C.M.1986 E:19

Toxicity of environmental toxicants on the marine nematode Monhystera disjuncta. A comparison between developmental rate, fecundity and mortality as toxicity-indices.

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ABSTRACT

The toxicity of heavy metals (Cd, Co, Cu, Cr, Hg, Ni, Zn), acid-iron waste, pentachlorophenol and γhexachlorocyclohexane on Monhystera disjuncta is studied in laboratory assays. High levels of toxicants (1mg/l) are necessary to cause acute effects on J_2-larvae. The relative toxicity as measured by a developmental test and fecundity was compared with threshold concentrations obtained from the acute tests. Fecundity is the most sensitive criterion. When LC 50 values are ranked and compared with the other criteria, they correlate significantly with minimum effect concentrations causing mortality and developmental inhibition (when only the metals are considered), which are also correlated between them. Ranking of toxicity based on fecundity does not correlate with ranking based on mortality or development. Monhystera disjuncta exhibits high resistance to pollutants when compared with other benthic organisms. Therefore toxicants causing harmful effects to nematodes have to be considered as extremely dangerous.

INTRODUCTION

The goal of ecological surveys on natural faunal assemblages is to relate scarcity or absence of sensitive taxa to pollution. To demonstrate the causality of such relationships, however, is not a simple task. As presence/absence of species is the result of a variety of physical, chemical and biological processes (Andrewartha & Birch, 1954), adverse effects caused by pollution are not easily distinguished from natural variability. This is certainly the case for nematodes where effects of pollution and sediment-texture are very difficult to separate (Heip et al., 1985). In the same paper, Heip et al. (1985) concluded that the interpretation of observed changes in stuctural community parameters such as diversity is difficult, especially because the pre-pollution situation is mostly unknown. In nature, pollution-induced changes in community structure are probably the result of a complex set of multiple interactions, such as synergystic and antagonistic effects between constituents of effluents (Babich & Stotzky, 1983) influenced in turn by fluctuating abiotic parameters (Bryant et al., 1984) and by biological activity, e.g. bioaccumulation (Slowik, 1981) and biodegradation (Doelman et al., 1985). Therefore it is hard to study simple dose-response effects in natural conditions. Neither do such studies yield quantitative information abouth no effect levels (NEL's) of individual contaminants. Consequently the effects of single chemical agents, such as heavy metals, on nematode viability can only be determined properly under laboratory conditions.

Experiments dealing with the adverse effects of pollutants on organisms can be divided into two categories: 1) lethal short term tests in either 48 or 96 h and 2) assays in which sublethal effects over a longer period (several weeks) are studied. Acute tests end with the determination of LC 50 values (concentration of toxicant at which 50% of the organisms studied die). Mostly these levels are much higher than those found in nature, implying that short-term acute tests are inappropriate to assess and predict long-term biological effects in moderately polluted situations. Recently, Ward (1984) has shown that LC 50's are bad predictors of ecological effects and that acute toxicity is only a minor

factor in structuring a seagrass faunal community. Brown's (1981) critisism goes even further. He simply states that the determination of LC 50's is irrelevant for purposes of environmental protection. To predict 'safe' concentrations from 96 h LC 50's, an application factor of 0.01 is often used (Reish & Carr, 1978). This, of course, is rather thricky, unless the factor corresponds to all or nearly all possible differences between acute and sublethal toxicity indices. Most investigators, however, have published only on acute effects and therefore it remains unknown whether there exists a correlation between the two types of response. When not, the usefulness of the arbitrary application-factor concept is highly questionnable.

Another flaw of short-term tests is that standard procedures which have been developed for easily cultured, so-called 'weed' species usually are not suitable to test important indigenous species. From an ecological point of view, the latter are without doubt more important, especially in the case of point-discharges. Nevertheless, short-term tests using standard-species remain an important tool for fast toxicity-screening and for inter-species comparisons. The application of LC 50's is limited to these purposes and their use in hazard assessment is not at all advocated.

In sublethal tests an array of possible responses can be studied in relation to different toxicants-levels: respiration rates (Bakke & Skjoldal, 1977), colonization ability (Chapman & Long, 1983), alternations of an organisms physiological condition (O:N ratio) (Widdows, 1985), changements in adenylate energy charge and ATP-ase activity (Verschraegen et al., 1985), scope for growth (Widdows, 1985). Two toxicity-criteria, often used, are of major interest in this context: the life-history parameters development time until adulthood and fecundity. They are the key-factors determining a species' potential productivity, and are thus related to the intrinsic rate of natural increase, r_m, which is considered as a measure of fitness (Snell, 1978). The exact determination of r_m requires the construction of the age-specific survival rate (l_x) and age-specific fecundity schedules (m_x), which is a tedious and very time-consuming task. To reduce time and consequently cost of the experiments, we will study the influence of environmental toxicants on daily fecundity and success in reaching adulthood. These criteria are obviously related to m_x and l_x-data. The results obtained from the fecundity and developmental

assay will be compared with those obtained from acute tests.

