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The influence of soil characteristics on the toxicity of cadmium for *Folsomia candida*, *Eisenia fetida* and glutamate mineralisation

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SAMENVATTING

In de hier beschreven experimenten werd getracht relaties vast te stellen tussen bepaalde bodemeigenschappen, met de nadruk op de pH, en de toxiciteit van cadmium voor de regenworm *Eisenia fetida*, de springstaart *Folsomia candida* en de mineralisatie van glutamaat door bodemmicro-organismen.

Het onderzoek werd uitgevoerd met drie grondsoorten, nl. een kunstmatig samengestelde (OECD) grond, een zandige grond uit Panheel en een humeuze zandgrond uit Wageningen. Cadmium werd bij een lage pH minder sterk geadsorbeerd aan de gronden dan bij een hoge pH, hetgeen resulteerde in hogere cadmium concentraties en chemische activiteiten in poriewater en grondextracten bij een lage pH. Er werd een lineaire relatie gevonden tussen enerzijds de logaritme van de evenwichts Cd^{2+} activiteit in oplossing (poriewater of extract met ionsterkte 0,01) en anderzijds de pH. De hellingcoëfficiënt van de deze relatie was in de orde -0,4 tot -0,9. Deze waarde komt goed overeen met soortgelijke waarden in de literatuur. Er was echter geen duidelijke relatie tussen de CEC, het lutum gehalte en het organische stof gehalte enerzijds en de concentratie of activiteit in de oplossing anderzijds. Er was ook geen duidelijke relatie tussen opgeloste organische koolstof in de bodemoplossing en de verhouding tussen concentratie van vrije, ongecomplexeerde Cd^{2+} en totaal cadmium in oplossing.

De LC_{50} voor *Eisenia* en *Folsomia* vertoonde een tendens om toe te nemen met stijgende pH. Dit effect werd ook waargenomen voor effecten van cadmium op de mineralisatie van glutamaat door bodemmicro-organismen, behalve bij Panheelgrond. In dit geval was de toxiciteit van cadmium lager bij lagere pH.

Indien effecten van cadmium op het gewichtsverlies van de wormen, en de gewichtstoename en populatiegroei van de springstaarten werden gerelateerd aan de concentratie en activiteit in poriewater en bodemextracten, dan was de toxiciteit van cadmium altijd lager bij hoge pH (4.5-7.8) dan bij lage pH (3.2-4.9). Het verschil was niet altijd significant.

Deze lagere toxiciteit zou kunnen duiden op een beschermend effect van H^+ ionen op de toxiciteit van cadmium bij lage pH.

SUMMARY

In this study we tried to establish a relationship between soil characteristics, with emphasis on soil pH, and the toxicity of cadmium to the earthworm *Eisenia fetida*, to the springtail *Folsomia candida* and to the mineralisation of glutamate by soil micro-organisms. The study was carried out with three soils: an artificial composite (OECD) soil, a sandy soil from Panheel and a humic sandy soil from Wageningen.

At low pH cadmium was less strongly adsorbed to the soils than at high pH, resulting in higher concentrations and Cd^{2+} chemical activities at low pH in pore water and soil extracts. A linear relationship could be found between the logarithm of the liquid (pore water or extract with 0.01 M ionic strength) equilibrium activity and the pH. The slope was -0.4 to -0.9. This value was in good agreement with values found in the literature. There was no clear relationship between Cation Exchange Capacity (CEC), clay content and organic matter content at one side and the liquid equilibrium concentration or Cd^{2+} activity at the other side. Also, there was no clear relationship between the dissolved organic carbon in the soil solution and the ratio of free, uncomplexed Cd^{2+} and total cadmium.

The LC_{50} for the earthworm *Eisenia fetida* and the springtail *Folsomia candida* tended to increase with increasing pH. This effect was also observed for effects of cadmium on mineralisation of glutamate, except for the Panheel soil, in which the toxicity was lowest at the lowest pH.

If effects of cadmium on weight loss of worms, or weight and population growth of springtails were related to concentrations or activities in pore water or soil extracts, the toxicity of cadmium was always lower at low pH (3.2-4.9) than at high pH (4.5-7.8). The difference was not always significant.

This observed effect may indicate a protecting action of H^+ ions against toxicity of cadmium at low pH.

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1. INTRODUCTION

The toxicity of heavy metals for soil organisms is generally determined in laboratory tests carried out under standard conditions. As an example, the well-known test with the earthworm *Eisenia fetida* in an artificially composed soil may be mentioned (OECD, 1984). However, tests on other animals may require other types of soils. In order to compare the results of tests carried out in different soils, it is necessary to know the influence of the various soil characteristics like pH, organic matter, clay content, redox potential, etc. on the toxicity of heavy metals.

Based on the results of such standard laboratory tests, concentration limits for heavy metals are established for field conditions. To deal with the soil characteristics that may be different from those in the test, correction factors are applied. In the Netherlands such correction factors are used for soil organic matter and clay content. These are based on statistical relationships between concentrations of metals in different, relatively clean soils and clay and organic matter content (Lexmond, 1987). These relationships have been used to develop correction formulas to derive concentration limits for heavy metals in soil (De Bruin and De Walle, 1988). The formulas were, however, not intended to be used to extrapolate ecotoxicological test results from one soil to another, as the correction factors do not take into account the effect of soil characteristics on the bioavailability of metals. In order to normalize test results obtained with different soils, other correction formulas may be necessary. Moreover, no correction method is available for the soil pH, which strongly influences the availability of metals in soil.

In a literature review (Vonk *et al.*, 1994) the effects of soil properties on the availability and toxicity of heavy metals to soil organisms (including plants) were summarised.

It appeared that a general insight in the effects of soil characteristics on the toxicity of heavy metals for soil organisms was lacking. This was mainly due to the limited number of data reported. More data were found on the relationship between soil properties and internal concentrations in soil organisms (including plants). For soil invertebrates the pH had a stronger influence on internal concentration than other factors and there was probably a relationship between the concentration in the soil (pore) water and the internal concentration. Also indications were found that a low pH did not always result in increased internal concentrations. Also in this case reliable data were mostly lacking.

Because knowledge of the physico-chemical behaviour (solubility, speciation, adsorption, etc.) of metals in soil was deemed crucial for insight in their bioavailability to organisms, a literature study was also carried out on this subject (Vonk, 1995). This study revealed that ion-exchange and adsorption to solid soil phases were more important than precipitation reactions and that adsorption to oxides, if present, was more important than to clay. A possible relationship between the concentration in the soil water phase and the pH could be derived, based on many similar literature data.

This study focuses on the relationship between the distribution of cadmium in soil and its effects on terrestrial organisms (micro-organisms, Collembola, earthworms). Starting point for this study forms the so-called pore water hypothesis (Figure 1.1). According to this hypothesis, the main route of uptake of metals in terrestrial organisms is via the aqueous phase: the pore water. As a consequence uptake and effects are mainly determined by the concentration of cadmium in the pore water, in equilibrium with adsorbed cadmium. As it is hypothesized that free Cd^{2+} ions will be the most important cadmium species determining toxicity, speciation of cadmium in the pore water will be of importance for its uptake and effects. The characteristics of the pore water (such as pH and ionic composition) may affect uptake as well.

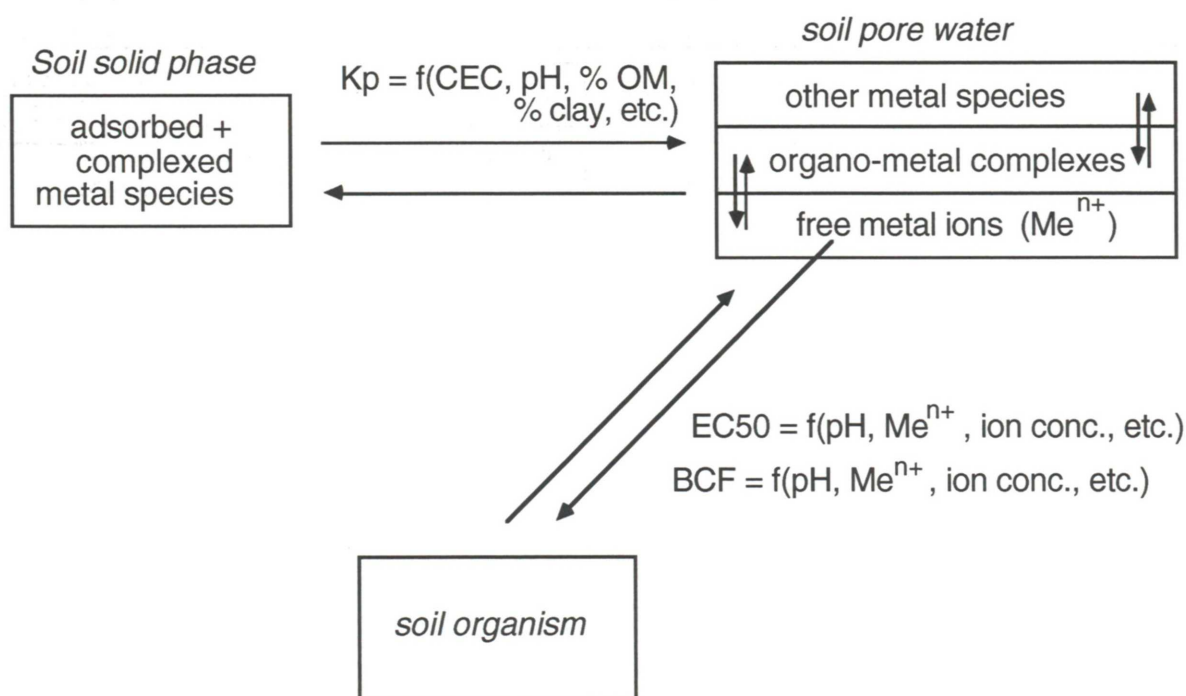


Figure 1.1 Pore water hypothesis for heavy metal uptake and effects on terrestrial organisms.

The study was designed as much as possible to test this hypothesis. Therefore, soils with different contents of organic matter and clay were chosen, because these soil constituents were known to adsorb metals and decrease their bioavailability.

The pH of each soil was adjusted artificially to three different levels, because the pH was thought to have a strong influence on the bioavailability of metals. Moreover, the pH may also affect the uptake processes of soil organisms. A low pH was expected to give a higher bioavailability of the metal.

Heavy metals are known to reside in the soil in pools with different availabilities to organisms, which is reflected by their extractability by different solvents. In this study attention was paid to this phenomenon by determining cadmium concentrations in pore water obtained by centrifugation, as well as in soil extracts of different ionic strength. For extraction we used water, a simulated soil solution (0.003 M CaCl_2 , 0.001 M KCl, 0.001 M NaCl), 0.01 M CaCl_2 and 0.1 M CaCl_2 . In the aqueous phase speciation of the metal by dissolved organic matter could lower its bioavailability. Therefore DOC and the concentration of free, uncomplexed cadmium ions were measured in pore water and extracts.

The effects of cadmium on three types of soil organisms, the oligochaete earthworm *Eisenia fetida*, the springtail *Folsomia candida*, and the indigenous population of soil micro-organisms were related to concentrations of total cadmium concentration and free Cd^{2+} ions in different extracts and pore water. For micro-organisms the effect of heavy metals on the process of mineralisation was studied rather than the organisms themselves, which is of course impossible. Because of financial and time constraints, plants were not included in the project. The organisms chosen were considered to be representative for different behaviour of organisms in soil and thus for the different routes of exposure to soil pore water.

Because of the relation of this research with the project on "the validation of laboratory toxicity data to field conditions" (Notenboom and Posthuma, 1995), zinc would have been the choice as metal to be studied. However, for the aim of the study it was necessary to be able to measure the (chemical) activity¹ of the free metal ion in the pore water rather than total concentrations, because of the strong indications that this activity is related to the rate of uptake and hence to the internal concentration in the organism. Since no specific methods to determine Zn^{2+} do exist, the choice for the present project had to be cadmium.

¹ Chemical activity of ions is explained in Part 2

The choice of the soils was also directed by the fact that the OECD composite artificial soil is the most standardized and widely used soil in toxicity tests with soil animals, and is the prescribed soil type for tests with earthworms (*E. fetida*) and Collembola (*F. candida*). The soil from Heel (province of Limburg, The Netherlands, here further named Panheel soil) obtained via the Panheel Gravel Comp. was used in both field and laboratory studies in the "Validation" project mentioned above. A limited program was also carried out with a humic sandy soil from Wageningen

This research project was carried out by co-operation of three research groups: TNO Institute of Environmental, Energy and Process Innovation Research, Delft, TNO Nutrition and Food Research Institute, Division Toxicology, Delft and Vrije Universiteit Amsterdam, Department of Ecology and Ecotoxicology.

2. MICRO-ORGANISMS

2.1 Methods and materials

2.1.1 Preparation of the soils

OECD artificial soil was prepared as described in the guideline for earthworm toxicity testing (OECD, 1984; NNI, 1988). It contained 10% organic matter (ground, pulverised Sphagnum peat, sieved over 1 mm), 20% kaoline clay and 705 quartz sand. pH was adjusted by adding calcium carbonate in different amounts. For the lower pH (pH = 3.9) no calcium carbonate was added. The soils were then pre-incubated for about 3 weeks at 22°C to establish the required pH (3.9;5.7;7.1). The water content was 35%.

"Panheel" soil was obtained from a bulk store at Vrije Universiteit Amsterdam. The moist part (about 6% moisture) of the store was chosen. It was brought to a moisture content of 15% (i.e. 40% of Water Holding Capacity) and preincubated for several weeks to restore microbial activity.

The pH(KCl) was ca. 5.2. Its organic matter content was 2.9% and its clay content 1.9%. It was brought to a pH of 3.9 by addition of 1 g of sulphur powder per kg soil and incubation for 1 week. A pH of 7.3 could be established by adding 1.5 g CaCO₃ to 1 kg soil. Further proceedings were as described above.

Wageningen humic sandy soil was obtained from Experimental farm Droevendaal, The Netherlands. Its pH(KCl) was 4.9, and it contained 3.8% organic matter and 1.9% clay. It was processed as described above.

Cadmium chloride, CdCl₂ (99%, Aldrich) was mixed thoroughly in with 250 g batches of the soils and preincubated a further 2 weeks. In the Panheel soil the concentrations were: 0, 3.2, 10, 32, 100 and 320 mg/kg; in the Wageningen and OECD artificial soil the concentration was 0, 10, 32, 100, 320, 1000 mg/kg

Homogeneity in the soil (32 mg/kg) was checked in OECD artificial soil by analysing five 1 g samples for total cadmium: the mean was 29.0 ± 3.9 mg/kg.

2.1.2 Effects of cadmium on glutamate mineralisation

The effect of cadmium on micro-organisms in soil was studied by their ability to mineralise sodium glutamate. Triplicate 50 g (dry wt.) batches of preincubated cadmium-containing soil were amended with 100 mg sodium glutamate in talcum (ca. 1 g). Thereafter CO₂-free, wet air was led through the samples. In the outstreaming air the concentration of CO₂ was measured every hour. The experiment was carried out at 22°C.

A scheme of the apparatus used is given in Figure 2.1. The procedure is described by Vonk and Matla (1993).

Inhibition of CO₂ output by cadmium was evaluated as follows. The time point of maximum CO₂ output in the control (no CdCl₂ added) was taken as the reference time. The cumulative CO₂ output (ml CO₂/kg soil) until the reference time was then calculated for all samples from the CO₂ concentrations and the air flow-rate. These data were fitted with a log-logistic model (Haanstra *et al.*, 1985) in SYSTAT to calculate the EC₅₀ value:

$$CO_2^i = CO_2^0 / (1 + \exp(b * (\ln i - EC_{50})))$$

where:

- i = cadmium concentration in soil (mg/kg)
- CO₂ⁱ = cumulative output of CO₂ at concentration i (ml/kg soil)
- CO₂⁰ = uninhibited CO₂ output (ml/kg soil)
- b = the slope parameter of the concentration-response curve

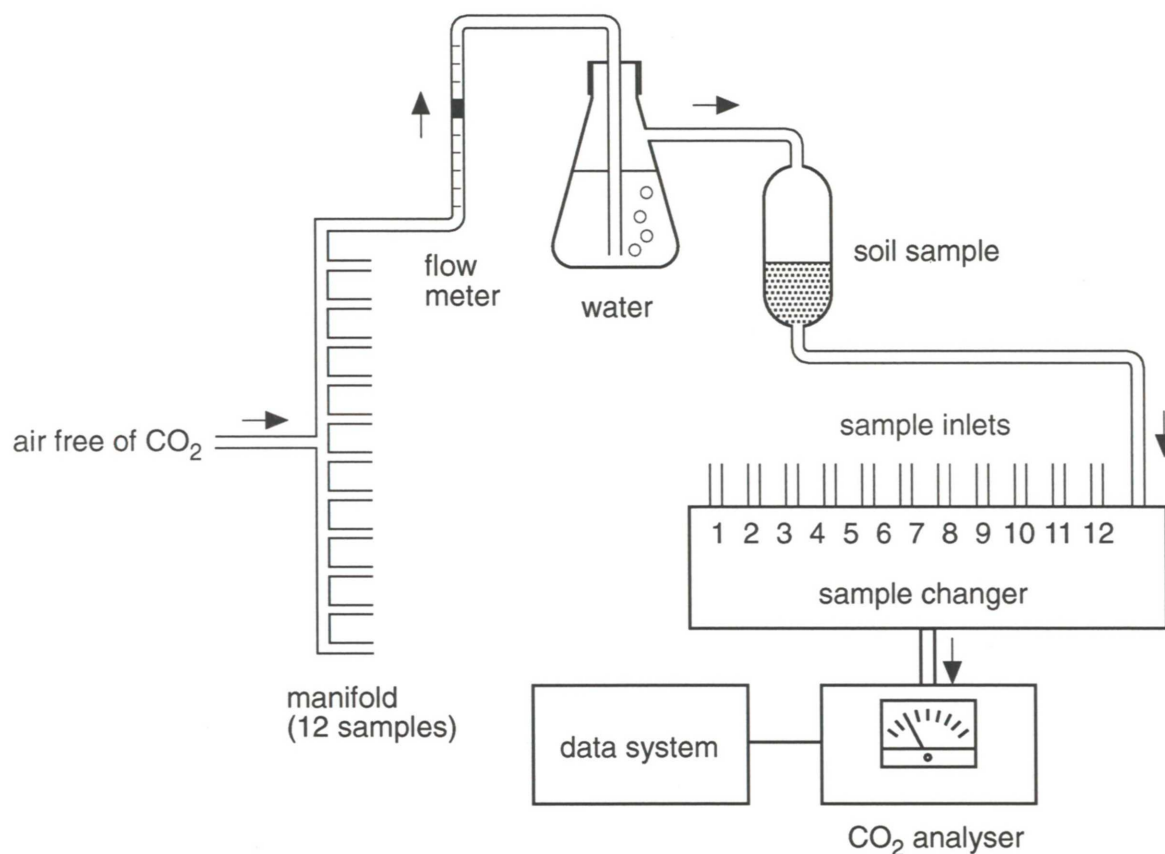


Figure 2.1 Flow-through system for measuring CO₂.

