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Antagonistic and Synergistic Toxic Effects of Pb and Cd in a Simple Foodchain: Nematodes Feeding on Bacteria or Fungi

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Soil pollution with heavy metals may affect the functioning of the soil biota by inhibiting the decomposition of organic matter (Doelman and Haanstra 1979) and may also influence foodchains. This paper presents the results of an investigation how the reproduction of soil nematodes can be influenced by feeding on bacteria or fungi contaminated with lead and cadmium.

MATERIALS AND METHODS

The nematodes Mesorhabditus monhystera (Ordo Rhabditida, Goodey 1963) and Aphelenchus avenae (Ordo Tylenchidae, Goodey 1963) were supplied by the department of Nematology, Agricultural University in Wageningen. M. monhystera was reared on a mixed culture of gutbacteria, isolated from this species. A. avenae was reared on mycelium of the fungus Alternaria solani or Alternaria porri.

The bacteria, as food for M. monhystera, were grown in liquid medium (yeast extract 1%; glucose 1%; pH 6) at 25°C. The washed (3 times in demineralized water) bacteria suspensions were incubated for 2.5 hours in salt solutions of 0, 20, 200, 1000 $\mu g.g^{-1}$ Pb $(NO_3)_2$, 2, 20, 200 $\mu g.g^{-1}$ $CdCl_2$ respectively, or in combined Pb/Cd solutions: 20/2, 200/20 or 1000/100 $\mu g.g^{-1}$. Equal amounts of bacteria biomass were transferred to petridishes with wateragar to which 5 pregnant nematodes of a similar age were added. Reproduction was determined by counting the number of nematodes on day 5, 8, 12, 19 and 22. The experiment was carried out in tenfold. Preliminary experiments had shown that the reproduction of nematodes feeding on bacteria biomass of single strains was much lower than on mixed strains isolated from the gutsystem.

The fungus A. solani, as a food for A. avenae, was grown at 25°C on maltagar (15%) to which Pb and Cd were added separately in concentrations of 0, 1, 10, 25 $\mu g.g^{-1}$ (as $CdCl_2$) and 10, 100, 250 $\mu g.g^{-1}$ Pb (as $Pb(NO_3)_2$) respectively, or combined in a Pb/Cd ratio of 10/1, 100/10, 250 $\mu g.g^{-1}$. The agar was adjusted to pH 6 and covered with thin plastic foil (Cuprophane dralysene foil, PT 300, Enka). After 4 days of growth of the added punch of fungus on agar (Ø 5 mm) 5 pregnant nematodes of a similar age were added. Reproduction was determined by counting the

nematodes on day 7, 10, 14, 17 and 21. The experiment was carried out in threefold and repeated with A. porri. The quantity of heavy metals taken up by the bacteria biomass was calculated by subtracting the supernatant quantity from the original quantity. The quantity taken up by the fungus was determined by separating the mycelium and the foil. the mycelium was left overnight in 20 ml H_2O_2 (36%) and boiled after adding concentrated HNO_3. Then, the pH was brought at 3.5 - 4. All heavy-metal concentrations were determined with an atomic absorption spectrophotometer (Pye Unicum 191).

The size of the population exposed to Pb and Cd was expressed as a percentage of the control. Multiplying these two percentages provides the expected size of the population when the combination of Pb and Cd acts additionally. If the measured size of the population is smaller than the expected size, synergism is involved. Antagonism is involved if the combined effect is larger than the expected effect.
Since the uptake of the cell wall (bacteria eaten by M. monhystera) depended on whether the metals were added separately or in combination (Table 1) a correction factor was necessary. The reduction caused by 1 $\mu g.g^{-1}$ was calculated for each day the population size was determined. For example, when the reproduction was known on a range 7.6 - 34.5 $\mu g.g^{-1}$ Pb its reduction by 1 $\mu g.g^{-1}$ Pb was calculated, then the population size was calculated when the Pb concentration was 8.8 $\mu g.g^{-1}$ Pb (the quantity Pb taken up when Pb and Cd were added as a combination). In analogous calculation for the Cd group was assessed whether the toxic action was additional, antagonistic of synergystic.

