Effects of soil properties on food web accumulation of heavy metals to the wood mouse (*Apodemus sylvaticus*)

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Soil properties significantly affect accumulation of heavy metals to wood mice so; risks cannot be based on total concentrations.

**Abstract**

Effects of soil properties on the accumulation of metals to wood mice (*Apodemus sylvaticus*) were evaluated at two sites with different pH and organic matter content of the soil. pH and organic matter content significantly affected accumulation of Cd, Cu, Pb and Zn in earthworms and vegetation. For Cd, Cu and Zn these effects propagated through the food web to the wood mouse. Soil-to-kidney ratios differed between sites: Cd: 0.15 versus 3.52, Cu: 0.37 versus 1.30 and Zn: 0.33–0.83. This was confirmed in model calculations for Cd and Zn. Results indicate that total soil concentrations may be unsuitable indicators for risks that metals pose to wildlife. Furthermore, environmental managers may, unintentionally, change soil properties while taking specific environmental measures. In this way they may affect risks of metals to wildlife, even without changes in total soil concentrations.

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1. Introduction

Wildlife is generally orally exposed to soil pollutants through food web accumulation. Numerous studies are available that relate soil concentrations of metals to concentrations in wildlife, in order to assess the risks that they may pose (Beyer et al., 1985; Hamers et al., 2002; Hendriks et al., 1995; Pascoe et al., 1996; Rogival et al., 2007; Van den Brink et al., 2003; Wijnhoven et al., 2007). In addition to field based studies, modelling approaches have been developed to assess food web accumulation of contaminants (Gorree et al., 1995; Hendriks et al., 1995; Hunter et al., 2003; Kooistra et al., 2001). Studies are generally based on total concentrations of pollutants in the soil, although the bioavailable fraction has also been used to base risk assessments on (Rogival et al., 2007; Torres and Johnson, 2001). It has been shown that bioavailability is affected by soil properties like pH, organic matter content ([OM]) or clay content ([clay]) (Bradham et al., 2006; Hobbelien et al., 2004; Ma, 2004; Spurgeon et al., 2006; Tack et al., 1996; van Wezel et al., 2003). Nevertheless, little is known of such effects on the food web accumulation of metals to wildlife in terrestrial food webs. This is in contrast to aquatic food webs, for which effects of for instance pH and dissolved organic content on accumulation patterns to predators are reviewed (Scheuhammer, 1991).

Several studies on terrestrial small mammals report linear or log-linear relationships between total metal concentrations in soil and in wildlife (Sharma and Shupe, 1977; Shore, 1995), but, others report no or only weak relationships (D’Havé et al., 2007; Torres and Johnson, 2001), or relationships that are dominated by other factors such as age or bodyweight (Wijnhoven et al., 2007). Only few papers report on levels of bioavailable fraction based on specific extraction approaches (Rogival et al., 2007). When reviewing the literature on accumulation of metals, it is evident that no clear relationships between total soil concentrations and animal tissue concentrations can be derived. Nevertheless, total soil concentrations of contaminants are very often used to derive their risks for wildlife. Furthermore, it is unknown whether effects of soil properties on the availability of metals at the lower trophic levels are propagated to the higher trophic levels. To address this we studied accumulation of metals to the wood mouse (*Apodemus sylvaticus*) and its food items at two sites. Both sites are historically contaminated with similar levels of metals, but with different soil properties like pH and [OM]. In the current paper we discuss effects that soil properties have on the accumulation of metals in organisms lower in the food web, and whether these effects propagate to the wood mouse, higher in the food web. Since the design of the study was limited to the two sites we used simple food web accumulation models to interpret the results in a more generic context.

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2. Materials and methods

2.1. Site description

2.1.1. Heteren

Heteren is the name of a floodplain area of the Neder-Rijn River near the village Heteren in The Netherlands (51°16'01N, 5°24'07E). This floodplain floods regularly and sediments are deposited in the area. Historically, these sediments were contaminated with heavy metals and other contaminants from upstream industrial areas (van Den Brink et al., 2003). This process resulted in a spatially variable levels of contaminants (Kooistra et al., 2001). Soils of these floodplains are further characterised by relatively high pH-levels and high clay content. This site is managed under an agricultural regime, with pastures and cornfields. Fields are surrounded by hedges and trees.

