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Effects of pollutants on life-history parameters of the marine nematode *Monhystera disjuncta*

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The toxicity of heavy metals (Cd, Co, Cu, Cr, Hg, Ni, Zn), acid-iron waste, pentachlorophenol, and γ-hexachlorocyclohexane on the marine nematode *Monhystera disjuncta* has been studied in laboratory assays. High levels of toxicants (> 1 mg/l) are necessary to cause acute effects on J2-larvae. The relative toxicity as measured by development retardation and reduction of fecundity is compared with threshold concentrations obtained from the acute tests. Fecundity is the most sensitive indicator. LC 50 values correlated significantly with the minimum concentrations of metals causing mortality and developmental inhibition. Ranking of toxicity based on fecundity does not correlate with ranking based on mortality or development rate. *Monhystera disjuncta* exhibits high resistance to pollutants compared with other benthic organisms.

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Introduction

The scarcity or absence of sensitive taxa, as judged by ecological surveys, is frequently used as a means of detecting the effects of pollution. However, to establish causal relationships is not simple. The distribution of species is the result of multiple processes (Andrewartha and Birch, 1954), and the influence of pollution is not easily distinguished from natural variability. This is true for nematodes, on which the effects of pollution and sediment are difficult to separate (Heip *et al.*, 1985). In nature, pollution-induced changes in community structure are the result of a complex set of interactions, such as synergism and antagonism between constituents of effluents (Babich and Stotzky, 1983) influenced in turn by abiotic factors (Bryant *et al.*, 1984) and biological processes, such as bioaccumulation (Slowik, 1981) and biodegradation (Doelman *et al.*, 1985). Therefore, natural complexity hampers the study of individual dose–response relations in the field.

Consequently the effects of individual chemicals are usually assayed in laboratory conditions. Nevertheless, predicting safe limits from laboratory tests presents other problems, because these limits depend on the criterion used (Reish and Carr, 1978) and are often time-dependent (Vranken *et al.*, 1985; Best and Morita, 1983). Thus, the inappropriateness of short-term acute toxicity tests (LC 50 tests) for predicting long-term biological effects in moderately polluted situations is well known (Samoiloff *et al.*, 1980; Brown, 1981; Ward, 1984). Some consider the LC 50 test only important for fast toxicity screening, for broad effect-ranking of chemicals and for inter-species comparisons. Here we will show that for some chemicals the lowest concentrations having a detectable effect on mortality are not much different from those determined using criteria such as fecundity and development which would be expected to be more sensitive.

Theoretically, the intrinsic rate of natural increase r_m, which represents the ability of a species to increase in numbers in a particular environment (Andrewartha and Birch, 1954) and which is considered as a measure of fitness (Snell, 1978), is directly related, *inter alia*, to the physical conditions of the environment. Changes in r_m might therefore be useful for predicting the environmental impact of toxicants (Sabatini and Marcotte, 1983). However, an accurate determination of r_m requires the construction of age-specific survival (l_x) and fecundity (m_x) schedules, which is very time-consuming (Vranken, 1987). To reduce experimental time, we have studied the influence of chemicals on daily fecundity and success in reaching adulthood (criteria obviously related to m_x and l_x, respectively). For the criteria studied, the smallest concentrations having a detectable effect are estimated.

Monhystera disjuncta, the test species used, is a bacterivorous cosmopolitan nematode species which is very abundant in some biotopes (Trotter and Webster, 1983). Its advantages as a bioassay organism have been discussed by Vranken *et al.* (1984a, b). The test period of 96 h will be assessed in relation to the life-cycle of the species. Procedures presented are also applicable to

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brackish water and terrestrial nematodes (Vranken *et al.*, 1985; Coomans and Vanderhaeghen, 1985).

Materials and methods

Isolation and simple culture techniques

Monhystera disjuncta was obtained from the Sluice Dock of Ostend, a marine lagoon near the Belgian North Sea coast. A few millilitres of sediment were inoculated in four spots made in 14 cm diameter petri-dishes filled with (DIFCO) bacto-agar (1% in water from the natural habitat). After 1–2 weeks incubation, the nematodes (and other organisms) invade the agar surrounding the detritus spots. Adults of *M. disjuncta* were picked out of the agar individually with a needle to start the pure cultures. Agnobiotic cultures of *M. disjuncta* (cultures with an unknown number of different food organisms) were set up in small petri-dishes (diameter 3.5 cm) filled with 4 ml 0.4% bacto-agar. This agar was made up with Sluice Dock water and enriched with 1% Vlasblom medium (0.278 g $FeSO_4.7H_2O$, 3.0 g $NaHPO_4.2H_2O$, 30.0 g $NaNO_3$, 0.47 g $MnCl_2.4H_2O$, 50.0 g glycine in 1 l aq. dest.: Vlasblom 1963) and 0.5% of a 15 g/l solution of $Na_2SiO_3.9H_2O$. Salinity was measured with a refractometer and kept between 29‰ and 31‰ by the addition of distilled water when necessary. The agar was inoculated with a few drops of paper-filtered water from the Sluice Dock. Bacteria grew rapidly on the medium. After a few days the petri-dishes were inspected. Dishes showing development of fungi were discarded. The rest were inoculated with adult *M. disjuncta* from the extraction plates. These stock cultures were renewed at regular intervals (at least once each month) by transferring at least 20 females and 10 males, sampled at random from different cultures, to new petri-dishes. 4–5 stock culture dishes were simultaneously kept. The Sluice Dock was routinely sampled and extraction dishes set up so that wild animals could regularly be added to the laboratory stock.

