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TOXICITY OF COPPER, MERCURY AND LEAD TO A MARINE NEMATODE

G. VRANKEN & C. HEIP

Marine Biology Section, Zoology Institute, State University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Toxicity of copper, lead and mercury to the nematode Diplolaimella spec 1 is studied. Mortality responses obtained demonstrate high resistance to heavy metals. Population growth parameters as the intrinsic rate of natural increase and net-reproductivity are significantly depressed at copper-concentrations which cause no juvenile mortality. The lowest concentrations tested caused significant inhibition of development rate in both sexes. For this particular nematode species suppression of fecundity and developmental inhibition are more reliable criteria, determining non-exceedable limits with regard to environmental safety. Our tests show that nematode productivity may be significantly depressed at copper levels found in some areas of the North Sea.

INTRODUCTION

Marine nematodes are the most abundant animals in marine sediments and have several features that are advantageous in ecotoxicological research in the laboratory : they have a short life-span (Vranken & Heip, in press), a high fecundity (Vranken & Heip, 1983), represent several trophic levels (herbivores, bacterial feeders and carnivores) and at least some species are easily cultured (Heip et al., 1985). However, the effect of only a very limited set of chemical agents to only a few species has been tested up till now (Bogaert et al., 1984; Howell, 1984; Vranken et al., 1984b). From these few experiments it appears that nematodes are relatively resistant to pollutants. This is corroborated by evidence from field surveys which have shown that nematode density is not affected substantially by raw domestic sewage (Vidakovic, 1983), heavy metals (Tietjen, 1977, 1980) and acid iron waste (Lorenzen, 1974).

In this paper we present results from tests on the species Diplolaimella spec 1, a new species described by Jacobs & Vranken (in prep.) and misidentified as Monhystera microphthalma in previous papers. First, a series of acute (static) and sublethal (chronic) tests on the toxicity of copper, mercury and lead is described, in which both mortality and development rate are studied simultaneously. Nematodes moult four times before becoming adult and the success in reaching a particular stage has been proposed as a sensitive indicator of toxicity (Samoiloff et al., 1980). We used a slightly different approach and have determined generation time and preadult mortality as a function of metal concentration. These tests are presented as a supplement to the few data available and are intended to check whether the resistance of nematodes to toxicants is indeed true. Second, we will also report on sublethal effects of copper on fecundity, because mortality is not a

very sensitive index of toxicity. Fecundity, the number of eggs produced, has been used because it is known to be a sensitive criterion in toxicity testing (Bryan, 1980; Reish & Carr, 1978).

As a combination of fecundity and development rate (generation time) the intrinsic rate of natural increase r_m of the population can be calculated. This has been done by Tietjen & Lee (1984) to measure the impact of PCB's, PAH's and heavy metals on the nematodes Chromadorina germanica and Diplolaimella punicea. Because r_m values relate also to the productivity of the population, EC-50 values, defined as the concentration at which there is a 50 % inhibitory effect on population growth, are excellent tools to predict the environmental impact of toxicants (Hummon & Hummon, 1975; Sabatini & Marcotte, 1983; Vernberg & Coull, 1981). For several nematodes, a good correlation between r_m values and the rate of increase in the field has been found (Heip et al., 1978; Smol et al., 1980; Romeyn et al., 1983). Since nematodes are part of the diet of macrofauna, some fish and commercial crustaceans such as Crangon crangon, and since a significant part of the energy flow through benthic systems passes through the nematodes (Platt & Warwick, 1980),any factor influencing nematode productivity may be of more global importance.

MATERIAL AND METHODS

The species Diplolaimella spec 1, previously misidentified as Monhystera microphthalma, is a new species not as yet described. It was sampled in the Dievengat, a polyhaline water pond, situated near the Nature Reserve 'het Zwin' in north-western Belgium. Adults are about 1 mm long and weight circa 0.5 µg wet weight. Stock-cultures were maintained agnotobiotically on 0.5 % bacto-agar (DIFCO) enriched with 1 % Vlasblom-medium (Vranken et al., 1984a) and 1 % silicate

($Na_2SiO_3.9H_2O$; 0.053 M stock-solution). Stock-cultures were kept in vented petri-dishes of 5 cm diameter.

