The influence of dietary nitrogen and phosphorus on Cd accumulation in the woodlouse Porcellio scaber Latr

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Abstract

Bioaccumulation of potentially toxic metals by litter-feeding invertebrates is determined not only by the concentration of metal in the diet, but also by the flux of food through the body. Nitrogen and phosphorus are the main elements regulating food consumption and so are expected to affect the bioaccumulation of trace elements such as cadmium. To test this idea, we applied a three-factor orthogonal experimental design to estimate the effects of nitrogen, phosphorus and cadmium additions to the food on cadmium accumulation by the terrestrial isopod Porcellio scaber. Cd, N and P were added to milled poplar litter in concentrations of 0, 10 and 20 μg g⁻¹ for cadmium, 0, 0.875 and 1.75% for nitrogen and 0, 0.2 and 0.4% for phosphorus. Observations were made for daily food consumption (estimated from faecal pellet production) and weekly Cd accumulation over a period of 4 weeks. Dietary Cd decreased consumption in the first 2 weeks of the experiment, but this effect disappeared later. Phosphorus had a significant positive effect on consumption in the second and the third week of the experiment. Accumulation of Cd was determined mainly by the Cd concentration in the food, but the effect was stimulated by P and diminished by N additions. Analysis of the data by regression using response surfaces confirmed that N and P had opposite influences on both Cd accumulation and food consumption. The data underline the importance of measuring consumption and food quality when conducting bioaccumulation and ecotoxicity experiments with soil invertebrates exposed through the diet. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bioaccumulation; Cadmium; Heavy metals; Isopoda; Nutrition; Soil invertebrates

1. Introduction

Food is an important source of contaminants as well as nutrients for soil animals. An elevated concentration of heavy metals in food commonly leads to an increased concentration in the body (Reichle et al., 1970; Martin and Coughrey, 1982; Pokarzhevskii, 1985; Hopkin, 1989). However, metal accumulation depends not only on the concentration in food but also on the rate of food passage through the body. Metal accumulation should be considered as a balance between input and output processes (Pokarzhevskii, 1985; Hopkin, 1989; Fågerström, 1991).

Nitrogen and phosphorus are the main elements determining animal productivity and food requirements for animals, including invertebrates (Dunger, 1958a,b; Prosser, 1973; House, 1974; Satchell, 1983; Gunnarsson, 1987). Within certain limits, low concentrations of N and P in the food will stimulate consumption, as is well known from livestock farming practice. We have suggested earlier (Pokarzhevskii, 1985; Pokarzhevskii and Van Straalen, 1996) that N
and P regulate the passage of food through the animal body and that hence these elements could also affect heavy metal uptake and accumulation.

To test the idea of N and P regulating trace metal accumulation it is necessary to manipulate dietary N and P concentrations at a constant level of heavy metal. In most studies these factors are considered separately. This is due to the problem of how to determine the influence of more than two interacting factors simultaneously in a single experiment. A suitable method for such an experiment is a multi-factorial design. This approach has been used in many fields of science especially in the determination of optimal conditions for technological processes including industrial microbiology and agrochemistry (Nalimov and Chernova, 1965; Adler et al., 1973; Mead, 1988). We have used the method for ecotoxicological and radioecological studies some years ago (Terytze et al., 1989; Pokarzhevskii et al., 1993). A similar approach was applied by Loch et al. (1993) to assess the influence of different factors on Cr accumulation by ryegrass. Multifactorial response surface analysis assumes that the distances between the levels of a factor are equally spaced and can be represented by codes such as $-1$, 0 and $+1$. This simplifies the construction of orthogonal regression models of interactions between factors.

Soil animals such as earthworms, woodlice and diplopods are good subjects for testing the hypothesis concerning the influence of N and P on trace metal accumulation. These invertebrates consume soil and litter and, although the attractiveness of the food depends on microbial infestation, they do not pick out particular species growing on the food (bacteria, microfungi, etc.), as do microarthropods. This allows N and P compounds to be added directly to soil or litter without taking into account reallocations within the food, as in the case with microarthropods. In a pilot study (Pokarzhevskii et al., 1994) we found that N and P influenced Cu accumulation in the earthworm Octolasium lacteum (Oerley).