The nematode species studied, Monhystera disjuncta, is a bacterivorous cosmopolitan species which can easily be cultured under laboratory conditions (Vranken et al., 1984a). The advantages of M. disjuncta for an in vivo toxicological assay have been discussed by Vranken et al. (1984 a&b). Procedures presented are also applicable to other brackish-water nematode species (Vranken et al., in press) and even terrestrial nematodes. The test procedure described by Coomans & Vanderhaeghen (1984) for Caenorhabditis elegans is in fact based on our work on M. disjuncta.

MATERIAL AND METHODS

Monhystera disjuncta has been isolated from the Sluice Dock of Ostend, a marine lagoon near the Belgian North Sea coast. For isolation and simple agnotobiotic maintenance techniques we refer to previous studies(Vranken & Heip, in press and Vranken et al., in press).

Medium

Small vented petri-dishes (35 X 10mm, Falcon) are filled with 4 ml 0.5% sterile bacto-agar (DIFCO) suspension in buffered artificial seawater (ASW) (30°/$_{oo}$S, 5 mM Tris buffer) after Dietrich & 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 stigma sterol (Merck); 0.2 g dehydrosterol (Merck) and 100 ml ethanol. The sterol-mixture is prepared by adding 10 ml of the above mixture to 100 ml distilled water. Hereafter the ethanol is evaporated and the mixture is autoclaved during 20 min. at 1.2 bar.

After the medium has cooled down, a central ring-shaped excavation (Ø : 15 mm) is 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 excavation is filled with ± 0.02 ml of an Alteromonas haloplanktis suspension containing 10^{11}cells/ml.

Food preparation

Erlenmeyer flasks (100 ml) were filled with 50 ml heart infusion broth suspended in artificial seawater (ASW) and than sterilized. The medium is inoculated with the bacterial strain Alt. haloplanktis

under a horizontal laminar air flow bench and incubated during 48 h at room-temperature and rotated in a rotary shaking machine at 125 rpm. Bacterial cells were harvested by centrifugation at 6000 rpm during 15 min. The pellet is resuspended in sterile ASW and added to the cultures.

Toxicity tests

Juvenile stage 2 larvae of M. disjuncta (4.5 days old) were exposed to cadmium($CdCl_2$.2 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 γhexachlorocyclohexane (γHCH, Sigma). For the metals, stock-solutions of 1000 mg/l (as metal-ion) were prepared in 1 liter distilled water using analytical grade salts. Then a series of 100 ml solutions with concentrations ten times higher than the final test-solutions were prepared. 5 ml of each solution is 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 immediately at the desired test-concentrations.

Stock-solutions of PCP and γHCH were prepared by solving 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 TiO_2-waste, two parallel experiments were run. In the first, the waste was added to natural seawater (NSW) and the bacterial cells used as food were grown previously during 48 h in identical waste-concentrations. In the second set, the toxicity of the waste was tested in buffered (5 mM Tris) ASW. For practical purposes, the food was grown in metal-free medium, because at pH = 7.5 to 8, the iron present in the waste-water precipitates as ironhydroxide ($Fe(OH)_3$). This precipitate has a brown colour and hampers observation. Except with the iron-waste assay in ASW, the Alt. haloplanktis suspension (containing ± 10^{11}cells/ml) added to the cultures was grown during 48 h at the same drug doses as used in the tests.

Active J_2-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 4 replicates. After

96 h the number of dead juveniles and adults were counted in all test-cultures. Death is operationally defined as lack of movement after stimulation with a needle. The daily egg-production of at least 10 adult females, obtained from the different test-concentrations was thereafter determined by direct counts. Fecundity assays were run during a period of 96 hours. All experiments were done at 17°C and in the dark.

Statistical analysis.

LC 50 values were calculated by minimum logit Chi-square procedure (Hewlett& Plackett, 1979). After fitting a linear relation between the logit of the mortality response and the $\log_{10}$dose, predicted frequencies were compared with experimental frequencies by goodness of fit analysis (Pearson's Chi-square). When the data were heterogeneous, the variances of both LC 50 values and the slopes were corrected by the heterogeneity factor proposed by Hewlett & Plackett (1979). In the γHCH-test, mortality could not be linearized by logit nor 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 & Rohlf, 1981). Minimum effective concentrations (MEC's) based on mortality and development were estimated by a log likelihood test (G-test, Sokal & Rohlf, 1981). The raw data to determine the MEC's based on development were obtained by counting the number of new adults in all test-cultures when at least 50% of the juveniles reached adulthood in the blanks (after 96 h). The lowest drug concentration at which the first significant difference appears, when compared with the blank, is the MEC. Fecundity-data were examined by ANOVA. MEC's based on this criterion were determined by a posteriori tests by calculating 95% comparison intervals (Sokal & Rohlf, 1981).