RESULTS AND DISCUSSION

The amounts of heavy metals bound by the bacteria differ between Pb and Cd and between the various concentrations and combinations (Table 1).

Table 1 Quantities of heavy metals ($\mu g.g^{-1}$) taken up by bacteria

Pb	7.6	(20)	34.5	(200)	110	(1000)
Cd	0.23	(2)	4.4	(20)	12.7	(100)
Pb/Cd	8.8/0.33	(20/2)	20/0.7	(200/20)	105/7	(1000/100)

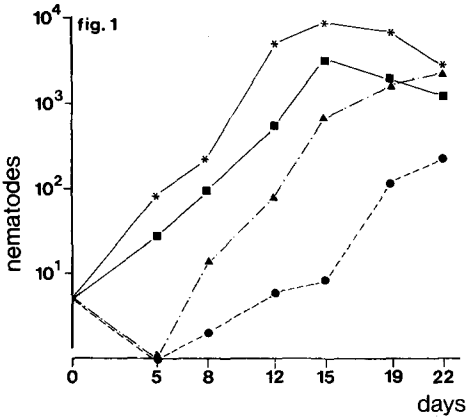
( ) The heavy-metals concentration ($\mu g.g^{-1}$) in the incubation solution.

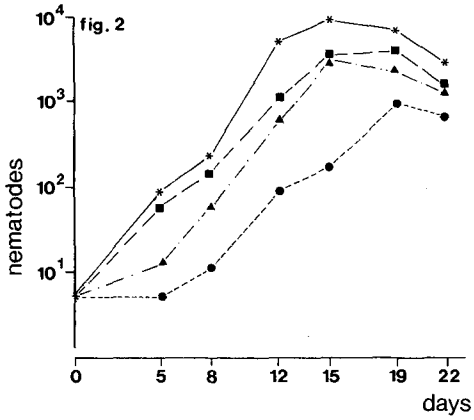
For sake of clarity the influence of the heavy metals on the reproduction of Mesorhabditus monhystera is given in Table 2 and Figures 1-3.
The differences between the control and the contaminated categories were statistically ($P < 0.05$ - Wilcoxon) except for 0.23 $\mu g.g^{-1}$ Cd and 110 $\mu g.g^{-1}$ Pb on day 5; for 1000/100 $\mu g.g^{-1}$ Pb/Cd on day 5, 19 and 22; for 4.4 $\mu g.g^{-1}$ Cd on day 8, 15 and 22 and for 34.4 $\mu g.g^{-1}$ Pb on day 19 and 22. Thus in the beginning and at the end of the experiment the values were least signifi-

Table 2 The influence of cadmium and lead, separately and simultaneously, taken up by bacteria on the population size of the nematode Mesorhabditus monhystera during time

Concentration ($\mu g.g^{-1}$)		time (days)											
		5		8		12		15		19		22	
Cd	Pb	$\bar{N}$	± S.E.	$\bar{N}$	± S.E.	$\bar{N}$	± S.E.	$\bar{N}$	± S.E.	$\bar{N}$	± S.E.	$\bar{N}$	± S.E.
-	-	81	13	224	31	4795	610	8691	1072	6502	978	2754	436
0.23	-	48	11	114	24	1067	188	3487	320	3972	476	1373	252
4.4	-	13	4	47	13	578	145	3369	605	2129	309	1021	231
12.7	-	7	4	11	4	88	43	106	64	443	234	463	173
-	7.0	14	12	27	11	95	27	527	160	3144	688	1081	236
-	34.5	1	0	15	4	80	44	665	373	1763	536	2005	370
-	110	1	0	2	1	6	3	9	3	117	61	208	89
0.33	8.8	10	5	51	12	298	75	1668	382	2388	458	1303	293
0.70	20	1	0	9	3	40	17	385	170	942	288	1134	432