2.1.3 Determination of cadmium in the different soil fractions

Extraction

After the glutamate mineralisation test the triplicate soil samples were pooled and 20 g sub-samples were extracted in duplicate with 100 ml of either 0.1 M CaCl₂ or 0.001 M NaCl / 0.001 M KCl / 0.003 M CaCl₂. The latter could be a simulation of the ionic strength in soil solution (Gerritse and Van Driel, 1984). Extraction was carried out by shaking for at least 20 h. This is considered to be sufficient for a distribution equilibrium. Extracts were, if necessary, centrifuged and filtered over 0.45 µm.

Determination of cadmium

Cd^{2+} ion activity was measured directly in the soil/extractant suspension with a Metrohm ISE cadmium electrode. The millivoltage was read against a reference calomel electrode, and compared with a calibration line from known solutions of CdCl_2 , corrected for Cl complexation and ionic strength. The calibration curve was a straight line against $\log [\text{Cd}^{2+}]$ from 0.1 to 500 mg Cd/l. Before every measuring series calibration was carried out with 1 and 10 mg Cd/l. The measurements were not very accurate, because of the logarithmic concentration response. At low concentrations of Cd^{2+} other metals may disturb. The detection limit is ca. 0.04 mg/l.

Total cadmium in clear extracts was measured by standard procedures with a Perkin Elmer 5100 Flame Atomic Absorption Spectrometer.

Pore water

Pore water was obtained by centrifugation of 300 g soil samples in plastic tubes with a perforated bottom and a reservoir. The bottom was lined with filter paper.

2.2 Results

2.2.1 Adsorption behaviour of cadmium in different soils

In the extracts obtained with low salt concentration (0.005 M) and in pore water the total Cd concentration and the Cd^{2+} activity were determined. The cadmium Cd^{2+} activity (a_{Cd}) is defined as

$$a_{\text{Cd}} = f \cdot C_{\text{Cd}}$$

where:

f is the activity coefficient and

C_{Cd} is the total concentration of free, not-complexed Cd^{2+} ions.

Actually, a_{Cd} is the concentration of Cd^{2+} ions which take part in chemical equilibria, such as adsorption phenomena and probably also in uptake processes by biota.

f can be calculated by the Debye-Hückel approach:

$$-\log f = 0.51 \cdot z^2 \cdot I^{1/2} / (1 + 0.33 \cdot k \cdot I^{1/2})$$

where:

k is a constant depending on the effective size of the ion ($Cd = 5$)

I is the ionic strength of the solution, $I = 0.5 \sum C_i \cdot z_i^2$

(C_i and z_i are concentration and charge of each ion, respectively)

a_{Cd} can be measured by a cadmium-specific ion selective electrode, as was done in this study.

Table 2.1 lists the total concentration of cadmium and the Cd^{2+} activity in extracts of the different soils at three pH's used for the study of effects of cadmium on glutamate mineralisation by soil micro-organisms. Soil samples were also extracted with 0.1 M $CaCl_2$. This can be regarded as a measure for the exchangeable part, and therefore for totally available cadmium. These values are also listed in Table 2.1. In Part 3 similar experiments are reported with the same soils but slightly different pH's. Other types of soil extractions (aqueous extraction, 0.01 M $CaCl_2$) were also used in the experiments reported in Part 3.

From Table 2.1 it appears that the Cd^{2+} activity in the $CaCl_2/KCl/NaCl$ extracts ("extractable part") was generally of the same order and mostly slightly lower than the total cadmium concentration, except for the Panheel soil at pH 7, where considerable lower activities were measured. From the chloride concentration measured in the extracts (ca. 0.008 to 0.01 M) and the activity coefficient f it could be calculated (Boekhold *et al.*, 1993) that the free Cd^{2+} activity was at most 40-50% of the total cadmium concentration.

Dissolved Organic Carbon (DOC) was also measured and is given in Table 2.2. It was higher at higher pH in Panheel soil and considerably higher at lower pH in the OECD artificial soil. Yet there was no clear relationship between DOC and Cd^{2+} activity.

As expected, concentrations and activities in the $Ca/K/Na$ extracts were strongly influenced by the pH of the soils. The distribution of cadmium between the solid soil phase and the aqueous phase was calculated from the nominal amount of cadmium added and the equilibrium concentration in the extract. If no cadmium activities were available total concentrations were taken. For the Panheel soil pH 7, the Cd^{2+} activities of lower concentrations were calculated by proportionality. Adsorption isotherms are given in Figure 2.2 and 2.3. Adsorption of heavy metals onto soil can generally be well described by the empirical Freundlich equation:

$$C_s = K_f \cdot a_{Cd}^{1/n}$$

or: $\log C_s = \log K_f + 1/n \cdot \log a_{Cd}$

where

C_s	=	cadmium content adsorbed to the solid phase (in mg/kg)
a_{Cd}	=	cadmium activity in the solution at equilibrium (in mg/l)
$1/n$	=	a dimensionless exponent
K_f	=	the Freundlich adsorption constant

The double logarithmic plot of the data in Figure 2.2 and 2.3 shows a good fit with this Freundlich equation. In the OECD soil the points of low concentration (3.2 mg/kg) were omitted from the regression analysis carried out with STATGRAPHICS, because they clearly deviated. The calculated parameters of the Freundlich equations and the regression coefficients are given in Table 2.3.

The cadmium concentrations in the 0.1 M calcium chloride extracts were, as expected, much higher than in the 0.005 M Ca/K/Na extracts. They were much less influenced by the pH than the corresponding cadmium concentrations in the aqueous extracts. The "available" cadmium varied from 20-40% (pH 7.3) to 80% (pH 4.3) in the Panheel soil and from 50-60% (pH 7.1) to > 90% (pH 3.9) in the artificial OECD soil.

From the above data a relationship between pH and adsorption could be derived. Relationships were derived for four different concentrations in each soil type (Figure 2.4 and 2.5). Although only three pH's were available, a linear relationship between the pH and the logarithm of Cd^{2+} activity was strongly suggested. The following approximate relations were calculated by linear regression:

Artificial OECD soil:

$$\log a_{Cd} = -0.58(\pm 0.05) \cdot pH + \log C_s + 0.53(\pm 0.30), r^2 = 0.92, p < 0.001$$

Panheel soil:

$$\log a_{Cd} = -0.65(\pm 0.02) \cdot pH + \log C_s + 1.44(\pm 0.14), r^2 = 0.99, p < 0.001$$

It should be stressed that these relationships were obtained with simulated soil solutions.

A similar exercise with pore water from the Panheel soil in the *Eisenia fetida* experiment (Part 4) gave the following relationship:

$$\log a_{Cd} = -0.90(\pm 0.07) \cdot pH + \log C_s + 2.0(\pm 0.4), r^2 = 0.94, p < 0.001$$

In Part 3 relationships have been found between K_f and soil properties.

Table 2.1 Cadmium concentrations and activities in extracts of soils (average of duplicates).
Between brackets : percentages of total cadmium in the soil.

Panheel soil									
Nominal Cd in soil (mg/kg)	pH 4.4			pH 5.7			pH 7.3		
	Extract*** (mg/l)		CaCl ₂ extract (mg/l)	Extract (mg/l)		CaCl ₂ extract (mg/l)	Extract (mg/l)		CaCl ₂ extract (mg/l)
	Total Cd	Cd activity		Total Cd	Cd activity		Total Cd	Cd activity	
0	0.03	-*	0.05	0.01	-	0.03	0.002	-	-
3.2	0.11 (18)**	0.13 (20)	0.42 (75)	0.03 (7)	-	0.31 (55)	0.02 (4)	-	0.11 (19)
10	0.35 (20)	0.7 (40)	1.4 (80)	0.03 (1.5)	-	0.92 (53)	0.03 (1.5))	-	0.46 (26)
32	1.5 (27)	1.4 (23)	4.7 (84)	0.11 (1.9)	0.08 (1.4)	3.4 (60)	0.07 (1.3)	-	1.35 (24)
100	3.7 (21)	3.8 (21)	14.3 (82)	0.40 (2.3)	0.33 (1.8)	10.8 (62)	0.34 (2.0)	-	5.4 (31)
320	11.7 (21)	11.5 (21)	44 (79)	2.2 (4.0)	1.8 (3.2)	40 (71)	0.90 (1.6)	0.14 (0.25)	22 (40)

* Below detection limit

** Percentage

*** 0.005 M Ca/K/Na extract

Table 2.1 Continued.

OECD Artificial soil									
Nominal Cd in soil (mg/kg)	pH 3.9			pH 5.6			pH 7.1		
	Extract*** (mg/l)		CaCl ₂ extract (mg/l)	Extract (mg/l)		CaCl ₂ extract (mg/l)	Extract (mg/l)		CaCl ₂ extract (mg/l)
	Total Cd	Cd activity		Total Cd	Cd activity		Total Cd	Cd activity	
0	0.02	0.08	0.04	0.01	-	-	0.04	-	-
10	0.22 (14)	0.2 (13)	1.3 (89)	0.04 (2.7)	-	0.95 (63)	0.02 (1.3)	-	0.56 (37)
32	0.66 (14)	0.5 (11)	4.0 (83)	0.10 (1.1)	-	3.6 (76)	0.02 (0.3)	-	2.2 (46)
100	2.4 (16)	1.7 (11)	12.5 (84)	0.44 (3.0)	0.2 (1.4)	10.2 (68)	0.06 (0.4)	0.04 (0.3)	8.3 (56)
320	9.5 (20)	7 (15)	42 (87)	1.8 (3.7)	0.9 (1.8)	34 (61)	0.36 (0.7)	0.27 (0.6)	26 (54)
1000	44 (29)	23 (17)	123 (83)	10 (6.7)	4.8 (3.2)	108 (73)	2.8 (1.9)	2.2 (1.4)	96 (64)

* Below detection limit

** Percentage

*** 0.005 M Ca/K/Na extract

Table 2.1 Continued.

Wageningen humic sand, pH 6.2			
Nominal Cd in soil(mg/kg)	Extract (mg/l)		CaCl ₂ extract
	Total Cd	Cd activity	
0	0.02	-	0.02
32	0.17 (3.1)	0.1 (1.8)	3.3 (59)
100	0.54 (3.1)	0.3 (1.7)	13.4 (77)
320	3.0 (5.4)	1.7 (3.1)	37 (67)

Table 2.2 *Dissolved Organic Carbon (DOC) in the Ca/K/Na extracts and pore water of Panheel and OECD soils.*

Cd concentration in soil(mg/kg)	Panheel soil			OECD soil			Wageningen soil
	pH 4.4	pH 5.7	pH 7.3	pH 3.9	pH 5.6	pH 7.1	pH 6.2
Extract (mg C/kg soil)							
0	110	141	347	223	308	735	81
3.2	104	-	403	-	-	-	-
10	98	-	381	242	-	840	-
32	110	134	369	-	242	-	74
100	110	-	575	203	-	-	104
320	104	113	347	-	294	725	77
1000	-	-	-	315	256	670	-
Pore water* (mg C/kg soil)							
	pH 3.5	5.6	6.8	3.9	5.0	6.7	
0	48	24	31	242	363	434	1
3.2	-	-	-	215	350	-	-
10	-	-	-	-	-	-	-
32	-	-	-	231	383	341	8
100	-	26	34	-	-	-	21
320	54	-	-	190	297	308	-
1000	54	30	40	-	-	-	-
3200	52	30	32	195	314	242	3

* Pore water was obtained from soils used in the experiments with earthworms.

Table 2.3 *Freundlich parameters for the adsorption of cadmium to Panheel soil and OECD artificial soil..*

Parameter	Panheel soil			OECD soil		
	pH 4.4	pH 5.7	pH 7.3	pH 3.9	pH 5.6	pH 7.1
K	21	180	2700	52	330	670
1/n	0.96	0.93	1.07	0.92	0.73	0.69
r	0.99	0.94	0.98	0.99	1.00	0.98

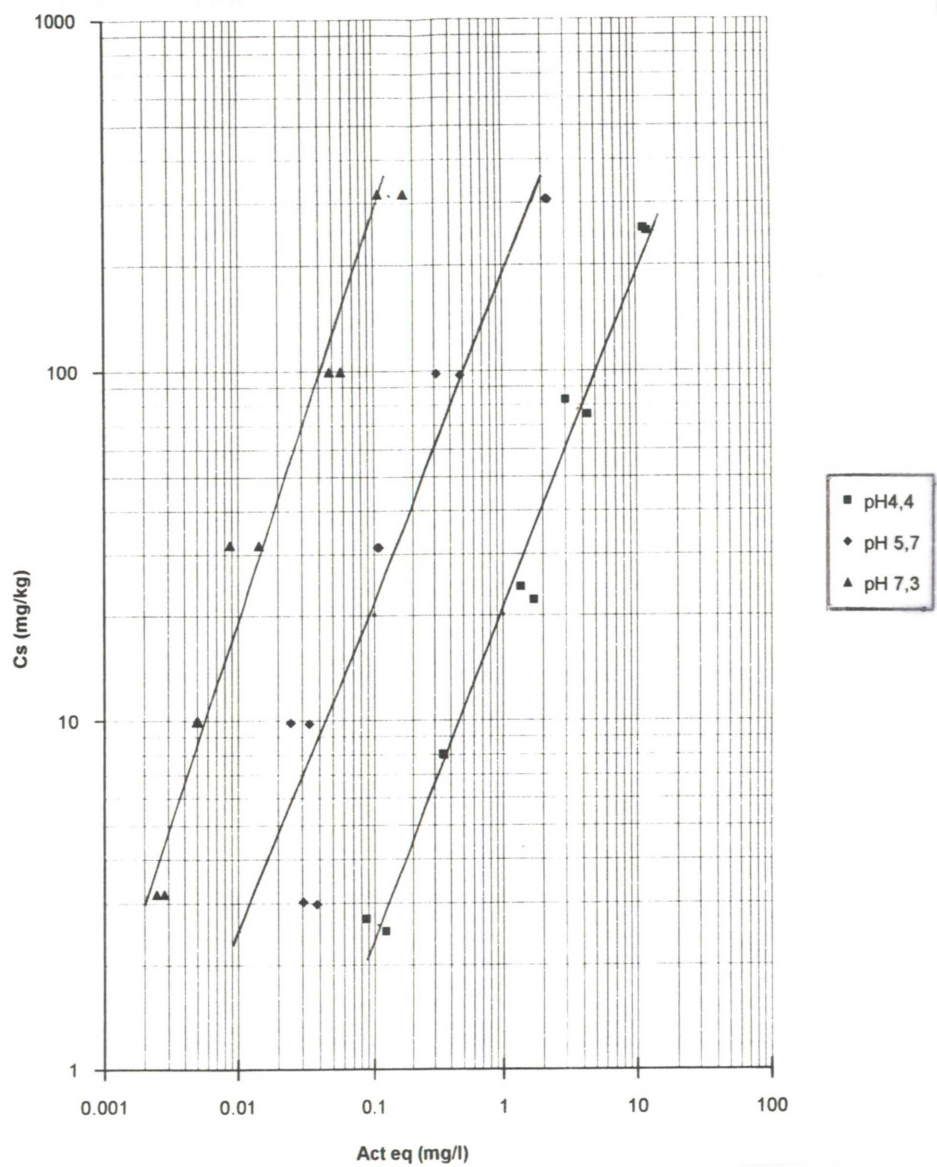


Figure 2.2 Adsorption isotherms of Cd^{2+} sorption to Panheel soil..
 $\text{Act}_{eq} = \text{Cd}^{2+}$ activity in the equilibrium solution; $C_s = \text{cadmium concentration in soil}$.