RESULTS

Acute Toxicity

Acute toxicity to M. disjuncta of metals, TiO_2-waste, the biocide PCP and the insectide γHCH are presented as LC 50 values (Table 3 &4). The mortality responses at the individual concentrations tested are compiled in Table 2. The responses of M. disjuncta J_2-larvae ranged only over one order of magnitude. Except mercury and copper, none of the agents tested caused substantial mortality at concentrations lower than 10 mg/l. The most toxic metal is copper (96 h LC 50= 2.4 mg/l), followed by mercury (96 h LC50 = 5.6

mg/l). Zinc, cadmium, hexavalent chromium and cobalt are less toxic than the first two. Their LC 50 values ranged between 10 mg/l and 100 mg/l. Nickel is the least toxic metal, with an LC 50 of 103 mg/l. This metal did not have an effect at concentrations lower than 30 mg/l. Acute short-term toxicity testing results in the following rank order of,toxcity: Cu > Hg > Cr > Zn > Cd > Co > Ni. Values of MEC based on juvenile mortality result in the following rank order of toxicity: Cu > Hg > Co > Cr > Zn > Cd > Ni. Except for the position of cobalt, both sequences are the same (Spearman's r_s=0.79; $P < 0.05$; N=7). The concentration of Co which causes a significant increase in mortality is 10 mg/l whereas its LC 50 is 94 mg/l. This illustrates the very smooth dose-response pattern for this particular metal (slope f=3.2; Table 3). For copper a very steep relationship was found between mortality and concentration (slope f=11.8 $\pm$ 1.0; Table 3). This means that deleterious effects caused by Cu are situated in a very small range: between 1 mg/l and 2.5 mg/l. The slopes of the dose-mortality curves of Zn, Hg, Cr and Ni have intermediate and nearly identical values (Table 3).
Acid-iron waste, tested in NSW, did not cause mortality up to a dilution of 0.3 ml waste/ 1 NSW. At 0.4 ml waste/l NSW mortality steeply rose to 90%. This corresponds with a sharp decline in pH : at 0.3 ml waste/l, pH is 6.7, whereas at 0.4 ml waste/l, pH is 5.2, which demonstrates that an increase in acidity significantly influences mortality (F_s=1706; df= 1,10; P<0.001). The 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 water is 100% toxic at a dilution of 0.5 ml waste water/l. The dose-response relation caused by the waste water diluted in NSW is extremely steep. No dose response relationship 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 toxicity of the biocide PCP and the insecticide ɣHCH is comparable to that of the most toxic metals (Cu and Hg). Concentrations of 1 mg/l HCH and 2.5 mg/l PCP did not cause any mortality. The LC 50's of PCP& ɣHCH are respectively 4.8 and 6.7 mg/l. The steepest dose-response relationship was found for the highly toxic PCP. As a result the MEC of ɣPCP as measured by the percentage mortality is somewhat higher than the LC 50.

Developmental assay.
Developmental inhibition of J_2 larvae caused by the metals, acid-iron waste water and the organics is shown in Table 5. Hexavalent chromium, although only intermediately toxic in the acute tsts, is extremely effective in inhibiting development. 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 developmental 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 concentrations which cause a significant developmental inhibition are at least similar (Cu and Hg) and for most metals (Zn, Cd, Cr and Ni) smaller than those found in the acute tests. The ratio's of the MEC's based on mortality and development ranged between 1 and 20. The ratio is maximal for Cr, intermediate for the intermediately and relatively non-toxic metals (Zn, Cd and Ni) and identical to 1 for Cu and Hg.
Similar results were obtained for PCP, ɣ HCH and the acid-iron waste. Lindane inhibits growth at 1mg/l, a concentration 2.5 times less than the acute toxicity threshold.
The toxicity thresholds of acid-iron waste tested in NSW are identical for both development and mortality. As for mortality, developmental rate is influenced by pH, the MEC is 5 times smaller in NSW than in buffered ASW. The EC 100 of the waste water diluted in buffered ASW is more than 25 times higher than in NSW.
The relative toxicity rank order of all substances tested as determined by developmental inhibition only correlates significantly with the rank order obtained using MEC mortality as a criterion.

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. Except for copper, the concentration range tested caused a significant reduction in fecundity (ANOVA, Table 8). At the highest test-level of Cu, a concentration identical to the MEC as measured by mortality, reproductive impairment was only 24%. For copper and mercury MEC's as measured by reduction in egg-production, are identical to the MEC's obtained in the acute and developmental assays. The position of Cu and Hg with regard to their relative toxicity as measured by suppression of fecundity is

intermediate to low. Chromium and zinc are the most toxic metals followed by cadmium. Ni requires, as for the other criteria, the highest concentration to inhibit reproduction. Toxicity ranking of the metals based on reduction of fecundity does not correlate with any of the other criteria studied (Table 9 & 10). For Zn and Ni EC 50's were estimated (Table 7). Their values were almost (Ni) and more (Zn) than one order of magnitude less than the corresponding LC 50 values. For PCP and γHCH 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

