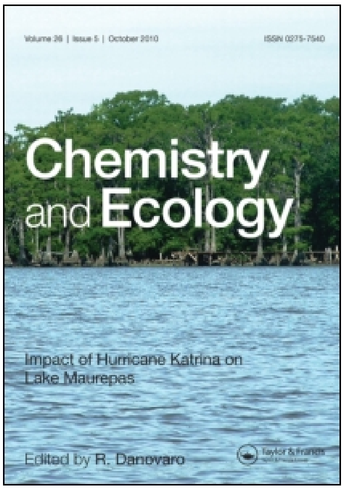

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Metal accumulation risks in regularly flooded and non-flooded parts of floodplains of the River Rhine: Extractability and exposure through the food chain

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Metal accumulation risks in regularly flooded and non-flooded parts of floodplains of the River Rhine: Extractability and exposure through the food chain

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Ecotoxicological risks of sediment contamination in floodplains are supposed to be highest in the regularly flooded parts. Therefore, in risk assessments, the non-flooded parts are neglected or considered to be reference areas. We investigated the metal extractability and levels in important food sources for vertebrates, viz. grass shoots and earthworms, in flooded as well as non-flooded parts and compared these with total metal concentrations. A comparison of these areas in the moderately polluted 'Afferdensche en Deestsche Waarden' floodplains along the River Rhine showed that total Zn, Pb, and Cd concentrations were highest in the regularly flooded parts. However, $CaCl_2$-extractable Zn concentrations were highest in non-flooded areas, and those of Pb and Cd were equal in both areas. Total Cu concentrations were not significantly different between the two areas, but $CaCl_2$-extractable Cu concentrations were highest in the regularly flooded areas. The metal concentrations in grass shoots of non-flooded areas were equal to (Zn, Cu, Cd) or higher than (Pb) those in regularly flooded areas. Zn concentrations in earthworms in regularly flooded areas were higher, but concentrations of Cu, Pb, and Cd were not. Ecotoxicological risk assessments require analysis of the total and potentially bioavailable metal concentrations in soils as well as concentrations in biota. This study shows that the less contaminated non-flooded areas in moderately polluted floodplains cannot be neglected in metal accumulation studies and cannot be used as pristine reference areas.

Keywords: Contaminated floodplains; Metal accumulation; Non-flooded areas; Food web; Bioavailability; Risk assessment

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1. Introduction

Due to emissions in the past, the sediments of many large European rivers and their floodplains have become polluted with a variety of organic and inorganic substances. Although the quality of the river water and suspended matter has recently improved [1], large quantities of metals (e.g. zinc, copper, lead, and cadmium) are still present in the floodplain soils due to the persistent character of these pollutants and the large amounts adsorbed to suspended matter deposited in recent decades [2, 3]. It has often been hypothesized and shown that metals accumulate in the floodplain food webs, where they can have toxic effects at different trophic levels [4–6]. Vertebrates (mammals and birds of prey) might be particularly at risk due to their positions in food webs. Traditionally, research on contaminant loads in substrates and biota, ecotoxicological risk assessments, and floodplain management as regards contaminants have focused on the regularly flooded parts, as contaminants are largely supplied during floods. The highest total metal quantities are often found at low elevations in floodplains, or in parts with low water velocities during floods, as this is where the clayey sediments deposit [7–10].

It is known that the risk of heavy-metal accumulation in food webs is not necessarily simply related to total metal concentrations in soils, as bioavailability and the exposure of and uptake by biota are also important [11–16]. However, environmental regulations in most industrialized countries are based on total soil concentrations (an exception being Switzerland) [17]. In ecotoxicological risk assessment, as well as for management purposes and priority assessment for sanitation, the binding capacity of the substrate is partly taken into account by including special rules for certain pH, organic matter, and/or clay levels [11, 17–19]. For instance, according to Dutch regulations, total metal concentrations are calculated relative to a standard soil containing 10% organic matter and 25% lutum (particles $<2\,\mu$m) on a dry-weight basis [20] to correct for binding capacity and hence for availability. Such calculations to determine the risk to biota probably yield useful estimations for many soil types, but the outcomes are highly uncertain in the clay-rich soil types with pH values around 7–8 [6, 18, 21], which are found in the floodplains of the large lowland rivers in the Netherlands and elsewhere [16, 22]. In these situations, soluble or extractable concentrations of metals in soils could be more relevant to the accumulation risk [11, 23, 24]. Moreover, correlations between total and extractable concentrations might be weak or absent under those conditions. The $CaCl_2$-extractable concentration is often mentioned as a better indicator of site-specific accumulation risks than the total concentration, as it is positively related to the bioavailability of heavy metals for several organisms [18, 25, 26].

As the non-flooded parts of floodplains are generally expected to contain lower total metal concentrations than the regularly flooded parts, these non-flooded parts are often assumed to be unimportant in ecotoxicological risk assessment. They are assumed to contain natural background concentrations [9] or are not taken into account at all [4, 5]. However, these elevated parts may also be polluted to some extent, especially in floodplains where they consist of constructed elevations, often built from substrates excavated from the floodplain itself [27]. In addition, these areas have no deposition of sediments rich in clay and organic matter, which would reduce the chemical and biological availability of metals. Constructed elevations, including dikes (embankments) and non-flooded parts used for industry or housing, are numerous in the semi-natural and constructed floodplains along the large lowland rivers in the Netherlands.