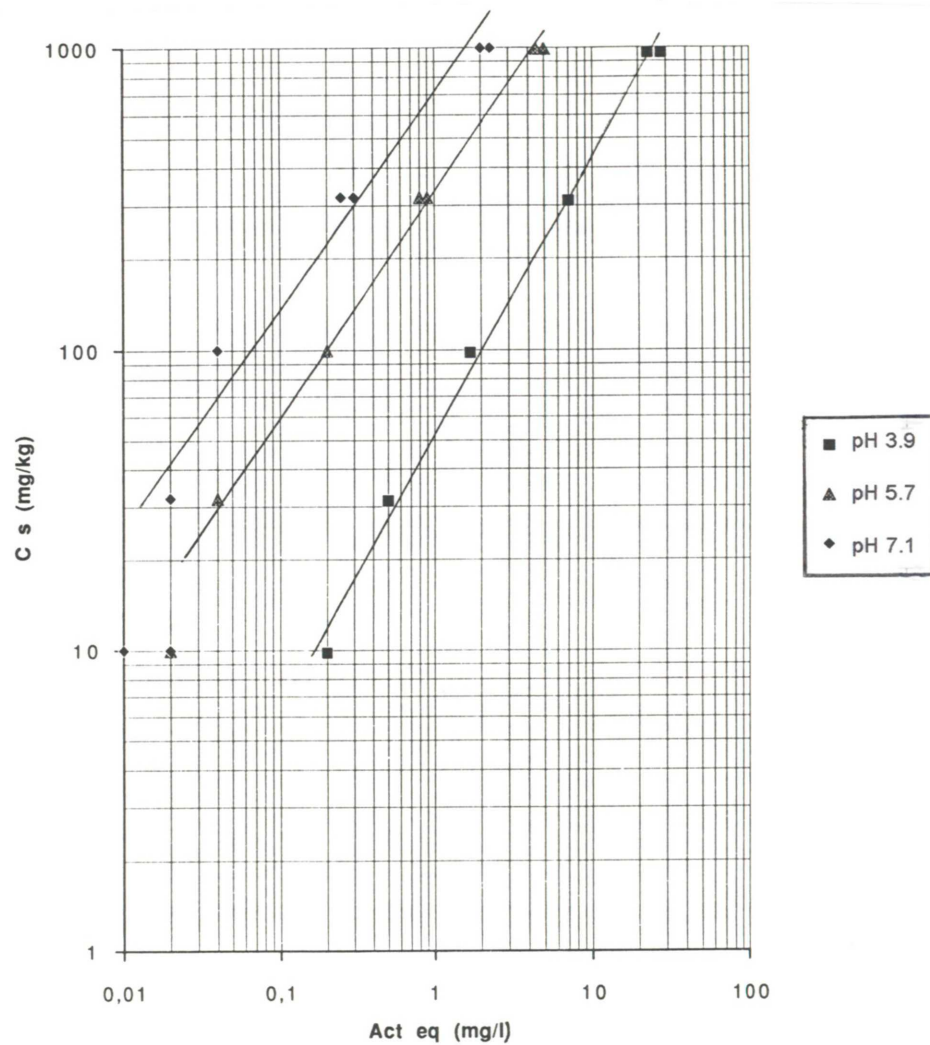


Figure 2.3 Adsorption isotherms of Cd^{2+} sorption to OECD artificial soil.
 $\text{Act}_{eq} = \text{Cd}^{2+}$ activity in the equilibrium solution; $C_s = \text{cadmium concentration in soil}$.

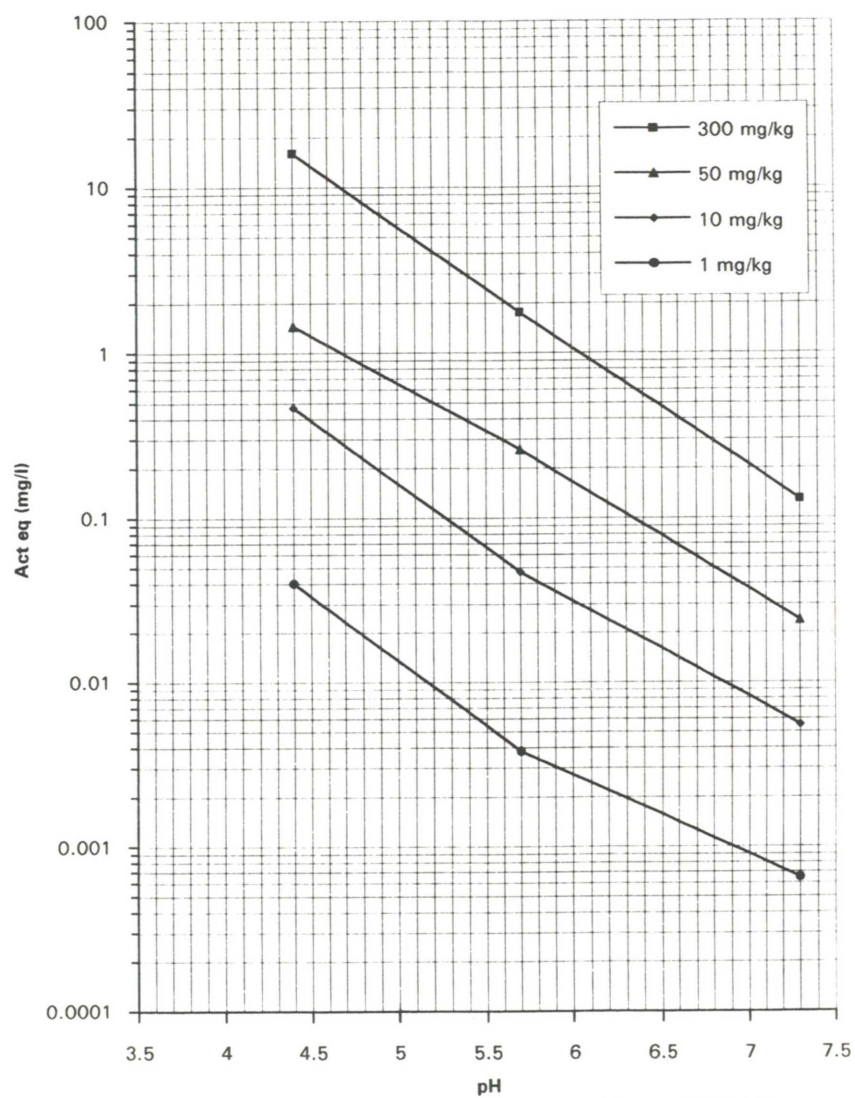


Figure 2.4 Relation between pH and the Cd^{2+} activity in Panheel soil at four soil concentrations.

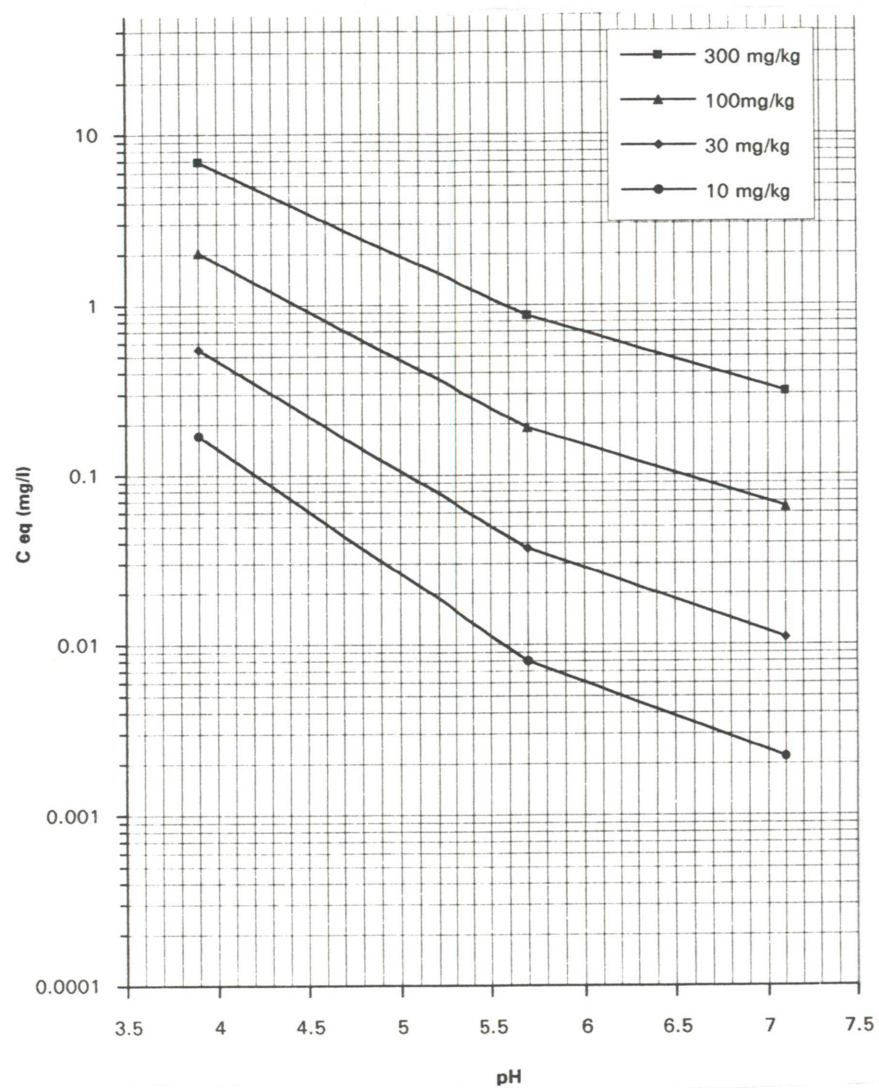


Figure 2.5 Relation between pH and the Cd^{2+} activity in OECD artificial soil at four soil concentrations.

2.2.2 The relationship between cadmium in soil pore water and in extracts

The concentrations and activities of cadmium were also measured in pore water of OECD soil and Panheel soil used in an experiment on the toxicity and uptake of Cd by earthworms at three different pH's described in part 4 (Table 4.4). By comparing Tables 2.1 and 2.4 a comparison can be made between these concentrations and equilibrium concentrations after shaking (extraction) with an aqueous solution of low salt strength. It has been hypothesised that these equilibrium concentrations should correspond with the pore water concentrations since the adsorption equilibrium is independent of the ratio between solid and liquid phase. Cd^{2+} activities in the pore water were much lower at higher concentrations than total cadmium concentrations because of higher chloride concentrations and higher ionic strength (in these experiments Cl^- has not been removed from the soil).

Table 2.4 shows that the total cadmium concentrations in pore water were higher than the determined equilibrium concentrations after shaking. One explanation could be that the total salt concentration in porewater is higher than the salt concentration in the extraction solution. For instance, Cd^{2+} added to the OECD artificial soil will be exchanged by Ca^{2+} , thus leading to about 0.01 M Ca^{2+} in soil with 320 mg Cd/kg. In the extraction procedure 0.003 M Ca^{2+} was applied.

Table 2.4 Cadmium concentrations (mg/l) in pore water of soil samples used in the toxicity experiments with *Eisenia fetida*.

OECD Artificial soil						
Nominal concentration (mg/kg)	pH ^a 3.9		pH 5.0		pH 6.7	
	Total Cd	Cd^{2+} activity	Total Cd	Cd^{2+} activity	Total Cd	Cd^{2+} activity
0	0.03	0.6	0.83	0.6	0.18	-
0.32	0.25	0.25	0.26	0.14	0.68	0.12
3.2	0.14	2.0	0.10	0.25	0.05	-
32	0.83	1.0	0.32	0.18	0.16	0.07
320	51	18	9.8	5.0	0.48	0.7
3200	3200	190	1540	110 ^b	750	55 ^c

^a Average pH measured in pore water ^b pH 4.2 ^c pH 5.7

Table 2.4 Continued.

Nominal concentration (mg/kg)	Panheel soil							
	pH ^a 3.5		pH 5.6		pH 6.8		Wageningen pH 6.0	
	Total Cd	Cd ²⁺ activity	Total Cd	Cd ²⁺ activity	Total Cd	Cd ²⁺ activity	Total Cd	Cd ²⁺ activity
0	0.41	1.5 (4.3) ^d	6.7	1.0 (2.0)	2.1	0.7 (0.4)	0.32	0.3
32	46	19 (16)	48	(4.3)	2.7	1.1 (0.8)	1.4	1.3
100	155	50 (34)	35	6.0 (5.0)	1.59	0.7 (0.8)	6.8	4.5
320	710	100 (90)	93	21 (16)	14.6	3.0 (3.4)	56	17
1000	2800	200 (200)	1020	80 (53)	350	30 (20)	870	115
3200	10800	1100 (1000)	7500	300 ^b (210)	5000	170 ^c (160)	8500	530 ^d

^a Average pH measured in pore water ^b pH 5.0 ^c pH 6.1 ^d pH 5.2.

^d () = measured after the toxicity experiment

2.2.3 Effect of cadmium on glutamate mineralisation by soil micro-organisms

CO₂ formation from glutamate in soil showed a clear dose-response effect to cadmium chloride. In Figure 2.6 as an example the effect of different concentrations of CdCl₂ on the evolution of CO₂ from Panheel soil is given. CO₂ formation from glutamate in the OECD artificial soil had not been reported before, but its microbial activity was sufficient to carry out these experiments. In Figure 2.7 an example of a dose-response curve is given. Generally, the data could be well fitted by a log-logistic model, from which EC₅₀ values could be calculated.

Table 2.5 gives the EC₅₀ values obtained with the two soils at three pH's and with the humic sandy soil at pH 6.2. In Figure 2.8A,B the toxicity data of the three pH's are represented on a linear scale.

Toxicity of cadmium to glutamate mineralisation increased in Panheel soil from pH 4.4 to 5.7 and did not change if the pH was further increased. However, contrastingly in OECD soil the toxicity decreased from pH 3.9 to pH 7.1.

Table 2.5 *EC₅₀ values (with 95% confidence limits) for the effect of cadmium on glutamate mineralisation in soils at different pH.*

Soil	Panheel soil			OECD artificial soil			Wageningen soil
pH	4.4	5.7	7.3	3.9	5.6	7.1	6.2
EC ₅₀ Calculated on: nominal concentration (mg/kg)	>320	130 (92-183)	102 (65-158)	283 (137-586)	586 (438-784)	1100 (674-1795)	132 (97-180)
Conc in Ca/K/Na extract (mg/l)	>12	0.26 (0.04-1.8)	0.25 (0.14-0.46)	6.5 (1.2-34)	4.8 (2.8-6.5)	2.7 (1.5-4.9)	0.86 (0.52-1.4)
Cd ²⁺ activity (mg/l)	>12	0.25 (0.08-1.3)	-	4.8 (1.3-18)	2.1 (1.2-3.6)	2.0 (1.0-4.0)	0.44 (0.23-0.8)

As was expected the EC₅₀ values soil were generally higher in OECD artificial soil than in Panheel soil, reflecting the stronger adsorption of cadmium to the OECD soil and the lower activity measured in the soil solution. The OECD artificial soil had a much higher organic matter and clay content than the Panheel and Wageningen soils, and therefore adsorbed cadmium more strongly. The differences in cadmium toxicity may also reflect differences in the sensitivity of the microbial populations for cadmium.

No relation could be found between the Cd total concentrations or activity in Ca/K/Na extracts and the toxicity of cadmium in the Panheel soil except that cadmium was less toxic at pH 4.4. In the OECD soil there was a positive relation between the toxicity and the concentration in the soil, although only the difference between pH 3.9 and 7.1 was statistically significant. Based on Cd²⁺ activity or total cadmium concentration in the Ca/K/Na extract there was no significant influence of the pH. The toxic Cd²⁺ activity giving 50 % inhibition of mineralisation was obviously 2-5 mg/l in the soil pore water.

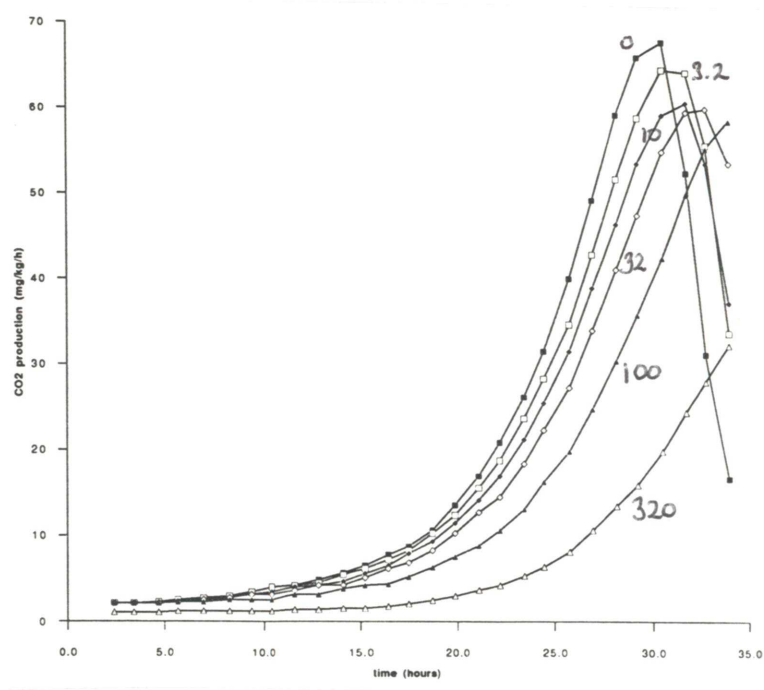


Figure 2.6 Effect of cadmium on CO₂ production from glutamate in Panheel soil at pH 5.7.

