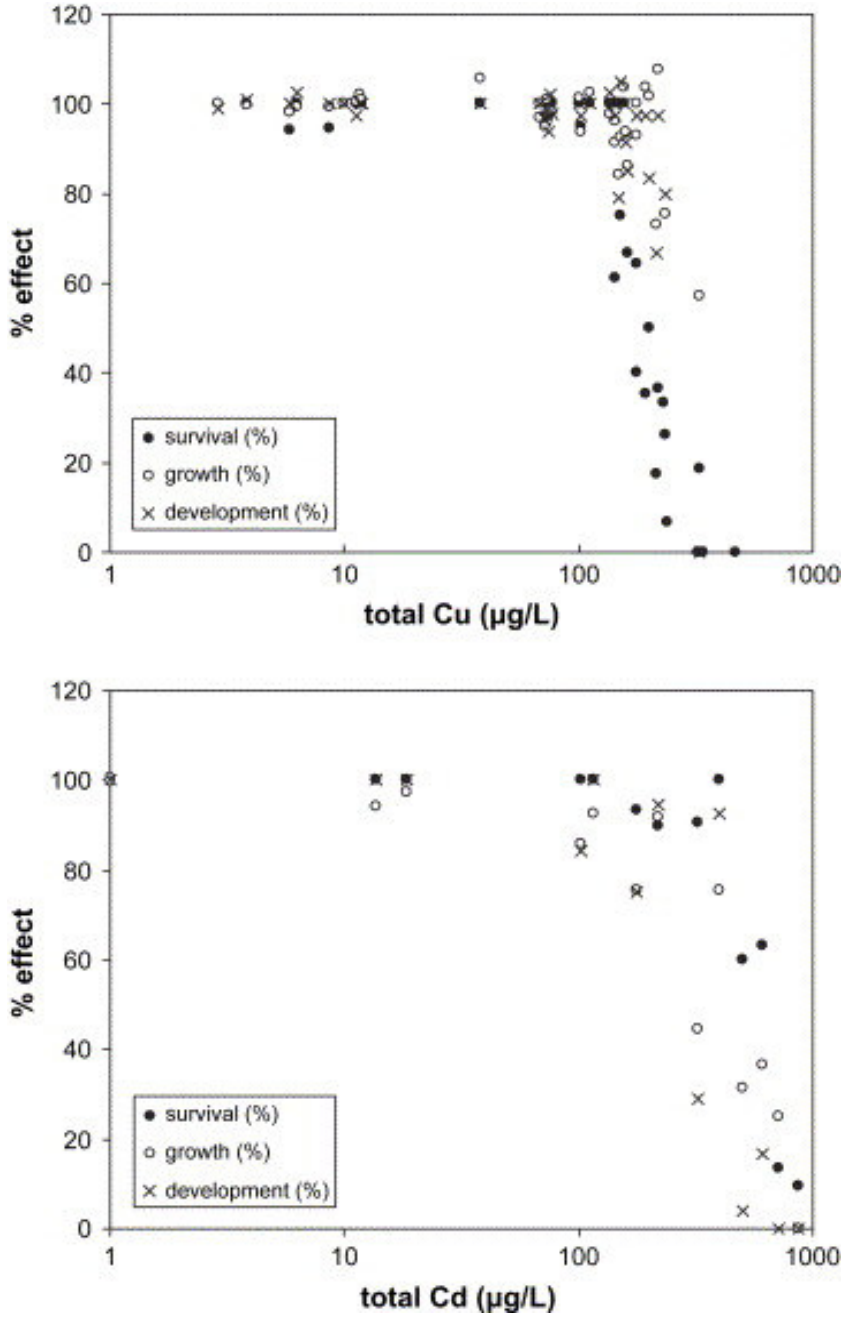

Fig. 4. Survival, growth and development of *C. sphaericus* after 96 h exposure to copper (top) and cadmium (bottom).

Table 2.

Median effect concentrations for survival (LC_{50}) and growth and development (EC_{50}) for *Chydorus sphaericus* after 96 h of exposure to copper and cadmium (95% confidence limits are given in parentheses)

Metal	LC_{50} (µg/L)	EC_{50} growth (µg/L)	EC_{50} development (µg/L)
Cu	191 (184–198)	358 (303–413)	258 (240–276)
Cd	594 (545–641)	409 (322–496)	359 (251–467)

3.4. Ammonia toxicity

Control survival was at least 80%. Clear dose–response relationships were observed for survival of chydorids exposed to ammonia, either expressed as total ammonia or as NH_3 (Fig. 5). The corresponding LC_{50} and LC_{10} values are listed in Table 3. Since ammonia is one of the most frequently occurring confounding factors in sediment toxicity tests the present effect concentrations should be used as a reference for sediment toxicity tests with *C. sphaericus*.