Woodlice (Crustacea: Isopoda: Oniscoidea) are convenient animals for such ecotoxicological experiments as conditions for their rearing and breeding and their physiological features are well known (Sutton, 1972; Warburg, 1987; Hopkin, 1989; Van Straalen and Donker, 1994). They survive well in captivity, readily consume litter including contaminated materials and are one of the best investigated soil invertebrates in ecotoxicology (Drobne, 1997). Hopkin et al. (1993) suggested the use of the woodlouse Porcellio scaber (Latreille) as a monitor of bioavailability of metals in terrestrial ecosystems.

Based on the hypothesis formulated above, we expected that manipulation of the N and P contents in the food would affect accumulation of trace metals. We conducted a multi-factorial experiment, exposing isopods to cadmium-contaminated food at various levels of N and P, to test this expectation.

2. Materials and methods

2.1. Animals

Isopods (Porcellio scaber) were taken from the laboratory culture of the Department of Animal Ecology of the Vrije Universiteit, Amsterdam, which originated from the forest Spanderswoud. The size of animals varied between 30 and 70 mg fresh weight. Specimens of both sexes were used in the experiment, excluding gravid females.

2.2. Experimental design

We chose an experimental design with three levels for every factor; this allows the inclusion of second order interactions in the regression model. The following levels of N, P and Cd were applied in the experiment. The first level for each element was the background concentration in the original leaf litter used as food. The second level for N corresponded to addition of 0.875% N (on a dry mass basis), for P an addition of 0.2%, and for Cd $10\mu g g^{-1}$. The third level for N was an addition of 1.75%, for P 0.4%, for Cd $20\mu g g^{-1}$. The treatments are designated by the codes $-1$, 0 and $+1$; the codes are used as independent variables in the regression. The additions were selected in accordance with previous data on the toxicity of the compounds. Nitrogen was added as NH$_4$NO$_3$, P as Ca(H$_2$PO$_4$)$_2$·H$_2$O and Cd as Cd(NO$_3$)$_2$·4H$_2$O. The acute toxicity of NH$_4$NO$_3$ is relatively low; for mammals the LD$_{50}$ varies from 3500 to 6000 mg kg$^{-1}$ of animal body mass (Hazardous Chemical Compounds, 1988). The increase of N in the food was in the range known for natural N concentrations in leaf
litter (up to 3%, see Van Wensem et al., 1992). The toxicity of calcium phosphate is very low, effects on larvae of *Tribolium confusum* Duval being observed at a concentration of nearly 3% P in food (Chaudhary and Lemonde, 1962). Effects of Cd contamination on food consumption were marked at concentrations above 20 µg g⁻¹ in food (Donker, 1992). Hence only the highest Cd concentration chosen was close to the effective limit for isopods.

2.3. Food preparation

Senescent poplar leaves from an uncontaminated reference site (measured Cd content about 0.2 µg g⁻¹ dw) were ground in a coffee mill and sieved through a 0.5 mm mesh. The powder thus obtained was mixed with an equal volume of 1% glucose solution to immobilize added N by stimulation of microfloral development. After this procedure the food was dried for 2 days at 50°C in a desiccator. After drying, part of the food was used for acclimating the animals to the experimental food and the rest (240 g on a dry mass basis) was divided into three portions. The first portion was used as it was, without further additions. A solution of Cd(NO₃)₂·4H₂O was added to the other two portions to obtain the second and third levels of cadmium. All portions were then divided into ten small portions of 8 g each. Phosphorus as a 0.244% Ca(H₂PO₄)₂·H₂O solution and nitrogen as a 0.75% NH₄NO₃ solution were added to every sample corresponding to the experimental design. To obtain the second and third levels, 26 or 52 ml of the corresponding solution was applied to an 8 g portion of the food. Calcium carbonate as a 0.11% suspension was added at 52 ml per portion to the portions without and 26 ml per portion to the portions with mean addition of Ca phosphate, to equalize the Ca concentration of the food and to eliminate a possible influence of Ca added with the phosphate. Nitrogen added with cadmium nitrate was not compensated for because it did not increase the N content of the food significantly; it contributed only 0.06% to total N at the second level of N application and 0.03% to total N at the third level of N application. After adding all compounds the food was dried at 50°C and stored at room temperature. Directly before feeding a part of the dried food was mixed with distilled water at a ratio of 1:3 to obtain a dispensable paste.