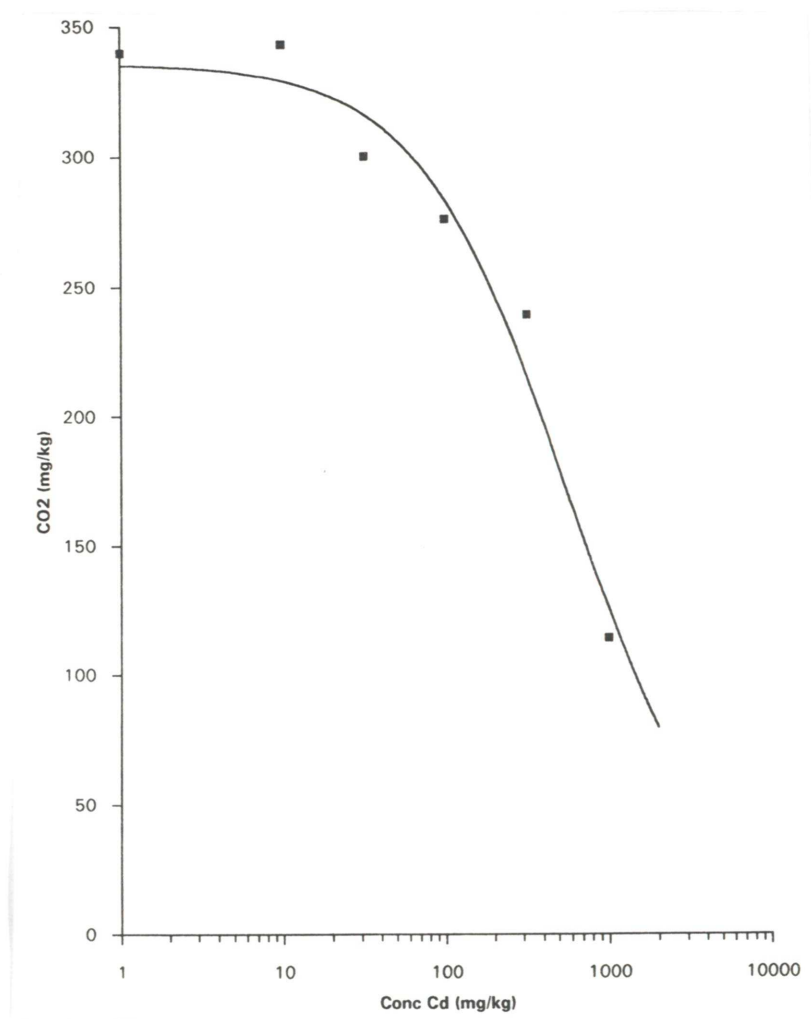


Figure 2.7 Dose response curve for the effect of cadmium on cumulative CO₂ output in OECD artificial soil at pH 5.6.

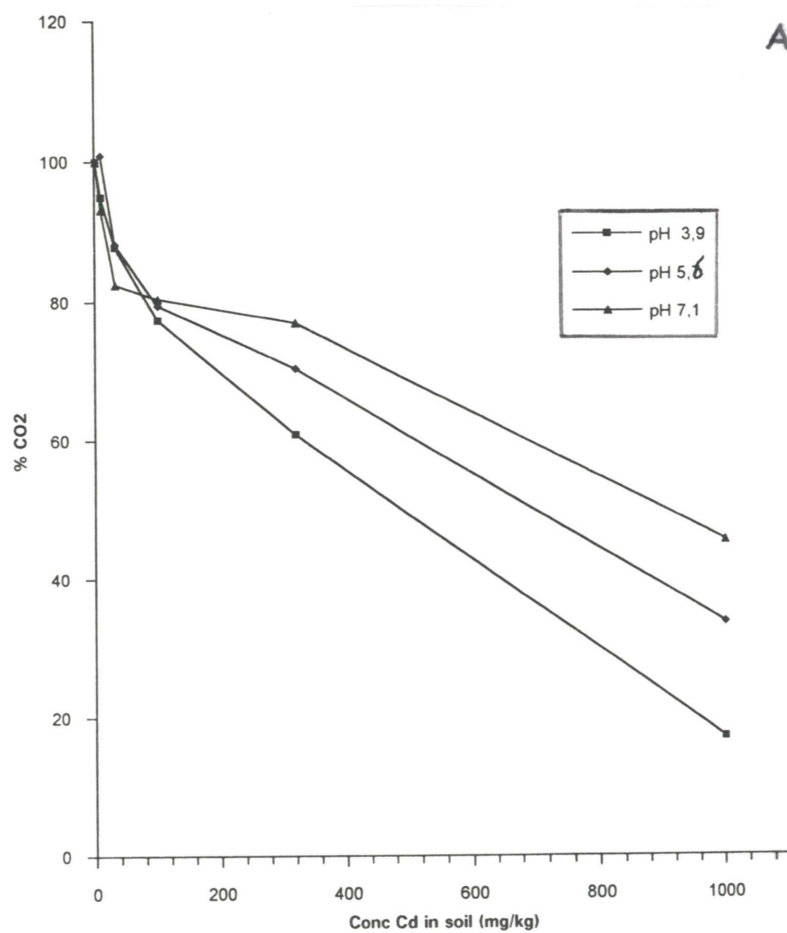


Figure 2.8 Dose response curves for inhibition of glutamate mineralisation at three pH levels.
A: Artificial soil; B: Panheel soil.

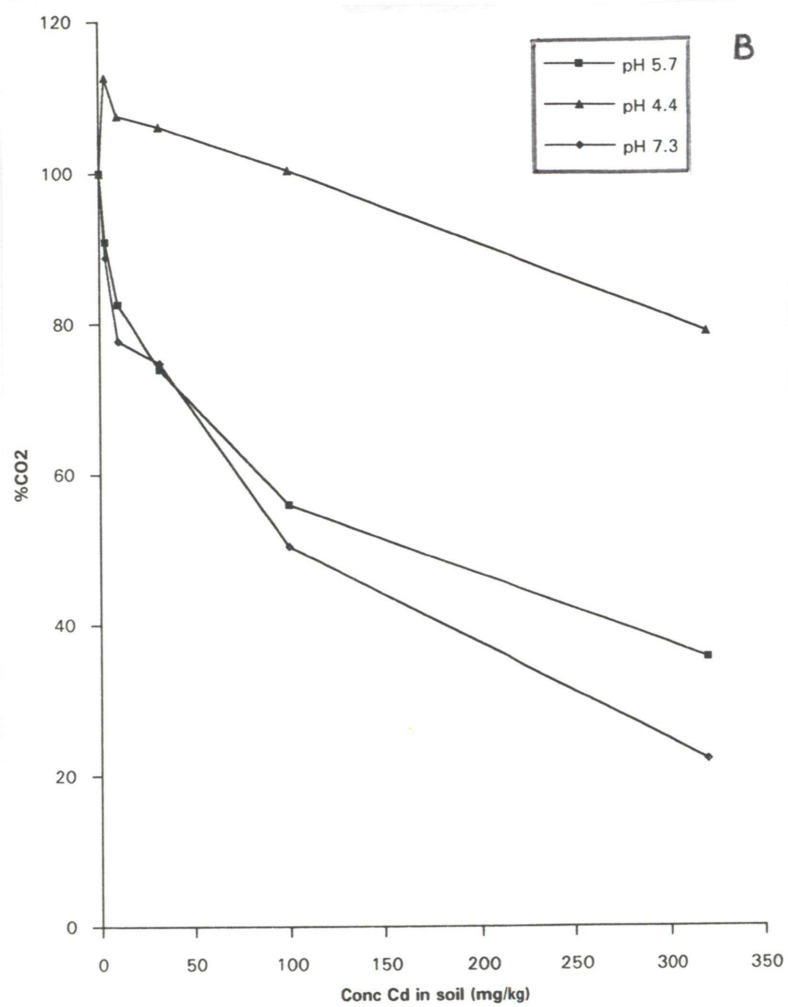


Figure 2.8 Continued.

3. SPRINGTAIL *FOLSOMIA CANDIDA*

3.1 Methods and materials

3.1.1 Test Animals

In the experiments, collembolans of the species *Folsomia candida*. (Willem 1902) were used. The animals were cultured in the laboratory, in pots with a bottom of plaster of Paris mixed with 10% charcoal, at 18°C and a light/dark cycle of 12:12 hours. The breeding culture originated from arable land at the experimental farm "The Lovinckhoeve" at Marknesse, The Netherlands.

The experiments were started with synchronised animals, having the same age (10-12 days). To obtain synchronised juveniles, 10 adults were incubated in culture pots to lay eggs and removed after two days. The first juveniles emerged after two weeks. From each synchronisation, starting weight of ten animals was determined.

The food used in the culture, during the synchronisations and in the experiments was a commercial baker's yeast (Dr. Oetker).

3.1.2 Preparation of the soils

Experiments were performed using an artificial soil according to OECD (1984), and two natural soils: Panheel soil and Wageningen soil. The first two soils were tested at three different nominal pH (1 M KCl) levels: 3.5, 5.0 and 6.5. The Wageningen soil is only tested at its own pH of approximately 5.0.

The **artificial soil** was made of 70% quartz sand, 20% kaolin clay and c. 10% air dried peat. The peat was finely ground and sieved over 1.0 mm. The amounts of CaCO₃ (Baker analyzed) needed to obtain the desired pH were derived from pH calibration curves. The substrates were thoroughly mixed to obtain a homogeneous soil.

The **Panheel soil** contains 89% sand, 7.5% silt, 1.9% clay and 2.4% organic matter. It was used at its own pH of 5.2, at a pH of 6.5 (obtained by adding CaCO₃) and at a pH of 3.5 (obtained by adding HCl (9%)).

The **Wageningen soil** contains 86% sand, 10% silt, 1.9% clay and 3.8% organic matter and was only tested at its own pH of 4.9.

3.1.3 Water Holding Capacity

For each soil the Water Holding Capacity (WHC) was determined. For this purpose, 20 g dry soil was placed in a pot with a gauze bottom covered with filter paper, incubated in a water layer for three hours, and subsequently placed on wet quartz sand to drain for 2 hours. The resulting water content is the WHC, which is expressed as % of the dry soil weight.

In the tests, artificial soil was moistened to about 50% of the WHC and Panheel and Wageningen soils to 30% of the WHC.

3.1.4 Cation Exchange Capacity

To determine Cation Exchange Capacity (CEC) of the test soils, 20 g wet soil was mixed with 50 g sieved quartz sand and placed in a 2 cm Ø perspex tube. This tube had some cotton wool on the bottom with 1 cm quartz sand on it, and the soil layer was covered with another 1 cm layer of quartz sand. The soil was saturated with K^+ by leaching with a 1M KCl solution. The excess K^+ was removed by leaching the soil column with 60% ethanol, subsequently the soil column was eluted with a 0.5 M $CaCl_2$ solution. The $CaCl_2$ fraction was collected and analysed for K^+ concentration by flame Atomic Absorption Spectrophotometry (AAS) on a Perkin Elmer 1100B AAS. CEC was expressed as the amount of K^+ retained by the soil (in mmol/kg). These measurements were done in duplicate for each soil and pH level.

3.1.5 Contamination of the soils

The artificial soil was contaminated by adding a solution of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ (BDH, AnalaR) in deionized water. The Panheel and Wageningen soils were contaminated with a solution of $CdCl_2 \cdot H_2O$ (Baker) in deionized water. The amounts of water used were sufficient to moisten the soils to the desired moisture level.

The nominal cadmium concentrations added to the soils were 0-50-100-200-400-800-1600 mg Cd/kg dry soil. This was done approximately three weeks after the artificial soil was prepared and brought to the different pH levels, and approximately six weeks after the adjustment of pH levels in the Panheel soil.

Two weeks before the start of the toxicity experiments, soils were placed in 5 cm Ø tubes (with a 0.45 µm filter in the bottom) and flushed with deionized water to get rid of the excess chloride which was added together with the cadmium. Soils were flushed with twice as much water as was present in the soil (at 50 and 30% of WHC for artificial and natural soils, respectively). The eluate was collected, the volume was determined the next day and analysed for cadmium concentration and conductivity. The soils were put in the stove at 50°C to retain the desired moisture content.

3.1.6 Experimental design

Five test containers were prepared for each control and treatment group, with 25 g wet soil in each container. Additional containers, containing 10 g wet soil, were prepared for pH and soil moisture measurements at the beginning and at the end of the experiment.

At the start of the experiment, 10 juvenile (10-12 day old) *F. candida* were placed in each test container, and some grains of dried baker's yeast were added. All the test containers were randomly placed on trays and incubated in a climate room at a temperature of 18°C, a relative air humidity of 75% and a light/dark cycle of 12:12 hours. All test containers were opened and closed twice a week for aeration. Once a week some additional yeast was added if necessary. After four weeks, the test containers were sacrificed for analysis of the numbers of adults and juveniles, body weight and accumulation of cadmium in the adults. The Wageningen soil was sacrificed after five weeks, because the initial body weight was lower than in the other experiments and no juveniles were seen after four weeks.

3.1.7 Finishing the tests

To each container, 100 ml of deionized water was added and the soil/water mixture was stirred thoroughly but carefully to let all the animals present float to the surface. The water surface was then photographed on diapositive material and juveniles were counted using an electronic pen by projecting the slide on a desk-top slide projector. After photographing, the surviving adults were counted by picking them from the water surface. Ten animals per test concentration were weighed to the nearest microgram using a Sartorius S4 supermicro-balance to obtain the wet weight. Subsequently, the animals were lyophilised at -40°C for 24 hours. After determination of the dry weight, the animals were individually digested in a 300 µl HClO₄/HNO₃ mixture (1:7) as described by Van Straalen and Van Wensem (1986)

and cadmium concentrations in their bodies were measured using a Perkin Elmer 1100B AAS equipped with a graphite furnace at a wavelength of 228.9 nm.

3.1.8 Cadmium speciation in the test soils

To determine total soil concentrations, dry soil samples were digested in a microwave using a mixture of deionized water, HCl and HNO₃ (1:1:4). After digestion, the solution was diluted to 25 ml and analysed for cadmium by flame AAS on a Perkin Elmer 1100B AAS at a wavelength of 228.9 nm.

Water soluble cadmium concentrations were determined by shaking dry soil samples with deionized water (1:10) for two hours. The resulting soil suspension was filtered over a 0.45 µm filter. Extractable cadmium concentrations were determined by shaking dry soil samples with a solution of 0.003M CaCl₂, 0.001M NaCl and 0.001M KCl for 2 hours at a soil/liquid ratio of 1:5. After determination of pH and conductivity (only in the case of water soluble cadmium), cadmium concentrations in the solutions were measured by flame AAS. The Cd²⁺ activity was measured using an ionspecific electrode (Orion Research Model 94-48). The concentration of total soluble organic carbon (DOC) was measured on a C-analyzer (Rosemount Analytical Dohrmann DC190).

To determine exchangeable cadmium, dry soil samples were shaken with solutions containing 0.01M CaCl₂ or 0.1M CaCl₂ for 2 hours at a soil/liquid ratio of 1:10. The solutions were filtered over a paper filter, and analysed for cadmium concentrations as described above.

Pore water was sampled by centrifugation of wet soil samples for one hour at 4000 rpm. The pore water was 0.45 µm filtered. After measurement of pH, conductivity, DOC, Cd²⁺ activity and total cadmium concentrations were determined as described above.

3.1.9 Calculations and statistical analysis

To determine the sorption of cadmium to the test soils, the relationships between total concentrations and water soluble cadmium concentrations and the relationship between total concentrations and pore water cadmium concentrations were fitted applying Freundlich's equation:

$$\log(C_s) = \log(K_f) + 1/n * \log(C_e)$$

in which:

- C_s = cadmium content adsorbed to the solid phase (in mg/kg)
- C_e = cadmium concentration in the solution at equilibrium (in mg/l)
- $1/n$ = a dimensionless exponent
- K_f = the Freundlich adsorption constant

LC₅₀ values for the effect of cadmium on the survival of *F. candida* were calculated using the trimmed Spearman Karber method (Hamilton *et al.*, 1977/1978). LC₅₀ values were only calculated on the basis of total soil concentrations.

For the calculation of EC₅₀ values for the effect of cadmium on growth (fresh and dry weight) and reproduction (the number of juveniles produced), the logistic model described by Haanstra *et al.* (1985) was used. EC₅₀ values were based on total, water soluble, extractable and exchangeable soil concentrations, pore water concentrations and internal cadmium concentrations in the animals.

3.2 RESULTS

3.2.1 Soil characteristics

Table 3.1 shows the measured pH(KCl) and cation exchange capacity (CEC) of the test soils.

Table 3.1 Actual pH-KCl and cation exchange capacity (CEC) and moisture content of the soils used in the cadmium toxicity tests with *Folsomia candida*.

Soil	Nominal pH	Actual pH	CEC (mmol K ⁺ /kg)	Moisture content (%)
Artificial soil	3.5	3.7	52	55
	5.0	5.0	75	55
	6.5	7.1	86	50
Panheel	3.5	3.8	73	15.5
	5.0	5.2	106	14.5
	6.5	6.2	130	13.5
Wageningen	5.0	4.9	75	14

As can be concluded from Table 3.1, CEC of the artificial and Panheel soils increased with increasing pH.

Table 3.2 shows the actual cadmium concentrations measured in the test soils after percolation with water to remove excess chloride.

Table 3.2 Actual cadmium concentrations in the test soils used in the cadmium toxicity tests with *Folsomia candida*.

nominal cadmium (mg/kg)	actual cadmium concentration (mg/kg) in soils (at nominal pH)						
	artificial soil			Panheel soil			Wageningen
	pH 3.5	pH 5.0	pH 6.5	pH 3.5	pH 5.0	pH 6.5	pH 5.0
0	0.18	0.60	0.28	0.41	0.32	0.16	0.36
50	37.1	35.3	28.1	30.7	64.1	59.0	50.0
100	69.2	73.8	71.9	60.3	111	112	94.1
200	114	138	170	93.4	137	108	191
400	269	274	325	171	365	394	376
800	596	887	618	454	554	744	658
1600	1089	1273	1410	818	1207	1307	1425

From Table 3.2, it can be concluded that the actual cadmium levels deviate somewhat from the nominal ones. Elution of the soils to get rid of the excess chloride has led to a considerable loss of cadmium, which was highest in the low pH soils. Most striking is the loss in Panheel soil at pH 3.5, in which almost 50% of the applied cadmium was leached from the soil. Lowest loss was recorded in the Wageningen soil, where on average only 7.5% of the applied cadmium eluted from the soil with water. In all further calculations, the actual soil concentrations mentioned in Table 3.2 will be used. The concentrations of cadmium measured in the eluates (not displayed here) were in agreement with the decrease of total soil concentrations, indicating that the loss indeed is due to a removal of freely available cadmium by the elution with water.

