TRACE METALS IN FLOODPLAIN LAKE SEDIMENTS

SEM/AVS AS INDICATOR OF BIOAVAILABILITY AND ECOLOGICAL EFFECTS

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Trace metals in floodplain lake sediments

SEM/AVS as indicator of bioavailability and ecological effects

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ABSTRACT

Floodplains in The Netherlands have historically accumulated large amounts of pollutants including trace metals. Plans are made to reconstruct floodplains for use as storage basins to attenuate river peak discharges and rehabilitate its natural reserves and at the same time. It is recognized that the ecological risk of polluted sediments depends on the bioavailable fraction of pollutants rather than on total content. In reduced sediments, metals may bind strongly to sulfides leading to insoluble metal sulfide precipitates. This metal binding mechanism can be assessed by the SEM (Simultaneously Extracted Metals)/AVS (Acid Volatile Sulfide) measurement method. This method is insufficiently validated for use in field-based ecological risk assessment, especially for systems with large spatial and temporal variability like floodplains. The main goals of this thesis are to evaluate the applicability of the SEM/AVS method for the estimation of bioavailable trace metals in floodplain sediments with regard to variability and ecological meaning and to explore the effects of trace metals to benthic communities in floodplain lakes.

For the routine measurement of AVS in floodplain lake sediments, an existing diffusion method was adapted and optimised including an evaluation of the effect of reaction time, sample weight and passive sample aeration on AVS recovery. This adapted diffusion method is well suited for the routine measurement of AVS concentrations in floodplain sediments.

In situ AVS formation and degradation in two floodplain lakes was compared this to AVS formation rates in laboratory experiments in initially oxidized sediments. In the field, AVS concentrations were correlated to surface water temperature, oxygen concentrations, and sediment composition. AVS formation was approximately linear during the growing season. Degradation rates in autumn differed drastically between the two lakes due to different degradation mechanisms (resuspension by fish and dessication). In the laboratory, concentrations of AVS with and without sulfate addition were similar during the first weeks. Sulfate addition increased the final AVS concentration suggesting that AVS formation was sulfate-limited in these floodplain sediments.

Temporal variation of sediment characteristics including DOC, AVS, and solid and dissolved trace metals were studied in a floodplain lake during one year. Metal concentrations in sediment indicated a gradual improvement of sediment quality, while dissolved metal concentrations were highest in top layers. Inundation had a mobilizing effect due to enhanced sediment resuspension and oxygenation. Water table lowering combined with organic matter decomposition led to immobilization due to sulfide formation. The chemistry of the sediment was consistent with

model calculations, especially for macro-elements, illustrating the importance of seasonality for metal risk assessment.

The spatial variability of sediment, environmental conditions, total trace metals, AVS and SEM in a floodplain lake was studied using common statistics and geostatistical methods. Total and extractable trace metal concentrations and most sediment characteristics were more or less constant, while AVS concentrations were highly variable and showed a strong spatial dependence due to differences in lake depth, total sulfur and redox conditions. The spatial pattern of SEM-AVS deviates from total or normalized trace metal patterns with consequences for risk assessment.

Redox changes in the sediment during bioassay studies may change metal availability and animal exposure considerably. We studied the effect of sampling method and sample pretreatment on bioassay response, using AVS concentrations as a proxy for sediment disturbance. Storage and pretreatment affect AVS but not SEM levels of the sediment. It is concluded that to preserve initial conditions it is best to sample the sediment with an Ekman grab, immediately store it in a jar without headspace, and freeze it as soon as possible. Bioassay response of *Asellus aquaticus* was related to SEM and AVS characteristics of the sediment.

In situ effects of trace metals and common environmental variables on benthic macroinvertebrate communities were studied in ten floodplain lakes. Alternative measures of trace metal availability were evaluated, including total and normalized metals, SEM, combinations of SEM and AVS, and metals accumulated by a detritivore invertebrate species. Accumulated metal concentrations correlated positively with sediment trace metals and negatively with surface water DOC. A maximum of sixty-eight percent of the variation in benthic community composition could be explained. SEM-AVS and individual SEM concentrations significantly contributed to the explanation of the community composition, while total, normalized, and accumulated metals were not significantly linked to community composition, showing the ecological relevance of SEM and SEM-AVS for benthic communities in these floodplains.

Altogether, the research described in this thesis shows that the use of the SEM/AVS method is promising for ecological risk assessment purposes if consideration is given to the variability of the underlying process.

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CHAPTER 1

GENERAL INTRODUCTION

Overview

Floodplains in The Netherlands have historically accumulated large amounts of pollutants, the majority of which are heavy metals. Plans are undertaken to restore floodplains as nature reserves and at the same time as storage basins to attenuate river peak discharges. It is recognized that the ecological risk of polluted sediments depends on the bioavailable fraction of pollutants –the actual exposure of organisms to pollutants, possibly leading to toxicological effects- rather than on total pollutant content. The redox status of the sediment strongly influences the speciation, and therefore the bioavailability of metals. In reduced sediments metals may bind strongly to sulfides leading to insoluble metal sulfide precipitates. This metal binding mechanism can be assessed by the SEM (Simultaneously Extracted Metals)/AVS (Acid Volatile Sulfide) measurement method. This concept still is insufficiently validated for use in field-based ecological risk assessment, especially for systems with large spatial and temporal variability like floodplains.

The main goals of this thesis are a) to evaluate the applicability of SEM/AVS as a tool to estimate bioavailability of trace metals in floodplain sediments and b) to explore the actual risks of trace metals to benthic communities in floodplain lakes. In the next sections, an overview of the pollution history, floodplain rehabilitation plans, current and new approaches to incorporate bioavailability of trace metals, focusing on the SEM/AVS method, is given. In the last section, the main research questions and the thesis outline are described in detail.

1.1 Floodplains

1.1.1 Metal pollution

Since the industrial revolution, emission of both natural and anthropogenic chemicals has led to worldwide contamination of aquatic sediments. As The Netherlands are one of the main deltas of Europe, much of the pollution carried by the rivers Rhine and Meuse has accumulated in sedimentation areas in this country. Although the water quality has improved much since the 1970-80s, the sediment is still loaded with contaminants. For instance, water systems like the Ketelmeer, IJsselmeer (Winkels, 1997), Volkerak/Zoommeer, Hollands Diep (Koelmans, 1994), and the Biesbosch area (Van den Berg *et al.*, 1998) have acted as main sinks for contaminated sediments. Also sedimentation areas such as floodplains along the large Dutch rivers, Rhine and Meuse, have accumulated various contaminants (e.g., Beurskens *et al.*, 1994; Kooistra *et al.*, 2005; Schröder *et al.*, 2005). Most sediments in Dutch floodplains are moderately to heavily polluted, sometimes exceeding the upper sediment quality criteria. Trace metals, especially zinc, determine the overall sediment quality in many floodplain areas (Anonymous, 1996). Trace metal pollution in floodplain soils can be 6 to 10 times higher than natural background concentrations (Middelkoop, 2002). Elevated metal concentrations have been detected in soil organisms inhabiting floodplains (Hobbelen *et al.*, 2004) and in aquatic organisms (Kraak *et al.*, 1999; De Haas *et al.*, 2002, 2004) and these may pose ecotoxicological effects when these concentrations exceed critical threshold values (Vink & Hendriks, 1999). For instance, on the species level, menthum deformations and limited growth and survival have been observed in benthic invertebrates (Van Hattum *et al.*, 1996; Kraak *et al.*, 1999; De Haas *et al.*, 2005). Higher organisms like fish (Traas, 2003; Moermond *et al.*, 2004), mice (Hendriks *et al.*, 1995) and owls (e.g., Van den Brink *et al.*, 2003; Kooistra *et al.*, 2005; Leuven *et al.*, 2005) may exhibit adverse effects due to biomagnification in the food chain. Also, impacts on higher biological levels of organization have been observed. Metal concentrations are found to have an impact on benthic community structure and species abundance (e.g., Den Besten et al, 1995; De Jonge *et al.*, 1999; Peeters *et al.*, 2000, 2001) and to negatively influence the carrying capacity of benthic ecosystems (De Lange *et al.*, 2004).

1.1.2 Rehabilitation plans

Rivers and adjacent floodplains are hydrologically dynamic systems. In The Netherlands and Germany, river peak discharges have increased over the last years, causing a high frequency of inundations (e.g., 1992, 1993 and 1995). Therefore, a new line of policy called 'Ruimte voor de Rivier' has been developed aiming at increasing the water storage capacity (Anonymous, 1997). To achieve this, extensive reconstruction activities along the rivers Rhine and Meuse are necessary in The Netherlands. One of the suite of potential measures is to allow inundations in designated areas by lowering floodplains, combined with the construction of side channels and lakes (e.g., Van Stokkom *et al.*, 2005). At the same time this will enhance the possibilities for natural development of floodplain areas, which belong to the most diverse habitats of the Netherlands (e.g., De Bruin *et al.*, 1987). Excavated sediments should then be stored or re-used within the river system. Historical pollution of these sediments, however, poses a problem in the implementation of this policy. After all, lowering of floodplains implies displacement of polluted sediments. Old sediment layers with relatively high pollutant levels may surface, leading to possible risks and limitations for developing specific ecotopes. In order to deal with the large amounts of polluted sediments, the policy line 'Actief Bodembeheer Rivierbed' has been developed (Anonymous, 1996). The main goal of this policy is to deal pragmatically with polluted sediment, while ensuring that the toxicological risks in designated areas are not increased (‘stand still’ principle). It may include, for instance, the storage of polluted sediments in deep clay or sand pits within the riverbeds. When polluted sediments are excavated and subsequently stored, e.g. in sub-surface depots, mobilisation of contaminants in groundwater or surface water may occur depending on local circumstances.

Therefore, a careful risk evaluation of floodplain reconstructions in the Netherlands is necessary in order to gain knowledge about the consequences of the displacement of metal polluted sediment. Hence, information about possible environmental risks posed by trace metals in sediments is crucial. In current risk assessment, total concentrations of pollutants are used to judge the environmental risk. Total concentrations, however, often do not relate to the actual ecological risk (Ankley *et al.*, 1996; Sijm *et al.*, 2001; Cornelissen & Kamerling, 2003). More realistic assessments of ecological risk imply that only pollutant fractions that actually pose a risk for biota should be taken into account (e.g., Sijm *et al.*, 2001). New quality criteria are needed that respect this actual availability of pollutants. The urge for data on bio-availability and related risks under natural or potential conditions is high. An important confounding issue for risk assessment in dynamic systems such as floodplains is how spatial and temporal variability in the availability of pollutants should be dealt with. In the next section, current sediment quality criteria, the bioavailability concept, and new developments in the assessment of bioavailability of metals are discussed.

1.2 Current Sediment Quality Criteria and bioavailability

1.2.1 Current assessment

Current standards are mainly derived from (aquatic) toxicity tests with single species (e.g., Cornelissen & Kamerling, 2003). The standards are expressed as total concentrations in sediments. This is done on the basis of a fixed ratio between solid and dissolved concentrations of the pollutant (a distribution coefficient) including several safety factors. For organic contaminants, a partitioning coefficient K_p is defined, which is based on sorption of these substances on organic matter (e.g., Jonker, 2004). For trace metals, a distribution coefficient K_d is used based on sorption of metals on organic matter and clay minerals. Sediments are corrected to a ‘standard’ sediment containing 25 % clay and 10 % organic matter to enable comparison of the pollution degree of soils or sediments with differing compositions. The minimum quality for sediments is expressed as the maximum permissible risk (MPR), which is based on species sensitivity distributions derived from (mainly) laboratory aquatic toxicity testing. The negligible concentration (NC) is defined as 0.01 * MPR. In The Netherlands, sediments are divided in classes ranging from 0 to 4 according to their pollution degree. Important threshold values between pollution classes are the target value and the intervention value. In Table 1.1, Dutch standards for trace metals in surface water (dissolved concentrations and concentrations in suspended solids) and sediments are presented.

Table 1.1. Minimum Quality (MPR, Maximal Permissible Risk) and target values for water and sediment, including background concentrations (4th National Policy Document on Water Management, 1998).

Concentrations	**Background in salt water**	**Surface water (dissolved) µg/l**		**Surface water (total conc.) [a] µg/l**		**Sediment (dry weight base) [b] mg/kg**	
Metals	*North Sea*	*Target value*	*MPR*	*target value*	*MPR*	*target value*	*MPR-sediment*
Cd	0.03	0.08	0.4	0.4	2.1	0.8	12 [c]
Cu	0.3	0.4	1.5	1.1	3.9	36	73
Ni	--	3.3	5.1	4.1	6.3	35	44
Pb	0.02	0.2	11	3.1	225	85	530 [c]
Zn	0.4	2.8	9.4	12	40	140	620
As	--	0.8	25	1	32	29	55
Cr	--	0.2	8.7	1.6	84	100	380 [c]

a) standardized for surface water containing 30 mg/l of suspended solids (composed of 20 % organic matter and 40 % clay)

b) standardized for sediment containing 10 % organic matter and 25 % clay

c) MPR = intervention value

Current first tier risk assessment is based on total concentrations in sediment. A disadvantage of basing standards on total metal content, which are based on K_d estimates is that these coefficients are highly conditional, i.e. in reality they cannot be considered constants and inherently do not account for changes in chemical conditions (e.g., redox changes) or variable sediment or water composition (e.g., Radovanovic & Koelmans, 1998). Furthermore, an important aspect that is overlooked in first tier risk assessment is the fact that not all metal fractions are actually available to ecosystems When standards are exceeded, a second tier risk assessment can be performed, using chemical or biological methods addressing this pollutant availability (Sijm *et al.*, 2001; see next section).

1.2.2 Description of bioavailability

Assessment of actual effects of contaminants on biota has to take into account that only a fraction of pollutants is available to the ecosystem. The availability and the potential occurrence of subsequent effects of pollutants for ecological receptors can be divided in three steps (Landrum *et al.*, 1994): a) chemical availability (exposure, accessibility), b) biological availability (uptake) and c) toxicokinetics (transport to target sites, internal distribution and mode of action).

First, chemical speciation needs to be considered. There is strong evidence that the directly bioavailable concentration correlates well with the concentration of pollutant present in pore water (e.g., Peijnenburg *et al.*, 2005; Vijver *et al.*, 2003), especially the free metal ion. For most sediment dwelling organisms it is thus assumed that uptake of contaminants also takes place via the pore water phase. There are, however, several exceptions to this assumption. For instance, some organisms ingest particles and may thus be exposed to bound pollutant fractions. Vink (2002) and Hare *et al.* (2001) gave evidence that uptake by benthic organisms is partly dictated by free metal ion activities in the overlying surface water compartment in addition to the pore water route. The second step is uptake, which depends on species specific features such as uptake route. In addition to the fraction available for uptake in the soil or sediment, it is related to the behaviour of the organism and the ecological aspects of the organisms considered at risk (mode of food collection, habitat requirements). For instance, the route of uptake (dermal, oral) differs significantly among various (groups of) organisms, which further complicates generalization of the bioavailability concept (e.g., Vijver *et al.*, 2003). Furthermore, the mode of food intake (e.g., filter feeders, shredders, collectors) and the feeding behavior (e.g., detritivore, herbivore, carnivore species) determines if contaminants are actually ingested. Thirdly, after uptake, internal transport and allocation, interaction with specific receptors, and processes such as detoxification determine if toxic effects occur. This may even lead to the development of specific adaptations of whole communities of exposed organisms to pollution (PICT concept, e.g., Clements, 1999).

In this thesis, the focus is mainly on the first step: the (biogeo)chemical availability of trace metals in polluted floodplains. To understand the distribution of trace metals over solid phase and pore water, their geochemical behavior must be known. The next section briefly outlines the most important processes determining the chemical availability of sediment-bound trace metals in (natural) aquatic systems.

1.3 Chemical availability of trace metals in aquatic sediments

1.3.1 Speciation of metals

The chemical availability of trace metals in aquatic systems depends on their thermodynamic properties (chemical equilibria, dissolution rate, and sorption kinetics), on sediment properties (e.g., sorption capacity, pH), and on environmental conditions (temperature, hydrology, redox conditions). Trace metals can be present in an extremely complex range of different forms (species). This distribution of metals over distinct phases is called 'metal speciation' (e.g. Mattigod, 1981; Salomons and Stigliani, 1995; Calmano & Förstner, 1996). Various fractions can be distinguished: inert (bound in mineral form), exchangeable (sorbed/complexed) and dissolved, see Figure 1.1. Especially manganese oxides, iron oxyhydroxides and organic matter act as important scavengers of metals, by surface adsorption under aerobic conditions (Sigg, 1987; Koelmans, 1997). Additionally, in anoxic sediments with sufficient sulfate and organic matter, sulfate reduction may lead to the formation of inorganic sulfides, and metal-sulfide precipitates become important. Metals that cannot be bound by the solid phase are present in the interstitial spaces between sediment particles: the pore water. In this aqueous fraction, metals can be present in many forms, e.g., as complexes with dissolved organic matter and inorganic ligands (for example chloride, sulfate, carbonates, hydroxyl ions), and as free ions. Some dissolved species may be taken up while other species are not. This is the link between speciation and availability. It is widely accepted that in any case the free metal ion is available for uptake (Campbell *et al.*, 1995). The most important factors that influence metal speciation (and hence availability) are redox conditions and pH (e.g., Calmano *et al.*, 1993). They affect characteristics of main soil components that bind metals. For instance, at low redox potential, sulfides may form that can precipitate with trace metals. At low pH values, aqueous phase concentrations may increase because of competition of metal ions with H^+ ions at sorption sites.

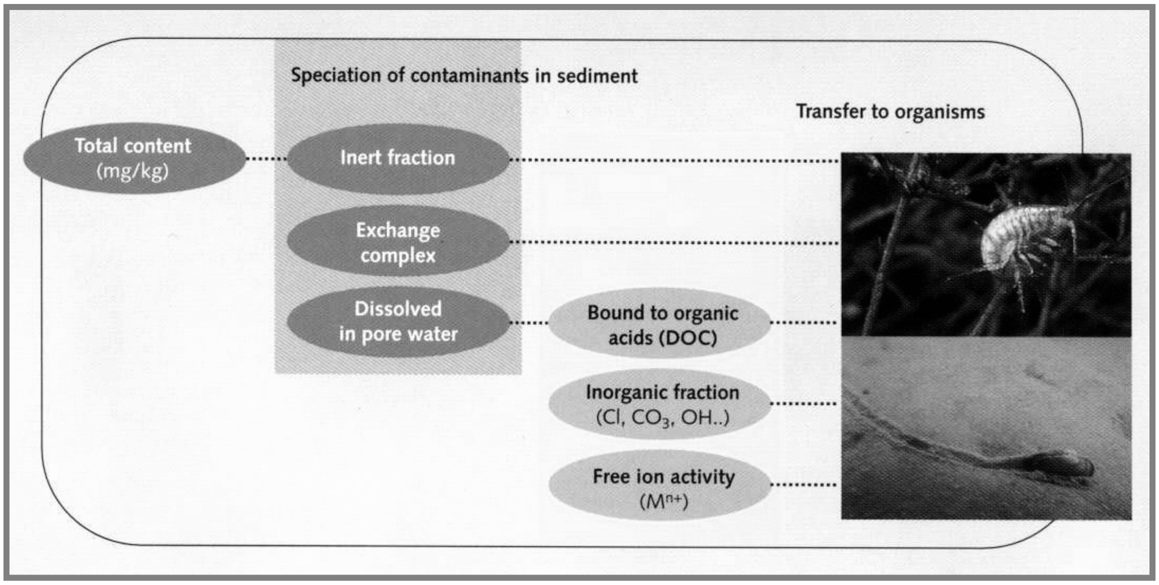

Figure 1.1. Schematic representation of the speciation of trace metals in sediment and the link towards availability (after www.sofie.nl, 2005)

1.3.2 Methods to assess metal availability

A variety of analytical and operationally defined methods have – recently – been developed to estimate chemically available concentrations of trace metals. These methods can be divided into a) sediment extractions using complexing agents or (weak) acids, and b) pore water extractions (directly or via an adsorbent). Below, a short overview of these methods and their applicability in the risk assessment of sediments is given**.**

Sediment extractions

1. Sequential extraction methods. Sequential extractions may give information about the distribution of metals over various fractions (e.g., extractable, reducible, organic/sulfidic) in soils and sediments and the role of pH and E_h in determining this distribution (e.g., Tessier *et al.*, 1979; Förstner *et al.*, 1989; Mann and Ritchie, 1993; Davidson *et al.*, 1998). The main disadvantage of this method is that it is not always clear what the various fractions mean with respect to bioavailability. Differences in operational definitions (e.g., different extraction times and extractant concentrations) may lead to incomparable results. Furthermore, the methods are laborious and redistribution of metals during the analysis may lead to artifacts (Nirel and Morel, 1990). Recently, new proposals for sequential extraction procedures have been developed by the

Standards, Measurements and Testing Programme (formerly BCR) of the European Commission (Rauret *et al.*, 1999) leading to a three-step sequential (EDTA, acetic acid, and *aqua regia*) extraction procedure for sediment analysis.

2. Singular (mild) extraction methods. Next, a variety of singular (mild) extraction methods that liberate (loosely) bound metal fractions followed by conventional metal analysis are employed, such as 0.43 M HNO_3 (Mann & Ritchie, 1993), 0.01 M $CaCl_2$ (Houba *et al.*, 1986). These extraction techniques are sometimes found to correlate well with observed effects -especially for plants- but they lack a theoretical basis. The 0.01 M $CaCl_2$ extraction was developed for soils but is in principle applicable to (anoxic) sediments when performed in oxygen-free conditions (like in a glove box or bag). Another singular extraction method is the SEM/AVS extraction.

3. SEM/AVS approach. One of the current methods to take bioavailability of trace metals in sediments into account is the SEM/AVS approach (e.g., DiToro *et al.*, 1992), which assumes precipitation of trace metals (measured as SEM) with sulfides (measured as AVS) in reduced (anoxic) environments. If SEM exceeds AVS, trace metals will potentially be present in the pore water, depending on the presence of other sorption sites in the sediment. In contrast, if SEM is smaller than AVS, virtually all metals will be precipitated. This concept has been tested in laboratory and field settings as an evaluation tool of toxicity risks. SEM-AVS has been found to give good predictions about pore water concentrations and absence of toxicity in test organisms (e.g., Ankley *et al.*, 1991; DiToro *et al.*, 1992). It does not directly account for partitioning of excess metals.

Pore water extractions

1. Pore water isolation and analysis. Total pore water metal concentrations can be determined after sampling by centrifugation or squeezing of sediments(e.g., Van den Berg, 1998), or *in situ* during sampling of pore water by ceramic suction cups or rhizons (Meijboom & Van Noordwijk, 1992). The sampling (isolation) and analysis of pore water, however, is rather sensitive to analytical and procedural uncertainty, due to the low concentrations involved and time related variation. This makes the analysis of pore waters as part of a routine risk assessment time-consuming and (thus) costly.

2. Direct measurement of free ion activity. The past decade, methods have been developed to directly measure free metal activity. Examples of these techniques are CHELEX columns (e.g., Vink, 2002), the Donnan Membrane Technique (DMT, introduced by Lampert (1982), and further developed by e.g., Temminghoff *et al.* (2000) and Weng *et al.* (2002)), and the Diffusive

Gradients in Thin films technique (DGT, e.g., Davison & Zhang, 1994; Ernstberger *et al.*, 2002) where metal ions are isolated using diffusion over a gel membrane.

CHELEX material can bind trace metal ions quickly and specific. CHELEX techniques, e.g., CHELEX microcolumns, must be applied very accurately, because if the extraction time is too long, also DOC or inorganic metal complexes may be measured (*Cornelissen & Kamerling*, 2003). Some uncertainty exists on the variation in dissociation velocities for different metals and sediment types. A further development using CHELEX and employing a very short extraction time of 1-2 seconds is the measure cell SOFIE (Sediment Or Fauna Incubation Experiment), where an undisturbed sediment core can be extracted *in situ* and redox conditions thus remain intact (Vink, 2002). Bioassays can be conducted simultaneously in the same setting. Sediment samples are taken in an undisturbed manner, including the overlying surface water. Gradients of metal species and micro/macro nutrients can be accurately measured over layer increments as low as 5 mm. This method is especially suited for mechanistic research and model development and can be applied in decision making processes (Cornelissen & Kamerling, 2003).

The (Wageningen) Donnan Membrane Technique (WDMT) involves a continuous flow system with a donor side and an acceptor side separated by a negatively charged membrane. These sides are continuously flushed with solution across the membrane, allowing a pseudo-equilibrium. The donor solution contains both ‘free’ and complexed metal ions. The concentration of the cations in the acceptor solution is either equal to the concentration of the ‘free’ cation concentration in the donor solution, or it can be calculated using simple correction factors (Temminghoff *et al.*, 2000). The Donnan Membrane Technique uses equilibrium times of 1-2 days (Weng *et al.*, 2002) and is relatively laborious and complex. It has not yet been applied to aquatic sediments (Cornelissen & Kamerling, 2003).

A third method is the DGT method (Diffusive Gradient in Thin Films, developed by Davison and Zhang in 1994). The DGT probe also contains a CHELEX layer, which is separated from the pore water solution by a hydrogel. Free ions can move towards the CHELEX through the gel. Complexed ions, however, can also pass the gel, which means that it is not yet possible to only detect free ions.

In conclusion, many methods exist, all with their specific advantages and disadvantages. For the assessment of environmental risks of metal polluted floodplain sediments, an analysis is needed that can be applied on a routine basis, addressing the specific processes ocurring in (alternately) reduced sediments. In the past decade, especially the SEM-AVS approach received considerable interest (e.g. DiToro *et al.*, 1992; Allen *et al.*, 1993; Ankley *et al.*, 1996; Hansen *et al.*, 1996), because precipitation with sulfides is the most important process regulating trace metal availability in reduced sediments. Further advantages of the method are that it is relatively simple and cheap, it is based on mechanistic understanding of metal speciation and bioavailability in anaerobic sediments, and it implicitly considers the total combined metal load (through considering ∑SEM) on an ecosystem instead of individual metals. These are the main reasons why the SEM-AVS method was selected for this study. This method will be discussed in more detail in the next section.

1.4 The SEM-AVS approach

1.4.1 Sulfide geochemistry and trace metals

Sulfide formation in sediments is mediated by the reduction of organic matter in the absence of oxygen and other electron acceptors. The redox sequence of organic matter reduction is as follows: first, aerobic bacteria use oxygen as electron acceptor. When all oxygen is consumed, nitrate is used, followed by manganese and iron, accompanied by a gradual decline in redox potential. When these constituents are depleted, sulfate reducing bacteria transform sulfate into sulfide (Stumm & Morgan, 1981), according to

$2\ CH_2O + SO_4^{2-} \rightarrow 2\ CO_2 + S^{2-} + 2\ H_2O$ (Goldhaber *et al.*, 1974)

followed by

$Fe^{2+} + S^{2-} \rightarrow FeS\ (s)$ (Berner, 1967)

In the past decade it has been recognized (e.g. DiToro *et al.*, 1992; Allen *et al.*, 1993; Ankley *et al.*, 1996; Hansen *et al.*, 1996) that the reactive iron sulfide fraction in anaerobic sediments has a large potential to decrease metal availability. Various processes may limit the formation of sulfides, either due to a lack of sulfate or a lack of organic matter (Smith & Melville, 2004) or the presence of other electron acceptors such as nitrates. Sulfate limitation is more important in freshwater than in marine sediments (Leonard *et al.*, 1993). In natural, unpolluted systems, sulfide mainly precipitates with Fe, leading to the formation of iron monosulfides ($FeS_{1.09-1.11}$), mackinawite (tetragonal $FeS_{0.94}$), and greigite (Fe_3S_4), together known as AVS. Morse & Rickard

(2004) state that also dissolved sulfide species may contribute importantly to this AVS pool. Although these metastable monosulfides act as precursors in the eventual formation of pyrite (FeS_2), which is the thermodynamically most stable mineral, they are very common in most reducing environments(Machemer *et al.*, 1993). Sulfides can also form precipitates with trace metals, which have considerably lower solubility constants than Fe-sulfides. Chemical equilibrium calculations and observations showed that the relative affinity for S^{2-} increases as Cu>Pb>Cd>Zn>Ni (see Table 1.2).

Table 1.2. Solubility products of metal sulfide minerals in increasing order for neutral pH values. For the net reaction $Me^{2+} + S^{2-} \leftrightarrow MeS\ (s)$, $\log K_{sp}$ is calculated from partial reactions. After DiToro *et al.*, 1990.

Metal sulfide	Sulfide mineral	$\log K_{sp}$
MnS	Alabandite	-19.15
FeS (am)	Amorphous	-21.80
FeS	Mackinawite	-22.39
NiS	Millerite	-27.98
ZnS	Wurtzite	-28.39
CdS	Greenockite	-33.42
PbS	Galena	-40.94
CuS	Covellite	-57.25

Thus, trace metals in pore water can be scavenged to a considerable degree and under anoxic conditions trace metal availability can be largely controlled by co-precipitation with sulfides. Some researchers, guided by DiToro *et al.* (1990, 1992) claim that the trace metals actually form precipitates with S^{2-} by solution of iron monosulfides. Others (e.g., Van den Berg, 1998; Morse & Rickard, 2004) argue that the metals mainly coprecipitate with FeS. The net replacement reaction can be described by the following equation, in which Me^{2+} represents a divalent trace metal ion:

$$FeS\ (s) + Me^{2+} \leftrightarrow MeS\ (s) + Fe^{2+}$$

or

$$FeS_m + Me^{2+} \leftrightarrow MeFeS_n\ (s)$$

Important issues for the relevance of this process for ecological risks posed by metal contaminated sediments are whether sufficient sulfide is present to bind all metals, and whether anoxic conditions are maintained in the whole water-sediment system all the time.

1.4.2 Measurement of AVS and SEM

The use of AVS as a tool to describe metal toxicity started at the beginning of the 1990's (e.g., DiToro *et al.*, 1990). There are two main methods used to extract and analyze this reactive and operationally defined sulfide pool. First, there is the purge-and-trap method, in which the sulfides are volatilized from the sediment with HCl in a first reaction vessel.The vessel is purged with N_2 transfering the sulfides to a second reaction vessel containing a sulfide trap, e.g., $AgNO_3$ (Morse *et al.*, 1987). Second, the diffusion method exists, in which the sulfide is trapped in a small reaction vessel containing a sulfide anti-oxidant buffer solution (SAOB). This small vessel is present within the first reaction vessel in which the volatilization step is carried out (Brouwer & Murphy, 1994). The subsequent determination of sulfide concentrations/amounts can be done gravimetrically, colorimetrically or with an ion specific electrode. The acid strength may differ among different methods from 1 to 6 M. Metals bound to sulfides are claimed to be unavailable for uptake by biota that are exposed via the pore water. Conversely, excess metals that cannot be bound to sulfides are assumed to be dissolved or sorbed to easily accessible surfaces, possibly leading to toxicity. Hence, for the evaluation of potential toxicity of the sediment, the concentration of AVS is compared to the sum of Zn, Cu, Pb, Cd, and Ni concentrations extracted from the sediment during the volatilization of the sulfides, both on a molar basis. These extracted metals are known as SEM. In this approach, the combined toxicity of metals is inherently based on additivity. The comparison of SEM and AVS concentrations can be made either by regarding the ratio of SEM/AVS (if SEM/AVS>1, possible toxicity) or the difference of SEM-AVS (if SEM-AVS> 0, possible toxicity). The latter method has the advantage that the magnitude of the excess is accounted for, which can be compared with toxicological threshold values for target organisms (e.g., NOEC, EC_{50}). This excess concentration thus is assumed to be present in pore waters. However, excess SEM may be bound to other fractions. Organic carbon is an important additional binding phase controlling interstitial water concentrations under anaerobic conditions, especially for Pb and Cu. Consequently, it is proposed to further correct SEM-AVS concentrations for organic C to account for organic sorption (Mahoney *et al.*, 1996, McGrath *et al.* 2002; DiToro *et al.*, 2005). This is done by normalizing the amount of excess metal to the fraction of organic carbon in the sediment. The resulting risk indicator then is (SEM-AVS)/f_{OC}.

Recently, new toxicity threshold values have been proposed for the SEM/AVS method that better predict the presence of toxicity (Sijm *et al.*, 2001; McGrath *et al.*, 2002; DiToro *et al.*, 2005). If SEM-AVS exceeds 2 µmol/g dw sediment and (SEM-AVS)/f_{OC} exceeds 150 µmol/g OC, the chance of toxic effects is considered to be large (> 90%) (McGrath *et al.*, 2004; DiToro *et al.*, 2005).

1.4.3 Validation of the SEM-AVS approach

There are some limitations to the use of the SEM/AVS concept in risk assessment of metal contaminated sediments**.** First, the SEM/AVS approach is obviously only applicable to anaerobic sediments, because in aerobic sediments other binding factors control bioavailability in the absence of AVS (Van den Berg, 1998). Furthermore, organisms may influence their habitat in search for food or oxygen. For instance, aquatic plants are found to oxidize their rhizospheres in order to capture essential nutrients, leading to metal release ions into the pore water due to sulfide oxidation (Förstner *et al.*, 1993, cited from Klaine *et al.*, 1993). Benthic invertebrates (Ciarelli *et al.*, 1999) and benthivorous fish like bream (e.g., Scheffer *et al.*, 2003) can bioturbate or resuspend sediment leaving it oxygenated. Finally, dissolved sulfides themselves may cause toxicity when present at high concentrations (Brouwer & Murphy, 1995). Further, the concept is only valid when exposed organisms mainly take up metals via the pore water. In this section, recent research into the validation of the method is described shortly.

Experimental proof of the validity of the SEM/AVS concept for the establishment of the absence of toxicity has been found by many authors in laboratory experiments, where both short-term and long-term exposures were tested using bioassays. For instance, the relation of SEM-AVS with bioaccumulation was shown in laboratory studies including full life-cycle experiments., e.g. with *Chironomus tentans* on Zn spiked sediments by Sibley *et al.* (1996), and with the estuarine amphipod *Leptocheirus plumulosus* on Cd spiked sediments by DeWitt *et al.* (1996). In a laboratory study by Hansen *et al.* (1996) SEM-AVS and Interstitial Water Toxic Units (IWTUs) accurately (99.2%) identified absence of sediment toxicity. Berry *et al.* (1996) showed in 10-day laboratory experiments on a variety of marine and freshwater benthic organisms that when AVS exceeded SEM, toxicity did not occur, because the interstitial water metal concentrations remained below those predicted to cause damage. Also life-cycle and longer experiments up to more than 1 year were performed with the same conclusions, i.e. SEM/AVS ratios and IWTUs are more accurate predictors of acute mortality than dry weight metal concentrations.

The relevance of the AVS concept for biological responses in the field has only been tested in a few studies, however. Hare *et al.* (1994) studied toxicity and accumulation of Cd from sediment by benthic invertebrates along a Cd gradient that was artificially created in the field, and evaluated the efficacy of AVS concentrations in improving the prediction of biological effects in the field. A high relationship between Cd concentrations in pore waters and AVS was observed. However, the relation of AVS to eco(toxico)logical endpoints was not uniform among species. The abundance of *Chironomus (salinarius gp.) sp.*, was significantly related to the Cd/AVS molar ratio. For other taxa no relationship between abundances or growth rates with Cd/AVS molar ratio was found. The authors explained this lack of toxicity by differing sensitivities to Cd for the animal species used in laboratory and field, and by the relatively low concentrations of Cd in excess of AVS even at the highest Cd/AVS molar ratio. Later studies by Warren *et al.* (1998) and Hare *et al.* (2001) concluded that the AVS concept adequately described Cd amounts in porewater and Cd accumulation in benthic taxa such as chironomids and oligochaetes. However, accumulation of Cd or (sub)lethal effects (abundance and growth) were not related to Cd/AVS ratios for other species, most probably because their feeding behavior implies little contact with the sediment. Detritivore species such as Oligochaeta, Chironomidae, and Asselids are in closer contact with sediments while they feed on organic detritus. Other functional feeding groups, for instance predators or filter feeders, are less dependent on sediment properties (Vos, 2001; Peeters et al, 2000). Altogether, it can be concluded that *in situ* relevance of SEM-AVS depends on organism behavior. More work on *in situ* validation is needed because a) only a few studies exist; b) little attention has been paid to the relevance at community level; c) multiple stress is a complicating factor in field situations. Furthermore, AVS levels are subject to variable environmental conditions, which implies that it is important to account for variations in AVS levels when using the concept in risk assessment. In relatively few studies, spatial as well as temporal variation have been assessed. Howard & Evans (1993) studied seasonal and spatial changes in sediment AVS in a seasonally anoxic mesotrophic lake. Results indicated that AVS increases during anoxic periods and that lake morphology is important due to different sedimentation of particles of varying grain sizes. They further observed seasonal variations in AVS concentrations. Besser *et al.* (1996) studied effects of spatial and temporal variation in AVS on the bioavailability of copper and zinc in freshwater sediments from the upper Clark Fork River of Montana (USA). Variation in concentrations of AVS was associated with differences in bioaccumulation of Cu and Zn and growth of larvae of *Chironomus tentans*. Mackey & Mackay (1996) found that spatial variability of AVS was high and suggested that seasonal variations further increase the observed variability in bioavailability. Van den Hoop *et al.* (1997) studied both spatial and seasonal variations of AVS and SEM in Dutch marine and freshwater sediments and observed seasonal variations in AVS and SEM concentrations. Van den Berg *et al.* (1998) studied vertical distribution of AVS and SEM in summer and winter in single cores from four locations in a recent sedimentation area of the river Meuse (The Netherlands) and concluded that the depth of the boundary between the suboxic and the anoxic layer changes seasonally with consequences for sulfide levels. Overall, it can be concluded that variability in AVS concentrations is an important factor to take into account when applying the concept in ecological risk assessment. A relevant question is how large this variation is in regularly inundated floodplains.

1.5 Scope and aim of thesis

The studies described in the above section show a part of the picture but lack a system oriented approach. Comparability among studies often is limited because different methods to measure AVS are employed. Thus, although it is clear that the concept has its merits, there is still a need for validation of the applicability of the AVS method in risk analysis of trace metal polluted sediments, especially for dynamic and variable field situations like floodplains. This thesis addresses the geochemical aspects of AVS and SEM in floodplain lake sediment, its spatial distribution in floodplain lakes and dynamics over time, the link with effects on single species (bioassays), as well as the impact of excess SEM on the *in situ* benthic community, in one consistent methodological approach. Using this approach the SEM-AVS concept is thus evaluated on various levels from geochemical through ecotoxicology towards ecology. Furthermore, the aspect of spatial and temporal variability is addressed. For the case studies in the field, several floodplain lakes located in the floodplain area Afferdensche and Deestsche Waarden along the Waal branche of the Rhine were selected. Occasionally, this selection was extended towards other floodplain lakes along the Rhine branches. The main goal of this research is a) to explore the actual risks of trace metals to benthic communities in floodplain systems and b) to evaluate the SEM-AVS concept as a tool to assess bioavailable concentrations of trace metals in dynamic floodplain sediments. These objectives can be divided into the following main research topics:

1. To assess the spatial and temporal variability of SEM-AVS in relation to total and dissolved trace metal concentrations in floodplain sediments
2. To assess the eco(toxico)logical relevance/significance of trace metals and SEM-AVS in floodplain lakes (link with bioaccumulation, impact in single species tests, impact on benthic communities)
3. To assess if the use of SEM-AVS improves risk analysis compared to classic/current risk indicators (is SEM-AVS a better predictor of ecological endpoints?)

1.6 Outline of thesis

This thesis covers the geochemical aspects of AVS in floodplain lakes (persistence and formation) and variation in both AVS and extracted and total metal concentrations (spatial and temporal) to facilitate the translation of AVS based risk analysis to biological endpoints (single species as well as benthic communities, both laboratory and field based). In order to process large numbers of sediment samples, a fast method to measure AVS was required. In The Netherlands, only the relatively laborious purge-and-trap method was operational (Van den Hoop, 1995; Van den Berg, 1998). Hence, a method for the determination of sulfides in freshwater wetlands with presumed variable concentrations of AVS was tested and implemented based on existing methods (Chapter 2). Persistence, degradation and formation of AVS were studied in field and lab experiments in order to estimate time scales and overall reaction rate constants for AVS formation (Chapter 3). Temporal variability of solid and dissolved trace metals in relation to sulfate-sulfide cycles due to seasonal dynamics, combined with fluctuations in water levels, was addressed in Chapter 4. Often, only a few localized point samples are taken to assess sediment quality. However, while bioavailability is dictated by local conditions, it is necessary to quantify spatial patterns on an ecologically relevant scale (benthic community habitat). Spatial within-lake variability of trace metals and sulfides was studied in a selected floodplain lake in the Afferdensche and Deestsche Waarden (Chapter 5). For the assessment of large-scale between-lakes variability a larger survey comprising ten floodplain lakes was conducted (Chapter 7). To use SEM/AVS as a tool in risk assessment, the relevance of the concept to different ecological endpoints is of major importance. Benthic invertebrate species and community are selected as ecological receptor because they are closely linked to sediment-bound contaminants and play an important role by supporting higher levels of the aquatic food chain. In Chapter 6, a translation of geochemical data to biological responses is tested using bioassays in a laboratory setting. Endpoints comprise survival and growth of *Asellus aquaticus*, a benthic organism that lives in close contact with sediment. In Chapter 7, an experiment on field scale is presented in which the link between the geochemical variables total metals, AVS and SEM with bioavailability (metal accumulation) and ecological endpoints on the community level is studied. In Chapter 8, the results are integrated and discussed with respect to recent scientific literature as well as developments in the field of risk assessment of metal contaminated sediments.

CHAPTER 2

MEASURING AVS IN FLOODPLAIN LAKE SEDIMENTS: EFFECT OF REACTION TIME, SAMPLE SIZE AND AERATION

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Abstract

For the routine measurement of acid volatile sulfide (AVS) in floodplain lake sediments, an earlier published diffusion method was adapted and optimised. We evaluated the effect of reaction time, sample weight and passive sample aeration on AVS recovery, and determined the method's reproducibility. The optimal reaction time was 4 h. Losses of AVS due to sample aeration did not occur within 15 min of sample-air contact. Relative standard deviation was <2.5% for known sulfide solutions, was between 10% and 15% for AVS concentrations in sediments and <12% for SEM concentrations in sediments, generally. Sediment samples with known SEM and AVS content were used to compare results of the developed method with the purge-and-trap method. There is a good agreement with the purge-and-trap method for SEM concentrations. AVS concentrations measured with the diffusion method are higher than with the purge-and-trap method. We conclude that the adapted diffusion method is well suited for the routine measurement of AVS concentrations in floodplain sediments.

2.1 Introduction

Sulfides can play an important role in controlling the speciation and the environmental risks of trace metals in aquatic sediments. When sediments are reduced, trace metals present in the interstitial water, can either adsorb to, (co)precipitate with or replace Fe and Mn in FeS or MnS minerals (Morse *et al.*, 1987; DiToro *et al.*, 1992). They are considered to be less mobile and less bio-available to benthic organisms, leading to a reduction of the environmental risk. Depending on the amount of sulfides present in the system, the mobility could even be very low, if all trace metals are removed from the pore water phase and immobilised as solid sulfide minerals. For this reason, the sulfide chemistry of sediments has received much attention in the past decade (Allen, 1993; Leonard *et al.*, 1995; Ankley, 1996; Huerta-Diaz *et al.*, 1998; Van den Berg *et al.*, 1998). Acid volatile sulfide (AVS) is operationally defined as the amount of sulfide that can be volatilised during a cold acid extraction. The AVS-bound metals (usually Zn, Cu, Cd, Ni and Pb are considered) are extracted at the same time and are therefore called SEM (simultaneously extracted metals). The ratio or the difference between AVS and SEM gives an indication of the potential sediment toxicity (DiToro *et al.*, 1990).

The most widely applied method for the determination of AVS and SEM is the purge-and-trap method, developed by Morse *et al.* (1987) and used by many others, e.g. Van den Hoop *et al.* (1997) and Van den Berg *et al.* (1998). In short, the method uses the volatilisation of sediment sulfide when acid is added to the sediment. Through purging with N_2 gas the H_2S is transferred from the sediment vessel into another vessel containing a NaOH buffer solution. The sulfide concentration in the NaOH can then be measured in various ways, e.g. polarographic, colorimetric, gravimetric or by means of a specific electrode.

However, the purge-and-trap method is laborious: a lot of glassware, gas and space is required, resulting in a relatively small number of samples that can be handled simultaneously (Brouwer and Murphy, 1994; Leonard *et al.*, 1996). This makes the purge-and-trap method less suitable for routine AVS measurements, where large numbers of samples must be analysed, e.g. when monitoring seasonal or spatial variation of AVS and SEM. In contrast, the diffusion method (Hsieh and Yang, 1989) has been proposed as an equally effective but much faster method than the purge-and-trap method (Brouwer and Murphy, 1994; Leonard *et al.*, 1996). The main difference with the purge-and-trap method is that the liberated gaseous H_2S is trapped in a small vial placed within the sediment/acid reaction vessel. The gas is subsequently absorbed by a sulfide anti-oxidant buffer solution (SAOB) inside the small vial. This causes a concentration gradient leading to further diffusion of the H2S towards the SAOB solution. The concentration in the SAOB solution is measured with an ion specific electrode (ISE) for sulfide, which also is a lot faster and simpler than the polarographic or colorimetric method.

Brouwer and Murphy (1994) and Leonard *et al.* (1996) described some variations of this diffusion method. Brouwer and Murphy (1994) used sediments from Lake Ontario with high AVS levels (up to 4 mg S/g dry sediment), established the acid strength needed to obtain similar AVS and SEM concentrations as in the purge-and-trap method, and determined the optimal reaction time and sample size. Leonard *et al.* (1996) used freshwater samples with much lower AVS concentrations but they did not specifically evaluate reaction time and the effect of sample mass. These experimental factors vary among sediment types and vessel volumes. The purpose of this study was to optimise the diffusion method for the routine analysis of AVS and SEM for floodplain lake sediments. AVS concentrations in floodplain lakes are assumed to be rather low compared to marine sediments and fluctuating due to the nature of the sediments and the seasonal change in environmental conditions due to hydrological processes (desiccation and inundation). We established the optimal reaction time, the recovery and the effect of sample size for our design. Also the effect of sample aeration was tested for a variety of sediments. There is a risk of sample oxidation during sample handling and manipulation, which could lead to the underestimation of the amount of AVS present in the sediment. We thus also examined the effect of passive aeration of the sample on the measured AVS concentration in order to establish the time scale at which oxidation starts to occur. Finally, the current adaptation of the diffusion method was compared with the purge-and-trap method, by comparing the results of the two methods for one set of sediment samples.

2.2 Material and methods

2.2.1. Sediments

Sediments with known AVS and SEM content (Van den Hoop *et al.*, 1997), as determined by a purge-and-trap method (Den Hollander and Van den Hoop, 1994), were obtained from the National Institute for Public Health and the Environment of the Netherlands (RIVM). These freshwater sediments originate from the Ketelmeer (lake), Nieuwersluis (small lake) and Kromme Rijn (a small river). The Ketelmeer sediment is fine grained, highly calcareous (12.5% calcium carbonate) and contains 3.9% of organic carbon (Buykx *et al.*, 1999; Van den Berg *et al.*, 2001). Floodplain lake sediments were collected by means of a Jenkins core sampler from various floodplains along the river Waal (The Netherlands) and stored either frozen or cooled until analysis. In general, these sediments are fine grained (over 25% of the particles is smaller than 2 lm) and have high organic matter contents (10–15%).

2.2.2 AVS measurement

A large glass jar of 800 ml with a height of 14 cm and an inner diameter of 8 cm was used as reaction vessel (see Figure 2.1). A small vial of ± 30 ml was glued to the inner wall. The cap was made airtight by means of a Teflon inlay.

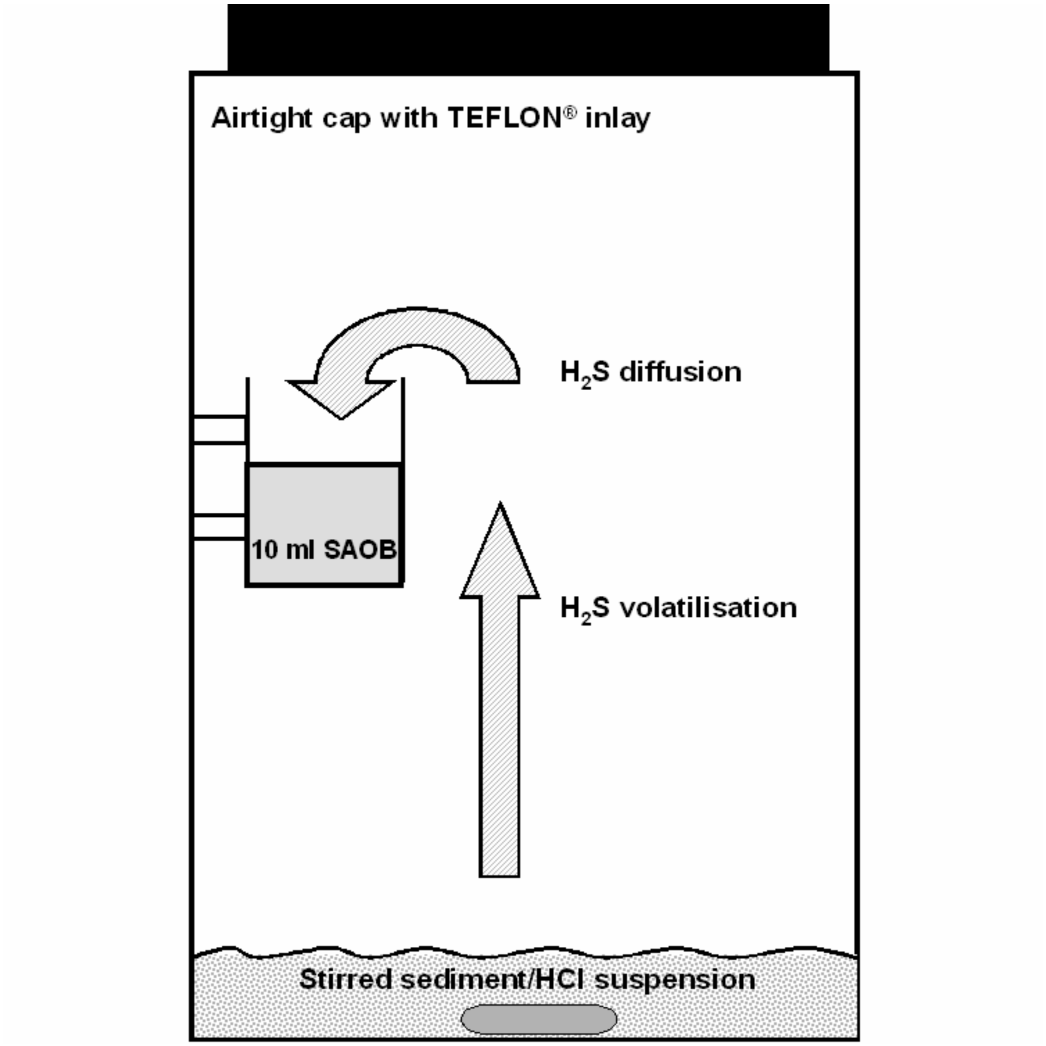

Figure 2.1. Experimental set-up of the reaction vessel.