$\bar{N}$ = average population size
S.E. = standard error of the mean





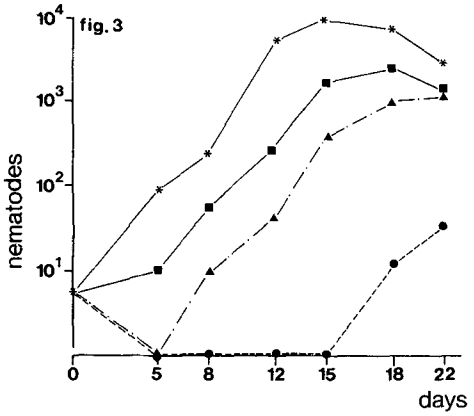


Figure 1 The influence of lead, taken up by bacteria, on the reproduction of the nematode Mesorhabditus monhystera; * — * = control, ■ — ■ = 7.6 µg.g^{-1} Pb, ▲ — ▲ = 34.5 µg.g^{-1}, ● — ● = 110 µg.g^{-1} Pb

Figure 2 The influence of cadmium, taken up by bacteria, on the reproduction of M. monhystera; * - * = control, ■ — ■ = 0.23 µg.g^{-1} Cd, ▲ — ▲ = 4.4 µg.g^{-1} Cd, ● — ● = 12.7 µg.g^{-1} Cd

Figure 3 The influence of lead, cadmium, simultaneously taken up by bacteria, on the reproduction of M. monhystera; * — * = control, ■ — ■ = 8.8/0.33 µg.g^{-1} Pb/Cd, ▲ — ▲ = 20/0.7 µg.g^{-1} Pb/Cd, ● — ● = 105/7 µg.g^{-1} Pb/Cd

cant. This can be explained by the fact that the same number of nematodes (5) was used at the starting point and by the decreasing reproduction at the end of the experiment. During day 8, 12 and 15 almost all values show a statistically reliable inhibition of the reproduction of the nematodes, due to heavy

metals. With Cd and Pb present in the food the nematode population reaches in general its maximal population size later in time (4 days or more) and, moreover, this maximum is reduced (approximately an average of 8700 nematodes per petridish versus 4000 or less).
Whether the combined action of Pb and Cd is additional, antagonistic or synergistic can be concluded from the data given in Table 3.

Table 3 Comparison of the action of Pb and Cd separately and simultaneously. The numbers reported are the measured (I) and corresponding calculated (II) reproduction percentages (in comparison to the controls)

$\mu g.g^{-1}$ Pb.Cd	I 8.8/0.33	II 7.6x0.23	I 20/0.7	II 34.5x4.4	I 105/7	II 110x12.7
day 5	12.3	23.8	1.32	0.21	1.34	0.08
day 8	22.6	21.2	4.0	1.53	0.06	0.04
day 12	6.1	2.32	0.82	0.19	0.02	0.002
day 15	18.9	14.2	4.5	2.91	0.01	0.002
day 19	36.7	16.9	14.5	0.58	0.17	3.92
day 22	47.3	22.8	47.2	27.0	1.30	1.63

At low concentrations the inhibition is less than expected which implicates antagonism. Only at the fifth day with the concentration combination 8.8/0.33 $\mu g.g^{-1}$ Pb/Cd synergism was involved (12.3% reproduction versus an expected 23.8%). At high heavy-metal concentrations the reproduction was so low (except on day 22) that little difference existed between observed and expected values.

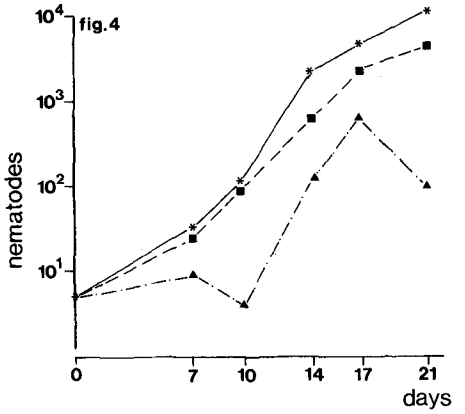
The metal concentrations in the fungus Alternaria solani were low (Table 4).

