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Adaptation to Soil Pollution by Cadmium Excretion in Natural Populations of *Orchesella cincta* (L.) (Collembola)

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Abstract. Population differentiation in *Orchesella cincta* (L.) (Collembola) populations, from various heavy metal contaminated sites, was studied by comparing cadmium excretion efficiency in first generation (F_1) laboratory individuals. Animals from sites with high metal concentrations in the litter and with a long history of contamination showed significantly higher excretion efficiencies than animals from low pollution, or reference sites. Differences found in the F_1 laboratory animals suggest evidence for genetic differences between the populations.

Beneficial and detrimental effects of cadmium excretion were studied in relation to body growth and cadmium concentrations. In chronically exposed animals from an unpolluted site, no physiological acclimation was observed. Excretion efficiency was negatively correlated with body concentrations of cadmium. No detrimental effects were found.

Whole-body equilibrium concentrations of cadmium and lead were similar in F_1 animals from the reference site and polluted sites. Significant differences in excretion efficiencies imply that the distribution of toxic metals over body compartments differs, tolerant populations having a higher proportion deposited in the gut. Body concentrations of zinc were consistently higher in animals from the polluted site, during both cadmium and zinc exposure. No detrimental effects of increased cadmium excretion on body concentrations of zinc were observed. Population comparisons of cadmium excretion efficiency data and growth reduction in F_1 laboratory animals showed that both parameters were inversely related. Cadmium and lead contamination were not the sole factors determining tolerance differentiation.

Heavy metal contamination may cause population differentiation. Local populations showing increased tolerance for heavy metals have been reported for terrestrial plants (Antonovics *et al.* 1971; Bradshaw and McNeilly 1981) and aquatic species (Klerks and Weis 1987). Tolerant populations either result from the development of tolerance during individual exposure (acclimation), from natural selection acting upon genetically based individual variation in tolerance (adaptation), or from a combination of both.

The recognition of adaptation in natural populations exposed to a contaminant is important. Apart from increasing tolerance for the contaminant, natural selection affects the ecological performance of future generations: there may be phenotypic or genetic costs of tolerance (Stearns 1989; Wilson 1988), and genetic variation may be decreased either in characteristics selected (Shaw 1988), or because of pleiotropic effects (Falconer 1981). To study adaptation, several authors have worked with characteristics of first generation (F_1) laboratory animals cultured in a clean environment (Klerks and Levinton 1989; Posthuma 1990; Donker and Bogert 1991). This approach is used here also. This paper focuses on the occurrence and consequences of genetic differentiation in natural populations of a litter dwelling collembolan, *Orchesella cincta* (L.). This species has been found at clean and heavy metal contaminated sites in North-Western Europe (Van Straalen *et al.* 1987). Selection may operate in this species, as total cadmium concentrations in the litter at some sites exceeded the No Observed Effect Concentration for growth or survival for reference animals (Van Straalen *et al.* 1989). Genetic differentiation has been shown for growth reduction by Posthuma (1990): F_1 laboratory animals from severely polluted sites showed less growth reduction during exposure to cadmium than animals from less polluted sites, and were considered to be more tolerant. For zinc, no differentiation of tolerance was found. Earlier, we have shown that field animals from highly polluted sites were more tolerant for Cd and Pb than animals from less polluted sites, as they showed increased excretion efficiency after 3 days of exposure (Van Straalen *et al.* 1987). However, in that study field animals were used, and differences between populations could not be attributed to either acclimation or adaptation.

Orchesella cincta is a species which exhibits an inherent degree of metal tolerance, related to the ion regulation system in the midgut epithelium. Following each moult, animals excrete the old midgut wall, which contains high amounts of assimilated metals fixed in granules (Van Straalen *et al.* 1987). Re-use of the fixed ions is doubtful (Humbert 1978). The concentration of non-fixed metals, that eventually cause the toxic effects of metals, depends on the balance of assimilation and excretion. From the population comparisons of growth reduction and excretion efficiency, it is hypothesized

Table 1. Sampling sites and characteristics of actual sampling locations within sites. Concentrations of metals in the organic layer of the forest floor are expressed on a dry wt basis. Population numbering is based on increasing levels of cadmium contamination

Site code	Location[a]	Site characteristics	Cd (nmol g^{-1})	Zn (μmol g^{-1})	Pb (μmol g^{-1})	Cu (μmol g^{-1})	Ca (μmol g^{-1})
1	Roggebotzand (NL)	Reference	3.8	0.5	0.3	0.1	248
2	Dalfsen (NL)	Reference	<5	1.2	0.5	0.5	38
5	Budel (NL)	Zinc smelter	45	14.5	1.5	0.7	46
6	Plombières (B)	Abandoned mine	244	75.2	54.9	2.7	421
7	Stolberg (G)	Lead smelter	557	23.9	41.1	20.2	286

[a] NL: The Netherlands; B: Belgium; G: Germany

that development of cadmium tolerance involves a change in metal distribution over body and midgut epithelium. This can be accomplished by increased excretion efficiency or by increased moulting frequency. In view of metal specificity of assimilation and elimination (Hopkin 1990) and metal specific effects on collembolan growth (Posthuma 1990), we chose to expose animals to either cadmium, lead or zinc.

The present study was designed with three objectives:

(A) To determine whether genetic components for population differentiation for cadmium excretion efficiency are present in natural populations of *O. cincta*.
(B) To determine characteristics of the excretion system under chronic exposure.
(C) To determine the consequences of increased excretion efficiency values to individuals and populations.

Data were obtained from three separate experiments, with short-term or long-term exposure, in one or more populations.

Material and Methods

Site Description

Animals were collected from randomly selected litter samples at three contaminated sites and two reference sites in September 1987 and in March 1988. At least 600 animals were captured at each site. Locations of sites, details of site characteristics and sampling techniques are described by Van Straalen *et al.* (1987) and Posthuma (1990). For convenience, populations are in increasing order of total cadmium concentrations of the litter, and numbered consecutively. The main contamination characteristics of the populations used are summarized in Table 1. Sites 1 and 2 are reference sites, 5 is low polluted, and 6 and 7 are heavily polluted. Sites 6 and 7 are naturally enriched with heavy metals (Ernst 1974).