2.1.2. Plateaux

The site Plateaux is located in the southern part of The Netherlands, south of the city of Eindhoven (51°57'49N, 5°44'28E). It is characterised by sandy soils with relatively low pH and organic matter content. At this site the soil is historically polluted by atmospheric deposition of heavy metals from a zinc-smelter nearby (Ma et al., 1983). Plateaux is a nature conservation area with low density grazing, with solitary trees and shrubs.

2.2. Collection of soil, vegetation and earthworms

Soil was collected from the top 20 cm, after removal of visible organic material like roots. The soil was stored in a poly-ethylene bag at −20 °C prior to metal analysis. The pH was assessed within 24 h of collection. Samples of vegetation were clipped with stainless steel scissors and also stored in poly-ethylene bags at −20 °C. Only above ground parts were collected, since wood mice predominantly feed on vegetable plant parts. Samples from stinging nettle (Urtica dioica), grasses and herbs were collected at both sites and analysed separately. At both sites stinging nettle was a dominant species. No other species were as dominant as stinging nettle, so the remaining species were grouped to either grass or herbs. The group of grasses included all monocotyledonous species; the group of herbs covered the dicotyledonous species, excluding stinging nettle. In Plateaux, additional samples were collected from grass seeds and berries because these items can be of importance in the diet of the wood mouse. Adult earthworms (Lumbricus rubellus) were hand collected in the upper 20 cm of the soil, when possible from the sampled soil. This was the only species that could be collected in high enough numbers at both sites for proper chemical and statistical analyses. Worms were starved on a filter paper for two days to remove their gut content. After this, the earthworms were stored in a glass jar at −20 °C. Before analysis all samples were weight to assess fresh weight. After this the samples were freeze dried and weight to assess dry weight. Wet-to-dry weight ratios were determined as the ratio between wet weight and dry weight.

2.3. Collection of wood mice

Wood mice (A. sylvaticus) were collected using Longworth live traps (Van Den Brink and Bosveld, 2005). Traps were baited with peanut butter and cat food, and contained hay as bedding. Animals are caught alive in these traps, so side-catches could have been released. Animals were sedated with CO2, and sacrificed by cervical dislocation. All procedures involving the handling of animals were conducted by certified persons, and reviewed by an Animal Ethics Committee according to Dutch legislation on the protection and welfare of vertebrate animals used for experimental and other scientific purposes. In Heteren, wood mice were captured between 20th and 28th of September 2006 and 27 animals in Plateaux between 10th and 25th of August 2006. In the lab, the animals were weighed and dissected under clean conditions.

2.4. Chemical analyses

Soil was dried at 40 °C, all other samples were freeze dried prior to chemical analysis. Samples of soil, earthworms and kidneys were destructed with aqua regia in a microwave in teflon vessels. Vegetation samples were destructed gistly with fluoric acid at room temperature to remove the silica-skeleton in the plants. After this, the samples were destructed with aqua regia similarly to the other samples (Novozamsky et al., 1996). Samples were analysed for cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) using inductively coupled plasma atomic emission spectrometry (ICP-AES). When concentrations were below detection limits, analyses were performed with inductively coupled plasma mass spectrometry (ICP-MS). All concentrations are reported as dry weight concentrations, unless stated otherwise. For quality assurance, reference samples from clay and sandy soils and from grass and strawberry were analysed (respectively ISE 989, ISE 949, IPE 100 and IPE 125, WEPAL, www.wepal.nl).