Since the lower parts of floodplains are frequently inundated (often more than once a year), the elevated areas function as important refuges for many animal species [28–30]. Substantial proportions of the populations of several species are assumed to forage on or inhabit the elevated areas for at least part of their lives. Recently, it has been shown that the densities of small mammals (e.g. voles, shrews, and mice) within Dutch floodplains were highest on the elevated parts throughout the year. Total numbers in the lower parts only started to exceed those on the elevated parts several months (up to more than half a year) after the floodplain became dry following a flood [30, 31]. Small mammals are important prey species for several predators [4, 32, 33], which generally forage in the areas with the highest prey densities. Hence, substantial parts of the total exposure to metal pollution of several vertebrate species will take place in the non-flooded areas. This makes it relevant to investigate to which concentrations of metals vertebrates are exposed.

To this end, we have compared the importance of the exposure of biota to metal concentrations in the topsoil of elevated non-flooded areas and regularly flooded areas. We expected that total concentrations of the investigated metals Zn, Cu, Pb, and Cd would be higher in the regularly flooded parts of the floodplain than in the non-flooded areas. We investigated whether the risks of metal accumulation in vertebrates were indeed highest in these regularly flooded parts, by comparing the regularly flooded and non-flooded areas in terms of total and $CaCl_2$-extractable soil concentrations. For most vertebrates, the available fraction in soils is only indirectly relevant, as food sources form the links for accumulation. Food species deal with contaminants in various ways: various species along the food chain may excrete contaminants, concentrate them in certain tissues, or immobilize them [34, 35]. It is therefore useful to analyse heavy-metal concentrations in important food sources, in addition to total or extractable soil concentrations. Two major exposure routes in the food chains of vertebrates are grasses and earthworms, which are also analysed. The following research questions were addressed in the present study:

(1) Are there any differences in total metal concentrations between flooded and non-flooded areas?
(2) Are there any differences in potentially bioavailable concentrations between these areas?
(3) Are there any differences in the metal concentrations in grasses and earthworms between regularly flooded and non-flooded areas?

The findings are discussed in relation to exposure risks of vertebrates in regularly flooded and non-flooded parts of floodplains.

2. Materials and methods

2.1 *Research area and sampling sites*

The research project was carried out at the ‘Afferdensche en Deestsche Waarden’ (ADW) floodplains (51° 54′ N, 5° 38′ E), situated 20 km west of the town of Nijmegen along the river Waal (the main distributary of the River Rhine in the Netherlands). These floodplains are characteristic of diffusely and moderately polluted floodplains in the Rhine delta and were a focal area in an extensive ecotoxicological research programme by the Netherlands Organization for Scientific Research (NWO-SSEO programme) [10, 16, 36]. Between May 2002 and November 2003, soil cores were taken from the top 10 cm soil layer at 58 sampling sites (figure 1). These sampling sites, 16 of which were situated in non-flooded areas, were characterized by various types of vegetation, soil, and land use [30]. At each of the sites, three or five samples were taken with line intervals of at least 10 m. Each sample was prepared from three soil cores taken within a 1 m^2 plot. The sites were initially selected for the monitoring

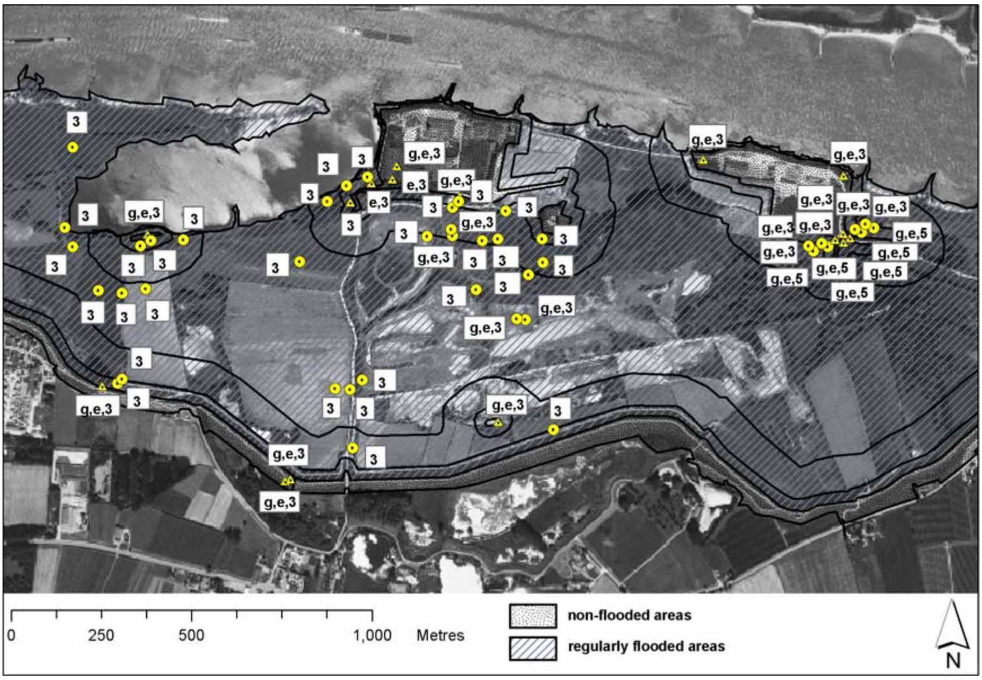

Figure 1. Location of the sampling sites at the 'Afferdensche en Deestsche Waarden' (ADW) floodplains, which are bordered on the north side by the river Waal and on the south side by the outer dike. The non-flooded areas (a quarter of the total area of 280 ha) are dot-shaded (sampling sites indicated by triangles), while the regularly flooded areas are hatch-shaded (sampling sites indicated by circles). The number of samples taken at each sampling site is indicated. When earthworms or grasses were sampled, this is indicated by 'e' and 'g,' respectively. The digital aerial photograph was put at our disposal by the Geometric Service of the Directorate-General of Public Works and Water Management.