2.4. Experimental procedures

Every animal was kept individually in a small plastic pot (5 cm in diameter and 4 cm in height) covered by a perforated lid. The bottoms of the pots were filled with a plaster of Paris layer up to 1/3 of the depth of the pot. Animals were put into the pots and every animal was provided with clean food (about 0.2 g fresh mass) for acclimation. A piece of pottery or plastic sponge was put into every pot as a humid shelter for the woodlouse. Pots were placed on trays in a climate chamber (temperature 20°C, relative humidity 75%, light to dark periods as 12:12, light period beginning at 8.00 h a.m.). After 10 days all experimental animals were weighed, the control food was removed from the pots and the experimental food was distributed (0.1 g per pot) in accordance with the experimental plan. Ten animals were selected at that moment as the reference group to estimate the initial Cd concentration of the animals. Every experimental group initially included 20 animals and the total number of pots was 540 (= 3 x 3 x 3 x 3 x 20).

Every pot was examined daily to check the animals, and to remove and count their faecal pellets. Faeces counting was used as an estimation of food consumption for each individual because for adult isopods a linear relationship holds between consumption and number of pellets (cf. Khalil et al., 1995). Faeces were removed daily to prevent coprophagy which can influence Cd accumulation. Food and water were added during inspections when necessary. The duration of the experiment was 4 weeks. Every week from every experimental group three to five woodlice (depending on death or escapes) were collected. Dead animals were removed during the inspections and were not taken into account. Animals were selected by random number allocation. Before chemical analysis the animals were kept without food for 12 h and then frozen.

2.5. Chemical analysis procedures

The selected experimental animals were weighed on a microbalance, their sex was determined under a dissecting microscope and they were then freeze-dried for 24 h. Every lyophilized animal was weighed again to determine the dry weight. It was then digested in a 2.5 ml pyrex tube using a mixture of concentrated HNO₃ and HClO₄ (7:1, Ultrex quality) at 160 and
185°C till dryness. Before Cd determination 1 ml of 0.1 N HNO₃ was added to every tube to dissolve the pellet. Samples with digestion mixture but without animals (one per 10 animal samples) and certified reference material (bovine liver, BSA, ref. Material no. 185) (one per 10 animal samples) were used to check the quality of the analytical procedures. The analytical results obtained for the reference material were usually within 10% of the certified value but in a few cases deviated up to 17%. No corrections were made for this. Food was digested in a microwave oven for 50 min in teflon tubes. Every tube contained a well ground dry sample of food (near 100 mg dwt), 4 ml concentrated HNO₃, 1 ml HCl and 1 ml demineralized water. After digestion the sample was made up to 25 ml with demineralized water.

Cadmium determinations were done by flame atomic absorption spectrophotometry (Perkin–Elmer 1100) for both isopods and food.

2.6. Statistical analysis

The experimental results were analyzed using the STATGRAPHICS 5.0, STATGRAPHICS PLUS for WINDOWS 2.1 and Lotus 1-2-3 1.0. software packages. The first two packages have special options for analysis of specific experimental designs. We analyzed the data as a three factor orthogonal experimental design (N, P and Cd in food as factors) and the response surface of the second order both for Cd accumulation in isopods and food consumption (faecal pellet production) was described by:

\[ y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 \]  

(1)

where \( y \) is Cd concentration in \( \mu g \text{g}^{-1} \) dwt or daily faecal pellet production, \( x_1 \) is nitrogen concentration, \( x_2 \) is phosphorus concentration and \( x_3 \) is cadmium concentration (all in coded form), and the \( b \) values are coefficients estimated from the data by regression. The model allows for linear effects of N, P and Cd (with coefficients \( b_1, b_2, \) and \( b_3 \)), two-way interactions between the factors (with coefficients \( b_{12}, b_{13}, \) and \( b_{23} \)) and non-linear, quadratic effects of the single factors (with coefficients \( b_{11}, b_{22}, \) and \( b_{33} \)). The model was considered to describe the data satisfactorily if the \( F \)-test for lack-of-fit had \( P > 0.05 \).