2.5. Statistical analyses

Differences between sites and vegetation types were analysed with Analysis of Variance (ANOVA), with Least Significant Differences (LSD) as post-hoc test, using Genstat version 11.1 (www.genstat.co.uk). Generally, data on concentrations in biotic samples are not normally distributed, a prerequisite for the use of parametric methods, like ANOVA. To obtain normality of the data they were transformed into their natural logarithm prior to statistical analysis. In those cases the geometric mean will be reported, combined with the range of observations. The number of observations is sometimes low, which could demand for non-parametric methods. In those cases both parametric (ANOVA) and non-parametric (Kruskal–Wallis ANOVA) tests have been applied. In no case the results differed between the tests, and for sake of unity we report the parametric ANOVA results.

2.6. Accumulation modelling

2.6.1. Earthworms

Ma (2004) reports a regression model Equation (1) that can be used to calculate concentrations in L. rubellus from total soil concentrations in combination with soil properties, using metal specific parameters for Cd, Cu, Pb and Zn (Table 1). The database on which the parameters are based was very extensive, and covered a wide range of soil types (Ma, 2004). This allows to apply these parameters at both sites.

\[
\log(\text{HM}_{\text{soil}}) = a + b \cdot \log(\text{HM}_{\text{soil}}) + c \cdot \text{pH} + d \cdot \log(\text{OM}) + e \cdot \text{epigeic}
\]

(Equation (1), Ma, 2004); epigeic worms live more or less in the litter. We consider L. rubellus to be epigeic (Ernest et al., 2008). [\text{HM}_{\text{soil}}]: heavy metal concentration in soil (mg/kg d.w.); [\text{OM}]: % organic matter in soil; [clay]: % clay content in soil. The r² differ between metals, with highest r² for Cd and Pb, and relatively low r² for Cu and Zn (Table 1).

2.6.2. Vegetation

For grass, accumulation models have been developed similar to the previous ones for earthworms (Equation (2), with metal specific parameters (Table 2). The parameters are for Cd and Zn are derived based on a Dutch national wide database, but for Cu the dataset was more or less limited to a dataset on floodplain soils (van Wezel et al., 2003). For Cd and Zn the parameters are applicable at both sites, for Cu the application of the models for Plateaux should be considered with some caution.

\[
\log(\text{HM}_{\text{vegetation}}) = a + b \cdot \log(\text{HM}_{\text{soil}}) + c \cdot \text{pH} + d \cdot \log(\text{OM}) + e \cdot \log(\text{clay})
\]

(Equation (2), van Wezel et al., 2003); [HMvegetation]: heavy metal concentration in vegetation (mg/kg d.w.); [HMsoil]: heavy metal concentration in soil (mg/kg d.w.); [OM]: % organic matter in soil; [clay]: % clay in soil.

For Pb no significant parameters could be established (van Wezel et al., 2003). This was related to the fact that Pb generally occurs in organic complexes, and is thus not available for uptake by vegetation. In this parameter set it can be seen that the effect of pH on the accumulation of Zn is only limited (pH parameter is only −0.09) while the effect of [OM] appears to be larger ([OM] parameter is 1.09), similar to (Tack et al., 1996). For Cu and Cd the relative contribution of pH in the accumulation patterns is larger.

2.6.3. Food web accumulation

In the current study the food web accumulation is modelled for Zn and Cd, an essential and non-essential metal. The modelling is not performed for Pb because no parameters are available to include soil properties in modelling the accumulation to grasses (see above). For essential metals the modelling was restricted to Zn due to lack of proper parameters to model the uptake of Cu by the wood mouse in the literature. The uptake of Cd and Zn by wood mice is modelled based on their Total Daily Intake (TDI, μg/day). TDI is calculated by Equation (3).

\[
\text{TDI} = \left( \text{fw} \times \text{HM}_{\text{seed}} + \text{fw}_{\text{worm}} \times \text{HM}_{\text{worm}} + \text{fw}_{\text{berry}} \times \text{HM}_{\text{berry}} + \text{fw}_{\text{grass}} \times \text{HM}_{\text{grass}} \right) \times \text{Cons}_{\text{rate}}
\]

(Equation (3), TDI calculation; fw: fraction of seed in diet; fwworm: fraction of earthworms in diet; fwberry: fraction of berries in diet;-fwgrass: fraction of grass in diet; \text{HM}_{\text{seed}}: geometric metal concentration in seed (mg/kg w.w.); \text{HM}_{\text{worm}}: geometric metal concentration in earthworm (mg/kg w.w.); \text{HM}_{\text{berry}}: geometric metal concentration in berries (mg/kg w.w.); \text{HM}_{\text{grass}}: geometric metal concentration in grass (mg/kg w.w.); Cons_rate: daily consumption rate (g/day).