The toxicity of heavy metals, acid-iron waste, PCP and γHCH to Monhystera disjuncta, as measured by mortality, developmental inhibition and suppression of fecundity was studied. Relatively high levels of these substances, exceeding 1 mg/l are necessary to cause immediate effects on J_2-larvae of the nematode. Fecundity is the most sensitive criterion for all substances tested. Large discrepancies between the three toxicity-indices were only found for the agents which are intermediately or relatively non-toxic on an acute basis. For the most toxic drugs (Cu, Hg & PCP) on an acute basis, there exists no difference in sensitivity between the three parameters studied. For the metals, development (MEC), correlates with mortality (LC 50 and MEC) when these effects are ranked. For all the substances tested, however, development (MEC's) only correlates with MEC's as determined by mortality. Fecundity does not correlate with mortality (MEC), nor with development. Hexavalent Cr is most toxic, both in the developmental and fecundity-test. Zn and Cd reduce reproduction more strongly than Cu and Hg. Ni is the least toxic metal for all criteria tested. These results are similar to those reported by Reish and Carr (1978) for two polychaetes (Ctenodrilus serratus and Ophryotrocha diadema): Cr, Zn, Cd and Pb were extremely effective in suppressing reproduction, when compared with 96 h acute tests. For Cu and Hg, on the contrary, the difference between the 96 h LC 50 data and reduction of reproduction was rather small. Samoiloff (1980) reported for the nematode Panagrellus redivivus that significant reduction in production

of offspring caused by Cd occurred at concentrations three orders of magnitude lower than the MEC as measured by survival. For Daphnia magna conflicting results are available concerning this aspect of Cu-toxicity. Biesinger & Christensen (1972) and Winner et al.(1977) found only small differences between the two criteria, whereas Blaylock et al.(1985) found considerable differences in Cu-toxicity depending on the criteria studied. For Diplolaimella spec 1 significant reduction in fecundity occurs at levels more than one order of magnitude less than the LC 50, which is highly in variance with the present observations.

Haight et al.(1982) studied the influence of 7 heavy metals on the length-growth and mortality of the free-living terrestrial nematode Panagrellus silusiae. Concentrations necessary to block growth ranged between 50 mg/l for Cu, Cd & Cr and 500 mg/l for Zn, and were considerably higher than the 72 h LC 50 data. Ni and Pb were without effect at the highest soluble concentrations and mercury was either ineffective in blocking growth or lethal. For this species effective growth inhibitory concentrations are considerably higher than those found for M. disjuncta for all metals tested. Samoiloff et al. (1980) present completely different results for the nematode Panagrellus redivivus. For this species growth inhibition is a more sensitive toxicity index than mortality. This also holds for the free-living marine monhysterid Diplolaimella spec 1 (Vranken & Heip, in prep.). The difference between the 2 toxicity-measures was 2 (Cu & Pb) and 1.5 (Hg) orders of magnitude. Vranken et al. (1984 a) studying mercury toxicity to M. disjuncta, found, as did Haight et al. (1982) that the three mercury-compounds tested were either without effect on development or lethal. More or less identical results were obtained by Bogaert et al. (1984) for the nematodes Diplolaimelloides bruciei and Diplolaimella spec 1. Only the percentage of successful moults of stage 4 larvae to adults is reduced at Hg levels which cause no mortality.

Athough relevant information is lacking on the uptake and loss by the nematodes of the substances tested, their bioavailability, their concentration in the food offered and on the complexation capacity of the growth-medium, we can conclude that M. disjuncta is relatively resistant to pollution. Similar high resistance of nematodes, especially to heavy metals is reported in other studies.

Feldmesser & Rebois (1966) reported LC 50 values for mixed populations of Panagrellus and Rhabditis between 53 and 40 mg/l. De Maeseneer (1968) 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 influenced, even in very acid soils. For three plant-parasitic nematodes, Xiphinema diversicaudatum, Aphelenchoides ritzemabosi and Pratylenchus penetrans, the 18 - 24 LC 50's of Cu are respectively 0.1, 4.1 and 2.6 mg/l (Pitcher and Mc Namara, 1972). A rather low LC 50 value for Cu of 0.06 mg/l is given by Hafkenscheid (1971) for the nematode Trichodorus pachydermus. The predatory nematode Mononchus aquaticus remained highly active during the first six hours after exposure to 63.5 ppm Cu (Bilgrami & Jairajpuri, 1984).
Although LC 50 values are only of limited importance in hazard assessment studies, they are a convenient criterion to compare on the hand the relative toxicity of harmful substances and on the other hand the relative susceptibility of different organisms.
In Table 11 the toxicity of heavy metals to M. disjuncta as measured in acute tests is compared with 3 free-living nematodes and with representatives of the major taxonomic groups living in benthic and epibenthic marine faunal assemblages. The ranking of the LC 50 values of M. disjuncta correlates only significantly with Panagrellus silusiae (r_s=0.829; P=0.05). Although copper is most toxic to both species the difference between the two species is slight to 1 order of magnitude. This is readily explained as in seawater the maximum solubility of coppersalts is 0.4 - 0.8 mg/l (Davenport & Redpath, 1984). At higher concentrations, excess copper will be precipitated as malachite (Bengtsson, 1978). For the other metals the differences between the two species range between 6.3 (Cd) and 1.1 (Cr) times. Enoplus communis is much more sensitive than M.disjuncta. The Blyth river population of Enoplus brevis, on the contrary is almost as resistant as M. disjuncta. In contrast, E. brevis, from Budle Bay,a site which is considered as relatively unpolluted, exhibits higher resistance to Zn, Cd & Cu than M. disjuncta. The LC50 values of the enoplids calculated by us are high exposure concentrations (Howell, 1984). At low exposure concentrations E. brevis from the Blyth survives better than Budle Bay animals. Howell (1984) explained this as