3. Results

3.1. Weight gain

Weight changes during the experiment were not significantly different between the groups. Body growth was slightly (not significantly) higher during the first week for animals fed litter without Cd than for individuals fed contaminated food, and then increased during the second week of the experiment in almost all groups. In other periods of the experiment the mean weight of the different groups varied between 95 and 105% of the initial weight.

3.2. Faecal pellet production

Faecal pellet production by individual animals fluctuated greatly from day to day and from week to week (Table 1). Overall, there was a trend of increasing pellet production from the first week to the fourth week. In the first 2 weeks of the experiment, Cd had a significantly negative effect on faecal pellet production (\( P < 0.001 \) in the first week, \( P = 0.007 \) in the second week). In the second and the third weeks, phosphorus had a significantly positive effect on consumption (\( P = 0.006 \) in the second week, \( P = 0.029 \) in the third week). In addition, the non-linear component of nitrogen was significant in the second (\( P = 0.037 \)) and the fourth week (\( P < 0.001 \)). At the end of the experiment daily pellet production was quite similar in all groups. It is also important to note that uncontaminated food after glucose addition was covered by a tuft of fungal mycelium in the beginning of the experiment while contaminated food had no visible fungal mycelium in the food.

The three-factor regression for faecal pellet production coincided with the data (non-significant lack of fit), however, it explained only 4–16% of the variability (Table 2). It is evident that there is a very large random component in the consumption, which cannot be explained by the dietary factors considered here. Nevertheless, the coefficients in Table 2 illustrate that the main factor determining faecal pellet production (food consumption) of woodlice was the Cd concentration of the food itself in the first 2 weeks (coefficient \( b_3 \)). This factor is negative for food consumption, while for Cd accumulation it is positive. The negative influence of dietary cadmium on pellet production was...
especially notable in the beginning of the experiment and disappeared later. Nitrogen and phosphorus began to play a main role in food consumption of isopods during the third and fourth week of the experiment. The effect of nitrogen included a negative nonlinear component, indicating an optimum curve rather than an overall increase or decrease; the effect of phosphorus was generally positive (Table 2). Because the full model cannot be plotted in a graph, we used three-dimensional versions to illustrate the effects of N and P on the pellet production of isopods only for the intermediate Cd level (code 0), see Fig. 1. In accordance with the statistical tests, the response surfaces illustrate the overall positive effect of phosphorus and the non-linear (quadratic) effect of nitrogen.

### 3.3. Cadmium accumulation in isopods

The mean initial cadmium concentration of isopods before the experiment was 17.0 μg g⁻¹ (standard error was 0.85). Cadmium concentrations increased with time and with Cd concentration in the diet (Table 1). Tests for the main (linear) effect of Cd were significant in all 4 weeks (P < 0.001). There was also a small but significant non-linear effect of Cd in the fourth week (P = 0.023). After the first week the Cd concentrations in the various groups were similar; only in the group without any addition to the diet was it lower than the initial concentration. In the course of the following 3 weeks the average cadmium concentration in isopods fed uncontaminated litter became higher.
Table 2
Estimated coefficients for the three dimensional response surfaces fitted to faecal pellet production or cadmium concentration in isopods as a function of cadmium, nitrogen and phosphorus concentrations in fooda