The sulfide specific electrode (Ag500 WTW 106292, Wissenschaftlich-Technische Werkstätte, Weilheim, Germany) was calibrated with a 0.1 M Na_2S solution (sodium sulfide nonahydrate, Sigma S-4766, Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands) diluted in SAOB, in a concentration range of 10^{-4}–10^{-2} M. This covers the range of AVS concentrations in the SAOB, liberated from the sediment samples. The SAOB solution was prepared analogously to the solution used by Brouwer *et al.* (1994), and contained 2 M NaOH (p.a., Merck 6498, E. Merck Nederland B.V., Amsterdam, The Netherlands), 0.1 M ascorbic acid (p.a., Merck 127, L (+) ascorbic acid) and 0.1 M EDTA (p.a., Merck 8418, Titriplex ®). The molarity of the Na_2S stock solution used for the calibration was measured daily by back titration with 0.02 M sodium thiosulfate (Merck 1.09950) after complexation with 0.02 M iodine (iodine volumetric standard 0.0513 N solution in water, Aldrich 40,535-3, Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands). A 10% starch solution (p.a., Merck 1252) was used as indicator. The detection limit for the ISE using 10 ml of SAOB and ± 5 g of dry sediment was 0.1 µmol S/g dry sediment. The ISE was calibrated at regular intervals to correct for drift of the signal. Calibration series were accepted when the R^2 of the relationship between the signal and the logarithm of the sulfide concentration was above 0.95. Furthermore, flow charts were used to check intercept and slope values of calibration series. To extract the AVS from the sediment samples, first 10 ml of SAOB was introduced into the inner vial of the reaction vessel. Sediment samples were homogenised before (replicate) subsampling. Then, the (sub)sample (5–10 g of wet sediment) was transferred to the vessel. 50 ml of 1 M HCl (37%, Riedel de Haen, 30721, Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was added carefully via the side of the large jar. The stirring bar was added promptly and the vessel was capped. The sediment suspension was stirred briskly but regularly to ensure that all sediment was in suspension. During the reaction, calibration series were prepared and the molarity of the sulfide stock solution was measured. After the measurement of the S^{2-} concentration, the SAOB solution was removed with a pipette and disposed off. Part of the sediment extract was filtered on 0.45 µm membrane filters (Schleicher and Schüell's, Hertogenbosch, The Netherlands) with a water jet pump. The first 10 ml were used to rinse the 50 ml PE bottles and their caps and were discarded; the next 20 ml of filtrate was collected for SEM determination. The SEM extract was diluted 1:10 (v/v) with Barnstead ® nanopure water, yielding a final acid concentration of 0.1 M HCl, and element concentrations were determined on ICP-MS (Elan 6000, Perkin–Elmer). Detection limits for SEM metals were $9.1*10^{-2}$ for Zn, $9.9*10^{-3}$ for Cu, $2.7*10^{-4}$ for Cd, $1.5*10^{-1}$ for Ni, and $5.5*10^{-6}$ for Pb, in µmol/g dry sediment. The high detection limit for Ni is caused by interference with the ICP-MS response of Cl atoms, which have approximately the same molar weight. During data analysis erroneous Ni concentrations, which occurred in 40% of the cases, were calculated from the average ratio of correct Ni measurements with Cu. Because Ni contributes only marginally to total SEM (<4%) this procedure is considered appropriate. The experimental design enables simultaneous measurements of about 10–15 samples. The measurement of two series a day is feasible, thus leading to a total of 30 samples per day.

2.2.3 Effect of reaction time

The optimal reaction time was established by measuring AVS and SEM concentrations after various reaction periods of various sample types:

(a) Barnstead ® nanopure water spiked with a fixed amount of Na_2S solution (74.3 µmol S) which is representative of sediment AVS concentrations.

(b) Floodplain lake sediment (originating from a floodplain along the river Waal, The Netherlands).

2.2.4 Effect of sample weight

The effect of sample weight was tested by evaluating the AVS response for a range of sediment wet weights from 2–14 g (± 1–7 g of dry sediment). The used sediment originates from the Kromme Rijn, The Netherlands. The reaction time used to test the effect of sample weight was between 1 and 2 h, based on the reaction time used by Brouwer and Murphy (1994) and Leonard *et al.* (1996).

2.2.5 Effect of aeration

Prolonged passive aeration of the sediment is expected to have an effect on the recovery of AVS. This was tested on Ketelmeer, Kromme Rijn and floodplain lake sediment. The passive aeration treatment consists of weighing in the sediment in the reaction vessel and leave it uncapped for a certain period of time. After the predetermined aeration time, 50 ml of 1 M hydrochloric acid was added, the vessel was capped and the 4 h reaction period started. AVS concentrations measured are compared to the AVS concentrations measured at zero aeration time.

2.2.6 Comparison with purge-and-trap method

Method comparison was achieved by measuring AVS and SEM with the current diffusion method using sediment samples which were also analysed with the purge-and-trap method as part of an earlier study (Van den Hoop *et al.*, 1997). These freshwater samples originate from various locations in the Netherlands: Ketelmeer (lake), Nieuwersluis (lake) and Kromme Rijn (river). After the determination of AVS and SEM contents, these samples were stored in flasks with nitrogen headspace at 4 °C.

2.3 Results and discussion

2.3.1 Effect of reaction time

The graph of the recovery of a Na_2S solution versus the reaction time (Figure 2.2a) shows that recovery increases fast between 0 and 1 h of reaction time, and after that increases slowly until a maximum is reached. At the optimal reaction time (4 h) the recovery is 94.5 ± 1.2% (n=3). The error of the replicated measurements is typically within 2.5%. We conclude that the amount of sulphur recovered is not significantly different from 100%. For floodplain sediment it took 2–3 h of reaction before the measured amount of AVS liberated from the sediment and trapped in the SAOB remained constant (see Figure 2b). However, according to former studies, the optimal reaction time was thought to be around 1 h for the purge-and-trap method as well as for the diffusion method. Probably the large jar volume caused a slower transport towards the SAOB. Because the required reaction time for AVS in floodplain sediments (Figure 2b) is longer than for Na_2S solutions (Figure 2a), we conclude that not the diffusion, but the acid extraction is the rate-limiting factor. The relatively slow extraction may be explained from a lower accessibility of the sulfide minerals as opposed to sulfide in solution, or from the simultaneous dissolution of other minerals, such as calcite, which is abundant in freshwater sediments (Van den Hoop *et al.*, 1997). To be on the safe side for all sediment types, we selected an optimal reaction time of 4 h. Results show that the maximal analytical error in replicate spikes is lower than 2.5%, whereas the error in replicate analyses of sediment samples in general is below 10–15%. Sediment heterogeneity, even after careful stirring of the sediment before subsampling, probably causes this larger error. The error in replicate SEM analyses is generally <12%. The amount of SEM extracted is not significantly different ($\alpha < 0.05$) between the different reaction times applied (see Figure 2b) which indicates a quick dissolution of the bound metals, be it from sulfides or from other binding phases. The quick and complete metal extraction seems to contradict the slower rate of sulfide extraction. However, the amount of SEM on a molar basis is larger than the AVS amount, which points to a substantial amount of metals bound in other phases than sulfide minerals, for instance adsorbed metals. This independency of SEM to extraction time also shows that prolonged exposure to acid does not yield increased SEM concentrations. This enables the comparison of SEM concentrations determined with the current method with results of methods with extraction periods of 1 h.

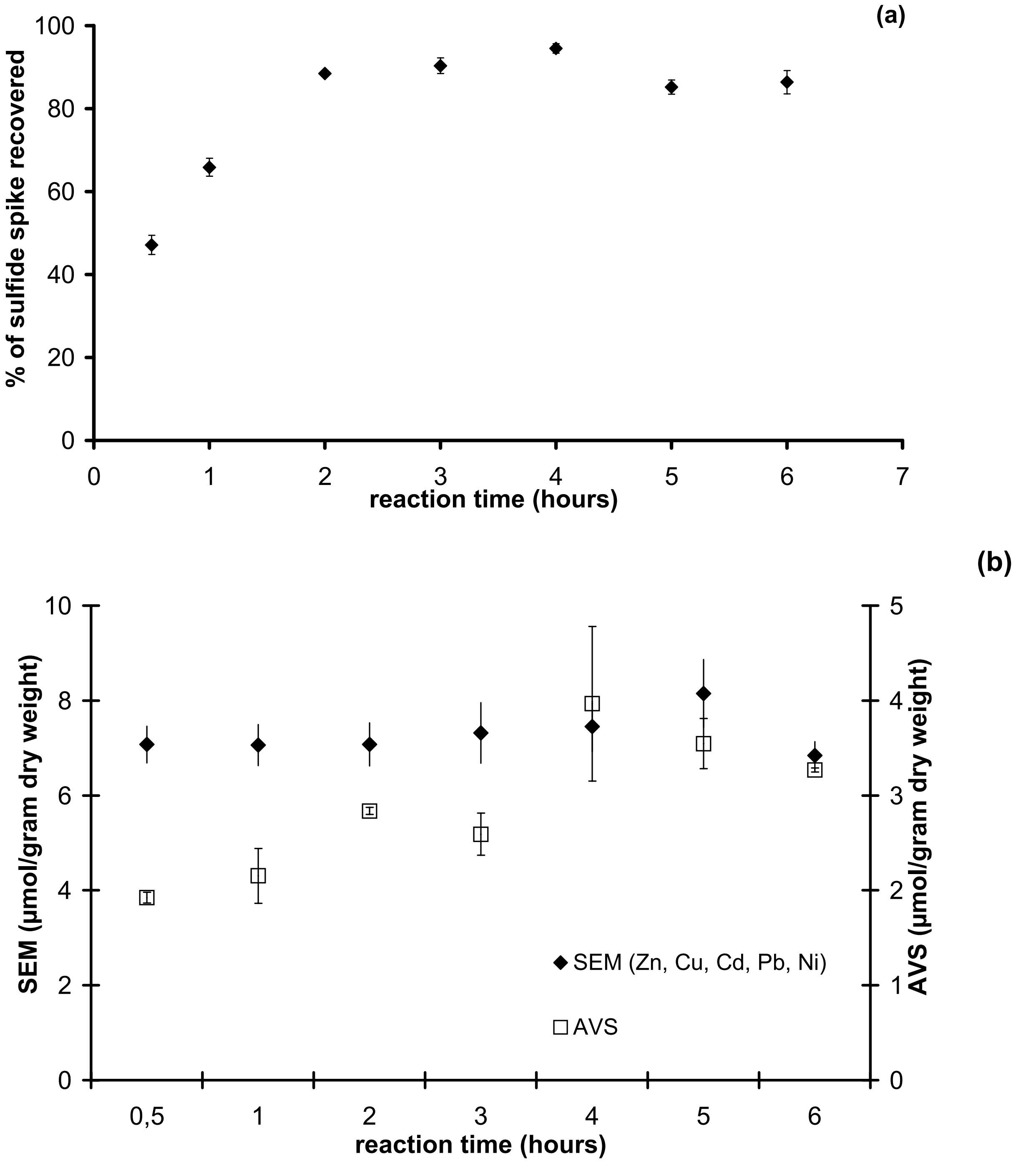

Figure 2.2. (a) Effect of reaction time (h) on sulfide spike recovery (n=3). Error bars represent S.D. (b) Effect of reaction time (h) on AVS and SEM (µmol/g DW) in floodplain lake sediment (n=3). Error bars represent S.D.

2.3.2 Effect of sample weight

The AVS measured as a function of sample weight is presented in Figure 2.3. The mass of sample used does not affect the measured concentration of AVS. So, the method is not sensitive to different masses within the range studied. However, at very large or very small sample sizes, a deviation of linearity is observed (not shown). At very small sample masses, the absolute small loss of sulfides that may occur during sample handling makes up a relatively large part of the sulfide present in that sample. This may lead to an underestimation of the AVS content. At very large samples, also a decrease in recovery is observed, although the 50 ml of HCl is in excess. Possibly, sample mixing in the reaction vessel becomes a problem. To avoid any over- or underestimation, we chose to use 5–10 g of wet sediment, which agrees to the weight used by Leonard *et al.* (1996).

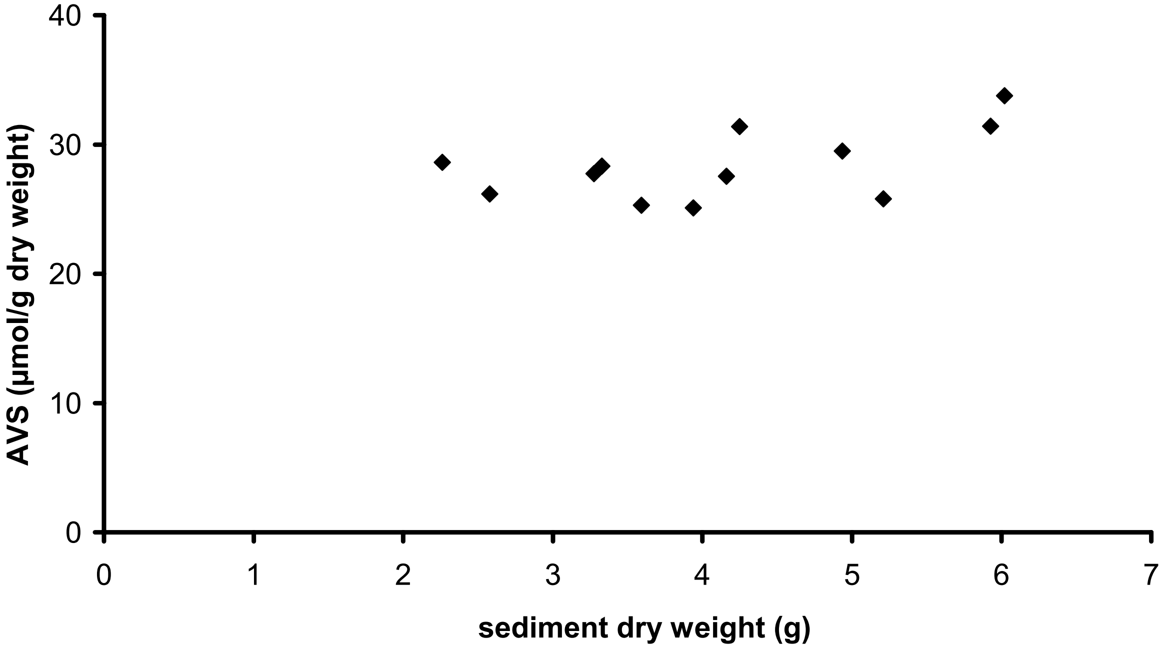

Figure 2.3. Effect of sample weight (g) on AVS concentration (µmol/g dry weight) in Kromme Rijn sediment. Error bars represent SD.

2.3.3 Effect of aeration

During passive aeration, the decrease in the measured AVS concentrations in the test sediments is very slow and becomes significant after half an hour of aeration (Figure 2.4).

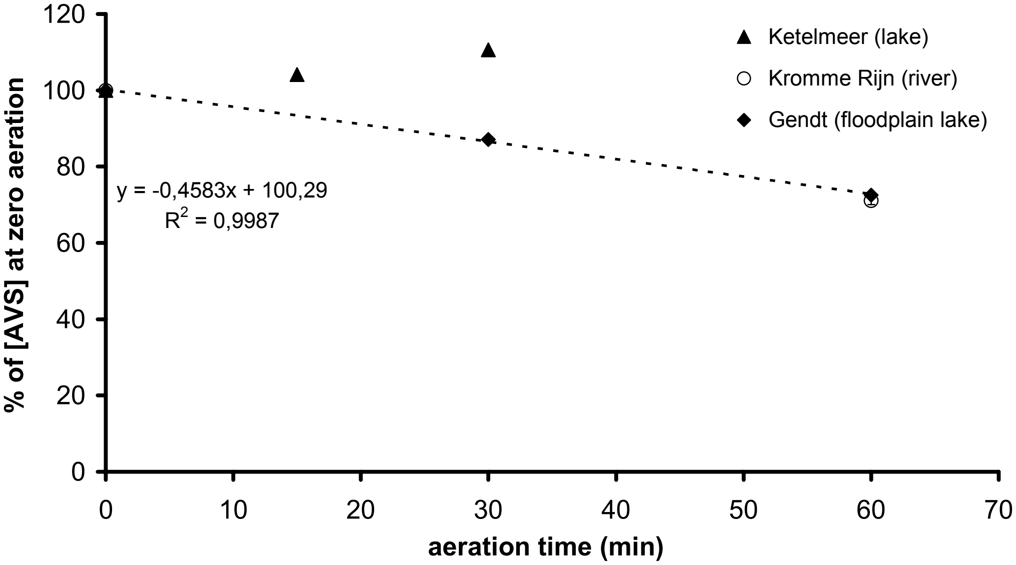

Figure 2.4. Effect of passive aeration on AVS concentration (µmol/g) in tested sediments (n=3). Error bars represent SD. Regression line is for Gendt and Kromme Rijn samples together.

The Kromme Rijn and Gendt sediments have an identical linear relative decrease of AVS upon aeration, which follows zero order kinetics with *k* = 27.6 % h^{-1}. The Ketelmeer sediment seems to behave differently, showing a relative increase. However, based on the absolute content, which was rather low (12–14 µmol/g), this increase is very small and falls within the analytical error. In summary, these results indicate that <1 min of air contact during the normal sample handling is not critical and, in accordance with findings of Leonard *et al.* (1996), that working under nitrogen atmosphere is not necessary.

2.3.4 Comparison with purge-and-trap method

Method comparison was established by measuring AVS and SEM in different types of freshwater sediments with the two methods. Data for the purge-and-trap method (1) were derived from an earlier study (Van den Hoop *et al.*, 1997). Data for the diffusion method (2) were measured during the current study in the same samples. Differences in SEM concentrations are small and indicate the comparability of the methods with regard to extraction strength and metal analysis. The diffusion method yields higher AVS concentrations than the purge-and-trap method. This higher yield was also found by Brouwer and Murphy (1994) (up to 75%) and Leonard *et al.* (1996) (up to 20%). They attributed this to the higher efficiency of the diffusion method compared to the purge-and-trap method. An additional explanation in this study may be that the prolonged anoxic storage of the samples caused an increase of AVS. See Table 2.1.

Table 2.1. Comparison of AVS and SEM concentrations obtained by the purge-and-trap method (1) and the developed diffusion method (2)

SAMPLE	METHOD [(a)]			
	PURGE-AND-TRAP (1)		**DIFFUSION (2)**	
	AVS	***∑SEM***	***AVS***	***∑SEM***
Ketelmeer (n=5)	10.0	10.5	26.8 ± 5.1(b)	11.3 ± 1.3
Nieuwersluis (n=6)	19.8	2.9	27.8 ± 8.1	3.5 ± 0.9
Kromme Rijn (n=6)	21.7	9.6	26.9 ± 1.6(b)	9.9 ± 0.7

a) The reaction time used in method (2) is between 1 and 2 h for the Nieuwersluis sample and 4 h for Ketelmeer and Kromme Rijn samples. The number of replicate measurements refers to method (2).

b) Value differs significantly ($\alpha < 0.05$) from value obtained with purge-and-trap.

2.4 Conclusions

The diffusion method in its current modification is appropriate for the determination of AVS and SEM in sediment samples with low (± 0.5–40 µmol/g dry weight) AVS concentrations. The method is accurate and allows for the measurement of up to 30 samples a day. No significant effect of sample weight is observed within a range from 5–10 g of wet sediment. Depending on the sediment, prolonged sample aeration showed negligible or a slow linear decrease in AVS concentrations. Results obtained with this method are well comparable to SEM concentrations obtained with the classical purge-and-trap method. AVS concentrations were higher using the diffusion method, probably partly because of the prolonged storage of reference samples. Hence, decisive conclusions on method comparability could not yet be drawn.

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CHAPTER 3

TEMPORAL DYNAMICS OF AVS AND SEM IN SEDIMENT OF SHALLOW FRESHWATER FLOODPLAIN LAKES

Based on: C. van Griethuysen, H.J. de Lange, M. van den Heuij, S.C. de Bies, F. Gillissen, A.A. Koelmans (2006)

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Abstract

Acid volatile sulfide (AVS) is an operationally defined sulfide fraction, which is considered important for trace metal fate in reduced sediments. Understanding AVS formation rates is important for the management of metal polluted sediment. However, little is known about the fate and dynamics of AVS in spatially and seasonally variable freshwater environments. We monitored *in situ* AVS formation and degradation and simultaneously extracted metals (SEM) in two floodplain lakes and compared this to AVS formation rates in laboratory experiments with the same sediment. In the laboratory experiments, the formation rates of AVS were studied at 20 °C for initially oxidized sediments that were (a) untreated, (b) enriched with extra sulfate, and (c) treated with sodium-azide (biocide). In the field, AVS concentrations were highly variable and were significantly correlated to surface water temperature and oxygen concentrations as well as to sediment composition. Between February and August, AVS formation was approximately linear at a rate of 0.07 $\mu mol*g^{-1}*d^{-1}$. Degradation rates differed drastically between the lakes due to different degradation mechanisms. In one lake AVS removal was caused by reworking and oxygenation of the sediments by bream (*Abrami brama)*, at a rate of 0.25 $\mu mol*g^{-1}*d^{-1}$. In the other lake AVS removal was caused by dessication, at a rate of ± 2.6 $\mu mol*g^{-1}*d^{-1}$. This illustrates the large differences that can be found between similar lakes, and the importance of biological processes. In the laboratory, concentrations of AVS with and without sulfate addition were similar during the first weeks, and increased at a rate of 0.15 $\mu mol*g^{-1}*d^{-1}$. However, sulfate addition increased the AVS concentration at the end of the experiment, whereas sodium-azide eliminated AVS formation, as expected. This suggests that AVS formation was sulfate-limited in the laboratory as well as in these shallow freshwater lakes.

3.1 Introduction

In the past decade, the role of precipitation of trace metals with sulfides in reduced sedimentary environments has received considerable attention. Sulfides are formed by microbial reduction of sulfate when degradation of organic matter takes place under reduced conditions, after oxygen, nitrate and manganese- and ferrous oxides are depleted (e.g., Stumm & Morgan, 1981). A clear prerequisite for the formation of sulfides is the supply of sulfate, which may be a limiting factor in freshwater environments as opposed to marine environments. Acid volatile sulfide (AVS) is an operationally defined reactive sulfide fraction that mainly comprises dissolved (hydrogen) sulfides and mackinawite (FeS), which may differ largely in its composition and capacity for precipitating trace metals in different sediments (Morse & Rickard, 2004). During the extraction of AVS, metals are simultaneously extracted from the sediment. These are referred to as SEM, the molar sum of all trace metals simultaneously extracted. When AVS is present in excess over

SEM, theoretically it will bind all reactive trace metals. Hence, the SEM-AVS difference is proposed to be an important indicator of bioavailability and as a consequence, the ecological risk of trace metals (DiToro *et al.*, 1992). Based on SEM-AVS differences, reliable predictions of the absence of toxic effects can be made (e.g., DiToro *et al.*, 1992; 2005; Casas & Crecelius; 1994; McGrath *et al.*, 2002). This concept has been widely tested and validated under laboratory conditions and was recently refined by taking the role of organic carbon into account (McGrath *et al.*, 2002; DiToro *et al.*, 2005). Field studies on the validity of relations between SEM-AVS or AVS/SEM and ecological endpoints are promising for use in ecological risk assessment. For instance, Hare *et al.* (1994, 2001) found a good relationship of SEM/AVS and pore water trace metal concentrations, whereas the relationship between SEM/AVS and species composition and metal accumulation was much weaker. Van Griethuysen *et al.* (2004) found clear indications that SEM-AVS and SEM concentrations correlated with benthic community composition and trace metal accumulation in Oligochaeta in a range of moderately polluted floodplain lakes. De Lange *et al.* (2004) also found significant correlations of benthic community structure with (SEM-AVS)/f_{OC}) in moderately to heavily polluted freshwater sediments in the Biesbosch Delta (The Netherlands). Increasing mortality and decreasing growth of benthic invertebrates with increasing (SEM-AVS)/f_{OC} was observed in bioassays conducted with the same sediment (De Lange *et al.*, 2005).

Conditions leading to the formation and persistence of AVS in aquatic sediments are likely to be complex when chemical properties of sediment and pore water vary seasonally and spatially, as is the case in shallow freshwater floodplain lakes. Earlier studies mainly focused on spatial variation of AVS (Howards & Evans, 1993; Otero & Macias, 2002; Van Griethuysen *et al.*, 2003/Chapter 5). In these studies the supposed relation of AVS concentrations to water depth, sediment composition, redox potential and surface water oxygen concentrations was confirmed. In some studies, attention was paid to temporal variation but investigations were often restricted to e.g. bi–annual sampling (Van den Hoop *et al.*, 1997; Van den Berg *et al.*, 1998; Otero & Macias, 2002). The expected seasonal pattern in Western Europe and similar climatic regions is one in which highest AVS concentrations occur in summer due to the enhanced anoxia in surface water and sediments, combined with higher microbial activity at higher temperatures (Van den Hoop *et al.*, 2000). However, there still is a lack of knowledge on rates of AVS formation and persistence in freshwater environments in relation to seasonal cycles. This knowledge is needed to evaluate the applicability of this concept for assessing ecological risks of trace metals in floodplains. After all, non-toxic sites in summer may become toxic to benthic invertebrates during winter when AVS levels are decreasing. Relevant processes affecting AVS concentrations in this type of shallow aquatic environments are sediment oxidation by inundation, sediment resuspension or desiccation*,* and reduction of the sediment, leading to formation of sulfides.

In rain and melt water dominated river systems, like the Rhine system in The Netherlands, water levels are usually elevated in winter and low in summer. However, large deviations from this pattern may occur due to varying climatic conditions, influencing the fate of AVS in floodplains and therewith the availability of trace metals.

The objectives of this study were to evaluate AVS dynamics and time scales for AVS formation in natural floodplain sediment. To this end, we performed a field study as well as a laboratory experiment. For the field study, two adjacent floodplain lakes were selected and monitored in different seasons and under varying hydrological conditions. The laboratory study was performed to evaluate the rate of AVS formation under controlled conditions in floodplain sediment taken from the same floodplain area. The experiments included testing whether the natural sulfate source is rate limiting for AVS formation process.

3.2 Material and Methods

3.2.1 Field sampling

The two studied floodplain lakes (Deest 3 and Deest 4) are located in the Afferdensche and Deestsche Waarden along the river Waal, The Netherlands (Figure 3.1). These lakes are very shallow (typically <1.0 - 2.5 meters) and do not exhibit seasonal turnover or stratification.

The monitoring period included various seasons and years including inundations and a period of desiccation in order to achieve a data set covering periods of AVS formation and degradation. In Deest 4, triplicate bi-weekly to bi-monthly sampling was performed at the same location within 2 m2 over eight months. In Deest 3, monthly consecutive samples were collected for a period of 14 months from a transect running parallel to the shore so that water depth and sediment composition differences were negligible. Sediments were sampled by means of a Jenkins core sampler for measurements of AVS and SEM concentrations and redox potential. Intact sediment cores with overlying water were transported to the laboratory to preserve anoxic consitions, and sediments were sectioned and analysed within a day. The top 2 cm (in lake Deest 3) or the top 5 cm (in lake Deest 4) of the sediment were analysed. In a few cases, the 0-5 cm layer was further split up into 1-2 and 2-5 cm sections in the laboratory. Water depth was determined and water temperature, pH, electric conductivity, and oxygen concentration and saturation were measured in the field near the sediment surface with specific electrodes (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany, and OxyGuard International A/S, Birkerød, Denmark).

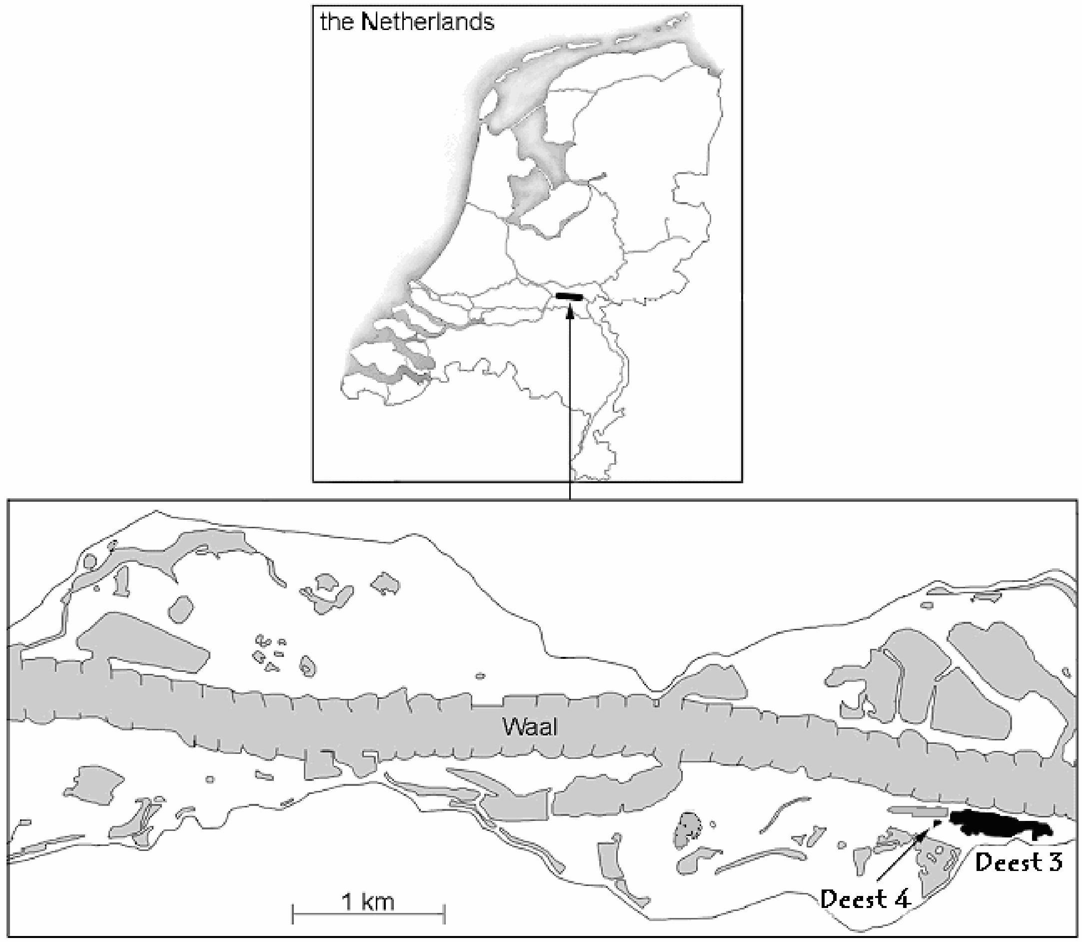

Figure 3.1. Location of the studied floodplain lakes in The Netherlands (After De Haas & Reuvers, 2001).

3.2.2 Analyses

After transport of the cores to the laboratory, redox potential of the sediment was measured with a redox combination electrode (SenTix ORP, WTW, Weilheim, Germany) that was inserted at 2-3 cm depth in the sediment. After that, overlying water was carefully removed. AVS concentrations were measured according to Van Griethuysen *et al.* (2002). Approximately 5 g of wet sediment was added to a reaction flask employed with a separate vial containing 10 ml sulfide anti-oxidant buffer (SAOB) solution. Then 50 ml of 1 M HCl was added to the sediment and the flask was tightly capped. Sulfide liberated from the sediment as H_2S was trapped in the SAOB solution and measured with a sulfide specific electrode (ISE Ag^+ 500, WTW, Weilheim, Germany). SEM concentrations were measured in 0.45 μm filtered acid sediment residues using ICP-MS (Elan 6000). Quality assurance of the AVS and SEM measurements was undertaken by analytical replication and checking of calibration lines by means of a flowchart. Sediments were further analyzed for clay content by laser diffraction on a Coulter LS 230 (Beckman-Coulter, Coulter Scientific Instruments, Miami, FL, USA) and organic matter content as loss on ignition after heating for 3.5 h at 550 °C.

3.2.3 Laboratory experiments

The goal of the experiments was to assess AVS formation rates for initially oxidized sediments (AVS removed) in a progressively reducing environment. From numerous other studies (e.g., Van den Berg *et al.*, 1998; Otero & Macias, 2002; Howard & Evans, 1993), a positive correlation of AVS concentrations with temperature was confirmed. The experiments therefore was carried out in a climate room at 20±1 °C to mimic summer temperatures when AVS is formed *in situ* in the lakes.

Sediment was collected in lake Deest 4 in January 2000 with perspex tubes that were employed either manually or with a stainless steel Jenkins sampler. Surface water used in the experiments was collected from the floodplain lake. About 5 to 6 kg of wet sediment was mixed thoroughly, spread out, left open to the air for two weeks, and mixed manually on a daily basis to ensure complete sediment oxidation. The sediment was not allowed to dry completely to prevent irreversible physical changes (Koelmans & Prevo, 2003). The degree of oxidation of the bulk sample was recorded with E_h measurements with a redox combination electrode (SenTix ORP, WTW, Weilheim, Germany). After this oxidation period, the homogeneous sediment was divided into three identical portions, which were treated differently.

The first sediment portion was not pretreated. In the second treatment, extra sulfate was added to the sediment to assess whether low sulfate concentrations were limiting AVS formation. The sulfate concentration in surface water of floodplain lake Deest 4 ranged from 4-100 mg/l during the period March 1998 – December 2000, and showed a clear seasonal pattern with depletion of sulfate in the summer and autumn (Roozen, 2005). The lowest measured field concentration of 4 mg/l corresponded to around 80 μmol S per gram DW that should be available for transformation to AVS in the experimental systems. Previous sediment analysis showed that average AVS concentrations were 15 µmol/g DW with maximum values up to 40 µmol/g DW in lake Deest 4 (Van Griethuysen *et al.* 2003, 2004/Chapter 5 and 7). To ensure that sulfate limitation could not occur, approximately 5 g Na_2SO_4 /kg wet sediment, corresponding to approximately 70 µmol/g S at a sediment dry weight fraction of 50 %, was added to this second sediment portion. If this added sulfate was completely reduced, this would result in almost threefold higher AVS concentrations than maximum concentrations measured at the field location. A third sediment portion was spiked with the biocide sodium-azide (3.5 g NaN_3/l = 4.375 g/kg wet sediment) to inhibit microbial activity, which served as a 'blank' experimental treatment.

After applying these pretreatments (natural sediment, sulfate addition, biocide addition), laboratory scale sediment-water systems were constructed by dividing each sediment portion into 18 samples of approximately 100 grams. These were transferred to a series of 250 ml PE bottles resulting in a sediment layer of approximately 5 cm. The bottles were completely filled with approximately 170 ml of floodplain lake surface water, flushed for 2 to 3 minutes with N_2 and sealed to simulate anoxic, stagnant water conditions in an inundated floodplain. In total, 54 (3 treatments x 6 time steps x 3 replicates) sediment-water systems were prepared. The bottles were kept in a glove bag filled with nitrogen gas to assure that no oxygen could penetrate the sediments. For each treatment, three bottles were removed from the glove bag after 1, 3, 7, 13, 21 and 37 days, and used for oxygen, redox, and AVS measurements. Oxygen concentration in the overlying water was measured with an oxygen electrode (OxyGuard International A/S). Redox potential of the sediment was measured with the redox combination electrode that was inserted to 2-3 cm depth in the sediment. After that, overlying water was carefully removed. Three sediment subsamples were taken from each carefully mixed bottle as analytical replicates and analyzed directly for AVS concentrations according to Van Griethuysen *et al.* (2002) as mentioned in the previous section. Relative standard deviations for replicates analyses typically were 10-15 % for both AVS and SEM.

3.3 Results and discussion

3.3.1 Field study

An overview of field variables for the whole data set is given in Table 1. AVS concentrations, oxygen concentration and saturation level, water level, water turbidity and sediment redox potential all showed a large range, with coefficients of variation (COV) larger than 60 % and up to 145 %. All other variables show a COV of around 10-20 % indicating that there was little temporal variation, nor spatial variation among the samples.

Table 1 Overview of field data

Variable	Unit	*N*	Median	Range	Standard deviation of mean	COV % [a)]
AVS [b)]	µmol/g DW	30	6.66	0.44 - 40.35	12.84	113
SEM [c)]	µmol/g DW	28	5.61	4.31 - 7.62	0.86	15
LOI [d)]	%	30	9.16	7.02 - 10.57	0.91	10
Clay	%	23	13.20	10.20 - 23.57	3.88	26
DW [e)]	-	30	0.31	0.22 - 0.48	0.06	20
depth	cm	30	65.00	0.00 - 400.00	112.25	101
pH	-	28	7.63	6.00 - 9.43	0.75	10
EC [f)]	µS/cm	28	547.50	403.00 - 659.00	68.99	13
O_2	mg/l	26	7.50	0.30 - 14.98	4.72	69
O_2sat [g)]	%	26	75.75	0.50 - 112.40	38.44	63
T	°C	28	11.45	2.10 - 30.30	7.55	58
E_h [h)]	mV	9	-151.00	-195.13 - +142.30	133.68	145
Turbidity	NTU [i)]	15	25.00	3.70 - 273.00	84.48	125

a) COV = Coefficient of Variation
b) AVS = Acid Volatile Sulfide
c) SEM = Simultaneously Extracted Metals
d) LOI = Loss On Ignition
e) DW = fraction dry weight
f) EC = Electric Conductivity
g) O_2sat = Oxygen saturation
h) E_h = redox potential
i) NTU = nephalometric turbidity unit

Deest 3

A clear seasonal variation in AVS and SEM levels was recorded in lake Deest 3 (Figure 3.2A). AVS formation in the field increases linearly in time between February and August (slope = 0.07 ± 0.01 µmol*g-1*d-1, R2 = 0.90, p=0.01). AVS degradation started abruptly in August and proceeded till mid September, at a rate of 0.25 ± 0.004 µmol*g-1*d-1 (R2 = 1.00, p<0.01). Subsequently, AVS concentrations remained low at an average level of ± 1.5 µmol/g DW, similar to concentrations in the beginning of 2003. The exact moment at which AVS concentrations reach a maximum thus appears to depend on seasonal features such as temperature (onset of favorable conditions for bacterial community, primary production), but also on oxygen regime and water depth. In floodplain lakes these may vary considerably within a year, but also from year to year. The observed increase in AVS concentrations may be explained by the temperature increase of the surface water, stimulating primary production and subsequent organic matter decomposition (leading to increased bacterial activity and oxygen consumption and thus reduced conditions). The sharp drop in AVS concentrations in August-September is most likely caused by a sudden oxygenation of the sediment. Organic matter decomposition, however, still takes place in these months due to favorable weather conditions and the abundance of decaying plant material. Furthermore, the sediment had not desiccated. Hence, another explanation is needed. The sharp decrease may be explained from increased sediment resuspension and subsequent sulfide oxidation, which resulted from a drop in water level. This drop in water level (from 1 m depth to less than 0.5 m) may have caused increased wind-induced resuspension. This is however not very likely, as the lakes are too small to be exposed to much wind fetch induced resuspension. More importantly, the drop in water level decreased the habitat of bream (Abrami brama), an abundant fish in rivers and floodplains in this area (Grift, 2001). Bream is well known for its resuspension of bottom material when foraging (e.g., Scheffer *et al.*, 2003). In Deest 3, the total sediment reworking by bream can be as much as 220 g*m-2*d-1, based on a daily resuspension capacity of 5 times the bodyweight of the fish (Breukelaar *et al.*, 1994) at maximum measured biomass values of 440 kg/ha (Grift, 2001). An increased resuspension is also consistent with a simultaneously observed large increase in turbidity of the surface water. Eventually these fish die (because of anoxia of the surface water and the decreased habitat) and as a result turbidity decreases again. AVS concentrations in October-March remained low because low temperature and limited organic matter degradation inhibit the formation of AVS. Concentrations of SEM (Figure 2A) are rather constant over time and exceed AVS values from October through April. This means that during this period sediments may become toxic and organisms could be exposed to trace metals via the pore water. The large seasonal variation of SEM-AVS emphasizes that limited SEM-AVS measurements in a system may easily lead to erroneous estimates of environmental risk from pore water toxicity. For instance, within two months (August – October) a safe SEM-AVS of –10 µmol/g DW changes into a potentially toxic value of +5.5 µmol/g DW. Seasonal variations also determine the ecological endpoints for which metal risks should be evaluated. In this lake, metals primarily may affect aquatic worms (Oligochaeta) and many larval invertebrate species that are present during winter as third or fourth instar stages (De Lange *et al.*, 2005). In this period, growth and reproduction are negligible due to low temperatures.

Deest 4

Variations of AVS and SEM levels in Deest 4 samples for various periods (November 1998, March 1999, September 2000, and September 2003 through April 2004) are shown in Figure 3.2B. AVS concentrations were in general higher than those observed in Deest 3. This is partly related to differences in the sampling methods.:For Deest 3, only the top 2 cm was sampled, while for Deest 4 the top 5 cm were collected. AVS concentrations generally increased downward in the first 10-20 cm of the sediment profile due to more reduced conditions (e.g., Van den Berg *et al.*, 1998). AVS concentrations were approximately 40 µmol/g DW in September 2003 and showed a dramatic drop in autumn 2003 as a result of oxidation, and hardly any recovery during the following winter and spring (Figure 3.2). The separate samples from September 2000 and November 1998-March 1999 confirm the general pattern of higher AVS levels in autumn as compared to winter and spring samples. Furthermore, September samples illustrate the spatial variability of AVS within one floodplain lake at one point in time.

The rate of AVS degradation in Deest 4 for the samples from autumn 2003 (late October) was ± 2.6 µmol*g-1*d-1, and there was negligible reformation of AVS during the period November – April. This degradation rate is ten times that calculated for Deest 3 (Figure 3.2A). We believe the rapid degradation resulted from the drop in water level in autumn 2003 due to a lack of rainfall, leading to desiccation of the sediment in mid October. AVS levels are high in autumn because of warm conditions and oxygen-depleted surface water, but as soon as the sample location becomes dry, AVS concentrations drop dramatically. After inundation of the floodplain in January 2004, AVS recovery was negligible because conditions for formation were unfavorable. Concentrations of SEM are relatively constant and generally lower than AVS levels in early autumn samples (Table 3.1). However, after complete desiccation of the lake (mid October 2003) and in the top layer samples (November 1998 and March 1999, 1-2 cm), trace metals may have potentially been released to groundwater through rainfall and infiltration and, after inundation of the lake, to surface and pore waters.

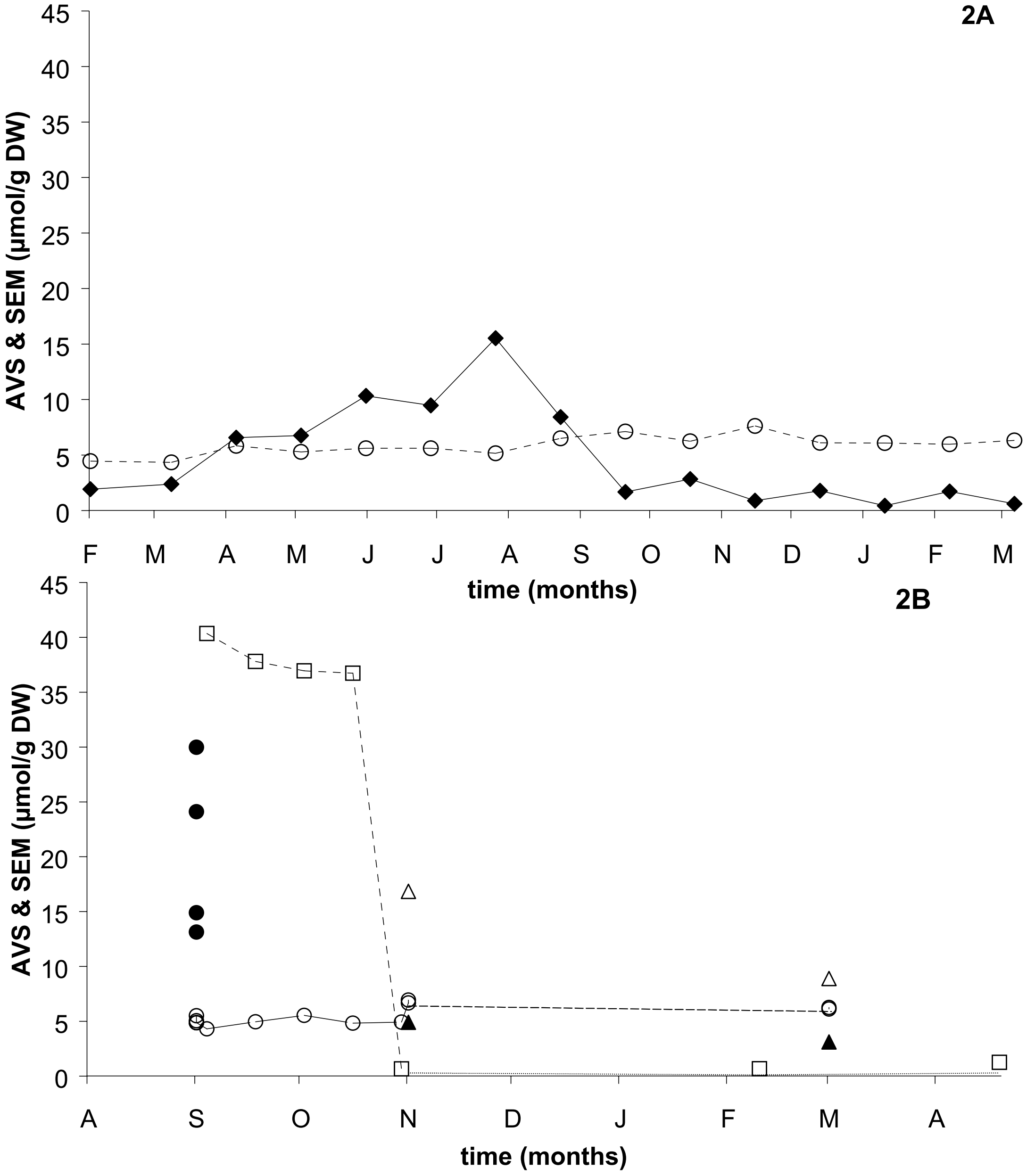

Figure 3.2. Temporal variation in AVS and SEM concentrations in floodplain lake sediment. Symbols indicate different sampling sessions. Mind the difference in the scale of the horizontal axis. Figure 2A: Deest 3 2003-2004 0-2 cm: ♦ AVS; ○ SEM; Figure 2B: Deest 4: □ AVS Fall 2003–Spring 2004 0-5 cm; ● AVS September 2000 0-5 cm, 4 locations within the lake; ▲ AVS November 1998 and March 1999 1-2 cm; Δ AVS November 1998 and March 1999 2-5 cm; ○ SEM.

Statistical analysis of field data

The above field observations indicate that AVS values are highly variable due to local differences in environmental conditions. In order to detect general relationships between AVS and environmental parameters, a Pearson correlation analysis was performed on all data (Table 2).

Table 2 Correlation of AVS with surface water, sediment and climatic variables. Bold: significant at p< 0.01, bold and underlined: significant at p < 0.005.

Variable	LOI	clay	DW	Depth	pH	EC	O_2	O_{2sat}	T	E_h	turbidity	SEM
AVS [a]	.02	**.69** [b]	**.45**	-.31	**-.74**	-.17	**-.77**	**-.79**	**.64**	**-.59**	**-.48**	**-.44** [c]
LOI [d]		.30	-.09	-.25	.07	-.18	-.09	-.15	-.12	-.31	.11	-.01
Clay			.39	.09	**-.50**	**-.61**	**-.72**	**-.80**	.26	-1.0	.00	-.09
DW [e]				-.33	**-.67**	-.03	**-.53**	**-.60**	.14	.49	-.09	**-.58**
Depth					.62	-.01	**.64**	**.62**	**-.41**	.13	**-.70**	.23
pH						.08	**.83**	**.83**	**-.47**	.35	-.24	.38
EC [f]							.21	.30	-.02	.60	-.07	.38
O_2								**.96**	**-.72**	.47	.10	.35
O_2sat [g]									**-.56**	.42	-.19	.38
T										-.50	-.44	-.30
E_h [h]											Nd [i]	-.05
Turbidity												**.53**

a) AVS = Acid Volatile Sulfide (µmol/g DW)
b) Bold and underlined: significant at $p \leq 0.05$
c) Bold: significant at $p \leq 0.10$
d) LOI = Loss On Ignition (%)
e) DW = fraction dry weight (-)
f) EC = Electric Conductivity (µS/cm)
g) O_2sat = Oxygen saturation (%)
h) E_h = redox potential
i) Nd = not determined (no observations)

Note that the good correlation (*r*=0.45, *p*=0.01) of AVS and fraction dry weight (DW) only represents vertical variation in the sampled sediment cores. Surface samples have a lower bulk density (i.e., a higher water content) and lower AVS contents because top layers are more oxic than deeper layers (e.g., Van den Berg *et al.*, 1998; Van Griethuysen *et al.*, 2005/Chapter 4). The negative relation (*r*=-0.48, *p*=0.07) between AVS concentrations and turbidity over the monitoring period (only Deest 3 samples) is consistent with our hypothesis of wind- and bream induced resuspension, explaining the AVS drop in August 2003. The significant negative relation of AVS and SEM (r=0.44, p=0.02) may originate from low AVS concentrations in autumn and winter, when SEM levels are slightly elevated. Further, SEM is higher in top layers than in deeper layers, whereas AVS levels show an opposite trend (compare Deest 4 November/March 1-2 cm samples with Autumn 2003 0-5 cm measurements). This is supported by the negative relation of DW and SEM (r=0.53, p<0.005) showing that top layers with low DW have higher extractable metal contents. Highly significant negative correlations were found between AVS and oxygen saturation of the surface water (r=-0.79, p≤0.01) and sediment redox potential (r=-0.59, p=0.10), while the correlation with clay-sized particles (< 2 µm) was positive (r=0.69, p≤0.01). This is all consistent with AVS formation in reduced, muddy sediments. Furthermore, the small particles may besides of clay minerals also consist of fine-grained organic material. A positive correlation of AVS levels with fine organic matter particles is plausible because AVS is formed during degradation of organic matter. There was no significant correlation of AVS with water depth. This contradicts several other studies regarding spatial variation of AVS that reported good correlation between AVS and water depth for samples from different locations but measured simultaneously (e.g., Howards and Evans, 1993; Van Griethuysen *et al.*, 2003/Chapter 5). Variation in water level in floodplains is often related to seasonal changes, i.e. inundation during winter and drought during summer. However, small differences in lake morphology may modify this general pattern and cause hydrological conditions to differ between lakes (one lake did dry up during the study, the other did not). There is a significant correlation (r=0.62, p≤0.01) between oxygen saturation and water depth, which is explained by the coincidence of low summer water levels with low summer oxygen levels due to the high biological oxygen demand. In clayey sediments, reduced conditions are established sooner explaining the relation of oxygen saturation and clay content (r=-0.80, p≤0.01). Deest 4 has higher clay contents and lower surface water oxygen concentrations than Deest 3 samples. Furthermore, there is a strong relation between oxygen and pH, which is indicative for primary production processes (production of oxygen and consumption of H^+ ions). This causes an indirect correlation between AVS and pH (r=-0.74, p≤0.01).