an adaptation to environmental realistic concentrations. Perhaps the high resistance of M. disjuncta may have evolved as the result of a similar adaptation as very high metal-levels are reported for sediments in the vincinity of the Sluice Dock (Bouquiaux & Herman, 1977). Monhystera disjuncta is also extremely abundant in heavily polluted sediments of the Southern Bight of the North Sea (Vincx, 1983). The harpacticoid Nitocra spinipes is except for Cu, at least 17 times more sensitive. Whether this can be used as a support for the use of the nematode/ copepod ratio for risk assessment is open to question. However, it proves unequivocally that the harpacticoid probably is the better in vitro bioassay. As similar high sensitiveness of harpacticoids to pollution, especially heavy metal-load, is also observed in the highly polluted Westerschelde estuary (Van Damme et al., 1984), the use of harpacticoids as bioindicators in survey studies merits more consideratiion. Further the interspecies comparison revealed high differences between on the one hand, larvae of Carcinus and Crassostrea and on the other, M. disjuncta with the former the most sensitive. The differences with the polychaete Nereis diversicolor, except for Cu and Hg, are rather small.
The growth-medium used is relatively poor in nutrients (Vranken et al., in press). The most active binding substances present in standard microbial growth media (Ramamoorthy & Kushner, 1975) are not included in our medium. Therefore the low toxicity of heavy metals and other compounds tested to M. disjuncta is in our opinion indicative for the general resistance exhibited by this particular nematode feeding-type to effects of pollution. This conclusion is corroborated by field-studies, which have shown that nematode-density is not effected substantially by raw domestic sewage (Vidakovic, 1983), heavy metals (Tietjen, 1977, 1980) and acid-iron waste (Lorenzen, 1974).
Ernst (1984) reviewed the toxicity of pesticides and organic chemicals to marine organisms. For ɣHCH, 96h LC50 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. Crangon crangon

Palaemon elegans and the blue mussel Mytilus edulis exhibit similar or higher resistance to PCP than M. disjuncta. In the present assays, PCP and γHCH, were solved in acetone before adding to the test-medium. The concentration of acetone at the highest exposure-concentration of both PCP and γHCH is about 40 mg/l. Although acetone toxicity to M. disjuncta is not tested, there is good reason to believe that such acetone concentrations are without effect: 18h LC50's of acetone are higher than 1 g/l (Bouwman et al., 1981), for example the 96h LC50 of acetone to Nitocra spinipes is 16.7 g/l (Lindén et al., 1979).
At a temperature of 17°C and in NSW of about 30°/$_{oo}$S, the LC50 value of the acid-iron waste tested (pH=1), which contains about 20% sulphuric acid and 2% iron (Roekens and Van Grieken, 1983), is 0.036% (pH=5.7; Fe= ± 7mg/l). At such a dilution Cr is the only metal present at a relatively high concentration of 120 µg/l. The other metal-constituents probably occur at levels far below their MEC : 14 µg/l Zn, 1 µg/l Cu, 1 µg/lPb and about 40 ng/l of both Cd & Hg (simplified after Pickaver, 1981). Acidification might of course increase metal-toxicity. In our opinion it is only the sulphuric acid component of the effluent which is harmful. To test this hypothesis pure sulphuric acid will be tested in a future-experiment. Possible synergistic interactions between the metal-constituents can then be evaluated. Under such test-conditions, a dilution of 1/500 (Fe concentration= 40 mg/l) caused developmental inhibition. After dumping in the sea the iron present in the effluent is oxidized and precipitated. The metals are coprecipitated forming mainly complexes with Fe and Ti (Lehtinen et al., 1984), which probably reduce their toxicity. The acid discharge is completely neutralized within less than 1h (Roekens & Van Grieken, 1983). After 20 min. the waste is 80,000 times diluted which is 160 times higher than the developmental threshold against M. disjuncta, and 80 times higher than the MEC as measured by fecundity. In the sea, the reduction in pH is only limited to the first seconds after discharge (after 1 min : pH>6). Therefore the assay done in buffered ASW is probably more useful for risk-evaluation of acid-iron waste with regard to nematodes. Newell et al. (1983) studied the benthos-community in the vincinity of TiO_2 outfall and

they found no evidence of faunal impoverishment although some stations were poor in meiofauna, but the differences were not considered significant in view of meiofaunal variability in numbers. However, Huys et al. (1984) report a significant decrease in taxonomic diversity in dumping areas of the Dutch coastal water. Temperature also seems to have a significant impact on the acid-waste toxicity. Lehtinen et al. (1984) reported at 21°C and 7°/$_{oo}$ salinity a 96h LC50 to adult Nitocra spinipes of 0.013% of the undiluted effluent, whereas at 4°C, 0.09% of the undiluted effluent results in 50% mortality. The results of their 13 day fecundity test (EC50=0.024 to 0.033% of the undiluted waste-water) can not be compared with the data of the Monhystera disjuncta fecundity assay because in the present work the egg-production is studied under buffered conditions. We suppose however that under natural conditions the effect of TiO_2 outfall have only a minor effect on the viability of M. disjuncta. In conclusion we may say that environmental toxicants that reduce the nematodes' viability at levels occurring in nature have to be considered extremely dangerous, since M. disjuncta is a relatively resistant species.