Monoxenic culture technique of the test species

The culture techniques for the toxicity tests in monoxenic cultures (cultures with a single species as food organism) were extensively described by Vranken *et al.* (1985). Small vented petri-dishes (35 × 10 mm, Falcon) were filled with 4 ml 0.5% sterile bacto-agar (DIFCO) suspension in buffered (pH: 7.5–8.0) artificial sea water (ASW) (30‰ S, 5 mM Tris buffer) after Dietrich and Kalle (1957) and mixed with 0.2 ml of a sterol mixture, with the following constituents (Van Fleteren, 1980): 0.2 g cholesterol (Fluka AG Buchs SG); 0.2 g ergosterol (Fluka AG Buchs SG); 0.2 g β-sitosterol (Merck); 0.2 g stigmasterol (Merck); 0.2 g dehydrosterol (Merck) and 100 ml ethanol. The sterol mixture was prepared by adding 10 ml of the above mixture to 100 ml distilled water. The ethanol was then evaporated and the mixture autoclaved for 20 min at 1.2 bar. After the medium had cooled, a central ring-shaped excavation (diameter: 15 mm) was made in the culture medium by pushing the top of a sterile test-tube through the agar towards the bottom of the petri-dish. The cavity was filled with a suspension of 0.02 ml of a bacterial strain belonging to the *Alteromonas haloplanktis* rRNA group, containing 10^{11} cells/ml. Nematodes isolated from field samples as described above, axenized for 24 h in agar containing 10 000 IU penicillin and 10 mg/ml streptomycine, were transferred with a needle to the monoxenic cultures. Different concentrations of metals and other toxicants were added to these monoxenic cultures for the toxicity tests.

Food preparation

Erlenmeyer flasks (100 ml) were filled with 50 ml heart infusion broth suspended in artificial sea water (ASW) and then sterilized. The medium was inoculated with the bacterial strain *Alteromonas haloplanktis* and incubated for 48 h at room temperature and rotated in a rotary shaking machine at 125 rpm. Bacterial cells were harvested by centrifugation at 6000 rpm for 15 min. The pellet was resuspended in sterile ASW and added to the cultures.

Toxicity tests

Juvenile stage 2 (J2) larvae of *M. disjuncta* (4.5 days old) were exposed to cadmium ($CdCl_2.2\frac{1}{2}H_2O$, Baker Chemicals BV, Holland); chromium ($Na_2Cr_2O_7.2H_2O$, Merck); copper ($CuCl_2.2H_2O$, Merck); cobalt ($CoCl_2.6H_2O$, Baker Chemicals BV, Phillipsburg); mercury ($HgCl_2$, UCB Belgium); nickel ($NiCl_2.6H_2O$, Merck) and zinc ($ZnCl_2$, UCB Belgium) salts, to titanium dioxide waste water (pH = 1, NL Chemicals Sa-nv Ghent, Belgium), the biocide pentachlorophenol (PCP, Merck) and to the insecticide γ-hexachlorocyclohexane (γ-HCH, SIGMA). For the metals, stock cultures of 1000 mg/l (as metal-ion) were prepared in 1 l distilled water using analytical grade salts. A series of 100 ml solutions with concentrations 10 times higher than the final test solutions were then prepared. Of each solution 5 ml was mixed with 42.5 ml 0.6% sterile (buffered) bacto-agar (60°C) and 2.5 ml of a sterol mixture. For cadmium the medium was prepared directly at the desired test concentrations. Stock solutions of PCP and γ-HCH were prepared by disolving 100 mg in 0.5 ml analytical grade acetone, which was then added to 999.5 ml ASW and diluted to the final test concentration. For $Ti-O_2$-waste, two parallel experiments were run. In the first, the waste was added to natural sea water (NSW) and the bacterial cells used as food were grown previously for 48 h in identical waste concentrations. In the second, the toxicity of the waste was tested in buffered (5 mM Tris)

ASW. For practical purposes, the food was grown in a metal-free medium, because at pH of 7.5 to 8, the iron present in the waste water precipitates as iron hydroxide ($Fe(OH)_3$). This precipitate has a brown colour and hampers observation.

With the exception of the iron waste assay in ASW, the *Alteromonas haloplanktis* suspension added to the cultures was grown for 48 h at the same toxicant doses as used in the tests. Active J2-larvae (4.5 days old) sampled at random from a synchronous cohort of *M. disjuncta* were tested in groups of 120 worms (per concentration) equally distributed among four replicates. After 96 h the number of dead juveniles and adults was counted under a stereoscopic microscope in all test cultures. Death was operationally defined as complete inactivity and lack of movement after stimulation with a needle. After less than 24 h, dead worms become very pale and soon completely lose the typical internal nematode turgor. Therefore after one day the initial counts of dead worms were checked for these criteria and corrected if necessary.