In summary, it is clear that oxygen saturation, temperature and clay content in particular are important variables for the fate and dynamics of AVS. By means of multiple linear regression, this relation can be made more explicit. Multiple regression with stepwise variable selection was performed (p for enter 0.05, for removal 0.10) yielding:

AVS (µmol/g DW) = 3.15 – 0.92*O_2 (mg/l) + 0.85*clay (%) (R^2adj=0.70)

We emphasize that this equation is highly conditional. For the estimation of AVS concentrations in these floodplain lakes, however, knowledge of sediment composition and the oxygenation conditions of the surface water appear to provide reasonable estimates.

3.3.2 Laboratory experiments

Formation of AVS was measured in the laboratory as a function of time for 37 days, for (1) untreated, (2) sulfate-added, and (3) sterilized systems. Initial average oxygen concentrations of all treatments were 1.0-1.5 mg/l and decreased over time to about 0.5 mg/l in the untreated and sulfate-added samples. Oxygen concentrations in treatment 3 remained at the initial concentration of 1.0-1.5 mg/l as expected due to the absence of microbial respiration. Redox potential (E_h) was on average +50 mV in all systems before they were flooded and decreased rapidly after inundation to values of around –60 mV. During the course of the experiment, it decreased until a value of –200 mV after about 15 days and then remained constant for treatments 1 and 2. Redox potential in treatment 3 remained higher (at around –80 mV) than in the other two treatments, supporting the theory that in the absence of a microbial community, there is no organic matter degradation as a driving force to lower the redox potential. The slight decrease over time in E_h is probably due to chemical oxygen consumption. Considerable formation of AVS occurred in treatment 1 and 2 (Figure 3.3). Final AVS concentration in treatment 1 was somewhat lower than those observed in the field (Figure 3.2, Van Griethuysen *et al.*, 2003, 2004/Chapters 5 & 7), which may indicate that maximum concentrations have not been reached yet, or that sulfate was limiting. The sulfate-added treatment yielded final AVS levels twice as high as the normal sediment treatment indeed suggesting sulfate limitation. AVS concentrations were similar during the first 21 days. The rate of AVS formation only became significantly higher in the sulfate-added treatment compared to the untreated samples at the end of the experiment (day 37, t-test on triplicate systems, $\alpha \leq 0.05$). This delay may be explained by a slow development of the bacterial community. Vink (2001) reported that it took three months before AVS formation started after storing Meuse floodplain soil under water. In a study of Harmsen *et al.* (2001), a time period of 50 days storage under water was needed to immobilize trace metals with sulfides in polluted sediment. We conclude that probably sulfate limitation occurred in the final stage of the experiment for the untreated systems. As expected, no significant increase in AVS concentration was observed in treatment 3 (Figure 3.3), consistent with the observed redox potential and oxygen levels.

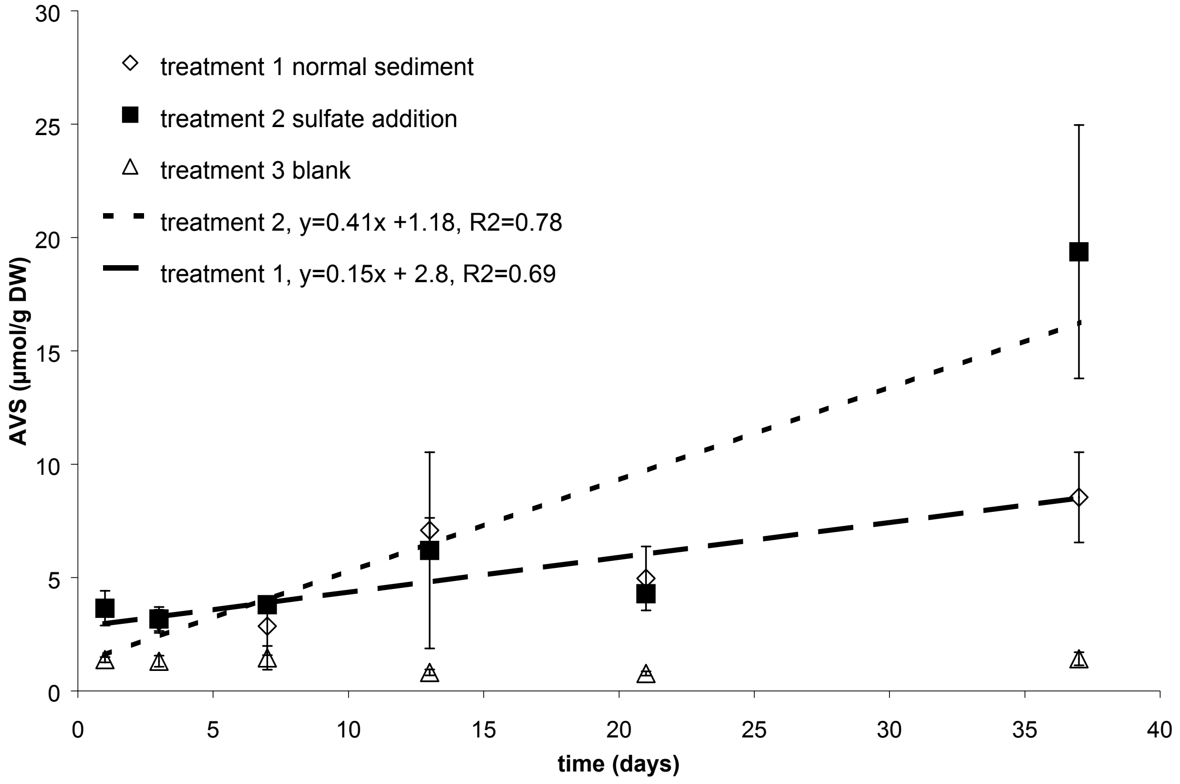

Figure 3.3. AVS formation in laboratory experiment. Symbols indicate different treatments: ◊ treatment 1: natural sediment; ■ treatment 2: sulfate addition; Δ treatment 3: azide addition (blank). The dotted lines represent overall linear regression curves for treatment 1 and 2. Error bars represent standard deviations (n=3).

Linear regression on the data from treatment 1 yields a slope of 0.157 ± 0.036 µmol*g^{-1}*d^{-1} (R^2=0.60, *p*=0.01). Treatment 1 thus has a slope twice as high than the one established for Deest 3 field data (0.07 µmol*g^{-1}*d^{-1}). This can be explained by less optimal AVS formation in the field as compared to the lab. For instance, in the latter case there is no oxygen supply from diffusion from the air and no bioturbation or resuspension by fish or invertebrates.

For treatment 2, AVS formation seemed to occur in two stages. The first stage was similar to treatment 1, as stated before. In the second stage, AVS formation seems to proceed much faster. If we fit a linear model to treatment 2 data for comparison, an overall slope of 0.407 ± 0.073

μmol*g^{-1}*d^{-1} is found ($R^2=0.66$, $p<0.005$). Eventually, sulfate addition thus seems to speed up the formation of AVS by more than a factor of two.

The current results are valid for wet sediments that have been partially oxidized by resuspension or desiccation, as does occur in the shallow freshwater floodplain lakes that we studied. Additional information on the development of a suitable bacterial community is needed to extrapolate these results to sulfide formation in dry soils that are subsequently stored under water.

3.4 Conclusions

Linear AVS formation and degradation rates observed in the field illustrate that AVS concentrations may vary considerably over time. Formation of AVS under West-European climate conditions is rather slow. In contrast, AVS degradation can occur much more abruptly and can lead to a sudden exceedance of proposed threshold values for metal toxicity. Oxygenation of dessiccated sediments results in a ten times faster degradation of AVS than partial oxygenation of wet sediments. Sediment type and surface water oxygen regime are the most important factors influencing AVS concentrations. The latter is linked to several processes including primary production (dependent on temperature) and resuspension. However, AVS concentrations cannot be predicted solely from seasonality in primary production, because other factors like water depth do not always have the same seasonal pattern.

Under optimized conditions in the laboratory, considerable AVS formation takes place within one month. Formation of AVS in untreated sediment in the laboratory occurred approximately twice as fast as in the field. Higher AVS concentrations were measured at the end of the experiment after sulfate addition, suggesting that sulfate was rate limiting in untreated sediments. For the ecological risk of trace metals in these environments, the results imply that, under favorable conditions, AVS levels can reach values higher than prevalent SEM concentrations within one to two months.

Acknowledgements

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CHAPTER 4

TEMPORAL VARIATION OF TRACE METAL GEOCHEMISTRY IN FLOODPLAIN LAKE SEDIMENT SUBJECT TO DYNAMIC HYDROLOGICAL CONDITIONS

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Abstract

Climate change and land use may significantly influence metal cycling in dynamic river systems. We studied temporal variation of sediment characteristics in a floodplain lake, including concentrations of dissolved organic carbon, acid volatile sulfide and trace metals. The sampling period included a severe winter inundation and a dramatic water level drop during summer. Temporal changes were interpreted using multivariate analysis and chemical equilibrium calculations. Metal concentrations in sediment increased with depth, indicating a gradual improvement of sediment quality. In contrast, dissolved metal concentrations were highest in top layers due to mobilization from oxyhydroxides and precipitation with sulfides in deeper layers. Inundation had a mobilizing effect as it stimulated resuspension and oxygenation of sediment top layers. Water table lowering combined with organic matter decomposition led to immobilization due to sulfide formation. The chemistry of the sediments was consistent with model calculations, especially for macro-elements. The results illustrate the importance of seasonality for metal risk assessment.

4.1 Introduction

Climate change and land use may have major impacts on the hydrology and biogeochemical cycles in European river systems. Because severe floods have become more frequent in The Netherlands over the past years (e.g. in 1993 and 1995), riverbeds are being enlarged to increase the discharge capacity. The possible consequences of climate change will have yet unpredictable effects on the quality of contaminated sediments and overlying waters of catchment areas and floodplains. Analyzing the complex interplay of geochemical and biological processes is crucial to predict sediment and soil quality changes under the anticipated climate change scenarios. Trace metals are distributed mainly over clay minerals, (dissolved) organic carbon, and sulfides, iron- and manganese (hydr)oxides, which are susceptible of redox changes. Redox processes driven by organic carbon degradation therefore play a key role in the mobility of sediment-bound contaminants (e.g. Paalman, 1997, Huerta-Diaz *et al.*, 1998, Van den Berg, 1998; Vink, 2002). Thus, spatial and temporal variability in these processes may have a large impact on the outcome of sediment quality monitoring programs. Several researchers studied temporal variation in trace metal concentrations and geochemical processes affecting trace metal distribution in various dynamic aquatic environments. For instance, Douglas and Adeney (2000) studied Fe/Mn hydroxide formation and related scavenging of trace metals in estuarine environments. In alternately reduced and oxidized environments like respectively dimictic lakes, estuaries and sedimentary basins, the temporal variation in (acid volatile) sulfides was studied by e.g. Howard and Evans (1993), Huerta-Diaz *et al.* (1998), Van den Berg *et al.* (1998), and Otero and Macias (2002). Furthermore, several studies addressed temporal variability of total trace metal concentrations in suspended solids and sediment from a perspective of historical deposition (e.g. Beurskens *et al.*, 1994 and Von Gunten *et al.*, 1997). Lau (2000) reported that total and soluble concentrations of nutrients and trace metals varied considerably with seasons in a coastal wetland. In fluvial systems, small-scale spatial and temporal variation may yield a variance in total trace metal concentrations of 25–30% (Birch *et al.*, 2001).

For ecological risk assessment purposes in floodplains along Dutch rivers, it is important to know the magnitude of temporal fluctuations of available trace metals. Most studies in related environments however only address vertical variation in sediment columns (e.g., Zwolsman *et al.*, 1993; Hindel *et al.*, 1996; Van den Berg *et al.*, 2001). Only few studies specifically address the effects of temporal variation in such systems. Apart from common seasonal changes in temperature and primary production, large fluctuations in water level are specific for floodplain systems. Successions of inundations and dry periods occur, which may lead to e.g. resuspension and oxidation of sediment top layers alternated by periods of reduction. In this study, we investigate the effect of temporal variations in redox processes on trace metal distribution in shallow floodplain lake sediments. Results are interpreted in terms of hydrological fluctuations like inundation in winter and water table lowering in summer. We hypothesize that inundation will lead to mobilization of metals due to for instance oxidation of sulfides and enhanced transport with DOC, whereas the combination of water level lowering and primary production will lead to immobilization due to sulfide precipitation. Furthermore, effects may be more pronounced in top layers due to more variation in diagenetic processes. Vertical major and trace element distribution in floodplain lake sediment were characterized in relation to redox transitions at five sampling moments differing in hydrological conditions, including a severe winter inundation. Data analysis was performed using multivariate statistics and equilibrium speciation modeling.

4.2 Material and methods

4.2.1 Study location

The study location is a small and shallow floodplain lake (0.2 ha) in the floodplain area Afferdensche and Deestsche Waarden in The Netherlands (Figure 4.1). The lake is a clay excavation pit, which was dug around 1930. The sediment is moderately polluted with trace metals Zn, Pb, Cu, and Cd, exceeding background concentrations (Crommentuijn *et al.*, 1997) by up to a factor of 3 for Zn (Koelmans and Moermond, 2000; Van Griethuysen *et al.*, 2003). The sediment can be characterized as clayey to silty and highly organic. For details on ecological features we refer to earlier studies in this floodplain lake (Koelmans and Moermond, 2000; Van Griethuysen *et al.*, 2004; Roozen, 2005).

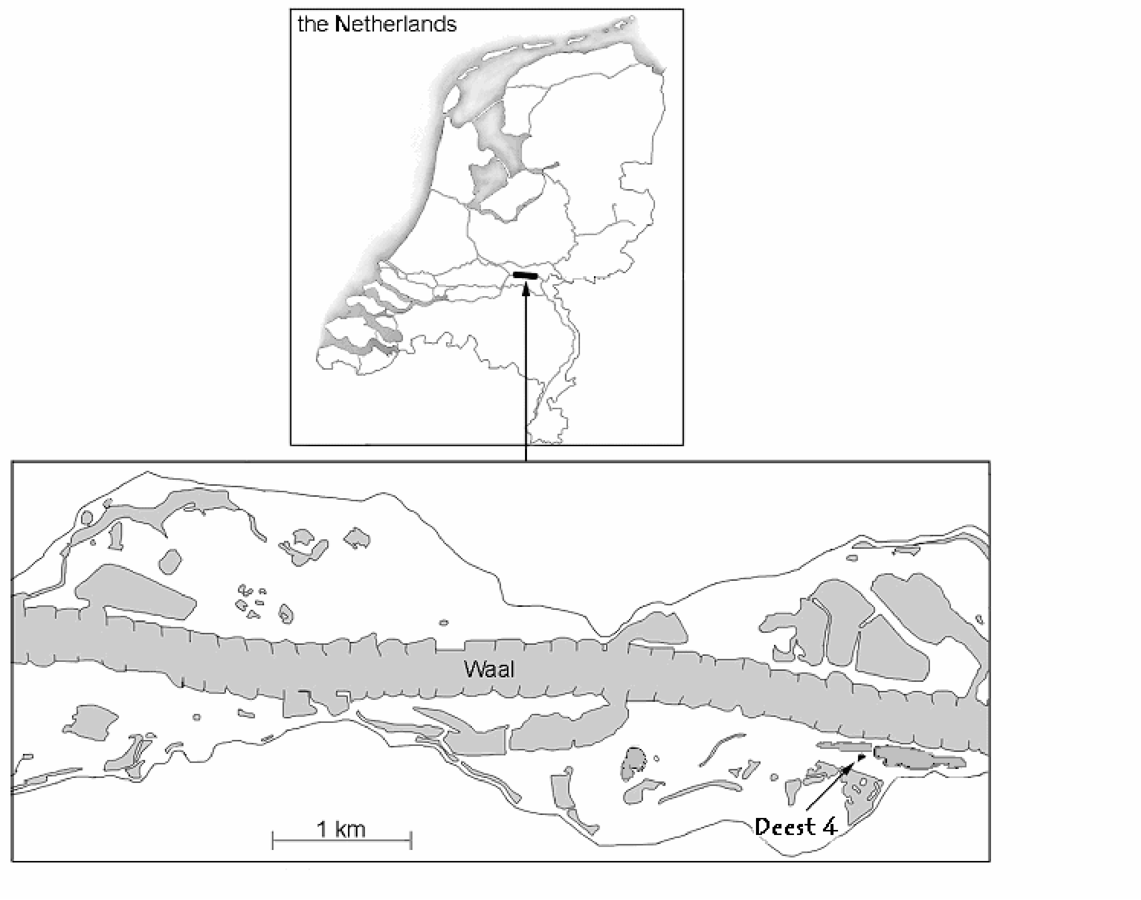

Figure 4.1. Location of the study area within The Netherlands (After De Haas & Reuvers, 2001). The arrow indicates the studied floodplain lake (5°40′ OL, 51°53′ NB). Samples were taken randomly within 10 m^2 in the upper left corner of the lake**.**

4.2.2 Sediment sampling

Sediment and surface waters were sampled in November 1998, March 1999, July 1999, September 1999, and January 2000 (see arrows in Figure 4.2). In this period, hydrological conditions within the river and the lake varied considerably (Figure 4.2).

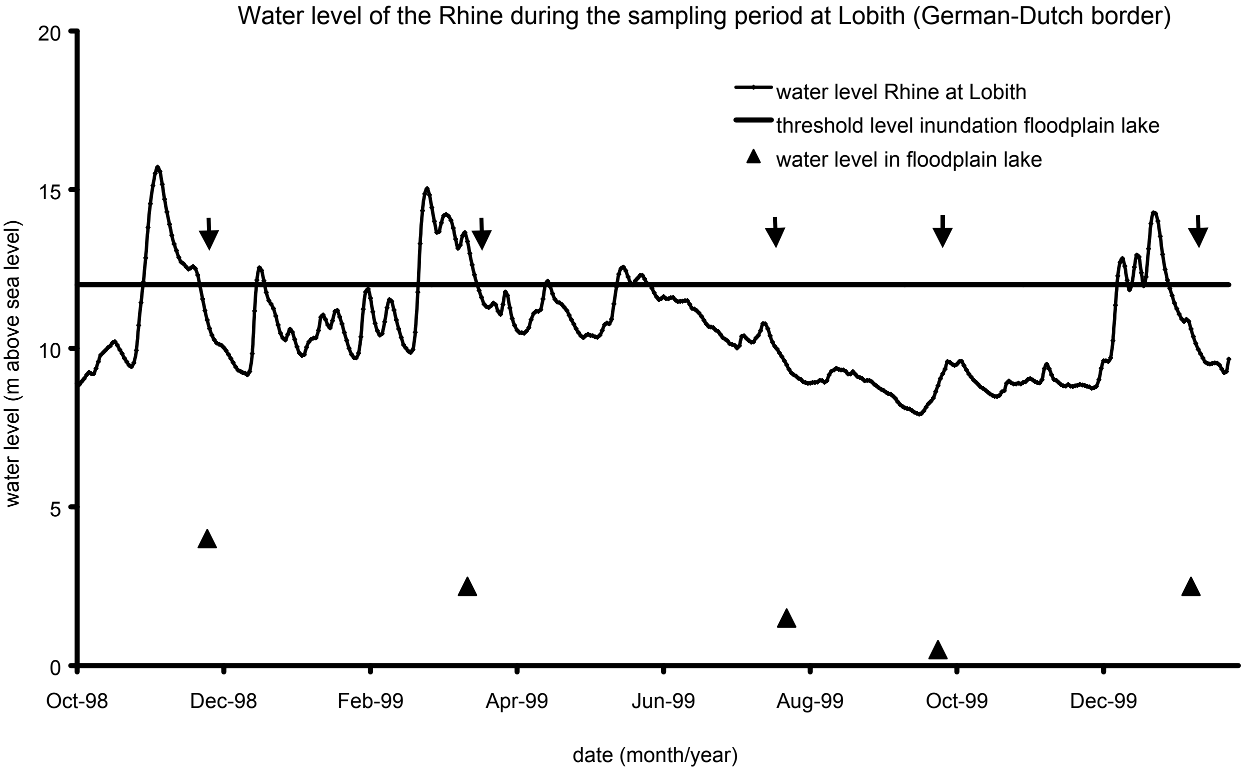

Figure 4.2. Hydrological regime of the river Rhine during the sampling period at Lobith (German–Dutch border). The floodplain lake is inundated at Rhine water levels above 12.00 m. Sampling moments are indicated with arrows.

At water levels exceeding 12 m above sea level at Lobith, inundation of the floodplain starts. Retreat of the water from the floodplain does not occur instantaneously after the water level in the river drops, but also depends on seepage and evaporation. Sampling moments represent autumn (large flood, water level in the lake >4 m), winter/spring (small flood, water level 2.5 m), spring/summer (warm, water level 1.5 m), summer/autumn (water level 0.5 m), and winter (small flood, water level 2.5 m) conditions. In each season, seven sediment cores were collected randomly within the same area of 10 m^2 using perspex cores as described in Van Griethuysen *et al.* (2003). Sampling depth was 15 cm. Three cores were used for the analysis of pore water composition and total concentration of trace metals and macro-elements. In November and March, the other three cores were used for the analysis of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) concentrations. In the water overlying the seventh core, surface water characteristics were measured in the field. Redox potential was measured in this core directly after returning to the laboratory (SenTix ORP electrode, WTW, Weilheim, Germany).

4.2.3 Analysis of sediment and pore water

Following sampling, sediment samples were extruded layer for layer from the cores and sliced into layers of 1–5 cm thickness (0–1, 1–2, 2–5, 5–10, 10–15 cm) with a stainless steel ruler and knife. These isolated layers from the first 3 cores were immediately transferred to acid rinsed high-density polyethylene (HDPE) centrifuge bottles (250 mL) that were immediately flushed with N_2 (Indugas, Vlaardingen, The Netherlands, >99,9% purity) and sealed. Bottles were centrifuged (Varifuge GL, Heraeus, Germany) for 20 min at 3000×*g* for the collection of pore water. Three subsamples were taken from the clear supernatant (≥10 mL) with an automatic pipette (Socorex, Lausanne, Switzerland) after discarding the first pipette volume. If non-settled particles were detected (July samples only), supernatants were filtered over a membrane filter (0.45 µm, Schleicher and Schuell,'s Hertogenbosch, The Netherlands). In that case the first pipette volume of the filtrate was discarded also. The first subsample with a volume of 1 mL was acidified by a 1:10 dilution with 0.1 M HNO_3 (p.a.) for subsequent analysis of trace metal and macro-element concentrations on inductively coupled plasma–mass spectrometry (ICP-MS, Elan 6000, Concord, Canada). Multiple procedural blanks consisting of nanopure water (Barnstead®, Dubuque, IA, USA) were analyzed in each series. In the second subsample, chloride, sulfate, nitrate, and ammonium were analyzed within one day using high-performance liquid chromatography (HPLC). Concentrations of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were analyzed in the third subsample on a TOC analyzer (Model 700, O.I. Analytical, College Station, Texas, USA). pH was measured in the remaining sediment suspension (SenTix 21 electrode, WTW, Weilheim, Germany).

For total sediment element analysis, subsamples were taken from the remaining sediment and *aqua regia* (1:3 v/v 14 M HNO_3/37% HCl) digested, and diluted 1:10 with nanopure water. Elements Al, Fe, Mn, Ca, S and Zn were analyzed on inductively coupled plasma–optical emission spectrometry (ICP-OES, Spectroflame, Spectro, Germany). All other trace metals were analyzed on ICP-MS. Analytical quality was assured by analyzing (*n*=5) a selected reference material, a moderately polluted river clay (ISE 970). Concentrations were within 90–100% of certified values. Analytical quality was further assured as described before (Van Griethuysen *et al.*, 2003/2004). Loss on Ignition (LOI) and grain size distribution were determined as in Van Griethuysen *et al.*, 2003 and 2004. Mineralogical composition was measured in selected sediment samples using X-ray diffraction using a Philips PW1820/1710 diffractometer, equipped with a graphite monochromator, using CoKα radiation generated at 40 kV and 30 mA.

For AVS and SEM analysis, the second three cores from the November and March sampling were stored frozen at −18 °C until analysis. Prior studies showed negligible effects of this storage method (Lasorsa and Casas, 1996; De Lange *et al.*/Chapter 6). After thawing, the cores were sliced in the same way as the cores used for pore water analysis. Determination of AVS and SEM and associated quality assurance was according to Van Griethuysen *et al.* (2002).

4.2.4 Data analysis

Normality of data was explored using box-and-whisker plots. Outliers (data exceeding the 75th percentile of the data distribution by more than 3 times the box length) occurred mainly in pore water concentrations (Zn, Cu, and Pb in January samples) and were discarded before statistical analysis. Relations among variables were assessed using principal component analysis (PCA) with Varimax rotation or correlation analysis. Differences between sampling moments and layers were tested with one-way ANOVA. Post hoc comparison of homogeneous subsets was performed using the Tukey-b criterion under the assumption of equal variances. Temporal effects on layers 0–1 cm and 10–15 cm were not evaluated because data either showed too much scatter due to imprecise separation from the overlying water layer or insufficient replicates were available to allow statistical testing. All statistics were performed with SPSS for Windows v. 10.0 (SPSS, 1997).

The validity of assumptions and possible explanations concerning the chemical composition of the sediment system was checked with the equilibrium speciation model ECOSAT® (Keizer & Van Riemsdijk, 2000). The model uses the NICA-Donnan model for adsorption of trace metals onto (dissolved) organic matter in addition to equilibrium calculations of minerals and aqueous phase complexes (e.g. Kinniburgh *et al.*, 1996; Weng *et al.*, 2001). Modeling was based on solid phase data because they showed less scatter than pore water data. At the start, all elements in the sediment were entered in the model as dissolved components, present at their (measured) total concentration. From there, aqueous metal speciation over organic and inorganic ligands was calculated. Dissolved organic matter (DOM) in the pore water was assumed to consist solely of humic acid, containing 56% carbon (Keizer & Van Riemsdijk, 2000). Saturation indexes for preselected minerals were calculated and those with the highest oversaturation were allowed to precipitate first. Results were compared with measured pore water data. The selection of minerals was adapted until the closest resemblance with measured values was reached. The preselection of minerals was based on X-ray diffraction data showing the presence of illite-type clay minerals, a common clay mineral in West-European soils (e.g. Breeuwsma, 1985). Macro-elements Al, Ca and Mg are commonly present in Dutch sediments as gibbsite (α-Al_2O_3), calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$) (Breeuwsma, 1985). As 95% of the Ca minerals were found present as calcite with up to 5% as dolomite, we included both calcite and dolomite as possible Ca minerals. Other minerals were not detected due to low quantities or a poor degree of crystallinity (i.e. ferrihydrite) but were selected based on literature reports. Reported candidate minerals to control Mn and Fe solubility in sediments are rhodochrosite ($MnCO_3$), pyrolusite (MnO_2), greenockite (MnS) for Mn, and siderite ($FeCO_3$), ferrihydrite ($Fe_5O_7(OH)\cdot 4H_2O$), goethite (FeOOH), vivianite ($Fe_3(PO4)_2$), pyrite FeS_2 and amorphous iron sulfide (FeS am) for Fe (Stumm and Morgan, 1981; Paalman, 1997; Wijdeveld & Smits, 1997; Huisman, 1998; Van den Berg, 1998). Selected trace metal minerals (not observed) were their respective (hydr)oxides, carbonates and sulfides. Solubility products for minerals and complexation constants for aqueous phase complexes were taken from the model database. For amorphous iron sulfide, the solubility product from DiToro *et al.* (1990) was adapted for use in ECOSAT using parameters from Lindsay (1979). Other model inputs were measured pH and redox potential.

4.3 Results and discussion

4.3.1 Sediment and water characteristics

Solid phase

An overview of the concentration ranges for selected variables is given in Table 4.1. Minimum and maximum values are given for the entire data set. In addition, ranges for 1–2 and 5–10 cm layers are given to provide an indication of variation with depth.

Solid phase variables typically have a Coefficient of Variation (COV) around 10% within each layer per season (not shown), which is in the order of analytical error. This indicates a low spatial heterogeneity among cores of individual sampling moments.

The sediment can be characterized as calcareous, silty to clayey, with rather high organic matter contents, a neutral pH and sub-oxic redox potential (E_h). Mineral composition (%<2 µm, Al, Ca, and S) is rather constant with depth. Mn (and Fe) solid phase concentrations are higher in top layers as compared to deeper layers. Mn – and to a lesser extent Fe- solid phase concentrations are clearly lower in the floodplain sediment than in suspended solids of the Rhine.

Table 4.1. Data ranges of selected variables for all layers (0–15 cm) and selected layers

Variable	Unit	All layers	1-2 cm	5-10 cm	Rhine [a]
Sediment					*Suspended solids*
< 2 µm	%	7.3-26.2	8.8-20.8	7.3-21.3	- [b]
< 63 µm	%	41.8-100.0	50.0-89.8	41.8-88.4	- [b]
LOI	%	6.5-22.8	6.9-12.4	6.5-10.4	3.1-5.8
Al	%	1.1-2.6	1.4-2.5	1.2-2.3	- [b]
Ca	%	1.9-5.0	2.8-3.9	1.9-4.1	- [b]
Fe	%	1.6-3.2	2.3-3.0	1.6-3.1	2.3-3.4
Mn	mg/kg	387-1039	614-1017	387-752	1358-2160
S	mg/kg	734-3790	1495-2822	734-2988	- [b]
Zn	mg/kg	255-915	291-445	255-536	170-614
Cu	mg/kg	39-118	45-64	41-75	40-92
Cd	mg/kg	0-10.4	0-2.3	1.5-3.6	0.6-4.8
Pb	mg/kg	67-297	68-115	86-132	51-119
Ni	mg/kg	28-112	33-60	30-43	39-57
Redox	mV	(-207)- (-100)	(-171)- (-107)	(-207)- (-131)	- [b]
AVS	µmol/g	0.7-73.0	0.7-6.1	8.5-41.4	- [b]
SEM	µmol/g	0.8-16.1	3.7-7.6	4.1-7.6	- [b]
Overlying water					*Surface water*
pH	-	7.4-8.0	-	-	7.7-8.2
EC	µS/cm	473-552	-	-	523-898
T	° C	4.8-21.1	-	-	5.3-23.1
Redox	mV	131-230	-	-	- [b]
O_2	mg/l	3.0-10.8	-	-	8.5-12.3
Pore water					*Surface water*
pH	-	6.3-8.1	6.5-7.5	7.2-8.1	7.7-8.2
Ca	mg/l	50-328	50-160	122-328	63-92
Fe	mg/l	0.5-28.1	0.5-14.9	0.7-25.5	0.46-6.65
Mn	µg/l	852-12455	1087-8915	966-7357	36-280
DIC	mg/l	7.3-104.7	19.0-53.6	32.7-104.7	- [b]
DOC	mg/l	3.5-35.2	5.5-26.5	12.3-30.6	1.6-4.4
Cl^-	mg/l	53-132	54-125	58-132	42-147
NO_3^-	mg/l	0-67.7	0-67.7	0-4.4	1.51-4.20
SO_4^{2-}	mg/l	0-56	0-18	0-43	39-73
NH_4^+	mg/l	0.1-17.6	0.9-8.6	4.7-16.9	0.02-0.20
Zn	µg/l	3.5-637.1	27.8-578.2	9.5-59.7	11-74
Cu	µg/l	2.6-179.6	3.7-179.6	3.2-13.2	2.7-14.5
Cd	µg/l	0.1-17.4	0.1-2.2	0.1-1.0	< 0.05-0.25
Pb	µg/l	0.5-197.1	0.5-98.5	2.8-27.0	1.9-14.5
Ni	µg/l	1.5-43.2	2.4-36.6	1.5-10.4	1.8-13.0

[a] Concentration range in Rhine water in 1999 at Lobith (International Rhine Committee, 2003). Concentrations in suspended solids (13–210 mg/L during 1999) are used for comparison with solid phase concentrations, whereas concentrations in surface water are used for comparison with pore water concentrations. [b] –, no data available.

Organic matter (OM) contents are generally highest in top layers (one-way ANOVA/independent samples *t*-test, $p<0.10$) although the OM content is rather patchy yielding large deviations between replicate cores. They are higher than in (Rhine) suspended solids. Generally, trace metal concentrations increase with depth (one-way ANOVA/independent samples *t*-test, $p<0.10$) confirming an overall sediment improvement consistent with the reported improvement of sediment quality in the past decades (Beurskens *et al.*, 1994). An exception is Ni that is present at near-background concentrations (Crommentuijn *et al.*, 1997) and often related to organic matter (e.g. Huisman, 1998 and Van Griethuysen *et al.*, 2003). Trace metals occasionally exceed environmental standards for Zn, Cu and Ni (maximum permissible risk is 620 mg/kg for Zn, 73 mg/kg for Cu, and 48 mg/kg for Ni (Crommentuijn *et al.*, 1997). Pollution levels are similar to those of silty polluted sediment from the Northern Delta Basin, i.e. Haringvliet and Volkerak Zoommeer (Van Hattum *et al.*, 1993) and the Biesbosch area (Van den Berg, 1998) in The Netherlands. Top layer sediment quality (0–1 cm layer, not shown in Table 4.1) is comparable to the suspended solids quality of the Rhine.

The progressively reduced conditions towards deeper sediment layers are reflected in rather high (up to 70 µmol/g) AVS concentrations at 15 cm depth (data from November 1998 and March 1999). In previous work, we found concentrations up to 40 µmol/g in mixed sediment from 0–5 cm depth (Van Griethuysen *et al.*, 2003). Concentrations of AVS in surface layers (1–2 cm) are low (<6 µmol/g) related to the higher E_h. Concentrations of SEM are rather variable for the whole data set, but are not different for the layers 1–2 and 5–10 cm. They are within the range commonly found in Dutch polluted freshwater sediments (Van den Hoop *et al.*, 1997; Van den Berg *et al.*, 1998, Van Griethuysen *et al.*, 2003 and Van Griethuysen *et al.*, 2004). Concentrations of SEM–AVS only exceeded 0 in the top 1 or 2 cm.

Surface and pore water

Surface water pH and electric conductivity (EC) show a small range and are comparable to Rhine water values. Seasonal changes in temperature will also affect the rate of biological and chemical processes like organic matter production (plant and algal growth) and degradation (microbial activity). As a result, oxygen concentration and redox potential show a dynamic behavior throughout the monitoring campaign; low in summer/autumn, high in winter/spring. Other studies in the same floodplain lake indicate dynamic seasonal behavior of redox sensitive ions like sulfate in the lake water (Roozen, 2005).

Pore water variables (Table 1) generally exhibit COVs of 10–30% for most macro-elements like DOC, Fe, Mn, and Cl^-, and 50% to more than 100% for trace metals, especially in the top layers. For the other layers, variation was also higher than observed for solid characteristics. This may be caused by larger analytical error, but also by spatial heterogeneity of pore water among cores at the same sampling moment. For instance, if vertical redox boundaries are slightly shifted between cores (vertical micro-variation), this may have large influences on redox sensitive variables. Moreover, pore water is subject to larger slicing artifacts than the solid phase (e.g. Koelmans *et al.*, 2000). Note that the resultant larger variation is accounted for in statistical data analysis and may therefore limit the detection of significant differences over time.

Pore water pH is neutral to slightly basic and somewhat lower than for Rhine water and lake surface water, especially in the top layer. Concentrations of Ca are higher than in Rhine water and increase with depth. The same trend is observed in DIC levels in the pore water. Fe and Mn concentrations in pore water are also higher than in Rhine water. Nitrate levels (NO_3^-) decrease quickly with depth (nitrate was in fact only detected in July top layer samples), while ammonium (NH_4^+) levels generally increase with depth. Ammonium and sulfate (SO_4^{2-}) are much higher respectively lower than in surface water. Dissolved trace metal concentrations are clearly higher in top layers (maximum in layer 1–2 cm) than in deeper layers, and also higher than in Rhine water.

4.3.2 Multivariate analysis

Solid phase

Principal component analysis on all data until 15 cm shows that 83.6% of the variation within the solid phase data can be attributed to three components (see Table 4.2). November samples and variables AVS and SEM were not included because of too many missing data. The first component explains 37.1% of the variation and consists of macro-elements (Fe, Al, Mn, Ca and S) and fine grain size fractions, representing mineral composition. Trace metals Cu, Cd, Pb, and Zn are highly correlated (r=0.70–0.95) and together explain 29.1% of the data variation (second component). Thus, they are not related to mineral composition, indicating an independent pollution source. This is confirmed by a very moderate regression of e.g. Al and Zn (r=0.24, p=0.04, n=73). Furthermore, trace metal concentrations increase downward in the sediment profile, while mineral composition is more or less constant with depth (Table 1). The third component (explaining 17.4%) consists of organic matter and Ni, illustrating the close association of this metal with organic matter (e.g. Huisman, 1998 and Van Griethuysen *et al.*, 2003). The appearance of mineral and organic variables in different Principal Components means that there is no dilution effect of organic matter on mineral characteristics, as is sometimes found in suspended solids studies (e.g. Koelmans, 1997). This is confirmed by the positive correlation between Al and organic matter (r=0.42, p<0.01, n=69).

Table 4.2. PCA on solid phase data after Varimax rotation. Only loadings ≥0.5 are indicated.

Component **% explained [a)]**	**1** **37.1**	**2** **29.1**	**3** **17.4**
Fe	.924		
Al	.896		
Ca	.870		
Mn	.835		
S	.744		
Clay	.659		
Pb		.972	
Cd		.942	
Zn		.897	
Cu		.815	
Ni			.872
LOI			.849

[a] Total explained variation: 83.6%.

Pore water

Conditions were assumed to be most reduced in September, because organic matter deposition and degradation is generally highest at the end of the growing season. We focused on this month to unravel associations of pore water characteristics with trace metal behavior. As this data set represents variation at one sampling time, PCA reflects variability over depth only. Redox potential was not included in the analysis because it was only measured in one core. However, it is taken into account indirectly by the concentrations of redox sensitive ions.

Three principal components explained 86.0% of the data variation (Table 4.3). The first (bipolar) component, explaining 38.2%, consists of DIC, DOC, NH_4^+ and Ca representing inorganic water characteristics (hardness) and organic matter degradation, with negative loadings for Cd and Zn. The second component explaining 33.3% shows a correlation of Pb and Cu with sulfate and chloride. The third (bipolar) component explains 17.5% of the variation in pore water composition and has positive loadings for Mn and Fe, and negative loadings for Cd and Zn. The trace metals Zn and Cd thus show complex multivariate behavior with loadings on two components.

Table 4.3. PCA on September pore water data after Varimax rotation. Only loadings ≥0.5 are indicated.

Component	**1**	**2**	**3**
% explained [a)]	**38.2**	**33.3**	**17.5**
Ca	0.91		
DIC	0.91		
Ammonium	0.91		
DOC	0.85		
Cd	-0.79		-0.55
Zn	-0.79		-0.52
Sulfate		0.90	
Pb		0.87	
Cu		0.93	
Cl-		0.77	
Mn			0.92
Fe			0.75

a) Total explained variation: 86.0%.

4.3.3 Statistical analysis of temporal variation

Temporal differences were tested with one-way ANOVA ($p<0.05$) for layers 1–2, 2–5 and 5–10 cm (Table 4.4). Figure 4.3 and Figure 4.4 provide an illustration of temporal trends for selected variables.

Table 4.4. Temporal differences for selected variables in separate layers (1-way ANOVA)

Variable	**1-2 cm**		**2-5 cm**		**5-10 cm**	
	p [a)]	Subsets [b)]	p	Subsets	p	Subsets
Solid phase						
Al	0.75	-	0.16	-	0.34	-
S	0.80	-	**0.01**	3<2,1	**0.008**	5,3,4<1
Ca	0.99	-	**0.001**	3,4<4,5,2<5,2,1	0.14	-
LOI	**0.008**	1< 2,3,5	**0.05**	-	0.17	-
Mn	0.42	-	0.52	-	0.32	-
Fe	0.51	-	**0.01**	4<2	0.32	-
Zn	0.08	-	**0.06**	4<2	0.44	-
Cu	0.16	-	**0.008**	4,5<3	0.69	-
Cd	**0.000**	1 < 5,4,3,2	0.71	-	**0.04**	5<1
Pb	0.06	-	**0.03**	5<3	**0.01**	-
Clay content	**0.005**	5,4<4,1<1,2	**0.00**	4,5,3<1,2	**0.008**	4,5,3< 1
Pore water						
pH	**0.033**	3<1,4	0.39	-	**0.01**	1,4<3
Ca	**0.003**	3,4<5	0.55	-	0.63	-
DIC	0.42	-	0.14	-	**0.02**	2,1,5<4
DOC	**0.03**	1<4	**0.005**	1,2,5,3<4	0.15	-
Fe	**0.003**	1<5,4	**0.009**	3,1<1,5<5,4	0.59	-
Mn	**0.03**	1<4	**0.04** [c)]	-	**0.01** [c)]	-
Cl^-	**0.00**	2,1,5,4<3	**0.00**	2,1,5,4<3	**0.00**	1,2,5,4<3
NO_3^-	0.21	-	0.05	-	0.11	-
NH_4^+	**0.03**	2,5<4	0.39	-	**0.008**	2,5<4
SO_4^{2-}	**0.04**	4<2	0.35	-	0.22	-
[Cu]	0.90	-	0.96	-	0.99	-
[Cd]	0.09	-	**0.02**	- [d)]	**0.04**	- [d)]
[Zn]	0.20	-	0.42	-	Insufficient data	-
[Pb]	0.07	-	**0.001**	1,4,2<5	0.25	-

a) Significant temporal differences ($p \leq 0.05$) are indicated in bold and homogeneous subsets of sampling moments are indicated.

b) 1=November 1998, 2=March 1999, 3=July 1999, 4=September 1999, 5=January 2000

c) Although there are significant temporal differences, different homogeneous subsets are not discerned.

d) Insufficient replication to calculate homogeneous subsets.

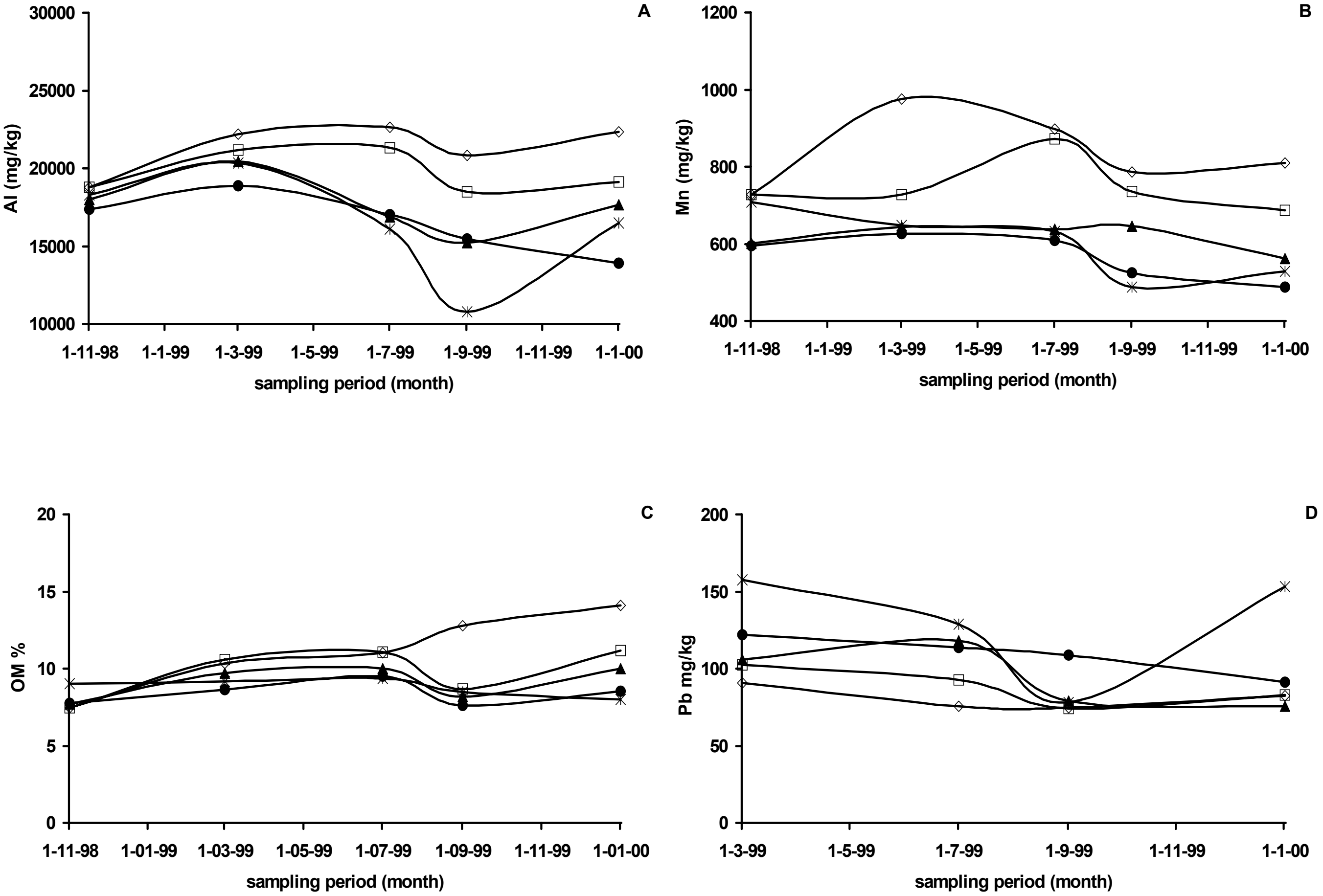

Figure 4.3. Typical temporal patterns for solid phase variables. Data points represent mean values per layer (*n*=3). Error bars have been omitted for reasons of clarity. A: Al (mg/kg); B: Organic matter (%); C: Mn (mg/kg); D: Pb (mg/kg). ◇0-1 cm, □ 1-2 cm, ▲ 2-5 cm, ● 5-10 cm, ✳ 10-15 cm

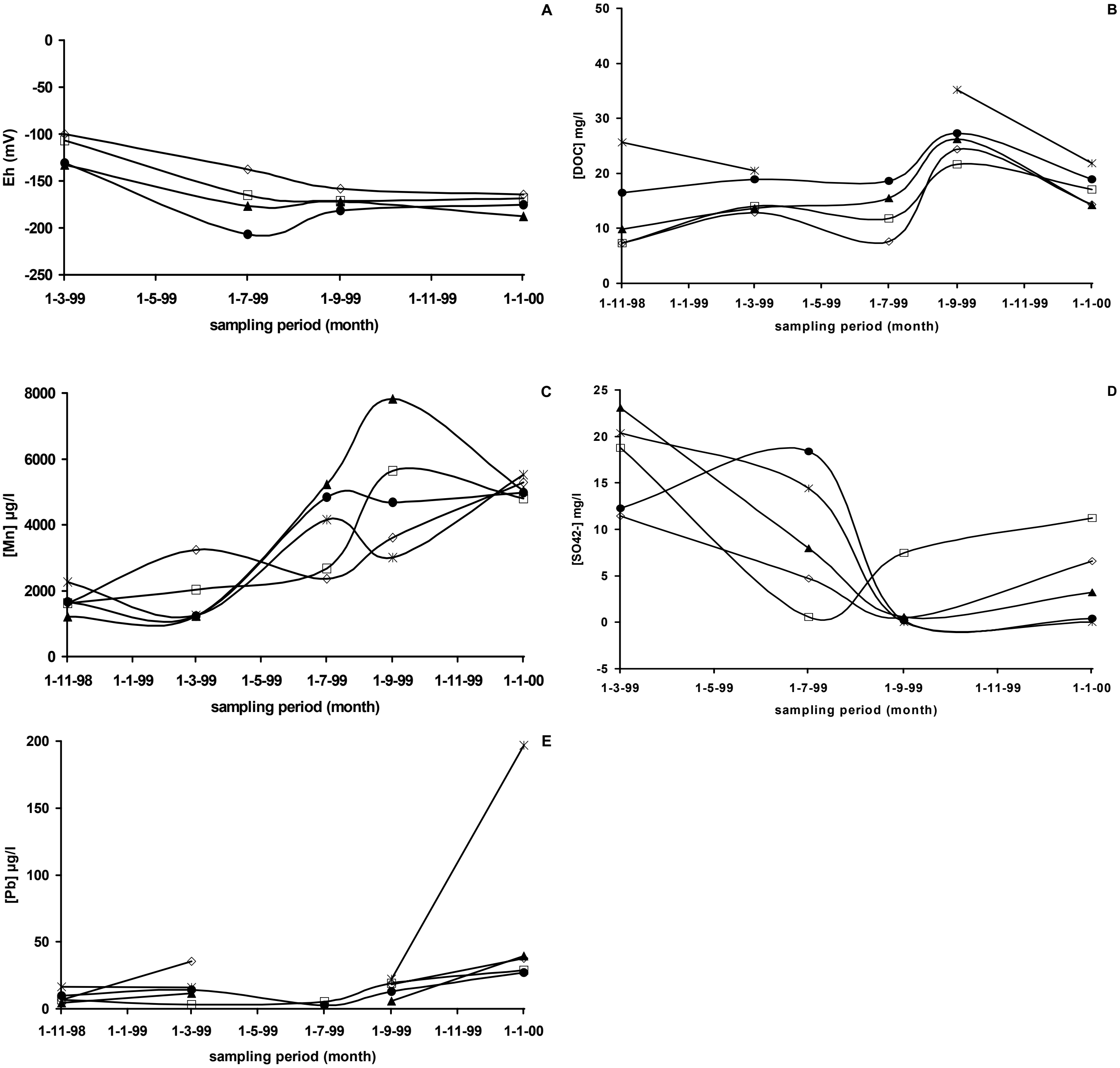

Figure 4.4. Typical temporal patterns for pore water variables. Data points represent mean values per layer (n=3). Error bars have been omitted for reasons of clarity. A: redox potential (mV, n=1); B: DOC (mg/L); C: Mn (µg/L); D: SO_4^{2-} (mg/L); E: Pb (µg/L). ◇0-1 cm, □ 1-2 cm, ▲ 2-5 cm, ● 5-10 cm, ✳ 10-15 cm

Solid phase

Mineral variables (Al, S, Ca, and Fe) show no significant trends for layer 1–2 (e.g. Al, Figure 4.3A). For the 2–5 cm layer however, S, Ca and Fe are significantly lower in the summer (July to September) samples (Table 4.4). For Fe this may be related to the dissolution of oxyhydroxides due to the shift in redox front (e.g. Douglas and Adeney, 2000). The content of organic matter (Figure 4.3B) is significantly (p=0.008, Table 4.4) lower for layer 1–2 cm in November than in March, July and January with intermediate September levels. For deeper layers, the temporal effect on organic matter becomes less clear. Mn concentrations are higher in spring and summer months in top layers (Figure 4.3C), but the trends are not significant due to large variation among replicate samples (Table 4.4).

Various trace metals show significant but different temporal trends (e.g. Pb, Figure 4.3D). The general pattern is that at the end of the series, trace metal levels (as well as Fe and clay) are significantly lower than at earlier sampling moments. For instance, in the 2–5 cm layer, mean concentrations of Zn are lower (p=0.056) in September (292 mg/kg) than in March (417 mg/kg). Pb and Cu show similar behavior. Mean Cu concentrations are lower (p=0.008) in September and January (43.5 respectively 47.5 mg/kg), than in July (61.7 mg/kg). Mean concentrations of Pb are lower (p=0.033) in January (75.6 mg/kg) than in July (118.1 mg/kg). In all layers, the content of clay particles also decreased significantly (p<0.008 for each layer) over time. This is also observed for the grain size fraction <63 μm in layers 2–5 and 5–10 cm (not shown). Hence, the trend in metal concentrations may be related to the progressively coarsening grain size of the sediment, as trace metals tend to attach to fine particles. Furthermore, the similar temporal behavior of e.g. Fe and Zn may point to an accumulation of trace elements in Fe oxyhydroxide minerals (Douglas & Adeney, 2000).

