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Metal accumulation in earthworms inhabiting floodplain soils

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Metal levels in earthworms show large variation among sites, among seasons and among epigeic and endogeic species.

Abstract

The main factors contributing to variation in metal concentrations in earthworms inhabiting floodplain soils were investigated in three floodplains differing in inundation frequency and vegetation type. Metal concentrations in epigeic earthworms showed larger seasonal variations than endogeic earthworms. Variation in internal levels between sampling intervals were largest in earthworms from floodplain sites frequently inundated. High and low frequency flooding did not result in consistent changes in internal metal concentrations. Vegetation types of the floodplains did not affect metal levels in *Lumbricus rubellus*, except for internal Cd levels, which were positively related to the presence of organic litter. Internal levels of most essential metals were higher in spring. In general, no clear patterns in metal uptake were found and repetition of the sampling campaign will probably yield different results.

Keywords: Field results; Soil organisms; Floodplain systems; Metals; Earthworms

1. Introduction

It is relatively unknown how metal levels in organisms inhabiting floodplains alter throughout the year. Despite high contamination levels, floodplain soil properties, such as high pH and high organic matter content, cause low soluble metal concentrations (Janssen et al., 1997a; Sauvé et al., 2000; Van Leeuwen, 1999). Soluble metal is believed to be an important source of exposure for most organisms (Van Gestel, 1997), especially for organisms having a water permeable epidermis (Vijver et al., 2003a,b). However, elevated metal concentrations in earthworms were detected in Dutch floodplains, whereas isopods and millipedes (having a less water-permeable epidermis) showed similar metal levels as in species from unpolluted reference areas (Hobbelen et al., 2004). The explanation of this controversy remained indistinct, probably because of the involvement of many interfering factors.

In literature, field studies in which internal and external metal concentrations are measured usually have a static character, while floodplain ecosystems by definition are subjected to fluctuating conditions. Inundation, which is by definition a dynamic process, is known to influence metal speciation in soils (Vink and Meeussen, 2007). We tested the hypothesis that this variation in speciation is also reflected in bioaccumulation. To investigate dynamics of metal bioaccumulation, the epigeic *Lumbricus rubellus*, and the endogeic species *Allolobophora chlorotica* and *Aporrectodea caliginosa* were monitored. The epigeic species have their habitat in the top layers of soil and in litter layers, whereas the habitat of endogeic species is predominantly in deeper soil layers (Bouché, 1977). Three different floodplain sites were selected, based on differences in inundation frequency and vegetation types. Internal metal concentrations in earthworms were related to external factors, such as soil properties, metal concentrations, river

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water composition, seasonal changes in vegetation, and inundation frequency.

2. Materials and methods

2.1. Research sites and abiotic parameters

Three sites along the rivers Nieuwe Merwede (coded M and P) and Waal (coded S) were selected. River Waal switches over to the Nieuwe Merwede, so comparable water characteristics are found. Geographical information on the sites and vegetation types are summarized in Table 1.

Cattle grazed the sites M, P and S. The sites are located on gradually sloping riverbanks, and are subjected to periodic inundation. Plots from which earthworms were sampled, measured 10 by 10 meter. Fluctuations in water level and inundation frequency are shown in Fig. 1.

Inundation patterns in the fresh water estuary floodplain M and P are influenced by sea tides and wind, an example of a typical cycle is given in Fig. 1 (inset). Water levels are monitored on an hourly basis by Rijkswaterstaat and are available on http://www.waterbase.nl. At all sites we measured inundation frequency, surface water composition of both rivers, and soil and pore water properties, metal concentrations in soil, pore water, litter, and organisms. Soil characteristics and total metal concentrations were determined in November 1999 and August 2000. Pore water was collected using different techniques. At all three sites, pore water was collected by centrifugation, and filtered over 0.45 μm before chemical analysis. In addition, at site M pore water was monitored *in situ* regularly from November 2000 to December 2001, using a permeable pore water sampler (Rhizon SMS-MOM, Rhizosphere Research Products, Wageningen, The Netherlands). Organic litter was collected three times in 2003 and 2004 for metal analyses. Due to logistic reasons, sampling was done at different times.