3.2.2 Extractable and exchangeable cadmium concentrations

Table 3.3 summarizes the pH values of the different soil extracts.

Table 3.3 Ranges of pH values measured in different soil extracts.

soil	pH	pH in extract				
		water soluble	extractable	exchangeable 0.01M CaCl ₂	exchangeable 0.1M CaCl ₂	pore water
Artificial	3.5	4.15-4.87*	3.81-3.89	3.79-3.88	3.42-3.48	3.80-4.41*
	5.0	4.99-5.85*	4.81-5.13*	4.52-4.96*	4.29-4.51	4.56-5.25*
	6.5	6.50-7.80*	6.15-7.16*	5.86-6.72*	6.11-6.57*	5.79-7.40*
Panheel	3.5	3.64-3.92	3.78-3.84	3.80-3.84	3.68-3.73	3.17-3.44*
	5.0	5.42-5.66	5.26-5.45	4.78-5.31	4.72-4.90	4.73-5.41*
	6.5	6.20-6.30	6.00-6.14	5.80-5.94	5.42-5.64	4.50-6.14*
Wageningen	5.0	5.22-5.57*	5.07-5.21	4.99-5.05	4.50-4.74	4.28-4.92

* highest values in control and lowest at highest test concentration

pH values measured in the different extracts (Table 3.3) are in agreement with the measured soil pH values given in Table 3.1. In a number of cases, pH decreased at high test concentrations. This trend was also noted for the soil pH (not indicated in Table 1), and may be explained by the excess Cd²⁺ ions, added to the soils at high concentrations, causing a release of H⁺ ions from the sorption sites on the soils.

DOC concentrations were measured in the water soluble and pore water extracts only; results are summarized in Table 3.4.

Table 3.4 *DOC concentrations (expressed as mg/kg dry soil) in water soluble and pore water extracts of the test soils.*

Soil	DOC (mg/kg dry soil $\pm$ SD) in fraction at pH					
	water soluble			pore water		
	3.5	5.0	6.5	3.5	5.0	6.5
Artificial	321 $\pm$ 41	387 $\pm$ 33	373 $\pm$ 29	59 $\pm$ 12	101 $\pm$ 19	82 $\pm$ 10
Panheel	164 $\pm$ 35*	171 $\pm$ 33*	186 $\pm$ 16	17 $\pm$ 3	10 $\pm$ 1.0	21 $\pm$ 2
Wageningen		150 $\pm$ 45*			2.9 $\pm$ 1.0#	

increasing with increasing soil concentration

* decreasing trend with increasing soil concentration

DOC concentrations in the water soluble fractions were not affected by soil pH in the artificial and Panheel soils. For all three soils, DOC concentrations were much higher in the water soluble extracts compared to the pore water. In addition, DOC concentrations were much higher in artificial soil extracts than in the extracts of both natural soils. In Panheel and Wageningen soils, DOC concentrations of the water extracts showed an increase with increasing cadmium concentrations, while in Wageningen soil DOC levels in the pore water decreased with increasing concentrations. In all other soils, DOC concentrations were not affected by cadmium concentrations. DOC levels were of the same order as reported in Part 2.

Tables 3.5-7 (page 56 and following) show the cadmium concentrations and Cd²⁺ activities measured in the different soil extracts and the pore water. In these tables, all values are expressed as percentage of the total measured soil concentrations. In Tables 3.A-C concentrations are given.

Highest relative amounts of cadmium were usually found in the controls; probably this is due the fairly low cadmium concentrations in the untreated soils in combination with the detection level of the analytical method applied. The control values will not be included in the further discussion of results in this section.

In a number of cases, and most particularly in artificial soil, the relative amounts of cadmium present in the different extracts increased at the highest soil concentration(s). Apparently, at these concentrations the binding capacity of the soil was exceeded resulting in a relatively high amount of cadmium in the water soluble, extractable, exchangeable or pore water fractions.

From Tables 3.5 and 3.6 (page 56 and following), it may be concluded that cadmium concentrations or Cd^{2+} activities in all fractions decreased with increasing soil pH. Water soluble cadmium concentrations were very high in Panheel soil at pH 3.5 (30-50%), and below 5% in all other soils. Only at high concentrations at pH 5.0, water soluble concentrations were somewhat higher (9-14%).

The amount of extractable cadmium (using 0.003M CaCl_2 , 0.001M NaCl and 0.001M KCl) was highest in Panheel soil at pH 3.5, and much lower in all other soils. The fraction 0.01M CaCl_2 exchangeable cadmium also decreased with increasing soil pH, and was particularly high at pH 3.5 in both artificial and Panheel soils. In artificial soil (Table 3.5), the fraction of 0.1M CaCl_2 exchangeable cadmium exceeds 100%; due to unexplained reasons, in these extracts more cadmium was measured than in the acid digests of the soil. From the data for the natural soils, it may be concluded that 0.1M CaCl_2 is very efficient in extracting cadmium from the soils, with 60-100% being recovered in this extract.

The concentrations in pore water show a trend which is more or less similar to the one found for water soluble cadmium concentrations. Again, highest values were found in Panheel soil at pH 3.5, while in all other soils concentrations often were below 1%.

In Table 3.8, the amount of cadmium present as free Cd^{2+} (activity) in the water soluble, soil solution and pore water is shown as % of the concentration in the different extracts. From this table, it may be concluded that the fraction of Cd^{2+} generally decreased with increasing soil pH. In artificial soil, a relatively large amount of water soluble and extractable cadmium was present as free Cd^{2+} , while the fraction free Cd^{2+} in the pore water of this soil was similar to that in the other soils.

3.2.3 Sorption of cadmium

Table 3.9 show Freundlich constants based on total cadmium concentrations and Cd^{2+} activities measured in aqueous extracts and pore water of the 7 soil*pH combinations used in this study.

Table 3.9 *Freundlich sorption constants for the sorption of cadmium onto three different soils at different pH's. K_f and $1/n$ values are calculated log-log plots of cadmium concentrations in soil versus total Cd and free Cd^{2+} concentrations in water extracts, extractable fractions and pore water.*

soil	pH	Freundlich constants for					
		K_f	$1/n$	r^2	K_f	$1/n$	r^2
artificial soil	3.5	water soluble Cd			water soluble Cd^{2+}		
		214	0.57	0.993	366	0.63	0.993
		316	0.64	0.993	517	0.60	0.964
Panheel	6.5	677	0.62	0.980	1787	0.69	0.917
		0.41	0.92	0.994	57.6	0.93	0.994
		25.1	0.58	0.974	693	0.56	0.978
Wageningen	6.5	22.2	0.70	0.982	1224	0.70	0.977
	4.9	23.2	0.60	0.978	994	0.65	0.931
		extractable Cd			extractable Cd^{2+}		
artificial soil	3.5	17.3	1.04	0.992	58.2	1.35	0.927
		33.2	1.11	0.959	259	1.32	0.888
		168	1.05	0.759	1265	1.09	0.579
Panheel	6.5	5.31	0.99	0.883	17.3	1.29	0.921
		35.1	1.08	0.932	248	1.26	0.863
		86.5	1.39	0.900	695	1.44	0.742
Wageningen	4.9	51.8	0.94	0.974	298	1.17	0.899
		pore water Cd			pore water Cd^{2+}		
artificial soil	3.5	41.3	0.54	0.998	77.5	0.64	0.998
		61.1	0.57	0.992	136	0.70	0.989
		189	0.47	0.974	423	0.53	0.938
Panheel	6.5	10.6	0.49	0.944	2.48	1.05	0.998
		58.0	0.42	0.994	115	0.48	0.998
		76.3	0.46	0.978	188	0.49	0.976
Wageningen	4.9	47.4	0.46	0.995	-	-	-

The amount of pore water extracted from the Wageningen soil was too small to allow for a measurement of the Cd^{2+} activity, therefore no K_f and $1/n$ values could be calculated.

For the artificial and Panheel soils, K_f values generally increase with increasing soil pH. For all soils, K_f values are higher when based on Cd^{2+} activities than on total cadmium concentrations in the aqueous extracts or pore water. The only exception forms Panheel soil at pH 3.5, where the K_f value based on Cd^{2+} activities in pore water is higher than that based on total pore water concentrations. The reason for this lies in the fact that free Cd^{2+} concentrations measured using an ion-specific electrode sometimes exceeded total cadmium levels measured by AAS (Table 3.8). Probably, in these cases almost all cadmium in the pore water was present as free Cd^{2+} .

From Table 3.9, it becomes obvious that in nearly all cases $1/n$ values are much smaller than unity with most values ranging between 0.4 and 0.7. This indicates that sorption was not linear, which is a normal phenomenon at high solution concentrations such as the one used in this study. Again, the only exception is Panheel soil at pH 3.5.

3.2.4 Toxicity of cadmium for *Folsomia candida*

Figure 3.1 shows graphs of the survival of *F. candida* after 4, 4 and 5 weeks exposure in artificial, Panheel and Wageningen soil, respectively. LC_{50} values are presented in Table 3.10.

Table 3.10 LC_{50} values (with 95% confidence limits) based on total soil concentrations for the effect of cadmium on the survival of *Folsomia candida* in artificial, Panheel and Wageningen soils.

soil	LC_{50} (in mg Cd/kg dry soil) at pH		
	3.5	5.0	6.5
Artificial	706 (595- 837)	933 (546-1594)	1189 (907-1558)
Panheel	794 (535-1180)	675 (538- 848)	> 1307
Wageningen	-	665	-

In Panheel soil at pH 6.5, survival at the highest test concentration (1307 mg Cd/g dry soil) was 58%, therefore no LC₅₀ value could be calculated. From Table 3.10, it may be concluded that LC₅₀ values in both artificial and Panheel soils tend to increase with increasing soil pH, although only the difference between pH 3.5 and 6.5 was statistically significant.

In Figure 3.2 and 3.3, graphs showing the effects of cadmium on the growth (fresh weight and dry weight) of *F. candida* are shown, while in Figure 3. 4 effects on reproduction are displayed. Tables 3.11-13 show the EC₅₀ values for the effect of cadmium on the fresh and dry body weight and the reproduction (number of juveniles) of *F. candida* after 4 (artificial and Panheel soils) or 5 weeks (Wageningen soil) exposure.

In the artificial soil, growth in the controls was significantly higher at pH 6.5. EC₅₀ values for the effect on fresh and dry weight decreased with decreasing soil pH when based on total soil concentrations. When expressed on the basis of water soluble, extractable, 0.01M CaCl₂ exchangeable or pore water cadmium or Cd²⁺ activities, EC₅₀'s increased with decreasing soil pH. EC₅₀'s on the basis of 0.1M CaCl₂ exchangeable concentrations were higher than those on the basis of total soil concentrations and did not show a significant correlation with soil pH. Finally, EC₅₀'s on the basis of internal body concentrations showed considerable agreement for the different pH levels in artificial soil.

From Figure 3.2 and 3.3, it may be concluded that in Panheel soil growth was strongly reduced at the low pH level, already in the control but even more pronounced at the lowest cadmium concentration in soil. This explains the extremely low EC₅₀ values for the effect on fresh and dry weight at pH 3.5, based on total and exchangeable soil concentrations in Table 3.12. It does not explain, however, the very low EC₅₀ value on the basis of internal cadmium concentrations in the animals; neither does it explain why the EC₅₀ values on the basis of water soluble, extractable and pore water cadmium and Cd²⁺ activities show the same increasing trend with decreasing pH as was observed in the artificial soil.

Both in the Panheel and the Wageningen soils, EC₅₀ values on the basis of internal

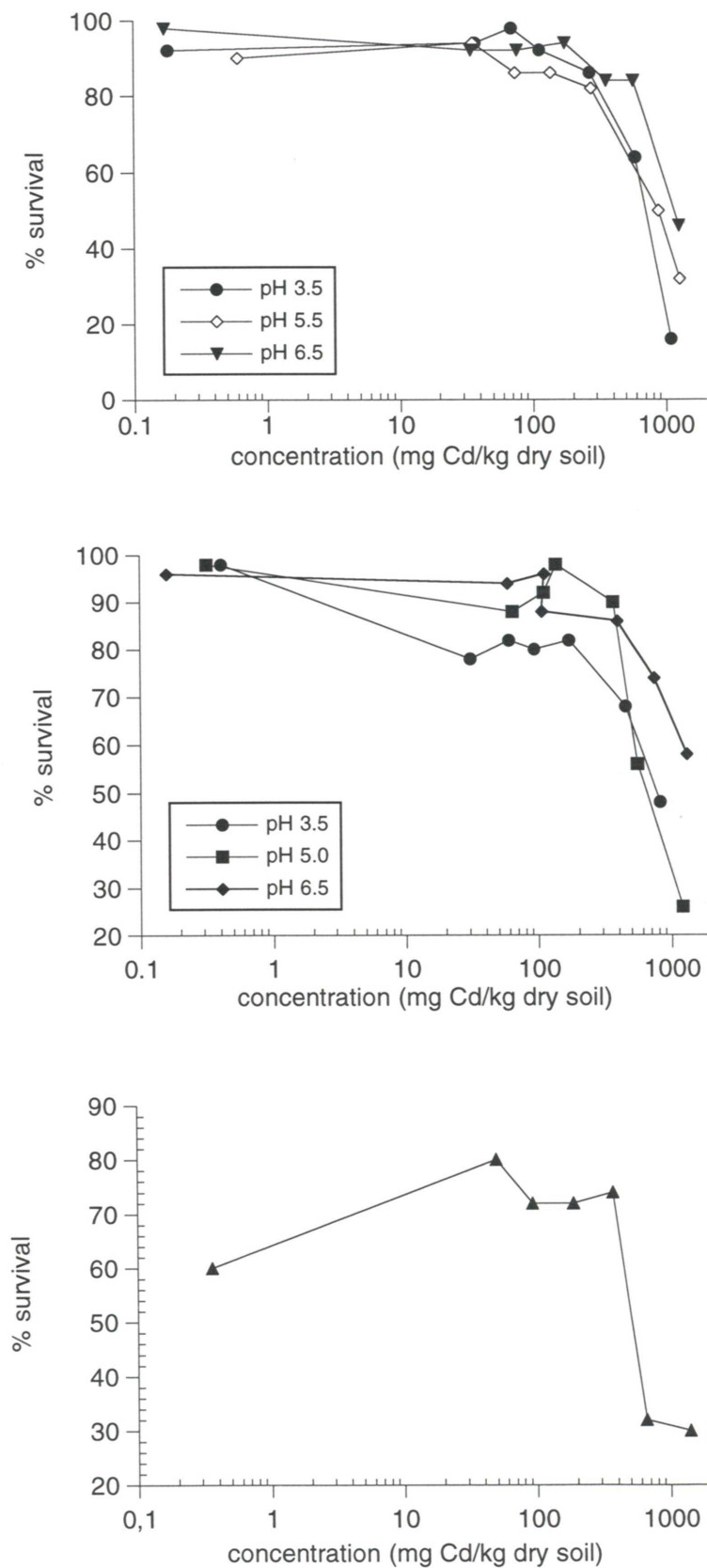


Figure 3.1 Survival of *Folsomia candida* after 4 weeks of exposure to cadmium in artificial soil (top) and Panheel soils (middle) at different pH and after 5 weeks of exposure in Wageningen soil (bottom). Graphs show the mean survival of 5 replicate test containers.

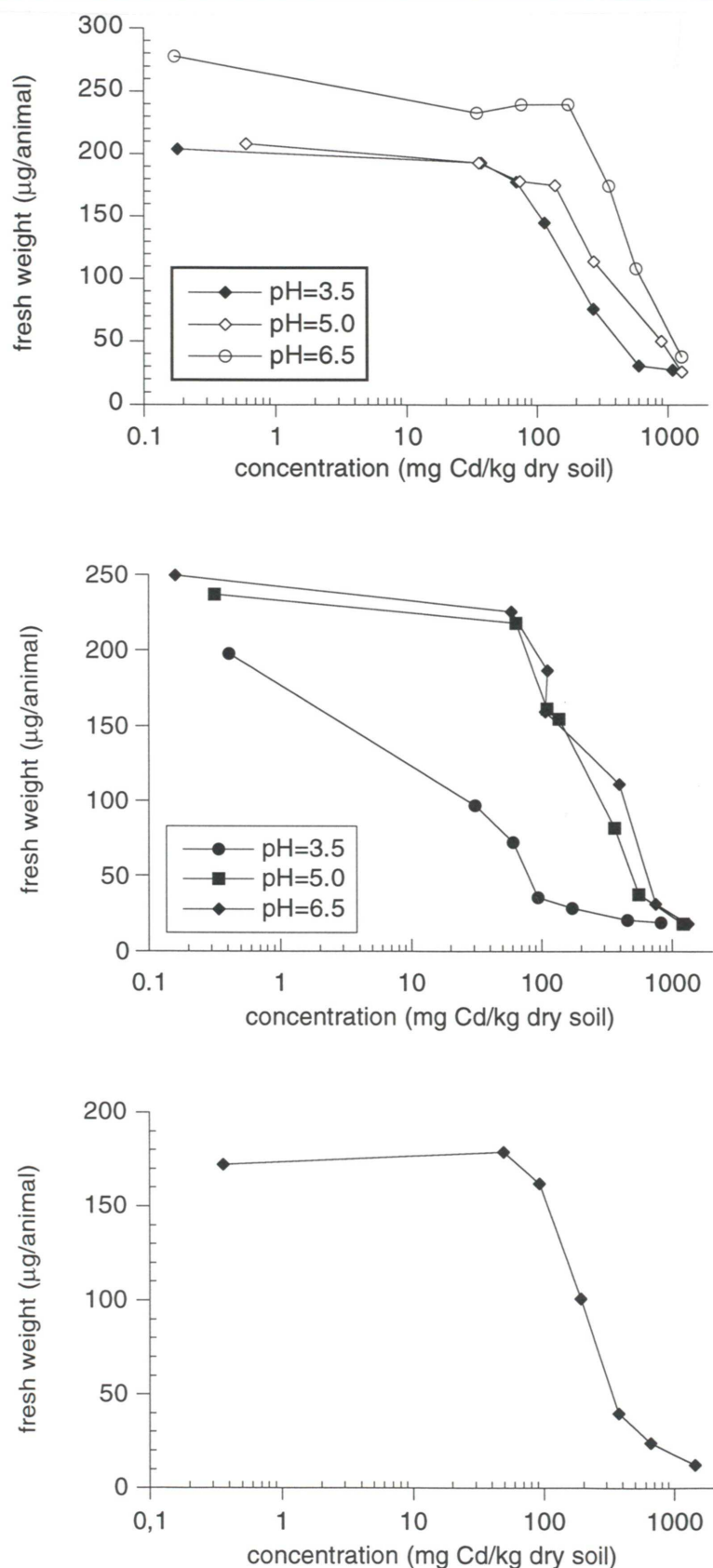


Figure 3.2 Effects of cadmium on the fresh weight of *Folsomia candida* after 4 weeks of exposure in OECD artificial soil (top) and Panheel soils (middle) at different pH and after 5 weeks of exposure in Wageningen soil (bottom). Graphs show the mean fresh weight of 10 animals per test concentration.