Copper ($CuSO_4.5H_2O$), lead ($Pb(C_2H_3O_2)_2.3H_2O$) and mercury (Hg_2Cl_2) toxicity was studied using eggs, juveniles and pooled pre-adult mortality as criteria. Experiments were performed in the dark in vented petri-dishes (Ø : 3.5 cm) at 20°C, 20°/₀₀ salinity and an initial pH = 7.5. Salinity was measured with a refractometer. The medium used for the experiments consisted out 0.5 % bacto-agar (DIFCO) made in Dietrich & Kalle (1957) seawater, enriched with 1 % Walne-Provasoli medium, plus 1 % amino-acids solution with glucose enrichment and 0.5 % silicate (see Vranken et al., 1984a for details). At each concentration 5 replicate experiments were run in which 125 gravid females were allowed to deposit eggs for 1 day. An unidentified bacterial mixture grown separately on bottoms free from metals was given as food. The number of eggs studied, varied between 268 and 348 for copper, 248 and 427 for mercury and 248 and 407 for lead.

Fecundity was studied under similar conditions as the lethal tests. At copper concentrations of 100 µg/l and 1 mg/l, 60 females and males which matured in the previously done acute tests, were divided at random over 5 replicates. Every 6 days eggs were counted and adults transferred to fresh cultures. At 500 µg/l copper no mortality-assay was performed. The adults (60 ♀♀ and ♂♂) for the fecundity-test at this concentration were taken from the 100 µg/l experiment.

Population growth r_m was calculated with Lotka's equation :

$$\sum_{x=0}^{\infty} e^{r_m x}.U_x = 1 \qquad (1)$$

U_x is the number of female-newborns produced per female of the preceeding generation, when that generation is in the age-interval (x, x+1). U_x is calculated as $U_x = Ne_x.p.l_x$, where Ne_x is fecundity of a female aged x, p the proportion females in the population (= 0.5) and

l_x the survival probability form the egg-stage onwards until age x. Net-reproductivity R_o, the multiplication rate per generation is calculated as :

$$R_o = \sum_{x=o}^{\infty} U_x \qquad (2).$$

Mean generation time T is determined as $T = \ln R_o / r_m$ (3). Minimum generation time, T_{min} is estimated as the period between identical stages of successive generations, equalling approximately the sum of embryonic and postembryonic time.

RESULTS

Acute toxicity tests

Copper (Table 1)

The lowest concentration inducing a significantly different hatchability ($P = 0.05$) whem compared with the blank (= MEC : minimum effective concentration) is 100 µg/l. For the juveniles MEC is 10 mg/l and for the pooled pre-adult mortality MEC is 100 µg/l. At 50 mg/l Cu, 5 eggs were deposited; none of these hatched.

Mercury (Table 1)

For both egg- and pre-adult mortality, MEC = 100 µg/l. At 1 mg/l 0.3 % of the juveniles did not reach adulthood. For the juveniles, MEC = 5 mg/l. At 10 mg/l pre-adult mortality is 42 % : 31 % of the eggs did not hatch and 16 % of the juveniles died before reaching maturity.

Lead (Table 1)

The MEC for non-hatching and pre-adult mortality is 5 mg/l. For juvenile mortality MEC is 10 mg/l.

Developmental assay

Development time prolongs significantly ($P < 0.001$; NESTED ANOVA) with increasing metal concentration (Table 2). The smallest levels tested caused a significant lengthening for the three metals. Developmental retardation of both sexes, with an exception at 20 µg/l Cu ($0.001 < P < 0.01$; ANOVA) is similar. Development time at the highest Cu level is significantly longer when compared with the other two metals.