ACKNOWLEDGEMENTS
This research is conducted under contract N° ENV-767-B of the Environmental Programme of the CEC and supported through the Concerted Actions Oceanography Project of the Ministry of Scientific Policy. C. Heip acknowledges a grant of the Belgian National Science Foundation (NFWO).

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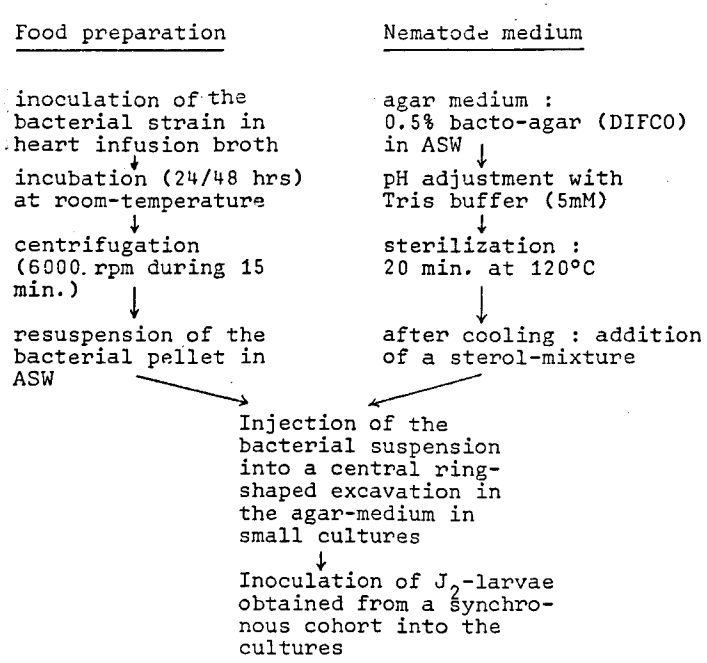
Table 1. Diagram of the experimental procedure used in the tests.



sterol-mixture: for composition see text

Table 2 : Percentage mortality (in parenthesis) after 96 hours at different metal/toxicant concentrations (mg/L) of 4.5 days old individuals of Monhystera disjuncta : the G/q statistic and an unplanned test : responses of underscored concentrations are homogeneous .
NSW : natural seawater ; ASW : artificial seawater

Metal/Toxicant	G/q	concentration and % mortality (in parenthesis)							
Zn^{2+}	825***	0.75(0)	5(0)	0(0)	1(4)	10(4)	20(37)	30(67)	50(86)	70(99)
Cd^{2+}	57***	0(0)	1(0)	5(0)	10(2)	25(22)				
Cu^{2+}	779***	0(0)	0.75(1)	1(2)	1.75(10)	2.5(60)	5(97.5)	10(100)		
Hg^{2+}	538***	0.5(0)	0(0)	1(0)	2.5(8)	5(36)	10(81)	7.5(82)		
Co^{2+}	936***	0(0)	1(0)	3(0)	6(0)	10(6)	200(65)	300(94)	400(100)	
Cr^{6+}	427***	0(0)	0.5(0)	0.75(0)	1(0)	5(0)	10(3)	15(13)	20(71)	30(71)
Ni^{2+}	284***	0(0)	5(0)	15(0)	25(0)	35(0)	50(7)	70(21)	90(44)	110(51)
PCP	866***	0(0)	0.5(0)	1(0)	2.5(0)	5(60)	7.5(97)	10(100)		
γ-HCH	497***	0(0)	0.1(0)	0.25(0)	0.5(0)	0.75(0)	1(0)	2.5(40)	5(37.5)	7.5(50)
TiO_2-waste (NSW) °	812***	0.3(0)	0.1(0)	0.2(0)	0(0)	0.4(90)	0.5(100)			
TiO_2-waste (ASW+buffer) °		no mortality; highest concentration tested : 10 ml waste / L medium								

° : concentrations in ml waste / L medium

Table 3 : Minimum logit chi - square analysis : regression of the logit of the mortality response (l) against the logarithm of the concentration (C) : $l = t + \beta \log C$; t : intercept ; β : slope ; m : 96 h LC 50 in mg / L ; SE : standard error ; CI : confidence interval ; χ^2 : Pearson's chi - square for goodness of fit .