Concentrations were in close agreement with those observed in the artificial soil. For all other expressions of EC_{50} , considerable differences between soil*pH combinations were observed, which did not decrease when EC_{50} was based on e.g. water soluble or pore water cadmium concentrations or Cd^{2+} activities.

From Figure 3.4, it may be concluded that reproduction was highly variable and probably not only affected by soil pH, but also by other soil properties. In artificial soil, control reproduction was much higher at pH 6.5 compared to the lower pH levels. In Panheel soil, reproduction was very high at pH 6.5 and 5.0, and much lower at pH 3.5. Finally, Wageningen soil showed a considerable reproduction in the control. But in both Panheel soil at pH 3.5 and Wageningen soil, reproduction was drastically reduced already at the lowest cadmium concentration. In the Panheel soil at pH 3.5, this effect is most extreme with no juveniles being produced at the lowest test concentration; as a consequence, EC_{50} values for this soil (Table 3.12) are less reliable.

From Tables 3.11-13, it may be concluded that, also for reproduction, EC_{50} values on the basis of internal body concentrations show the best agreement for the different soil*pH combinations tested. For extractable, water soluble and pore water cadmium and Cd^{2+} activities, there is a tendency of EC_{50} values to increase with decreasing soil pH, while EC_{50} 's on the basis of total soil concentrations tend to decrease with decreasing soil pH.

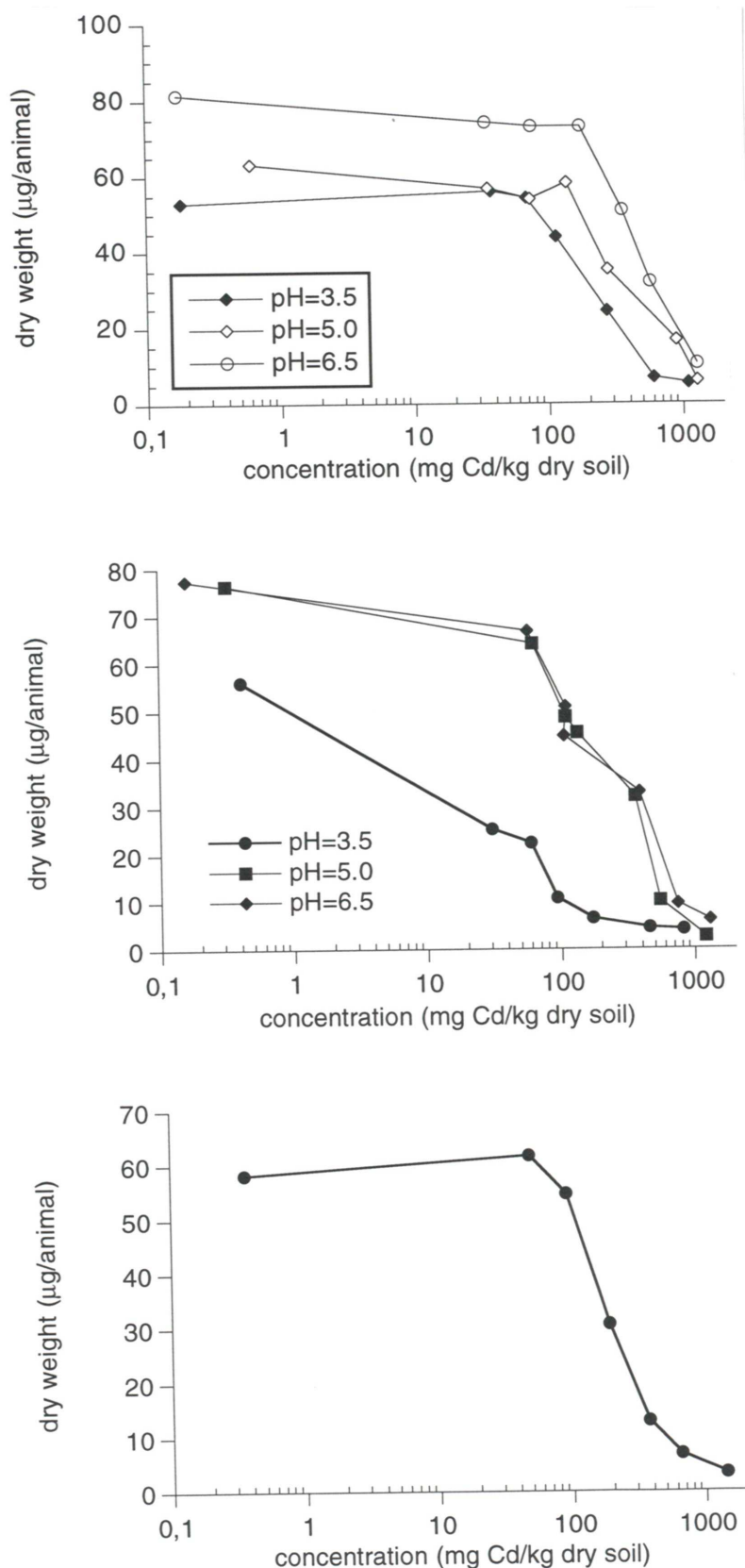


Figure 3.3 Effects of cadmium on the dry weight of *Folsomia candida* after 4 weeks of exposure in OECD artificial soil (top) and Panheel soils (middle) at different pH and after 5 weeks of exposure in Wageningen soil (bottom). Graphs show the mean dry weight of 10 animals per test concentration.

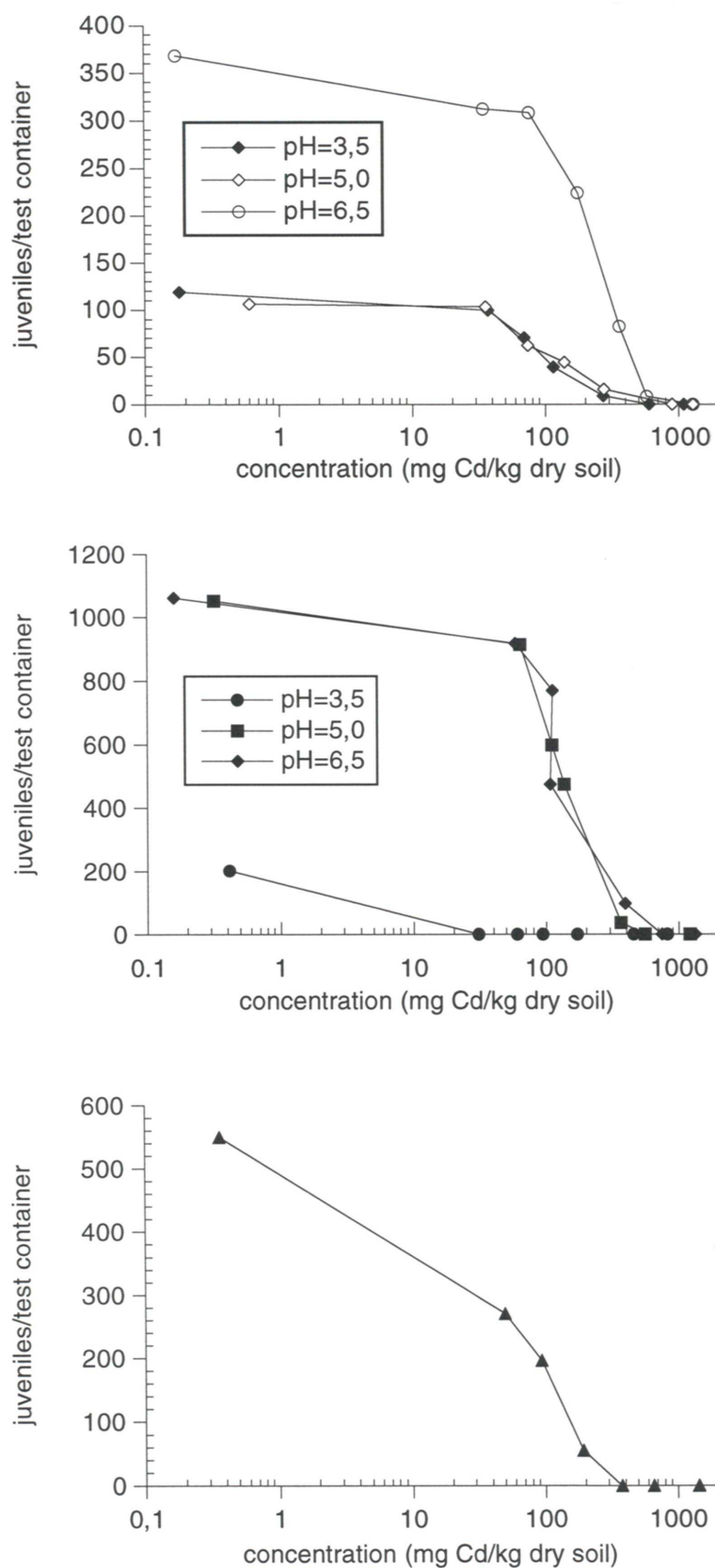


Figure 3. 4 Number of juveniles produced by *Folsomia candida* after 4 weeks of exposure in OECD artificial soil (top) and Panheel soils (middle) at different pH and after 5 weeks of exposure in Wageningen soil (bottom). Graphs show the mean number of juveniles of 5 replicate test containers.

3.2.5 Relationship with soil characteristics

From multiple linear regression analysis of K_f or $\log K_f$ values (Table 3.9) against soil pH and CEC (Table 3.1), results were obtained as given in Table 14.

Because $K_f = C_s/C_e^{1/n}$, the relationships between K_f and pH and/or CEC only holds for $C_e = 1$ mg/l.

The relationships between $\log K_f$ and pH and/or CEC can be interpreted as follows.

The best relationship between $\log K_f$ (extractable Cd^{2+}) and pH as given in Table 3.14 is:

$$\log K_f = 0.15 + 0.48 \cdot \text{pH} \quad (p = 0.003)$$

Because $\log K_f = \log C_s - 1/n \cdot \log C_e$ (see 3.1.9), this can be written as:

$$\log C_s - 1/n \cdot \log C_e = +0.48 \cdot \text{pH} + 0.15, \text{ or}$$

$\log C_e = n \cdot (-0.48 \cdot \text{pH} + \log C_s - 0.15)$. From Table 3.9 we find that $1/n$ is about 1.2, or $n \approx 0.8$

$$\log C_e = -0.38 \cdot \text{pH} + 2 \cdot \log C_s - 0.12$$

This relationship is well in line with the relationships derived in a different way in section 2.2.1

When Cd^{2+} activity in water soluble, extractable and pore water fractions were related to total soil concentrations and pH of the fractions, the following relationships were found. It should be noted that control values were omitted; with the control values included significance of the regression was much lower.

OECD:

water soluble : $\log \text{Cd}^{2+} = -0.351(\pm 0.052) \cdot \text{pH} + \log C_s - 1.106(\pm 0.303) \quad r^2 = 0.951;$
 $n = 18$

extractable : $\log \text{Cd}^{2+} = -0.471(\pm 0.025) \cdot \text{pH} + \log C_s - 0.127(\pm 0.134) \quad r^2 = 0.981;$
 $n = 18$

pore water : $\log \text{Cd}^{2+} = -0.513(\pm 0.046) \cdot \text{pH} + \log C_s + 0.474(\pm 0.246) \quad r^2 = 0.955;$
 $n = 18$

Panheel:

water soluble : $\log \text{Cd}^{2+} = -0.691(\pm 0.059)*\text{pH} + \log \text{Cs} + 0.814(\pm 0.314)$ $r^2 = 0.939$;
 $n = 18$

extractable : $\log \text{Cd}^{2+} = -0.597(\pm 0.037)*\text{pH} + \log \text{Cs} + 0.768(\pm 0.189)$ $r^2 = 0.965$;
 $n = 18$

pore water : $\log \text{Cd}^{2+} = -0.709(\pm 0.062)*\text{pH} + \log \text{Cs} + 1.935(\pm 0.288)$ $r^2 = 0.973$;
 $n = 16$

Table 3.14 Results of linear regression of K_f and $\log K_f$ values against soil pH (1M KCl) and CEC ($n=7$).

$K_f/\log K_f$	based on	constant	pH	CEC	r^2	p
K_f	water soluble	-235±201(n.s)	239_48	-9.45±2.33	0.865	0.018
	water soluble Cd^{2+}	-1490±382	448±72.7		0.883	0.002
	water soluble Cd^{2+}	-1409±384	511±92.8	-4.75±4.44(n.s.)	0.909	0.008
	extractable	-152±44.8	40.3±8.53		0.817	0.005
	extractable	-135±25.7	53.8±6.21	-1.02±0.30	0.954	0.002
	extract. Cd^{2+}	-1348±260	342±49.5		0.905	0.001
	extract. Cd^{2+}	-1265±210	406±50.8	-4.83±2.43(n.s.)	0.952	0.002
	pore water	-142±50.9	41.1±9.70		0.782	0.008
	pore water	-122±27.9	56.8±6.74	-1.18±0.32	0.950	0.003
	pore water Cd^{2+}	-354±118	99.0±22.2		0.833	0.011
	pore water Cd^{2+}	-303±43.1	133±10.2	-2.64±0.50	0.984	0.002
Log K_f *	water soluble Cd^{2+}	1.083±0.565(n.s)	0.324±0.108		0.645	0.030
	extractable	-0.31±0.37(n.s.)	0.37±0.071		0.842	0.004
	extractable	-0.27±0.41(n.s.)	0.40±0.098	-0.003±0.005(n.s.)	0.854	0.021
	extract. Cd^{2+}	-0.15±0.46(n.s.)	0.48±0.087		0.860	0.003
	extract. Cd^{2+}	-0.13±0.52(n.s.)	0.49±0.13	-0.001±0.006(n.s.)	0.861	0.019
	pore water	0.367±0.369(n.s)	0.263±0.070		0.738	0.013
	pore water	0.447±0.369(n.s)	0.325±0.089	-0.005±0.004(n.s)	0.798	0.041

* Other combinations of $\log K_f$ with pH and/or CEC showed no significant relationships.

In Table 3.15 EC_{50} values (tables 11-13) on the basis of total soil concentrations are related to soil pH and CEC (table 1).

Table 3.15 Results of linear regression of EC_{50} values against soil pH (1M KCl) and CEC (n=7).

EC_{50} for	constant	pH	CEC	r^2	p
fresh weight	-245±178(n.s.)	97±34.0		0.620	0.036
fresh weight	-179±117(n.s.)	148±28.2	-3.85±1.35	0.875	0.016
dry weight	-86.9±156(n.s.)	143±37.6	-4.64±1.80(n.s.)	0.784	0.046
reproduction	-138±68.8(n.s.)	46.5±13.1		0.716	0.016
reproduction	-123±67.8(n.s.)	58.6±16.4	-0.913±0.783	0.788	0.045

4. EARTHWORM *EISENIA FETIDA*

4.1 Methods and materials

4.1.1 Test animals

In the experiments oligochaete earthworms of the species *Eisenia fetida* (Savigny, 1826) were used. The animals were grown in a horse dung / garden soil (1:1) mixture at about 23°C. The mean weight per worm (about 0.5 g) and its standard deviation were measured at the beginning and at the end of the test. Before use, worms were preincubated for 7 days in the test soil with the required pH.

4.1.2 Preparation of the soils

Experiments were performed using an artificial soil according to OECD (1984), and two natural soils: Panheel soil and Wageningen humic sandy soil.

The **OECD artificial soil** was made of 70% quartz sand, 20% kaolin clay and c. 10% air-dried peat. The peat was finely ground and sieved over 1.0 mm. The amounts of CaCO₃ (Baker Analyzed) needed to obtain the desired pH were derived from pH calibration curves. The substrates were thoroughly mixed to obtain a homogeneous soil. The final pH's(KCl) measured before use were: 3.1, 5.4, and 6.9 The moisture content of the soil in the experiments was 55%.

The **Panheel soil** contains 89% sand, 7.5% silt, 1.9% clay and 2.4% organic matter. It was used at its own pH(KCl) of 5.4, at a pH of 6.9 (obtained by adding CaCO₃) and at a pH of 3.8 (obtained by adding H₂SO₄). Soils were not washed before use. The moisture content during use was 21%.

The **Wageningen humic sandy soil** contains 86% sand, 10% silt, 1.9% clay and 3.8% organic matter and was only tested at its own pH(KCl) of 4.9. The moisture content during use was 19%.