Laboratory Conditions

After capture, parent generation (P) animals were transferred to clean culture boxes. Each week, mass cultures were offered twigs covered with uncontaminated green algae, to prevent acclimation to heavy metals. Prior to feeding, animals and eggs possibly present on the twigs were killed by heating and drying at 40°C for four days. F_1 laboratory animals were used for experiments on population differentiation. All experiments were performed in a climate room (T: 20°C, R.H.: 75%, light/dark: 12/12).

Animals were reared individually in small culture boxes, and a freshly prepared thick slurry of green algae (*Pleurococcus* spec.) was offered each week on small paper discs. Algae were sampled from bark of *Acer* spec. at reference site 1, and had a background metal concentration of 5.4 nmol Cd, 0.3 μmol Pb, and 0.5 μmol Zn g^{-1} dry wt. Animals were offered clean, cadmium, lead or zinc contaminated food, concentrations varied for each experiment. Contaminated food was prepared with stock solutions of metal nitrate salts. A calculated volume of stock solution was added to the slurry, based on a dry matter content of 20%, to obtain the desired metal concentration. Metals are rapidly absorbed by the algae cells, which have a large binding capacity (Joosse and Verhoef 1983). If necessary, nitrate was balanced within an experiment by adding potassium nitrate to the food preparations with low metal concentrations. Further details of culturing conditions can be found in Posthuma (1990).

Determination of Excretion Efficiency

Standard cadmium excretion efficiency was determined individually by the standardized method described in Van Straalen *et al.* (1987). After moult, an animal was exposed to cadmium contaminated food for three days. The animal was transferred to a clean box, and following the next moult, the animal and the shed gut epithelium, *i.e.*, the gut pellet, were sampled for metal analysis. Gut pellets produced later than 7 days after the second moult, incomplete or broken were not used in calculations. Exuviae were not sampled. Excretion efficiency was calculated using the formula EE = 100P/(P + C) (in %), *i.e.*, the ratio of the amount of cadmium present in the gut pellet (P, in nmol $animal^{-1}$) and the assimilated amount (total nmol present in gut pellet and animal, (P + C), in nmol $animal^{-1}$).

Chronic cadmium exposure, *i.e.*, longer than one intermoult interval, may influence the relative contribution of both parameters. Therefore, the cadmium excretion efficiency calculated for chronically exposed animals will be distinguished from the standard value as chronic excretion efficiency. Chronic excretion efficiency probably resembles the natural situation of exposed animals more than the three day equivalent. The duration of exposure is given with the description of the experiments.

Metal Analysis

Body burden was determined for individuals with empty guts. Guts were emptied either during the procedure outlined above, or animals were deprived of food for 24 hours. Body concentrations were calculated on a dry weight basis: animals were freeze-dried and weighed on a Sartorius 4503 microbalance (accuracy: 1 μg). Samples to be analyzed for metal contents were completely digested with two treatments (HNO_3: 250 μl, Ultrex grade, Baker Chem., and H_2O_2: 200 μl, Aristar, both until dryness), in 500 μl pyrex tubes. Metal

content was determined using the methods described by Van Straalen and Van Wensem (1986).

Experimental Design

1. Population Comparison of Cadmium Excretion Efficiency: The experiments on population differentiation of standard cadmium excretion efficiency were started with 85 to 100 F_1 animals from each of the sites 1, 2, 5, 6 and 7. Animals were cultured from the parental generation caught in March 1988.

To obtain detectable amounts of cadmium in both P and C, the nominal cadmium concentration in the food was 0.356 μmol g^{-1} dry wt. Standard excretion efficiency data were inhomogeneous (Bartlett's test, Sokal and Rohlf 1981). The data were analyzed with the Kruskal & Wallis test, followed by *a posteriori* multiple comparisons (Conover 1980).

2. Cadmium Concentration and Body Growth in Reference Animals: Just hatched juveniles (210) of unknown sex were kept individually in small rearing boxes and fed with uncontaminated algae for 4 weeks. After this period animals received either blank (70 individuals) or cadmium contaminated food (140 individuals) for 6 weeks: the actual concentrations were 0.005 and 0.383 μmol g^{-1} dry wt. The high cadmium concentration was approximately ten times the No Observed Effect Concentration for growth of females in reference animals (Van Straalen *et al.* 1989). Four experimental groups were eventually distinguished, as sex dependency of characteristics (especially for body growth, Posthuma 1990) was taken into account. The sex of each animal was determined with Posthuma's (1990) method.

Individuals were weighed at the ages of 4, 6, and 10 weeks. The individual growth rate (μg $week^{-1}$) was determined by fitting a straight line to the data, which gave a reasonable description during this life stage. In the same period, exuviae were counted, and the moulting frequency was calculated (moults $week^{-1}$). Upon termination of the experiment, excretion efficiency was determined, either by following the standard procedure, with 3 days exposure (blank treatment), or by continuing exposure for three days during another moulting interval (chronic treatment). Body concentrations of Cd (nmol g^{-1}) after moulting were calculated from body contents and dry weight. Normality was tested by the method of Wilk and Shapiro (1968), homogeneity of variances with the method of Sokal and Rohlf (1981). Transformations of data were executed if necessary. Data were analyzed with a two-way ANOVA for each characteristic (independent variables being treatment and sex). Pearson product-moment correlation coefficients were calculated for parameters within all groups. All calculations were executed with the integrated SPSS statistical program.

3. Population Comparison of Cadmium, Lead and Zinc Kinetics: The experiment on the kinetics of cadmium and zinc was started with six week old F_1 juveniles of unknown sex, descended from the populations caught at reference site 1 and at the highly polluted site 7. The parental generation was caught in September, 1987. Each group consisted of 65 animals, reared individually. Animals were exposed to either cadmium or zinc during an accumulation period of 42 days, and to uncontaminated food during an elimination period of 28 days. Exposure levels were either low or high: actual metal concentrations in the food were 0.13 or 1.78 μmol g^{-1} for cadmium treatments, and 3.0 or 68.8 μmol g^{-1} dry wt for zinc treatments. Samples of individuals were taken on several days during accumulation and elimination, and analyzed for metal content. Cadmium concentrations were calculated for animals from the cadmium exposure groups. Zinc concentrations were calculated for all groups. The experiment on the kinetics of lead was performed with a similar method (Van Straalen and Van Meerendonk 1987), with animals descended from reference site 1 and mining site 6. The nominal lead concentration in the food was 9.66 μmol g^{-1} dry wt. Effects of exposure on growth were observed in the high cadmium treatment group only (Posthuma 1990).