of small mammal distribution and recolonization, generally based on vegetation structure, without any prior information on contaminant levels. As the site selection method was similar in non-flooded and regularly flooded areas, we assumed that our sampling scheme resulted in a representative comparison of the contaminant levels, distribution, and variability between the two areas. Both the non-flooded and the regularly flooded parts of the ADW floodplains included relatively sandy and more clayey sampling sites. The sandier parts are generally located in the excavated lower parts, and on the elevated grounds on which brick factories used to stand. The more clayey parts were generally the unexcavated lower parts, and the elevated dikes. All these areas were included in the sampling, because the sampling areas were randomly selected based on vegetation structure without any prior knowledge about substrate parameters.

At 27 of the sampling sites (15 non-flooded and 12 regularly flooded sites), we also collected all vegetation from 625 cm^2 plots ($n \geq 3$) situated at the sites where the soil cores were taken (figure 1). All grass shoots from the vegetation samples were selected to obtain mixed samples of all Gramineae. The dominant grass vegetation at the sampling sites was always dominated by one or several of the following species: *Poa pratenses*, *Agrostis stolonifera*, *Elytrigia repens*, *Lolium perenne*, and *Phleum pratense*. Thirteen grass samples of non-flooded areas were available, as grasses were absent from two of the sites. At the same 27 sites, all earthworms (Lumbricidae) were collected from 625 cm^2 plots ($n \geq 3$), down to a depth of 25 cm. The dominant earthworm species in the ADW floodplains are *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Allolobophora chlorotica*, and *Octolasion cyaneum*, which were also found in our samples in various combinations. Also *Lumbricus terrestris* is a common species in the ADW floodplains [36]. However, this species was not observed in the analysed earthworm samples. All grass and earthworm samples were collected in autumn, when no flooding had occurred for more than half a year, as both season and flooding events have large effects on species compositions and age assemblages, and therefore on metal concentrations [36]. All soil and grass samples were stored at 5 °C and treated (either oven-dried or suspended in $CaCl_2$ solution) within 3 d after collection. All earthworms were stored in 70% ethanol. Although preservation can have effects on dry weights [37] and metal concentrations [38], these effects should be similar for the samples from different sampling sites, as all earthworm samples were treated in the same way.

2.2 *Analyses and measurements*

We measured the moisture content, organic matter content, clay-silt to sand ratio, pH_{CaCl2}, total metal (Zn, Cu, Pb and Cd) concentrations and 0.01 M $CaCl_2$-extractable metal concentrations in all soil samples. The moisture content was determined by drying 5 g of wet soil (FW) for 24 h at 105 °C to measure the dry weight (DW). The moisture content (%) was subsequently calculated as $((FW - DW) \times 100)/DW$. The organic matter content (OM) was determined by scorching the dry soil for 4 h at 550 °C, after which the mineral weight (MW) was measured; $OM(\%) = ((DW - MW) \times 100)/DW$. The clay-silt to sand ratio was estimated by adding 50 ml of 35% H_2O_2 to 10 g of dry soil, to disrupt particle aggregations by digesting $CaCO_3$ and organic matter. After 2 d of incubation, the suspension was boiled while adding distilled water to keep the substrate in suspension. Sieving over a 53 μm mesh separated the clay-silt and sand fractions, and the suspensions were dried at 105 °C, after which the fractions were weighed.

The total metal concentration of the soil was measured after microwave extraction (using a Milestone 1200 microwave oven) of 0.2 mg of DW substrate in a mixture of 3.0 ml of 65% HNO_3 and 1.5 ml of 37% HCl. The samples were topped up to 50 ml, after which the metal concentration was measured using inductively coupled plasma–atomic emission spectrometry (ICP–AES). The 0.01 M $CaCl_2$-extractable fraction was determined as a measure of the potential metal availability. Six grams (FW) of substrate was mixed with 0.01 M $CaCl_2$ for 2 h in a soil:solution ratio of 1:10, after which the suspension was centrifuged at 12 000 rpm (5000 g) for 15 min. After pH_{CaCl2} had been measured in the substrate suspension in 0.01 M $CaCl_2$, the supernatant was filtered over a 0.45 μm mesh. A pH of 2 was obtained with a few droplets of 65% HNO_3, and the metal content of the sample was subsequently measured on the ICP-AES.

The grass shoot samples were oven-dried at 70 °C, after which they were cut into small pieces, ground in liquid nitrogen, and homogenized. The earthworm samples were also oven-dried at 70 °C. Approximately 0.2 g of grass shoots per sample and the entire earthworm samples (ranging between 0.2 and 0.6 g) were digested and analysed for metal concentrations as described for the soil samples.