Fecundity and demographic analysis

Egg-production is significantly influenced ($0.025 < P < 0.05$; ANOVA) in the range tested. Maximum production was reached after 31 days. Total fecundity drops from 147 eggs per female in the blank to 97 at 1 mg/l Cu (Table 3). Daily egg-production at 0.5 mg/l and 1 mg/l is significantly less ($P = 0.05$) when compared with the blank (Table 3). Both net-reproductivity ($0.005 < P < 0.01$; ANOVA) and r_m ($0.025 < P < 0.05$; ANOVA) decreased significantly with increasing concentrations. r_m decreased 9.7 % at 100 µg/l and 29 % at 1 mg/l Cu. At 1 mg/l this corresponds with a 43 % decrease in net-reproductivity (Table 4).

DISCUSSION

Three different responses : mortality during the pre-adult stages, development retardation and suppression of fecundity, were studied. For the first two responses a physiological standard, development time, was choosen as the duration of the experiment. As generation time increases with metal concentration (Table 2), exposure time in our experiments increases simultaneously. Using mortality as a toxicity ranking criterion, Diplolaimella spec 1, appears rather insensitive,

even when compared with larger marine invertebrates such as polychaetes (Reish, 1984), crustaceans (Ahsanullah et al., 1981) and bivalve molluscs (Bryan, 1984). For copper an LC 50 of 12 mg/l (95 % CI : 10.4-13.7), exceeding considerably its seawater solubility, was calculated from the pooled pre-adult mortality with the Reed-Muench method (Woolf, 1968). For mercury (I) the LC 50 is close to 10 mg/l and for lead it is higher than 10 mg/l, a value also more than 1 order higher than its maximal solubility. These figures are in contrast with those presented by Howell (1984) for two large free-living marine omnivorous/predacious nematodes, Enoplus brevis and E. communis. For a similar exposure duration as in our experiments (312 to 430 h) the LC 50's of Cu, Pb and Hg(II) of Enoplus brevis, the most resistant of the two enoplids, lies somewhere between 10 and 100 µg/l. Howell (1984), however did not mention whether he fed his animals during exposure. For other organisms such as Daphnia magna metal-toxicity drops by adding food (Biesinger and Christensen, 1972). We fed our animals on bacteria previously grown on bottoms without metals. This may reduce toxicity as it is known that some bacteria accumulate metals (Patrick & Loutit, 1976; Doyle et al., 1975; Berk & Colwell, 1981). The uptake of other bacteria, on the contrary, like Escherichia coli (Doyle et al., 1975) and gutbacteria of the nematode Mesorhabditis monhystera (Doelman et al., 1984) is very limited. In additional experiments with the closely related nematode species Monhystera disjuncta we could enhance mercury (II) toxicity when juveniles were fed E. coli cells previously exposed to 2.5 mg/l Hg(II). Mortality in this experiment was 56 %. When mercury was only added to the substratum mortality was 6 %. Hence it is clear that microbial activity influences toxicity in the Diplolaimella assays and contributes almost certainly to the discrepancies found with the Enoplus assay.