4.1.3 Contamination of the soils

The artificial soil was contaminated by adding a solution of CdCl₂ (Aldrich) in deionized water. The amounts of water used were sufficient to moisten the soils to the desired moisture level. Soils were thoroughly mixed with a hand mixer.

The nominal cadmium concentrations added to the soils were 0-0.32-3.2-32-320-3200 mg Cd/kg dry soil in the test with artificial soil and 0-32-100-320-1000-3200 mg Cd/kg in the experiments with Panheel and Wageningen soil. This was done approximately 2 weeks after the artificial soil was prepared and brought to the different pH levels.

4.1.4 Experimental design

Four glass test containers were prepared for each control and treatment group, with 800 g wet soil per container. In the experiment with Wageningen soil 675 g soil was used. Two additional series of containers without worms were prepared for pH and cadmium measurements in extracts and pore water at the beginning of the experiment. Control experiments at the end of the experiment (17 days) with soil from the containers with worms showed that the cadmium concentrations and pH were almost the same as in the containers without worms.

At the start of the experiment, 10 adult worms were placed in each test container. All the test containers were randomly incubated in a climatized room at a temperature of 20°C, a relative air humidity of 65% under continuous light. After 6 days the containers were examined for dead worms and their numbers counted. After 17 days, the test containers were sacrificed for determination of the numbers of surviving worms, body weight and accumulation of cadmium.

4.1.5 Determination of cadmium

Total cadmium concentration and Cd^{2+} activity were determined in the pore water and in 0.1 M CaCl_2 extracts of the test soils. Also, Cd^{2+} activity was determined. Total cadmium was determined in the worms.

Determinations in extracts were carried out as described in Part 2. Worms were digested in HNO_3 in a closed system in a magnetron oven.

Extractions and collection of pore water were carried out as described in Part 2.

4.1.6 Calculations and statistical analysis

For calculation of LC₅₀ values for the effect of cadmium on the survival of *E. fetida* the trimmed Spearman Karber method was used. EC₅₀ values for the effect of cadmium on weight loss were calculated with the logistic model described by Haanstra *et al.* (1985) (see Part 2). An approximate loss of weight of 50% of the original weight of the worms was found to be lethal. Therefore, this was chosen as the 100% effect. EC₅₀ values were based on total soil concentrations, on total cadmium concentrations and Cd²⁺ activities in pore water.

4.2 Results

4.2.1 Cadmium in pore water and soil extracts

The concentrations of cadmium and the Cd²⁺ activity found in pore water and in soil extracts are given in Part 2, Table 2.4. Extracts with 0.1 M CaCl₂ showed the same trend as observed in Parts 2 and 3: they extracted a good deal (30-80%) of the total amount of cadmium present (results not shown).

4.2.2 Toxicity of cadmium to *E. fetida*

In Table 4.1 the survival, LC₅₀, and EC₅₀ of effects of cadmium on the loss of weight of the worms are given. Loss of weight was slightly more sensitive than mortality. Because of the large spacing between the concentrations in the artificial soil, the number of surviving worms at each concentration is also given. For the artificial soil a large spacing factor (10) for the nominal cadmium concentrations was applied, because we expected large differences in toxicity due to pH. Because such differences were not found, the results of the experiment are difficult to interpretate. Toxicity of cadmium was higher at lower pH. No significant differences between pH 5.0 and pH 6.7 were found for the EC₅₀ values for weight loss.

In Panheel soil LC₅₀ values increased from pH 3.5 to pH 5.6/6.8. Also the EC₅₀ values for weight loss increased from pH 3.5 to pH 5.6/6.8. However, based on Cd²⁺ activities and on total cadmium concentration in pore water, cadmium was less toxic at pH 3.5 than at pH 6.8.

Table 4.1 *LC₅₀ values (with 95% confidence interval) for survival of Eisenia fetida by cadmium and EC₅₀ values for effect of cadmium on loss of weight.*

Nominal conc Cd (mg/kg)	Panheel soil			OECD artificial soil			Wageningen
	pH 3.5	pH 5.6	pH 6.8	pH 3.9	pH 5.0	pH 6.7	pH 6.0
	Surviving worms						
0	36	39	39	40	39	40	40
0.32	-	-	-	40	40	40	-
3.2	-	-	-	40	40	40	-
32	35	40	39	40	40	40	40
100	32	40	39	-	-	-	40
320	1	40	40	37	40	40	40
1000	0	0	2	-	-	-	0
3200	0	0	0	0	0	0	0
LC ₅₀ (mg/kg)	162 (134-195)	566 (**)	600 (551-653)	851 (703-1031)	1012 (**)	1012 (**)	566 (**)
EC ₅₀ for effect on weight loss based on:							
Total Cd soil (mg/kg)	143 (90-225)	393 (304-508)	343 (270-388)	*	410 (174-971)	358 (173-741)	332 (231-476)
Cd ²⁺ activity (mg/l)	44 (24-80)	19 (15-23)	3.6 (2.9-4.3)	*	5.4 (0.35-81)	1.9 (0.35-11)	17 (11-26)
Total Cd pore water (mg/l)	234 (101-543)	841 (73-9650)	15 (7.3-30)	*	12 (1.4-106)	75 (**)	47 (19-116)

* Could not be determined

** Confidence interval could not be determined

4.2.3 Accumulation of cadmium in *Eisenia fetida*

In Table 4.2 the internal cadmium concentration in the worms is compared with concentrations in the soil, total concentrations and Cd^{2+} activities in the pore water. The internal concentration shows a remarkably constant pattern, and is more or less independent of the pH. The internal concentration of cadmium at which the worms die is about 70-80 mg/kg fresh weight. The EC_{50} value for weight loss corresponds with an internal concentration of 60 to 70 mg/kg.

Table 4.2 Internal cadmium concentration (fresh weight, average of 2 determinations) in the worms compared with concentrations in the soil, total concentrations in the pore water and Cd^{2+} activities in the pore water (concentrations in soil: mg/kg; in pore water; mg/l).

Panheel soil											
pH 3.5				pH 5.6				pH 6.8			
Internal conc	Soil conc	Total pore water conc	Pore water activity	Internal conc	Soil conc	Total pore water conc	Pore water activity	Internal conc	Soil conc	Total pore water conc	Pore water activity
1	0	0.41	4.3	0.7	0	6.7	2	11	0	2.1	0.4
21	32	46	16	22	32	48	4.3	21	32	2.7	0.8
55	100	155	34	43	100	35	5	25	100	1.6	0.8
20*	320	710	90	68	320	93	16	70	320	15	3.4
-	1000	2800	200	-	1000	1020	53	-	1000	350	20

* One worm

Table 4.2 Continued.

OECD Artificial soil											
pH 3.9				pH 5.0				pH 6.7			
Internal conc	Soil conc	Total pore water conc	Pore water activity	Internal conc	Soil conc	Total pore water conc	Pore water activity	Internal conc	Soil conc	Total pore water conc	Pore water activity
0.2	0	0.03	0.6	0.2	0	0.6	0.83	0.4	0	0.18	-
2.9	3.2	0.14	2	-	3.2	0.25	0.1	3.5	3.2	0.05	-
16	32	0.83	1	21	32	0.18	0.32	20	32	0.16	0.07
63	320	51	18	68	320	5	9.8	65	320	0.45	0.7

Wageningen soil			
Internal conc	Soil conc.	Total pore water conc	Pore water activity
0.95	0	0.32	0.3
26	32	1.38	1.3
51	100	6.8	4.5
77	320	56	17

Table 3.5 Extractable and exchangeable cadmium and free Cd^{2+} concentrations in soil extracts and pore water at different pH's in artificial soil. All values are expressed as % of the total measured cadmium concentrations in the soils.

pH	Total Cd conc. (mg/kg)	Extractable and exchangeable concentration as % of total soil concentration							
		water soluble Cd	water soluble Cd^{2+}	extractable Cd	extractable Cd^{2+}	exchang. Cd 0.01M CaCl_2	exchang. Cd 0.1M CaCl_2	pore water Cd	pore water Cd^{2+}
3.5	0.18	33.3	75.8	44.4	76.7	6.25	12.5	1.11	21.7
	37.1	1.15	0.89	19.4	3.27	79.4	196	0.65	0.25
	69.2	1.69	0.77	20.1	6.04	91.6	175	0.88	0.3
	114	4.11	1.57	30.4	8.45	117	256	2.05	0.54
	269	5.61	2.06	29.1	6.37	81.8	207	3.12	0.72
	596	13.6	5.04	26.3	5.17	84.2	176	5.33	0.95
	1089	11.1	4.12	29.7	5.25	95.4	178	11.9	1.74
5.0	0.60	5.00	31.4	43.3	23.8	48.7	82.4	0.33	4.16
	35.3	0.99	0.58	8.61	1.45	43.6	177	0.37	0.17
	73.8	1.52	0.57	16.8	1.46	44.7	174	0.48	0.13
	138	1.89	0.58	11.3	1.68	54.4	192	0.65	0.14
	274	3.28	1.06	11.9	1.86	47.6	204	1.61	0.27
	887	3.67	1.10	10.8	1.38	43.1	107	1.95	0.32
	1273	9.27	7.28	18.2	2.34	65.7	147	6.04	0.70
6.5	0.17	35.3	68.3	47.1	25.2	0	6.61	0.59	4.71
	34.2	0.35	0.31	1.14	0.17	3.68	64.4	0.05	0.02
	76.2	0.49	0.15	0.81	0.14	4.59	62.0	0.05	0.01
	176	0.41	0.1	1.05	0.14	6.05	65.1	0.06	0.01
	359	0.60	0.11	1.73	0.21	11.4	94.8	0.13	0.02
	574	1.20	0.31	4.59	0.52	22.9	133	0.66	0.1
	1265	3.31	0.71	7.16	0.84	32.4	125	1.61	0.22

Table 3.6 Extractable and exchangeable cadmium and free Cd^{2+} concentrations in soil extracts and pore water at different pH's in Panheel soil. All values are expressed as % of the total measured cadmium concentrations in the soils.

pH	Total Cd conc. (mg/kg)	Extractable and exchangeable concentration as % of total soil concentration							
		water soluble Cd	water soluble Cd^{2+}	extractable Cd	extractable Cd^{2+}	exchang. Cd 0.01M CaCl_2	exchang. Cd 0.1M CaCl_2	pore water Cd	pore water Cd^{2+}
3.5	0.41	41.1	195	141	149	87.7	71.3	56.6	18.8
	30.7	29.3	14.2	56.7	16.0	70.2	83.8	2.56	3.01
	60.3	40.6	18.9	58.4	14.3	80.4	91.7	3.19	3.38
	93.4	41.3	19.8	64.2	14.9	75.0	93.2	32.0	3.22
	171	47.3	20.6	62.1	13.2	73.7	91.0	39.7	3.45
	454	44.3	22.1	64.8	12.0	69.6	93.2	36.0	2.74
	818	38.1	16.9	62	26.0	75.6	89.1	38.9	2.56
5.0	0.32	45.7	40.6	49.2	27.7	52.7	133	33.6	3.67
	64.1	1.39	0.37	6.43	1.03	28.6	70.2	0.21	0.04
	111	1.23	0.38	7.39	1.22	33.7	82.1	0.29	0.05
	137	1.18	0.39	8.1	1.37	-	86.3	0.32	0.06
	365	1.39	0.45	10.5	1.58	43.1	93.0	0.90	0.18
	554	3.13	1.00	15.6	2.32	49.3	101	2.85	0.32
	1207	8.93	3.17	23.9	3.02	53.4	91	9.82	0.83
6.5	0.16	7.03	56.3	87.8	60.1	28.1	98.4	34.3	3.37
	59	0.72	0.34	3.51	0.50	14.3	65.4	0.11	0.02
	112	0.89	0.28	3.29	0.43	15.1	69.6	0.09	-
	108	1.12	0.28	3.33	0.99	16.7	67.1	0.16	-
	394	0.89	0.28	4.06	0.56	21.1	68.0	0.26	0.04
	744	2.92	0.95	7.78	0.91	27.9	70.2	1.91	0.15
	1307	2.20	0.81	10.9	1.25	31.1	72.7	2.21	0.36

Table 3.7 Extractable and exchangeable cadmium and free Cd^{2+} concentrations in soil extracts and pore water in Wageningen soil.
All values are expressed as % of the total measured cadmium concentrations in the soils.

Total Cd conc. (mg/kg)	Extractable and exchangeable concentration as % of total soil concentration						pore water Cd
	water soluble Cd	water soluble Cd^{2+}	extractable Cd	extractable Cd^{2+}	exchang. Cd 0.01M CaCl_2	exchang. Cd 0.1M CaCl_2	
0.36	50.0	60.4	12.5	15.9	9.37	37.5	0.27
50.0	1.19	0.60	5.82	0.93	18.0	62.5	0.09
94.1	1.31	0.32	6.15	1.02	22.8	69.8	0.16
191	1.22	0.22	7.58	1.12	29.5	74.2	0.28
376	1.55	0.27	9.97	1.38	32.8	73.8	0.71
658	3.87	0.76	15.5	2.24	42.2	88.8	1.90
1425	9.99	1.63	20.2	2.51	43.6	80.6	5.08

Table 3.8 Fraction of cadmium in water soluble, soil solution or pore water extracts present as free Cd^{2+} .

pH	nominal conc. in soil (mg/kg)	Fraction of cadmium in extract present as Cd^{2+} (% of total cadmium concentration in extract)							
		artificial soil water soluble	Panheel soil extractable	Wageningen soil pore water	water soluble	extractable	pore water	water soluble	soil solution
3.5	0	228	173	1950	474	105	33.3		
	50	77.5	16.9	37.9	48.5	28.1	118		
	100	45.8	30.1	33.5	46.6	24.4	106		
	200	38.3	27.8	26.2	47.9	23.2	10.1		
	400	36.6	21.9	23.2	43.6	21.2	8.68		
	800	37.1	19.7	17.7	49.9	18.5	7.62		
	1600	37.2	17.7	14.6	44.4	42.0	6.59		
5.0	0	627	55.0	1247	88.8	56.4	10.9	121	127
	50	58.3	16.8	45.7	26.6	16.0	19.8	50.4	16.0
	100	37.6	8.69	27.1	30.9	16.6	17.4	24.3	17.0
	200	30.7	14.9	22.2	33.1	16.9	19.7	17.7	15.0
	400	32.3	15.6	16.8	32.4	15.0	20.5	17.2	14.0
	800	29.9	12.8	16.3	31.9	14.8	11.2	19.7	15.0
	1600	78.5	12.9	11.6	35.5	12.6	8.40	16.3	12.0
6.5	0	193	53.5	800	801	68.5	9.82		
	50	89.4	14.9	51.0	47.2	14.3	15.5		
	100	31.1	17.3	28.8	31.5	13.0	-		
	200	24.1	13.3	16.7	25.0	29.8	-		
	400	18.0	12.1	16.2	31.5	13.8	13.7		
	800	26.0	11.3	15.8	32.5	11.7	8.02		
	1600	21.4	11.7	13.8	36.8	11.5	16.3		

Table 3.11 *EC₅₀ values (with 95% confidence interval) for the effect of cadmium on the growth (fresh and dry weight) and reproduction of Folsomia candida in an artificial soil substrate.*

pH	EC ₅₀ in mg/kg on the basis of									
	total Cd	water soluble Cd	water soluble Cd ²⁺	extractable Cd	extractable Cd ²⁺	exchang. Cd 0.01M CaCl ₂	exchang. Cd 0.1M CaCl ₂	pore water Cd	pore water Cd ²⁺	internal Cd
3.5	fresh weight									
	200 (170-237)	10.2 (7.53-13.7)	3.33 (2.26-4.89)	58.6 (49.2-69.7)	14.3 (12.6-16.2)	191 (167-220)	445 (383-516)	5.35 (4.03-7.12)	1.23 (0.92-1.66)	104 (77.9-139)
	359 (287-449)	11.6 (8.48-15.9)	3.14 (1.88-5.24)	43.1 (34.8-53.5)	6.26 (5.07-7.73)	179 (144-222)	626 (538-728)	5.50 (3.77-8.00)	0.86 (0.56-1.31)	149 (108-206)
5.0	512 (445-589)	4.71 (3.30-6.73)	0.55 (0.15-2.10)	15 (10.2-22.3)	1.78 (1.21-2.63)	89 (65.6-121)	585 (476-719)	1.59 (0.90-2.81)	0.18 (0.06-0.53)	141 (120-165)
	dry weight									
	239 (186-307)	13.3 (8.85-19.8)	4.80 (3.17-7.26)	68.9 (54.3-87.4)	15.8 (13.2-18.8)	212 (175-255)	506 (416-616)	7.01 (4.73-10.4)	1.62 (1.14-2.31)	125 (93.1-168)
6.5	400 (287-557)	13.6 (8.85-20.9)	4.08 (2.52-6.59)	47.3 (34.2-65.4)	6.94 (5.22-9.24)	199 (145-274)	670 (556-808)	6.64 (4.03-11.0)	1.06 (0.64-1.77)	159 (113-223)
	494 (406-603)	4.40 (2.69-7.21)	0.37 (0.03-4.61)	14.1 (8.27-24.2)	1.63 (0.94-2.85)	82.9 (54.1-127)	554 (413-744)	1.5 (0.70-3.21)	0.14 (0.02-0.80)	140 (116-169)
	population growth									
3.5	81.5 (62.6-106)	1.74 (0.89-3.39)	0.60 (0.22-1.61)	19.0 (13.1-27.7)	5.46 (3.51-8.47)	79.3 (56.7-111)	165 (118-231)	0.98 (0.56-1.70)	0.23 (0.09-0.56)	47.4 (34.8-64.5)
	101 (76.3-134)	1.69 (1.09-2.61)	0.11 (0.001-14.0)	13.9 (11.2-17.3)	1.54 (1.06-2.24)	48.9 (35.0-68.2)	186 (136-253)	0.59 (0.37-1.07)	0.13 (0.07-0.22)	63.6 (49.6-81.5)
	223 (163-305)	1.04 (0.66-1.63)	0.17 (0.04-0.71)	2.51 (1.32-4.76)	0.33 (0.17-0.64)	15.9 (8.65-29.3)	158 (96.7-257)	0.16 (0.08-0.33)	0.008 (0.00-1.58)	59.4 (47.0-75.0)