2.3 *Calculations and statistics*

We calculated the location-specific reference value for the total metal concentrations in the soil, which is common practice for standard ecotoxicological risk assessment and priority determination for soil-management purposes. This means taking availability into account by compensation for the binding capacity of the substrate. The location-specific reference value was calculated, correcting for organic matter and lutum contents [1, 20, 39]:

$$RV_{ls} = \frac{RV_{ss} \times (a + (b + L) + (c \times OM))}{a + (b \times 25) + (c \times 10)}.$$

RV_{ls} = location-specific reference value for a metal (mg kg^{-1} DW); RV_{ss} = reference value of a metal for standard soil (mg kg^{-1} DW); L = % clay-silt (<53 μm) of DW; OM = % organic matter of DW; a, b, c = metal-specific values ('a' values are 50, 15, 50 and 0.4; 'b' values are 3, 0.6, 1 and 0.007; 'c' values are 1.5, 0.6, 1, and 0.021, for Zn, Cu, Pb, and Cd, respectively). Reference values used for standard soil were: 140 (Zn), 36 (Cu), 85 (Pb), and 0.8 (Cd) mg kg^{-1} DW [18, 19], at an organic matter content of 10%, and a lutum content of 25%. Instead of the lutum content (<2 μm), we used the clay-silt content in the calculations of the location-specific reference value, which yields higher values. However, we assume that there is a positive correlation between the lutum and the clay-silt content.

To assess correlations between the potentially bioavailable metal concentrations and the total metal concentrations and soil parameters, a principal-component analysis (PCA) was performed on the $CaCl_2$-extractable concentrations, after the gradient length for the dataset had been specified by means of a detrended correspondence analysis (DCA) using the Canoco for Windows software package (version 4) [40] including all individual samples ($n = 186$). To confirm the indications shown by the PCA, we executed multiple regressions on the log-transformed data using the stepwise method for each of the metals. As flooding is a categorical variable the way we measured it, this variable was excluded from the multiple regressions (results not shown). As grass shoots and earthworms were not collected at all of the sampling sites, we tested the homogeneity of variances using Levene statistics and possible differences in average total or $CaCl_2$-extractable concentration of metals and soil parameters using one-way ANOVAs between the sub-set of sampling sites where these biota were collected and the total set of sampling sites, using SPSS 11.5 for Windows. Average values and variances of metal concentrations and soil parameters were compared between regularly flooded and non-flooded areas with a t-test and F-test, using the average values per sampling site.

3. Results

3.1 *Soil characteristics*

For none of the soil parameters or metal concentrations tested were significant differences ($p \geq 0.05$) found between the sub-set of sampling sites where biota were collected and the total dataset of sampling sites. This was true for both regularly flooded and non-flooded areas. This means that the sub-sets were representative, in terms of average and variance, of the total datasets, for all parameters. The pH_{CaCl2} was significantly higher (t-test; $p < 0.001$) in the regularly flooded areas than in the non-flooded areas (table 1). The organic matter content was significantly higher (t-test; $p < 0.05$) in the flooded areas (based on the total dataset). The variation in the observed moisture content in particular was found to be higher in the regularly flooded areas than in the non-flooded areas (F-test; $p < 0.05$). The total metal concentrations in the soil were significantly higher in the flooded areas than in the non-flooded areas for all metals, except for Cu in the sub-set comparison. Differences in the $CaCl_2$-extractable concentrations were found for Zn, with a higher concentration ($p < 0.001$) in the non-flooded areas, and for Cu, with a higher concentration ($p < 0.05$) in the regularly flooded areas.

Table 1. Characterization and comparison of sets of soil samples (and sub-sets of samples from which grass and earthworm samples were taken or not) taken in the ADW floodplains.

	Non-flooded		Flooded		F-test		t-test	
	Total ($n = 16$)	Sub-set of sample sites ($n = 15$)	Total ($n = 42$)	Sub-set of sample sites ($n = 12$)	Total	Sub-set	Total	Sub-set
OM (%)	6.05 ± 2.65	6.19 ± 2.68	8.22 ± 3.77	8.26 ± 4.74	–	*	*	–
Clay (%)	49.4 ± 20.7	51.2 ± 20.1	53.6 ± 19.6	49.8 ± 27.1	–	–	–	–
pH_{CaCl2}	7.08 ± 0.27	7.05 ± 0.23	7.41 ± 0.12	7.38 ± 0.15	***	–	***	***
Moisture (%)	23.1 ± 5.27	23.1 ± 5.27	28.3 ± 12.2	28.3 ± 12.2	*	*	–	–
$[Zn]_{tot}$ (mg kg^{-1})	218 ± 148	222 ± 152	441 ± 282	395 ± 265	**	–	***	*
$[Zn]_{CaCl2}$ (mg kg^{-1}10)	11.6 ± 4.91	11.8 ± 4.95	2.87 ± 2.05	2.99 ± 2.36	***	**	***	***
$[Cu]_{tot}$ (mg kg^{-1})	30.9 ± 21.0	30.9 ± 21.7	61.0 ± 36.9	51.8 ± 39.0	*	*	***	–
$[Cu]_{CaCl2}$ (mg kg^{-1} 10^3)	63.6 ± 34.6	64.4 ± 35.6	94.8 ± 52.6	114 ± 59.3	–	–	*	*
$[Pb]_{tot}$ (mg kg^{-1})	79.9 ± 42.2	81.2 ± 43.4	138 ± 85.1	130 ± 74.9	**	–	**	*
$[Pb]_{CaCl2}$ (mg kg^{-1}10)	3.27 ± 3.62	2.70 ± 2.94	3.69 ± 2.56	4.20 ± 2.43	–	–	–	–
$[Cd]_{tot}$ (mg kg^{-1})	1.34 ± 0.97	1.36 ± 1.00	2.75 ± 2.24	3.75 ± 3.08	***	***	**	*
$[Cd]_{CaCl2}$ (mg kg^{-1}10)	18.8 ± 17.4	15.9 ± 13.4	21.4 ± 14.8	25.6 ± 13.7	–	–	–	–

Note: Average values ±S.D. for soil parameters and metal concentrations are shown, based on the average values per sampling site. Significant differences in variance (f) and average (t) value between non-flooded and flooded areas are shown for the total sample set and the sub-set of samples, with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and –not significant ($p \geq 0.05$).