The egg deposition of at least 10 adult females, obtained from the different test concentrations and transferred to fresh monoxenic cultures, was determined by direct counts under the stereomicroscope. Daily egg production was calculated from these counts as the mean number of eggs produced per female per day during the observation period. All fecundity tests were run during a period of 96 h.

Development time, or minimum generation time (the sum of embryonic and post-embryonic development time), of *M. disjuncta* under the optimum experimental conditions used (17°C and 30‰ S), is approximately 8.5 days. In the post-embryonic period nematodes moult four times and pass through four larval stages before reaching adulthood. The success and speed with which the different moults are completed have been used as criteria for measuring deleterious effects of chemicals (Samoiloff *et al*., 1980). In our tests, the J2-larvae of *M. disjuncta*, which are used to start the assays, need another four days (96 h) to complete the three remaining moults and become adult. Consequently, after the test period, maturation percentages higher than 50% are expected in the blank. In sublethal test conditions, smaller maturation percentages when compared with the control are the result of inhibitory effects caused by the chemicals tested on development rate. Therefore, maturation percentages obtained from such an experimental design provide information on development inhibition, and the assay will be referred to in this paper as a developmental assay.

The raw data to determine the MECs (Minimum Effective Concentrations, see below), based on the development response, were obtained by counting the number of new adults in all test cultures when at least 50% of the juveniles had reached adulthood in the blanks (after 96 h). The lowest toxicant concentration at which the first significant smaller number of adults, when compared with the blank, appears is considered as the MEC. This MEC depends on the concentrations tested. All experiments were done at 17°C in cooled incubators with a precision of 0.5°C, at 30‰ S and in the dark. The water hardness of the prepared Killian medium was 554°F.

Statistical analysis

LC 50 values were calculated by minimum logit chi-square procedure (Hewlett and Plackett, 1979). In the γ-HCH test, mortality could not be linearized by logit or probit transformation. LC 50 values for this compound were estimated by inverse prediction from a linear least squares regression of mortality, transformed into arc sin $\sqrt{\text{proportion}}$, against the logarithms of the concentrations (Sokal and Rohlf, 1981). Minimum effective concentrations (MECs), defined as the lowest concentrations tested which gave a significantly different response based on mortality and development time when compared with the blank, were estimated by a log-likelihood test (G-test, Sokal and Rohlf, 1981). The significance of the reduction in fecundity was examined by ANOVA. MECs based on reduction of fecundity were determined by unplanned comparisons between the mean fecundities by calculating 95% comparison intervals around the mean fecundities obtained at the different test concentrations (Sokal and Rholf, 1981).

Results

Acute toxicity

The mortality responses of J2-larvae of *M. disjuncta* at the individual concentrations of the chemicals tested are compiled in Table 1. LC 50 values and MEC values for mortality are given in Table 2. Apart from mercury and copper, none of the agents tested caused substantial mortality at concentrations lower than 10 mg/l. LC 50 values resulted in the following rank order of toxicity: Cu > Hg > Cr > Zn > Cd > Co > Ni. Values of MEC based on juvenile mortality resulted in the following ranking of toxicity: Cu > Hg > Co > Cr > Zn > Cd > Ni. With the exception of the position of cobalt, both sequences are the same (Spearman's $r_s = 0.79$; $p < 0.05$; $n = 7$), the MEC of Co being 10 mg/l whereas its LC 50 is 94 mg/l.

Figure 1 and Table 3 illustrate the very smooth dose-mortality response pattern for Co (smallest slope in Table 3). For copper, a very steep relationship was found between mortality and concentration (Fig. 1 and Table 3), the deleterious effects caused by Cu being manifest over a very small range from 1 mg/l to 2.5 mg/l (Fig. 1). The slopes of the dose–mortality curves of Zn, Hg, Cr and Ni are intermediate and nearly identical (Table 3).

Acid-iron waste (TiO_2) tested in NSW did not cause mortality up to a dilution of 0.3 ml waste/l NSW. The

Table 1. Percentage mortality (pooled results of four replicate cultures) after 96 h at different metal/toxicant concentrations (mg/l) of 4.5-day-old individuals of *Monhystera disjuncta*. Mortality in all the blanks was zero. Test concentrations inducing zero percent mortality are not shown, except for chemicals with a very steep dose–response relationship. NSW = natural sea water; ASW = artificial sea water