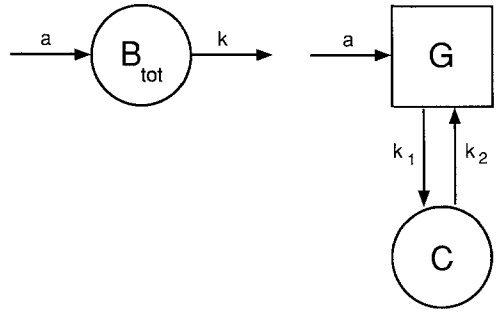

Fig. 1. Models for comparison of the net effect of assimilation and excretion in metal exposed *Orchesella cincta* populations. Gut contents were omitted, as animals had empty guts. (Left) General linear one-compartment model as applied by Janssen *et al.* (1991).

(1) $dB_{tot}/dt = a - kB_{tot}$ (for $0 < t \leq t_c$: $a > 0$; for $t > t_c$: $a = 0$)
(2) $B_{tot}(0) = 0$

(Right) Species-specific two compartment model, linear except for the reset of the midgut epithelium compartment at moulting and the discontinuous food intake.

(1) $dG/dt =$
$a - k_1G + k_2C$ (for $0 < t \leq t_c$: if $t < t_{m-n}$: $a > 0$; if $t_{m-n} < t < t_m$: $a = 0$ for $t > t_c$: $a = 0$)
(2) $dC/dt = k_1G - k_2C$
(3) $G(0) = G(t_m) = 0$
(4) $C(0) = 0$

B_{tot} = whole-body concentration of metal in the animal (nmol g^{-1} dry wt); G = metal burden in the midgut epithelium before moulting; C = metal burden in the animal, except midgut epithelium; a = assimilation rate constant (one-compartment) (nmol g^{-1} day^{-1}) or assimilation function (two-compartment); k_i = excretion rate constant of compartment i (day^{-1}); t = time (day); t_c = time at which animals were transferred to clean food; t_m = time of moult (intermoult interval: normally distributed parameter, mean 7 days); t_{m-n} = day at which feeding ceases, prior to moulting. For further explanation: see text

A linear one-compartment model (Figure 1, left) was fitted to the data. Parameter estimates of cadmium and lead kinetics were obtained simultaneously by a non-linear least squares procedure, following the methods of Janssen *et al.* (1991). This approach was chosen to obtain estimates of whole-body equilibrium concentrations, for population comparisons. Assimilation was assumed to be constant for the accumulation period, and zero during the elimination period. Initial concentrations of cadmium and lead were low compared to concentrations reached during exposure, and therefore not taken into account in parameter estimation. From the estimated excretion constant k (day^{-1}) a value for the excretion efficiency (%) was calculated with the formula $EE = 1 - e^{-dk}$ (d is the mean moulting interval (days) of exposed animals). Mean moulting intervals for exposed animals were determined in a separate experiment. The equilibrium concentration was determined with the formula $C_{eq} = a/k$. Half-lifes for cadmium were calculated by the formula $T_{1/2} = \ln 2/k$.

The one-compartment approach ignores discontinuities related to moulting dependent excretion and food intake (Joosse 1981). Therefore, changes of metal burden were simulated with a discontinuous

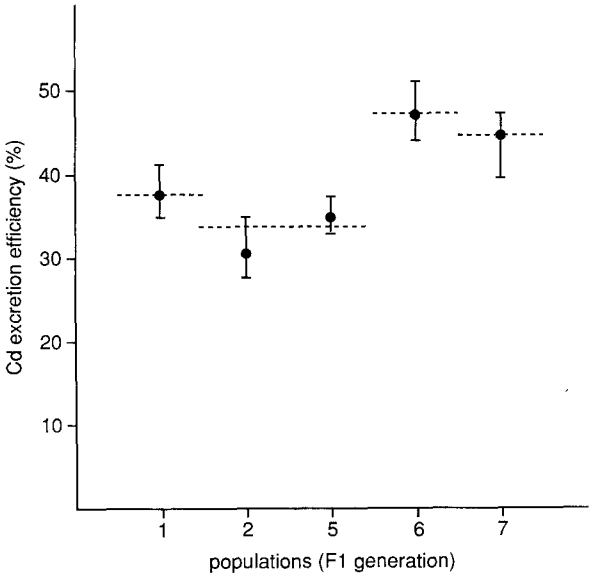

Fig. 2. Median cadmium excretion efficiency (±95% confidence interval) of 5 populations of *Orchesella cincta* in F_1 laboratory animals. Values which do not differ significantly are joined with horizontal broken lines. Populations are ordered from left to right according to increasing order of litter cadmium concentration. For site codes: see Table 1

two-compartment model (Figure 1, right). This species-specific approach was chosen to investigate the time-dependent distribution of metals within individuals. In this model, assimilation was assumed to be constant during feeding, and was zero for a few days during the preparation for moulting (40% of the moulting interval). At moulting, the amount in the gut epithelium was reset to zero (loss of the epithelium, excretion of the gut pellet), and the cycle was started again. The sequence of long and short intermoult intervals is related to periods of non-reproductive and reproductive instars. For further details: see Joosse (1981).

Normality and homogeneity for zinc data was tested as described above, and transformations were executed if necessary. Data were analyzed with a two-way ANOVA (independent variables being population and treatment).

Results

Population Comparison of Cadmium Excretion Efficiency

Prior to determining excretion differentiation, presence of avoidance differences was studied. Total cadmium assimilation for a 3 days feeding period (P + C) was similar for all populations, no indications of population dependent cadmium avoidance were found (one-way ANOVA, $P > 0.05$). Differences in cadmium avoidance between populations did not contribute to differentiation of standard cadmium excretion efficiency.