Metal/Toxicant	t	f (±SE)	m (95 % CI)	Pearson's χ^2
Zn^{2+}	-9.70	6.97(0.63)	24.6(22.7-26.6)	7.1($d\beta$=5; NS)
Cu^{2+}	-4.47	11.78(1.00)	2.4(2.2- 2.5)	6.6($d\beta$=4; NS)
Hg^{2+}	-5.30	7.06(0.98)*	5.6(5.0- 6.4)*	10.3($d\beta$=4;0.025<P<0.05)
Co^{2+}	-6.27	3.18(0.81)*	93.5(49 - 180)*	34.8($d\beta$=5;P<0.001)
Cr^{6+}	-11.38	8.64(2.09)*	20.7(17.5-24.6)*	28.4($d\beta$=6;P<0.001)
Ni^{2+}	-15.70	7.79(1.12)	103.3(94.4-113.1)	5.7($d\beta$=6; NS)
PCP	-11.77	17.37(2.25)	4.8(4.5- 5.0)	1.4($d\beta$=4; NS)
TiO_2-waste (NSW)	22.15	50.47(8.88)	0.36(0.35-0.38)°	2.0($d\beta$=3; NS)

° : ml waste / L medium

Table 4 : Regression of mortality (arc sin $\sqrt{\text{proportion}}$) against the logarithms of the γ-HCH concentration .
a : intercept ; b : slope (+ SE) ; R^2 : coëfficient of determination ; P : evaluates the significance of regression (F - test)

Metal/Toxicant	a	b	LC 50 (95 % CI)	R^2	P
γ- HCH	7.52	45.24(5.47)	6.7(2.6 - 19.1)	0.97	P<0.001

Table 5 : Percentage maturation (in parenthesis) after 96 hours at different metal / toxicant concentrations (mg/L) of 4.5 days old individuals of Monhystera disjuncta ; the G/q statistic and an unplanned test : responses of underscored concentrations are homogeneous.
NSW : natural seawater ; ASW : artificial seawater

Metal/Toxicant	G/q	concentration and % maturation (in parenthesis)
Zn^{2+}	1024***	0(91) 0.75(97) 1(91) 5(60.5) 10(2.5) 20(0) 30(0) 50(0) 70(0)
Cd^{2+} ⊕	194***	1(69) 5(69) 0(67) 10(11) 25(0)
Cu^{2+}	331***	0(61) 1(54) 1.75(42) 0.75(40) 2.5(0) 5(0) 10(0)
Hg^{2+}	584***	0.5(87) 0(85) 1(82) 2.5(24) 5(10) 7.5(1) 10(0)
Cr^{6+}	892***	0(97) 0.5(96) 0.75(79) 1(74) 5(0) 10(0) 15(0) 20(0) 30(0)
Ni^{2+}	680***	0(91) 5(87) 15(63) 25(39) 35(16) 50(0) 70(0) 90(0) 110(0)
PCP	703***	0.5(94) 0(92) 1(91) 2.5(87) 5(24) 7.5(0) 10(0)
γ-HCH	1066***	0.1(96) 0(95) 0.25(93) 0.75(88) 0.5(87) 1(73) 2.5(16) 5(0) 7.5(0)
TiO_2-waste (NSW) °	692***	0.3(94) 0.1(93) 0.2(93) 0(89) 0.4(0) 0.5(0)
TiO_2-waste (ASW+buffer) °	101***	0.1(92) 0(89) 1(88) 2(70) 10(43)

° : concentrations in ml waste / L medium
⊕ : experimental time period : 120 hours

Table 6 : Monhystera disjuncta : daily fecundity per female during the first 96 hours of the fertile life - period at different levels of metals / toxicants ; CI : confidence intervals ; Comp I : comparison intervals : non-overlapping intervals include significantly different fecundities ; Exp : experimental ; Est : estimated (when not specified experimental values are given) .

Metal/Toxicant	Concentration (mg/L)	Daily fecundity	95% CI	95% Comp I
Zn^{2+}	control	62.1	53.7 - 70.5	60.9 - 63.3
	0.75	47.4	44.8 - 50.0	46.2 - 48.6
	1	49.6	44.2 - 55.0	48.4 - 50.8
	5	5.0	3.4 - 6.6	3.8 - 6.2
Cu^{2+}	control	43.2		
	0.75	34.1		
	1	43.8		
	1.75	33.0		
Hg^{2+}	control	45.4	34.3 - 56.5	39.4 - 51.4
	0.5	35.1	28.2 - 42.0	29.1 - 41.1
	1	41.1	30.9 - 51.3	35.1 - 47.1
	2.5	32.0	24.5 - 39.5	26.0 - 38.0
Cr^{6+}	control	52.4	39.0 - 65.8	46.7 - 58.1
	0.5	44.5	34.4 - 54.7	38.9 - 50.2
	0.75	30.7	23.5 - 37.8	25.0 - 36.3
	1	36.9	25.7 - 48.1	31.3 - 42.6
Ni^{2+}		Exp. Est.	Est.(95% CI)	
	control	65.7 65.7	-	
	5	65.3 65.2	59.2 - 65.7	
	15	19.8 31.4	12.2 - 51.7	
	25	5.8 5.4	1.1 - 19.2	
	35	1.4 1.1	0.1 - 7.3	
PCP	control °	65.8	59.5 - 72.2	59.9 - 71.8
	0.5 °	58.7	48.8 - 68.5	52.7 - 64.6
	1 °	44.0	35.9 - 52.1	38.1 - 50.0
	5 °	10.2	2.1 - 18.3	4.2 - 16.1
PCP	control •	44.1	30.6 - 57.6	33.9 - 54.3
	0.5 •	49.9	33.9 - 65.9	39.7 - 60.1
	1 •	34.0	19.7 - 48.3	23.8 - 44.2
	5 •	9.6	0.0 - 21.5	0.0 - 19.8
γ-HCH	control	60.4	47.4 - 73.3	48.8 - 72.0
	0.5	44.4	31.8 - 57.0	32.8 - 56.0
	1	33.5	15.2 - 51.9	21.9 - 45.2
	2.5	26.2	4.4 - 47.9	11.2 - 41.2