Metal toxicant	Concentration (mg/l)	Mortality (%)	95% CI
Zn^{2+}	1	4	1.2–9.4
	10	4	1.3–9.2
	20	37	34.0–52.4
	30	67	42.7–75.8
	50	86	77.8–92.0
	70	99	95.1–99.95
Cd^{2+}	5	0	0–3.2
	10	2	0.1–6.4
	25	22	14.6–31.0
Cu^{2+}	0.75	1	0.05–4.8
	1	2	0.3–9.3
	1.75	10	5.3–16.8
	2.5	60	50.8–68.7
	5	97.5	93.6–99.7
	10	100	97.1–100
Hg^{2+}	2.5	8	3.7–14.7
	5	36	27.2–45.6
	7.5	82	73.8–88.5
	10	81	72.7–87.7
Co^{2+}	10	6	2.6–11.8
	200	65	54.4–74.7
	300	94	88.2–97.4
	400	100	97.5–100
Cr^{2+}	10	3	0.6–9.0
	15	13	6.9–21.8
	20	71	59.6–80.7
	30	71	59.6–80.7
Ni^{2+}	50	7	3.1–13.3
	70	21	13.4–30.4
	90	44	33.7–54.7
	110	51	40.1–61.8
PCP	2.5	0	0–2.5
	5	60	51.1–68.5
	7.5	97	92.1–99.2
	10	100	97.1–100
γ-HCH	1	0	0–2.5
	2.5	40	31.3–49.2
	5	37.5	29.4–47.2
	7.5	50	40.8–59.2
TiO_2 waste[1] (NSW)	0.3	0	0–2.5
	0.4	90	83.2–94.7
	0.5	100	97.5–100
TiO_2 waste[1] (ASW + buffer)	No mortality: highest concentration tested was 10 ml waste/l medium		

[1]Concentration in ml waste/l medium.

estimated LC 50 value is 0.36 ml waste water/l. The pH equivalence of this LC 50 is 5.7 (95% CI: 5.5–5.9). The acid-iron waste is 100% toxic at a dilution of 0.5 ml waste water/l. The dose–mortality relation caused by the waste water diluted in NSW is extremely steep (slope = 50.5; Table 3, Fig. 1). No mortality was observed when the acid-iron was diluted in buffered ASW. Concentrations up to 10 ml waste water per liter buffered ASW were tested.

The dose–mortality relationships of the biocide PCP and the insecticide γ-HCH are comparable to those most toxic metals (Cu and Hg; Fig. 1). Concentrations of 1 mg/l γ-HCH and 2.5 mg/l PCP did not cause any mortality. The LC 50s of PCP and γ-HCH are 4.8 and 6.7 mg/l, respectively. The steepest dose–mortality response was found for PCP (Table 3). As a result, the MEC of PCP as measured by the percentage mortality is somewhat higher than the LC 50 (Table 2).

Table 2. *Monhystera disjuncta*: list of metals and toxicants studied (first column), lethal concentrations (LC 50 values) (second column). Effective concentrations (EC 50 values) reducing daily egg production by 50% (third column) and smallest concentrations having a detectable effect (MEC = minimum effective concentrations) for three different criteria: mortality (column 4), development (column 5), and fecundity (column 6). All concentrations are in mg/l; CI = confidence interval; SW = natural sea water; ASW = artificial sea water

Metal toxicant	LC 50 (95% CI) mg/l	EC 50 (95% CI) mg/l	Mortality MEC (mg/l)	Development MEC (mg/l)	Fecundity MEC (mg/l)
Zn^{2+}	24.6 (22.7–26.6)	1.9 (0.8–4.3)	20	5	0.75
Cd^{2+}	37	–	25	10	1
Cu^{2+}	2.4 (2.2–2.5)	>1.75	1.75	1.75	1.75
Hg^{2+}	5.6 (4.7–6.7)	>2.5	2.5	2.5	2.5
Co^{2+}	94 (40–220)	–	10	–	–
Cr^{6+}	21 (16.8–25.7)	>1	15	0.75	0.75/1
Ni^{2+}	103 (94.4–113.1)	15 (7.0–28.8)	50	15	15
PCP	4.8 (4.5–5.0)	2.1 (0.5–7.0)	5	5	5
γ-HCH	6.7 (2.6–19.1)	1.6 (0.6–4.9)	2.5	1	1
TiO_2 waste (NSW)[1]	0.36 (0.35–0.38)	–	0.4	0.4	–
TiO_2 waste[1] (ASW + buffer)	–	–	10	2	1

[1] ml waste/l

Table 3. Minimum logit chi-square analysis: regression of the logit of the percentage mortality (M) against the logarithm of the concentration (C): M = a + blogC; a = intercept; b = slope; SE = standard error; χ^2 = Pearson's chi-square for goodness-of-fit

Metal/ toxicant	a	b (+s.e.)	Pearson's chi-square
Zn^{2+}	−9.7	7.0 (0.63)	n.s.
Cu^{2+}	−4.5	11.8 (1.00)	n.s.
Hg^{2+}	−5.3	7.1 (0.98)	*
Co^{2+}	−6.3	3.2 (0.81)	***
Cr^{6+}	−11.4	8.6 (2.09)	***
Ni^{2+}	−15.7	7.8 (1.12)	n.s.
PCP	−11.8	17.4 (2.25)	n.s.
γ-HCH[1]	7.5	45.2 (5.47)	–
TiO_2 waste (NSW)	22.2	50.5 (8.88)	n.s.