3.2 *Corrected metal concentrations relative to site-specific reference values*

Total concentrations of each of the metals in the top 10 cm layer varied across the sampling sites in the ADW floodplains. The average concentrations for each sampling site for Zn ranged from 57 to 1166 mg kg^{-1} DW, those for Cu from 7.9 to 147 mg kg^{-1} DW, those for Pb from 13

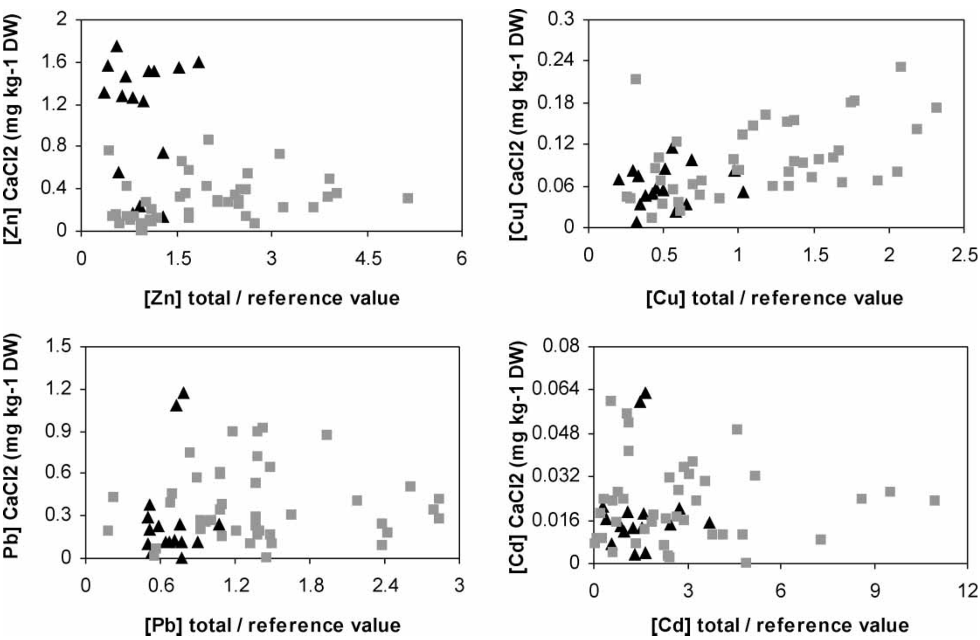

Figure 2. Scatter plots showing the $CaCl_2$-extractable concentration (in mg kg^{-1} DW) of each of the metals (Zn, Cu, Pb, and Cd) related to the total metal concentration divided by the location-specific reference value. This reference value is the background value of each metal for the Netherlands corrected for the organic matter and lutum contents. The non-flooded sampling sites are indicated by black triangles, and the regularly flooded sampling sites by grey squares.

to 359 mg kg^{-1} DW, and those for Cd from 0.07 to 8.3 mg kg^{-1} DW. The metal concentrations at many sampling sites exceeded the reference values (figure 2). This was the case for 64, 41, 55, and 72% of the sites for Zn, Cu, Pb, and Cd, respectively. For Cu and Pb, these were all, except for one, regularly flooded sampling sites. All sampling sites situated to the right of the graph for Zn and Cd were also from regularly flooded areas. The values for sampling sites in the non-flooded areas were generally situated to the left side of the plot, which means that only 33, 7, 7, and 67% of the sampling sites were above the reference values for Zn, Cu, Pb, and Cd, respectively. A comparison of the $CaCl_2$-extractable concentrations shows that the positions in the plot of the sampling sites in the non-flooded areas were similar to those of the regularly flooded areas for Pb and Cd (figure 2 and table 1). The $CaCl_2$-extractable concentrations for Zn were actually higher for the non-flooded sampling sites than for the regularly flooded sampling sites.

3.3 *Metal concentrations related and compared*

The PCA shows that the $CaCl_2$-extractable Zn concentrations are almost entirely explained by the horizontal axis, an aggregation of the 'flooded' and pH parameters (figure 3). The higher $CaCl_2$-extractable Zn concentrations were found at the non-flooded sites, and a negative correlation with pH was found. The total Zn concentration, a parameter more dominant in the vertical axis was of no importance for the $CaCl_2$-extractable Zn concentrations. The $CaCl_2$-extractable Pb concentrations appeared to be positively correlated to the total concentration, while relations for the other metals (Cu and Cd) were weaker. The results for Zn and Pb were confirmed by multiple regression analyses. Significant correlation was found between the $CaCl_2$-extractable concentrations and the independent variables for which the t-values of the independent variables were largest for the above-mentioned variables. An exception is the factor 'flooded' for Zn, as we did not include this factor in the multiple regressions. This factor is in the significant multiple regression equation partly substituted by clay (−) and $[Zn]_{tot}$(+).