Excretion efficiency varied considerably between individuals, and within populations, distributions were somewhat skewed. The observations are summarized in Figure 2. The Kruskal & Wallis test demonstrated that cadmium excretion-efficiencies differed between the populations ($P < 0.001$). The *a posteriori* multiple comparisons test showed that the excretion efficiencies of the populations from reference site 2 and low pollution site 5 did not differ significantly. The other populations showed significantly higher excretion efficiencies, ranging from 38% for reference site 1 to above 45% for the extremely polluted sites 6 and 7. Animals descended from sites with background contamination (1 and 2) had a significantly different performance (7.8%). The Pearson correlation coefficients between median cadmium excretion efficiency and site contamination (logarithmically transformed) were 0.81 and 0.90 ($n = 5$, $P < 0.05$), for cadmium and lead respectively. The correlation between cadmium excretion efficiency and zinc concentration in the litter was not significant.

In conclusion, excretion efficiency was highest at sites where increased tolerance would be most expected (*i.e.*, 6 and 7), but cadmium and lead were not the sole factors determining excretion efficiency differences.

Cadmium Concentration and Body Growth in Reference Animals

The effects of chronic exposure on growth and excretion parameters are shown in Table 2 (observations) and Table 3 (ANOVA results). For the parameters growth rate and dry weight, a significant interaction between sex and treatment was found. Chronic exposure to cadmium negatively affected growth in females only. Males moulted more frequently than females in both treatment groups, but chronic exposure negatively affected moulting frequency in both sexes. It is concluded that cadmium reduced growth in females and that there was no evidence for physiological acclimation under chronic exposure by means of an increased moulting frequency.

The cadmium contents of the gut pellet depended on both sex and treatment. Following chronic exposure, the amount of cadmium excreted by males increased more than that excreted by females, compared to three days exposure. The body concentration of cadmium was only dependent on treatment. Males and females showed a similar increase in body concentration following chronic exposure. Excretion efficiency was dependent on both sex and treatment, females showed decreased efficiency following chronic exposure, whereas males showed no response. It is concluded that in chronically exposed animals the absolute amounts of cadmium passing through the excretion system increased, but that there is no evidence for physiological acclimation by means of increased excretion efficiency. Furthermore, standard excretion efficiency provides a reasonable estimate of excretion efficiency under chronic exposure.

Within-population correlations between excretion and growth characteristics, classified according to sex and treatment, are shown in Table 4. Correlations are presented separately for each group, due to the significant effects of sex and treatment on all characteristics (Table 3). Growth rate data are not shown, as growth rate and dry weight showed a similar correlation pattern.

In blank-treated animals, which were eventually exposed for 3 days at the end of the experiment, the total assimilated amount of cadmium was positively correlated with dry weight and excretion efficiency, especially in females. Ref-

Table 2. Growth and cadmium excretion characteristics in *Orchesella cincta* from a reference site, data separated according to sex and treatment. Blank treatment: uncontaminated food, followed by three days of exposure during the excretion efficiency procedure; chronic treatment: Cd exposure of 0.383 μmol.g^{-1} dry wt in the food during weeks 4–10 after hatching, followed by the excretion efficiency procedure

Treatment		Blank (+3 days cadmium)		Chronic cadmium	
Characteristic	Sex	Females	Males	Females	Males
Growth rate		0.128	0.085	0.109	0.088
(μg week^{-1})		(0.004)[a]	(0.004)	(0.004)	(0.003)
Dry weight (μg)		0.303	0.171	0.236	0.169
		(0.011)	(0.005)	(0.010)	(0.005)
Moulting frequency		1.108	1.196	1.045	1.130
(week^{-1})		(0.026)	(0.032)	(0.021)	(0.019)
Body concentration of cadmium		49.7	50.8	95.6	107.9
(nmol g^{-1} dry wt)		(4.7)	(4.1)	(5.6)	(6.3)
Cadmium contents gut pellet		8.928	4.017	9.030	7.590
(nmol pellet^{-1})		(1.551)	(0.535)	(0.876)	(0.415)
Excretion efficiency (%)		34.5	30.0	28.9	30.7
		(1.8)	(2.2)	(1.3)	(1.1)
n[b]		31	22	53	59

[a] Standard error is given in parentheses; [b]n = number of observations

Table 3. Two-way ANOVA (independent variables being sex and treatment) for growth and cadmium excretion characteristics in *Orchesella cincta* from a reference site. Treatments as in Table 2

Characteristics		Growth rate (μg week^{-1})		Dry weight (μg)		Moulting frequency (week^{-1})	
Transformation		None		Logarithmic		None	
Anova	df	M.S.	F	M.S.	F	M.S.	F
Main effects							
Sex	1	0.032	57.430***	0.204	100.497***	0.303	13.783***
Treatment	1	0.003	5.117*	0.032	15.983***	0.147	6.699*
2-way interaction							
sex * treatment	1	0.004	6.918**	0.023	11.419***	0.001	0.004[n.s.]
Residual	161	0.001		0.002		0.022	
Total	164	0.001		0.004		0.024	

Characteristics		Body concentration cadmium (nmol g^{-1})		Cadmium contents gut pellet (nmol pellet^{-1})		Excretion efficiency (%)	
Transformation		Logarithmic		Logarithmic		Logarithmic	
Anova	df	M.S.	F	M.S.	F	M.S.	F
Main effects							
Sex	1	0.439	2.290[n.s.]	0.548	7.091**	2.289	0.027[n.s.]
Treatment	1	14.161	73.857***	0.618	8.009**	273.816	3.216[n.s.]
2-way interaction							
sex * treatment	1	0.009	0.045[n.s.]	0.364	4.712*	349.592	4.106*
Residual	161	0.192		0.077		85.137	
Total	164	0.281		0.084		87.445	

[n.s.] $P > 0.05$; *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$

erence animals with a high growth rate potentially have a high molar turnover of cadmium, and uptake and excretion of cadmium seem to be correlated. However, excretion efficiency, *i.e.*, excretion standardized by the assimilated amount, was not correlated with growth and moulting frequency.