<table>
<thead>
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<th>Metal</th>
<th>a</th>
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<th>d</th>
<th>e</th>
<th>r²</th>
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<td>−0.534</td>
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<td>−0.461</td>
<td>−0.347</td>
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<tr>
<td>Cu</td>
<td>0.936</td>
<td>0.499</td>
<td>−0.061</td>
<td>−0.311</td>
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</tr>
<tr>
<td>Zn</td>
<td>2.800</td>
<td>0.224</td>
<td>−0.064</td>
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</table>
Concentrations of earthworm and grass were analysed in the two studied areas. Berries and grass seeds were only analysed in Plateaux. Ratios between concentrations in berries, grass seed and grass were calculated for Plateaux and used to calculate heavy metal concentrations for berries and seeds in Heteren. It was assumed that the ratios between berries, grass seed and grass were similar between the sites. Vegetation and earthworm specific dry-to-wet weight ratios were assessed to calculate the concentration on a wet weight base.

Wood mice predominantly feed on seeds and vegetative parts of plants, but may supplement this with other food items, including animal species like earthworms and insects (Canova and Fasola, 1993; Rogers and Gorman, 1995; Rogival et al., 2007; Tew et al., 2000; Watts, 1968). Watts (1968) reports a seasonal shifting diet, which varied with the seasons. Watts (1968) reports a seasonal shifting diet, which varied with the seasons. The latter study was performed on set aside land, which may be most comparable to the sites in our study. Wood mice consumed a high fraction of grasses in spring and early summer, while later in the summer the amount of seeds increased (Rogers and Gorman, 1995). In spring and summer, the diet also contained animal material, both insects and other. The choice of diet items in the modelling was limited to the items for which metal data are available in order to being able to compare the modelling results with measured data. The diet composition used in the current study is presented in Table 3. Earthworms were included in the same ratio as “other animal” in Rogers and Gorman (1995) in order to reflect non-vegetarian uptake, apart from insects. Insects like beetles have a specific accumulation pattern, because metals may be excreted, for instance during metamorphosis (Scheifler et al., 2002). For insects no suitable accumulation model could be selected for application, and furthermore no site specific data on metal concentrations in insects were available for validation. Therefore, insects were not included in the diet used for modelling.

Table 2 lists the soil concentrations and soil properties per site. The pH and OM percentage were significantly higher in the soil from the floodplains near Heteren when compared to the Plateaux (p < 0.001). Levels of Pb and Zn did not differ significantly between the sites (p > 0.05), while concentrations of Cd were significantly lower near Heteren (p < 0.001). In contrast to this were the levels of Cu significantly higher in soils from the floodplains (p < 0.001).

3. Results

3.1. Soil

Concentrations of heavy metals in earthworms differed significantly for all metals between sites (p < 0.001 for Cd, Cu and Pb, p = 0.003 for Zn). Most concentrations were higher in the area near Plateaux, except for Cu which showed higher concentrations in Heteren (Table 5).

3.3. Vegetation

The vegetation types included were stinging nettle, grasses and herbs (Table 6). Concentrations of metals in the vegetation differed between sites, although this was not always significantly for all three vegetation types. Cd concentrations were significantly higher in all vegetation types from the Plateaux (p < 0.001 for grass and herbs, p = 0.003 for stinging nettle), comparable to Zn (p = 0.012 for stinging nettle, p = 0.027 for grass, p < 0.001 for herbs). For Cu this was the opposite; concentrations in stinging nettle from Heteren were significantly higher (p < 0.001). Concentrations of Pb were generally lowest in berries and highest in grass, although the differences are not always significant. Cu concentrations are similar for all three types (p > 0.05).