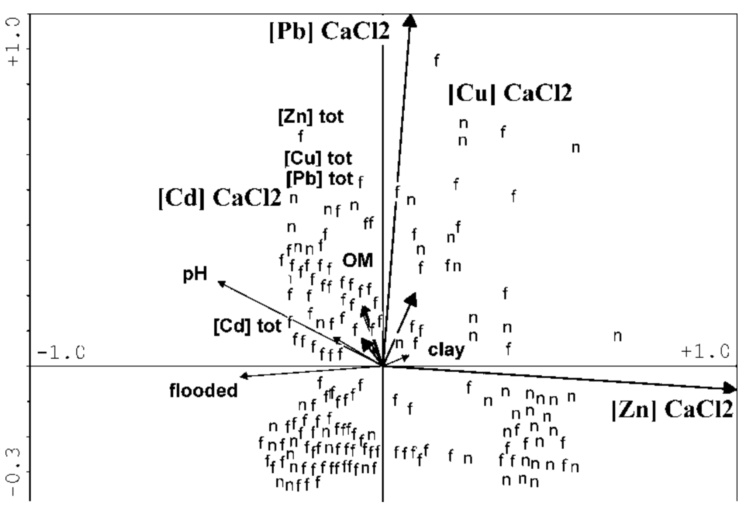

Figure 3. Principal-component analysis of the $CaCl_2$-extractable soil concentrations ($[Me]_{CaCl2}$) of 186 samples. $[Me]_{tot}$: total metal concentration in the soil, shown for Zn, Cu, Pb, and Cd; OM: organic matter content; clay: clay content (<50 μm); pH: pH_{CaCl2}; flooded: parameter which equals 1 for regularly flooded sampling sites (f) and 0 for non-flooded sampling sites (n).

In general, organic matter and clay content appeared to be poor indicators of the presence of high $CaCl_2$-extractable concentrations. Figure 3 also shows that for Zn, the non-flooded sampling sites are clustered at the high $CaCl_2$-extractable concentrations, while the flooded sampling sites are clustered at the low $CaCl_2$-extractable Zn concentrations. However, several sampling sites show the opposite positioning in the graph, and trends are less clear for the other metals.

The total metal concentrations were significantly ($p < 0.05$) higher in the regularly flooded areas than in the non-flooded areas (figure 4 and table 1). This difference remained when we related the total concentrations to the calculated location-specific reference values. The average uncorrected metal concentrations were close to the corrected reference values, but average Zn and Cd concentrations in regularly flooded areas were, respectively, 1.9 and

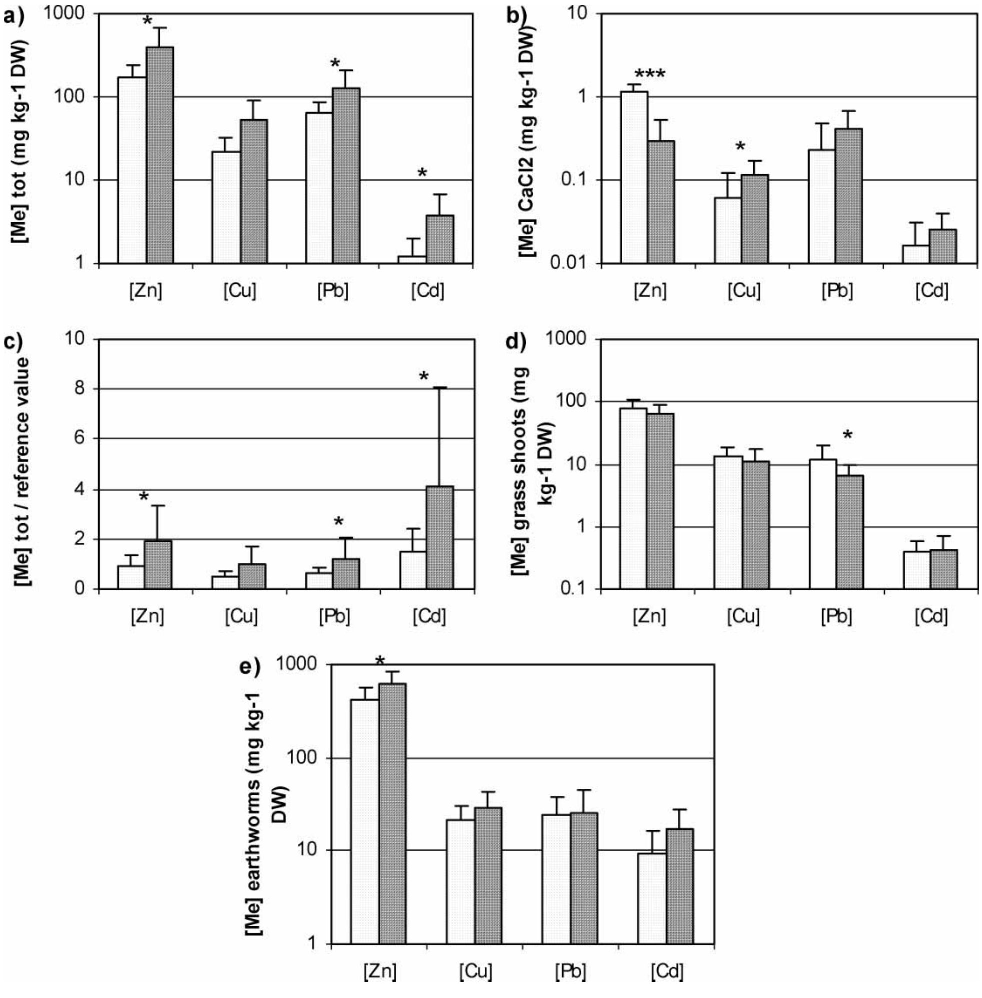

Figure 4. Graphs showing differences in metal concentrations (Zn, Cu, Pb, and Cd) between non-flooded (light bars) and regularly flooded (dark bars) sampling sites. Metal concentrations are shown as (a) total metal concentration (mg kg^{-1} DW); (b) $CaCl_2$-extractable metal concentration (mg kg^{-1} DW); (c) total metal concentration divided by the reference value corrected for organic matter and clay-silt content; (d) metal concentration in grass shoots (mg kg^{-1} DW); (e) metal concentration in earthworms including their digestive tract contents (mg kg^{-1} DW). Significant differences are indicated by asterisks ($^{*}p < 0.05$, $^{***}p < 0.001$), with $n = 15$ for non-flooded sampling sites (except for (d), where $n = 13$) and $n = 12$ for regularly flooded sampling sites.