In summary, variation of S, Ca, Fe and Mn show seasonality possibly related to redox processes, while metals have a negative trend explained from a lowering of clay contents.

Pore water

The redox potential provides a good indication of the oxygenation conditions in the pore water at the various sampling moments (Figure 4.4A). In March 1999 (no data for November) the sediment is much more oxidized than at all other sampling moments as a result of the severe winter inundation.

In the surface layer (1–2 cm) [Ca] is higher (p=0.003) in January samples than in July and September samples. In deeper layers, there is no temporal effect on [Ca]. DOC concentrations

(Figure 4.4B) show a clear maximum in September for the 1–2 cm (*p*=0.026) as well as for the 2–5 cm layer (*p*=0.005). Maximum values of NH_4^+ (not shown) occur in September in the 1–2 cm layer (*p*=0.033) and the 5–10 cm layer (*p*=0.008). Concentrations of dissolved Mn (Figure 4.4C) and – to a lesser extent – Fe are also significantly higher in September and – for Fe- in January than in November. This occurs in all layers for Mn (*p*=0.01–0.04), and in the layers 1–2 and 2–5 cm for Fe (*p*=0.03 and 0.009, respectively). This observation is consistent with temporal trends in solid Fe and Mn. Minimum values of SO_4^{2-} (*p*=0.04, see Figure 4.4D) occur in September in the 1–2 cm layer in accordance with frequent observations that rates of sulfate reduction are typically highest in summer due to increased organic matter production and higher bacterial activity (e.g. Besser *et al.*, 1996). Temporal trends in dissolved trace metals Cu and Zn were not significant due to large variation among replicates. Significant variation for Cd (all layers) points to a maximum in January but differences between sampling moments cannot be assigned due to insufficient replication. January samples also had significantly higher Pb concentrations (*p*=0.001) than November, March, and September samples (no data for July) in layer 2–5 cm (Figure 4.4E). These higher metal concentrations in winter are consistent with metal mobilization from metal sulfides (AVS) that formed in spring and summer (Van Griethuysen *et al.*, 2005/Chapter 3).

In summary, the general pattern for dissolved species is that DOC and redox sensitive ions like NH_4^+, Fe, Mn, and SO_4^{2-} all show significantly different concentrations in summer (July, September) than in winter (November, March, January) samples, which can be explained from organic matter breakdown and subsequent redox cycling. This pattern is quite comparable with that of other freshwater sediments in the Rhine-Meuse delta (Paalman, 1997; Van den Berg, 1998) and probably has consequences for metal fixation in the sediments (discussed in next sections).

4.3.4 Equilibrium modeling of solid and solution phase composition

Model results obtained with NICA-Donnan in ECOSAT® were compared with measured pore water contents from March, July, September, and January samples.

Macrochemistry

The modeled precipitation of minerals is summarized in Figure 4.5 and Table 4.5.

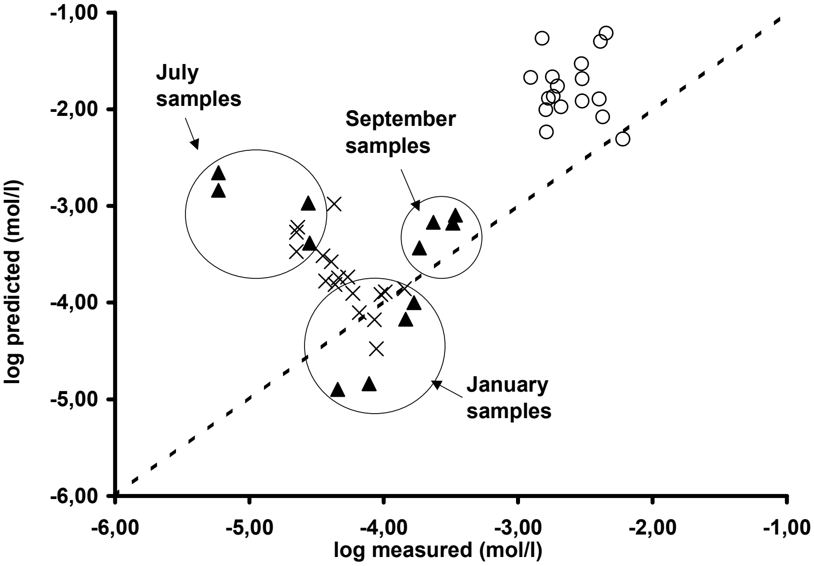

Figure 4.5. Modeled versus measured pore water macro-element concentrations in averaged layers. Dotted line represents 1:1 relation. Sample codes: ○ Ca, ▲Fe, × Mn.

Table 4.5. Modeled mineral stability in different months for 0–15 cm[a]

Element	March	July	September	January
Ca	$CaCO_3$ + $CaMg(CO_3)_2$	$CaCO_3$ + $CaMg(CO_3)_2$	$CaCO_3$ + $CaMg(CO_3)_2$	$CaCO_3$ + $CaMg(CO_3)_2$
Fe	α-FeOOH	FeS+$FeCO_3$ (0-5 cm); $FeCO_3$ (5-10 cm) [b)]	$FeCO_3$	α-FeOOH
Mn	$MnCO_3$	$MnCO_3$	$MnCO_3$	$MnCO_3$
Zn	$ZnCO_3$ (0-10 cm); α-ZnS (10-15 cm)	α-ZnS	α-ZnS	α-ZnS
Cu	CuOH (0-2 cm); CuS (2-15 cm)	CuS	CuS	CuS
Pb	$PbCO_3$	PbS	$PbCO_3$ (0-1 cm); PbS (1-10 cm)	PbS
Cd	$CdCO_3$ (0-10 cm); CdS (10-15 cm)	CdS	CdS	CdS

a) Calculated with ECOSAT® (Keizer et al 2000) with average chemical conditions (Eh, pH, total element contents) as input. Selection of minerals was based on X-ray diffraction, literature and best agreement with measured values

b) If not mentioned otherwise, the mineral precipitated in all layers

Aluminum precipitated as α-Al_2O_3 and Mg always precipitated as $CaMg(CO_3)_2$, which is consistent with X-ray diffraction results. The combined precipitation of $CaMg(CO_3)_2$ and $CaCO_3$ often resulted in a slight overestimation of Ca contents in pore water compared to field data (Figure 4.5). Manganese was always controlled by $MnCO_3$ precipitation, consistent with results of Saulnier and Mucci (2000). Modeled dissolved Mn values were somewhat higher than measured Mn pore water contents, suggesting that in the field probably other minerals, e.g. Mn-(hydr)oxides co-exist (see Figure 4.5). Iron often was controlled by $FeCO_3$ precipitation resulting in pore water contents close to measured values (Figure 4.5). However, striking temporal differences in predicted Fe mineral composition did occur (Table 4.5). In January and September, Fe pore water concentrations were in agreement with the precipitation of FeOOH and $FeCO_3$. The model underestimated Fe pore water concentrations in January samples, suggesting that reduction of Fe-hydroxides or oxidation of Fe sulfides occurred in the field. In March, the precipitation of FeOOH was chosen based on the oxidized conditions (Fe was not measured). For July samples, a co-existence of $FeCO_3$ and FeS (am) is found in top layers. This implies that formation of AVS in summer is plausible. It is well known that stability fields of FeS and $FeCO_3$ overlap (e.g. Stumm and Morgan, 1981). In our case, co-existence of FeS and $FeCO_3$ occurs only at pH values ≤ 7 (July top layers) consistent with precipitation of reactive Fe with sulfides in acidic environments (e.g. Huerta-Diaz *et al.*, 1998). At higher pH values in deeper layers, no FeS precipitation is predicted. However, dissolved Fe in equilibrium with FeS and $FeCO_3$ was always much higher than measured Fe pore water concentrations. This may be due to kinetic constraints or to additional immobilization of Fe by other (not modeled) processes, like adsorption to sediment organic matter or co-precipitation. Calculations show that the observed minimum aqueous Fe concentrations in summer (Table 4.4) can be explained by reduction to sulfides in summer having a lower solubility product than (hydr)oxides and carbonates that are dominant during winter. Formation of metal sulfides in summer is confirmed by current and literature data on AVS in these floodplains (Table 4.1; Van Griethuysen *et al.*/Chapter 3) and by observed higher pore water Pb and Cd concentrations in winter.

In summary, the macrochemistry of the sediment could be modeled quite well and appears to be close to equilibrium with selected minerals. This supports the validity of modeling assumptions. Most macro-elements are present as one mineral form, except for Fe, where predicted minerals varied with seasons.

Trace metals

In general, the model predicts that major fractions of trace metals in the pore waters were sorbed to DOC (around 50% for Pb and over 90% for other trace metals), especially in the deeper layers. Depending on redox conditions, stable minerals with either sulfides or with carbonates (copper with hydroxides) were predicted (Table 4.5). Generally, at more reduced conditions (increasing depth, summer), metal sulfides become dominant, which confirms the aforementioned observations of measured AVS and trace metal pore water concentrations. In the most oxidized samples of March, only sulfides of Zn are stable in samples from deeper layers. In July, September and January, metal sulfides precipitate to various extents depending on depth. In top layers, carbonates are most important, especially in March (most oxidized month). Copper shows a different speciation, precipitating mostly as sulfide and never as carbonate. In oxidized March samples CuOH precipitates leading to pore water contents similar to measured values. In other months, when CuS is precipitating, modeled pore water contents are always much lower than measured concentrations. One explanation for this discrepancy is that field DOC-Cu affinity constants differs from those used in the model, where it was assumed that DOC consists of pure humic acid.

When precipitation of sulfides is calculated at low pE and pH (e.g. in January), indeed the lowest trace metal concentrations are measured. However, predicted values still are much lower than measured values (up to 2×10^5 times) except for Cd, where predicted pore water concentrations are relatively close to measured values. At high pH (e.g. deepest layer July, September) modeled pore water contents agree better with measured contents. When solid sulfides or carbonates are predicted at high pe values, modeled trace metal pore water concentrations are generally higher (up to 10000 times) than measured values, indicating non-equilibrium or conceptual discrepancy between model and field. Obvious reasons for the discrepancies between model and field observations are non-equilibrium, incomplete system definition or data scatter. Co-precipitation of heavy metals with FeS implies oversaturation of the pore water with respect to discrete sulfide precipitates (e.g. Van den Berg, 1998). Situations of over – as well as undersaturation (which are both found in this study) are possible because precipitation is often kinetically controlled (Stumm and Morgan, 1981; Paalman, 1997). Furthermore, there may be uncertainties in system definition, which will be larger for trace metals than for major ions present in excess, like Fe and Mn.

In summary, the model provided a good description of macrochemistry and mineral composition of sediments. For trace metals, useful backup for assumptions concerning their geochemistry was obtained, but uncertainty in system definition and data was too large for a quantitative agreement.

Still, a general seasonal shift of mainly carbonates towards sulfides in summer was clearly indicated for most metals as a result of redox changes over time and depth.

4.3.5 Integrated discussion of processes affecting temporal changes in floodplain lake sediment geochemistry

In the previous sections primary results of measurements, statistical analyses and model calculations are presented and discussed in detail. In this section we summarize and relate the key processes in the lake to hydrological changes.

Macrochemistry

All mineral variables are related (PCA, Component 1) and thus will show similar vertical and temporal patterns. In general, these variables show small but significant temporal variation with lower values in summer samples than in winter samples. Temporal variation in e.g. calcite in the layer 2–5 cm suggests net calcite dissolution during reduced conditions (summer) and net calcite formation during oxidized conditions in winter (Table 4.4). The increase of DIC and Ca concentrations with depth (Table 4.1) points to redox related calcite dissolution in the sediment. The content of clay, which is related to mineral characteristics, shows a downward trend over time for all layers implying that incoming particles are coarser than the bed sediment.

Organic matter dynamics

Sediment organic matter is higher in top layers because of deposition of fresh material on top of the sediment. This difference between top and deeper layers is becoming more pronounced during the monitoring period due to the build-up of the organic layer over time (Table 4.4). The production of organic acids during organic matter breakdown explains the lower pH in top layers. The significantly higher (up to a factor 2) DOC concentration in September top layers (1–2, 2–5 cm) as compared to all other sampling moments can be explained by the decomposition of freshly deposited organic matter at the end of the growing season. The concurrence of maximum DOC and NH_4^+ in deeper layers (Table 4.1) and their positive correlation in PCA suggests that they have a common source in decomposition of organic matter.

Redox cycling

E_h measurements indicate a basically anoxic environment throughout the monitoring period. The large inundation in the first winter period (November 1998) followed by the small flood in March

1999 resulted in a higher redox potential of the sediment in March 1999 compared to later sampling moments. Inundation causes oxygen intrusion into the sediment by e.g. wave action and resuspension (e.g. Simpson *et al.*, 1998). Combined with a low biological activity during winter, conditions then become oxidized. During summer, conditions get progressively more reduced due to temperature increase speeding up microbial processes, primary production and subsequent degradation of deposited organic matter. It appears to depend on the magnitude of the winter flood whether conditions become oxidized again. In January 2000, the much smaller flood (Figure 4.2) only had a small influence on redox conditions (Figure 4.4A).

In all samples, pore water hardly contained any nitrate indicating that the redox status of the sediment is always at least in the phase of nitrate reduction. Higher ammonium and lower nitrate concentrations in pore versus surface water indicate consumption of nitrate, next to production of ammonium due to organic matter breakdown (e.g. amino acids). Concentrations of ammonium also indicate progressive reduction towards deeper and more reduced layers.

Reductive dissolution in summer/autumn and immobilization by oxidation in winter/spring explains the behavior of Fe and Mn, for which we provide the following evidence. First, Fe concentrations are lower in the reduced sediment than in suspended solids. Second, pore water concentrations are higher than dissolved concentrations in Rhine water. Third, PCA and correlation analysis indicate the same reductive dissolution patterns. Concentrations of dissolved Fe are positively related to AVS (r=0.64, p=0.01, n=15), which points to reductive dissolution of Fe-oxides in reduced samples with high AVS concentrations. Furthermore, significant temporal changes occur for both Fe and Mn in pore water. These can be explained by reduction of oxides in September and less oxidizing conditions in the winter of 1999–2000 than in 1998–1999 due to a less dramatic inundation (Figure 4.2). Pore water Mn concentrations increase significantly from November to September, which can be explained by progressive dissolution of Mn-oxides during the growing season. A similar pattern is observed for Fe except for a striking minimum in the 2–5 cm layer in July samples. The solid Fe concentration in the layer 2–5 cm is significantly lower in September samples than in March suggesting dissolution of Fe (hydr)oxides and precipitation of reduced Fe as sulfide in deeper layers. Thus, the observed temporal behavior suggests that Fe and Mn cycling is related to reductive dissolution. Model calculations showed that mainly Fe and Mn carbonates are dominant in most samples and that Fe- and Mn-(hydr)oxide minerals are less important. However, the equilibrium model does not account for the dynamic conditions in the lake that promote cycling of Fe and Mn as (hydr)oxides and sulfides. Concentrations of sulfate also indicate progressive reduction towards deeper layers. Lower sulfate in pore water than in surface water indicate the consumption of sulfate in the sediment. The occurrence of lowest SO_4^{2-} in top layers of September samples compared to other sampling moments shows that the strongest sulfate reduction took place preceding that period. The low concentrations of AVS in the top layers of November and March samples are consistent with the aerobic nature of this layer. This may be enhanced by oxidation and a slow recovery after the inundation in November 1998.

In pore waters, temporal effects on redox-dependent geochemical processes are clearly visible and appear related to both inundation regime and primary production in the floodplain lake. In general, these reduction and oxidation processes are more variable in upper layers than in deeper layers. This suggests a dynamic fate of trace metals (dissolution, precipitation, and adsorption) especially in the top 5 cm, where also biological activity is concentrated.

Trace metal dynamics

Temporal effects on *solid phase* trace metals are observed especially for the 2–5 cm layer. This layer has very dynamic redox conditions (Figure 4.4 A, C, D), where redox zones of Mn, Fe, and sulfate reduction overlap. This may lead to repeated precipitation and dissolution of metals as oxides or sulfides, which even affects solid phase concentrations. Each metal appears to show a specific response to these temporal changes.

Pore water concentrations of layer 0–1 cm may approach surface water concentrations due to diffusive fluxes towards the cleaner surface water (e.g. Vink, 2002). The occurrence of maximum concentrations in top layers points to mobilization of metals under aerobic conditions and immobilization when reduced conditions prevail (e.g. Van den Berg, 1998; Vink, 2002). Observed temporal effects on pore water concentrations of trace metals are not significant because of high spatial and/or analytical variability among replicate samples. However, patterns of pore water concentrations may be explained by relationships of trace metal concentrations with other pore water variables. Complexation with DOC and precipitation with sulfides are relevant geochemical processes affecting concentrations of trace metals in pore waters. DOC is important in keeping metals in solution by complexation, especially for Cu (Radovanovic & Koelmans, 1998; Van den Berg, 1998; Vink, 2002). In our study, trace metal-DOC complexes are important for speciation within pore water as shown by model calculations. Significant relations with DOC are confirmed for Pb in November (r=0.74, p=0.002, n=15) and March (r=0.66, p=0.008, n=14). For Cu, relations are significant for all pooled samples (r=0.41, p=0.02, n=35) and in March samples (r=0.69, p=0.002, n=15). A highly significant correlation is also found for between DOC and Fe (*r*=0.63, *p*=0.000, *n*=45) for all samples. For November 1998 samples this correlation is extremely high (*r*=0.94, *p*=0.000, *n*=15). The association of Fe with DOC (Brezonik *et al.*, 2003) may be more important than complexation of e.g. Cu by DOC under prevailing field conditions (Fe present in excess). Overall, association of (trace) metals and DOC appears to be more important during winter conditions.

The association of Cu and Pb with sulfate in PCA points either to the formation of Pb and Cu complexes with sulfate, or to dissolution of Pb and Cu sulfides at aerobic conditions. In deeper layers, low Pb/Cu and low sulfate concentrations occur, indicating the formation of metal sulfides, while in aerobic top layers these sulfides are oxidized. The negative association of Zn and Cd with Fe and Mn might indicate replacement of Fe by Zn and Cd in sulfides (e.g., DiToro *et al.*, 1990) or simultaneous dissolution of Fe- and Mn-oxides and precipitation of Cd- and Zn-sulfides. In November samples, Zn pore water concentrations are negatively correlated with AVS concentrations (*r*=−0.96, *p*=0.04, *n*=4) consistent with ZnS formation. Sphalerite formation was also indicated by model calculations for the other months (for March only in the deepest layer). In September, pore water Cu (*r*=0.68, *p*=0.01) and Pb (*r*=0.65, *p*=0.02) are positively correlated with sulfate, supporting the role of sulfide precipitation.

In summary, there are only small temporal effects on solid phase trace metals occurring mainly in the 2–5 layer, which is most susceptible to redox shifts. Direct evidence for temporal changes of trace metals in pore water could not be established. However, trends in processes governing trace metal behavior (previous section) and temporal variation in solid trace metal concentration together suggest that trace metals are more mobile and available during winter than during summer conditions in floodplain lake systems.

4.4 Conclusions

Total trace metal concentrations increased with sediment depth indicating an improvement of sediment quality over the last years. In contrast, dissolved trace metals concentrations were highest in the top layers, due to sulfide precipitation in deeper layers and metal mobilization from oxyhydroxides in top layers. Metal sulfide formation was important in deeper layers and in summer seasons when degradation rate of organic matter was highest. In fact, trace metal behavior was driven by dynamic sulfur, Fe and Mn seasonal redox cycles. Inundation had a mobilizing effect due to the flux of oxygen towards and resuspension of the sediments, while water table lowering combined with primary production and organic matter decomposition led to immobilization. All direct observations and indirect inferences on the occurrence of chemical phases were consistent with equilibrium model calculations. For macro-elements a good agreement between modeled and observed solid and solution phase composition was found. Combined model results, pore water measurements and AVS measurements point to a strong short-term variability (seasonality) in the way metals are immobilized. For ecological risk assessment in floodplain lakes, this short-term variation should be considered in relation to variations in species sensitivities during their annual life cycles. For instance, risks in summer may be lower than in winter, where metal sulfides may not precipitate leaving them available for benthic invertebrates.

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CHAPTER 5

SPATIAL VARIATION OF METALS AND AVS IN FLOODPLAIN LAKE SEDIMENT

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Abstract

In risk assessment of aquatic sediments, much attention is paid to the immobilizing effect of acid volatile sulfide (AVS) on trace metals. The difference of AVS and simultaneously extracted metals (SEM) gives an indication of metal availability. In floodplain sediments, where changing redox conditions occur, AVS may play a major role in determining variation in metal availability. The importance of spatial heterogeneity has been recognized in risk assessment of trace-metal-polluted sediments. However, little is known about spatial variation of available metal fractions.We studied spatial variability of sediment, environmental conditions, total contaminant concentrations, and available metals (as SEM-AVS or SEM-AVS/*f*OC) in a floodplain lake. The top 5 cm of sediment was sampled at 43 locations. Data were analyzed with correlation and principal component analysis as well as with geostatistical methods. Trace metal and SEM concentrations and most sediment characteristics were more or less constant within 10%. In contrast, AVS concentrations were much more variable and showed a strong spatial dependence due to differences in lake depth, total sulfur pools, and redox potential (Eh), which resulted in crucial differences in trace-metal availability within the lake. The spatial pattern of SEM-AVS deviates from total or normalized trace-metal patterns. This particularly has implications for risk assessment of sediments prone to dynamic hydrological conditions, where AVS concentrations are also variable in time.

5.1 Introduction

In the past decade, much attention has been paid to the immobilizing effect of sulfide on trace metals in aquatic sediments (DiToro *et al.*, 1990; Ankley *et al.*, 1991; Allen, 1993). Sulfide controls trace-metal concentrations in pore water by forming precipitates with trace metals from solution, thus reducing environmental availability and mobility of trace metals. This sulfide is measured as acid volatile sulfide (AVS), which is operationally defined as the amount of sulfide extracted and volatilized from anaerobic sediment with hydrochloric acid (generally, 1 M is used). Availability can be evaluated by comparing AVS concentrations with concentrations of simultaneously extracted metals (SEM). SEM is defined as the sum of the molar concentrations of all metals that are extracted during the acid volatilization step and have solubility products lower than those of FeS and MnS. Commonly, SEM is calculated by taking the molar sum of the five most important trace metals (Zn, Cu, Cd, Pb, and Ni), but other metals could be included if they contribute significantly to total SEM concentrations. When SEM exceeds AVS, trace metals become potentially available because excess trace metals can be released to the pore water. Much research has focused on the relationship between AVS and SEM and toxicity under laboratory conditions. The AVS/SEM concept has shown its value to estimate toxic effects on benthic organisms in laboratory and field studies (DiToro *et al.*, 1990; Berry *et al.*, 1996). It has been proven to be important for reduced sediments and is more and more incorporated in sediment management strategies (e.g., U.S. EPA, 1998). Hare *et al.* (1994, 2001) studied potential limitations of the AVS concept to describe effects on various benthic organisms. They found that the AVS approach predicts effects well for most detritivores (mainly chironomids and oligochaetes) but not for other functional groups.

AVS can only be formed when the sulfur supply is sufficiently large and conditions within the sediment favor sulfate reduction, i.e., in moderately to strongly reducing environments (redox potential $[E_h]$, 2100 mV). It has been suggested that spatial and temporal variability may limit applicability of the AVS concept in natural systems. When AVS concentrations are spatially variable under field conditions, availability of trace metals can thus be variable, e.g., within one lake. Several studies addressed the issue of vertical variability of AVS (Besser *et al.*, 1996; Van den Hoop *et al.*, 1997; Van den Berg *et al.*, 1998, 2001). Few studies, however, concerned variability of AVS among locations in the field. Van den Hoop *et al.* (1997) compared AVS in point samples from different water systems. Van den Berg *et al.* (1998) reported highly variable AVS concentrations for four locations in a sedimentation area of the Rhine and Meuse rivers (The Netherlands). Howard and Evans (1993) evaluated trends in AVS by means of observations along transects. Although several authors (e.g., Winkels, 1997; Chang *et al.*, 1998) provide information on the spatial distribution of sediment characteristics and total concentrations of pollutants in lakes and rivers, only one study concerned a whole-surface spatial analysis of AVS (in mangrove sediments) (Mackey & Mackay, 1996).

In summary, there is a lack of data concerning whole-surface spatial variability of AVS and SEM for aquatic systems. Especially in floodplain systems, where hydrological conditions can vary largely in both space and time, it is hypothesized that, due to varying redox states and microbial community distribution, AVS will be highly dynamic in both space and time. Ecological risk analysis requires information on spatial distribution of risk-related variables. For instance, the response of detritivore macroinvertebrate species, which is the most abundant functional group, to sediment-bound contaminants depends on the gradients in environmental availability of trace metals rather than on total concentrations (Peeters *et al.*, 2000). The aim of this study is to explore differences between spatial variation in total metal concentrations and sediment bulk characteristics versus spatial variation in redox conditions and available metal concentrations. The difference between SEM and AVS is used here as an estimator for the availability of trace metals. This highlights implications of spatial heterogeneity on two current approaches to assess sediment quality with respect to trace metals, i.e., the approach based on total metals and normalization on clay or organic carbon content and the approach based on the correction for precipitation with sulfides. We also pay attention to a recently proposed combined normalization on sulfide and organic carbon, accounting for sulfide precipitation under reduced conditions and for binding to organic carbon under oxidized conditions (Sijm *et al.*, 2001).

5.2 Materials and methods

5.2.1 Study area

The study was performed in a small (0.2-ha) floodplain lake located in the floodplain area Afferdensche en Deestsche Waarden, along the river Waal, The Netherlands (see Figure 5.1).

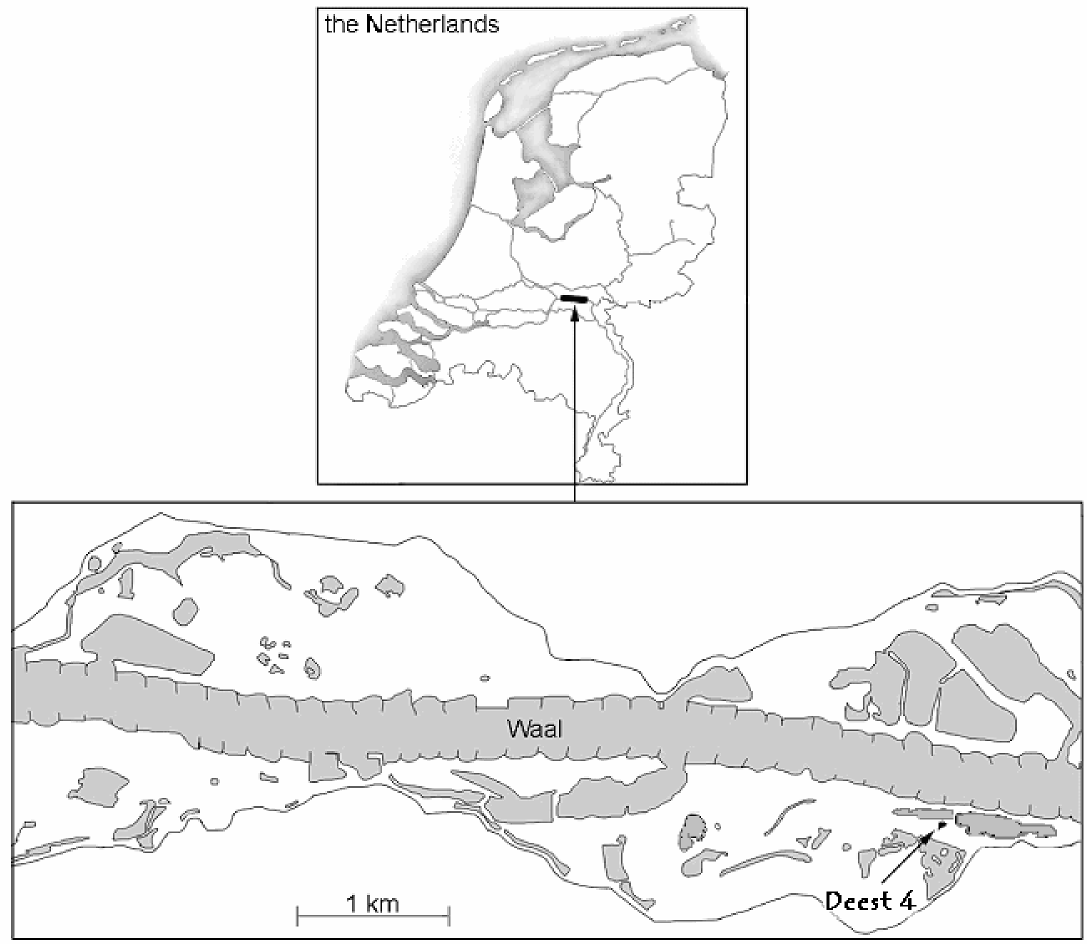


Figure 5.1. Map of The Netherlands with the location of the study area (after De Haas & Reuvers, 2001).

This area is polluted with trace metals and hydrophobic organic contaminants, resulting from pollution of the river Rhine, which reached its maximum during the 1960s and 1970s (Beurkens *et al.*, 1994; Vink & Winkels, 1994; Zwolsman *et al.*, 2000). Vegetation in the lake is characterized by a dense mat of submerged macrophytes (mainly *Potamogeton* species) accompanied by a small border of helophytes (*Butomus*) (Roozen, 2005). The benthic community is relatively species-rich compared with other floodplain lakes and is dominated by detritivore species, mainly consisting of oligochaetes and chironomids (Koelmans & Moermond, 2000). The floodplain is used for grazing cattle, which also have access to the lake borders. Fluctuating hydrological conditions cause a dynamic behavior of the water table within the lake, which leads to periodic shifting of borders. Sampling took place in January 2000, after winter inundation of the floodplain, at a surface water temperature of 5.5 °C, an electric conductivity of 523 μS, and an O_2 concentration of 11.5 mg/L (91% saturation). Water pH was 7.89.

5.2.2 Sediment sampling and analysis

To analyze spatial variability, several sampling grids are possible. In this study, no zonation or gradients were expected except for a possible gradient perpendicular to the river. However, due to the small dimensions of the studied lake, this gradient was assumed to have a minor influence on the spatial distribution of the variables studied. We therefore decided to sample at regular 2- to 4-m intervals on a predetermined regular grid, which was tuned to the shape of the triangular lake (Figure 5.2). Geographical position of corner points as well as actual lake boundaries were determined by a global positioning system (Trimble 4000SE; Trimble, Sunnyvale, CA, USA). Coordinates of in-between sample locations were accurately determined by sampling at marked intervals along ropes spanned across the lake. Lake depth was measured at 31 locations during the 1999/2000 winter, which were subsequently kriged to derive the values at the sample locations of this study. Fortythree sediment cores were sampled by means of a stainless steel Jenkins core sampler using perspex tubes with an inner diameter of 5 cm and a length of 50 cm. Sediment sample depth was at least 5 cm to include the biologically most important layer. The tubes remained completely filled with surface water and were sealed to preserve anoxic conditions. After careful transportation to the laboratory, cores were stored at 4° C until analysis, which took place within two weeks after sampling.

A set of 24 cores was selected for the determination of redox potential and concentrations of AVS and SEM. Redox potential was measured in the laboratory at a depth of 2 to 3 cm with a redox combination electrode (SenTix ORP; Wissenschaftlich-Technische Werkstätten, Weinheim, Germany). After that, the layer of interest (i.e., 0–5 cm) was separated from the rest of the core by slicing. In representative subsamples from these 24 cores, SEM and AVS were determined in duplicate using a recent modification of the diffusion method (Brouwer & Murphy, 1994; Leonard *et al.*, 1996) by Van Griethuysen *et al.* (2002/Chapter 2). In short, sulfides are dissolved and volatilized from the sediments with 1 M HCl and subsequently trapped into a buffer solution. Concentrations of AVS were measured with a sulfide-specific electrode (Ag 500;Wissenschaftlich-TechnischeWerkstätten) and a double-junction reference electrode (R 502; Wissenschaftlich-Technische Werkstätten). The detection limit was 0.1 µmol/g dry sediment and accuracy was between 10 and 15% (Van Griethuysen *et al.*, 2002/Chapter 2). Simultaneously extracted metal concentrations were analyzed in the remaining sediment suspension after filtration over a 0.45-µm membrane filter (Schleicher & Schuell, s-Hertogenbosch, The Netherlands). Metals were detected using inductively coupled plasma-mass spectrometry (ICP-MS, Elan 6000, Perkin-Elmer Sciex Instruments, Concord, ON, Canada) (Van Griethuysen *et al.*, 2002/Chapter 2). Representative subsamples of all 43 sediment cores were used for the other analyses. Sediment was digested in aqua regia (2.5 h at 100 °C) in order to determine macroelements and trace-metal total contents. All glassware was rinsed with 4 M HNO_3 before use and all reagents used during analysis of element concentrations were of at least analytical grade. Barnsteadt® (Barnstead, Dubuque, IA, USA) nanopure water was used for dilution of extracts. Macroelements and Zn were measured on inductively coupled plasma-optical emission spectrometry (ICP-OES, Spectroflame, Spectro, Kleve, Germany), and other trace metals were measured on ICP-MS (Elan 6000, Perkin-Elmer Sciex Instruments). Analytical quality was assured by comparison (n=5) with metal contents of a selected reference material (ISE 970), a moderately polluted clayey fluvial sediment, kindly donated by the Central Laboratory of Soil Quality (Wageningen University, Wageningen, The Netherlands). Recovery was within 90 to 100% for trace metals compared with certified values. Analytical quality of aqua regia and SEM extractions was further assured with numerous analytical blanks and multiple random duplicate analyses. Loss on ignition (LOI) was determined by drying overnight at 105 °C and subsequent heating for 3.5 h at 550 °C. Organic carbon content (OC) was taken as 0.58 * LOI (Page, 1982). Grain-size distribution was determined by laser diffraction on a Coulter 230 (Beckman-Coulter, Coulter Scientific Instruments, Miami, FL, USA) at 10% obscuration of the laser beam (Buurman *et al.*, 1996).

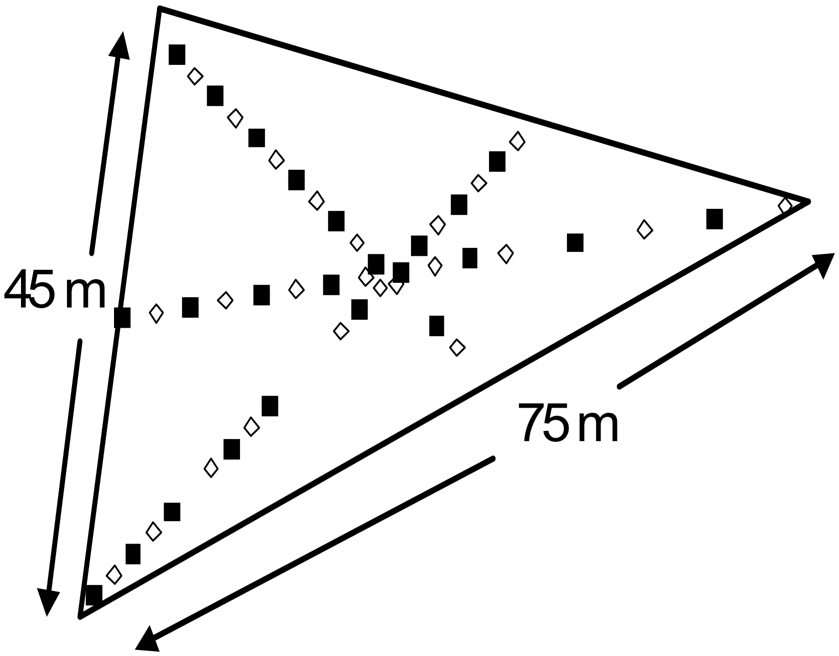


Figure 5.2 Sample locations within the lake. Open diamonds, macrochemistry and total trace metals; filled diamonds, additional analyses on AVS and SEM, depth, and redox potential.

5.2.3 Data analysis

Statistical data analysis was performed with the SPSS 10.0 for Window software package (SPSS, 1997). Normality of data was tested by examining skewness and kurtosis values as well as testing with the Kolmogorov–Smirnov test ($\alpha = 0.05$). Extreme values, situated at more than three times the interquartile ranges (box length) away from the 25th or 75th percentile value of that variable were removed. Pearson correlation analysis and principal components analysis on standardized variables using the Varimax rotation option were performed to reduce data and to assess association between sediment characteristics, trace metals, and chemical conditions. Principal components analysis describes the variation of a set of multivariate data as a reduced set of uncorrelated variables, each of which is a particular linear combination of the original variables. A spatial analysis was performed on selected variables. This selection includes variables influencing environmental conditions (depth, E_h), sediment characteristics (OC and clay fraction, macroelements), and trace-metal variables (total and normalized concentrations, SEM, and AVS). Spatial variability was assessed using kriging, which is a frequently used geostatistical method in soil science (Journel & Huijbregts, 1978). Some applications for aquatic environments exist (Leenaers, 1989; Marnette & Stein, 1993; Wehrens *et al.*, 1993; Winkels & Stein, 1997; Stein &

Staritsky, 1995). The theory behind geostatistics (the theory of regionalized variables) assumes that the variance of a variable at two points in an area increases with increasing distances between those two points and that this variance is independent of the position of this pair of points. So points close to one another are more likely to have similar values than points situated further apart (Journel & Huijbregts, 1978). In short, the procedure is as follows. In the first place, spatial variability for a variable *z* is characterized and quantified in a variogram $\gamma z(h)$, which is defined by half the variance of the difference of $z(x)-z(x+h)$, where *h* is the distance interval (called lag) between locations. A variogram is calculated at a given distance *h* as:

$$\gamma\, z(h) = 1/(2\mathrm{N}z(h))^* \, \Sigma\, [z(x_i) - z(x_{i+h})]^2 \qquad \text{for } i = 1 \text{ to } \mathrm{N}z(h) \qquad (1)$$

in which $z(x_i)$ and $z(x_{i+h})$ are the *i*th pair of observations of variable *z* with a distance *h* between observation points, where $\mathrm{N}z(h)$ is the total number of observation pairs (Journel & Huijbregts, 1978; Leenaers, 1989; Winkels & Stein, 1997). A practical rule provided to enable the estimation of a variogram is to have at least 30 observation pairs within each lag distance (Journel & Huijbregts, 1978). Variograms are characterized by three parameters: nugget, sill, and range. The nugget C_0 refers to the variance among observations at zero distance. The sill C_0+C is the maximum variance between observations, where C is the variation between nugget and maximum variance. The range *r* is the distance between observations at which this maximum variance is achieved. Beyond this distance, variance no longer increases; there is no spatial dependency between sample points separated by a distance larger than the range. In the current study, samples were taken at distances of 2 to 4 m. Therefore, it would not be appropriate to draw conclusions about variation occurring at smaller scales. Lag width is therefore set at 4 m. Variogram values were calculated with the program SpatAnal (Stein & Staritsky, 1986).

Second, appropriate models were fitted through the experimental variograms, based on weighted least squares with the program WLSFIT (Heuvelink, 1992), assuming isotropy (i.e., no gradients in a specific direction are expected). The model that fitted best to experimental variances was chosen from linear, Gaussian, exponential, and spherical models (Journel & Huijbregts, 1978). If a variable showed very little or spatially independent variance, a linear model with zero slope was assigned to that variable. Model significance was assessed using analysis of residual variance compared with variance due to regression (*F*-test). Models were not accepted when the significance level was above 0.15. Finally, modeled variances were used to create contour plots with SURFER (Golden Software, 1999), using ordinary point kriging as the interpolation method. For each location in the lake (represented in SURFER by a grid node), values measured in the neighborhood of that grid node were used for interpolation of the variable value, taking a minimum of four and a maximum of eight observations around each grid node.

5.3 Results and discussion

5.3.1 Sediment and environmental data

Sediment and environmental data are presented in Table 5.1. The sediment can be characterized as clayey to silty (9–18% < 2 µm) with rather high (4–7%) OC contents. According to Dutch quality standards, the sediment is moderately polluted with trace metals, which is characteristic for floodplains subject to diffuse pollution. Concentrations are below the Dutch maximum permissible risk (Crommentuijn *et al.*, 1997; Ministry of Transport and Public Works, 1998). Redox potential varies between approximately -100 and -220 mV, indicating a moderately to strongly reduced environment favoring sulfate reduction. AVS concentrations range from 0.2 to 40 µmol/g dry sediment, which is a common concentration range in Dutch freshwater systems (Van den Berg *et al.*, 1998, 2001; Van den Hoop *et al.*, 1997). The concentration of SEM is almost constant around 5 µmol/g dry sediment. The major contributor to SEM is Zn, which makes up around 80%. Percentages of total metal content present as SEM are 78.8 % for Zn, 56.1 % for Cu, 90.9 % for Cd, and 81.6 % for Pb. These percentages are somewhat lower than in a study by Van den Berg *et al.* (1998), who found that almost all (85–96%) Zn, Cu, Cd, and Pb was present as SEM. They, however, used an acid extraction strength of 6 M HCl for the analysis of SEM, in contrast with 1M HCl used in this study. Especially for Cu, they also noticed a significantly lower extractability in 1 M HCl as compared with 6 M HCl. The percentage of total Ni extracted as SEM was only 23 ± 3%, which agrees with their study, where also a low percentage compared with other metals was found. Apparently, Ni is primarily bound to other phases than those extracted with 1 or even 6 M HCl, e.g., incorporated in clay mineral structures. In 4 out of the 24 locations, SEM exceeded AVS above the proposed threshold value of 1.7 µmol/g (Sijm *et al.*, 2001), indicating a potential availability of metals. The maximum value of SEMAVS corrected for the fraction OC (f_{OC}) was less than 80 µmol/gOC and thus did not exceed the proposed toxicity limit of 100 to 150 µmol/gOC (Sijm *et al.*, 2001).

5.3.2 Statistical analysis

In Table 5.1, descriptive data statistics are shown. The Kolmogorov–Smirnov test revealed that all variables except Zn and P are normally distributed. After logarithmic transformation, Zn and P still deviated from a normal distribution. Therefore, three extreme Zn and one extreme P concentrations were removed before statistical and spatial analyses were performed, yielding a normal distribution for these variables as well. Most variables show a coefficient of variation of ± 10%, meaning that the sediment is not very heterogeneous. However, some variables show larger ranges, like total S, AVS, and the clay fraction (grain size fraction < 2 μm).

Table 5.1 Description of sediment and environmental data $^{a)}$

Variable	**Unit**	**Minimum**	**Maximum**	**Average**	**Standard deviation**	**COV $^{b)}$**
Depth $^{c)}$	m	1.7	2.4	2.2	0.2	9
E_h $^{c)}$	mV	-213.6	-117.8	-170.4	22.4	13
OC $^{d)}$	%	4.1	7.1	5.6	0.6	10
Clay fraction	%	8.9	18.1	14.1	2.5	17
Al	%	1.7	2.9	2.3	0.3	12
Ca	%	2.8	4.6	3.8	0.4	9
Fe	%	2.3	3.3	2.9	0.3	9
Mn	mg/kg	535.9	1035.2	835.7	112.5	13
P $^{e)}$	mg/kg	1119.0	1717.9	1492.8	151.1	10
S	mg/kg	1097.4	2856.1	1919.3	401.8	21
Cr	mg/kg	65.2	94.9	83.2	5.9	7
Zn $^{f)}$	mg/kg	346.2	509.5	419.4	37.8	9
Cu	mg/kg	48.1	67.3	56.2	4.8	9
Cd	mg/kg	1.4	2.2	1.8	0.2	12
Pb	mg/kg	75.8	106.8	91.3	6.8	7
Ni	mg/kg	34.0	47.1	40.8	3.4	8
AVS $^{c, g)}$	μmol/g	0.2	40.6	15.3	9.8	64
SEM $^{c, h)}$	μmol/g	4.6	6.1	5.3	0.4	8
SEM-AVS $^{c)}$	μmol/g	-34.5	5.2	-10.0	9.7	97
(SEM-AVS)/f_{OC} $^{c,i)}$	μmol/g OC	-625.1	81.2	-189.6	177.5	94

a) *n* = 43 unless indicated otherwise; b) COV = coefficient of variation (SD/average·100%); c) *n* = 24; d) OC = organic carbon; e) *n* = 42 (extreme value 2331.0 mg/kg removed); f) *n* = 40 (extreme values 1043.5, 646.8, 670.5 mg/kg removed); g) AVS = acid volatile sulfide; h) SEM = simultaneously extracted metals; i) f_{OC} = fraction organic carbon

Analysis of principal components divides the standardized data into four rotated (VARIMAX rotation) components, which together explain 82% of the variation within the data set. Factor loadings ≥0.5 are shown in Table 5.2.

Table 5.2 Principal components analysis (PCA) [a)]

Component	**1**	**2**	**3**	**4**
% explained	**34.52**	**20.69**	**19.44**	**7.47**
Fe	0.94			
Al	0.91			
OC [b)]	0.91			
Mn	0.87			
Ni	0.80			
P [c)]	0.78			
Ca	0.77			
Clay	0.58		0.54	
S	0.53		0.75	
Pb		0.93		
Cd		0.91		
Cr		0.85		
Zn [d)]		0.64		
Cu		0.71		
Depth [e)]			0.93	
AVS [e,f)]			0.85	
E_h [e)]			-0.65	0.61
SEM [e,g)]				0.51

a) Performed with VARIMAX rotation. Only loadings ≥0.5 are presented. *n*=43 unless otherwise indicated; b) OC = organic carbon (%); c) *n*=42; d) *n*=40; e) *n*=24; f) AVS=acid volatile sulfide; g) SEM=simultaneously extracted metals

Some variables show high loadings on more than one component. In component 1 (explaining 34.5%), the main mineral and organic fractions (Al, Fe, P, Mn, Ca, S, OC, and the clay fraction) are represented. The occurrence of Ni in this first component is due to the fact that Ni is present at natural background levels, 35 mg/kg in standard soil (Crommentuijn *et al.*, 1997), thus fluctuating in the same way as Mn or Fe concentrations and depending on sediment characteristics. Nickel can be strongly associated with minerals, i.e., bound to Mn-(hydr)oxides

(Zwolsman *et al.*, 1996), or with the organic matrix (Huisman, 1998), which is also indicated by the relatively high correlation ($r \geq 0.65$ at $p < 0.01$) of Ni with Mn, Fe, Al, and clay. Correlation of other trace metals (Zn, Cu, Cd, Pb, and Cr) with bulk variables is low to moderate ($r = 0.35$ at $p < 0.05$ [Pb with Al] to 0.54 [Cu with Fe] at $p < 0.01$). These trace metals form the second component, explaining 20.7%. AVS concentrations are highly correlated with depth and total S ($r = 0.75$ and 0.80 respectively, at $p < 0.01$) and moderately correlated with the clay fraction ($r = 0.53$ at $p < 0.05$). They are represented by the third component, which explains 19.4%. Redox potential is negatively associated to the other variables in this component, meaning that, when E_h is lower (more reduced conditions), AVS, depth, clay content, and S are higher. The observation that not only AVS but also total S is related to depth and Eh can be explained by various processes. In reduced environments, S^{2-} is formed from SO_4^{2-}. This sulfide can react with Fe, leading to precipitation of FeS or FeS_2. So an accumulation of total S as reduced mineral S species occurs more at locations dominated by reduced conditions. Sulfide may, however, also react directly with organic matter to form carbon-bonded sulfur that is known to accumulate in peat and lake sediment (Schlesinger, 1991). This also explains why total S correlates negatively with E_h, provided that locations with low E_h at the time of sampling are likely to be reduced most of the time. This is likely because it also concerns the deeper parts, where disturbance of reduced conditions is less probable than in the shallow parts of the lake. At the time of sampling, the redox potential throughout the lake is low enough to facilitate sulfide formation. Another S source is dead plant material. If this were an important pathway for S accumulation, a relationship with redox potential would not seem logical. It is, however, possible that plant primary production is more abundant in the deeper parts of the lake, which would lead to an indirect relation between redox potential and sediment S content. Total S shows only a marginal loading on component 1, whereas OC has a very high loading on this component. As correlation analysis also shows that S and OC are poorly associated ($r = 0.17$), we conclude that organically derived S is of minor importance in this floodplain lake. Reduction of surface-water sulfate and subsequent precipitation of iron sulfides will be the main process involved. The last component, explaining 7.5%, is made up by E_h and SEM. Simultaneously extracted metals show only a marginal loading on this component and do not load on any of the other components. Correlation analysis, however, shows that SEM is moderately correlated with Fe (r=0.47, p<0.05). This might indicate SEM binding with either iron sulfides due to (co)precipitation or with iron (hydr)oxides due to adsorption or both. Note that we have a mixed sample from the top 5 cm. The sediment thus may have aerobic and anaerobic parts due to oxygen penetration in the top layer.

The measured SEM will therefore not necessarily be related to iron sulfides. Under aerobic conditions, these metals will probably partly be bound to iron (hydr)oxides.

5.3.3 Spatial analysis

At sample distances larger than 40 m, the number of observation pairs decreases rapidly. In variogram model fitting, the maximum distance regarded was therefore 40 m, consisting of 10 lags with a lag width of 4 m. Model parameters used for variogram estimation, based on the weighted least squares method, as well as model significance are shown in Table 5.3. For most variables, the Gaussian model gave the best description of spatial variance within the data. This model is commonly used in kriging, together with spherical and linear models (Winkels, 1997; Leenaers, 1989). Examples of variograms for selected variables from each component are shown in Figure 5.3. Variogram form relates to model parameters described in Table 5.3. Numbers in the graphs represent the number of observation pairs within the considered lag on which the variogram value is determined. When fitting an appropriate model, this number of observation pairs is taken into account by the weighted least squares procedure.

No clear spatial dependency could be detected for most bulk characteristics (Mn, Fe, Al, clay), clay-normalized trace metals, and SEM concentrations. In these cases, the best fitting realistic variogram shows a constant variation at increasing distance between observations (linear model). For linear models, the steepness of the slope indicates the amount of variability within the variable considered. A zero slope implies that there is no spatially dependent variation for that variable. In Gaussian models, the amount of spatial variation can be evaluated by comparing the modeled nugget (C_0) and sill (the sum of C_0 and C) values, the minimal variation and the maximal variation within the modeled range (r in Table 5.3). Some variables only show a variation factor of 2 (e.g., S, Cu), while for other variables, the maximum variation can be 10 times or more as high as the minimum variation at zero distance (e.g., AVS, SEM-AVS). Ranges can be used to judge the scale of underlying processes determining variation in a variable. These processes may be small scale, e.g., for E_h and Cu, where the range is approximately 5 to 10 m, indicating a patchy pattern, or large scale, for e.g., depth, OC, and AVS, indicating a more gradual variation. Comparison of variograms of total trace metals (Figure 5.3e and 5.3f) and OC normalized trace metals (Figure 5.3g and 5.3h) shows that, for Zn, variograms look very similar. For Cu, however, the steepness of the curve decreases after normalization, as does the variogram shape. Variograms of AVS and SEM-AVS (Figure 5.3j and 5.3l) are nearly identical, which indicates that the spatially independent variation in SEM (Figure 5.3k) has very little influence on the spatial variability of the composite variable. The variogram parameters of SEM-AVS/f_{OC} (Table 5.3) show that there is only a slightly diminishing effect on the relative difference between nugget and sill compared with SEM-AVS, indicating that only little variability is removed.