[1] For γ-HCH the arcsine transformation gave the best fit (r^2 = 0.97; $p < 0.001$)
n.s. = not significant.
* = $0.01 < p < 0.05$; ** = $0.001 < p < 0.01$; *** = $p < 0.001$

Developmental assay

The influence of the different toxicants on the maturation percentages of J2-larvae of *M. disjuncta* is shown in Table 4. Hexavalent chromium is extremely effective in inhibiting development rate. Chromium is the only metal studied which is toxic in the µg/l range. The relative toxicity rank order of the metals as measured by development inhibition is comparable to the sequence obtained when using mortality (LC 50 and MEC) as toxicity index (r_s = 0.829; $p = 0.05$). The MECs for development inhibition are either similar (Cu and Hg) or, for most metals (Zn, Cd, Cr and Ni), smaller than those found in the acute tests. The ratios of the MECs based on mortality and development ranged between 1 and 20. The ratio is greatest for Cr, intermediate for the relatively non-toxic metals (Zn, Cd and Ni) and unity for Cu and Hg. Similar results were obtained for PCP, γ-HCH and the acid-iron waste. Lindane inhibits development at 1 mg/l, a concentration 2.5 times less than the MEC for mortality. The toxicity threshold of acid-iron waste tested in NSW is identical for both development and mortality. As in the case of mortality, development rate is influenced by pH, the MEC being five times smaller in NSW than in buffered ASW. The EC 100 of the wastewater diluted in buffered ASW is more than 25 times higher than in NSW. The relative toxicity rank order as determined by development inhibition correlates significantly only with the rank order obtained using MEC mortality as a criterion (Table 5). It does not correlate with the rank orders obtained using LC 50 and MEC fecundity as criteria.

Fecundity

In Table 6 the daily number of eggs produced per female in the first 4 days of the fertile period is shown for all chemicals. With the exception of copper, the concentration range tested caused significant reduction in fecundity (ANOVA). At the highest test level of Cu, a concentration identical to the MEC as measured by mortality, the daily egg production is reduced by 24%. For copper and mercury, MECs as measured by reduction in egg production are identical to the MECs obtained in the

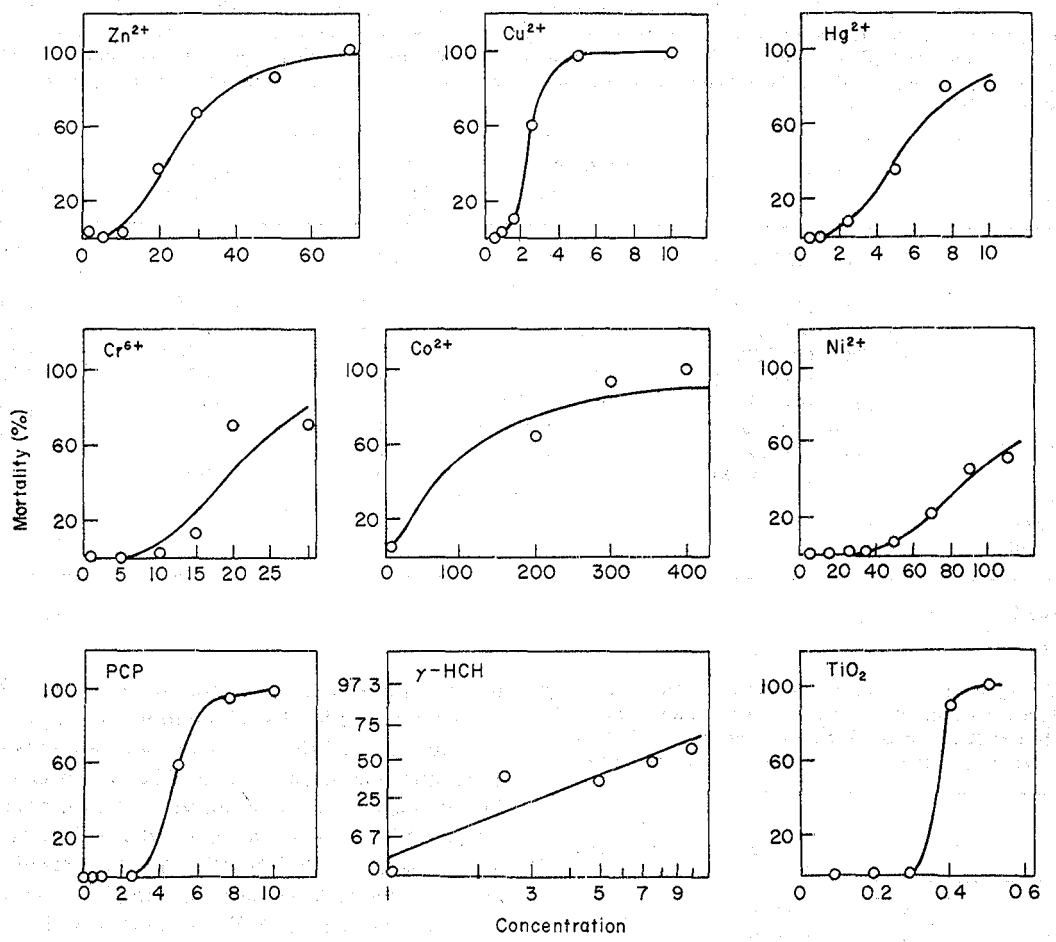

Figure 1. *M. disjuncta*: dose–response relationships for J2-larvae after 96 h. Curves were fitted using coefficients of Table 3.

acute and developmental assays. The positions of Cu and Hg as determined by their suppression of fecundity is intermediate to low. Chromium and zinc are the most toxic metals followed by cadmium. Ni is, as for the other criteria, the least toxic metal. Toxicity ranking of the metals based on reduction of fecundity does not correlate with any of the other criteria studied (Table 5). For Zn and Ni EC 50s were estimated (Table 2); their values were almost (Ni) and more (Zn) than one order of magnitude smaller than the corresponding LC 50 values. For PCP and γ-HCH the MEC is 1 mg/l. In buffered ASW a concentration of 0.1% acid-iron waste significantly depressed fecundity. The EC 50 was estimated roughly as 0.09% of the undiluted effluent.