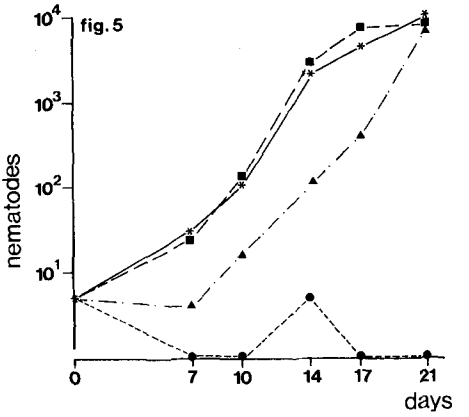
Table 4 Quantities of heavy metals ($\mu g.g^{-1}$) taken up by fungi

Pb			0,082	(10)	2,471	(100)
Cd	0,030	(1)	0,094	(10)	0,122	(25)
Pb/Cd	0,068/0,037	(10/1)	0,342/0,054	(100/10)		

( ): The heavy-metal concentrations ($\mu g.g^{-1}$) in the malt agar

The average influence of these concentrations on the production of Aphelenchus avenae is given in Figures 4, 5 and 6. These values showed a large standard error which seemed to be common with nematodes and especially the combination fungi-nematodes provided large standard errors. Possible differences in sensitivity towards heavy metals of the various larval stages (Croll 1970) may have contributed to these large standards errors. High metal concentrations also restricted fungal growth, resulting in lack of food at higher concentrations. However, at





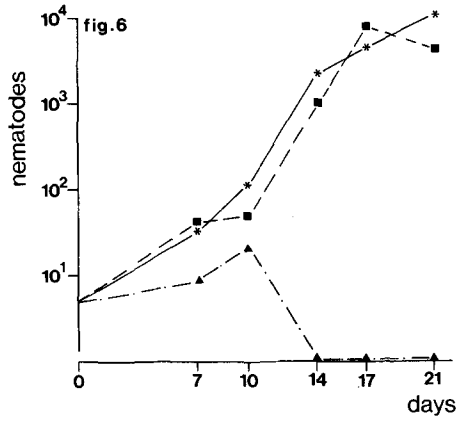


Figure 4 The influence of lead, in agar and taken up by fungi, on the reproduction of the nematode Aphelenchus avenae; * — * = control, ■ — ■ = 10 $\mu g.g^{-1}$ Pb, ▲ — ▲ = 100 $\mu g.g^{-1}$ Pb

Figure 5 The influence of cadmium, in agar and taken up by fungi, on the reproduction of A. avenae; * — * = control, ■ — ■ = 1 $\mu g.g^{-1}$ Cd, ▲ — ▲ = 10 $\mu g.g^{-1}$ Cd, ● — ● = 25 $\mu g.g^{-1}$

Figure 6 The influence of lead and cadmium, simultaneously added to agar and taken up by fungi, on the reproduction of A. avenae; * — * = control, ■ — ■ = 10/1 $\mu g.g^{-1}$ Pb/Cd, ▲ — ▲ = 100/10 $\mu g.g^{-1}$ Pb/Cd

10 $\mu g.g^{-1}$ Cd fungal growth was limited but the number of nematodes was considerable. Therefore, lack of nutrient may be neglected as a factor, at least in the middle of the experiment. Concentrations of 2.471 $\mu g.g^{-1}$ Pb in the fungus which served as food for nutrient A. avenae strongly influenced their reproduction, just as 0.122 $\mu g.g^{-1}$ Cd did. The strong inhibition at

0.342/0.054 $\mu g.g^{-1}$ Pb/Cd after the tenth day indicated that Pb plus Cd intensified their mutual influence.

Both varieties of experiments show that Pb and Cd, when taken up by microorganisms (which is probably adsorption in the case of bacteria and absorption in the case of fungi) have an inhibitory effect on the reproduction rate of the nematodes Mesorhabditus monhystera and Aphelenchus avenae. They also show the possibility of a synergistic interaction of the metals. Also other soil microorganisms may bind heavy metals and, when used as food, this would adversely affect the reproduction of various nematode species.

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