Chronically exposed animals showed a similar pattern of correlations, probably due to the correlation between cadmium uptake and excretion. Contrary to the situation in 3-day exposed animals, the amount and concentration of cadmium contained in the body after a moult in chronically exposed animals was significantly lower in animals with a high excretion efficiency, especially in males. As this may be partly determined by autocorrelation, a compartment model was used to obtain independent estimates of excretion and equilibrium body concentrations.

It is concluded that the excretion mechanism can handle high concentrations of cadmium over a long time, without being seriously affected. Furthermore, the ANOVA data suggested that there may be positive effects of total excretion on growth in chronically exposed animals, especially males. This effect was not present in within group correla-

Table 4. Pearson correlation coefficients between individual characteristics of *Orchesella cincta* from a reference site. Top/right triangle: blank treatment animals, eventually exposed to cadmium for 3 days. Bottom/left triangle: chronically treated animals

Characteristics[a]		Dry wt	MF	C	C/dry wt	P	W.B.	EE
Dry wt	♂	—	0.40*	0.43*	0.07	0.49*	0.49*	0.32
	♀	—	0.22	0.72***	0.43**	0.59***	0.68***	0.10
MF	♂	0.05	—	0.07	−0.11	0.20	0.15	0.22
	♀	0.30*	—	0.06	0.00	−0.03	0.05	−0.17
C	♂	0.25*	0.33**	—	0.92***	0.69***	0.94***	0.11
	♀	0.42***	0.02	—	0.82***	0.83***	0.92***	0.20
C/Dry wt	♂	−0.19	0.31**	0.89***	—	0.57**	0.80***	0.02
	♀	−0.21	−0.20	0.79***	—	0.68***	0.75***	0.15
P	♂	0.36**	0.27*	0.59***	0.44***	—	0.88***	0.77***
	♀	0.37**	−0.10	0.66***	0.46***	—	0.94***	0.67***
W.B.	♂	0.28*	0.33**	0.96***	0.83***	0.76***	—	0.39*
	♀	0.49***	0.03	0.95***	0.68***	0.82***	—	0.42***
EE	♂	0.08	−0.06	−0.48***	−0.53***	0.41***	−0.26*	—
	♀	0.05	−0.15	−0.24*	−0.31*	0.53***	0.01	—

[a] MF = moulting frequency; C = cadmium contents body; P = cadmium contents gut pellet; W.B. = whole body contents (sum of P and C); EE = excretion efficiency; *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$

tions, probably due to the correlated increases of assimilation and excretion of cadmium.

Population Comparison of Cadmium, Lead, and Zinc Kinetics

First generation laboratory animals contained background concentrations of cadmium and lead before exposure. Upon exposure, cadmium and lead accumulated rapidly in both populations (Figure 3). Whole-body concentrations varied widely in both populations during the accumulation phase, which was not predicted on the basis of a linear one-compartment model. Analysis of variance of these concentration data did not reveal population differences, as the significant interaction (population * time, $P < 0.05$) may be attributed to asynchronicity of moulting rather than to differences in accumulation behaviour between populations. We used the two-compartment model with discontinuous input, to simulate the cadmium contents of animals in the course of the moulting cycles (Figure 4). The simulation demonstrated that the feeding and moulting cycle in *Orchesella cincta* introduces large fluctuations in the cadmium burden of a complete animal (dotted line). The interior burden behaves in a more smooth manner (solid line).

The one-compartment model was used to obtain estimates of excretion constants and equilibrium concentrations. Estimated model parameters are shown in Table 5. For both metals, a maximum likelihood ratio test (Cox and Hinkley 1974) showed that estimated parameters for both populations were similar. An analysis of trends showed that assimilation and excretion constants for reference animals were lower than for animals from polluted sites, for both metals. The difference between the excretion efficiencies calculated from the estimated k-values was similar to difference of excretion efficiency data determined directly (Figure 2, cf. Van Straalen *et al.* 1987), the level was slightly increased for cadmium. This may be a consequence of algae food characteristics: metal binding characteristics may vary between subsequent samplings due to age or season. The equilibrium whole-body metal concentrations from polluted sites seemed to be slightly lower compared to animals from the reference site.

During the high treatment, cadmium in the body reached concentrations of 300–400 nmol g^{-1} dry wt. At these concentrations growth was reduced in susceptible populations (Posthuma 1990); excretion caused a fast decrease in concentration when exposure ceased.

No clear accumulation and elimination pattern was observed for zinc concentrations in zinc treated animals. Therefore, the time-series data were pooled within populations. The observations are summarized in Table 6. A two-way ANOVA (population, treatment, Table 7) showed a main effect for the factor population. Animals descended from polluted site 7 consistently had a higher body concentration of zinc than animals descended from reference site 1. This difference was not caused by weight differences between the populations, Figure 5 shows that the body concentrations of zinc are not related to body weight. For zinc exposed animals, a main effect for treatment is also present. Exposure to 68.8 μmol g^{-1} dry wt caused a higher median zinc concentration than exposure to 3.0 μmol g^{-1} dry wt. This was similar for both populations (Table 6). The increase in zinc concentration in the animals (0.4 μmol g^{-1}) was small compared to the difference between treatment concentrations.

Zinc kinetics for animals exposed to cadmium showed similar differences between the populations (Tables 6, 7). Animals from the polluted site have a higher zinc concentration than animals from the reference site. Zinc concentrations in the animals were not influenced by cadmium exposure.