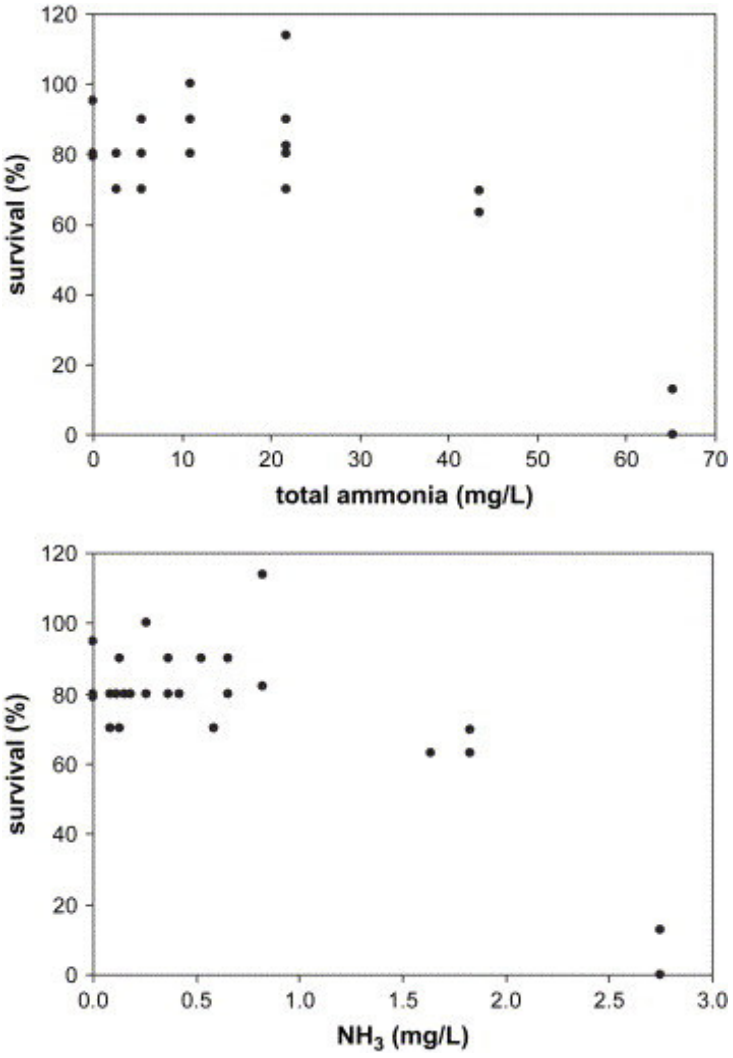

Fig. 5. Survival of *C. sphaericus* after 96 h exposure to ammonia, expressed as total ammonia (top) and as NH_3 (bottom).

Table 3.

LC_{10} and LC_{50} values for *Chydorus sphaericus* after 96 h of exposure to ammonia at pH 8, expressed as total ammonia and as NH_3 (95% confidence limits are given in parentheses)

	LC_{10} (mg/L)	**LC_{50} (mg/L)**
Ammonia	36 (26–49)	46 (42–51)
NH_3	1.38 (1.04–1.85)	1.90 (1.70–2.13)

3.5. Sediment toxicity

Control survival was at least 80%. The field collected sediments caused a clear response gradient (Fig. 6) since five samples, C and E–H, caused a significant ($p < 0.05$) lower survival than in the controls and the sediments G and H caused even complete mortality. In the samples A–D enough larvae survived the 96 h exposure to measure growth, which was significantly ($p < 0.05$) lower than in the corresponding controls. There was, however, no significant ($p > 0.05$) correlation between survival (Fig. 6) and the factor tox (Table 1). This was mainly caused by sample G, which contained relatively low concentrations of the measured toxicants, but caused complete mortality. Omitting this sediment from the statistical analysis revealed a significant negative correlation ($p < 0.05$; $r = -0.76$) between survival and the factor tox. The NH_4^+ concentrations were below detection limits in all treatments.

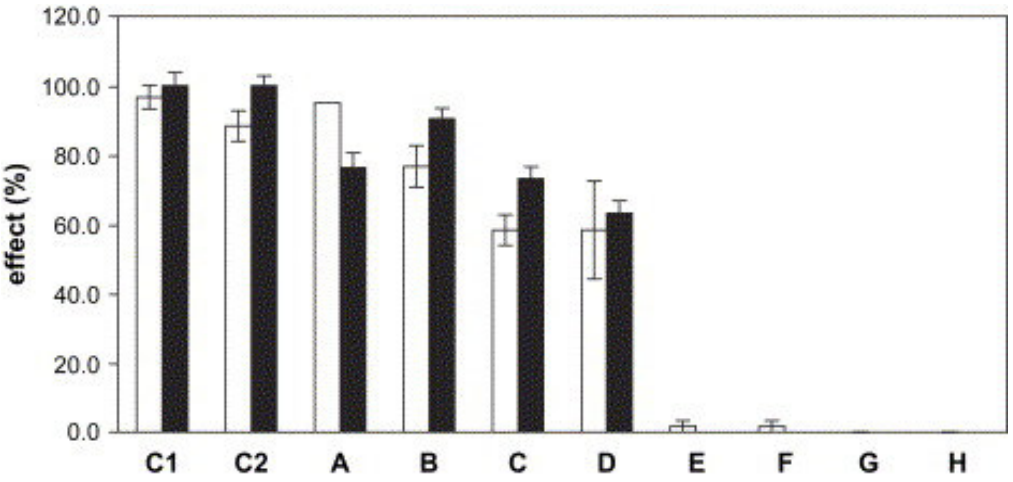

Fig. 6. Survival (white bars) and growth (black bars) of *C. sphaericus* after 96 h exposure to sediments from the Wenumse Beek. A–H field samples, C1 water only control, C2 water and sand control. Error bars represent standard errors.