° : first 96 h period of the fertile life-period

• : second 96 h period of the fertile life-period

Table 7 : 96 h minimum effect concentrations (MEC : concentrations at which the first significant differences, compared with the blanks, appear) of different metals / toxicants obtained from mortality responses, a developmental assay and fecundity suppression.

Metal/Toxicant	Criterion		
	Mortality (MEC : mg/L)	Development (MEC : mg/L)	Fecundity (MEC : mg/L)
Zn^{2+}	20	5	0.75
Cd^{2+}	25	10	1 *
Cu^{2+}	1.75	1.75	1.75
Hg^{2+}	2.5	2.5	2.5
Co^{2+}	10	-	-
Cr^{6+}	15	0.75	0.75/1
Ni^{2+}	50	15	15
PCP	5	5	1/5
γ-HCH	2.5	1	1
TiO_2-waste ° (NSW)	0.4	0.4	-
TiO_2-waste ° (ASW+buffer)	>10	2	1 +

* : Boffé (pers. comm.)

° : in ml waste / L medium

+ : Verschraegen (pers. comm.)

NSW : natural seawater

ASW : artificial seawater

Table 8 : Influence of metals / toxicants, examined by ANOVA (F_s - statistic), on fecundity during the first 96 hours of the fertile life - period . P : significance level ; EC 50 (mg/L) : effective concentration reducing fecundity with 50 % when compared with the blank ; df : degrees of freedom ; NS : not significant .

Metal/Toxicant	ANOVA : F_s (df)	P	EC 50 (95% CI) (mg/L)
Zn^{2+}	3661 (3,4)	P<0.001	1.9 (0.8-4.3)
Cu^{2+}	0.8 (1,2)	NS	>1.75
Hg^{2+}	4.4 (3,12)	0.025<P<0.05	>2.5
Cr^{6+}	15.0 (3,8)	0.001<P<0.005	>1
Ni^{2+}	117 (1,2)	0.005<P<0.01	15 (7.0-28.8)
PCP			
first period	70 (3,16)	P<0.001	2.1 (0.5-7.0)
second period	12.5 (3,16)	P<0.001	-
γ-HCH	6.6 (3,14)	0.005<P<0.01	1.6 (0.6-4.9)

Table 9 : Relative toxicities of metals to Monhystera disjuncta as measured by different toxicity criteria.
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 10 : Correlation between the relative toxicity rank order of metals to Monhystera disjuncta as measured by different toxicity indices : LC 50 ; MEC (M) ; MEC (D) and MEC (F) .

Criteria	Spearman's r_s	P	N
LC 50 / MEC(M)	0.786	P 0.05	7
LC 50 / MEC(D)	0.829	P=0.05	6
MEC(M) / MEC(D)	0.829	P=0.05	6
LC 50 / MEC(F)	0.116	NS	6
MEC(M) / MEC(F)	0.116	NS	6
MEC(D) / MEC(F)	0.464	NS	6

Table 11 : Toxicity of heavy metals to Monhystera disjuncta (mg / L), measured as 96 h LC 50 's in comparison to other organisms.

Metals	Monhystera disjuncta	Panagrellus silusiae	Enoplus communis	Enoplus brevis		Nitocra spinipes	Carcinus maenas	Nereis diversicolor	Crassostrea virginica
	96 h LC 50 (J2-larvae)	72 h LC 50 (J2-larvae)	96 h LC 50* B.B.	96 h LC 50* B.R.	B.B.	96 h LC 50 (A)	48 h LC 50 (L)	192 h LC 50*	48 LC 50 (L)
Zn^{2+}	24.6	20.0	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+}

L : larvae ; A : adults ; B.B. : Budle Bay (unpolluted) ; B.R. : Blyth River (polluted).

References : P.silusiae in Haight et al. (1982) ; E.communis & E.brevis in Howell (1984) ; N.spinipes in Bengtsson (1978) ; C.maenas in Connor (1972) ; N.diversicolor in Bryan (1980) ; C.virginica in Calabrese et al (1973).