Several studies indicate that the free metal ion is the toxic species

(see Borgmann, 1983; Moore & Ramamoorthy, 1984 for a review). Canterford & Canterford (1980) have demonstrated this with the marine diatom Ditylum brightwellii : Cu, Zn, Cd & Pb toxicity decreased as the concentration of EDTA, a strong chelating agent, increased. Mercury-toxicity, on the other hand was independent of EDTA, which is expected as in seawater Hg(II) froms strong covalent compounds with chloride ions (Moore & Ramamoorthy, 1984). In freshwater mercury complexation by EDTA lowers its toxicity (Whitton, 1967). The medium used to culture Diplolaimella spec 1 is rich in substances, such as phosphates, nitrates and organics like amino-acids, vitamins and sodium EDTA (Vranken et al., 1984a) which bind a reduce toxicity of heavy metals (Ramamoorthy & Kushner, 1975; Knezovich et al., 1981). With the mussel Mytilus edulis, on the contrary, prior complexation of cadmium enhances its uptake (George & Coombs, 1977) and therefore probably its toxicity. Concerning the uptake of dissolved organics by nematodes, conflicting results exist in the literature. Two predacious/omnivorous nematodes Pontonema vulgaris (Chia & Warwick, 1969) and Adoncholaimus thalassophygas (Lopez et al., 1979) are able to assimilate labeled glucose, whereas the bactivorous Pellioditis marina is not capable of doing it (Tietjen & Lee, 1975). Howell (1983) suggested that, besides food intake, a significant route of metal uptake is via the cuticle. Without having quantitative knowledge on the influence of complexation on metal-toxicity to nematodes and without knowing the exact route of metal-uptake we cannot assess the importance of these factors in relation to the low toxicity-levels observed. Nevertheless we may conclude that Diplolaimella spec 1 is a rather insensitive species. Experimental evidence exists that this also holds for other nematode species (Pitcher & McNamara, 1972; Samoiloff et al., 1980; Haight et al., 1982). Field observations again corroborate this statement as in highly polluted sediments in the North Sea, representatives

of the same feeding-type as Diplolaimella spec 1 become extremely dominant (Heip et al., 1984).

Compared with mortality, development inhibition is a much more sensitive toxicity index for Diplolaimella spec 1, which is similar to what Samoiloff et al. (1980) reported for Panagrellus redivivus. Data obtained by Haight et al. (1982) for P. silusiae are in variance with the previous observations. The effective concentrations inhibiting growth of P. silusiae were much higher than the LC 50's. Vranken et al. (1984b) studying mercury toxicity to Monhystera disjuncta noticed an 'all-or-none' response : some individuals developed as fast as those in the blank, whereas the others died very quickly. So far it seems advisable to supplement developmental tests by other short-term assays. A possible alternative is to count the numbers reaching adulthood in a fixed period of time, e.g. when at least 50 % of the individuals matured in the blank. These figures are typical for two different responses : mortality and developmental inhibition. When using this as measure we obtain EC 50's (concentrations which induce a 50 % maturation inhibition when compared with the blank) of 28 µg/l copper, 93 µg/l mercury (I) and 60 µg/l lead.

For Diplolaimella spec 1 significant reduction in fecundity occurs at levels more than one order of magnitude less than the LC 50, e.g. for the net-reproductivity the EC 50 is 1.4 mg/l Cu^{2+} (95 % CI : 0.7-3.0 mg/l Cu^{2+}). Reish & Carr (1978) found for two polychaetes differences of the same magnitude between these two types of tests, except for copper and mercury where the differences were rather small. Analogous small differences were obtained by Biesinger & Christensen (1972) and Winner et al. (1977) for Daphnia magna. For this species the 72 h LC 50 value of copper was not much different from the concentration which inhibited the intrinsic rate of natural increase. This is highly in contrast with our findings. Others (Blaylock et al,

1985) working with Daphnia magna, under copper stress also, observed large differences between mortality and reproduction, with the latter the most sensitive biological endpoint. Similar results were obtained by Moraitou-Apostolopoulou & Verriopoulos (1979) for the marine copepod Acartia clausi again under copper stress.

When pre-adult mortality is not too high, r_m approximates daily weight-specific productivity, namely the production-biomass ratio (P/B) (Vranken & Heip, in press). Mean annual temperature during the yearly growth period of Diplolaimella spec 1 is about 16° C. At this temperature daily P/B equals 0.112 Joule.Joule^{-1}.day^{-1}, resulting in a yearly P/B of approximately 22. At a Cu concentration of 1 mg/l, a level found in some sediments of the Belgian coastal area of the North Sea (Heip et al., 1984), yearly P/B reduces to 15.5. Consequently it is clear that heavy-metal load may lower the productivity of bottom-dwelling organisms such as nematodes.