4.1 times the location-specific reference values. Of the $CaCl_2$-extractable metal concentrations, only those for Cu were significantly higher in the regularly flooded areas than in the non-flooded areas ($p < 0.05$). The opposite was found for Zn, with significantly higher $CaCl_2$-extractable concentrations ($p < 0.001$) in non-flooded areas than in regularly flooded areas. The metal concentrations in grass shoots were similar in non-flooded and regularly flooded areas, except for the Pb concentrations, which were higher in non-flooded areas ($p < 0.05$). Significant differences in metal concentrations in earthworms were only found for Zn, with the highest concentrations in the earthworms from regularly flooded areas ($p < 0.05$).

4. Discussion

Heavy metals are not distributed homogeneously over floodplains, and human interference in the form of constructions and excavations has increased the variability in soil metal concentrations in floodplains even more [9, 10]. Our measurements confirm that the ADW floodplains are moderately polluted, as the average topsoil concentrations for all metals were below the testing level (Classes 0–2, according to the Dutch soil quality standards [16, 18]). Only three sampling sites were Class 3 contaminated, that is, had contaminant levels between the testing and intervention levels. Two of these sites were contaminated with Cd, one with Zn. Our comparison of the regularly flooded and non-flooded sites showed that in spite of the large variation in total metal concentrations within both areas, the regularly flooded areas were significantly more polluted with Zn, Pb, and Cd than the non-flooded areas (figure 4). For all metals, including Cu, the most polluted sites were situated in the regularly flooded areas (figure 2).

When metal pollution is the result of deposition of contaminated sediments, the most contaminated areas generally also contain higher clay and organic matter contents [7]. As these metal-binding substances reduce the chemical availability of metals, and therefore possibly the bioavailability, we compared the regularly flooded and non-flooded areas relative to the site-specific reference values used in the Dutch system of soil legislation [18, 19]. This correction yielded results similar to those obtained with total soil concentrations. This means that, in agreement with risk-assessment studies and the focus of policy making, any ecotoxicological effects are expected to be generally the result of exposure of biota in the regularly flooded areas, rather than in non-flooded areas.

On the other hand, the $CaCl_2$-extractable soil concentrations did not show a significant positive correlation with the total metal concentrations divided by the location-specific reference values (figures 2 and 3). Only for Cu might there be a positive trend, but Zn showed the opposite tendency, with the highest $CaCl_2$-extractable concentrations at the sites with the lowest total concentrations. The sites with the highest $CaCl_2$-extractable Zn concentrations were all situated in the non-flooded areas.

Other factors than the total concentration might be more important for the potential bioavailable concentration of heavy metals in moderately polluted floodplain soils. For Zn in particular, pH might be of importance (figure 3), as pH was significantly lower in the non-flooded areas than in the regularly flooded areas (table 1). As regards Zn, Pb, and Cd, the non-flooded areas showed a wide range of values, including the highest levels, of $CaCl_2$-extractable concentrations, even though the total concentrations were significantly lower (figures 2 and 4). This indicates that either cleaner substrates were used for the construction of the non-flooded terrains, or there was little or no input of contaminants after the construction of the non-flooded areas. At several elevated locations, however, the conditions were different in such a way that the percentage chemically available metals of the total concentration were higher than in the flooded areas. This might be due to drier substrates and slightly lower organic matter contents (table 1), but this could not be verified by our data. Another reason might be a weaker influence of river water (pH around 8) with its pH-stabilizing effect, and a greater influence of rainwater (pH values around 5–6 [41]), but this hypothesis was not tested in this study.

Two important exposure routes to heavy metals in floodplains for first-, second-, or third-order consumers in food webs are those via vegetation and via earthworms [6, 42]. The metal concentrations in the grass shoots were not higher in the regularly flooded areas than in the non-flooded areas, and Pb concentrations in the grass shoots were highest in the non-flooded areas. Hence, the risk of heavy-metal accumulation in vegetation and herbivorous species feeding on above-ground plant parts seems to be at least similar, or even higher, in the non-flooded areas than in the regularly flooded areas. On the other hand, the metal concentrations observed in grass shoots were generally lower than the lowest recorded critical tissue concentrations for vegetation [22]. The differences in $CaCl_2$-extractable concentrations of Zn and Cu observed in the soil were not reflected in the vegetation. This could be the result of $CaCl_2$-extractable concentrations not being very high, as is generally the case in Dutch floodplain soils. In addition, Zn and Cu, essential elements for plant species, are probably regulated to a certain extent [34, 43]. Our measurements cannot explain the higher Pb concentrations in grass shoots from non-flooded areas, though poor correlations between salt extractions and Pb uptake by vegetation have been recorded before [23, 34].