<table>
<thead>
<tr>
<th>Week (d.f.)</th>
<th>b₀</th>
<th>b₁</th>
<th>b₂</th>
<th>b₃</th>
<th>b₁₂</th>
<th>b₂₃</th>
<th>b₁₁</th>
<th>b₂₂</th>
<th>b₃₃</th>
<th>Lack-of-fit (P-value)</th>
<th>R² (%)</th>
<th>R² (adj. to d.f.)</th>
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</thead>
<tbody>
<tr>
<td>Daily faecal pellet production of isopods</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 (501)</td>
<td>7.98</td>
<td>0.01</td>
<td>0.11</td>
<td>−1.32</td>
<td>−0.20</td>
<td>0.03</td>
<td>0.33</td>
<td>0.78</td>
<td>−0.08</td>
<td>0.64 · 0.88</td>
<td>7.8</td>
<td>6.1</td>
</tr>
<tr>
<td>2 (377)</td>
<td>9.60</td>
<td>0.03</td>
<td>0.72</td>
<td>−0.71</td>
<td>−0.52</td>
<td>0.02</td>
<td>−0.47</td>
<td>−0.94</td>
<td>0.33</td>
<td>0.53 · 0.64</td>
<td>6.8</td>
<td>4.5</td>
</tr>
<tr>
<td>3 (271)</td>
<td>10.41</td>
<td>0.07</td>
<td>0.66</td>
<td>0.59</td>
<td>−0.11</td>
<td>0.15</td>
<td>−0.18</td>
<td>−0.42</td>
<td>−0.50</td>
<td>0.29 · 0.75</td>
<td>3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>4 (127)</td>
<td>14.21</td>
<td>0.17</td>
<td>0.19</td>
<td>0.00</td>
<td>0.98</td>
<td>0.80</td>
<td>−0.36</td>
<td>−3.34</td>
<td>−0.32</td>
<td>−0.18 · 0.82</td>
<td>15.71</td>
<td>9.3</td>
</tr>
<tr>
<td>Cd concentrations in isopods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 (125)</td>
<td>21.71</td>
<td>0.10</td>
<td>1.55</td>
<td>3.11</td>
<td>−0.80</td>
<td>−1.69</td>
<td>−1.39</td>
<td>1.32</td>
<td>−0.89</td>
<td>−0.42 · 0.76</td>
<td>25.0</td>
<td>19.1</td>
</tr>
<tr>
<td>2 (133)</td>
<td>23.64</td>
<td>−0.53</td>
<td>0.19</td>
<td>7.53</td>
<td>−1.06</td>
<td>−1.00</td>
<td>−0.51</td>
<td>1.33</td>
<td>1.46</td>
<td>1.88 · 0.72</td>
<td>42.7</td>
<td>38.5</td>
</tr>
<tr>
<td>3 (109)</td>
<td>28.64</td>
<td>−0.08</td>
<td>0.57</td>
<td>8.11</td>
<td>2.65</td>
<td>−0.15</td>
<td>1.58</td>
<td>0.01</td>
<td>−0.73</td>
<td>4.23 · 0.19</td>
<td>40.0</td>
<td>34.6</td>
</tr>
<tr>
<td>4 (125)</td>
<td>34.28</td>
<td>−1.50</td>
<td>−0.26</td>
<td>13.60</td>
<td>−2.35</td>
<td>−0.47</td>
<td>−1.86</td>
<td>0.43</td>
<td>−1.00</td>
<td>0.25 · 0.31</td>
<td>60.8</td>
<td>57.8</td>
</tr>
</tbody>
</table>

*a See Eq. (1) for the model. The interpretation of the coefficients is as follows: b₀ = effect independent of any factor, b₁ = linear effect of N, b₂ = linear effect of P, b₃ = linear effect of Cd, b₁₂ = effect due to interaction between N and P, b₁₃ = effect due to interaction between N and Cd, b₂₃ = effect due to interaction between P and Cd, b₁₁ = quadratic effect of N, b₂₂ = quadratic effect of P, b₃₃ = quadratic effect of Cd

Fig. 1. Response surfaces for the effects of nitrogen and phosphorus on daily faecal pellet production of isopods during the experiment, for the intermediate Cd level (code 0) in week 1 (A), week 2 (B), week 3 (C) and week 4 (D). Nitrogen and phosphorus are given as codes and increase from −1 to +1.
than the initial value. There were small, but significant, interactions between nitrogen and cadmium in the first week ($P = 0.039$) and between phosphorus and cadmium in the third week ($P = 0.045$). These interactions illustrate that dietary nitrogen diminished the positive trend for Cd, while phosphorus reinforced it. As in the case of faecal pellet production, the data show that phosphorus has a stimulatory effect and nitrogen a mostly negative effect on Cd accumulation.