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Table 1. Diplolaimella spec. 1. Egg mortality (e), juvenile mortality (j) and preadult mortality in percentages at different metal concentrations (mg/l)

metal	significance (G/q test)	life stage	concentration (mg/l) 0	0.1	1	5	10	50
copper	***	e	3	12*	17	-	25	100
	***	j	1	0	0	-	10*	
	***	preadult	3	12*	17	-	33	100
mercury	***	e	3	10*	11	15	31	
	***	j	0	0	0	5	16	
	***	preadult	3	10*	11	19	42	
lead	***	e	3	5	8	10*	17	
	***	j	0	0	0	0	9*	
	***	preadult	3	5	8	10*	25	

*** : $P < 0.001$

* : MEC-value ($P \leq 0.05$)

\- : not tested

Table 2. Diplolaimella spec. 1. Development time (T_{min}) in days of females and males with 95 % confidence interval (CI) in parantheses at different copper, mercury and lead levels; N = number of individuals studied, the concentrations are in mg/l

		T_{min}			
	metal (mg/l)	♀♀	N	♂♂	N
copper	0	12.9 (± 0.23)	156	12.8 (± 0.20)	180
	0.02	14.0 (± 0.27)	114	14.6 (± 0.22)	131
	0.04	15.3 (± 0.28)	121	15.4 (± 0.29)	134
	0.06	14.8 (± 0.23)	142	15.0 (± 0.22)	149
	0.1	14.8 (± 0.24)	120	14.8 (± 0.21)	121
	1	17.0 (± 0.42)	107	17.0 (± 0.46)	116
	10	17.6 (± 0.51)	102	17.9 (± 0.44)	114
mercury	0	12.7 (± 0.19)	213	13.0 (± 0.22)	181
	0.1	14.3 (± 0.29)	126	14.2 (± 0.24)	140
	1	15.2 (± 0.28)	186	15.1 (± 0.25)	194
	5	15.0 (± 0.35)	109	15.2 (± 0.37)	119
	10	16.1 (± 0.61)	69	16.3 (± 0.60)	75
lead	0	12.7 (± 0.19)	213	13.0 (± 0.22)	181
	0.1	14.3 (± 0.25)	183	14.0 (± 0.23)	195
	1	14.5 (± 0.32)	123	14.6 (± 0.29)	129
	5	15.0 (± 0.39)	115	15.3 (± 0.33)	146
	10	16.3 (± 0.44)	90	16.2 (± 0.47)	96

Table 3. Diplolaimella spec. 1. Total and daily egg-production per female at different copper concentrations (mg/l); R^2 = coefficient of determination; in parantheses : ± 95 % confidence limit, n = number of observations

Cu	Total egg-production	Daily egg-production	R^2	n
0	147	4.9 (± 0.7)	0.99	6
0.1	132	4.2 (± 0.9)	0.98	6
0.5	112	3.8 (± 0.9)	0.97	6
1	97	3.5 (± 1.0)	0.96	6

Table 4. Diplolaimella spec. 1 : demographic characteristics at different copper concentrations (mg/l)

copper	Ro	estimated Ro ± 95 % CI	r_m (day^{-1})	estimated r_m ± 95 % CI	T (days)	estimated T ± 95 % CI
control	71	70 ± 7.0	0.186	0.181 (0.158-0.207)	23	23.1 ± 2.6
0.1	58	60 ± 4.7	0.168	0.176 (0.156-0.198)	24	23.6 ± 2.3
0.5	50	49 ± 4.8	0.159	0.155 (0.141-0.171)	25	25.4 ± 1.9
1	41	41 ± 6.9	0.132	0.133 (0.112-0.158)	28	27.7 ± 3.3