Discussion

For all three criteria studied, a 96 h period was chosen as exposure time. This is meaningful for assays with *M. disjuncta*, (a) because cumulative egg production by nematodes, even in the presence of cadmium, copper and nickel, is a linear function of time (Vranken and Heip, 1986; Verschraegen *et al.*, 1986; Vranken, 1987) and (b) because the J2-larvae used need exactly another 4 days to become adult (Vranken *et al.*, 1988). Therefore the exposure time, which equals the time necessary to complete the life-cycle, has a physiological meaning for the species. J2-larvae were chosen because the J1-larvae are too fragile to manipulate and because the eggs are known to be highly resistant (Khan and McFadden, 1980). Moreover, in a preliminary experiment with methyl mercury chloride, no clear-cut causal relationship was found between the egg mortality pattern and the test concentrations used (Vranken *et al.*, 1984a, b). This is probably due to the fact that the nematode egg-shell is the only nematode structure containing chitin (Bird, 1980), and therefore a structure which is less permeable to some substances than the highly metabolically active cuticle (Bird, 1980). A significant reduction in the viability of the eggs in the sublethal concentrations used to study fecundity is not to be expected. A 96 h test period was also chosen

Table 4. Percentage maturation (pooled result of four replicate cultures) after 96 h at different metal/toxicant concentrations (mg/l) of 4.5-day-old individuals of *M. disjuncta*. NSW = natural sea water; ASW = artificial sea water. Confidence limits of the percentages were calculated using the binomial distribution.

Metal/ toxicant	Concentration (mg/l)	% maturation	95% CI
Zn^{2+}	Control	91	84.4–95.4
	0.75	98	93.6–99.7
	1	91	84.5–95.4
	5	61	51.7–69.7
	10	2.5	0.3–8.7
	20	0	0–2.5
	30	0	0–2.8
	50	0	0–2.8
	70	0	0–2.5
Cd^{2+} [1]	Control	67	54.2–78.2
	1	69	56.8–79.5
	5	69	56.0–80.1
	10	11	4.6–21.4
	25	0	0–2.8
Cu^{2+}	Control	61	51.8–69.7
	0.75	40	31.3–49.2
	1	54	55.2–63.0
	1.75	42	33.2–51.2
	2.5	0	0–2.8
Hg^{2+}	Control	85	77.3–90.9
	0.5	87	79.6–92.4
	1	82	73.9–88.4
	2.5	24	16.5–33.0
	5	10	5.2–17.1
	7.5	1	0.04–5.0
	10	0	0.0–2.6
Cr^{6+}	Control	97	92.0–99.3
	0.5	96	90.6–98.7
	0.75	79	70.4–86.0
	1	74	65.2–81.5
	5	0	0.0–3.5
	10	0	0.0–3.3
Ni^{2+}	Control	91	84.3–95.5
	5	87	79.3–92.6
	15	63	53.6–71.8
	25	39	30.0–48.5
	35	16	9.7–24.2
	50	0	0–2.6
	70	0	0–3.0
PCP	Control	92	85.7–96.1
	0.5	94	88.1–97.4
	1	91	84.5–95.3
	2.5	87	79.8–92.3
	5	24	17.0–32.3
	7.5	0	0.0–2.5
γ-HCH	Control	95	89.3–98.1
	0.1	96	90.7–98.7
	0.25	93	86.9–96.8
	0.5	87	79.7–92.4
	0.75	88	80.9–93.1
	1	73	64.1–80.7
	2.5	16	10.0–23.7
	5	0	0–2.5
TiO_2 waste /NSW[2]	Control	89	82.0–93.9
	0.1	93	86.9–96.8
	0.2	93	86.9–96.8
	0.3	94	88.1–97.5
	0.4	0	0–2.5
TiO_2 waste /ASW[2]	Control	89	82.0–93.9
	0.1	92	85.5–96.2
	1	88	80.6–93.3
	2	70	60.9–78.1
	10	43	33.8–52.6

[1] Experimental time is 120 h
[2] Concentrations in ml waste/l medium

Table 5. Relative rank order of toxicity for the different criteria studied. MEC = minimum effective concentration at $p < 0.05$; D = development; M = mortality; F = fecundity.