It is concluded that whole-body concentrations of cadmium and lead changed similarly in the populations. Excretion efficiency differences between populations imply that, in tolerant populations, a higher proportion of cadmium and lead is deposited in the gut. Zinc appears to be regulated within narrow limits; these are population specific, not in-

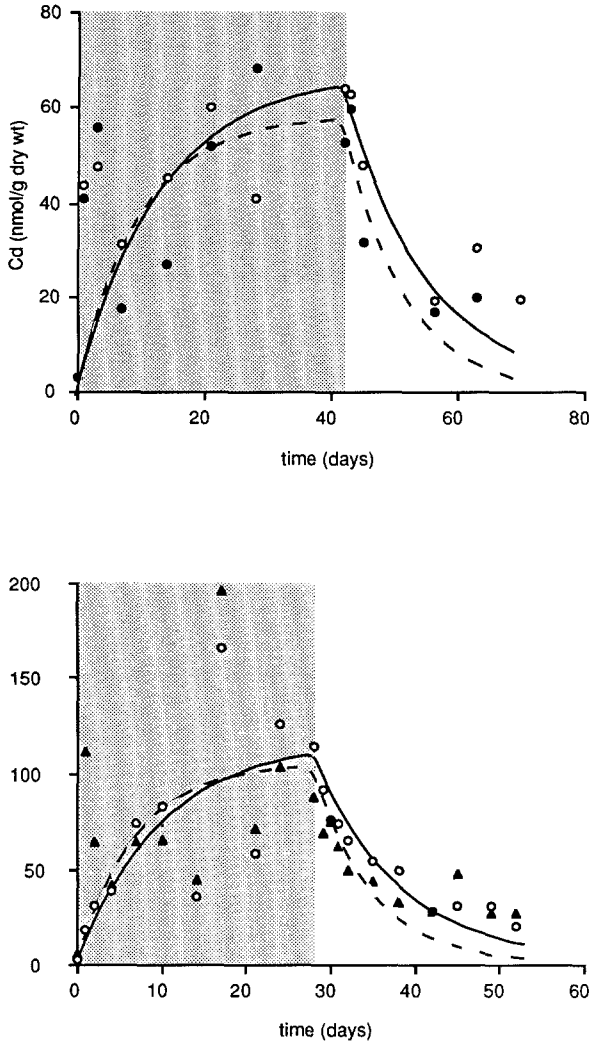

Fig. 3. Mean cadmium (top) and lead (bottom) concentrations in *Orchesella cincta* (dry wt base) during accumulation (shaded) and elimination, for F_1 laboratory animals from the reference site 1 (open circles), mining site 6 (black triangles) and industrial site 7 (black circles). Standard errors were omitted for clarity. Smooth lines are based on the one compartment model fitted to the data; broken lines represent the mine or smelter population. For parameter estimates: see Table 5

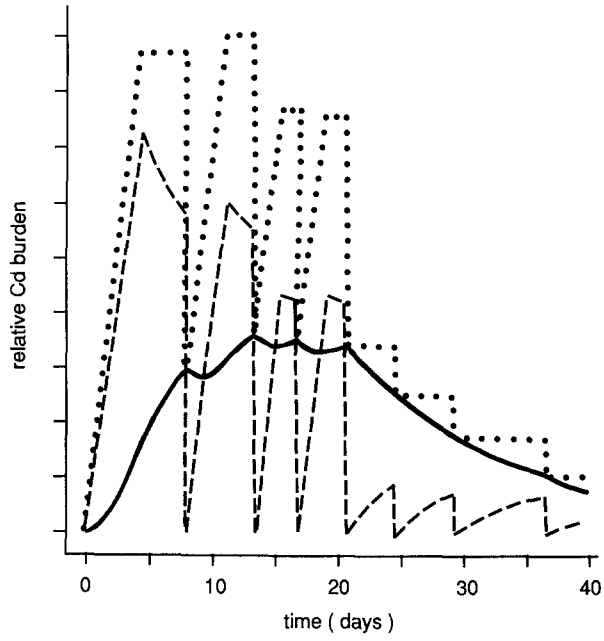

Fig. 4. Typical simulation result for an individual during accumulation (4 moulting intervals) and elimination based on the discontinuous two-compartment model, with $k_1 = k_2 = 0.1\ day^{-1}$. Values for the discontinuous ingestion function and moulting intervals were derived from Joosse (1981). Whole-body burden is constant before moulting, as assimilation ceases. Cadmium burdens were simulated for separate compartments, and expressed on an arbitrary scale. Dotted line: whole-body (body with gut epithelium); broken line: gut epithelium; solid line: body without gut epithelium

fluenced by cadmium exposure and show little effect regarding zinc treatment. Detrimental effects of increasing cadmium excretion upon zinc regulation were absent.

Growth Reduction and Excretion Efficiency

Population means for cadmium excretion efficiencies were compared to population means for indices of growth reduction (IGR) for populations for which data on both variables were available, viz. 1, 5, 6, and 7 (Figure 6). The latter data were recalculated from individual growth reduction (IGR) indices, which were determined by Posthuma (1990) for two cadmium treatments. Using weight gains, an IGR was calculated, which expresses an individual's growth reduction upon the start of cadmium exposure at 6 weeks of age. Growth reduction was either a consequence of the natural deflection of the growth curve (low exposure group), or of cadmium exposure, superimposed on natural deflection (high exposure group). To obtain homogeneity, data were transformed logarithmically. The relative growth reduction index expresses tolerance, and is calculated as the ratio of the transformed means of both parameters within a population.

The data on growth reduction and cadmium excretion efficiency in F_1 laboratory animals showed a close correlation (Figure 6). Animals from the high pollution sites had higher excretion and lower growth reduction than animals descended from the reference site. Animals from low pollution site 5 were less tolerant than animals from the reference site. Males show a similar pattern with higher variation due to low sample sizes. An exact value for the correlation cannot be given, since parameters were not measured simultaneously in one individual.

Discussion

The current study indicated the presence of a genetic component for population differentiation in *O. cincta* with respect to cadmium excretion efficiency and cadmium, lead, and zinc kinetics in chronically exposed animals. Acclimation probably did not contribute to differentiation, as chronic

Table 5. Parameter estimates of a linear one-compartment model for metal kinetics in *Orchesella cincta* (F_1 generation) from a reference population (1), a mining population (6) and an industrial population (7). Top: exposure to cadmium (0.13 μmol g^{-1} dry wt food, actual). Bottom: exposure to lead (9.66 μmol g^{-1} dry wt food, nominal)