2.2. Collection of organisms

From November 2000 to February 2004, earthworms of the species *Lumbricus rubellus* were collected from the floodplain sites M and P at three to five months intervals. Endogenic earthworm species were collected from November 2000 to May 2002, also site S was visited during these years. At all sites collection was random within the 10 × 10 m plot. At least 5 individuals of each pedo-ecological group were taken. After taking to the laboratory, the earthworms were allowed to defaecate on wet filter paper for 48 h. Wet weights were determined, followed by storage in a freezer at −18 °C until analyses.

2.3. Chemical analyses

Animals and litter were freeze-dried and dry weights were determined. Lyophilized organisms were digested in concentrated HNO_3 solution using a Mars5 destruction microwave oven and measured by ICP-MS (Perkin Elmer, SciEx ELAN 6000, Concord, Ontario, Canada). Soil samples were dried, sieved (<2 mm), and freeze dried before analysis at −50 °C for 18 h at −0.04 kPa. As, Cd, Cr, Cu, Pb, Zn, Mn, Fe concentrations in aqua regia (NEN 6465, 1992) were measured by ICP-AES (Spectros, Specto Flame) and ICP-MS. Certified reference material Dolt-2 (BCR, Brussels, Belgium), and blancs were treated similar as the samples. No systematic correction was applied to the body residue analyses, since recovery of the standard addition was within acceptance limits (90–110%). Measured metal concentrations of the standard reference were within performance acceptance limits (95–105%). The organic carbon content of the soils was analysed by wet oxidation with $K_2Cr_2O_7$ (Wallinga et al., 1992). Clay content was measured by sedimentation according to Houba et al. (1996).

2.4. Statistics

Internal metal concentrations of organisms were tested for outliers using the Grubbs test (Funk et al., 1985). The test is used to detect data outliers from a univariate and normally distributed data set. For each group of animal species, homogeneity of data was tested using Fmax, followed by analysis of covariance (ANCOVA) to correct metal accumulation for body weight (dependent = internal concentration and covariable = weight and factor = time). For each group of animal species, one-way ANOVA tested the significance of time of measurement (dependent = internal concentration and factor = time). This test was followed by a post-hoc test (Tukey) to analyse for significance of the differences that can be attributed to seasonality, specifically ($p < 0.05$). All statistics were performed using the software packages Systat 9.0 and SPSS 10.0.

3. Results

Sites M, P, and S differed in inundation frequency (Fig. 1). Due to the influence of sea tides, M and P were flooded periodically during the year, but were only permanently inundated during some winter periods. Flooding of site S was long-lasting and occurred usually in winter and early spring, and lasted for up to three months, depending on rainfall.

Water composition did not differ between the rivers Waal and Nieuwe Merwede. Soil characteristics and total metal concentrations are summarized in Table 2. Pollution levels of the three floodplain soils are specified as pollution class 4. This means that concentrations of one or more metals exceed the regulatory standards according to the Dutch regulations (4th National Policy Document). To compare metal levels with the quality criteria, a correction for clay and organic matter content was applied as described by Vegter (1995).

Site P is the most polluted location followed by site M and site S. Concentrations of Zn, Cd, Cu, Cr and Pb exceed the intervention values in most cases at sites M and P, and target values at site S. The metals exceeding these criteria are marked in Table 2. A summary of the pore water compositions at the different sites is given in Table 3.

Compared to the control and reference soils, only Cd concentrations in pore water of site M were elevated (Hobbelen et al., 2004). Pore water concentrations of all other metals were within boundary range of reference values. Both methods used for pore water sampling, i.e. rhizon and centrifugation,

Table 1
Characteristics of the floodplain sites selected for determining dynamics of metal concentrations in earthworms

Code	Location	*x*-coordinates	*y*-coordinates	Height above NAP (cm)	Rivers	Vegetation type
M	Lage Hof	111.314	420.428	80	Nieuwe Merwede	Grassland
P	Ruitersplaat	111.235	420.000	100	Nieuwe Merwede	Mixed vegetation
S	Stiftse Waard	155.240	427.360	457	Waal	Grassland

x and *y* coordinates were determined using a GPS-system calibrated at Amersfoort, The Netherlands. NAP = Dutch reference mean sea level. Mixed vegetation includes reed *Phragmites australis*, herbs e.g., *Urtica dioica*, *Symphytum officinale* and *Valeriana officinalis* and shrub species, e.g., *Salix*. The grassland vegetation mainly consists of grass and some *Phragmites australis*.