LC 50:	Cu > Hg > Cr > Zn > Cd > Co > Ni
MEC (M):	Cu > Hg > Co > Cr > Zn > Cd > Ni
MEC (D):	Cr > Cu > Hg > Zn > Cd > Ni
MEC (F):	Cr = Zn > Cd > Cu > Hg > Ni

Table 6. *Monhystera disjuncta*: daily fecundity: number of eggs produced per female during the first 96 h of the fertile life period at different levels of metals/toxicants; CI = confidence intervals, * = first value significantly different from the blank at ($p < 0.05$).

Metal/ toxicant	Concentration (mg/l)	Daily fecundity	95% CI
Zn^{2+}	Control	63	41.5–83.5
	0.75	48*	40.5–54.5
	1	50	36.2–62.8
	5	5	1.1–8.7
Cu^{2+}	Control	43	36.9–49.5
	0.75	34	29.8–38.5
	1	44	38.7–48.9
	1.75	33*	23.6–42.4
Hg^{2+}	Control	45	34.3–56.5
	0.5	35	28.2–42.0
	1	41	30.9–51.3
	2.5	32*	24.5–39.5
Cr^{6+}	Control	49	31.2–66.2
	0.5	43	30.7–54.9
	0.75	31*	23.7–38.9
	1	33	29.2–36.0
Ni^{2+}	Control	66	59.5–72.2
	5	65	59.2–65.7
	15	31*	12.2–51.7
	25	5	1.1–19.2
	35	1	0.1–7.3
PCP	Control	66	59.5–72.2
	0.5	59	48.8–68.5
	1	44*	35.9–52.1
	5	10	2.1–18.3
γ-HCH	Control	60	47.4–73.3
	0.5	44	31.8–57.0
	1	34*	15.2–51.9
	2.5	26	4.4–47.9

Table 7. Toxicity of heavy metals to *Monhystera disjuncta*, measured at 96 h LC 50s, compared with other organisms. All concentrations are in mg/l.

Metals	*Monhystera disjuncta* 96 h LC 50 J2-larvae	*Panagrellus silusiae* 72 h LC 50 J2-larvae	*Enoplus communis* 96 h LC 50* B.B.	*Enoplus brevis* 96 h LC 50* B.R.	*Enoplus brevis* 96 h LC 50* B.B.	*Nitocra spinipes* 96 h LC 50 adults	*Carcinus maenas* 48 h LC 50 larvae	*Nereis diversicolor* 192 h LC 50*	*Crassostrea virginica* 48 h LC 50 larvae
Zn^{2+}	24.6	20	0.38	>100	>100	1.45	1.0	30	0.31
Cd^{2+}	37	5.85	0.2	10	>100	1.8	–	100	3.8
Cu^{2+}	2.4	0.28	0.1	1.6	5	1.8	0.6	0.27	0.103
Hg^{2+}	5.6	2.81	<0.01	5	0.08	0.23	0.014	>0.1	0.0056
Cr^{6+}	20.7	18.5†	–	–	–	–	–	10	10.3†
Ni^{2+}	103.3	28.6	–	–	–	6.0	–	–	1.18

*Stage not mentioned
†Cr^{3+}
B.B.: Budle bay; B.R.: Blyth River
References: *P. silusiae* in Haight *et al.* (1982); *E. communis* and *E. brevis* in Howell (1984); *N. spinipes* in Bengtsson (1978); *C. maenas* in Connor (1972); *C. virginica* in Calabrese *et al.* (1973).

for comparative purposes, as this period is often used in toxicity work, even for species with much longer life-cycles (Reish *et al.*, 1983). Therefore the present results have the advantage of being comparable to a large number of published data.

Relatively high levels of toxic agents are necessary to cause immediate effects on the J2-larvae. Fecundity is the most sensitive criterion used. Relatively large discrepancies between the three toxicity indices were found only for the agents which are intermediately or relatively non-toxic on an acute basis. This means that the difference between 96 h LC 50 and the threshold of a sublethal effect is relatively small, although experimental time might be too short to evaluate the sublethal effects unequivocally.

Reish and Carr (1978) and Petrich and Reish (1979) found for two polychaetes, *Ctenodrilus serratus* and *Ophryotrocha diadema*, differences of the same magnitude between acute and fecundity tests for most heavy metals studied, except for copper and mercury, where the difference between the 96 h LC 50 test and reduction in fecundity was small. Samoiloff (1980) reported for the nematode *Panagrellus redivivus* that significant reduction in fecundity caused by Cd occurs at levels three orders of magnitude lower than the MEC as measured by mortality. Similarly, for the nematode species *Diplolaimella dievengatensis* significant reduction in fecundity caused by copper occurs at levels more than one order less than the LC 50 (Vranken and Heip, 1986), a result in sharp contrast to the present findings. Haight *et al.* (1982) studied the influence of seven metals on the length-growth and mortality of the free-living terrestrial nematode *Panagrellus silusiae*. With the exception of copper (LC 50 = 280 µg/l), all LC 50 values were higher than 2 mg/l (Table 7). Concentrations necessary to block growth were higher than 50 mg/l and hence considerably higher than the LC 50 data.