The coefficients of the factorial response regression are presented in Table 2. The regressions explained 24–61% of the variability of Cd accumulation in isopods and there was no significant lack of fit. The signs of the coefficients demonstrate the strongly positive effect of dietary Cd ($b_3$ and $b_{33}$), and the generally negative effect of nitrogen ($b_1$ and $b_{13}$). There were no significant residual differences between experimental and model data and standard deviations commonly overlapped differences between experimental and model data. This is confirmed by the non-significant $F$-values for the $F$-test for lack-of-fit (Table 2). Because the full model cannot be plotted in a graph, we used three-dimensional versions for the intermediate level of Cd, to illustrate the effects of N and P on Cd in isopods (Fig. 2). The regression describes a slow increase in Cd accumulation after the first 2 weeks of the experiment and a significant increase in accumulation over the last 3 weeks.

3.4. Correlations among response variables

The Cd concentrations in isopods were correlated with other variables in several cases. Correlation coefficients between Cd and faecal pellet production, dry and fresh mass of animals, ratio dry mass to fresh mass (water content), and weight gain were significant at a $P$-value smaller than 0.05, if we consider the groups fed food with different levels of contamination.
separately (Table 3). In the groups fed contaminated litter (Cd = 0, Cd = +1), animals with a high consumption also had a high Cd concentration. In the groups fed clean food (Cd = −1) there was no such correlation (Table 3); in these animals a weak, but significant negative correlation was present between internal Cd and dry body mass, weight gain and fresh/dry mass ratio.

### 4. Discussion

Our experiment has demonstrated that nitrogen and phosphorous may play a role as dietary factors that affect contaminant accumulation in animals. The two nutrients appeared to have opposite effects on Cd accumulation. The three-dimensional response regressions showed that phosphorus addition to food stimulates Cd accumulation (except at high N in food), while nitrogen additions decreased it. There are, however, no simple linear impacts of the nutrient additions in diet. The coefficients of the second order (b<sub>11</sub> and b<sub>22</sub>) had signs opposite to the first order coefficients (b<sub>1</sub> and b<sub>2</sub>) and this reflects the complicated nature of the effects. The effects also varied with time: interactions between nitrogen and phosphorus increased with time of exposure although in the last week the interaction decreased accumulation. The effects of nitrogen and phosphorus on Cd accumulation were, however, relatively small in comparison with dietary Cd itself, although this effect was small after the first week and began to increase after the second week. The higher the cadmium content in food the smaller were the effects of dietary nitrogen and phosphorus on Cd concentration in isopods. This may be explained from the physiology of Cd in isopods and the impact of dietary quality on food consumption.

As we supposed when designing our experiment Cd accumulation in isopods is connected directly with food consumption (faecal pellet production). Cd accumulation is however more strongly correlated with the accumulated food consumption over 4 weeks than with daily food consumption. The animal body acts as an integrator and so accumulation is more stable than intake rate. This is well known from radioecological and ecotoxicological practice (Pokarzhevskii, 1990; Fågerström, 1991; Pokarzhevskii and Van Straalen, 1996). In addition, Cd is hardly eliminated from woodlice in accordance with the total volume of food consumed (total number of pellets) more than with the flow of food through the gut (daily faecal pellet production).

The time-varying and partially opposite effects of nutrient additions on Cd accumulation by isopods suggest that these effects are not related only to food consumption as such but also derive from interactions in the gut (nutrient amendments changing the bioavailability of Cd), or from physiological interactions (nutrient amendments changing the metabolism of Cd). In studies with laboratory rats, calcium/phosphorus additions to the diet decreased Cd assimilation (Groten et al., 1991); and in studies with Japanese quail, uptake of Cd was affected by the Zn status of the diet (McKenna et al., 1992). The interaction between Cd and N (significant in the first week of the experiment) may relate to the synthesis of cadmium binding proteins in isopods. Up to now a metallothionein has not been found in Porcellio scaber; however other
proteins have been found to bind cadmium in isopod bodies (Donker et al., 1990; Dallinger, 1993), and the synthesis of these proteins may be alleviated by a high availability of nitrogen in the diet. Phosphorus may act as an internal immobilizing factor by precipitating metals in intracellular deposits (Prosi et al., 1983).