3.4. Wood mice

In the two areas, several species of small mammals were caught, but only wood mice were sampled in high enough numbers to allow for statistical analyses. In Table 8, concentrations of metals in kidneys of wood mice are listed. Concentrations of Cd were significantly higher in wood mice from the Plateaux (p < 0.001). Concentrations of Pb and Zn did not differ significantly between sites (p > 0.05).
Plateaux are in the same range as in an earlier study performed 25 years ago in the same region (Ma et al., 1983). Current soil properties in Plateaux are also similar to those earlier study performed 25 year ago in the same region (Ma et al., 1983). OM and pH in Heteren are in the same range as in another study concerning river floodplains of the river Waal (Hendriks et al., 1995). This indicates that Heteren can be considered as a representative for river floodplains of the rivers Waal and Neder-Rijn in the Netherlands, while Plateaux may stand for the situation in the Kempen in the South of The Netherlands, which is an area that is contaminated by a nearby zinc smelting factory (Ma et al., 1983).

4.2. Accumulation from soil to earthworm

When addressing the accumulation of the different metals from soil to earthworms, it is evident that only Cd and Zn show higher concentrations in the worms compared to soil. Accumulation of Cd, Pb and Zn in earthworms is higher in Plateaux than in Heteren, for Cu this difference in accumulation is less obvious. Such differences in accumulation have been reported before, indicating that total soil concentrations are not the only factor governing the accumulation of metals (Janssen et al., 1997). pH and OM affect the bioavailability of metals considerably (Bradham et al., 2006; Janssen et al., 1997; Ma et al., 1983; Sample et al., 1999; Spurgeon et al., 2006) which may account for the elevated accumulation of metals in the earthworms at Plateaux, the site with the lower pH and OM (Table 9). When applying the accumulation model (Equation (1) and Table 1), the differences in measured Cd concentrations are also reflected in the modelled concentrations (Table 5), although the modelled difference is larger. This is similar for Pb. The measured Cu concentrations are significantly lower in earthworms from the Plateaux in comparison to Heteren, but this is not reflected in the modelled concentration. This may be due to the fact that the predictive power of the Cu specific parameters is relatively low (r² = 0.46, Table 1). The difference in measured Zn concentrations between the sites is also shown in the modelled Zn concentrations, although the difference between the measured concentrations appears to be larger.

These modelling results show that accumulation patterns of Cd, Pb, and Zn from soil to earthworm are affected by soil properties, in addition to total soil concentrations. For Cu this is less the case, perhaps this is due to other soil properties that may be more important in governing the uptake, for instance dissolved organic carbon (Janssen et al., 1997; Ma, 2005).
In Table 6 it is shown that the differences between the sites as found for Cd and Zn are also reflected in the modelling based on Equation (2) and Table 2, similar to the earthworms. For Cu, the modelling results appear to differ between the sites, but this is not the case for the measured concentrations. This lack of agreement between modelled and measured concentrations in case of Cu was also noticeable in the earthworm modelling. This may point to other soil properties than the ones measured here, that may affect Cu accumulation in vegetation as well as in earthworms (i.e. dissolved organic carbon). Nevertheless, it can be concluded that also for vegetation the differences in accumulation patterns for Cd, Zn and likely also Cu can be described better with inclusion of the appropriate soil properties. This however appears to be less applicable for Pb.

### 4.4. Food web accumulation from soil to wood mice

In earlier paragraphs it is shown that the accumulation of metals in earthworms and vegetation is affected by total concentrations in the soil and soil properties like pH and [OM]. It is however unclear whether effects of soil properties are propagated through the food web to the wood mouse, especially for essential metals which are physiologically regulated by organisms. Several other factors may also affect the food web accumulation, for instance the foraging ecology of the receptor (e.g. diet composition, food preferences, searching behaviour), the habitat preferences of the species (e.g. spatial distribution, prey availability), and the spatial/temporal occurrence of food items (van den Brink, 2004). These ecological factors, in combination with physiological feedback mechanisms like metabolism and excretion of contaminants may obscure direct relationships between soil concentrations and properties on the one hand and food web accumulation patterns on the other. In Fig. 1, the concentrations of Cd, Cu, Pb and Zn in soil and kidneys of wood mice are shown for the two sites. This figure illustrates that the metal specific accumulation patterns differ considerably between sites, except for Pb. It is evident that apart from Pb, the mice/soil ratios for the other metals are higher in Plateaux when compared to Heteren, ranging from 1.24 times (Pb) to 24.05 times in case of Cd. These differences indicate different patterns of accumulation, i.e. the efficiency of accumulation is much higher at the Plateaux.