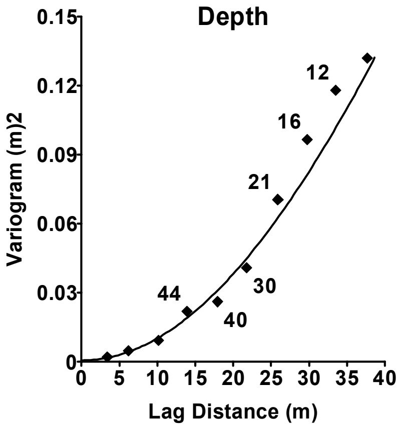
Spatial patterns of selected sediment and environmental variables are presented in Figure 5.4. While spatial patterns of E_h and Cu (Figure 5.4b and 5.4f) are patchy, most variables show gradually changing spatial patterns (e.g., depth, OC, S, Zn, Zn/OC, Cu/OC, AVS, and SEM-AVS in Figure 5.4a, c, d, e, g, h, i, and j).

Table 3 Model parameters for spatial analysis a)

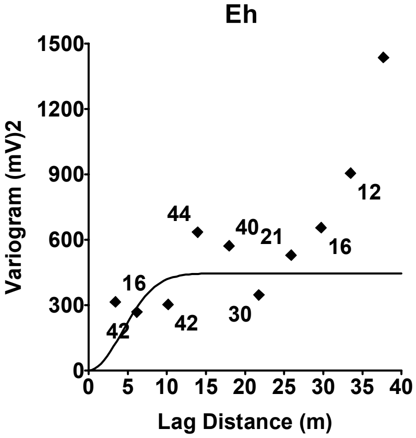
Variable	**Model**	***C₀***	***C***	***r***	**Slope**	**Significance**
Depth b)	Gaussian	$6.58*10^{-4}$	0.783	90.2		0.00025
E_h b)	Gaussian	$1.00*10^{-3}$	445	6.0		0.0026
OC c)	Gaussian	0.198	2.14	89.2		0.0012
Clay fraction	Linear	5.27			0	n.s. h)
Al	Linear	$7.23*10^{+6}$			0	n.s.
Ca	Linear + sill	$5.22*10^{+6}$	$9.74*10^{+6}$	19.7		0.0048
Mn	Linear	$1.12*10^{+4}$			0	n.s.
Fe	Linear	$5.42*10^{+6}$			0	n.s.
P d)	Gaussian	$1.21*10^{+4}$	$1.06*10^{+4}$	6.0		n.s.
S	Gaussian	$6.37*10^{+4}$	$1.28*10^{+5}$	10.1		0.0012
Cr	Gaussian	20.5	26	19.6		0.047
Zn e)	Gaussian	732	1700	27.1		0.092
Cu	Gaussian	13.4	10.2	4.75		0.14
Cd	Gaussian	0.024	0.0293	12.9		0.038
Pb	Gaussian	27.7	24.9	12.3		0.061
Ni	Linear	10.5			0	n.s.
Zn/OC e)	Gaussian	62.7	163	34.4		0.0045
Cu/OC	Linear	0.578			$2.56*10^{-2}$	0.00036
Zn/clay e)	Linear	34.5			0	n.s.
Cu/clay	Linear	0.436			0	n.s.
AVS b,f)	Gaussian	44.8	330	45.3		0.027
SEM b,g)	Linear	0.131			0	n.s.
SEM-AVS b)	Gaussian	42.4	440	53.4		0.023
(SEM-AVS)/f_{OC} b)	Gaussian	$1.42*10^{+4}$	$1.45*10^{+5}$	52.6		0.013

a) *n*=43 unless indicated otherwise; b) *n*=24; c) OC=organic carbon; d) *n*=42; e) *n*=40; f) AVS=acid volatile sulfide; g) SEM=simultaneously extracted metals; h) n.s.=not significant.

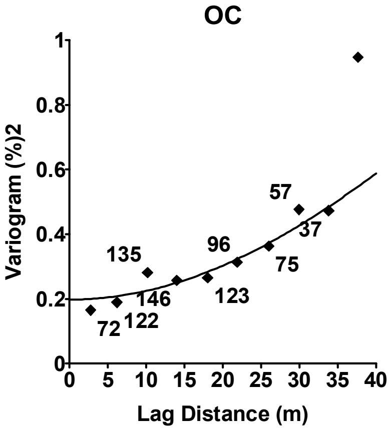
a)


Depth
Variogram (m)2
Lag Distance (m)
0.15
0.12
0.09
0.06
0.03
0
0 5 10 15 20 25 30 35 40
12
16
21
30
44
40

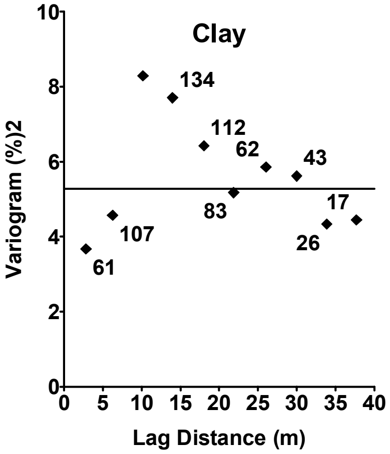
b)


Eh
Variogram (mV)2
Lag Distance (m)
1500
1200
900
600
300
0
0 5 10 15 20 25 30 35 40
44
40
21
16
12
16
42
42
30

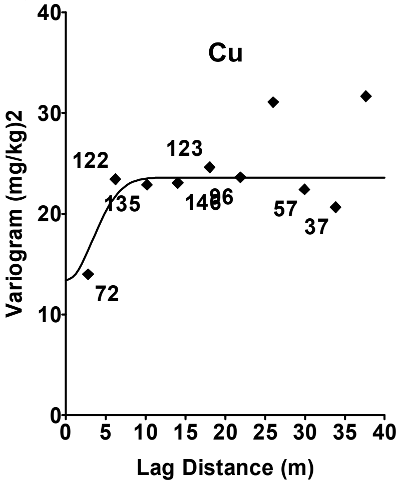
c)


OC
Variogram (%)2
Lag Distance (m)
1
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40
57
37
96
75
135
146
123
72
122

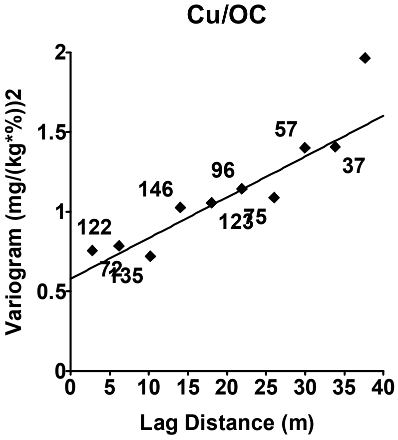
d)


Clay
Variogram (%)2
Lag Distance (m)
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40
134
112
62
43
83
17
107
26
61

e)


Cu
Variogram (mg/kg)2
Lag Distance (m)
40
30
20
10
0
0 5 10 15 20 25 30 35 40
122
123
135
146
96
57
37
72

f)


Cu/OC
Variogram (mg/(kg*%))2
Lag Distance (m)
2
1.5
1
0.5
0
0 5 10 15 20 25 30 35 40
57
96
37
146
122
123
75
72
135

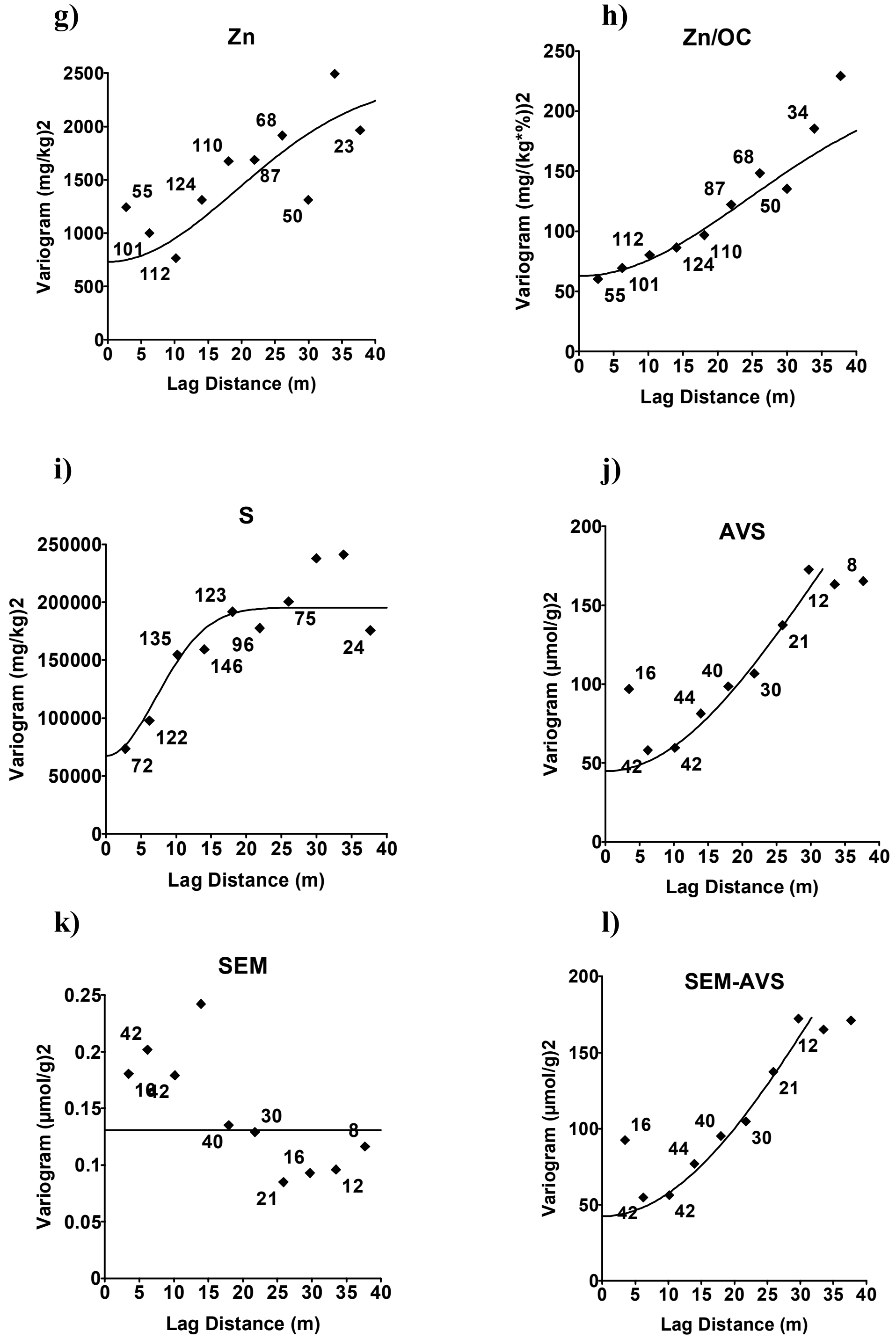
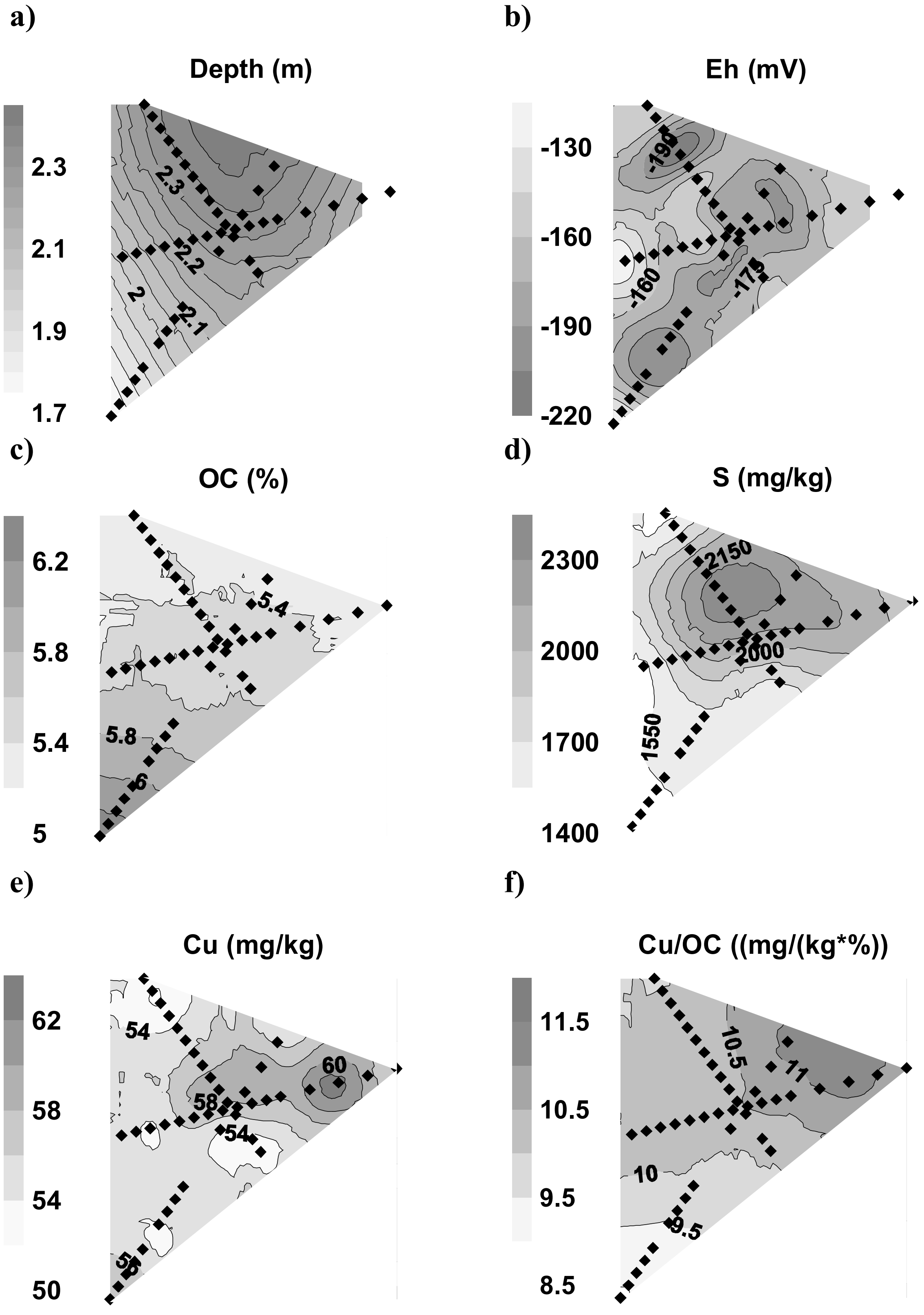


Figure 5.3. Experimental variograms. a) depth, b) Eh, c) OC, d) clay, e) Zn, f) Cu, g) Zn/OC, h) Cu/OC, i) total S, j) AVS, k) SEM, l) SEM-AVS. The filled diamonds indicate the number of observation pairs in the considered lag. OC=organic carbon; AVS=acid volatile sulfide; SEM=simultaneously extracted metals.


a)
Depth (m)
2.3
2.1
1.9
1.7
2.3
2.2
2
2.1
b)
Eh (mV)
-130
-160
-190
-220
-190
-160
-175
c)
OC (%)
6.2
5.8
5.4
5
5.4
5.8
6
d)
S (mg/kg)
2300
2000
1700
1400
2150
2000
1550
e)
Cu (mg/kg)
62
58
54
50
54
60
58
54
56
f)
Cu/OC ((mg/(kg*%))
11.5
10.5
9.5
8.5
10.5
11
10
9.5

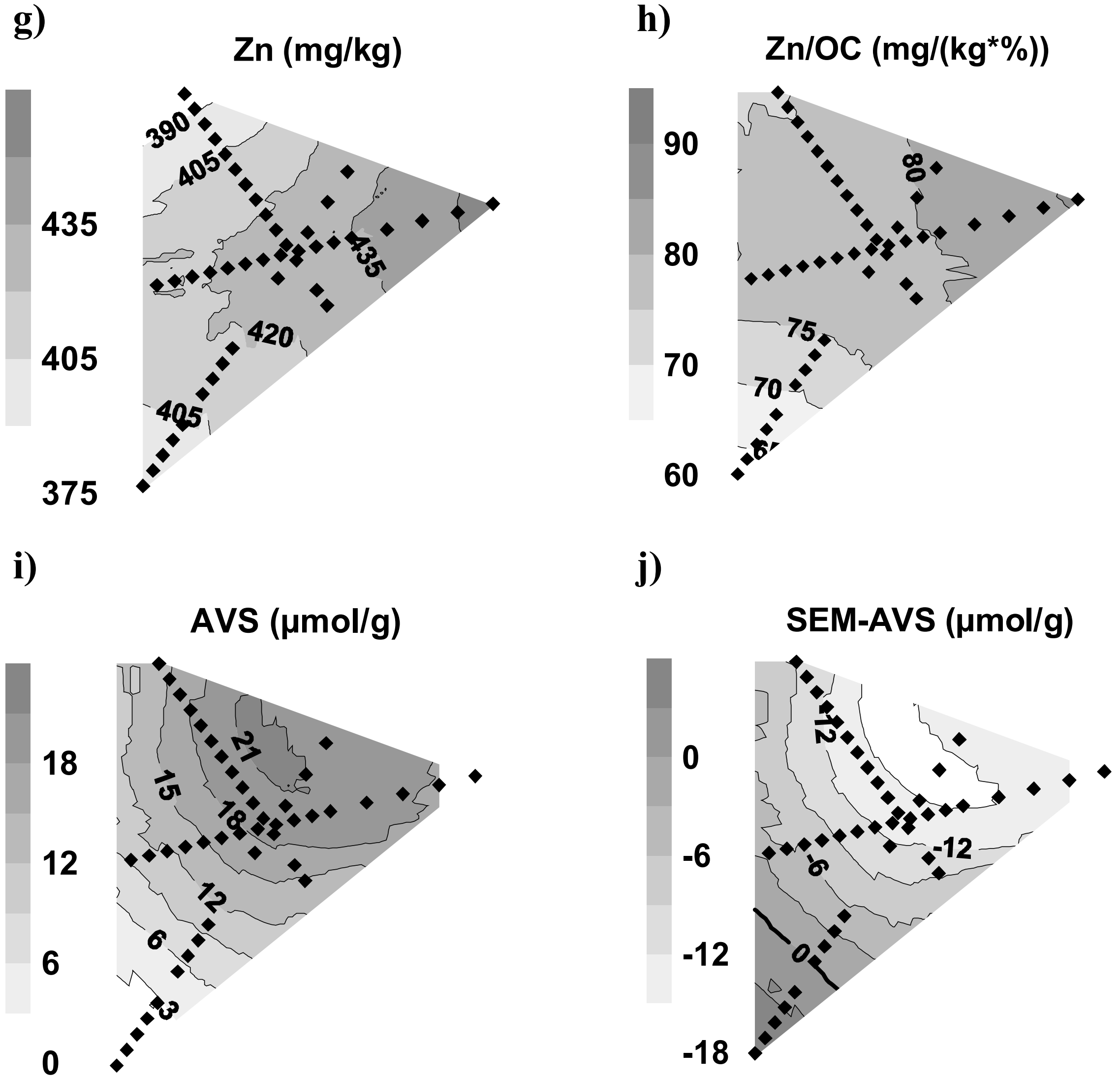


Figure 5.4. Contour plots for a) depth, b) E_h, c) OC, d) total S, e) Zn, f) Cu, g) Zn/OC, h) Cu/OC, i) AVS, j) SEM-AVS. The bold line in figure 4j indicates the boundary between positive and negative SEM-AVS values. OC = organic carbon; AVS = acid volatile sulfide; SEM = simultaneously extracted metals.

The pattern of lake depth (Figure 5.4a) clearly shows that the southwestern corner is more shallow and that depth increases relatively slowly compared with the other corners. The redox potential shows a very patchy pattern, which may be reflective of the high dynamics in redox processes. Organic carbon is highest in the southwestern corner, possibly due to a larger primary production in this part that is terrestrial most of the year. Total S increases to the central part of the lake. For total trace-metal concentrations, illustrated by Zn and Cu (Figure 5.4e and 5.4f) and also Cd and Pb, the general trend is an increasing concentration toward the eastern part of the lake. After normalization on organic carbon, the highest values still occur in the northeastern part (Figure 5.4g and 5.4h), but the spatial pattern becomes more gradual. So part of the spatial variability in trace-metal concentrations disappears after normalization on organic carbon. The spatial pattern of AVS (Figure 5.4i) resembles the spatial patterns of depth and total S (Figure 5.4a and 5.4d), which is consistent with the high correlation between these variables. The relationship of AVS with depth was also found by Howard and Evans (1993) in mesotrophic lakes of about 15-m depth. They concluded that AVS varied spatially depending on lake morphology causing sediment focusing (settling of fine sediment fraction). In our case, we cannot confirm a spatial relationship between AVS and clay because clay content shows no clear spatial dependency (Table 5.3), but a correlation between these variables was confirmed in PCA (Table 5.2). Redox conditions favor sulfate reduction throughout the whole lake, so a clear spatial correlation between E_h and AVS is not necessarily expected. There is, however, still an overlap of E_h minimum and AVS maximum values (Figure 5.4b and 5.4i). Apparently, AVS has accumulated more in the most reduced parts because, even when redox conditions are changing due to varying water tables and seasonal impacts, these spots remain relatively reduced. Discrepancies between patterns can be explained by the fact that redox potential E_h is based on a single moment recording, while E_h levels are highly dynamic. Accumulated AVS concentrations reflect long-term environmental conditions. The history of anoxia during which AVS could form is probably different in places with different E_h. Another reason for discrepancies between AVS and E_h patterns is that AVS concentration is measured as an average over the 0- to 5-cm layer while E_h is recorded in the middle of these 5 cm. AVS is known to vary with sediment depth (Van den Berg *et al.*, 1998, 2001; Van Griethuysen *et al.,* 2005/Chapter 4). Because of the lack of (spatial) variation in SEM, the spatial variation in SEM-AVS (Figure 5.4j) shows an opposite pattern to that of AVS.

5.3.4 Implications for risk analysis

Current risk assessment of polluted soils and sediments is often based on normalization on clay and organic carbon content, which is justified because organic carbon and clay provide important sorption surfaces under aerobic conditions. This normalization procedure, however, does not account for precipitation of trace metals with sulfides under reduced conditions. When we evaluate the spatial pattern of total or organic carbon normalized trace metals (Figure 5.4e through 5.4h), the highest concentrations occur in the northeastern corner of the lake. Kriging of clay-normalized metal concentrations results in a variogram for which no significant model can be found (see Table 5.3). This means that no spatially dependent variation can be detected.

AVS concentrations increase sharply from the southwestern to the north-northeastern part of the lake. This large gradient can be explained by lake depth, i.e., the southwestern corner of the lake is very shallow and only inundated during the winter period. This means that during a large part of the year, oxygen can penetrate deeper into the sediment. Therefore, AVS is subject to frequent periods of degradation and formation, especially in this corner. At the moment of sampling (January), AVS formation may not have been completed due to kinetic constraints, which may be caused by a lack of recruitment by sulfate-reducing bacteria or by a slowly dropping redox potential. The phenomenon of slow AVS kinetics is also supported by findings of Vink (2001), who showed that sulfide formation did not occur within a sixmonth period in terrestrial floodplain sediment stored under water. In a parallel study (Van Griethuysen *et al.*, 2006/Chapter 3), we observed that it took days to weeks to yield significant sulfide formation in floodplain lake sediments that were oxidized for a short period and subsequently reduced. Recruitment by benthic infauna can, however, occur faster than these time scales (Peeters, Wageningen University, personal communication), which would lead to exposure to elevated trace-metal pore-water concentrations. Because SEM is relatively constant across the lake, the SEM-AVS pattern is almost completely opposed to the AVS pattern. The SEM-AVS concentrations are smaller than zero throughout most of the lake, but they increase to values greater than zero in the southwestern part, indicating a potential availability of trace metals. The pattern of SEM-AVS (Figure 5.4j) shows a zero boundary line separating two regions within the lake where SEM-AVS is either positive or negative. This means that sediment in the northern (major) part of the lake should be considered nontoxic for benthic detritivores. In contrast, AVS is not high enough to bind all SEM in the southwestern part of the lake. Thus, potential ecological risk from metals is highest in this corner. The concentration of SEM-AVS exceeds the proposed limit of 1.7 to 2 µmol/g below which no toxic effects are to be expected (Sijm *et al.*, 2001). The proposed toxicity limit for SEM-AVS/f_{OC} is 100 to 150 µmol/g OC (Sijm *et al.*, 2001). Trespassing of this limit does not occur in this floodplain lake sediment.

5.4 Conclusions

The results of this study clearly show that common alternative sediment quality criteria like SEM-AVS and (normalized) total metal concentrations may yield different priority settings for contaminated sites due to differences in spatial variability of the underlying variables. Small-scaled spatial variation is essential to address in risk assessment of trace metals. It is demonstrated that—even at almost constant metal or SEM concentrations—metal-associated risks may vary among sites because of spatial variation in environmental conditions like redox and subsequently AVS. The availability of trace metals shows a different and much larger spatial heterogeneity than total or normalized trace-metal concentrations. This is ignored when environmental conditions and occurrence and importance of metal-sulfide precipitation are not considered in risk assessment. Spatial analysis further illustrates the limited use of risk estimates for water bodies based on single or a small number of observations.

Acknowledgements

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CHAPTER 6

SAMPLING METHOD, STORAGE AND PRETREATMENT OF SEDIMENT AFFECT AVS CONCENTRATIONS WITH CONSEQUENCES FOR BIOASSAY RESPONSE

Based on: H.J. de Lange, C. van Griethuysen, A.A. Koelmans (2006)

Submitted to *Environmental Toxicol Chem*

Abstract

One of the major problems with laboratory-bioassays testing field-collected sediment is the preservation of the original chemical and physical state of the sediment. Redox changes in the sediment may change metal availability and animal exposure considerably. We studied the effect of sampling method and sample pretreatment on bioassay response. We used Acid Volatile Sulfide (AVS) concentrations as a measure for the disturbance of the sediment. AVS and Simultaneously Extracted Metal (SEM) concentrations were compared to bioassay responses with the benthic macroinvertebrate *Asellus aquaticus*. Storage conditions appeared to affect AVS but not SEM levels of the sediment. Freezing is the best way to preserve AVS levels. The different sediment pretreatments affected AVS but not SEM levels of the sediment. AVS declined during storage at 4ºC and after sieving the sediment. The best way to pretreat the sediment for use in a bioassay in order to maintain initial AVS conditions was to sample the sediment with an Ekman grab, immediately store it in a jar without headspace, and freeze it as soon as possible. The ranking of the 7 lakes based on SEM levels and bioassay response was similar to the previously established ranking based on total metals and organic contaminants. Bioassay response of *Asellus aquaticus* was related to SEM and AVS characteristics of the sediment.

6.1 Introduction

Sediment contamination is a serious problem in river sedimentation areas, such as floodplain lakes, delta areas and estuaries. Sediments in Dutch floodplain areas are moderately to heavily polluted with trace metals and organic contaminants like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Den Besten *et al.*, 1995; Reinhold-Dudok van Heel *et al.*, 1999; De Haas *et al.*, 2002; De Lange *et al.*, 2004; Van Griethuysen *et al.*, 2004). The relationship between sediment contamination and effects on biota has been studied through various approaches. These include studies into the effects of sediment contamination on in situ macroinvertebrate communities (e.g., Van Griethuysen *et al.*, 2004; Schlekat *et al.*, 1994; Pinel-Alloul *et al.*, 1996; Peeters *et al.*, 2000 and 2001), and effects on single species in situ and in laboratory bioassays (e.g., De Haas *et al.*, 2002; Chappie & Burton, 1997; US-EPA, 2000; OECD, 2001; Den Besten *et al.*, 2003). The usefulness of bioassay results in predicting in situ effects may be limited (Peeters *et al.*, 2001), because biotic responses are usually attributed to total contaminant concentrations, which give good estimates of the degree of pollution, but may not be fully available to biota. Several extraction techniques, such as Tenax extraction for organic contaminants (Cornelissen *et al.*, 2001) and the SEM-AVS method for trace metals (DiToro *et* *al.*, 1990), have been applied to estimate the toxicant concentrations that are available for benthic organisms. Accordingly, these concentrations are usually termed bioavailable concentrations.

Acid Volatile Sulfide (AVS) concentrations can provide insight in the chemical state of a sediment. Sulfides are formed in reduced sediments and are susceptible to oxidation by e.g. resuspension or desiccation of sediment. Therefore, the common precaution for AVS analysis, is to minimize contact with oxygen and to perform analyses as soon as possible. Furthermore, AVS is important in controlling trace metal availability, since sulfides precipitate with metals and become unavailable for organisms. AVS concentrations can be compared to Simultaneously Extracted Metal concentrations (SEM) to evaluate potential sediment toxicity. (SEM-AVS)/f_{OC}, the difference between extracted metals and sulfides, normalized on the fraction organic carbon, is proposed as a proxy for trace metal bioavailability (DiToro *et al.*, 2005). Sediments with (SEM-AVS)/f_{OC} values below 150 (µmol g^{-1} OC) are considered to be safe. The range of uncertainty is between 150 and 3400 (µmol g^{-1} OC), and sediments with (SEM-AVS)/f_{OC} values above 3400 (µmol g^{-1} OC) are considered acute toxic. De Lange *et al.* (2005) indeed found a good relation between bioassay response of the water louse *Asellus aquaticus* and (SEM-AVS)/f_{OC} concentrations in Biesbosch sediments, whereas Van Griethuysen *et al.* (2004) and De Lange *et al.* (2004) found such relations at community level.

Pretreatment of field-collected sediment often includes sieving, stirring and storage. This however can have effects on chemical and physical properties of the sediment studied, while usually the aim of bioassay testing is to get insight in sediment toxicity under *in situ* conditions. Hence, the sampling protocol should aim at bringing a small part of “the field” back to the laboratory without disturbance. However, practical limitations will arise because sampling will inevitably disturb the natural condition of the sediment. For example, sampling and sieving of the sediment will result in changes in redox conditions of the sediment causing a loss in AVS (Bull & Williams, 2002) which may affect the bioavailable metal concentrations (Lasorsa & Casas, 1996), or result in changes in DOC mobility also affecting metal behaviour (Koelmans & Prevo, 2003). Indigenous fauna that may prey on or compete with the test organism should be removed from the sediment. Commonly used methods are sieving or freezing the sediment. This will affect physico-chemical properties of the sediment. Which method is best to remove endemic species should be determined on a case-by-case basis and is specific to the organism used in toxicity tests (Day *et al.*, 1995). Storage of the sample may also change physico-chemical properties of the sediment. Temperature and duration of storage can influence sediment toxicity. One study recommends fresh storage (4 °C) for no longer than a few days, and frozen storage (-20 °C) for prolonged storage of field sediments (Beiras *et al.*, 1998). However, another study showed no change in biological responses after extended storage of toxic field sediments at 4 °C (Defoe & Ankley, 1998). All these studies show that sediment treatment and sediment storage may alter sediment toxicity, and consequently biotic response. No study so far has combined these three elements. Our aim was to do so, therefore we studied the effect of sampling method and sample pretreatment on trace metal availability and bioassay response. We used AVS concentrations as a measure of disturbance of chemical conditions in the sediment. AVS and SEM concentrations were used to infer trace metal availability and were compared to bioassay responses. The benthic macroinvertebrate *Asellus aquaticus* was used as bioassay organism. This organism is robust and widely used in trace metal and PAH bioaccumulation studies with sediment (e.g., Van Hattum *et al.*, 1996; Peeters *et al.*, 2000a).

To summarize, our objectives were:
1) to study the effect of sediment storage on AVS and SEM concentrations;
2) to study the effect of sediment pretreatment on AVS and SEM concentrations and bioassay response;
3) to investigate the relation between AVS and SEM concentrations and bioassay response for a range of sediments.

6.2 Materials and methods

6.2.1 Study area

Sediment from 7 floodplain lakes along the river Waal was sampled in July 2002 (Figure 6.1). The lakes were selected from a large set of Dutch floodplain lakes (Roozen *et al.*, 2003; Van Geest *et al.*, 2003) and have been subject of earlier ecotoxicological studies (De Haas *et al.*, 2002; Koelmans & Moermond, 2000). Sediment of lake Deest Oost 4 (DeO4), also included in the previously mentioned 7 lakes, was used for the AVS storage and sediment pretreatment experiments (1 and 2). This lake has also been subject of a study into spatial variation of AVS (Van Griethuysen *et al.*, 2003). Sediment of all seven lakes was used to investigate the relation between AVS and SEM concentrations and bioassay response (experiment 3).

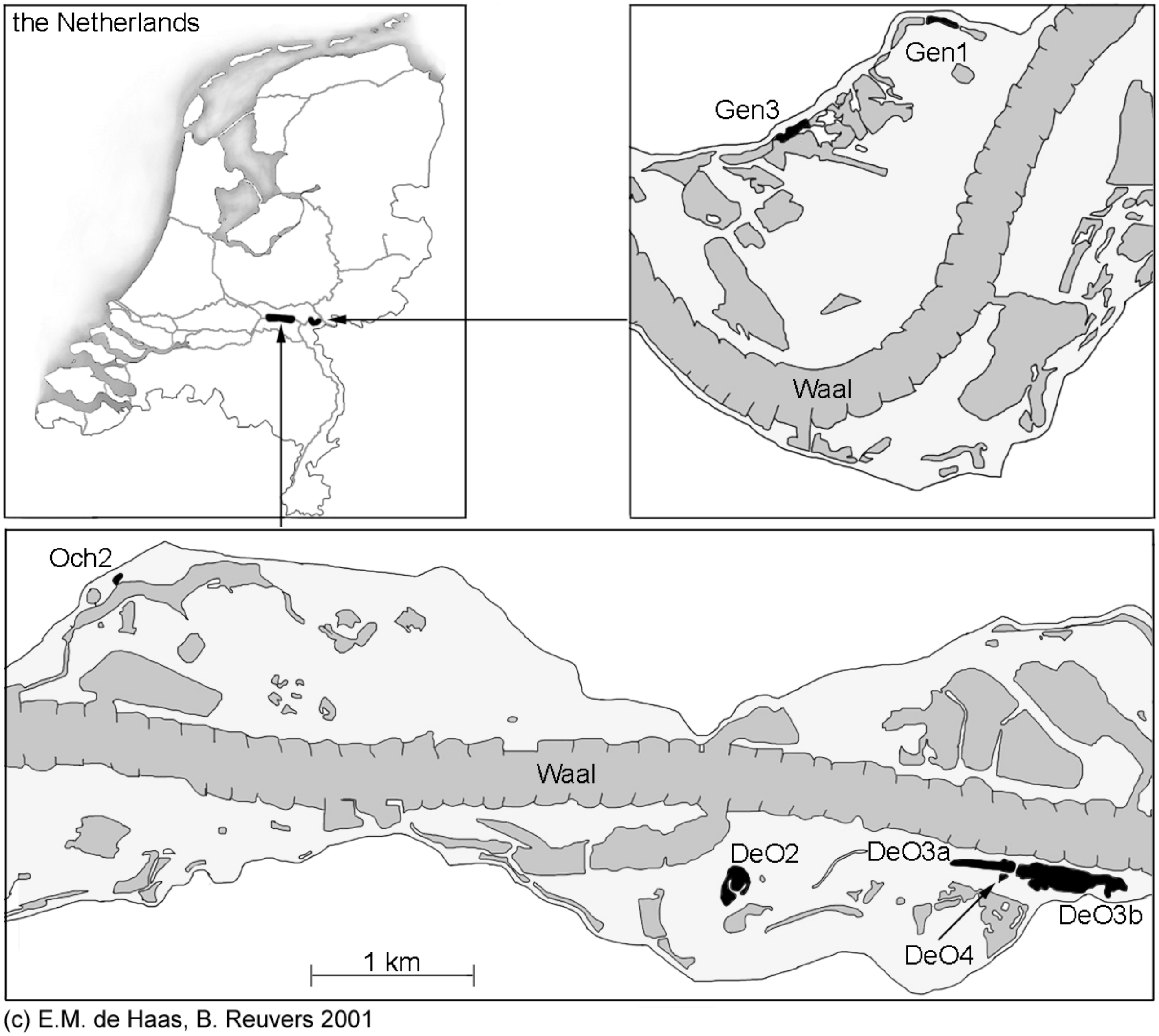

Figure 6.1. Sampled floodplain lakes along the River Waal, The Netherlands. Locations are Ochten 2 (Och2), Gendt 1 (Gen1), Gendt 3 (Gen3), Deest Oost 2 (DeO2), Deest Oost 3A (DeO3a), Deest Oost 3B (DeO3b) and Deest Oost 4 (DeO4).

6.2.2 Sediment sampling and handling

In experiment 1, the effect of different types and periods of sample storage on AVS and SEM concentrations was examined (no bioassay was performed in experiment 1). Lake DeO4 was sampled manually with Jenkins core samplers. Cores were transported back to the laboratory and stored cool or frozen, according to Table 6.1. Four replicate cores were analyzed for each storage condition. AVS analysis was done on two layers: 0-2 cm and 2-5 cm.

Table 6.1. Storage conditions of sediment cores in Experiment 1: AVS storage study

Storage duration	4 °C	-20 °C
1 day (initial measurement)	X	
2 weeks	X	
4 weeks	X	X
12 weeks	X	X

In experiment 2, sediment from DeO4 used in the sediment treatment experiment was manipulated according to Table 6.2. The core samples were obtained from experiment 1. The grab samples were obtained using an Ekman grab. The upper 5 cm of the sediment was sampled, quickly mixed in the field and stored in PE bottles without headspace. Sediment in the sieving treatment was transported back to the laboratory in a 3 L bucket, and sieved over a 500-µm sieve. Storage was either cool (4 °C) or frozen (-18 °C). The sediments were analyzed for AVS, SEM, and % organic matter (OM), and a bioassay with *Asellus aquaticus* was performed.

Table 6.2 Treatment of sediment in Experiment 2 used in bioassay; storage duration was 1 month.

Sample code	Sampling device	Sieving	Storage temperature
core-freeze *	Jenkins corer	no	-20 °C
core-cool *	Jenkins corer	no	4 °C
grab-freeze	Ekman grab	no	-20 °C
grab-cool	Ekman grab	no	4 °C
sieve-freeze	Ekman grab	yes	-20 °C
sieve-cool	Ekman grab	yes	4 °C

* same samples as in Experiment 1 were used.

For experiment 3, sediments from the 7 floodplain lakes were sampled with an Ekman grab, quickly mixed in the field and stored in PE bottles without headspace to maintain redox conditions as much as possible. Samples were frozen (-18 °C) within 4 hours after sampling. The sediments were analyzed for AVS, SEM, grain size fractions and % organic matter (OM), and a bioassay with *Asellus* was performed.

6.2.3 Sediment analyses

Concentrations of AVS and SEM were determined according to Van Griethuysen *et al.* (2002). In short, sulfides were volatilized with 1 M HCl and detected with a sulfide specific electrode (Ag500, WTW, Weilheim, Germany). Detection limit was 0.1 µmol/g dry sediment and accuracy was between 10 and 15 % (Van Griethuysen *et al.*, 2002). Simultaneously Extracted Metal concentrations were analyzed in the remaining sediment suspension after filtration over a pre-rinsed 0.45 µm membrane filter (Schleicher & Schuell, 's Hertogenbosch, The Netherlands) using ICP-MS (Elan 6000, Perkin Elmer Sciex Instruments, Concord, ON, Canada). Organic matter content was determined as loss on ignition and converted into fraction OC by dividing % OM with 0.01724 (Van de Guchte *et al.*, 2000). Grain size fractions were determined for sediments used in the survey by sieving a known mass of sediment over different sieve mesh sizes (2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.050 mm). Each size fraction was dried (overnight at 105 °C), weighed, ashed (3 h at 550 °C) and weighed again. Size fractions were calculated as weight percentages.

6.2.4 Asellus aquaticus bioassay

Survival and growth of the waterlouse *A. aquaticus* on field sediments was determined in a 28-d experiment. Bioassays were performed 1 month after sampling. Asellids in the size class 4-6 mm were obtained from a non-polluted pool in Doorwerth, the Netherlands. Sediments were thawed at 4 °C four days before the start of the experiment. Samples were placed in a glovebag under nitrogen, to ensure anaerobic conditions, and were thoroughly mixed. Thirty ml of sediment was placed in a 100-ml jar, with 15 replicate jars for each sediment. Immediately after removal from the glovebag, 40 ml oxygen-free (nitrogen purged) Dutch Standard Water (DSW; NNI, 1980) was added. The samples were then exposed to air to allow passive reaeration of water and sediment in each jar. Control treatment contained 30 ml quartz sand (50- to 500-µm grain size) mixed with 10% organic material (decomposing poplar leaves, 500- to 2000-µm size range), and 40 ml oxygen-free DSW. After ten days, when oxygen levels were > 70% saturation, 1 *A. aquaticus* was added in each jar. The jars were placed in a temperature controlled room with a temperature of 17 ± 0.5 °C in dim light (6 µmol m^{-2} s^{-1}) at 16:8 L:D rhythm. Initial and final length of each individual was measured with a Leica Image Analyser. Growth was calculated by subtracting the individual initial length from the individual final length. Growth and survival are expressed as % of growth and survival in control treatment. Standard errors of survival were computed using a Jackknife procedure (Meyer *et al.*, 1986).

6.2.5 Statistical analyses

Treatment effects were analyzed with ANOVA, after a check for homogeneity of variances with Levene's test. Differences in survival of *Asellus* were tested with a z-test (according to Meyer *et al.*, 1986). Relations between sediment, water, and pollution characteristics and bioassay responses were evaluated using principal component analysis (PCA) with VARIMAX rotation. All statistical analyses were conducted using SPSS® 11.5 for Windows (SPSS, Chicago, IL, USA).

6.3 Results

6.3.1 Experiment 1: effect of storage conditions on AVS and SEM

The different storage conditions had an effect on AVS (Figure 6.2). AVS in the top layer (0-2 cm) increased significantly compared with the initial measurement during cool storage, probably as a result of oxygen consumption and prolonged sulfate reduction in the sediment core. The bottom layer (2-5 cm) did not show significant alterations from the initial measurement. Freezing of the sediment did not significantly affect AVS compared with initial measurements in any of the layers. AVS concentrations were significantly higher in the bottom layer than in the top layer, which is consistent with known vertical patterns. SEM concentrations varied slightly between storage conditions, without significant differences between time, layer or storage temperature (Table 6.3).

Table 6.3. Storage effects on SEM concentration ± SD. No significant effects observed between storage conditions or layers (ANOVA p>0.05).

Storage condition	**SEM (µmol/g DW) Layer 0-2 cm**	**SEM (µmol/g DW) Layer 2-5 cm**
Initial measurement	5.31 ± 1.22	5.55 ± 0.23
2 weeks at 4 °C	5.37 ± 1.55	5.05 ± 1.23
4 weeks at 4 °C	4.09 ± 0.18	4.63 ± 0.30
8 weeks at 4 °C	4.04 ± 0.21	4.75 ± 0.40
4 weeks at -18 °C	3.90 ± 0.09	4.59 ± 0.27

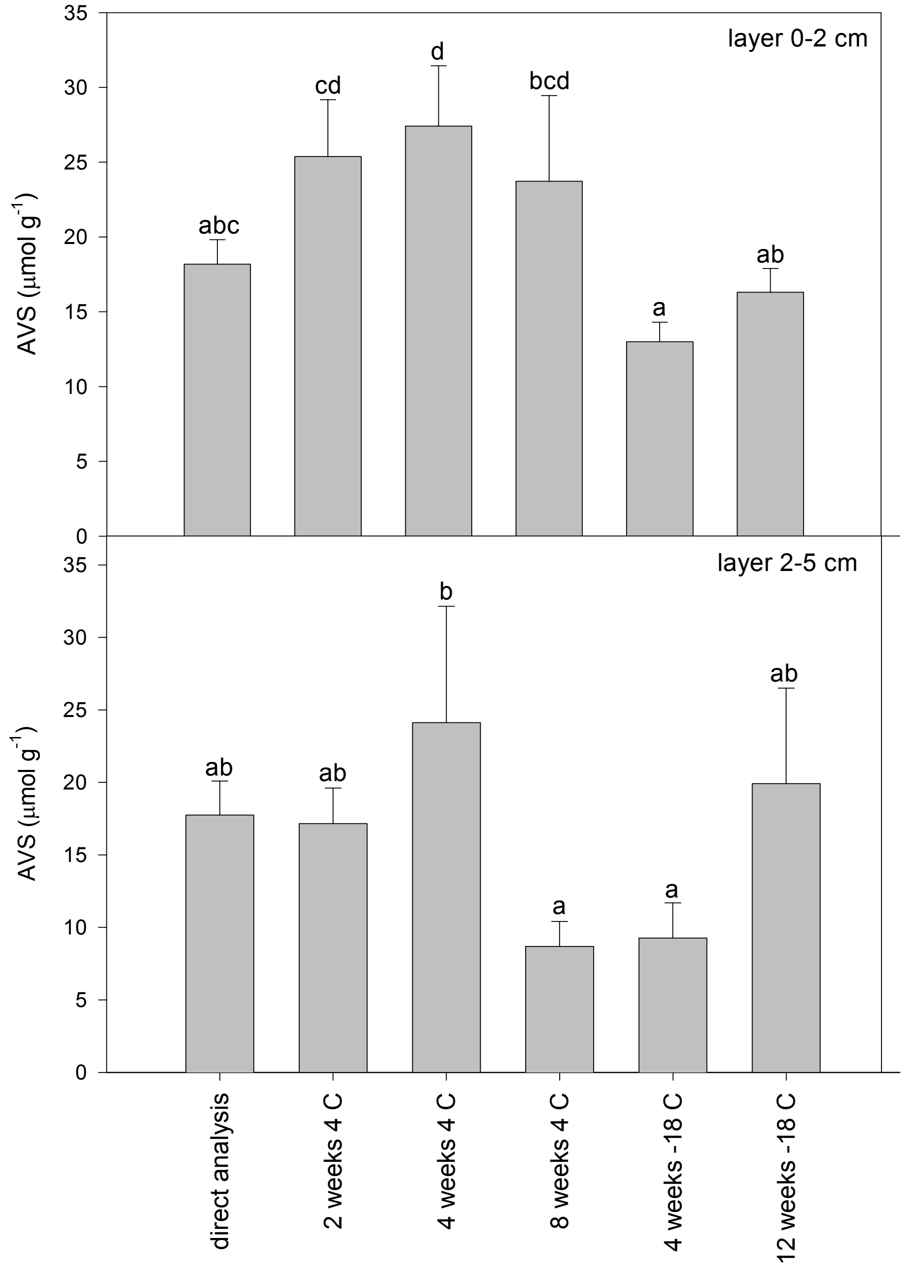

Figure 6.2. Effect of different storage conditions on AVS concentration of layer 0-2 cm (upper panel) and layer 2-5 cm (lower panel); error bars = SD; lower case letters indicate homogeneous groups (ANOVA + posthoc Tukey test, $p<0.01$).

Calculated values for (SEM-AVS)/f_{OC} showed an inverse pattern to AVS (Figure 6.3).

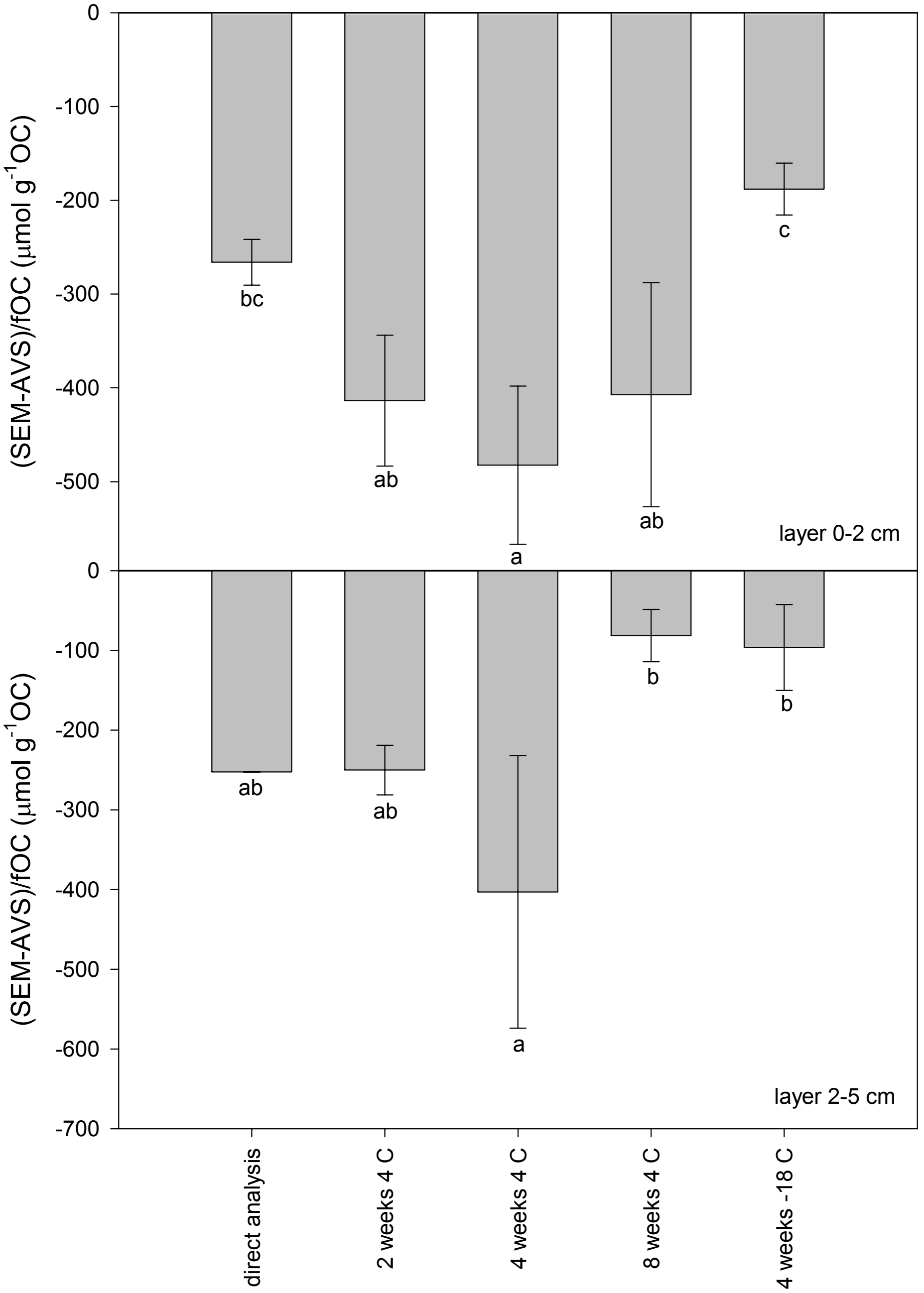

Figure 6.3. Effect of different storage conditions on (SEM-AVS)/f_{OC} of layer 0-2 cm (upper panel) and layer 2-5 cm (lower panel); error bars = SD; lower case letters indicate homogeneous groups (ANOVA + posthoc Tukey test, $p<0.01$).