Metal	Population	Parameter[a] a (±s.d.) (nmol g^{-1} d^{-1})	k (±s.d.) (day^{-1})	Equilibrium conc. (a/k, nmol/g dry wt)	$T_{1/2}$ (day)	d (days)	EE_{est} (%)	χ^2 (df = 2)
Cd	1	5.11 ± 1.04	0.076 ± 0.018	67	9.1	7.0	41.3	
	7	6.07 ± 1.71	0.103 ± 0.030	59	6.7	7.3	52.9	
	All data	5.56 ± 0.95	0.088 ± 0.016					$1.010^{n.s.}$
Pb	1	11.42 ± 1.40	0.098 ± 0.013	117	7.1	7.0	49.6	
	6	15.06 ± 2.90	0.143 ± 0.028	105	4.8	7.0	63.2	
	All data	12.66 ± 1.41	0.113 ± 0.014					$2.106^{n.s.}$

[a] a = assimilation rate constant; k = elimination rate constant; $T_{1/2}$ = half-life; d = moulting interval of exposed animals; EE_{est} = estimated excretion efficiency, calculated as $1 - e^{-dk}$; χ^2 = likelihood ratio-test statistic for differences between populations; $^{n.s.}$ = not significant

Table 6. Zinc concentrations (μmol g^{-1} dry wt) in *Orchesella cincta* (F_1 generation) from a reference population (1) and a contaminated population (7), exposed to a cadmium concentration of 0.13 (low) or 1.78 (high) μmol g^{-1} dry wt food, or to a zinc concentration of 3.0 (low) or 68.8 (high) μmol g^{-1} dry wt food

Treatment	Treatment	Cadmium		Zinc	
	Population	1	7	1	7
Low	Mean	1.346	1.857	1.104	1.878
	(S.E., n)[a]	(0.053, 46)	(0.074, 40)	(0.063, 45)	(0.105, 34)
High	Mean	1.284	1.718	1.513	2.157
	(S.E., n)	(0.098, 42)	(0.092, 34)	(0.060, 40)	(0.071, 34)

[a] S.E. = standard error; n = number of observations

Table 7. Two-way ANOVA (independent variables being population and treatment) for zinc concentrations (μmol g^{-1} dry wt) in *Orchesella cincta* (F_1 generation) from a reference population and a contaminated population (populations and treatments as in Table 6)

Treatment Transformation	Cadmium Logarithmic			Zinc Logarithmic		
Anova	df	M.S.	F	df	M.S.	F
Main effects						
Population	1	1.454	47.688^{***}	1	2.834	86.418^{***}
Treatment	1	0.091	$2.990^{n.s.}$	1	0.858	26.160^{***}
2-way interaction						
population * treatment	1	0.001	$0.043^{n.s.}$	1	0.064	$1.950^{n.s.}$
Residual	158	0.030		149	0.033	
Total	161	0.040		152	0.057	

$^{n.s.}$ = not significant: $P > 0.05$; $^{***}P < 0.001$

exposure did not cause an increase in excretion efficiency or moulting frequency. Benefits of development of tolerance seemed to be related mainly to decreased growth reduction upon exposure, probably caused by decreased body concentrations of cadmium. Costs of tolerance were absent, or obscured by multiple interactions between characteristics. The history of site contamination does not appear to be the sole factor determining population differentiation in *Orchesella cincta*.

The toxic effects of exposure eventually depend on the concentration of the metal in an animal at the target site. Accumulation of metals in terrestrial invertebrates is avoided or postponed by three mechanisms, which may occur together in a single species: behavioural avoidance of uptake, compartmentalization and excretion (Hopkin 1989). Metal avoidance has been shown in springtails (Joosse and Verhoef 1983; Tranvik and Eijsackers 1989), snails (Russell *et al.* 1981) and isopods (Van Capelleveen 1987). Compartmentalization may consist of metal accumulation in an innocuous form within organs and cells (Taylor and Simkiss 1984); *e.g.*, granules in hepatopancreas cells in isopods (Hopkin and Martin 1982) and metallothionein in fruit flies (Maroni *et al.* 1987). Selection for increased tolerance may improve the functioning of these mechanisms in exposed populations.

In *O. cincta*, avoidance of uptake did not significantly contribute to population differentiation: assimilated amounts (3 day exposure) or estimated assimilation rates (chronic ex-

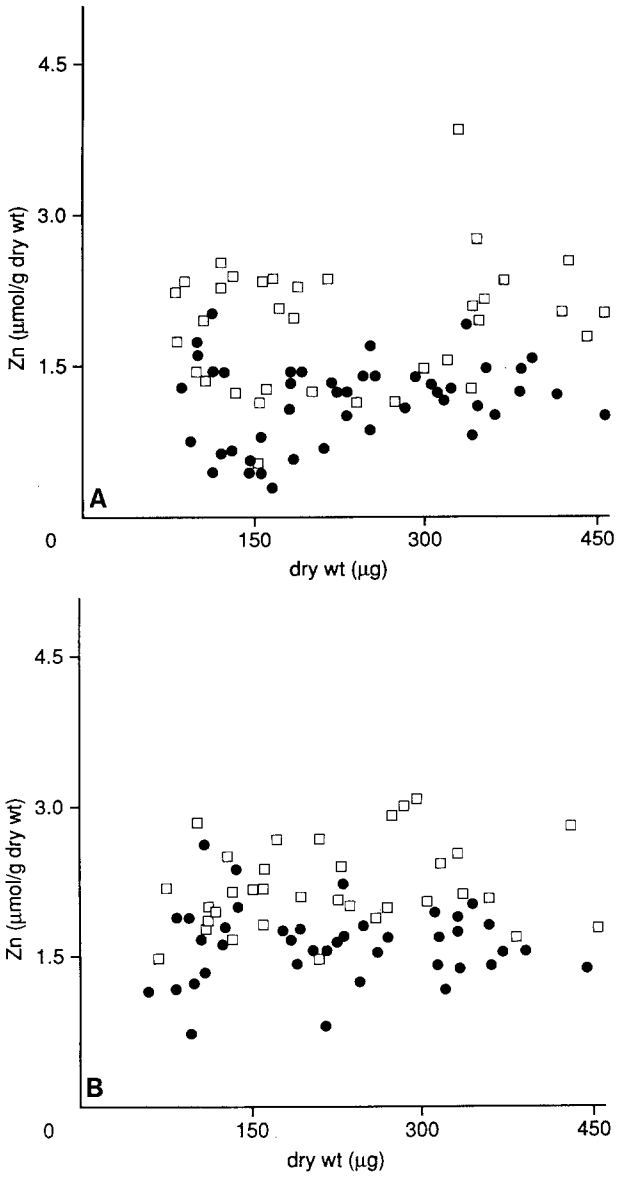

Fig. 5. Mean zinc concentrations (µmol g^{-1} dry wt) in F_1 generation *Orchesella cincta* from reference site 1 (points) and industrial site 7 (squares), exposed to a zinc concentration of 3.0 µmol g^{-1} (top) or 68.8 µmol g^{-1} (bottom) dry wt in the food. Slopes of regression lines of body concentration on weight are not significantly different from zero. For site codes: see Table 1