Table 3.12 *EC₅₀ values (with 95% confidence interval) for the effect of cadmium on the growth (fresh and dry weight) and reproduction of Folsomia candida in Panheel soil.*

pH	EC ₅₀ in mg/kg on the basis of									
	total Cd	water soluble Cd	water soluble Cd ²⁺	extractable Cd	extractable Cd ²⁺	exchang. Cd 0.01M CaCl ₂	exchang. Cd 0.1M CaCl ₂	pore water Cd	pore water Cd ²⁺	internal Cd
	fresh weight									
3.5	26.3 (19.0-36.3)	7.19 (4.52-11.4)	0.52 (0.036-7.58)	13.5 (8.79-20.7)	3.00 (1.47-6.13)	18.6 (13.1-26.5)	22.4 (16.0-31.3)	2.40*	0.59 (0.32-1.09)	-
5.0	208 (177-243)	2.72 (2.11-3.5)	0.46 (0.21-0.99)	19.2 (15.4-23.9)	2.95 (2.37-3.67)	75.8 (62.1-92.6)	183 (154-218)	0.0092 #	0.067 (0.020-0.22)	120 (95-151)
6.5	242 (197-297)	2.58 (2.08-3.20)	0.41 (0.20-0.82)	9.83 (7.49-12.9)	1.53 (1.18-1.98)	46.7 (36.4-59.9)	166 (135-204)	0.17 (0.011-2.68)	0.060 (0.034-0.12)	112 (82.5-153)
	dry weight									
3.5	26.4 (16.4-42.1)	7.08 (3.46-14.5)	0.66 (0.017-25)	13.8 (7.4-25.4)	3.22 (1.31-7.90)	18.3 (10.7-31.3)	22.2 (13.4-36.7)	1.71*	0.61 (0.25-1.47)	3.42 (0.25-47.6)
5.0	197 (153-252)	2.43 (1.55-3.80)	0.29 (0.052-1.60)	17.9 (12.5-25.5)	2.70 (1.85-3.96)	70.7 (51.4-97.3)	172 (130-228)	0.0029 #	0.030 (0.0016-0.57)	109 (81.5-145)
6.5	200 (147-272)	2.08 (1.44-3.00)	0.15 (0.019-1.30)	7.52 (4.78-11.8)	1.05 (0.56-1.97)	37.1 (25.3-54.5)	137 (100-187)	-	0.039 (0.0056-0.28)	103 (72.6-145)
	population growth									
3.5	10.1 #	4.06 #	1.89 #	9.29 #	3.07 #	7.55 #	11.3 #	0.34 #	0.21 #	112 #
5.0	125 (111-140)	1.51 (1.36-1.66)	0.47 (0.39-0.55)	9.72 (8.34-11.3)	1.62 (1.38-1.90)	39.6 (34.9-44.9)	105 (91.7-121)	0.26 (0.10-0.68)	0.06 (0.044-0.090)	97.7 (88.2-108)
6.5	112 (98.7-126)	1.12 (0.97-1.29)	0.31 (0.28-3.01)	3.69 (3.29-4.14)	0.68 (0.49-0.93)	17.7 (15.6-20.1)	76.4 (66.6-87.7)	0.26 (0.10-0.68)	0.021 (0.011-0.039)	88.7 (83.4-94.2)

* EC₅₀ estimated by linear regression; # 95% confidence interval extremely large; - no value could be calculated

Table 3.13 *EC₅₀ values (with 95% confidence interval) for the effect of cadmium on the growth (fresh and dry weight) and reproduction of Folsomia candida in Wageningen soil.*

EC ₅₀ in mg/kg on the basis of								
total Cd	water soluble Cd	water soluble Cd ²⁺	extractable Cd	extractable Cd ²⁺	exchang. Cd 0.01M CaCl ₂	exchang. Cd 0.1M CaCl ₂	pore water Cd	internal Cd
fresh weight								
223 (195-255)	2.92 (2.45-3.48)	0.42 (0.27-0.66)	18.1 (15.0-21.9)	2.64 (2.21-3.15)	66.6 (56.4-78.6)	165 (143-189)	0.80 (0.59-1.09)	89.3 (61.2-130)
dry weight								
206 (176-242)	2.66 (2.18-3.24)	0.41 (0.27-0.63)	16.3 (13.0-20.3)	2.39 (1.95-2.94)	60.4 (49.5-73.8)	152 (128-179)	0.67 (0.47-0.96)	90.0 (61.3-132)
population growth								
49.0 (41.7-68.9)	0.44 (0.19-1.01)	0.26 (0.18-0.36)	3.08 (2.29-4.16)	0.45 (0.30-0.69)	9.91 (7.04-14.0)	34.0 (25.5-45.3)	0.048 (0.027-0.087)	45.6 (39.6-52.6)

Table 3.A Extractable and exchangeable cadmium concentrations and Cd^{2+} activity in soil extracts and pore water at different pH's in artificial soil. All values in mg/l.

pH	Total Cd conc. (mg/kg)	water soluble Cd	water soluble Cd^{2+}	extractable Cd	extractable Cd^{2+}	exchang. Cd 0.01M CaCl_2	exchang. Cd 0.1M CaCl_2	pore water Cd	pore water Cd^{2+}
3.5	0.18	0.006	0.014	0.016	0.030	0.001	0.002	0.010	0.14
	37.1	0.043	0.033	1.44	0.24	2.95	7.27	0.85	0.32
	69.2	0.12	0.053	2.78	0.84	6.34	12.1	2.17	0.73
	114	0.47	0.18	6.94	1.92	13.3	29.2	8.62	2.26
	269	1.51	0.55	15.6	3.43	22.0	55.7	30.4	7.04
	596	8.11	3.00	31.3	6.16	50.2	105	124	21.9
	1089	12.1	4.49	64.7	11.4	104	194	469	68.3
5	0.6	0.003	0.019	0.053	0.030	0.029	0.049	0.010	0.080
	35.3	0.035	0.020	0.61	0.10	1.54	6.25	0.47	0.22
	73.76	0.112	0.042	1.27	0.22	3.30	12.8	1.29	0.35
	138	0.261	0.080	3.11	0.47	7.51	26.5	3.84	0.85
	274	0.899	0.29	6.55	1.02	13	55.9	15.9	2.66
	887	3.26	0.976	19.2	2.45	38.2	94.9	64.9	10.6
	1273	11.8	9.27	46.3	5.96	83.6	187	283	32.9
6.5	0.17	0.006	0.012	0.017	0.010	0	0.001	0.01	0.031
	34.2	0.012	0.011	0.079	0.010	0.13	2.20	0.06	0.033
	76.2	0.037	0.011	0.12	0.020	0.35	4.72	0.15	0.040
	176	0.072	0.018	0.37	0.050	1.06	11.5	0.42	0.070
	359	0.22	0.039	1.26	0.15	4.09	34.0	1.84	0.30
	574	0.69	0.18	5.28	0.59	13.1	76.3	14.3	2.26
	1265	4.19	0.90	18.1	2.12	41.0	158	76.7	10.6

Table 3.B Extractable and exchangeable cadmium and free Cd²⁺ concentrations in soil extracts and pore water at different pH's in Panheel soil. All values in mg/l.

pH	Total Cd conc. (mg/kg)	water soluble Cd	water soluble Cd ²⁺	extractable Cd	extractable Cd ²⁺	exchang. Cd 0.01M CaCl ₂	exchang. Cd 0.1M CaCl ₂	pore water Cd	pore water Cd ²⁺
3.5	0.41	0.017	0.080	0.12	0.12	0.036	0.029	2.53	0.84
	30.7	0.90	0.43	3.48	0.98	2.16	2.57	8.50	10.0
	60.3	2.45	1.14	70.4	1.72	4.85	5.53	19.9	21.1
	93.4	3.86	1.85	12.0	2.78	7.01	8.70	343	34.5
	171	8.09	3.53	21.2	4.51	12.6	15.6	709	61.5
	454	20.1	10.0	58.8	10.9	31.6	42.3	1843	140
	818	31.2	13.9	101.4	42.5	61.8	72.8	3496	230
5	0.32	0.015	0.010	0.031	0.018	0.017	0.043	1.61	0.18
	64.1	0.089	0.020	0.824	0.13	1.83	4.50	1.87	0.37
	111	0.14	0.040	1.64	0.27	3.74	9.11	4.84	0.84
	137	0.16	0.050	2.21	0.38	-	11.8	6.46	1.27
	365	0.51	0.17	7.68	1.15	15.7	33.9	50.6	10.4
	554	1.73	0.55	17.31	2.57	27.3	56.0	242	27
	1207	10.8	3.82	57.7	7.29	64.5	110	1671	140
6.5	0.16	0.001	0.010	0.028	0.019	0.004	0.016	0.95	0.090
	59	0.042	0.020	0.41	0.059	0.84	3.86	0.99	0.15
	112	0.10	0.030	0.74	0.096	1.69	7.80	1.73	-
	108	0.12	0.030	0.72	0.21	1.80	7.25	2.50	-
	394	0.35	0.11	3.19	0.44	8.31	26.8	14.1	1.92
	744	2.17	0.70	11.6	1.35	20.8	52.2	223	17.8
	1307	2.88	1.05	28.4	3.27	40.6	95	424	69.1

Table 3.C Extractable and exchangeable cadmium and free Cd^{2+} concentrations in soil extracts and pore water in Wageningen soil. All values in mg/l.

Total Cd conc. (mg/kg)	water soluble Cd	water soluble Cd^{2+}	extractable Cd	extractable Cd^{2+}	exchang. Cd 0.01M CaCl_2	exchang. Cd 0.1M CaCl_2	pore water Cd
0.36	0.018	0.022	0.009	0.01	0.003	0.014	0.03
50.0	0.06	0.03	0.582	0.09	0.9	3.13	1.64
94.1	0.12	0.03	1.16	0.19	2.15	6.57	4.01
191	0.23	0.042	2.9	0.43	5.63	14.2	15
376	0.58	0.1	7.5	1.04	12.3	27.7	73.9
658	2.55	0.5	20.4	2.95	27.8	58.4	415
1425	14.2	2.32	57.6	7.16	62.1	115	1836

5. DISCUSSION

This study was intended to test current hypotheses on the relationship between the concentration of metals in soil and their toxicity to soil organisms, as discussed in the introduction section. Soil properties like the content of clay and organic matter (o.m), pH and ionic strength of the pore water, are known to determine adsorption equilibria and chemical speciation. Moreover, factors like pH and ionic strength might also influence uptake by organisms.

In our investigation we found slight differences in soil adsorption of cadmium at comparable pH by soils with different clay and organic matter contents. Generally, the OECD artificial soil, containing more o.m. and clay, adsorbed more cadmium than the other soils. However, these differences could not be explained by the differences in clay and o.m. contents, nor by the differences in CEC. Probably this is caused by the type of o.m. in the artificial soil (ground peat), which differs from the mor type humic substance in the other soils. Moreover, kaolin clay used in the OECD artificial soil is known to possess only weak metal adsorbing properties compared to other clay types. Therefore, the suitability of the OECD artificial soil as a reference test soil may be doubted, because a straightforward extrapolation of test results to other soils is difficult.

The pH had, as expected, had a considerable influence on adsorption. With decreasing pH the concentration of cadmium in the water phase increased. This is in agreement with many observations reported in the literature. A relationship could be established between the logarithm of the liquid equilibrium cadmium concentration and the pH of the liquid phase. This relationship agrees with similar reports in the literature (for a review see Vonk, 1995). The coefficient of the relationship ($\Delta \log a_{Cd}/\Delta pH$) was of the same order (about 0.6) as reported in the literature (Gerritse and Van Driel, 1984; Scheffer and Schachtschabel, 1982). For a soil pH correction factor for physico-chemical behaviour of cadmium in soil, e.g. leaching, the following approach could be useful.

If the soil cadmium concentration in a reference soil with pH 6 is C_{ref} , than in a similar soil (same content of clay and o.m) with a pH = pH_x the cadmium concentration with a similar physico-chemical behaviour is:

$$C_x = C_{ref} \cdot 10^{0.6(pH_x - 6)}$$

However, it should be realised that this formula does not account for the amount of cadmium that is actually available and taken up by organisms. As will be clear from further discussions given below, for ecotoxicological extrapolation this formula would give over-compensation.

We found also that the ionic strength (I) of the pore water is an important factor in determining adsorption of cadmium to soils. The amount of cadmium in the liquid phase increased in the following order: extraction by water (I=0); 0.005 M $\text{CaCl}_2/\text{KCl}/\text{NaCl}$ (I= 0.01); 0.01 M CaCl_2 (I=0.03); 0.1 M CaCl_2 (I=0.3). It was not the aim of the present investigation to study the effect of ionic strength on cadmium availability in soils, but in future studies this might be an important item of attention.

It is remarkable that the proportion of Cd^{2+} activity to the total cadmium concentration was relatively high in the extracts and in the pore water of the artificial soil. Differences at high concentrations could be explained on the basis of Cl^- concentrations. For the Panheel soil there was no agreement between the ratios of total cadmium to Cd^{2+} measured in the different laboratories. The cause of this difference is not known. No relationship was found between the percentage of Cd^{2+} and the concentration of dissolved organic carbon (DOC) in the pore water. Such a relation had been expected, because of reported complexation between cadmium and DOC. Possibly the nature of the DOC in the soils studied differs.

This was one of the first studies in which the relationship between Cd^{2+} activity and uptake/toxicity was studied. We had hoped to find clear relationships, but, as discussed below, it became evident that such a relationship does not exist.

The effect of cadmium on the mineralisation of glutamate in the artificial soil was clearly related to the concentration of Cd^{2+} in the pore water. Mineralisation was 50% inhibited by a Cd^{2+} activity of 2-5 mg/l. However, in the Panheel soil no such relationship could be observed, and the low pH obviously protected the microbial population against the cadmium toxicity.

Similar protecting effects were observed for *Eisenia fetida*, as 34 mg Cd^{2+} /l in the extract caused a mortality of only 22% at pH 3.5, whereas 20 mg Cd^{2+} /l caused a mortality of 95% at pH 6.8. Similar effects at low pH were also observed for relative weight loss of the earthworms.

From Part 3, Tables 3.11 and 3.12 a general trend appears that the EC_{50} values for the effect on *Folsomia candida* growth and reproduction based on water soluble, extractable and pore water concentrations and activities are higher at pH 3.5 than at pH 6.5, albeit the difference is not always statistically significant. This could point to a protective effect of a low pH on cadmium toxicity.

The protective effect of H^+ ions on toxicity could be *via* the uptake mechanism, e.g. by competition of H^+ for Cd^{2+} on the uptake receptors. Similar effects have been described for plants growing in cadmium containing nutrient solutions at different pH (Hatch *et al.*, 1988). This phenomenon has also been observed in micro-organisms (Van Beelen and Fleuren-Kemilä, 1993 ; see for a review: Vonk *et al.*, 1994) .

The protective effect of H^+ ions is supported by the independency of internal concentrations in *E. fetida* and *F. candida* from the soil pH. However, it cannot be excluded that the worms take up cadmium directly from the solid soil phase instead from the pore water, e.g. from the (0.1 M $CaCl_2$ extractable) exchangeable part. For *F. candida* this seems improbable, since it does not take up solid soil phase. In fact the correlation of internal concentration with the exchangeable part is better than with other fractions (results not shown). To reject the latter possibility further research would be necessary.

From the results obtained at three different pH levels, it may become obvious that, especially in the case of *F. candida*, the lower pH itself was already stressful for the test organisms. In fact, the combined effects of high H^+ and cadmium concentrations were tested. It should be further noted that in the studies with *F. candida* the soils were flushed with water to remove excess chloride. With this also nutrients may have leached from the soils, thus creating another stress factor. Both factors may explain the high sensitivity of *F. candida* in Wageningen soil and in Panheel soil at low pH.

In this study, fairly high soil concentration concentrations had to be tested to establish concentration-response relationships for the test organisms used. It should be realised that relationships between soil characteristics and bioavailability will not always be linear and that deviations of linearity will especially occur at increasing soil and solution concentrations.

Internal concentrations found for the effects of cadmium on *E. fetida* and *F. candida* appeared to be relatively similar for different soils used, thus supporting the proposal of Van Wensem *et al.* (1994) to use internal rather than external concentrations for the derivation of soil quality criteria.

The Dutch policy for soil protection distinguishes between soils using a so-called soil type correction factor. This factor is based on differences in organic matter and clay content between soils. From the present study and the literature studies reported before (Vonk *et al.*, 1994; Vonk, 1995), it becomes clear that pH may strongly affect cadmium toxicity.

The data found in this study are, however, not very consistent, both between soils and for different organisms used. Neither is it possible to formulate a uniform correction factor to eliminate differences in toxicity between soils. It may be concluded that the relationship between metal speciation in soils and uptake and effects on soil organisms is rather complex.

It may be speculated that other heavy metals will show a similar behaviour as cadmium. Zinc is known to resemble cadmium in its behaviour, be it that earthworms seem to be capable of regulating their body concentration of zinc (Van Gestel *et al.*, 1993). The latter phenomenon probably also holds for copper. Copper, nickel and lead are known to form stronger complexes with organic matter than cadmium, which might influence the effect of soil characteristics on their uptake by soil organisms.

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