A significant decrease of (SEM-AVS)/f_{OC} in the top layer occured during cool storage after 4 weeks, compared with initial measurement. The bottom layer did not show significant alterations from the initial measurement. (SEM-AVS)/f_{OC} values were significantly higher in the bottom layer compared with the top layer, in a similar pattern as AVS concentrations.

6.3.2 Experiment 2: effect of sediment pretreatment on Asellus bioassay results

Survival of *Asellus* in the control was 93%; and average control growth was 0.70 mm. Survival on the different pretreated sediments differed, with the lowest survival on grab-freeze sediment (Figure 6.4). This treatment did however support the highest growth (Figure 6.4). AVS concentrations differed between the sediment pretreatments. The highest AVS level was observed in the grab-freeze treatment, which decreased slightly during the experiment. AVS levels declined more in all other treatments during the experiment (Table 6.4). The prolonged anoxic conditions during the bioassay in the grab-freeze treatment may have caused the poor survival on this sediment type (Figure 6.5).

Table 6.4 AVS and SEM characteristics after different sediment treatments in Experiment 2.

Sample code	**AVS start bioassay (µmol/g DW)**	**AVS end bioassay (µmol/g DW)**	**SEM start bioassay (µmol/g DW)**	**(SEM-AVS)/f_{OC} start bioassay (µmol/g OC)**
core-freeze *	9.4	7.4	4.1	-196
core-cool *	24.1	10.4	4.3	-435
grab-freeze	30.9	21.9	4.3	-633
grab-cool	30.5	9.2	4.4	-591
sieve-freeze	25.5	13.1	5.0	-557
sieve-cool	29.3	11.5	5.7	-643

* Same samples as in Experiment 1 were used. Both layers (0-2 and 2-5 cm) were mixed prior to AVS analysis and use in bioassay.

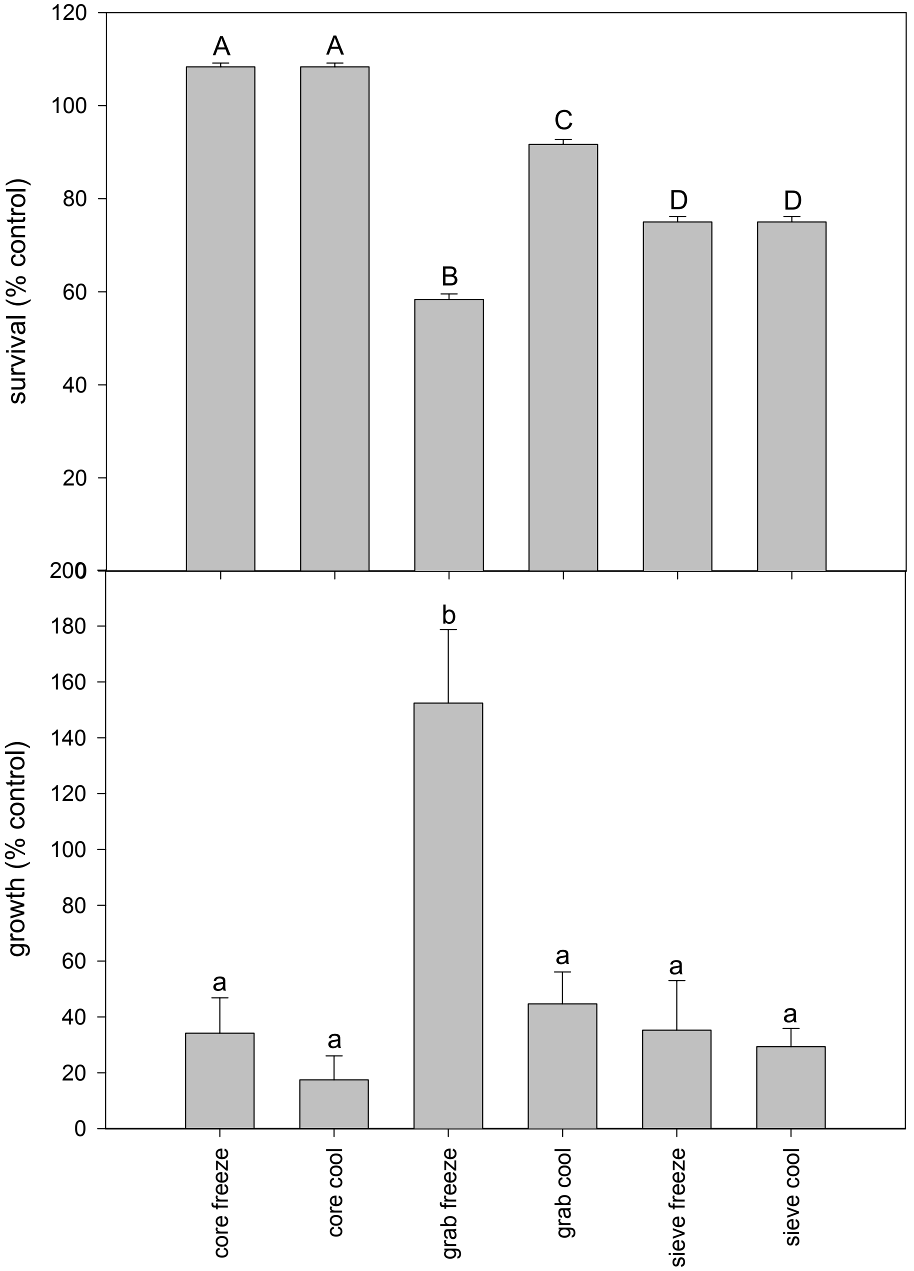

Figure 6.4. Survival (upper panel) and growth (lower panel) of *Asellus aquaticus* on different pretreated sediments. Error bars indicate standard errors; upper case letters indicate homogeneous groups after z-test ($p<0.01$), lower case letters indicate homogeneous groups after ANOVA + posthoc Tukey ($p<0.05$).

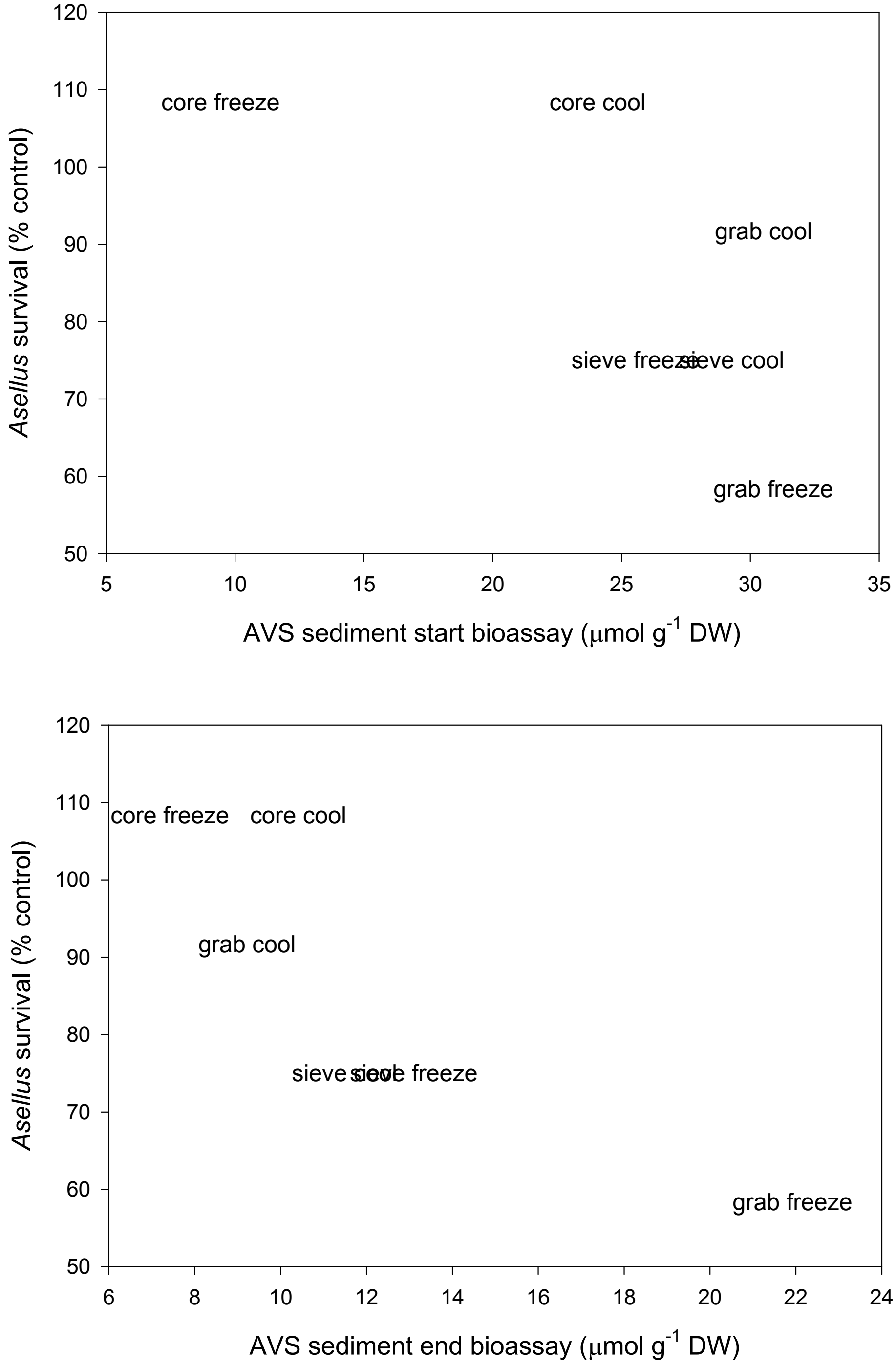

Figure 6.5. *Asellus* survival plotted against AVS concentration in sediment at start (upper panel) and end (lower panel) of bioassay. Labels indicate treatments.

6.3.3 Experiment 3: Results of Asellus bioassay on different floodplain lake sediments

Highest survival and growth are found on sediment from Gendt 1 and Gendt 3, which are the least contaminated sites (Figure 6.6). Survival of *Asellus* in the control was 87%; and average control growth was 3.78 mm. Lower survival and growth on DeO2 sediment may be caused by low organic matter content, which mostly consisted of ultrafine organic matter (Table 6.5). Survival and growth are both negatively correlated with (SEM-AVS)/f_{OC} (Figure 6.7), although values are well below the threshold level of 150 (µmol g^{-1} OC) proposed by DiToro *et al.* (2005).

Table 6.5. Sediment characteristics and AVS and SEM concentrations of different floodplain lake sediments used in Experiment 3.

Location*	**AVS (µmol/g DW)**	**SEM (µmol/g DW)**	**(SEM-AVS)/ f_{OC} (µmol/g OC)**	**Grain size fraction > 500 µm**	**OM (% DW)**	**OM % < 50 µm**
DeO2	10.5	1.3	-424	0.8	4.3	81.2
Gen1	58.8	1.5	-1199	4.6	8.4	57.0
Gen3	43.0	3.5	-732	3.2	10.1	59.3
Och2	20.4	4.2	-307	2.2	11.3	69.2
DeO3A	20.2	4.8	-342	1.2	10.0	74.2
DeO3B	25.9	3.9	-571	1.1	7.8	67.2
DeO4	32.7	4.0	-673	1.8	8.3	63.6

* see Figure 1 for lake codes

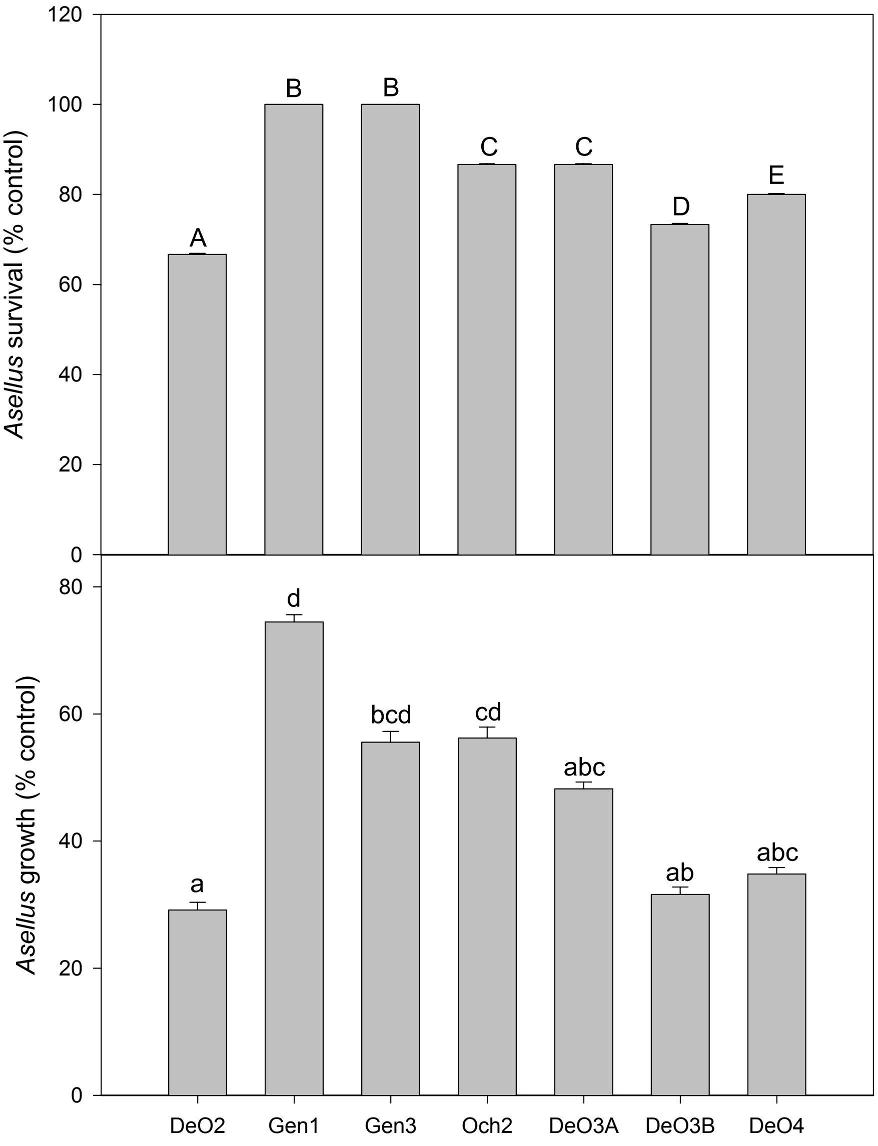

Figure 6.6. Survival (upper panel) and growth (lower panel) of *Asellus aquaticus* on sediment of different floodplain lakes. Lake codes as in Figure 1; error bars indicate standard errors; upper case letters indicate homogeneous groups after z-test ($p<0.01$), lower case letters indicate homogeneous groups after ANOVA + posthoc Tukey ($p<0.05$). Sediments are ordered in increasing level of pollution (DeO2<<DeO4).

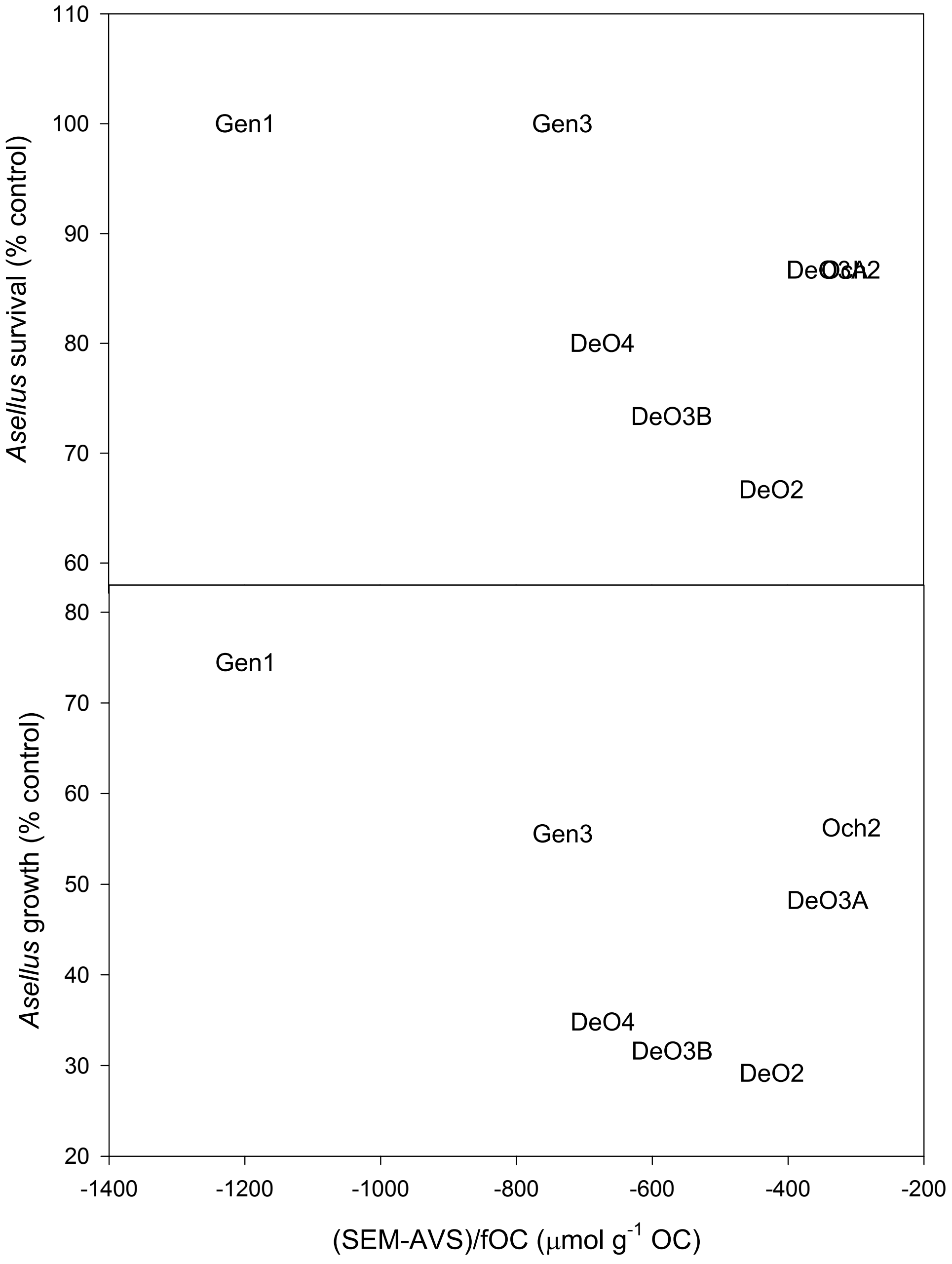

Figure 6.7. Survival (upper panel) and growth (lower panel) of *Asellus* versus (SEM-AVS)/f_{OC}. Labels indicate lake codes (see Figure 6.1).

6.4 Discussion

Storage conditions had some effects on AVS concentration, especially in the layer 0-2 cm. AVS levels increased during cool storage, probably caused by oxygen consumption and prolonged sulfate reduction in the sediment core. No significant alterations from the initial measurement were observed in the layer 2-5 cm or in the frozen storage treatment. Since SEM concentrations were not affected by storage condition, the (SEM-AVS)/f_{OC} of the sediment was solely determined by fluctuations in AVS. Our results suggest that after storage the highest ecological risk can be found in the deeper layers, although values are well below the threshold level of 150 (μmol g^{-1} OC) proposed by DiToro *et al.* (2005). The increase in AVS during cool storage in the upper layer will result in a decreased risk of trace metals for biota. This is relevant if the sediment would be used in bioassays, since prolonged storage would result in an underestimation of ecological risk.

The different sediment pretreatments resulted in differences in AVS levels. The grab-freeze treatment was the treatment with the lowest level of manipulation. This treatment resulted in the highest levels of AVS, with only a slight decline during the 4 weeks of the bioassay experiment. The other treatments, which all included some form of manipulation or disturbance, all resulted in lower AVS levels at the start of the bioassay, and a large decline in AVS levels during the bioassay. Homogenization of sediment results in loss of AVS (Bull & Williams, 2002). This is clearly expressed in the sediment from the cores, which were first divided for AVS and SEM measurements in separate layers and later pooled for the assay, resulting in lower AVS concentrations at the start of experiment 2 compared to those measured in experiment 1 (viz. Table 6.3 and 6.4). Sulfide levels are best maintained when samples have minimal exposure to oxygen, and are stored at 4 °C or frozen (Lasorsa & Casas, 1996). A comparative laboratory study showed that the decrease in AVS is already significant after 30 minutes of passive aeration (Van Griethuysen *et al.*, 2002).

The high levels of AVS and concurrent low oxygen levels may have caused the low survival of *Asellus* (47%) on the grab-freeze treated sediment. However, the same sediment treated the same way in experiment 3 resulted in a survival of *Asellus* of 80 %. AVS and SEM concentrations were similar in experiment 2 and 3. Thus, there seems to be some variation in *Asellus* performance between experiments, as indicated by this observed difference in survival on the same sediment. Also, growth of *Asellus* in the control was higher in experiment 3, compared with experiment 2. This may be explained by the average length at the start, which was 4.5 mm in experiment 3, and 5.8 in experiment 2. By normalizing the growth results on % of control growth, we tried to overcome the difference between experiments.

Reduced sulfur species in sediment may be a direct cause of toxicity in contaminated anoxic sediments (Brouwer & Murphy, 1995; Wang & Chapman, 1999). A comparison of sulfide toxicity to freshwater invertebrates showed that *Asellus* was the most tolerant species to sulfide (Wang & Chapman, 1999). The indirect positive effect of AVS is the immobilization of metals. From our results, we cannot unambiguously distinguish between potential negative effects of sulfide toxicity and/or oxygen deficiency and potential positive effects of decreased metal toxicity.

Growth was highest on the grab-freeze treated sediment; all other treatments had a significantly lower growth. The grab-freeze sediment manipulation is probably the best preservation of the organic matter content and size fractionation. Apparently, the higher AVS levels did not have a direct negative effect on *Asellus* growth, but an indirect positive effect by immobilizing metals (lower (SEM-AVS)/f_{OC}, Table 6.4). Survival and growth of *Asellus* in experiment 3 were both negatively correlated with (SEM-AVS)/f_{OC} (Figure 6.7), although values are well below the threshold level of 150 (μmol g^{-1} OC) proposed by DiToro *et al.* (2005). This finding suggests that either the threshold level has been set too high, because apparently effects can be observed at lower values, or that other sediment factors (e.g., other pollutants) co-inciding with (SEM-AVS)/f_{OC} cause the observed effects, thus interfering with the observed correlation. However, our finding does indicate that AVS can be used as a proxy for sediment disturbance, which is correlated with bioassay results.

SEM concentrations are indicative for contamination in the 7 lakes, since they follow the same ranking as previously established based on total concentrations (De Haas *et al.*, 2002; Koelmans & Moermond, 2000). Highest SEM concentration in this study was 5 μmol g^{-1}, which is low compared with other studies in floodplain areas: concentrations in the Biesbosch were up to 25 μmol g^{-1} (De Lange *et al.*, 2004), concentrations in ten different floodplain lakes along the rivers Rhine, IJssel and Waal in the Netherlands were up to 14 μmol g^{-1} (Van Griethuysen *et al.*, 2004).

Combining the results of the 7 lakes of this study with the data of De Haas *et al.* (2002) in a principal component analysis (PCA) gives us the opportunity to assess in what way different sediment characteristics are related to biotic responses. The total dataset consisted of 16 sediment food and contaminant characteristics, and growth and survival in three separate bioassays; all measured for the 7 lakes. The bioassays were conducted with *A. aquaticus* (this study), the mayfly nymph *Ephoron virgo* (De Haas *et al.*, 2002), and the chironomid larva *Chironomus* *riparius* (De Haas *et al.*, 2002). The bioassay with *E. virgo* and *C. riparius* was both unfed and fed during the bioassay. The PCA analysis showed a clear distinction between the three bioassays, each species is loaded on a different component (Table 6.6).

Table 6.6. Results of Principal Component Analysis with Varimax rotation. Bioassays and sediment characteristics were measured in different studies; superscript refers to reference: a = this study, b = De Haas *et al.* (2002).

Variables	**Component**			
	1	**2**	**3**	**4**
cumulative variance explained	30.9 %	57.3 %	78.9 %	94.3 %
Asellus survival ^{a}			0.871	
Asellus growth ^{a}			0.784	
Ephoron survival unfed ^{b}	-0.940			
Ephoron growth unfed ^{b}	-0.895			
Ephoron survival fed ^{b}	-0.888			
Ephoron growth fed ^{b}	-0.881			
Chironomus survival unfed ^{b}				-0.836
Chironomus growth unfed ^{b}		0.699		
Chironomus survival fed ^{b}				-0.889
Chironomus growth fed ^{b}		-0.965		
OM ^{b}		0.842		
grain size > 500 µm ^{a}			0.924	
OM < 50 µm ^{a}			-0.948	
AVS ^{a}			0.988	
SEM ^{a}		0.621		
(SEM-AVS)/f_{OC} ^{a}			-0.870	
Cd ^{b}	0.707			
Cu ^{b}				0.701
Zn ^{b}	0.886			
PAH ^{b}	0.924			
PCB ^{b}	0.847			
P ^{b}				0.809
Chlorophyll ^{b}		0.944		
FA ^{b}		0.938		
PUFA ^{b}		0.913		
CO_2 ^{b}		0.932		

Response of *A. aquaticus* is negatively related with bioavailable metals ((SEM-AVS)/f_{OC} and AVS). Response of *E. virgo* is negatively related with total contaminants. Response of *C. riparius* is positively related with food and not with contaminants. This distinction between the three species may be explained by differences in exposure and uptake routes of contaminants (via water, sediment particles or food), differences in ecology of the three species (behaviour and feeding) and/or by differences in sediment pre-treatment (stirring, sieving) (De Lange *et al.*, 2005). Sediment pretreatment in the *Asellus* bioassay was aimed to maintain redox conditions as much as possible. Apparently this was successful since *Asellus* responded mainly to the AVS and SEM characteristics of the sediment, and not to total contaminants. The positive relation of *Chironomus* with food and lack of relation with contaminants, is in agreement with previous findings that food abundance may mask negative effects of sediment contamination for *Chironomus* (De Haas *et al.*, 2002; Ristola *et al.*, 1999).

6.5 Conclusions

Storage conditions affect AVS levels, but not SEM levels of the sediment. Freezing is the best way to store a sample in order to preserve initial AVS concentrations. Although the different sediment pretreatments affected AVS concentrations, they did not affect SEM levels of the sediment. AVS levels declined during cool storage, and after sieving the sediment. The best way to pretreat the sediment for use in a bioassay and maintain initial AVS conditions was to use the Ekman grab to sample the sediment, immediately store it in a jar without headspace, and freeze it as soon as possible.

The ranking of the 7 lakes based on SEM levels and bioassay response was similar to the previously established ranking based on total metals and organic contaminants. Bioassay response of *Asellus aquaticus* was related to SEM and AVS characteristics of the sediment.

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CHAPTER 7

TRACE METAL AVAILABILITY AND EFFECTS ON BENTHIC COMMUNITY STRUCTURE IN FLOODPLAIN LAKES

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Abstract

Effects of contaminants on communities are difficult to assess and poorly understood. We analyzed in situ effects of trace metals and common environmental variables on benthic macroinvertebrate communities in floodplain lakes. Alternative measures of trace metal availability were evaluated, including total metals, metals normalized on organic carbon (OC) or clay, simultaneously extracted metals (SEM), combinations of SEM and acid-volatile sulfide (AVS), and metals accumulated by detritivore invertebrates (Oligochaeta). Accumulated metal concentrations correlated positively with sediment trace metals and negatively with surface water dissolved OC. Sixty-eight percent of the variation in benthic community composition was explained by a combination of 11 environmental variables, including sediment, water, and morphological characteristics with trace metals. Metals explained 2 to 6% of the community composition when SEM-AVS or individual SEM concentrations were regarded. In contrast, total, normalized, and accumulated metals were not significantly linked to community composition. We conclude that examination of SEM or SEM-AVS concentrations is useful for risk assessment of trace metals on the community level.

7.1 Introduction

Trace metal pollution can cause adverse effects on organisms, populations, communities, and ecosystems in natural aquatic systems. In the sedimentation area of the river Rhine (The Netherlands), diffuse water pollution started during the industrial revolution and reached its maximum during the 1960s and 1970s. This pollution consists of trace metals, which sometimes exceed natural background values by 5 to 10 times (Winkels, 1997), and organic contaminants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Effects on benthic invertebrate communities already have been demonstrated for various sedimentation areas (Den Besten *et al.*, 1995; Reinhold-Dudok van Heel *et al.*, 1999). The environmental availability of trace metals and potential ecological risks for benthic invertebrates are of concern for currently undertaken floodplain rehabilitation projects. Various mechanisms determine the environmental availability of trace metals. Trace metals imported with suspended solids tend to attach to fine mineral and organic particles, which generally results in a good relation between trace metals and organic carbon (OC) and clay content (Koelmans, 1997). Therefore, normalization on OC or clay fractions is useful when aerobic soils and sediments are compared. In reduced sediments, trace metals can precipitate with sulfides and become unavailable to organisms. These reactive sulfides can be measured as acid volatile sulfide (AVS) and compared to concentrations of simultaneously extracted metals (SEM). Several studies show good correlation between concentration of combinations of SEM and AVS and pore-water metal concentrations and the presence of toxicity for test organisms (Ankley *et al.*, 1991; DiToro *et al.*, 1992; Berry *et al.*, 1996; Ankley, 1996). Other studies also pointed out some drawbacks, such as vertical and spatial heterogeneity of AVS (Van den Berg *et al.*, 1998; JS Lee *et al.*, 2000; Van Griethuysen *et al.*, 2003) or the neglect of diffusive processes in the determination of pore-water concentrations (BG Lee *et al.*, 2000). Metals accumulated in organisms also provide information on bioavailability. In nature, organisms are exposed to multiple, simultaneously operating stressors (Peeters *et al.*, 2000). Pollution is just one, albeit an important factor. Furthermore, mixtures of pollutants make it difficult to ascribe observed effects to one or more substances. Recently, a specific multivariate statistical approach has been used to quantify the impact of pollutants on benthic communities in relation to the effects of other factors (Pinel-Alloul *et al.*, 1996; Peeters *et al.*, 2000, 2001). This approach, the variance partitioning method, can be applied to assess the contribution of pollutants relative to the effect of other variables, for example, water, sediment, and other habitat characteristics. The variance partitioning method allows for the separation of effects of trace metals from effects of ecological or environmental variables. Furthermore, the method evaluates the impact of pollutants by using the in situ community. According to the pollution-induced community tolerance concept, communities may be adapted to pollution (Clements, 1999). In that case, variance partitioning will underestimate the ecological impact that the pollutants would have on communities that are not adapted and thus can be seen as a conservative estimate of effects. Variance partitioning can be used to evaluate the potential of different measures of trace metal availability in explaining effects on benthic community structures. Results of Peeters *et al.* (2000) indicate that taking into account metal sorption onto OC and clay improves the separation of effects by metals and environmental variables. The main goal of this study was to examine the role of trace metals compared to other environmental conditions in structuring benthic communities in floodplain lakes. Furthermore, we evaluated which method of estimating environmental availability was most successful in explaining trace metal effects on community composition. To that end, we examined total, OC- and clay-normalized, SEM and AVS, and accumulated concentrations of trace metals and relations between these variables.

7.2 Materials and methods

7.2.1 Study area

A wide range in metal concentrations was achieved by selecting lakes according to inundation frequency. Trace metal content of floodplain sediments is positively correlated with inundation frequency (Rang *et al.*, 1987; Van Griethuysen, unpublished data). To maximize variation in metal concentrations, lakes from different branches of the river Rhine were selected. Lakes of similar size (<1 ha) and morphology (depth <2 m) were selected. To enable sampling of sediments and benthic macrofauna, lakes should not be completely covered with vegetation. Sampling was done in September 2000, four to six months after winter inundation to guarantee isolation from the river. Ten lakes were selected: three lakes along the river IJssel, four lakes along the Waal, and three lakes along the Nederrijn (The Netherlands; see Figure 7.1 for locations, and Table 7.1 for lake characteristics).

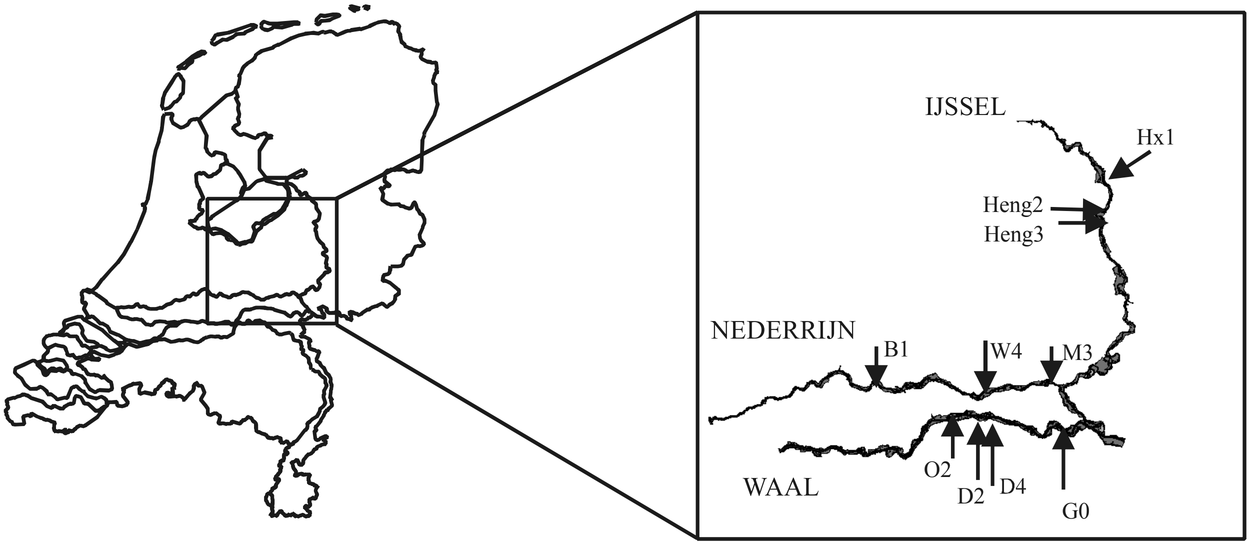

Figure 7.1. Location of studied floodplain lakes in The Netherlands. Lake locations are indicated with arrows. Abbreviation of sample locations: Hx1 = Herxen 1; Heng2 = Hengforderwaard 2; Heng3 = Hengforderwaard 3; G0 = Gendt 0; O2 = Ochten 2; D4 = Deest 4; D2 = Deest 2; B1= Beusichem 1; W4 = Wolfswaard 4; M3 = Meinerswijk 3.

In some cases, more than one lake was selected in the same floodplain. Four locations per lake were sampled to account for spatial variability in lake depth, grain size distribution, organic matter content, and redox conditions (Howards & Evans, 1993; Van Griethuysen *et al.*, 2003).

Table 7.1. Characteristics of studied floodplain lakes in The Netherlands

Floodplain lake	**Age (years)**	**Inundation class** [a)]	**Distance to river (m)**	**Lake area (ha)**	**Transparency** [b, c)] **(cm)**	**Depth** [c)] **(m)**
River IJSSEL						
Herxen 1	64	5	101	0.14	60	1.0
Hengforderwaard 2	> 300	4	48	1.00	31	1.1
Hengforderwaard 3	34	4	95	0.94	39	0.5
River WAAL						
Gendt 0	67	2	584	0.73	20	1.1
Ochten 2	144	2	757	0.19	149	1.5
Deest 4	75	4	167	0.19	30	0.5
Deest 2	5	2	425	1.13	28	0.3
River NEDERRIJN						
Beusichem 1	> 300	5	45	0.80	23	0.4
Wolfswaard 4	53	2	806	0.74	29	0.4
Meinerswijk 3	62	3	202	0.17	37	0.4

a) Inundation frequency class: 1 = inundated <2 d/year; 2 = 2–20 d/year; 3 = 20–50 d/year; 4 = 50–150 d/year; 5 = >150 d/year; b) Measured as Secchi depth; c) Averaged over four observations within each lake.

Sampling of surface water, sediment, and fauna

Surface water was assumed to be completely mixed because of the limited size and depth of these lakes; therefore, only one sample was collected at each location. Surface water transparency was measured with a Secchi disk before water and sediment sampling. Water temperature, pH, electric conductivity, and oxygen concentrations near the sediment surface were measured in the field with specific electrodes (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany, and OxyGuard International A/S, Birkerød, Denmark) at each location. Five hundred–milliliter water samples were collected in glass bottles for total OC (TOC) and dissolved OC (DOC), and total inorganic carbon (TIC) and dissolved inorganic carbon (DIC) analysis, and in polyethylene bottles for major element analysis. Before sampling, each bottle and lid were soaked overnight in 4 M HNO_3 (analytical grade, Merck, Darmstadt, Germany) and thoroughly rinsed with Barnstead® Nanopure water (Barnstead, Dubuque, IA, USA). Water samples collected for the analysis of major elements were filtered on the day of collection over 0.45-mm membrane filters (Schleicher & Schuell, 's-Hertogenbosch, The Netherlands) and diluted two times with HNO_3 (analytical grade, Merck) yielding a 0.1 M HNO_3 solution. Sediment and benthic invertebrate samples were taken in triplicate at four locations within each lake, leading to 40 samples, each consisting of three subsamples. Sample locations always included the center of the lake, whereas the other three locations were closer to the shores or corners. Sediment cores were taken with Perspext tubes of 5-cm diameter and 60-cm length. This was done manually or with a stainless steel Jenkins core sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands), depending on water depth. Cores were taken without headspace to maintain prevailing oxygen conditions.Fauna samples were taken with an Eckman grab with a surface area of 225 cm^2 (Hydrobios, Kiel, Germany) and sieved in the field over a brass 500-µm mesh sieve (BV Metaalgaas Twente, Hengelo, The Netherlands). After transport to the laboratory, samples were stored overnight at 4 °C, to be sorted and identified the next day.

Analysis of surface water, sediment, and fauna

On the day of sampling, unfiltered samples were analyzed for TOC and TIC, and DOC and DIC concentrations were determined after filtration over 0.45-µm membrane filters (Schleicher & Schuell) by using a TOC analyzer (Model 700, O.I. Analytical, College Station, TX, USA). Particulate OC is calculated as TOC-DOC. Acidified surface water samples were analyzed by inductively coupled plasma–mass spectrometry (ICP-MS; Elan 6000, Perkin-Elmer, Boston, MA, USA) for major element composition. After transport to the laboratory, the upper 5 cm of each core was isolated and stored in a 250-ml polyethylene bottle at -18 °C until analysis. Concentrations of AVS and SEM were determined according to van Griethuysen *et al.* (2002). In short, sulfides were volatilized with 1 M HCl and detected with a sulfide-specific electrode (Ag500, Wissenschaftlich-Technische Werkstätten). Simultaneously extracted metal concentrations were measured by ICP-MS in the remaining acidified sediment suspension after filtration. Total macroelement and metal concentrations were defined as those digested in a mixture of concentrated HCl and HNO_3 for 2.5 h at 100 °C (aqua regia digestion). Major elements and Zn were measured by inductively coupled plasma–atomic emission spectroscopy (ICP-AES Spectroflame, Spectro, Kleve, Germany), and Cd, Cu, Ni, and Pb were measured by ICP-MS. Analytical quality was assured by using a certified reference sample (International Soil-Analytical Exchange no. 970, n = 5). Recovery was 90 to 100% for each trace metal. Practical detection limits against replicated procedural blanks were 0.05 mg/kg for Cd, 2.15 mg/kg for Cu, 0.38 mg/kg for Ni, 1.51 mg/kg for Pb, and 7.0 mg/kg for Zn at a dry sample weight of 2.5 g. Grain size distribution was measured with laser diffraction on a Coulter LS 230 (Beckman-Coulter, Coulter Scientific Instruments, Miami, FL, USA). Content of OC was estimated as 0.58 times loss on ignition (loss on ignition after heating for 3.5 h at 550°C). Sediment pH was measured in a 0.01 M $CaCl_2$ extract with a pH electrode (SenTix 21, WTW, Weilheim, Germany). Organisms retained in the fauna samples were picked manually in the laboratory, sorted, counted, and identified live with available keys. The three replicate samples were combined. Species diversity was expressed in number of species and abundance was expressed in individuals per square meter. Oligochaetes were separated from other taxa for the analysis of trace metal contents. Oligochaetes were selected because of their suitability for the evaluation of sediment trace metal availability (Chapman *et al.*, 1999; Hare *et al.*, 2001) and the recommendation for their use in laboratory bioaccumulation studies by U.S. Environmental Protection Agency. Because they feed on detritus, they are more susceptible to sediment-related metal sources than organisms that feed on plants or food in the water column. Furthermore, oligochaetes are very common in soft-bottomed freshwater systems, which ensures sufficient material for analyses. After gut clearance for 20 h in aerated field water at 20 °C, organisms were collected manually, rinsed with 0.1 M HNO_3, and freeze-dried. A maximum of 25 mg of freeze-dried material was weighed in 2-ml Eppendorf vials with caps and digested on a microdestruction plate (Techne Driblock ® DB-3D, Thistle Scientific, Glasgow, UK) in a mixture of H_2O_2 and HNO_3 and finally dissolved in 2 ml of Barnstead ® Nanopure water. Samples were diluted 10 times with 0.1 M HNO_3 before analysis by ICP-MS. All reagents used for this digestion were of ultrapure quality. Practical detection limits against replicated procedural blanks were 1.21 mg/kg for Cd, 3.12 mg/kg for Cu, 0.12 mg/kg for Pb, and 5.48 mg/kg for Zn at a sample weight of 25 mg.

Data analysis

Relations between lake, sediment, water, and pollution characteristics were evaluated by principal components analysis (PCA) with Varimax rotation (SPSS, 1997). Relations between pollution concentrations and sediment characteristics were studied in more detail with linear regression and correlation analysis (SPSS, 1997). To judge the availability of trace metals, various relations of SEM and AVS were used. Ratios of $SEM/AVS > 1$ as well as differences of $SEM-AVS > 0$ indicate that trace metals may be present in the pore water and available to organisms (Ankley *et al.*, 1991; DiToro *et al.*, 1992; Berry *et al.*, 1996; Ankley, 1996). Recently, a correction of SEM-AVS for the fraction OC ((SEM-AVS)/f_{OC}) has been proposed to improve the prediction of availability, while it accounts for sulfide precipitation under reduced conditions as well as for sorption of excess metals to organic matter (US-EPA, 2000). Concentrations of SEM are calculated by summing five individual trace metals extracted with 1 M HCl (i.e., Zn, Cu, Cd, Pb, and Ni). Individual extracted metals are indicated as, for example, Zn-SEM. Sorbed and loosely bound metals will be determined together with sulfide minerals. Metals incorporated in crystal lattices of clay minerals that will never become available to the environment are not included in SEM. Therefore, SEM concentrations probably are better indicators of metal availability than are total concentrations. The relation of metals in oligochaetes to pollution concentrations and other environmental conditions was evaluated with stepwise linear regression (SPSS, 1997). Sediment and water characteristics that may influence metal accumulation were included in the stepwise linear regression procedure. Mineral sediment characteristics can be important as sorption sites for metals. Sediment Ca reduces toxicity and bioavailability of trace metals (Van Hattum *et al.*, 1996). AVS, total S, and organic C account for metal-sulfide precipitation and metal binding by organic matter. Overlying water characteristics influence trace metal speciation in surface water (Van Hattum *et al.*, 1991). Water transparency and lake depth give additional habitat characterization and may indirectly influence metal accumulation. Each alternative metal availability measure was tested in the regression model to evaluate which one accounts best for the influence of sediment trace metals on metal accumulation. Species diversity (total number of species) and abundance (total number of individuals per square meter) were summarized and evaluated for the different lakes. Functional feeding groups were distinguished to characterize the communities present. Relations of the community composition with environmental variables, including trace metals, were evaluated by using multivariate statistics. Before multivariate analyses, species abundance data were $\ln(x + 1)$ and abiotic data were $\log(x+1)$ transformed. Variables with negative values (SEM-AVS and (SEM-AVS)/f_{OC}) were raised with the lowest negative value to yield positive values for all samples. Downweighting of rare species was performed to give less weight to species that are only incidentally present. The length of the gradient within the species data was analyzed by using a detrended correspondence analysis (Hill, 1979). The gradient was short to moderate and, therefore, the linear response model, redundancy direct analysis, was regarded as appropriate (Ter Braak & Smilauer, 1998). Scaling was focused on intersample distance, species scores were divided by standard deviation, and data were centered by species. A set of environmental variables of importance for benthic organisms was selected from morphological, sediment, and surface water characteristics (Table 7.2).

Table 7.2 Environmental variables selected for multivariate analysis

Variable	**Type**	**Motivation**
OC $^{a)}$	Sediment	Food source and metal sorption
Fraction < 63 µm	Sediment	Food source and metal sorption
AVS $^{b)}$	Sediment	Redox indication and metal precipitation
pH $^{c)}$	Sediment	Habitat and metal speciation
TOC $^{d)}$	Water	Sorption of metals and food (including algae)
pH $_{bottom}$ $^{e)}$	Water	Chemical environment
EC $_{bottom}$ $^{e)}$	Water	Also (related to DIC content)
$O_{2\ bottom}$ $^{e)}$	Water	Oxygen supply near sediment surface
Transparency $^{f)}$	Habitat	Turbidity, type and amount of food
Depth	Habitat	Influences grain size and microclimate
Lake area	Habitat	Colonization possibilities

a) OC = organic carbon, taken as 0.58 * loss on ignition; b) Acid-volatile sulfide; c) pH determined in $CaCl_2$ extract; d) Total organic carbon in water column (sum of dissolved and particulate organic carbon); e) Measured close to sediment surface; f) Measured as Secchi depth.

This selection was based on a forward selection procedure (contribution and significance) and on a motivation of variable importance for invertebrate species. Multiple analyses were performed with these environmental variables (variable subset 1) in combination with one variable accounting for metal availability (variable subset 2). We divided subset two into nine separate measures accounting for trace metal availability: total metal concentrations, OC-normalized metal concentrations, clay-normalized metal concentrations, individual SEM, SEM, SEM/AVS, SEM-AVS, (SEM-AVS)/f_{OC}, and concentrations in oligochaetes. On several occasions, a high collinearity was found among variables within a subset or among subsets, indicated with high (> 20) variation inflation factors (VIFs). High VIF values result in interference in the analysis. Therefore, variables with highest VIF values were removed from the selection until all variables had a VIF value below 20 (Ter Braak & Smilauer, 1998). Four axes were extracted and scores were calculated for samples, species, and variables. Ordination diagrams representing the first two axes were used to indicate the main structure of the multivariate data. The total contribution of the environmental variables together with each separate metal variable to the explanation of the species composition was calculated. To determine the relative contribution of a certain variable or a group of variables to the explanation of species composition, partitioning of the variance was applied (Borcard *et al.*, 1992). The contribution of each separate subset was calculated, as well as the contribution of the two subsets together. This gives an overestimation of the contribution of each one of these subsets, because variation shared between variables from the two subsets is not accounted for. To know the actual (pure) contribution of a subset, the effect of variables from the other subset must be subtracted. This can be done in a partial redundancy direct analysis, where one subset is entered as a covariable set (Ter Braak & Smilauer, 1998). For example, the relative contribution of subset A is calculated as the contribution of subset A minus the contribution that is shared with subset B by including subset B as a covariable set in the analysis. Any variation shared between subsets A and B is calculated as the difference of the variance explained by subset A, with and without subset B as a covariable. In this study, the first subset consists of environmental variables of importance for benthic organisms. The second subset each time consists of one of the variables indicating trace metal pollution. Thus, variance partitioning gives insight into the contribution of environmental variables alone (subset 1), trace metals alone (subset 2a–i), and variation shared by both subsets and unexplained variation.

7.3 Results and Discussion

7.3.1.Geochemistry of water and sediment

Surface water characteristics are presented in Table 7.3. Water pH is near neutral to alkalic ranging from 6.75 to a maximum of 9.47. The range in electric conductivity (300–600 µS/cm) is normal for a mixture of river water and rainwater and agrees with earlier studies on similar water systems (Van den Brink, 1990). Higher values are possibly related to closer contact with river water via seepage or connections by small ditches. Large differences in oxygen concentrations occur (0.6–12.6 mg/L), illustrating the dynamic conditions in the lakes. Low concentrations in Deest 4 and Beusichem can be explained by the shallowness of the water column combined with a high biological or chemical oxygen demand. The DOC and TOC concentrations in these lakes also are very high, consistent with the observed oxygen concentrations. An overview of sediment characteristics per lake is provided in Table 7.4. General characteristics show a large range from sandy, OC-poor, and oxic sediments (e.g., part of Herxen, Hengforderwaard 3, and Deest 2) to clayey, OC-rich, and reduced sediments (Hengforderwaard 2, all Waal lakes except Deest 2, and Nederrijn lakes). Grain size differences also are reflected in macroelement concentrations such as Fe, Mn, and Ca.

Table 7.3. Ranges of surface water characteristics for each floodplain lake in The Netherlands a). Abbreviation of sample locations: Hx1 = Herxen 1; Heng2 = Hengforderwaard 2; Heng3 = Hengforderwaard 3; G0 = Gendt 0; O2 = Ochten 2; D4 = Deest 4; D2 = Deest 2; B1= Beusichem 1; W4 = Wolfswaard 4; M3 = Meinerswijk 3.

Lake	**pH**	**EC** b)	**O_2**	**Transparency** c)	**Depth**	**DIC** d)	**DOC** e)	**TIC** f)	**TOC** g)
	-	µS/cm	mg/l	m	m	mg/l	mg/l	mg/l	mg/l
River IJSSEL									
Hx 1	7.28–7.50	524.5–524.5	6.0–7.6	0.60–0.60	0.60–1.15	30.63–31.29	6.31–6.77	32.34–32.95	7.06– 7.73
Heng2	7.39–7.51	512–514	7.4–10.0	0.25–0.40	0.90–1.35	28.51–29.51	8.22–9.67	29.13–31.10	10.54–13.26
Heng3	7.41–7.48	536–567	5.1–5.8	0.35–0.40	0.40–0.80	35.82–36.35	7.78–7.97	38.31–38.88	8.67–9.06
River WAAL									
G0	7.75–8.35	608–614	7.5–12.6	0.20–0.20	0.80–1.35	29.66–30.74	7.54–8.88	30.81–32.83	10.04–10.47
O2	8.41–8.85	417–418	9.4–11.3	1.40–1.55	1.40–1.55	8.12–18.44	8.47–9.55	17.93–18.70	8.95–9.00
D4	6.75–7.12	403–411	0.6–1.2	0.25–0.40	0.40–0.60	27.05–27.39	11.33–11.83	29.08–29.57	12.53–13.44
D2	7.73-9.47	314–348	4.1–11.2	0.20–0.30	0.20–0.40	10.31–15.00	7.50–8.07	10.03–15.48	9.87–10.43
River NEDERRIJN									
B1	7.02–7.73	423–488	2.9–10.3	0.15–0.30	0.25–0.60	30.30–35.50	10.95–13.35	31.77–40.44	14.18–18.29
W4	7.34–7.72	535–543	5.6–9.2	0.25–0.30	0.25–0.55	8.78–40.19	10.79–11.05	41.24–43.02	11.81–13.76
M3	7.27–7.82	458–459	8.0–8.8	0.33–0.45	0.33–0.45	33.13–34.11	8.89–9.57	34.93–35.51	9.94–10.38

a) Underlined numbers indicate minimum and maximum concentrations in the total data set.

b) Electric conductivity.

c) Determined as Secchi depth.

d) Dissolved inorganic carbon.

e) Dissolved organic carbon.

f) Total inorganic carbon.