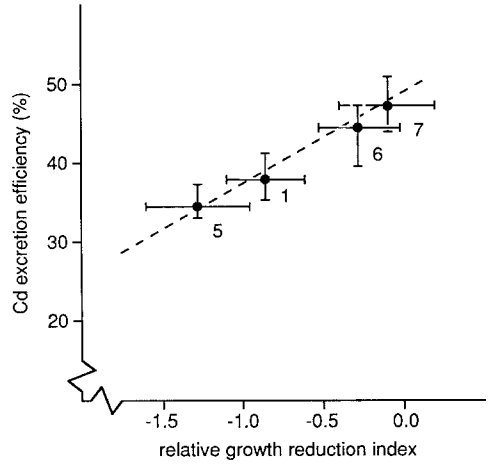

Fig. 6. Median excretion efficiency of 4 populations of *Orchesella cincta* in relation to mean relative growth reduction indices of females from the same populations. All data are based on observations of F_1 laboratory animals (data for growth reduction recalculated from Posthuma 1990). (Males show similar results). Horizontal bars indicate standard errors, vertical bars indicate 95% confidence intervals. For site codes: see Table 1

posure) did not show an adaptive difference. The other mechanisms of tolerance, compartmentalization and excretion, are mediated by the midgut epithelium. The flow of metals through an animal strongly depends on the characteristics of this compartment, due to its dual role in ion regulation and excretion. A two-compartment model of *O. cincta* was formulated, based on a study of the ultrastructure of the gut in *Orchesella villosa* (Geoffroy) (Dallai 1966) (See Figures 1 and 4). The pattern of inter-individual variation of whole-body concentrations was comparable for experimental results and the results of computer simulations of this model. The distribution of metals over body compartments can be interpreted at moulting. Directly before a moult, the metal burden of an individual can be partitioned between gut epithelium and body on the basis of excretion efficiency values. Time-dependent metal distribution is further illustrated in Figure 4. The similar estimates for the whole-body equilibria, in combination with significantly higher excretion at moulting imply that a larger proportion of cadmium is fixed in the gut in adapted populations. From both the compartment results and the individual correlations, there is evidence that tolerance development is (at least partly) accomplished by a change in metal kinetics. This mechanism may also explain the low copper concentration in *Onychiurus armatus* (Tullberg) found in field animals at the most polluted sites around a brass mill (Bengtsson and Rundgren 1988), and the occurrence of reproducing Collembola at a natural pollution site with 10–15% lead (dry wt) in humus (Hågvar and Abrahamsen 1990).

Beneficial differences in metal kinetics have been reported for field animals from exposed populations of isopods (Hopkin and Martin 1982; Van Capelleveen 1987; Hopkin 1990), centipedes (Hopkin and Martin 1984) and snails (Beeby and Richmond 1987) and for copper regulation in F_1 laboratory isopods (Donker and Bogert 1991). The development of tolerance, however, may be linked to detrimental effects for other characteristics. This expectation is based on life-history theory (Stearns 1989), and can be extended to ecotoxicology (Calow 1989). Phenotypic detrimental effects of heavy metal tolerance have been hypothesized for several invertebrate species (Ireland and Richards 1977; Joosse *et al.* 1983; Van Capelleveen 1987; Morgan *et al.* 1990). For *O. cincta,* metal excretion was hypothesized to be an energy demanding process (Joosse and Verhoef 1983). In the present study, however, no detrimental effects of increased cadmium excretion on body growth were found within population comparisons. Instead, inter-population comparisons revealed that an increased median excretion efficiency coincided with a smaller mean reduction of the growth rate upon

exposure. Furthermore, a change of cadmium excretion abilities may also have affected the excretion of essential nutrients. In tolerant animals a higher concentration of zinc was found.

The population descended from site 5 seemed to be less tolerant in terms of excretion efficiency than expected from site contamination. It is well known that genetic differentiation may be caused by selection for the characteristic itself, by selection for genetically correlated characteristics, or by other environmental factors correlated with the factor under investigation (Sober 1984; Endler 1986; Bendell-Young *et al.* 1986). In worms (Ireland 1975; Morgan and Morgan 1988), isopods (Beeby 1978) snails (Beeby and Richmond 1987, 1988, 1989) and fruit flies (Christie *et al.* 1983), metal toxicity partly depends on calcium, probably due to interference with granule formation (Taylor and Simkiss 1984). As the reference populations experienced different calcium concentrations in the habitat and had different excretion efficiencies, it can be hypothesized that the calcium status of the forest floor is one of the factors influencing metal adaptation in *O. cincta*. Additional observations were made on F_1 animals from Overpelt, Belgium. Animals were captured at a site comparable to site 5 for both calcium status and contamination history (cadmium and zinc from a zinc smelter), and excretion efficiency values appeared to be similar to site 5. Comparison of populations from sites of similar calcium status (Table 1) showed that the most tolerant populations are present at the most polluted sites (viz. sites 5 and Overpelt compared to 2, and 7 compared to 1). This hypothesis should be tested further to elucidate whether or not the populations from site 5 and Overpelt are more tolerant than animals from similar, but not contaminated sites.

The contribution of selection to genetic differentiation should be analyzed further by determining the additive genetic variation of tolerance characteristics in a reference population. A significant additive genetic variation for excretion efficiency forms a basis for adaptation and shows that directional selection is low or absent in such populations. The present results, giving evidence for a genetic component of differentiation, imply that a selectable amount of tolerance variation is present in uncontaminated populations of *Orchesella cincta*.

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