4. Discussion

The first step in the development of a sediment toxicity test using the benthic cladoceran *C. sphaericus* was to start a laboratory culture and optimize their performance under control conditions. The test species was easy to culture and high survival, growth and development were obtained when feeding the animals a mixture of *N. perminuta* and *Urtica* at 20 °C. As expected, developmental rate increased with increasing temperature, as observed previously for *C. sphaericus* (Keen, 1973, Bottrell, 1974, Meyers, 1984 and De Eyto and Irvine, 2001). At the highest tested temperature (25 °C) we found the highest developmental rate, but

mortality also increased. Therefore the most suitable temperature for the chydorid toxicity test was considered to be 20 °C. The choice of this temperature facilitates comparisons, since most standard toxicity tests are also performed at 20 °C.

De Eyto and Irvine (2001) observed that *C. sphaericus* grew equally well on all the food types offered: algae, detritus or filtered pond water, and explained this by its generalistic mode of feeding. Our findings agree only partly with theirs. Indeed, *C. sphaericus* grew and developed well on all food types, yet significant better growth was obtained when feeding the animals a mixture of *N. perminuta* and *Urtica*.

The sensitivity of *C. sphaericus* (96 h LC_{50} values of 594 μg/L and 191 μg/L for cadmium and copper, respectively) is similar to that of daphnids: Biesinger and Christensen (1972) found an LC_{50} for copper (48 h) for *D. magna* of 60 μg/L, using softer water than the present study. Stuhlbacher et al. (1993) found LC_{50} (48 h) values for cadmium for *D. magna* between 24.4 and 355 μg/L, depending on clone and preconditioning. Schubauer-Berrigan and Dierkes (1993) found LC_{50} values for *Ceriodaphnia dubia* for copper and cadmium to range from 9.5 μg/L to 500 μg/L and from 5 μg/L to 780 μg/L, respectively. A major influence on the LC_{50} values in their study was the pH of the medium.

Koivisto et al. (1992) and Bossuyt and Janssen (2005) reported lower LC_{50} values for copper for *C. sphaericus* (7.6 μg/L and 20–38 μg/L, respectively) than in the present study. However, Koivisto et al. (1992) gave green algae as food (*Scenedesmus* sp.) which is not optimal as observed in the present study, while Bossuyt and Janssen (2005) did not feed the animals at all. Here, the chydorids were fed a surplus of optimised food, which may have reduced the bioavailability of the metals as well as the sensitivity of the chydorids. However, when exposed to sediment bound toxicants, which was the purpose of the present test, chydorids may be exposed via several different exposure routes and Dekker et al. (2002) observed the effects of cadmium on *Chydorus piger* at relatively low concentrations in the sediment. The sensitivity of *C. sphaericus* to total ammonia (96 h LC_{50} value of 46 mg/L) is within the range reported for daphnids (25–189 mg/L) (Postma et al., 2002).

Reinhold-Dudok van Heel and Den Besten (1999) and Lahr et al. (2003) concluded that the chronic *D. magna* reproduction test with pore water was the most sensitive standard test to monitor sediment toxicity. Yet, Lahr et al. (2003) also reported that the results of none of the tests correlated with the sediment pollution classes deduced from chemical analysis. One of the reasons for these discrepancies could be the use of pelagic test organisms exposed to pore water. Alternatively, exposing sediment inhabiting organisms to whole sediment samples allows testing in their natural habitat. This way, the organisms are exposed not only to toxicants in the pore water but also to sediment bound toxicants. This may alter toxicity, since Simpson and King (2005) demonstrated that the rate of copper accumulation and the mode of copper toxicity were different for dissolved and particulate phases. Consequently, the discrepancies between results from sediment toxicity tests and chemical analyses may be reduced. In the present study, exposing benthic cladocerans to whole sediment samples, indeed a significant negative correlation between survival and toxicant concentrations was observed (when one outlier was ignored).

In conclusion, the toxicity test developed in the present study using the benthic cladoceran *C. sphaericus* has several advantages, such as the ease of maintaining cultures and the small amounts of material and space needed for the cultures and

experiments. The duration of the test is very short compared to other tests including reproduction, because the life cycle of *C. sphaericus* lasts only four days, compared to the seven days for *D. magna* (Tessier and Consolatti, 1989). These advantages may reduce test costs, making *C. sphaericus* a very suitable test species for routine laboratory sediment toxicity testing.

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