Table 7.4. Ranges of sediment variables for each floodplain lake in The Netherlands [a)]

Floodplain lake	LOI [b)]	Clay	Cd	Cu	Ni	Pb	Zn	Fe	Ca	Mn	S	AVS [c)]	SEM [d)]
	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	mg/kg	mg/kg	µmol/g	µmol/g
River IJSSEL													
Herxen 1	0.4 -7	1-23	0.4-5.4	6-83	6-40	11-134	53-654	0.3-2.7	0.3-3.9	59-808	365-3807	3.3-26.6	0.8-9.6
Hengforderwaard 2	7-12	15-28	1.8-3.1	38-70	28-48	73-117	341-653	1.9-3.2	3.1-4.8	504-987	1485-2653	10.4-20.5	4.0-8.4
Hengforderwaard 3	0.6-6	0.6-6	0.1-2.8	2-49	7-33	4-92	17-372	0.5-2.2	1.1-4.2	87-491	126-957	0.9-3.5	0.2-1.3
River WAAL													
Gendt 0	9-10	15-23	0.5-0.7	19-29	32-39	29-43	149-244	2.0-3.0	4.4-6.8	1221-1597	1649-2316	12.9-35.7	1.3-2.0
Ochten 2	11-12	24-26	1.2-1.3	43-47	42-47	72-79	326-368	3.1-3.4	3.4-4.8	790-1082	1814-2073	16.0-21.2	3.7-5.3
Deest 4	9-10	15-24	1.6-2.4	52-56	37-43	85-94	423-448	2.6-3.0	3.8-4.2	732-872	1905-2592	13.1-30.0	4.9-5.5
Deest 2	3-4	4-11	0.1-0.3	6-15	10-21	10-23	46-107	0.7-1.5	1.1-3.4	166-386	448-950	5.5-17.4	0.5-1.3
River NEDERRIJN													
Beusichem 1	13-18	21-26	3.0-5.7	69-105	44-51	145-236	618-939	2.9-3.1	1.7-2.8	435-575	2469-4240	18.4-48.8	7.7-13.5
Wolfswaard 4	16-28	21-24	1.6-2.4	38-52	34-43	77-104	295-392	2.2-2.8	2.7-3.7	561-993	3447-8427	12.1-30.9	3.9-8.3
Meinerswijk 3	9-13	19-22	6.4-7.6	123-142	39-47	217-250	840-947	2.6-3.4	3.4-4.5	835-1232	2171-2871	5.3-18.0	11.3-14.0

a) Underlined numbers indicate minimum and maximum concentrations in the total data set.

b) Loss on ignition.

c) Acid-volatile sulfide.

d) Simultaneously extracted metals (sum of extracted Zn, Cu, Cd, Ni, and Pb).

Large differences in general characteristics can be present within a lake, for example, OC and clay fraction in Herxen. Large differences also occur in trace metal levels (Table 7.4). Trace metal concentrations are lowest in Hengforderwaard 3 (IJssel branch), and highest in Meinerswijk (Nederrijn branch). Total trace metal concentrations recalculated to standard soil containing 10% organic matter and 25% clay exceed Dutch maximum permissible risk levels (Crommentuijn *et al.*, 1997) for Zn (620 ppm) and Cu (73 ppm) at various locations in Herxen, Deest 4, Beusichem, and Meinerswijk lakes. For Pb, the maximum permissible risk level (250 ppm) is exceeded in lake Meinerswijk. A first interpretation of relations between sediment, water, and lake morphological characteristics, together with accumulation in oligochaetes, explained 85% of the total variance with six components when using PCA (Table 7.5). Component 1, which explains 36.3% of the variance, shows high loadings of sediment total and extracted trace metals associated with OC, clay, P, and S and Pb accumulation in oligochaetes. Component 2 explains 14.1% of the variance and includes clay, OC in sediment and in water (DOC), and AVS relating to the redox state of the sediment. Apparently, finer sediment with more OC has higher AVS concentrations and more dissolved organic components. Metal concentrations in oligochaetes are negatively related to this second component, which appears consistent with a lower availability due to sorption to DOC, OC, or clay, and precipitation with sulfides. Component 3 represents water hardness (Ca, DIC, and electric conductivity), and explains 11% of the variance. Calcium, Mn, and Ni (component 4) explain another 10% of the variance and represent sediment mineral characteristics. Component 5 represents chemical processes in surface water (pH and oxygen concentration) and explains 7.5% of the variance. Transparency and depth are both negatively related to particulate OC concentrations in component 6 (which explains 7% of the variance) and can be interpreted as a turbidity component. The PCA results confirm that variation in the geochemical data is dominated by the pollution gradient.

Table 7.5. Principal components of environmental data [a)]

Component	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
% explained	36.3	14.1	10.9	10.0	7.5	7.0	85.8
Cu	0.98						
Cd	0.98						
Pb	0.97						
Zn	0.97						
Ni	0.69	0.52					
Cd-SEM [b)]	0.98						
Zn-SEM [b)]	0.97						
Pb-SEM [b)]	0.96						
Cu-SEM [b)]	0.94						
P	0.85						
< 2 µm [c)]	0.56	0.56		0.52			
OC [d)]	0.54	0.74					
S	0.52	0.69					
$\text{Pb}_{\text{oligochaete}}$	0.50	-0.57					
$\text{Cu}_{\text{oligochaete}}$		-0.71					
$\text{Zn}_{\text{oligochaete}}$		-0.61					
AVS [e)]		0.69					
DOC [f)] $_{\text{bottom water}}$		0.65					
$\text{Ca}_{\text{bottom water}}$			0.97				
EC [g)] $_{\text{bottom water}}$			0.93				
DIC [h)] $_{\text{bottom water}}$			0.85				
Ca				0.95			
Mn				0.90			
$\text{Oxygen}_{\text{bottom water}}$					0.94		
$\text{pH}_{\text{bottom water}}$					0.79		
Transparency						0.88	
Depth						0.66	
POC [i)] $_{\text{bottom water}}$						-0.70	

a) VARIMAX (SPSS, Chicago, IL, USA) rotation was applied. Only factor loadings ≥0.5 are shown; b) Simultaneously extracted metal; c) Fe and Al are highly correlated to <2 µm ($r^2 > 0.95$) and therefore were left out; d) Organic carbon; e) Acid-volatile sulfide; f) Dissolved organic carbon; g) Electric conductivity; h) Dissolved inorganic carbon; i) Particulate organic carbon.

Although the PCA shows that pollutant levels and sediment characteristics are related (component 1), striking differences in total trace metal distribution in relation to OC and clay are visible among branches. IJssel and Waal samples show clear linear relationships between total trace metals on the one hand and OC and clay on the other hand. However, for Nederrijn samples, no relation is found between these sediment characteristics and trace metal concentrations (Figure 7.2). The same pattern occurs for Cu, Pb, and to a somewhat lesser extent, also for Cd. Regressions of Zn, Cu, and Pb with either OC or clay show coefficients of determination (r^2) of 0.90 or higher for IJssel samples and 0.54 to 0.64 for Waal samples. For Cd, the r^2 for IJssel samples is 0.71 (with clay) to 0.83 (with OC), and for Waal samples r^2 is 0.38 (clay) to 0.42 (OC). Because sediment characteristics such as grain size and OC content govern contaminant concentrations from diffuse sources, applying a correction of trace metals for OC or clay usually leads to homogeneous pollution levels, even though total concentrations may vary considerably. For Waal and IJssel samples, this is demonstrated by the linear relationship in Figure 7.2.

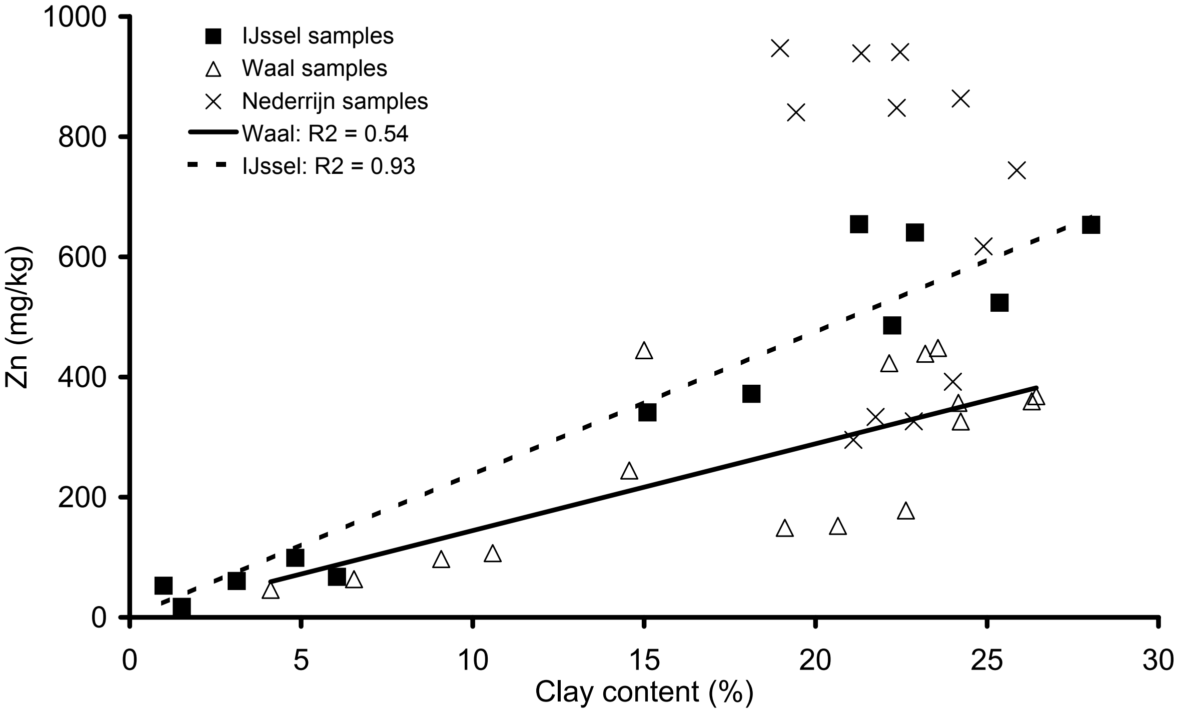

Figure 7.2. Relation of metal concentrations with sediment characteristics: Zn and clay content.

In most Nederrijn samples however (lakes Meinerswijk and Beusichem), trace metal concentrations are situated above the regression lines for Waal and IJssel branches. Natural background concentrations for Dutch soils and sediments (Crommentuijn *et al.*, 1997) are exceeded more than five times in these samples, suggesting the presence of a local source of trace metals in these floodplain lakes. Concentrations of total SEM vary between 0.2 µmol/g (Hengforderwaard 3) and 14.0 µmol/g (Meinerswijk) and correlate well (Pearson *r* ranges from 0.74 for Ni to 0.96 for Zn and Pb; $p < 0.01$) to total concentrations of each metal. Total SEM also is related to OC, fine grain size fractions, and AVS ($r = 0.44$ for AVS to 0.65 for OC; $p < 0.05$), suggesting that extracted metals originate not only from precipitates with sulfide but also from other sediment sorption phases, such as OC and clay minerals. Individual SEM concentrations are related to total metal concentrations. Linear regression shows that 75% of total Cd concentrations ($r^2 = 0.80$), 53% of total Cu ($r^2 = 0.82$), 88% of total Pb ($r^2 = 0.87$), only 8% of total Ni ($r^2 = 0.79$), and 69% of total Zn ($r^2 = 0.87$) are extracted with 1 M HCl. The order of magnitude of these percentages agrees with earlier studies (Van den Hoop *et al.*, 1997; Van den Berg *et al.*, 1998; Van Griethuysen *et al.*, 2003). According to the SEM-AVS criterion, trace metals are not available at most locations. Thirty-eight out of 40 samples show an excess of AVS over SEM, with AVS varying between 0.9 and 48.8 µmol/g, and SEM between 0.2 and 14.0 µmol/g. In lake Meinerswijk, which also has the highest total metal concentrations, SEM exceeds AVS at two locations. Theoretically, toxic effects can be expected from this value onward. Recently, practical toxicity threshold values were derived from a variety of experiments (US-EPA, 2000). For SEM-AVS, this proposed threshold value is 1.7 µmol/g, which also is exceeded at these two locations. For SEM-AVS/f_{OC}, the proposed value is 100 to 150 µmol/g OC, which is exceeded only once. Therefore, trace metal effects on organisms could only be expected in lake Meinerswijk according to the AVS/SEM concept. However, it should be kept in mind that SEM-AVS concentrations are measured as an average over 5 cm. In more oxidized top layers, the threshold values may be exceeded more frequently.

Accumulation in Oligochaeta

Minimum and maximum accumulated concentrations in oligochaetes are shown for each lake in Table 7.6.

Table 7.6. Ranges of accumulated metal concentrations in oligochaetes for each floodplain lake and correlation with sediment metal availability measures [a]

Floodplain lake	**Cu** (mg/kg DW [b])	**Pb** (mg/kg DW [b])	**Zn** (mg/kg DW [b])
River IJSSEL			
Herxen 1	20.3-68.0	5.8-8.1	128.7-169.1
Hengforderwaard 2	15.9-39.5	6.1-9.5	119.5-169.8
Hengforderwaard 3	16.6-57.3	4.5-11.3	96.0-158.7
River WAAL			
Gendt 0	10.8-36.4	3.6-5.9	116.9-141.2
Ochten 2	26.2-31.1	3.8-8.3	126.7-149.3
Deest 4	15.4-19.2	3.3-4.5	98.3-106.3
Deest 2	1.6-55.1	1.1-5.8	121.3-261.5
River NEDERRIJN			
Beusichem 1	18.4-35.9	2.9-5.4	107.2-167.5
Wolfswaard 4	4.3-14.4	4.4-7.2	82.1-99.2
Meinerswijk 3	36.4-62.8	6.9-26.4	177.7-206.8
Correlation [c]			
Total metal		0.45 **	
Metal/OC [d]		0.70 **	0.39 *
Metal/clay	0.57 **	0.54 **	
SEM [e]/AVS [f]	0.53 **	0.73 **	
SEM-AVS		0.53 **	
SEM-AVS/f_{OC}	0.37 *	0.54 **	

a) Underlined numbers indicate minimum and maximum concentrations in the total data set.; b) DW = Dry weight; c) Correlation coefficients (Pearson *r*) of accumulated metals with sediment metal availability measures for the whole data set. Only significant correlations are given: ** = $p < 0.01$, * = $p < 0.05$; d) OC = Organic carbon; e) SEM = Simultaneously extracted metals (sum of Zn, Cu, Pb, Cd, and Ni); f) AVS = Acid volatile sulfide.

Accumulated Cd concentrations were all below or near the detection limit (1.21 mg/kg) and are not reported. Metal concentrations in oligochaetes are comparable with concentrations reported for other water systems within The Netherlands (Van Hattum *et al.*, 1991, 1996). Ranges of accumulated concentrations (Table 7.6) are much lower than ranges of total sediment concentrations (Table 7.4), especially in the case of Zn, where the range of accumulated concentrations in oligochaetes is only a factor of three, whereas the range was more than 50 for total sediment concentrations. Accumulation is better correlated to OC-, clay-, or AVS-corrected metal concentrations than to total metal concentrations (Table 7.6), as also was indicated with PCA (Table 7.5). Accounting for OC and clay binding improves the relation of sediment metals with accumulation, probably because the uptake pathway (food) for oligochaetes is accounted for. An improvement of the relation between accumulation and sediment concentrations by accounting for AVS as observed for Pb is consistent with AVS field validation studies (Hare *et al.*, 1994, 2001; Warren *et al.*, 1998). These studies concluded that the AVS theory adequately described Cd porewater chemistry and Cd accumulation in sedentary taxa such as chironomids and oligochaetes. However, accumulation of Cd or (sub)lethal effects (abundance and growth) were not related to Cd/AVS ratios for other species, probably because their feeding behavior implies little contact with the sediment. Accumulation of Cd was better predicted by surface water Cd concentrations than by sediment Cd concentrations (Hare *et al.*, 2001). However, metal concentrations in the sediment still contributed approximately 50% to the observed Cd accumulation in oligochaetes and chironomids (Hare *et al.*, 2001). Linear stepwise regression with various trace metal availability variables, together with water and sediment characterictics, could explain about 50% of the variation in accumulation (Table 7.7).

Table 7.7. Linear regression of metal accumulation in oligochaetes, best fitting combinations only [a)]

Dependent variable	**Metal variable** [b)]	r^2_{adjusted}	**Regression equation**
Accumulated Zn	Zn-total	0.50	$321.41-15.03*DOC+0.066*Zn-0.17*EC_{\text{bottom water}}$
Accumulated Cu	Cu/clay	0.52	$59.33+6.41*Cu/clay-5.14*DOC$
Accumulated Pb	SEM/AVS	0.57	$8.91+9.11*SEM/AVS-0.61*DOC$

a) Stepwise regression with significance criterion $p < 0.05$ for entrance in model, $p > 0.10$ for removal. The following variables were regarded: Al, P, S, Ca, Mn, Fe, acid-volatile sulfide (AVS), organic carbon (OC), grain size fraction < 2 µm, pH bottom water, electric conductivity (EC) bottom water, O_2 bottom water, transparency, lake depth, dissolved inorganic carbon, dissolved organic carbon (DOC), particulate organic carbon, Ca bottom water, and alternative measures for metal availability.

b) Metal (Me) variable included in best explaining regression model, chosen from total Me, Me/OC, Me/clay, total simultaneously extracted metals (SEM), individual SEM-Me, SEM/AVS, SEM-AVS, and (SEM-AVS)/f_{OC}.

Only the equation for the metal availability variable explaining most variation for a particular metal is given in Table 7.7. An explanation for the moderate coefficients of determination (r^2) for at least Cu and Zn is that regulation of metal concentrations may occur in the body. For essential metals, the relationship between exposure concentrations and accumulation may be nonlinear. Accumulation is positively correlated with sediment pollution, but decreases with increasing levels of DOC in the bottom water. Dissolved OC is well known to bind trace metals and lower their availability. Results from this study show that this binding mechanism also occurs in the field. Dissolved OC has more influence on modeled accumulated concentrations for Cu and Pb than for Zn, which can be explained by stronger binding of Cu and Pb to DOC compared to Zn. For Zn, electric conductivity also is included in the model. Accumulation of Zn is less at high electric conductivity values, suggesting that inorganic complexes play a role in the speciation of Zn. In summary, all models show that surface water variables are at least as important for the amount accumulated in oligochaetes as sediment metals, especially for Zn. Hare *et al.* (1994, 2001) reported that effects on Oligochaeta in Cd-spiked sediments were only partly (50%) explained by sediment trace metal pollution and also were related to surface water variables. Our present findings show that this is also the case for other trace metals at pollution levels occurring in the field.

Community

Species community composition varies largely among lakes. A summary of species richness and abundance for each lake is given in Table 7.8. For a complete overview of all taxa, refer to the Appendix. Sixty-five taxa were identified. Abundance ranges from 1,348 individuals/m2 (Wolfswaard 4) to 28,063 (Beusichem 1) individuals/m^2. The number of taxa varies from 9 in lake Gendt 0 to 35 in Deest 2. Most taxa in the present study are common for Dutch fluvial lakes (Van den Brink, 1990). Oligochaetes and chironomids are the most dominant taxa. Most species belonging to these taxa have a detritivore feeding mode and are common in freshwater systems. Because they inhabit the sediment, they are potentially exposed to sediment pollution. Detritivores make up approximately 75% of the total abundance in all samples. However, herbivore species (e.g., *Radix auricularia* [Mollusca]) are dominant (up to 71% of all organisms) in lakes Deest 2 and Deest 4, whereas they are virtually absent in all other lakes. *Sialis lutaria* (Megaloptera) is the most dominant carnivorous species, with the highest abundance in Meinerswijk. It was also found in Ochten 2, Deest 4, and Beusichem 1. This species prefers muddy environments, which agrees with the clayey character of these lakes. Furthermore, the presence of carnivorous species suggests a high biomass production in these lakes, because these species will only be present if sufficient food is available.

Table 7.8. Main taxonomic groups of benthic invertebrates in 10 selected floodplain lakes in The Netherlands (individuals/m^2) [a)]

Floodplain lake	OLIG	CHIR	HIRU	MOLL	CRUS	EPHE	ODON	HEMI	MEGA	COLE	TRIC	DIPT	Total of main groups
River IJSSEL													
Herxen 1	3900	733		7	4	4				4		56	4633
Hengforderwaard 2	2078	204		11								259	2281
Hengforderwaard 3	2433	796		41		48						7	3230
River WAAL													
Gendt 0	4089	526		167								167	4615
Ochten 2	6222	1615	52	507	74				15		4	70	7837
Deest 4	4774	981	374	5037	41	4	70		30	44	7	330	5756
Deest 2	1330	156	115	1678	7	4	4	137		85	104	200	1485
River NEDERRIJN													
Beusichem 1	23870	1426	267	941	44	281			48		7	885	25296
Wolfswaard 4	970	81	19			81		7				174	1052
Meinerswijk 3	7041	2978	7	226		44			248			148	10693

a) OLIG = Oligochaeta; CHIR = Chironomidae; HIRU = Hirudinea; MOLL = Mollusca; CRUS = Crustacea; EPHE = Ephemeroptera; ODON = Odonata; HEMI = Hemiptera; MEGA = Megaloptera; COLE = Coleoptera; TRIC = Trichoptera; DIPT = Diptera other than Chironomidae.

The distribution of the taxa over the lakes and their relation to abiotic variables is indicated in ordination diagrams for the analysis on subset 1 and 2d (i.e., environmental variables together with extracted metals, see next section) of species with sample points (Figure 7.3a) and species with variables (Figure 7.3b). Axes represent the main two axes of the extraction procedure in the RDA. Sample locations of the same lake are sometimes heterogeneous with regard to species composition and abiotic variables (e.g., Herxen 1). Deest lakes (Deest 2 and Deest 4) clearly are situated far away from the other samples (Figure 7.3a). This is caused by the presence of herbivore species (e.g., *Radix auricularia* and *Mystacides* sp.). IJssel lakes all are located in the lower left corner, showing that they have a similar macroinvertebrate community. Meinerswijk 3, Ochten 2, and Beusichem 1 samples are related with regard to their community composition. Relations of macroinvertebrates and their environment are shown in Figure 7.3b. Arrow length indicates the magnitude of the contribution of each variable in explaining community structure. Arrow direction indicates in which way the variable influences community structure. Species can be projected perpendicular to these arrows to determine their ranking according to a specific variable. When the projection of a species is located near the arrowhead of a variable, this species is associated with higher values of that variable. When its projection is close to the arrow tail or even situated opposite the arrow direction, occurrence of the species is not related to that variable or is negatively associated with that variable (Ter Braak & Smilauer, 1998). Electric conductivity and oxygen concentration in the surface water is very important in structuring the community, as represented by the long arrows. Sediment characteristics (OC, AVS, and fine grain sizes) all point in the same direction. Species associated with these muddy, reduced sediments are, for example, oligochaetes and chironomid species. The Pb-SEM, Cd-SEM, and Cu-SEM concentrations coincide with the vertical axis (Figure 7.3b), where samples from lake Meinerswijk 3 also are located (Figure 7.3a). Variables such as pH in water and sediment are less important. Lake surface area apparently also is important, which may be related to wind influence, water level fluctuations, and refugee or colonization possibilities. The partitioning of the variance in community structure is shown in Table 7.9.

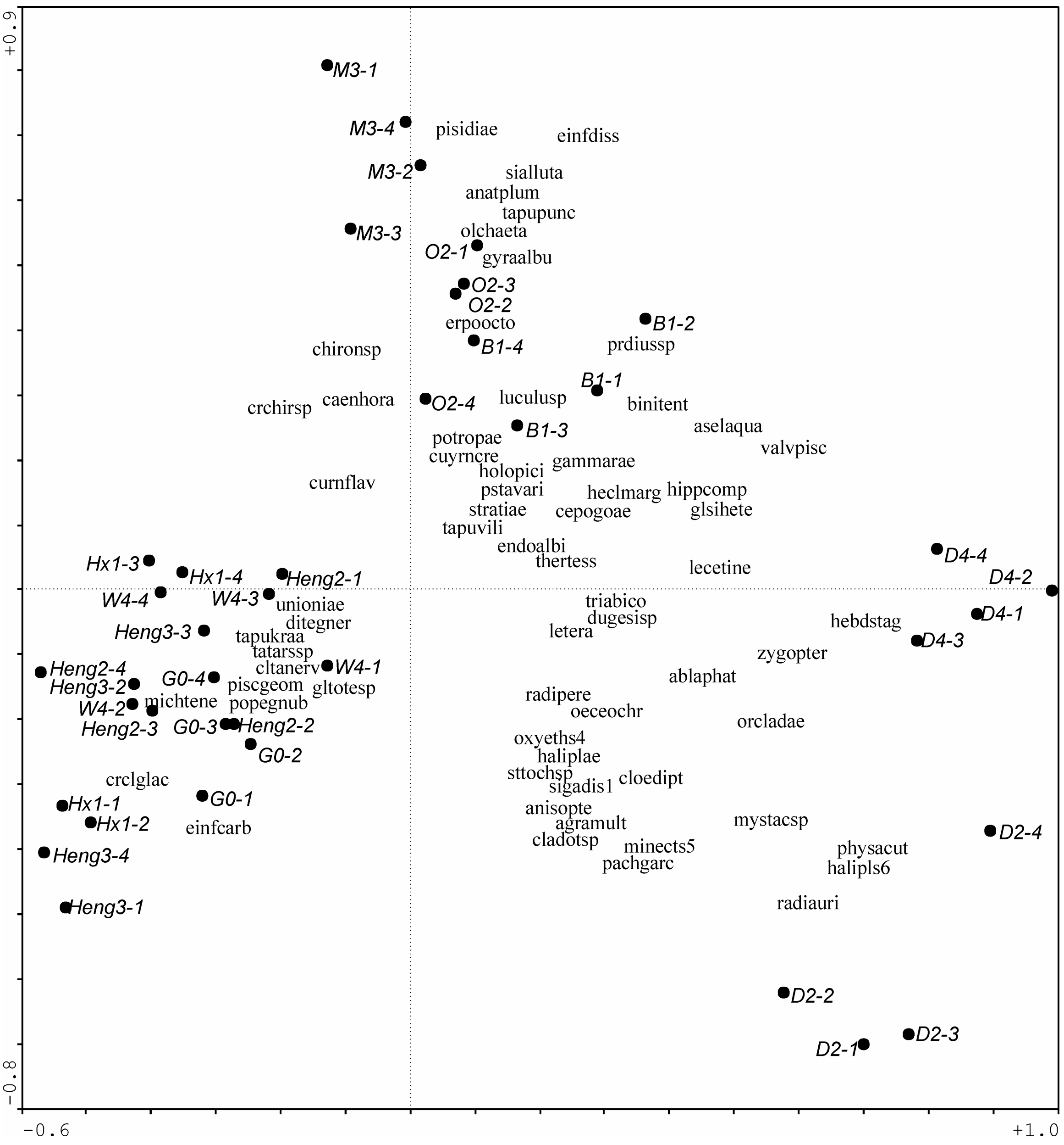

Figure 7.3. (**a**) Ordination of samples with species after partial redundancy direct analysis. Axes represent the first two axes of the ordination analysis. Sample locations (italic) are indicated with points; species are indicated with eight-letter codes, which are abbreviations of their scientific names (see the Appendix). Abbreviation of sample locations: Hx1 = Herxen 1; Heng2 = Hengforderwaard 2; Heng3 = Hengforderwaard 3; G0 = Gendt 0; O2 = Ochten 2; D4 = Deest 4; D2 = Deest 2; B1 = Beusichem 1; W4 = Wolfswaard 4; M3 = Meinerswijk 3. The numerals 1, 2, 3, or 4 after a hyphen indicate the separate samples within a lake.

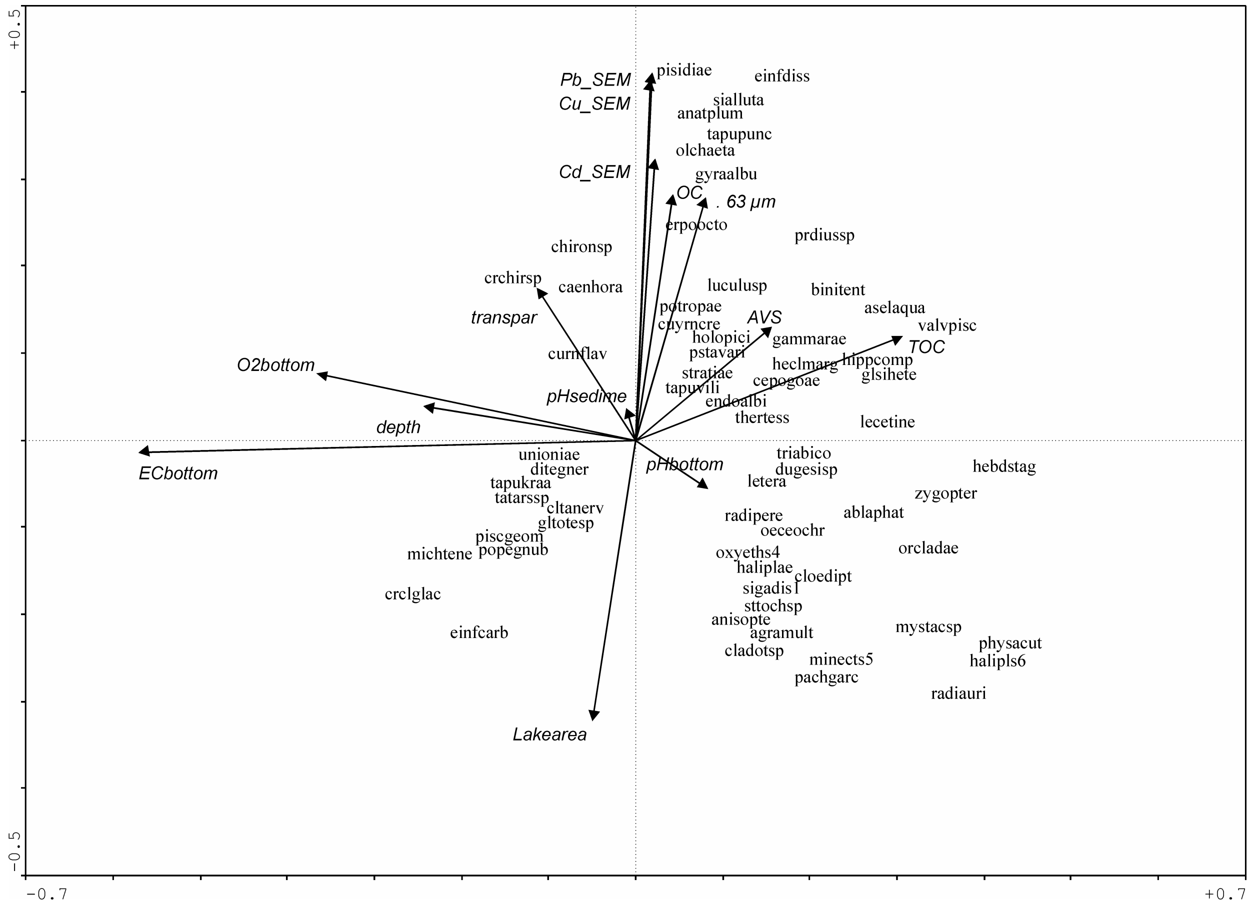

Figure 7.3. (**b**) Ordination of selected environmental variables and extractable metals with species after partial redundancy direct analysis. Axes represent the first two axes of the ordination analysis. Variables (italic) are shown as arrows; species are indicated with eight-letter codes, which are abbreviations of their scientific names (see the Appendix). Abbreviation of variables: transpar = transparency; ECbottom = electric conductivity of surface water near the sediment; O2bottom = oxygen concentration of surface water near the sediment; depth = lake depth; pHbottom = pH of surface water near the sediment; pHsedime = pH determined in $CaCl_2$ extract of sediment; OC = organic carbon in sediment (0.58 * loss on ignition); .63 mm = grain size fraction < 63 µm; AVS = acid volatile sulfide; TOC = total organic carbon in surface water; Lakearea = lake surface area; Pb_SEM = individual extracted Pb concentration; Cu_SEM = individual extracted Cu concentration; Cd_SEM = individual extracted Cd concentration. SEM = simultaneously extracted metals.

Table 7.9. Variance partitioning of selected environmental variables and alternative trace metal measures. Note that the sum of the variance of all individual variables within a group does not necessarily equal the variance of the group. Subset 1 represents environmental variables alone, subset 1+2 represents environmental variables together with a specific trace metal measure: 2a: total metal concentrations; 2b: OC-normalized metals; 2c: clay-normalized metals; 2d: individual extracted metals; 2e: SEM; 2f: SEM/AVS; 2g: SEM-AVS; 2h: SEM-AVS/ fOC; 2i: accumulated metals

Source of variation	**Subset 1**	**Subset 1+ 2a**	**Subset 1+2b**	**Subset 1+2c**	**Subset 1+2d**	**Subset 1+2e**	**Subset 1+2f**	**Subset 1+2g**	**Subset 1+2h**	**Subset 1+2i**
Environmental variables	62.1	55.0	58.1	57.5	51.7	54.4	55.2	60.1	58.8	50.0
AVS $^{a)}$	1.7 $^{NS\ c)}$	1.8 ^{NS}	1.8 ^{NS}	1.9 ^{NS}	1.7 ^{NS}	1.7 ^{NS}	1.3 ^{NS}	1.8 ^{NS}	1.5 ^{NS}	1.3 ^{NS}
OC $^{b)}$	2.7	2.8	2.1 ^{NS}	2.9	2.8	2.6	2.7	2.1	2.6	2.2 ^{NS}
Fraction < 63 µm	2.2	2.1 ^{NS}	1.8 ^{NS}	2.3	2	2.2	1.9 ^{NS}	2.2	2.2	1.5 ^{NS}
pH sediment	1.1 ^{NS}	1.1 ^{NS}	1.1 ^{NS}	1.1 ^{NS}	1.1 ^{NS}	1.0 ^{NS}	1.1 ^{NS}	1.2 ^{NS}	1.1 ^{NS}	1.0 ^{NS}
pH water	3	1.8 ^{NS}	1.6 ^{NS}	1.8 ^{NS}	1.2 ^{NS}	1.4 ^{NS}	1.7 ^{NS}	2.8	2.9	2.1 ^{NS}
EC water	4.3	4.3	4.1	4.1	3.7	4.1	3.9	3.1	4.4	2.8
O_2 water	5.7	2.9	3.0	3.2	1.6	2.6	2.5	5.6	5.6	2.0 ^{NS}
Depth	3.1	2.9	3.1	3.1	2.9	3.0	3.0	3.0	3.0	3.1
Transparency	4.0	4.1	4.0	4.1	3.2	4.0	3.9	3.9	4	3.9
TOC	5.5	5.7	5.2	5.4	3.7	5.4	5.5	3.5	5	5.1
Lake surface area	5.1	4.3	3.8	3.6	3.4	4.1	3.4	5.1	5	3.8
Trace metals		1.8 ^{NS}	1.5 ^{NS}	1.5 ^{NS}	5.7	1.4 ^{NS}	1.1 ^{NS}	2.4	1.4 ^{NS}	4.0 ^{NS}
Zn		- $^{d)}$	1.5 ^{NS}	1.5 ^{NS}	-					1.4 ^{NS}
Cu		-	-	-	1.8 ^{NS}					1.7 ^{NS}
Cd		1.8 ^{NS}	-	-	2.0 ^{NS}					$^{e)}$
Pb		-	-	-	2.0 ^{NS}					0.9 ^{NS}
∑SEM $^{f)}$						1.4 ^{NS}				
SEM/AVS							1.1 ^{NS}			
SEM-AVS								2.4		
SEM-AVS/f_{OC}									1.4 ^{NS}	
Variation shared	0.0	7.1	4.0	4.6	10.4	7.7	6.9	2.0	3.3	12.2
Unexplained	37.9	36.1	36.4	36.4	32.2	36.5	36.8	35.5	36.5	33.8
Total variation	100	100	100	100	100	100	100	100	100	100

a) Acid Volatile Sulfide; b) Organic Carbon, calculated as 0.58 * Loss On Ignition; c) not significant at $p < 0.05$; d) – means that variable is not included due to high VIF value; e) Cd in oligochaetes below detection limit; f) Simultaneously Extracted Metals.

The environmental variables denominated in Table 7.2 together explain 62.1% of the biological variation (Table 9, first column). This is very high in comparison with other studies. Approximately 20% of the biological variation was due to the considered ecological variables in a similar study in a Canadian fluvial lake (Pinel-Alloul *et al.*, 1996). In the Dutch North Sea Canal (Peeters *et al.*, 2000), common variables explained 45% of the community variation, whereas in the Rhine–Meuse Delta and river branches, their contribution was about 15% (Peeters *et al.*, 2001). Each following column in Table 7.9 represents an alternative measure of metal availability. Because of high VIF values, it was often impossible to include all metals in the analysis. In those cases, the contribution of one or more trace metals was then assumed to be representative for all metals. This is realistic because the occurrence of high VIF values is based on the high correlation between the variables. Total explained variation increased when the analysis was performed on environmental (subset 1) and metal (subset 2a–i) variables together. Maximum explained variation of 67.8% was achieved when separate extractable (SEM) metals (subset 2d) were included. This agrees with a study of Lee *et al.* where accumulation of metals in clams and polychaetes was best related to SEM concentrations, irrespective of AVS levels (BG Lee *et al.*, 2000). Percentages of explained variation by environmental and pollution variables are in the same range as found in other studies. In the Dutch Rhine–Meuse Delta and Dutch river branches, 45 to 72% of the variation in species composition could be explained by ecological and contaminant variables together (Peeters *et al.*, 2001). The contribution of environmental variables (subset 1) decreased if metals also were included in the analysis. This decrease in contribution depends on which metal subset is analyzed and is caused by shared variation between environmental variables and trace metals. This means that the influence of environmental variables partly coincides with the effects of trace metals and vice versa, and that their contribution cannot be separated. The pure contributions of trace metals range from 2.4 to 5.7% in this study. These contributions all were significant and of similar magnitude as other individual ecological factors. Accordingly, metals can be considered an important factor in structuring floodplain lake communities. In Rhine–Meuse Delta and river branches (Peeters *et al.*, 2001), 4 to 9% of the variation in species composition could be attributed solely to trace metals, whereas this value was up to 11.4% for the North Sea Canal (Peeters *et al.*, 2000). However, total pollutant concentrations in the present study are in the same range as levels in the North Sea Canal study (Peeters *et al.*, 2000). Environmental variables are likely to show a higher contribution to community variation in our study than in the North Sea Canal study because geographically different regions are regarded, whereas the North Sea Canal is one environmental entity in which merely salinity and sediment composition are variable.

When evaluating the contributions of different trace metal availability measures, only two methods appear to contribute significantly to the explanation of community structure (Monte Carlo permutation test, $p \leq 0.05$). These are the methods based on individual extracted metals (subset 2d, Cu-SEM, Cd-SEM, and Pb-SEM, explaining 5.7% at $p = 0.04$), and SEM-AVS (subset 2g, explaining 2.4% at $p = 0.05$). A further correction of SEM-AVS on OC content does not link metal pollution with community structure. A striking observation is that the contributions of total, normalized, and SEM metal concentrations in sediments as well as concentrations in Oligochaeta are not significant. Thus, approximating bioavailable concentrations by accumulation in Oligochaeta does not yield a better explanation of metal effects on the community than does studying environmentally available concentrations. This can be explained in several ways. First, exposure of oligochaetes to trace metals, which takes place via the sediment for a substantial part, is not representative for other benthic organisms. This is consistent with the earlier discussed studies on Cd in spiked sediments (Hare *et al.*, 1994, 2001; Warren *et al.*, 1998). Second, although no relation between oligochaete abundance and accumulated concentrations was observed, other more sensitive taxa may suffer toxic effects, leading to a decrease in abundance or even disappearance. A third explanation may be adaptation of the community to the pollution (Clements, 1999).

Shared variation between environmental and trace metal contribution is smallest, only 2%, when the combination with SEM-AVS is regarded. This means that effects on community structure can be ascribed well to the separate groups. Although SEM-AVS critical values are only exceeded occasionally, they have a significant effect on the studied community. We can only speculate about the causal relation between SEM-AVS and the community. The community possibly is still recovering from a period in which AVS concentrations were lower, whereas measured SEM-AVS concentrations indicate that little effect should be expected. Furthermore, we did not take into account the vertical variability in AVS concentrations. The SEM-AVS values will be higher in the top millimeters to centimeters. Several species probably are predominantly present in this environment and thus exposed to higher concentrations of dissolved metals. Individual extracted metals (SEM) explain most of the variation, which suggests that this fraction is related better to available concentrations than total or normalized concentrations. The individual contribution of environmental variables shows that surface water and habitat characteristics are the most important variables. Much of the variation explained by the group of environmental variables is in fact variation shared among these variables, as can be derived from Table 7.9 by summing up the contributions of the individual variables, and comparing that with the contribution of the environmental variables as a group. When trace metals are included, individual contributions of trace metals and sediment characteristics such as OC, AVS, and grain size ≤63 µm often become insignificant in the analyses, which points to shared variation between environmental variables and trace metals. The fact that at the low levels of metal stress, SEM- and AVS-related variables still were the best estimators of this stress illustrates the usefulness of the SEM/AVS approach for in situ community-based risk assessment.

The ordination as shown in Figure 7.3b discussed before shows that the arrows of the trace metals point in the same direction. This suggests that species composition is affected in a similar way. In contrast, metal arrows point in different directions when the effects of all other variables are subtracted (see Figure 7.4). The distribution of functional feeding groups now shows that they are affected in different ways. Species situated at the left and upper side of the diagram (e.g., *Sialis lutaria* [carnivore] and *Einfeldia dissidens*, Pisidiae [filter feeder]) apparently are less sensitive or less exposed to Cu and Pb than species at the lower right side. Strictly detritivore species (indicated in Figure 7.4 with italic characters) are concentrated in the lower left corner, which suggests a particular sensitivity to Cd. In contrast, herbivore species are found in the upper right quadrant, associated with extractable Cd. As was expected based on their feeding mode, these species are rather indifferent to sediment-bound metals. This specific response of functional feeding groups when only pollutants are regarded agrees to results of Peeters *et al.*. (2000), which suggests that detritivore species are most affected by trace metals.

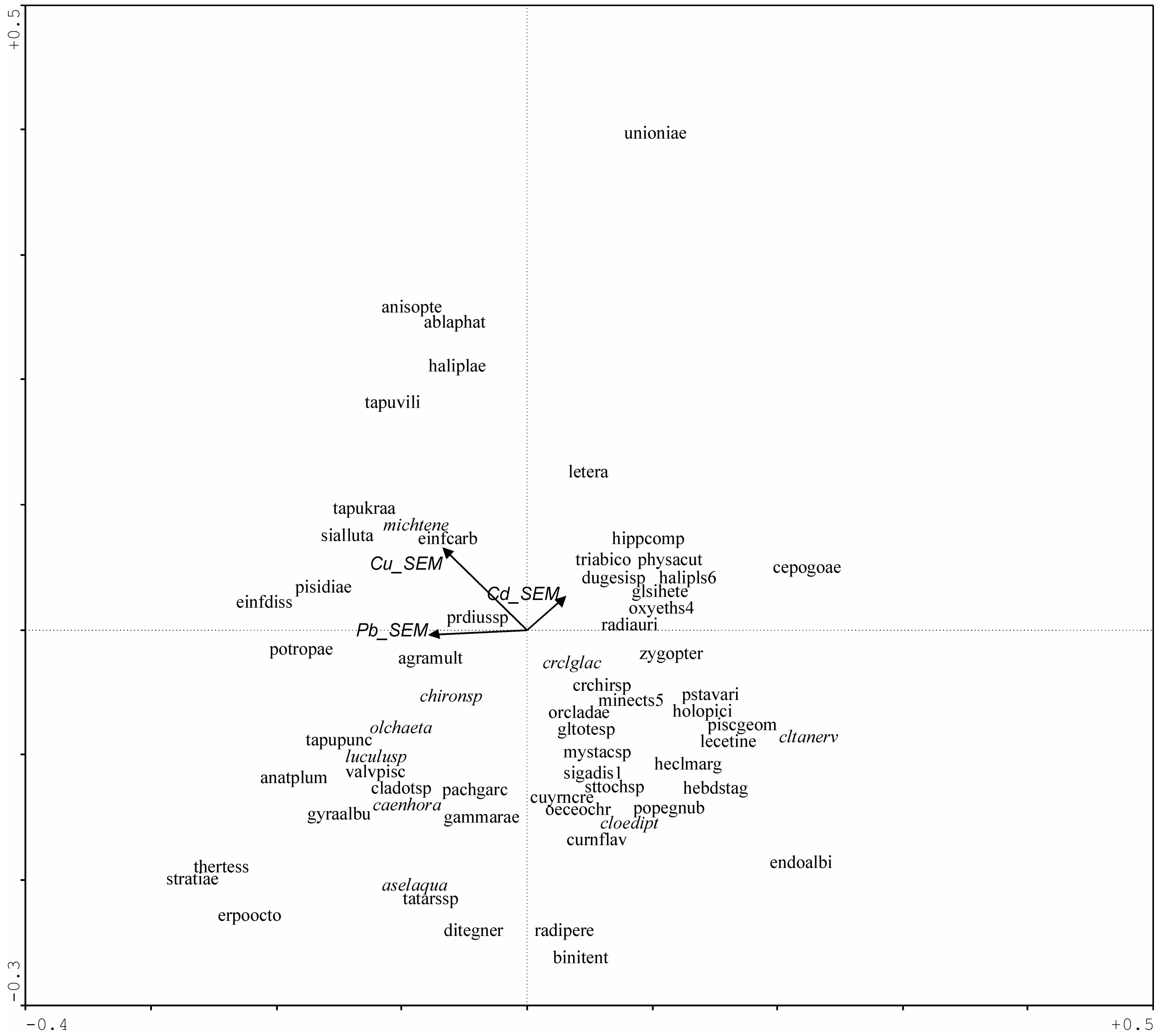

Figure 7.4. Ordination of species and extractable metals after removal of the effect of environmental variables with partial redundancy direct analysis. Axes represent the first two axes of the ordination analysis. Variables (italic) are shown as arrows; species are indicated with eight-letter codes, which are abbreviations of their scientific names (see the Appendix). Detritivore species are indicated in italics. Abbreviation of variables: Pb_SEM = individual extracted Pb concentration; Cu_SEM = individual extracted Cu concentration; Cd_SEM = individual extracted Cd concentration. SEM = sum of all simultaneously extracted metals (Zn, Cu, Pb, Ni, Cd).

7.4 Conclusion

Geochemical distribution of trace metals in floodplain lake sediments differs among Rhine branches. All methods to assess environmentally available trace metals (totals, normalized, and SEM-AVS) show a similar pattern, with only few locations exceeding threshold values. Trace metal accumulation in oligochaetes is positively correlated to concentrations of trace metals in the sediment but also strongly negatively correlated to DOC concentrations in surface water. Water quality variables, and to a somewhat lesser extent, sediment and other habitat variables, clearly are important in determining community structure in floodplain lakes. However, trace metals also have an important effect on benthic community structure. Approximately 6% of the biological variation can be attributed solely to trace metals in the form of extractable metals (individual SEMs). The SEM-AVS concentrations also significantly explained community composition for 2.4% of the variation. These percentages must be considered an underestimation of the true impact, because part of the effect of trace metals is still hidden in the variation shared with environmental variables. The functional feeding group of detritivores appears most affected by trace metals. Extracted metals (SEM) and SEM-AVS concentrations better explain community structure than do total or normalized concentrations, which supports the use of accounting for available fractions in ecological risk assessment. Approximating bioavailability of trace metals for benthic invertebrates by evaluating accumulation in Oligochaeta does not yield a better explanation of community structure than using environmentally available concentrations.

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Appendix I List of full and abbreviated scientific names of macroinvertebrate taxa.

Species	Code
TURBELLARIA	
Dugesia sp	dugesisp
OLIGOCHAETA	
Oligochaeta	olchaeta
Lumbriculus sp	luculusp
HIRUDINEA	
Helobdella stagnalis	hebdstag
Theromyzon tessulatum	thertess
Eropdella octoculata	erpoocto
Hemiclepsis marginata	heclmarg
Glossiphonia heteroclita	glsihete
Piscicola geometra	piscgeom
MOLLUSCA	
Valvata piscinalis	valvpisc
Physa acuta	physacut
Bithynia tentaculata	binitent
Gyraulus albus	gyraalbu
Pisidiidae	pisidiae
Radix auricularia	radiauri
Radix peregra	radipere
Hippeutis complanatus	hippcomp
Unionidae	unioniae
CRUSTACEA	
Asellus aquaticus	aselaqua
Gammararidae	gammarae
EPHEMEROPTERA	
Caenis horaria	caenhora
Cloeon dipterum	cloedipt
ODONATA	
Zygoptera	zygopter
HEMIPTERA	
Micronecta sp nymphs	minects5
Sigara distincta	sigadis1
MEGALOPTERA	
Sialis lutaria	sialluta
Haliplidae	haliplae
Haliplus sp	halipls6
TRICHOPTERA	
Agraylea multipunctata	agramult
Cyrnus crenaticornis	cuyrncren
Species (Continued)	**Code**
Cyrnus flavidus	curnflav
Holocentropus picicornis	holopici
Leptocerus tineiformis	lecetine
Oecetis ochracea	oeceochr
Mystacides sp	mystacsp
Oxyethira sp (pupa)	oxyeths4
Polycentropodiae	potropae
Triaenodes bicolor	triabico
LEPIDOPTERA	
Lepidoptera	letera
DIPTERA	
Ceratopogonidae	cepogoae
Stratiomyidae	stratiae
Chironomidae:	
Ablabesmyia phatta	ablaphat
Anatopynia plumipes	anatplum
Chironomus sp	chironsp
Cladotanytarsus sp	cladotsp
Clinotanypus nervosus	cltanerv
Cryptochironomus sp	crchirsp
Cryptocladopelma gr. *Laccophila*	crclglac
Dicrotendipes gr *nervosus*	ditegner
Einfeldia carbonaria	einfcarb
Einfeldia dissidens	einfdiss
Endochironomus albipennis	endoalbi
Glyptotendipes sp	gltotesp
Microchironomus tener	michtene
Orthocladiinae	orcladae
Polypedilum gr. *Nubeculosum*	popegnub
Procladius s.l.	prdiussp
Psectrotanupus varius	pstavari
Stictochironomus sp	sttochsp
Tanypus kraatzi	tapukraa
Tanypus punctipennis	tapupunc
Tanypus villipennis	tapuvili
Tanytarsus sp	Tatarssp

CHAPTER 8

SUMMARIZING DISCUSSION

8.1 Introduction

Climatic change may result in increased flooding frequencies and flood durations in lowland river systems. Hence, plans are developed to restore floodplains as nature reserves and at the same time as storage basins for river peak discharges. However, floodplains in The Netherlands are historically loaded with organic pollutants and trace metals. The ecological risk of metal polluted sediments in floodplains depends on the available contents of metals rather than on total contents. The redox status of the sediment strongly influences the bioavailability of metals. In reduced sediments, metals are known to bind strongly to sulfides leading to insoluble metal sulfide precipitates. This sulfide and bound metals can be assessed by the SEM/AVS measurement method. In dynamic systems such as floodplains, the persistence and spatial and temporal patterns of AVS may be highly variable. Validation of the SEM/AVS method by comparison with eco(toxico)logical endpoints is crucial to evaluate the applicability of this method for the assessment of ecological risks. Main goal of this research was to evaluate ecological risks of trace metals in floodplain sediments, and test the applicability of SEM/AVS as a tool to estimate bioavailability. Therefore, main research aims were related to both analytical aspects and the ecological significance of the variables AVS and SEM:

1. To assess the spatial and temporal variability of SEM and AVS in relation to total (and dissolved) trace metal concentrations in floodplain sediments
2. To assess the eco(toxico)logical relevance of trace metals, SEM and AVS in floodplain lakes
3. To assess whether the use of SEM and AVS improves risk analysis compared to conventional methods

In this chapter, the main results are summarized and their implications for the practice of risk assessment of polluted sediments are discussed in relation to recent findings and river restoration problems. Recently, several evaluations of the SEM/AVS concept have been made. For instance, the Dutch Institute for Public Health and the Environment (RIVM) (Sijm *et al.*, 2001) evaluated the usefulness of SEM/AVS in second tier risk assessment and concluded that normalizing on AVS is usefull for Zn. Cornelissen & Kamerling (2003) evaluated current procedures and techniques for risk assessment of contaminated sediments, and concluded that the SEM/AVS method provides a useful tool to assess the probability of risks in a first screening. For the subsequent assessment of possible effects of excess metals, however, other methods should be applied. DiToro *et al.* (2005) propose a refinement accounting for the partitioning of excess SEM, which can deal with the above mentioned comment.