The effect of dietary Cd on Cd concentration in isopods increased with exposure time while the effects of nitrogen and phosphorus were relatively stable during the experiment. The significance of dietary nitrogen and phosphorus on Cd concentration in isopods was nevertheless noticeable during the experiment, except during the second week. The direct effect of nitrogen addition increased (coefficient $b_1$) while the effect of phosphorus decreased (coefficient $b_2$) during the experiment (Table 2). This was apparently connected with changes of dietary nutrient impact on food consumption of isopods. Dietary Cd content had a significant negative influence on food consumption during the first 2 weeks of the experiment while nitrogen and phosphorus effects (coefficients $b_{11}$ and $b_{22}$) appeared only after the second week (Table 2). There are opposing tendencies in the phosphorus impacts on food consumption and Cd accumulation in isopods whereas the nitrogen impacts partially coincided. This is reflected in the curvature of the response surfaces for faecal pellet production and Cd concentration of isopods which have different cross-sections.

The decreased Cd concentration in isopods in the first week of the experiment (in comparison to the concentration before acclimation) is possibly due to an interaction of Ca with Cd uptake. Extra calcium carbonate was added to the low calcium phosphate treatment to equalize calcium levels over all phosphate additions. As a consequence, the Ca concentration of the food during exposure to Cd was considerably higher than that during the acclimation period. The drop in Cd uptake observed in the first week of the experiment suggests that adding Ca to food lowers the availability of Cd. The influence of calcium on accumulation of heavy metals in invertebrates is well known (Hopkin, 1989; Beeby, 1990).

Cadmium in the food at 20 $\mu$g g$^{-1}$ decreased consumption and Cd accumulation in isopods during the first 2 weeks although Donker and Bogert (1991) did not observe an influence on food consumption of cadmium concentration up to 20 $\mu$g g$^{-1}$. The reason may be due to differences in the nutritional value of food. We used pure poplar litter while in the previous experiments the poplar leaf diet was amended with 10% dog food; this material makes the feed more attractive to isopods and effects of cadmium on food may be less evident under these conditions. It must be added that in our experiment the effect of Cd on consumption was limited to the first 2 weeks. Later in the experiment consumption converged to a value similar for all groups and phosphorus and nitrogen began to influence consumption.

The growth of the isopods in our experiment was slow, which is due to the use of adult animals. There were no effects of cadmium nor of N and P on the growth of Porcellio. This accords with the highest Cd concentration (20 $\mu$g g$^{-1}$) being well below the no-effect concentration for Cd effects on growth of isopods (65–95 $\mu$g g$^{-1}$, Van Wensem et al., 1992; Crommentuijn et al., 1995). Our results are also in agreement with Lavy (1996), who found no effect of additions of peptone (increasing the N-content of poplar leaf litter from 2.4–3.1%) on the growth of Porcellio.

The regression models (Table 2) described the experimental data satisfactory ($F$-tests for lack of fit were not significant), although only 4–16% of the variability of faecal pellet production and 24–61% of the variability in Cd concentration in isopods was actually explained. This confirms the suggestion made above, that the impact of dietary factors on Cd concentration in isopods is not strongly related to their impact on food consumption, but also to bioavailability of Cd in the gut and physiological interactions.

In our hypothesis we supposed that nitrogen and phosphorus content in food would be the main factors determining Cd accumulation in isopods due to changes in the nutritional value of the food and a decrease in food consumption. The picture, however, turns out to be more complicated. First of all, dietary cadmium is itself a factor affecting food consumption. Secondly, nitrogen and phosphorus have opposite effects on Cd accumulation up to the third week of the experiment (N has variable effects but overall tends to decrease Cd accumulation, P generally increases Cd accumulation). Thirdly, the relative importance of these effects changes with exposure time. It is clear that our knowledge of dietary factors that influence metal bioavailability and accumulation by isopods is still meagre.
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