As we were interested in exposure and accumulation risks to vertebrates, we analysed heavy-metal concentrations in earthworms. We included the substrate in their digestive tract, as species preying on earthworms will be exposed to the metal burdens in the bodies of the earthworms plus the amounts in the digestive tracts. The differences between flooded and non-flooded areas in metal concentrations in earthworms were not as large as those observed for the total soil concentrations, and were only significant for Zn. The risk of accumulation of heavy metals in predators of earthworms thus seems to be higher in regularly flooded areas than in non-flooded areas, especially for Zn, but did not differ as much as would be expected from the total soil concentrations. It has been established that heavy-metal uptake generally takes place through the skin of earthworms, and should therefore be related to pore water concentrations [16, 44–46]. $CaCl_2$-extractable concentrations should thus reflect the accumulation risk for earthworms better than total concentrations. However, as we analysed earthworms containing substrate in their digestive tracts, the risk of accumulation of heavy metals in the earthworms themselves is probably similar in the non-flooded areas as in regularly flooded areas.

Total metal concentrations can also be relevant to direct exposure of vertebrates. Several species ingest substantial amounts of soil in floodplains and are thus exposed to the total concentrations in their digestive tracts. Herbivores, for instance, will ingest certain amounts of substrate attached to plant materials (especially to the roots but also the above-ground parts). Insectivores and carnivores ingest substrate attached to subterranean preys, to prey killed on the ground and to furs, or substrate ingested with the water they drink or substrate that may be present in the digestive tracts of prey species, as is the case with preying on earthworms. Their internal environment will determine which fraction is available to the organism itself and whether a weak or strong extraction method reflects the availability of heavy metals ingested with soil.

In this study, the soil was sampled by taking cores from depths of 0–10 cm. For most species, this is the relevant contact zone in which exposure or uptake takes place in the case of direct exposure. This soil layer is, however, not always the most polluted layer. It has been shown that the most polluted layers in several floodplains can nowadays be found at depths between

10 and 25 cm [2, 36]. Grasses root to depths of below 10 cm [22], and some variation in the vertical distribution of pollution occurs, introducing some variation in the relation with the concentrations measured in soil and grass. In addition, the rooting depth of grasses is probably influenced by soil characteristics. Similar aspects may have played a role in the heavy-metal concentrations we measured in earthworms [35]. Most earthworms and species are active in the top layer, but there will also be some exposure below 10 cm [36]. The earthworm species composition and the depths at which they are active are especially influenced by the presence of vegetation, soil characteristics, and the groundwater table [42].

A comparison between metal concentrations in soil and relatively immobile biota such as plants and earthworms is justified because heavy-metal exposure of them will take place in the immediate vicinity of the sampling sites [33]. To minimize the risk that any difference in concentrations in plants we found between regularly flooded and non-flooded areas is due to variations in heavy-metal uptake between plant species, we collected only Gramineae. Most factors influencing the metal concentrations in grass shoots, such as the species, the age of the plants and metal metabolism with storage in different parts, were assumed to be more or less similar between the sampling sites. We also assumed this for earthworms, realizing that a certain variation in species metabolism differences and age distributions will be present.

Focusing floodplain risk assessments and soil management for metal contamination on the regularly flooded parts alone means that an important part of the exposure risk in food webs is ignored. Calculations assuming background levels of metals in non-flooded areas, or the assumption that risks are minimal as total concentrations are much lower than in regularly flooded areas, underestimate the risks to several floodplain species, at least in moderately polluted floodplains. This is even truer in view of the recent finding that important terrestrial species in the floodplain food webs are exposed for extensive periods mainly in the non-flooded areas, since that is where they are most numerous [30, 31]. As $CaCl_2$-extractable concentrations of Zn were higher in the non-flooded areas than in the regularly flooded areas, and there were only minor differences in Pb and Cd concentrations, it is especially the ecotoxicological risks for plant species, herbivores, and predators foraging on herbivores which may be underestimated if these areas are not taken into account in risk assessments and floodplain soil management. This is supported by the similar metal concentrations, and even higher Pb concentrations in grass shoots from non-flooded areas as in those from the regularly flooded areas. Even the ecotoxicological risk to the food chains based on earthworms and insects should be reconsidered, as an important part of the exposure may also take place in non-flooded areas, and differences in accumulation appear to be not as large as expected from the total soil concentrations of Cu, Pb, and Cd. The regularly flooded areas in moderately polluted floodplains probably only offer significantly higher ecotoxicological risks for vertebrates when they ingest soil in substantial amounts.

5. Conclusion

Our results show that, as expected, the regularly flooded parts of the ADW floodplains have significantly higher total Zn, Pb, and Cd concentrations in the topsoils than the non-flooded areas. The $CaCl_2$-extractable Zn concentrations are, however, significantly higher in the non-flooded parts, whereas the $CaCl_2$-extractable Pb and Cd concentrations are not significantly different between the two areas. Significant differences in Cu were only found for the $CaCl_2$-extractable concentrations, which were highest in the regularly flooded areas. As regards soil parameters, the pH was significantly lower in the non-flooded areas than in the regularly flooded areas, which might partly explain the differences in availability of metals. Our comparison of major exposure routes for food webs showed that the metal concentrations in grass shoots were similar in the regularly flooded and non-flooded areas, while those for Pb were actually significantly higher in the non-flooded areas. Significant differences in metal concentrations in earthworms, analysed with their digestive tract contents, were only detected for Zn. The present study casts doubt on the usefulness of corrected total metal concentrations in contaminated floodplain soil policies, in spite of the correction for binding capacity (lutum and OM content). Risk assessments and management of heavy-metal contamination in floodplains should consider both total and extractable concentrations in the soil and concentrations in biota. Furthermore, the study shows that exposure to metals in non-flooded areas of moderately polluted floodplains cannot be neglected.

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