Morse & Rickard (2004) criticize the general applicability of the concept because AVS -being operationally defined and mainly comprising dissolved (hydrogen) sulfides and mackinawite (FeS)- may differ largely in composition and capacity of precipitating trace metals for different sediments (early diagenesis versus mature sediments). They conclude that the sediment sulfide system is complicated and that therefore AVS measurements have no straightforward interpretation. Furthermore, not all metal sulfide minerals are dissolvable in 1 M HCl. Zn, Pb and Cd are virtually completely extractable in 1 N HCl while Cu and Ni are dissolvable in nitric acid only. This implies that the latter metals do not react with the reactive sulfide pool that makes up AVS, but rather with a variety of sulfide minerals with and without Fe associated with the pyrite fraction (Morse & Luther, 1999). Another limitation already addressed above is that SEM/AVS in itself only predicts the absence or lack of toxicity but not the occurrence and magnitude of toxicity and that excess SEM may partition to different sediment constituents for different sediment types and environments. It appears that organic carbon is the most important sediment constituent to be taken into account (e.g., DiToro *et al.*, 2005).

There is some doubt about the biological relevance of the AVS/SEM concept. AVS only is formed in substantial amounts in reduced sediments, whereas many species mainly reside in oxic sediment layers, and are predominantly exposed to overlying water instead of pore water (Hare *et al.*, 1994, 2001). Recently however, Burton *et al.*. (2004) evaluated the relevance of AVS in field situations as part of an EU assessment programme and judged positively on the potential of SEM/AVS for the assessment of ecological risks. In this discussion we briefly incorporate the outcome of these parallel studies in the synthesis of our results.

8.2 Spatial and temporal variability of trace metals and AVS in floodplain lakes

Little is known about fate and dynamics of AVS in spatially and seasonally variable environments. Some studies have been conducted in The Netherlands, but these often focused on detailed study of a very limited number of samples (Van den Berg, 1998; Van den Berg *et al.*, 1998) or the geographical distribution in different water systems (Van den Hoop *et al.*, 1997). For the practice of ecological risk assessment, it is necessary to consider spatial and temporal variability at the scale of the aquatic community at risk, for instance the macro-invertebrate community in a floodplain lake. As AVS only persists under anoxic conditions, and floodplains are known to have very dynamic hydrological and thus redox conditions through time, but also in vertical and lateral direction, it is very important to get insight into the degree of this variation. In this research, AVS variability has been studied for the first time in the Dutch riverine system on both a seasonal and a small-scale spatial basis.

8.2.1 Spatial variability of trace metals and AVS

In Chapter 3, spatial variability of sediment properties, environmental conditions, total contaminant concentrations, and available metals (as SEM-AVS or (SEM-AVS)/f_{OC}) was studied in a floodplain lake. The top 5 cm of sediment was sampled at 43 locations. Trace metal and SEM concentrations, and most sediment characteristics were more or less constant within 10%. In contrast, AVS concentrations were much more variable and showed a strong spatial dependence due to differences in lake depth, total sulfur pools, and redox potential (E_h), which resulted in crucial differences in trace-metal availability within the lake. The spatial pattern of SEM-AVS deviated from total or 'standard soil' normalized trace-metal patterns as traditionally used in metal risk assessment. Consequently, the AVS-based method attributed the highest risk to a different corner of the lake than risk assessment based on standard soil normalisation. This illustrates the importance of combining such assessments, rather than relying on one methodology.

8.2.2 Temporal variability of AVS

In Chapter 4, *in situ* AVS formation and degradation and SEM were monitored in two floodplain lakes, and compared to AVS formation rates in laboratory experiments with the same sediment. In these laboratory experiments, formation rates of AVS were studied at 20 °C for initially oxidized sediments that were (a) untreated, (b) enriched with extra sulfate, and (c) treated with sodium-azide (biocide). In the field, AVS concentrations were highly variable and were significantly negatively correlated to oxygen concentrations, and significantly positively correlated to surface water temperature and to fine sediment particles. Between February and August, AVS formation was stimulated and increased linearly in time. Degradation rates differed drastically between the lakes due to different degradation mechanisms. In one lake, AVS removal was most probably caused by reworking and oxygenation of the sediments by bream. In the other lake, AVS removal was caused by dessication during a period of drought, at a tenfold higher rate. This illustrates the large differences that can be found between similar sediments, and the importance of biological processes. In the laboratory, concentrations of AVS with and without sulfate addition were similar during the first weeks, and increased at a rate twice as high as compared to the field situation. Because conditions for AVS formation were optimal in the lab as compared to the field, this is not surprising. However, sulfate addition increased the AVS concentration at the end of the experiment, whereas sodium-azide eliminated AVS formation, as expected. This suggests that AVS formation was sulfate-limited in laboratory and field conditions. This is in line with the general view that the sulfate source often limits sulfide production in freshwater sediments (e.g., Leonard *et al.*, 1993; Smith & Melville, 2004).

8.2.3 Temporal variability in trace metal concentrations

In Chapter 5, we monitored temporal variation in sediment and pore water variables including Dissolved Organic Carbon (DOC)-, AVS-, and trace metal concentrations in a floodplain lake. Also, vertical variation in variables and processes was considered by studying various layers of the sediment. The sampling period included a severe winter inundation and a dramatic water level drop during summer. Solid metal concentrations increased with depth indicating a gradual improvement of sediment quality in time. Dissolved metal concentrations were highest in top layers due to mobilization from oxyhydroxides and precipitation with sulfides in deeper layers. Inundation (in winter) had a mobilizing effect due to a) the flux of oxygen towards sediments and b) resuspension of sediment top layers. Conversely, water table lowering combined with organic matter decomposition (in summer) led to immobilization due to sulfide formation.

Overall, considerable spatial and temporal variation in AVS levels is observed. In order to make conservative risk estimates, AVS concentrations may be determined per season. For a robust indication of available metals in the biologically active layer, a mixed 5 cm layer could be considered. In that case, the use of a grab sampler is recommended in order to preserve AVS levels as much as possible (Chapter 6, see section 8.4). The experiments on formation and degradation of AVS show that after sudden events, like severe pertubation, drought or inundations, it may take 2 to 3 months before sufficient AVS has been formed to bind all SEM when conditions are favourable.

8.3 Ecological relevance of the SEM/AVS concept

8.3.1 Relation of SEM/AVS with eco(toxico)logical endpoints

Many literature reports provide validation of the SEM/AVS concept with respect to ecotoxicological endpoints in the laboratory (e.g., DiToro *et al.*, 1990, 1992; Ankley *et al.*, 1991;

Brouwer and Murphy, 1994; Ankley *et al.*, 1996). Very recently, DiToro *et al.* (2005) published a proposal to normalize excess SEM on OC content to improve the prediction of the magnitude of toxic effects. The applicability of AVS for ecological risk assessment of trace metal polluted sites should, however, also be validated using the relation with *in situ* ecological endpoints. Many studies report either a) AVS and SEM concentrations, b) SEM/AVS and bioassay results, c) SEM/AVS and field surveys but, to our knowledge, none integrate all using the same methodology. Alternatively, results from different studies could be combined, but this is often hampered by differences in sediments and methodology (Morse & Rickard, 2004). Both AVS (e.g., Lasorsa & Casas, 1996) and bioassays are very susceptible to handling procedures in the lab. Hence, the current research offers a good opportunity to provide such an integration, as chemical, ecotoxicological (survival, growth, bioaccumulation) and ecological (community structure) endpoints are studied using one, consistent methodology. This TRIAD-like approach is not new in itself, but in earlier studies often total contaminant concentrations are the starting point, not bio-available concentrations like SEM-AVS (e.g., Den Besten *et al.*, 1995; Reinhold-Dudok van Heel et al, 1999). A complicating factor in the interpretation of TRIAD studies is that other stressors than metals contribute to the observed ecological effects. Therefore it is useful to quantify the relative contribution of all environmental factors to the observed effects (Peeters *et al.*, 2000, 2001).

Hence, we studied relations of bioavailable metal concentrations as determined with SEM-AVS with bioassay responses (Chapter 6, survival and growth of *Asellus aquaticus*), bioaccumulation (Chapter 7) and benthic community structure (Chapter 7) in a selection of 7 to 10 floodplain lakes with different pollution characteristics in relation to environmental factors other than pollution. Results are summarized below and discussed in the light of other (recent) studies.

8.3.2 Relation with bioassays

In Chapter 6 (experiment 2), different alterations of the sediment from floodplain Deest 4 were made in order to determine their effects on AVS concentrations. Subsequently, AVS and SEM concentrations were compared to bioassay responses. It appeared that higher AVS concentrations coincide with a lower survival of *A. aquaticus*, which was explained by the anoxic conditions that prevail when AVS is high. Higher AVS resulted in higher growth of the organisms, which is explained by the lower resulting metal stress as SEM-AVS and (SEM-AVS)/f_{OC} are low when AVS concentrations are higher. SEM alone had no effect on survival and a slightly negative

effect on growth. In Biesbosch sediments, where the range of both SEM and AVS concentrations was larger (De Lange *et al.*, 2004), the positive effect of AVS on growth was not found.
When the response of *Asellus aquaticus* (a benthic invertebrate) was compared to the response of *E. virgo* (a water insect) from a parallel study in the same 7 floodplain lakes (De Haas *et al.*, 2002) in a Principal Component Analysis (PCA), it appears that *A. aquaticus* experiences a protecting effect of AVS (endpoint growth in experiment 3).

8.3.3 Relation with bioaccumulation

The field study described in Chapter 7 comprised a set of 10 floodplain lakes in the Rhine river catchment. Part of this field study focused on the amount of metals accumulated by detritivore invertebrates (Oligochaeta). The relation between accumulation and explanatory environmental variables as well as exposure concentrations of trace metals was analyzed with multiple linear regression. The variable data set for metals included total metals, metals normalized on organic carbon (OC) or clay, SEM, and combinations of SEM and AVS. Accumulated metal concentrations correlated positively with sediment trace metals and negatively with surface water DOC. 1 M HCl extractable trace metals (i.e., SEM for individual metals or total SEM) were better in explaining accumulated metals than *Aqua regia* -extractable ('total') metal content. This agrees to results of Snape *et al.* (2004) who found that a four hour extraction with 1 M HCl allows equilibrium dissolution of relatively labile metals correlating best with biological data in Antarctic marine sediments. SEM also correlated best with community structure, as will be discussed in the next section. Apparently, SEM better represents the exchangeable, potentially toxic fraction than 'total' metal fractions. Apart from sediment-bound metals, accumulation of metals in oligochaetes was also related to surface water variables. This is in accordance with findings of Hare *et al.* (1994; 2001), who conclude that, dependent on feeding pattern, benthic species may receive part of the metal contamination from surface water. For Cu and Zn there were indications of regulation within the organisms, which is a commonly observed phenomenon for these metals.

8.3.4 Relation with in situ community structure

The major part of the study in Chapter 7 focused on unraveling *in situ* effects of trace metals and common environmental variables on benthic macroinvertebrate communities in floodplain lakes. This completes the TRIAD of linking chemical (measurements of AVS and SEM), ecotoxicological (bioassays with AVS and SEM) and ecological observations (this section). The relation between community structure and various alternative measures of trace metal availability was evaluated. Explanatory variables included total metals, metals normalized on organic carbon (OC) or clay, SEM, combinations of SEM and AVS, and metals accumulated by detritivore invertebrates (Oligochaeta). Sixty-eight percent of the variation in benthic community composition was explained by a combination of 11 environmental variables. Metals explained 2 to 6% of the community composition when SEM-AVS or individual SEM concentrations were regarded. In contrast, total, normalized, and accumulated metals were not significantly linked to community composition. These results show that there is an effect of trace metal pollution on *in situ* communities, but only when the available portion of the pollution as quantified by SEM and AVS is regarded. During the survey, SEM exceeded AVS levels at only two locations and exceeded the proposed thresholds of SEM-AVS =1.7 µmol/g DW (McGrath *et al.*, 2004) with values of 4.2 and 6.0 µmol/g DW. The threshold based on (SEM-AVS)/f_{OC} (150 µmol/g OC, McGrath *et al.*, 2002; DiToro *et al.*, 2005) was not exceeded (highest value was 115 µmol/g OC). It may however be assumed (based on observations and calculations presented in Chapter 5) that AVS levels were at maximum value during the survey (late summer). Therefore, the relation of community structure with SEM-AVS or SEM may indicate long term (chronic) effects on benthic species due to metal pollution. Another explanation is that we measured average AVS of 5 cm layers. In these layers locally much higher excess SEM levels may have existed. During spring, the effect of metals is expected to be even more pronounced. Hare *et al.* (2001) reported that depending on their feeding behaviour, benthic species partly receive contaminants from the surface water, in addition to sediment-bound contamination. For our suite of benthic organisms, SEM and AVS concentrations together explained a substantial part of the variation in community structure. SEM-AVS better describes effects on benthic species and communities than total contaminant concentrations and the analysis showed that especially detritivore species are affected by metals. This is in line with observations by Hare *et al.* (2001) who also found that the proportion of metals taken up from sediments was largest in detritivore species. Studies linking SEM/AVS to community effects are scarce, but recently Burton *et al.* (2005) performed a long-term field validation study. They investigated if threshold values as proposed by DiToro and others (e.g., McGrath *et al.*, 2002, DiToro *et al.*, 2005) properly describe toxic effects in field sediment. Four European locations in freshwater lakes or riverine systems were selected, with high and low AVS levels. Sediments were then spiked with zinc (high, low, no dose) resulting in ranges from high to low ratios of SEM-AVS. Sediment was placed back in colonization or toxicity trays and response of in situ benthic community was analyzed. NOELs were found to be near a SEM/AVS ratio of 2 or at (SEM-AVS)/f_{OC} values of 100 µmol/g OC. Toxic effects always occurred at SEM/AVS > 8, and occasionally at ratios ranging from 2 – 8. This 'uncertainty range' corresponds to our findings where effects of metals on the community are significant at maximum SEM-AVS concentrations of 4.0 – 6.0 µmol/g DW. These results validate the usefulness of the concept for field situations.

A TRIAD approach was used earlier in studies in The Netherlands, using total contaminant concentrations without taking bioavailability into account. Reinhold-Dudok van Heel *et al.* (1999) analysed data from ecological and ecotoxicological research together in order to gain more insight into the biological effects caused by sediment pollution in the delta of the rivers Rhine and Meuse. They found that sediment toxicity was positively correlated with the levels of heavy metals. The most important factors determining species composition were the presence of solid substrates, depth, current velocity, erosion, grain size distribution and sediment organic matter content. Den Besten *et al.* (1995) used a TRIAD approach to demonstrate causal relations between effects on the macrozoobenthos community structure, effects demonstrated in bioassays and sediment pollution in 46 sites in the delta of the rivers Rhine and Meuse (The Netherlands) and identified 25 sites as areas where pollution can be held responsible for effects in the field.

In these studies, the quantitative role of ecological and pollution factors was not evaluated. In more recent studies by Peeters *et al.* (2000, 2001) this quantification was made for the Rhine/Meuse Delta and for the North Sea Canal. Percentages of explained variation by environmental and pollution variables in our study are in the same range as found in these studies. In the Rhine–Meuse Delta and river branches, 45 to 72% of the variation in species composition could be explained by ecological and contaminant variables together (Peeters *et al.*, 2001), with 4 to 9% of the variation in species composition attributable solely to trace metals. This contribution of pollutants was up to 11.4% for the North Sea Canal (Peeters *et al.*, 2000). Thus, values from these studies are comparable (Rhine-Meuse Delta) or slightly higher (North Sea Canal) than the values found in our study. Environmental variables are likely to show a higher contribution to community variation in our study than in the North Sea Canal study because geographically different regions are regarded, whereas the North Sea Canal is one environmental entity in which merely salinity and sediment composition are variable.

8.4 Use of SEM-AVS in risk assessment as opposed to traditional analysis

The drawbacks and merits of the SEM/AVS concept as compared to other methods can be divided in two aspects:

1. Analytical aspects (sampling, analysis)
2. Applicability of SEM/AVS in risk assessment

8.4.1 Analytical aspects

This item can be divided into sampling/storage of sediment samples and the actual chemical analysis.

Sample collection and storage of AVS in freshwater floodplains

An important drawback compared to the common practice of total metal content analysis is the sensitivity of AVS for disturbance. While for total content analysis disturbances like stirring/mixing, sieving and drying of the sediment have no consequences, AVS is susceptible to oxidation. Proper collection and storage of samples for the determination of AVS therefore is crucial. In Chapter 6, the effect of sampling method, storage conditions, and pretreatment on AVS concentrations was measured in relation to the effect on bioassay response. Traditionally, sediments are pretreated before using them in bioassays. This limits the extrapolation of results to the *in situ* field situation. The preservation of the original chemical and physical state of the sediment therefore is an important issue for the extrapolation of bioassay results as well as SEM/AVS results to the field. Due to redox changes in the sediment, metal availability and as a consequence animal exposure to trace metals may change considerably. Linking changes in AVS to changes in bioassay response as a result of sample alterations during sampling, pretreatment, and analysis has not been extensively explored.

A comparison was made between the effects on AVS concentrations of commonly applied methods to collect and store sediment samples. As for sediment collection, grab and core samples were compared, while for storage different temperatures were compared to the initial concentrations measured directly after sampling. Samples were either stored cool (4 ºC) or frozen at -18 ºC. Measurements were made at intervals of weeks (cool) to months (frozen). Storage conditions appeared to affect AVS levels, but not SEM levels of the sediment. Results showed that initial AVS concentrations were preserved best in grab samples stored frozen. Sediment pretreatments consisted of sieving versus non-sieving. Sieving affected AVS, but again, did not affect SEM levels. AVS concentrations increased during storage at 4 ºC in the presence of overlying water, and declined after sieving (500 µm) the sediment. The best way to pretreat sediment in order to maintain initial AVS conditions is using an Ekman grab for sampling, immediate storage in a jar without headspace, and freezing as soon as possible. This agrees with results of Lasorsa and Casas (1996).

Analysis of AVS in freshwater floodplains

Various methods or methodological set-ups exist for the determination of AVS and SEM. For instance, a variety of acid strengths, extraction times, and reaction vessels are used. As sediments geochemically are also very variable, this may yield much artefactual variability in reported AVS levels. In the literature, this variability in methodology has raised criticism. For instance, Morse and Rickard (2004) point to implications this may have when comparing results from different studies. This calls for standardisation and ringtesting of methods. Currently, two experimental set-ups can be discerned: the purge-and–trap method and the diffusion method. They differ in the way the sulfides are transferred after volatilization. Further, a variety of measurement techniques exist (e.g., gravimetric, polarographic, ion specific electrode). In Chapter 2, an existing method for the analysis of AVS and SEM based on diffusion was optimized for floodplain sediments with presumed highly variable concentrations due to the alternating hydrological conditions influencing redox state. Goal was to develop a method where many samples could be handled at a time and that could be used on a routine basis. For that reason, sulfides were detected using a sulfide specific electrode. The effects of sample size and reaction time were tested. Sample size had little effect, except at very low or high sample weights. The optimal reaction time was established to be 4 hours. This is longer than in other diffusion methods (Brouwer & Murphy, 1994; Leonard *et al.*, 1996), which is probably due to the larger jar we used. This may lead to longer diffusion times, but it nonetheless agrees to reaction times found by Snape *et al.* (2004). Results show that for our sediments SEM is constant over the period of extraction (1 – 4 h). Snape *et al.* suggest that this is due to the fact that the major part of the released SEM is not bound to sulfides but to other sediment constituents. This holds true for the sediment samples used in Chapter 2, where SEM levels exceeded AVS levels. However, the faster release of SEM may also be explained by the time lag in sulfide transfer from the sediment to the SAOB as compared to the spike solutions, implying that the actual dissolution of metal sulfides already occurs quickly, but the sulfide transfer to the trap solution is rate-limiting. Prolonged extraction with 1 M in any case had no consequences for the amount of metals extracted. In any case, the method was found suitable for the simultaneous analysis of several samples at appropriate sample weights (5-10 grams wet weight) and was used for all studies described in this thesis. Until a uniform protocol is defined, [SEM] and in particular [AVS] should be considered as operationally defined measures.

In conclusion, it is clear that in the different steps leading to determination of AVS and SEM concentrations, consideration of the sensitive properties of AVS is crucial. For conventional total metal content this is not necessary, but here information of metal bioavailability is overlooked. As described in Chapter 1, analytically sophisticated methods to take bioavailability into account are more difficult to apply in routine measurements than AVS and SEM analysis. Therefore, we recommend SEM/AVS as a quick and robust tool that may complement less detailed (total metals) or more detailed or specific methods (DGT, Chelex etc, see Chapter 1). In any case, international standardisation and ringtests would strengthen the analytical validity of the method. Obviously the use of reference material is hardly possible due to the labile nature of AVS and its susceptibility to oxidation.

8.4.2 Applicability in risk assessment

In this section, the results of this thesis with regard to the improvement of risk assessment using SEM/AVS as opposed to other methods are discussed. Further, the prospects for using AVS in risk assessment are given.

Applicability of SEM-AVS as opposed to other methods

In this thesis, data were gathered on the role of AVS in determining the bioavailability of trace metals. Information on AVS, SEM and relations between these quantities were compared to information based on total metal content (Chapters 5 and 7). In the spatial analysis (Chapter 5), it was shown that total metals can be distributed in a very different pattern than bioavailable metals. This may lead to very different estimates of ecological risk. Results in Chapter 7 indicate that bio-available metals are related better to eco(toxico)logical endpoints than total metals.

Based on these results and on those from parallel studies, we recommend to incorporate AVS and SEM measurements in the routine screening of metal polluted sediments. We suggest that SEM analysis can replace total metal analysis – at least for Zn, which is by far the most abundant metal- because of the fixed metal specific relation of SEM and total metal for individual metals (Chapter 5 & 7; Van den Berg, 1998; De Lange *et al.*, 2004). Based on data in Chapter 5 (one lake) and Chapter 7 (ten lakes) highly significant linear relations between 1 M HCl extracted

(SEM extraction) and *Aqua regia* -extracted ('total' metal) can be derived (*n* = 64). The correlations from data from this thesis practically coincide with those obtained for Biesbosch sediments by De Lange *et al.* (2004) (see Figure 8.1). The poor extractability of Cu and Ni compared to total metal analysis is in line with observations by e.g. Van den Berg (1998) and Luther and Morse (1999). For these metals, complexation by organic matter and inclusion in other sulfide minerals provides important binding additional to sulfide precipitation. Because these non-extractable metals and sulfides are not considered to be available to organisms, this does not affect the validity of the SEM-AVS approach. (cf DiToro *et al.*, 2005).

When the SEM/AVS method is compared with analytical methods for pore water, it appears that the analysis is more robust and less time consuming than for instance, DMT, DGT and Chelex (see Chapter 1). For routine sediment screenings, the SEM/AVS method is therefore considered to be more appropriate.

SEM/AVS method in current risk assessment

In the USA, sediment quality criteria already take AVS into account (US EPA, 2005). Currently, steps towards the incorporation of AVS in European policy are made. The SEM/AVS approach is recommended for the second tier risk assessment of zinc in Europe (Sijm *et al.*, 2001). For the moment, focus is on the use of SEM/AVS relations for Zn. This metal is one of the most problematic contaminants in our rivers and makes up over 80-90 % of the total molar SEM content in Dutch and Belgian sediments (Van den Berg, 1998; Van den Hoop *et al.*, 1997; VanGheluwe *et al.*, 2004; this thesis). In the draft Risk Assessment Report (RAR) of zinc in the EU, the use of AVS to describe bioavailable zinc concentrations is proposed for the second tier, including a correction to natural background concentrations. This second tier assessment is made when total concentrations exceed the MPR. If this occurs (SEMZn-AVS)/f_{OC} is considered, following the threshold values as proposed by the research group of DiToro (referenced in Sijm *et al.*, 2001; McGrath *et al.*, 2002). As non-essential metals like Pb and Cd are much more toxic than Zn, it should be considered to incorporate bioavailability as estimated by individual SEM–AVS for other metals too. Currently, the RARs for other metals are being prepared in the EU and the use of AVS for second tier assessment is discussed. Based on our results we recommend to include the SEM-AVS approach in the risk assessment schemes.

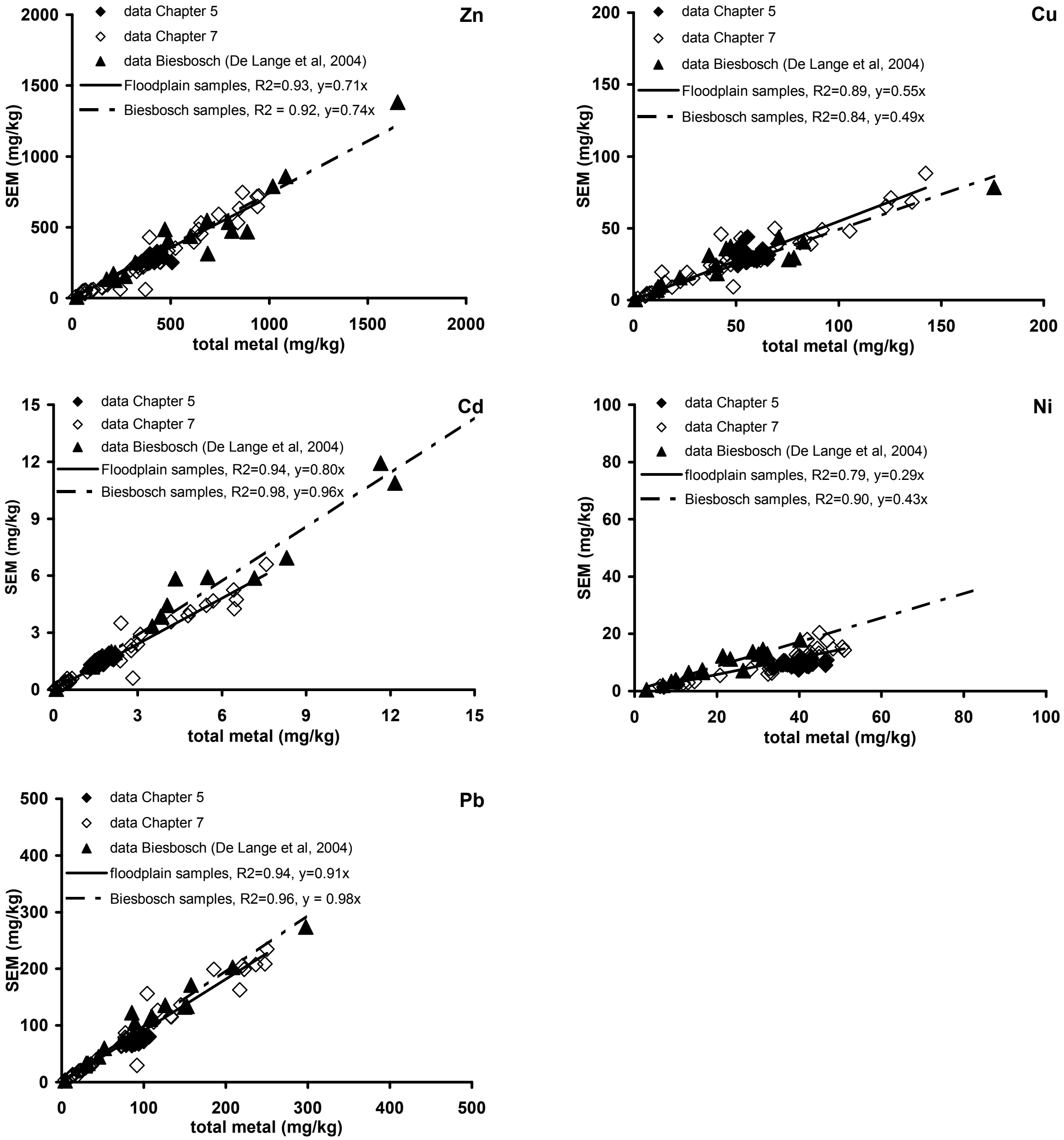

Figure 8.1 Linear relations between *Aqua regia* extractable (total) metals and 1 M HCl extractable metals (SEM) for individual metals Zn, Cu, Cd, Ni and Pb in floodplain sediment. Similar regressions for Biesbosch samples are added for comparison (after De Lange *et al.*, 2004).

8.5 Overall conclusion

The research described in this thesis indicates that AVS variability and as a consequence the bioavailable metal fraction is considerable through time and space and can be explained by variables related to redox conditions and sulfate supply. Furthermore, temporal variability is not one-to-one related to season but more specifically to typical events for floodplains like lake drawdown and drought, and inundation of the floodplain (Chapters 3-5) in Dutch river floodplains a significant effect of trace metals on invertebrate communities is observed, both on the individual as well as on the community level. This becomes particularly apparent when metal availability (SEM alone, or SEM divided or minus AVS) is taken into account (Chapters 6 and 7). Location and season specific risk assessment based on AVS and SEM often gives a different risk characterization than the conventional method based on total concentrations (Chapters 4 and 5), and are as good or better related to ecological endpoints (Chapters 6 and 7).

We conclude that examination of SEM and AVS concentrations is useful for risk assessment of trace metals on both the species level (depending on life history/functional feeding) as the community level, and may therefore contribute to our understanding of the effects of trace metals on aquatic ecosystems. Furthermore, for the management of contaminated sediments, the potential presence of AVS may be an important factor to decide upon storage possibilities. It should however be kept in mind that in deep storage depots, various factors, e.g., the role of primary production as an organic matter source, may be very different from the situation in shallow floodplain lakes. Furthermore, due to the large spatial and temporal variability in AVS, a worst case estimate of the bioavailability of trace metals should be made. In the determination of this estimate, the variables determining AVS concentrations and formation rates that were identified in this research should be taken into account.

Overall, the results from this research show that taking into account trace metal availability with the SEM-AVS approach contributes to a better understanding of trace metal fate and effects in floodplain sediments.

NEDERLANDSE SAMENVATTING

Inleiding

De uiterwaarden langs de grote rivieren in Nederland zijn in het verleden verontreinigd geraakt met grote hoeveelheden contaminanten (milieuvreemde stoffen). Deze verontreiniging is afkomstig van zwevende deeltjes in het rivierwater die bij de overstroming van uiterwaarden als sediment worden afgezet. Het maximum van de waterverontreiniging viel in de jaren '60 en '70 van de twintigste eeuw. De waterkwaliteit is nadien sterk verbeterd maar het al afgezette sediment blijft verontreinigd. Een aanzienlijk deel van deze sedimentverontreiniging bestaat uit zware metalen. Deze komen van nature in de aarde voor, maar door ertswinning en industriële en agrarische toepassingen zijn de gehalten aan het aardoppervlak sterk verhoogd. Naar aanleiding van de recente overstromingen in het rivierengebied (1993, 1995) heeft de overheid plannen ontwikkeld om de rivier meer ruimte te geven om zo de kans op nieuwe overstromingen te verkleinen (plan 'Ruimte voor de Rivier'). Eén van de mogelijkheden om dit te bereiken is het herinrichten van uiterwaarden. Hierbij zal sediment moeten worden afgegraven om meer bergingsruimte voor het rivierwater te creëren. Gelijktijdig kunnen meer specifieke riviergebonden natuurtypen worden gerealiseerd, onder andere door de aanleg van bijvoorbeeld nevengeulen of waterplassen. De vraag is echter hoe er moet worden omgegaan met het verontreinigde sediment dat zich in de uiterwaarden bevindt. Vanwege de grote hoeveelheden is het storten van alle sediment in depots praktisch en economisch niet haalbaar. In de beleidslijn Actief Bodembeheer Rivierbed is afgesproken pragmatisch om te gaan met dit sediment, met als uitgangspunt dat de lokale situatie niet mag verslechteren. In dit verband is het nodig te weten hoeveel risico de verontreiniging, in dit geval de zware metalen, oplevert voor het ecosysteem.

In Nederland zijn vervuilde bodems en sedimenten ingedeeld in klassen op basis van het totaalgehalte aan verontreiniging. Deze klassen zijn gebaseerd op evenwichtspartitie: een berekende vaste verdeling van zware metalen over de vaste sedimentdeeltjes en het poriewater (het water wat zich tussen de vaste sedimentdeeltjes bevindt). Dit zegt echter niet zoveel over het actuele risico van de verontreiniging, aangezien deze ook afhangt van hoe de verontreiniging in de bodem of het sediment zit (de biologische beschikbaarheid). Hier wordt rekening mee gehouden in de huidige risicobeoordelingsmethodiek door het toepassen van een correctie (normalisatie) voor de hoeveelheid klei en organische deeltjes, aangezien de zware metalen daaraan binden. Recente inzichten geven echter aan dat het actuele (werkelijke) risico vaak lager is dan ingeschat op basis van (genormaliseerde) totaalgehalten, omdat de concentraties in het poriewater – een belangrijke blootstellingsroute voor veel soorten benthische (sedimentbewonende) organismen- vaak veel lager is dan op basis van evenwichtspartitie zou worden verondersteld. Dit komt doordat andere bindingsvormen en de invloed van omgevingsfactoren zoals zuurgraad en zuurstofhuishouding (redoxpotentiaal) in de waterbodem en het oppervlaktewater bepalen wat de werkelijk biologische beschikbaarheid van de verontreiniging is. Hoe metalen verdeeld zijn in de bodem of het sediment wordt speciatie genoemd. In het sediment kunnen metalen gebonden zijn aan bijvoorbeeld organisch materiaal, ijzer- en mangaanoxiden, en sulfiden. Over het algemeen wordt er vanuit gegaan dat metalen biologisch beschikbaar zijn voor organismen als ze in het poriewater aanwezig zijn. Daarbij kunnen metalen in het poriewater ook weer gecomplexeerd of geadsorbeerd voorkomen; er is algemeen consensus dat in ieder geval het vrij opgeloste metaalion beschikbaar is voor opname. Bij zware metalen is de redoxtoestand van het sediment een belangrijke factor voor de mate van biologische beschikbaarheid. In gereduceerd (zuurstofloos) sediment worden metalen voor een aanzienlijk deel vastgelegd door sulfiden. Dit bindingsmechanisme kan worden gemeten met de SEM/AVS methode. Het principe achter deze methode is dat wanneer er meer AVS (reactief sulfide) is dan SEM (extraheerbaar metaal), de biologische beschikbaarheid van metalen laag is (weinig in poriewater). Bij hogere SEM concentraties dan AVS concentraties kunnen metalen potentieel beschikbaar zijn (ze kunnen dan wel aan andere bodemfracties gebonden zijn, afhankelijk van het type bodem en de omstandigheden). Deze methode is al veel onderzocht op laboratoriumschaal, maar nog niet uitgebreid gevalideerd in veldsituaties. Over de rol van dit proces in systemen met een grote ruimtelijke en temporele variatie zoals uiterwaarden is nog weinig bekend. Dit onderzoek had tot doel meer inzicht te verkrijgen in het vóórkomen van sulfides in uiterwaardsediment, de rol die sulfide speelt bij het vastleggen van zware metalen en de betekenis van dit proces voor de ecologische risico's van zware metalen.

De hoofddoelen van dit onderzoek waren om voor Nederlandse uiterwaardsystemen:
a) de toepasbaarheid van de SEM/AVS methode te evalueren als middel om biobeschikbaarheid van zware metalen in uiterwaardsediment te bepalen, met speciale aandacht voor de variabiliteit van AVS
b) de effecten van zware metalen op benthische levensgemeenschappen in uiterwaardplassen mede op basis van de SEM/AVS methode vast te stellen
c) de SEM/AVS methode te vergelijken met de klassieke risicobeoordeling op basis van het totaalgehalte aan zware metalen

Een groot deel van dit onderzoek werd uitgevoerd als veldstudie. Het studiegebied was een uiterwaardgebied langs de Waal, de 'Afferdensche en Deestsche Waarden'. Het meeste onderzoek is uitgevoerd in één uiterwaardplas (Deest 4). Voor enkele deelstudies zijn ook andere plassen bemonsterd in het Nederlandse stroomgebied van de Rijn.

Het onderzoek valt globaal in twee delen uiteen: studies naar de variabiliteit van zware metalen, SEM en AVS (Hoofdstuk 2-5), en studies naar de ecologische betekenis van SEM en AVS als chemische metingen aan biobeschikbaarheid (Hoofdstuk 6-7).

Variabiliteit van SEM en AVS

Om grote hoeveelheden monsters op AVS en SEM te kunnen onderzoeken is een snelle meetmethode vereist. In *Hoofdstuk 2* staat beschreven hoe een bestaande diffusiemethode om de hoeveelheid AVS en SEM te bepalen is aangepast aan de te verwachten variabele concentraties van sulfiden in uiterwaardsystemen. De experimenten zijn uitgevoerd met uiterwaardsediment, en een set sedimentmonsters afkomstig uit andere Nederlandse watersystemen is gebruikt ter validatie. De optimale reactietijd, het effect van monstergrootte en het effect van beluchting (aeratie) worden beschreven. De optimale reactietijd bleek 4 uur. Omdat relatief grote monsterhoeveelheden (± 5 gram) gebruikt kunnen worden, konden ook lage gehalten AVS worden bepaald. Contact met de lucht van minder dan een kwartier gaf geen verlies van AVS. Dit geeft aan dat het meten van AVS niet per definitie onder zuurstofloze omstandigheden hoeft plaats te vinden. De resultaten waren voor SEM vergelijkbaar met andere methoden, en lagen voor AVS iets hoger dan voor een andere veelgebruikte methode (purge-and-trap). Dit was ook bij eerder ontwikkelde diffusiemethoden het geval. De in dit hoofdstuk ontwikkelde methode is in het verdere onderzoek gebruikt.

In uiterwaardplassen kunnen redoxcondities snel variëren door typische processen voor dit soort systemen, zoals inundatie vanuit de rivier en droogval van de plassen. Omdat er nog weinig bekend is over karakteristieke tijdschalen van AVS vorming en afbraak, is het proces van sulfidevorming en –afbraak zowel in het veld als in het laboratorium bestudeerd (*Hoofdstuk 3*). Onder veldomstandigheden is een geleidelijke opbouw van de AVS concentratie gedurende enkele maanden in het groeiseizoen te zien, positief gecorreleerd aan de temperatuur en de sedimentsamenstelling (kleigehalte, pH en redoxpotentiaal) en negatief aan de zuurstofconcentratie van het oppervlaktewater. Afbraak van AVS vond plaats in de herfst door twee verschillende mechanismen: 1) droogval van de uiterwaardplas waardoor zuurstof in de bodem kon dringen (resulterend in abrupte afbraak) en 2) resuspensie (opwerveling van het sediment) door vis (resulterend in langzame afbraak). Deze verschijnselen zijn niet per definitie seizoensgebonden. In een laboratoriumexperiment met sedimentkolommen is bepaald hoe snel sulfiden gevormd worden onder optimale condities (temperatuur, redoxtoestand) na oxidatie van het sediment. Dit simuleert een gebeurtenis zoals een overstroming. Verder werd onderzocht of de hoeveelheid sulfaat limiterend is voor de vorming van AVS. Bij een zuurstofloze (gereduceerde) waterlaag bovenin de kolom duurt het ongeveer 3 weken voordat voldoende sulfide is gevormd om de aanwezige metalen kwantitatief te kunnen binden. Dit duidt erop dat de eerste tijd na een overstroming metalen nog niet volledig worden gebonden door sulfiden. De toediening van extra sulfaat resulteerde in meer AVS vorming, wat betekent dat de hoeveelheid sulfaat limiterend is voor de snelheid waarmee sulfiden gevormd worden in dit type sedimenten.

Om de variatie door de tijd van totale en beschikbare metaalgehalten te kunnen vaststellen en te bepalen hoe deze variatie ontstaat, zijn gedurende ruim een jaar om de paar maanden sedimentkolommen gestoken uit één uiterwaardplas, waaraan in het laboratorium metingen zijn uitgevoerd (*Hoofdstuk 4*). Deze periode omvatte een grote overstroming van de uiterwaard (november 1998) en een periode waarin de uiterwaardplas bijna droogviel (juli-september 1999). De samenstelling (macrochemie, milieucondities, metalen) van de vaste fase en het poriewater zijn gemeten in verschillende lagen van het sediment. De invloed van seizoensgebonden processen (resuspensie, organische stofdepositie, wijziging redoxcondities) was duidelijk terug te zien in de vaste fase van mangaan en ijzer (redoxgebonden in oplossing gaan en neerslaan) en in de macrochemie van het poriewater (sulfaat, nitraat, ammonium, DOC). Op basis van speciatieberekingen is geschat welke mineralen stabiel zijn en wanneer metaalsulfiden gevormd kunnen worden. Metalen zijn het meest in opgeloste vorm aanwezig in de winter, terwijl voor de zomerperiode neerslag van metalen met sulfiden wordt berekend. De berekeningen kloppen met het beeld dat in andere hoofdstukken is gevonden, met de hoogste AVS gehalten in de zomer/herfst, en de laagste in de winter.

Naast inzicht in de variatie van SEM en AVS in de tijd, is kennis van de ruimtelijke variatie van AVS en SEM van belang voor de interpretatie van mogelijke metaalrisico's. Dit is onderzocht in *Hoofdstuk 5*. De ruimtelijke variabiliteit van sedimenteigenschappen, milieucondities en totaal- en beschikbare (gemeten als SEM-AVS) metaalgehalten in een uiterwaardplas is bepaald door op 43 plaatsen monsters te nemen van de bovenste sedimentlaag (5 cm). De gegevens zijn met reguliere statistische methoden en met geostatistiek verwerkt. Hiermee kan een ruimtelijk, vlakdekkend beeld van de gegevens worden gemaakt aan de hand van contourlijnen. Totale concentraties metalen, SEM concentraties en de meeste sedimentkarakteristieken waren min of meer constant. AVS concentraties varieerden ruimtelijk sterk, wat was gerelateerd aan waterdiepte, de hoeveelheid zwavel in het systeem, en de redoxpotentiaal. De biologisch beschikbare metaalgehalten kunnen ruimtelijk dus sterk verschillen binnen een uiterwaardplas. De SEM/AVS methode geeft een andere inschatting van het risico dan totale of genormaliseerde metaalgehalten, waarbij de ruimtelijke variatie klein was. De resultaten geven aan dat het doen van een gering aantal puntmetingen geen juist beeld geeft van het actuele risico van zware metalen in uiterwaardsediment.

Ecologische betekenis van SEM en AVS

Voor het vertalen van chemische meetwaarden naar risico's voor organismen wordt vaak gebruikgemaakt van bio-assays (laboratoriumproeven met één soort organisme). De in deze proeven gebruikte sedimentmonsters worden meestal voorbehandeld, waardoor ze niet meer representatief zijn voor de veldsituatie. In *Hoofdstuk 6* is bestudeerd hoe verschillende behandelingen van het sediment voorafgaand aan de bio-assay de resultaten beïnvloeden, waarbij het voor oxidatie gevoelige AVS als maat voor de verstoring van het sediment werd gebruikt. Verder is gekeken of AVS en SEM van invloed zijn op de respons van de sedimentbewonende ongewervelde *Asellus aquaticus* (de waterpissebed) in een bio-assay met monsters uit zeven verschillende uiterwaardplassen. Monstername, opslag en voorbehandeling van sediment bleek een negatief effect op AVS gehalten te hebben. De ongestoorde veldsituatie wordt het beste benaderd als het sediment na monstername gelijk wordt opgeslagen in luchtdichte potten, niet wordt gezeefd, en wordt ingevroren tot gebruik in de bio-assay of chemische analyse. De volgorde van de verontreinigingsgraad van de zeven uiterwaardplassen op basis van de metingen aan SEM en de bio-assayrespons (overleving en groei van de testorganismen) kwam overeen met de volgorde die eerder was vastgesteld op basis van het totaalgehalte aan metalen en organische verontreiniging. Er was een verband tussen de bio-assayresultaten en het SEM en AVS gehalte. De gevonden effecten zijn echter niet alleen aan metalen toe te schrijven, aangezien een bio-assay een totaalbeeld geeft van het effect van alle verontreinigingen samen.

Mogelijke risico's van verontreinigende stoffen worden vaak op chemische metingen gebaseerd en ecologische effecten op laboratorium bio-assays. Of er werkelijk effecten in de veldsituatie optreden, kan alleen worden afgeleid uit veldwaarnemingen. In *Hoofdstuk 7* is aan de hand van een inventarisatie van een groot aantal milieuvariabelen inclusief (beschikbare) metalen en de soortensamenstelling van benthische organismen in tien uiterwaardplassen langs de Rijntakken onderzocht in hoeverre verschillende chemische bepalingen van de concentraties aan metalen (totale en genormaliseerde gehalten, SEM of SEM-AVS relaties, bio-accumulatie) in het milieu daadwerkelijk iets zeggen over de effecten op de benthische levensgemeenschap in uiterwaardplassen. De afhankelijkheid van de opname van metalen (bio-accumulatie) door Oligochaeten (benthische wormen) van de milieuvariabelen is bestudeerd door middel van lineaire regressie. De opname bleek positief gerelateerd aan het (beschikbare) gehalte metalen in het sediment en negatief aan bepaalde oppervlaktewaterkarakteristieken (o.a. de hoeveelheid organisch materiaal). Door middel van een multivariate statistische methode (variantiepartitie) kunnen de twee typen data (abiotisch en biotisch) aan elkaar worden gerelateerd en kan een uitspraak worden gedaan over het belang van de afzonderlijke variabelen voor het verklaren van de totale gevonden variatie in de samenstelling (aantallen en diversiteit) van de benthische levensgemeenschap. Een behoorlijk groot aandeel van de aanwezige variatie in soortensamenstelling (bijna 70 %) kon met elf milieuvariabelen worden verklaard. Zware metalen droegen voor 2 tot 6 % bij aan de verklaring van de variatie, maar alleen wanneer rekening gehouden werd met de beschikbare fractie (SEM-AVS en afzonderlijke SEM concentraties per metaal). Wanneer werd gerekend met totaal metaalgehalten, waarvan een deel niet biologisch beschikbaar is, werden geen significante bijdragen gevonden. De grootste effecten werden gevonden op detritivore (sediment-etende) soorten. De resultaten geven aan dat de SEM/AVS methode een ecologische betekenis blijkt te hebben. Het meten van SEM en AVS voor het vaststellen van ecologische risico's op het niveau van levensgemeenschappen is zinnig.

Conclusies

In de samenvattende discussie (*Hoofdstuk 8*) worden de algemene conclusies uit het gehele onderzoek bediscussieerd in het licht van andere recente bevindingen en ontwikkelingen in het beleid van vervuilde sedimenten. Op basis van de resultaten uit de deelonderzoeken wordt geconcludeerd dat de SEM/AVS methode bruikbaar is in de risicobeoordeling van met zware metalen vervuild uiterwaardsediment, mits rekening wordt gehouden met de variabiliteit. Voor wat betreft de relatie met ecologisch relevante effecten is aangetoond dat de SEM/AVS methode deze beter verklaart dan de klassieke beoordelingswijze op basis van totaalgehalten. Het beschreven onderzoek kan een bijdrage leveren aan verdere afwegingen op het gebied/beleidsterrein van de risicobeoordeling van verontreinigd uiterwaardsediment.

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Corine Zetten, januari 2006

CURRICULUM VITAE

Corine van Griethuysen werd geboren op 13 juni 1969 te Veenendaal. Na het VWO aan het Christelijk Lyceum te Veenendaal koos ze voor de opleiding Bosbouw met specialisatie Natuur- en Landschapstechniek aan de Hogere Bosbouw- en Cultuurtechnische School, de huidige IAHL, te Velp. Vervolgens volgde ze de studie Bodem, Water & Atmosfeer aan de Wageningen Universiteit, met specialisaties in Bodemvorming en Ecopedologie. Haar doctoraalonderzoeken betroffen de kleimineralogie van rivierterrassen van de Guadalhorce in Andalusië, Spanje, en het effect van verschralingsbeheer op de organische stofdynamiek van graslanden aan het Anloër Diepje (stroomgebied Drentsche Aa). Na het afronden van de studie (1996) werkte ze bij de vakgroep Bodemkunde en Geologie (WU) aan het project 'Laser grain size distribution of Oxisols from Brazil in relation to soil aggregation' en bij de Universidad de Santiago de Compostela, Facultad de Biología, Departamento de Edafología y Química Agrícola, Galicië, Spanje aan het project 'Trace metal distribution in the ombrotrophic peat of Chao de Lamoso, Galicia'. Aansluitend volgde de aanstelling als AIO bij de leerstoelgroep Aquatische Ecologie en Waterkwaliteitsbeheer aan de Wageningen Universiteit op het project ‘Actual risks of trace metals as a result of sediment displacement in the floodplains and catchment area of Dutch rivers’ (Februari 1998-Januari 2003). Het onderzoek dat tijdens deze aanstelling is uitgevoerd, is in dit proefschrift beschreven. Naast het promotieonderzoek heeft ze zich free-lance beziggehouden met het verzorgen van natuur-en milieueducatieprogramma’s voor met name middelbare scholieren bij de Stichting Veldstudie te Hei-en Boeicop. Eind 2003 is dochter Lieke geboren. Vanaf oktober 2004 is ze als Wetenschappelijk beoordelaar Milieu werkzaam bij het College voor de Toelating van Bestrijdingsmiddelen (CTB) te Wageningen. Ze woont in Zetten met Frits, zijn zoon Mats, en hun dochter Lieke.

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