Small differences between acute and development inhibition tests were also reported by Bogaert *et al.* (1984) for the nematodes *Diplolaimella bruciei* and *Diplolaimella dievengatensis*. For *Panagrellus redivivus*, on the contrary, growth inhibition is more sensitive than mortality (Samoiloff *et al.*, 1980). This also holds for the free-living brackish-water monhysterid *Diplolaimella dievengatensis* (Vranken and Heip, 1986). However, regardless of some conflicting results it is clear that compared with mortality, fecundity and development are the more sensitive toxicity criteria. The conflicting results reported by Haight *et al.* (1982) in the *P. redivivus* test may be due to additional nutritional stress. These authors did not feed their animals during the acute tests and it is known that metal toxicity falls when food is added (Biesinger and Christensen, 1972; Vranken and Heip, 1986).

Although information is lacking on the flux through the nematode of the substances tested, on their bioavailability and on their concentration in the food offered, it can be concluded that *M. disjuncta* is relatively resistant to heavy metals when compared with representatives of the major taxonomic groups living in benthic and epibenthic marine faunal assemblages (Table 7). Similar high resistance, especially to copper, is reported for non-marine nematodes. Feldmesser and Rebois (1966) obtained LC 50 values for mixed populations of *Panagrellus* and *Rhabditis* between 35 and 40 mg/l. De Maeseneer (1967) found that 200 ppm Cu caused 80% mortality with three longidorid species. However, the density of other nematode species such as *Pratylenchus crenatus*, *Rotylenchus robustus* and unidentified saprozoic species was not affected at this concentration, even in very acid soils. For three plant-parasitic nematodes, *Xiphinema diversicaudatum*, *Aphelenchoides ritzemabosi* and *Pratylenchus penetrans*, the 18–24 LC 50s of Cu are, respectively, 0.1, 4.1 and 2.6 mg/l (Pitcher and McNamara, 1972). The predatory nematode *Mononchus*

aquaticus remained highly active during the first 6 h after exposure to 63.5 mg/l Cu (Bilgrami and Jairajpuri, 1984).

The large marine predatory species *Enoplus communis* is an exception (Table 7). This species is much more sensitive compared with other nematodes (Howell, 1984). Thus, 96 h LC 50s for *E. communis* ranged between 10 µg/l Hg^{2+} and 380 µg/l Zn^{2+} (Table 7), which are relatively low values, even when compared with sensitive taxa such as copepods, amphipods (Reish *et al.*, 1983) and larvae of crustaceans (Martin *et al.*, 1981). In contrast to *E. communis*, the congeneric *E. brevis* exhibits almost the same resistance to heavy metals as does *M. disjuncta* (Howell, 1984). To explain this difference between congeneric species Howell (1984) suggested that animals from polluted localities were less susceptible to the toxicants tested than those from unpolluted sites.

Nematodes are the most abundant animals in marine sediments. A representative of the second most abundant meiobenthic taxon, the harpacticoid copepod *Nitocra spinipes*, is at least 17 times more sensitive to heavy metals, except Cu (Table 7). The harpacticoid copepod is therefore probably the better bioassay organism. Ernst (1984) reviewed the toxicity of pesticides and organic chemicals to marine organisms. For γ-HCH, 96 h LC 50 values ranged between 0.2 µg/l for the shrimp *Penaeus duorarum* and 0.1 mg/l for the sheepshead minnow *Cyprinodon variegatus*. The least sensitive organism with regard to lindane is still some 50 times more sensitive than *M. disjuncta*. For PCP, the median acute level is smaller than 1 mg/l for most organisms tested. Much larger invertebrates, such as *Crangon crangon*, *Palaemon elegans* and the blue mussel *Mytilus edulis*, exhibit almost the same resistance to PCP as *M. disjuncta*.

The LC 50 value of the acid-iron waste tested, which contains about 20% sulphuric acid and 2% iron (Roekens and Van Grieken, 1983), is 0.036% (pH = 5.7). At such a concentration, Cr is the only metal present at a relatively higher concentration of 120 µg/l, a value considerably lower than the MEC value. Although acidification increases metal toxicity, it is probably only the sulphuric acid component of the effluent which is harmful. In the sea the iron of the effluent is oxidized and precipated. The metals are co-precipitated by forming complexes with Fe and Ti (Lethinen *et al.*, 1984), which probably reduces their toxicity. The reduction in pH is limited to the first seconds after discharge from the barge into its wake (Roekens and Van Grieken, 1983), so that the assay done in buffered ASW is more useful for risk assessment. From this test it is concluded that the TiO_2 waste has only a minor effect on the viability of *M. disjuncta*. However, because of its relative resistance to toxicants, we conclude that the value of the species as a bioassay organism is limited.

The growth medium used to perform our tests with *M. disjuncta* lacks the most active binding substances present in standard microbial growth media (Ramamoorthy and Kushner, 1975). Therefore the present results are not artefacts of the methods used, but are in our opinion more indicative of the general resistance of bacterial eating nematodes to effects of pollution. Field observations corroborate this view as *M. disjuncta* is extremely abundant in heavily polluted sediments of the Southern Bight of the North Sea (Vincx *et al.*, 1984) and because species of the same feeding type as *M. disjuncta* become extremely dominant in highly polluted marine sediments (Heip *et al.*, 1985).

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