Rogival et al. (2007) found linear relationships between total metal concentrations and the bioavailable fraction in the soil and

![Fig. 1. Concentrations of Cd(a), Cu(b), Pb(c) and Zn(d) in soil (black bars) and kidneys of wood mice in Heteren and Plateaux (grey bars) (geometric means (mg/kg dry weight), for ranges and further details see Tables 4 and 8).](image-url)
levels in liver and kidneys in wood mice. Total concentrations and their bioavailable fractions in that study were highly correlated with each other, so it is likely that the ranges of soil properties were relatively small and did not play a major role in changing the bioavailability of the metals between sites. Nevertheless, the soil-to-kidney ratios of our study are in the same range as Rogival et al. (2007), although our ratios for Cd are in their lower range.

Modelling the concentrations using Equations (1)–(5) results in Cd concentrations similar to the measured concentrations in case of the Plateaux. The different modelling scenarios for Cd render similar trends between the sites, although the concentrations in the kidney from the Plateaux mice from Plateaux are slightly underestimated in case of the second scenario (scenario Cd_2, Table 8). The modelled Cd concentrations in the kidneys of the wood mice from Heteren are slightly too high for both scenarios, but due to the low measured concentrations this difference is relatively large (modelled concentrations at the 91–94th percentile of the distribution of the measured concentrations Table 8). The measured difference between the locations is reflected in the modelled concentrations, although the measured difference is somewhat larger. This is also the case for Zn for which the measured difference is larger than the modelled difference (Table 8). However, the measured difference was not significantly due to a high variability in Zn concentrations (p = 0.13). These results show that the differences in accumulation between the locations are larger for a non-essential metal like Cd than for Zn, an essential metal which is regulated. In the models, the uptake efficiency of Zn in the gut is depending on the concentrations of Zn in the food (cf. Weigand and Kirchgessner, 1980), which is part of the internal regulation of Zn through homeostasis. This is well described by simple BAFs or BMFs. These are applied to derive the concentrations in the soil, food items and the predator or herbivore. In Table 10 the ratios between the concentrations in the kidneys of the wood mouse and grass or earthworms are listed. Our results show that accumulation of metals in the lower food web is directly affected by soil properties that govern the bioavailability of these metals. This effect is propagated through the food web to wood mice, higher up in the food web. This is even the case for essential metals like Cu and Zn that are expected to be physiologically regulated to maintain homeostasis (Hunter and Johnson, 1982). This indicates that risks of metals or other pollutants for wildlife are not necessarily related to the total soil concentrations, which is currently often used in risk assessments. This illustrates the need to include bioavailability of pollutants while assessing such risks for wildlife. This study shows that accumulation is not well described by simple BAFs or BMFs. These are applied to derive generic environmental quality standards (EQSs) in for instance the Netherlands (Van Vlaardingen and Verbruggen, 2007). For generic EQSs a worst case scenario may be used, based on the most efficient food web route of accumulation, but also assuming that pollutants are bioavailable. When addressing site specific risks of pollutants, local concentrations and soil properties, and species specific information should be used to assess the local risks of the pollutants for the specific species residing in the area of concern. Furthermore, knowledge on the effects of soil properties on food web accumulation of pollutants is of importance in relation to land use management. Soil properties often vary with land use and land management, and it is possible that land managers affect risks associated with soil contaminants not because they change soil concentrations but because soil properties may change due to their activities, and thereby the bioavailability of the contaminants.

Acknowledgements

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References


### Table 10

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