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A B S T R A C T

The aim of this paper was to collect evidence for the effects of contaminants on biota in a highly dynamic river Rhine floodplain. To this purpose we reviewed the results of circa 10 studies performed in this floodplain. The floodplain was contaminated with elevated levels of cadmium, copper, PAHs, and PCBs and high levels of zinc which were at some sites above legislative values. The results showed that the present contaminants were accumulated by the floodplain inhabiting organisms, but meanwhile population and community effects were ambiguous. Only for the mayfly *Ephoron virgo* clear effects were detected at the level of the single floodplain. The absence of clear population and community effects is puzzling since at lower contaminant concentrations adverse effects were detected in other environments. Factors that may mask toxic effects include flooding and food quality and quantity. We conclude that given the site specific conditions, being an open, eutrophic system with a highly dynamic flooding pattern, assessment of the contribution of toxicants to observed population density or biomass and community composition requires 1] an increase in number of replicates; 2] a larger scale of investigation and 3] comparison to stable systems with comparable contamination levels.

1. Introduction

One of the sites in the Systems-oriented Ecotoxicological Research program was the river Rhine floodplain area "Afferdensche and Deestsche Waarden" (ADW). At this site more than 10 PhD students and researchers investigated the impact of contaminants at various levels of the ecosystem. The sediments of the embanked floodplains and the lower reaches of the river Rhine have been contaminated during the heavy water pollution in the 1960s and 1970s (Beurskens et al., 1993). Although recently deposited sediments contain considerably lower concentrations of contaminants, many floodplain lake sediments are still historically polluted with nutrients, metals, and hydrophobic organic contaminants (Beurskens et al., 1993). As a result of frequent inundations and (re)deposition of riverine sediments, the contamination has been spread over the terrestrial floodplain soils as well.

Results of chemical analyses (Van Vliet et al., 2005; Koelmans and Moermond, 2000) revealed that in some sites of the ADW contaminant levels exceed legislative values (De Haas and Kraak, 2008-this issue). However, studies on population density and biomass, and on species composition in the ADW floodplain show ambiguous effects of contamination (Ma et al., 2004; Roessink et al., 2006; Boivin et al., 2007; Van der Geest and Paumen, 2008-this issue). Also in floodplains in the Biesbosch and along the Scheldt river, where contaminant levels are generally higher, no significant relations between contaminants and population biomass and density of earthworms were reported (Hobbelen et al., 2004; Vandecasteele et al., 2004; Hobbelen et al., 2006). This triggers the question whether
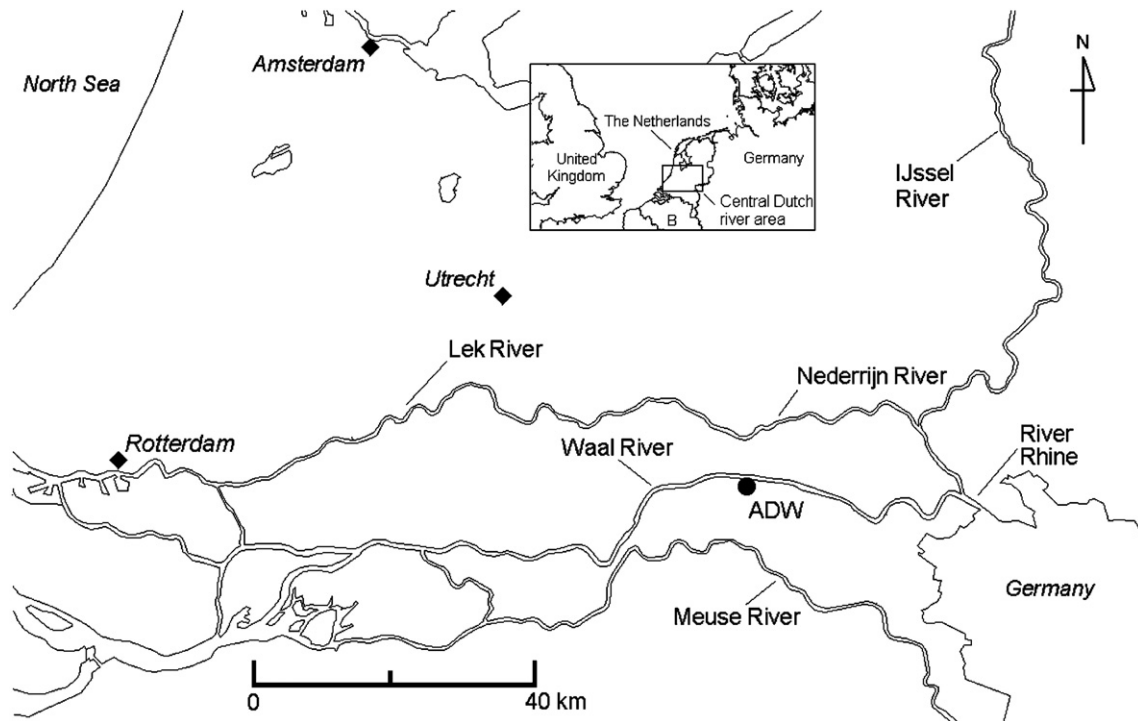
Fig. 1 – The lower River Rhine area, with the location of the Afferdensche and Deestsche Waarden (ADW) floodplain indicated (Thonon and Klok, 2007).

pollutant levels that locally exceed current legislative values indeed endanger local ecosystems.

Obviously, under field conditions a wide variety of abiotic (e.g. physical–chemical) and biotic factors (e.g. food availability, predation, competition) jointly determine the presence and abundance of species. Hence, the absence of species at polluted sites does not necessarily imply exclusion due to toxicity (Chapman et al., 2002), but could equally well be caused by one of the other aforementioned factors.

Moreover, in these highly dynamic environments frequent flooding can drastically change the terrestrial community, strongly reducing the abundance and biomass of earthworms (Zorn et al., 2005a). This highly dynamic nature of the floodplains impedes the probability to detect adverse effects of contaminants on biota (Klok et al., 2007), raising the question on the contribution of contamination to the observed population and community effects.

In this paper we review the results of the SSEO program obtained in the ADW floodplain, focusing on the ecological consequences of contaminant exposure at the species and community level. By combining (subtle) effects on ecological endpoints measured in different biota we aim to assess evidence for toxic impacts on floodplain inhabiting species.

2.   Site description

The floodplain Afferdensche and Deestsche Waarden (ADW), a small area of approximately 3 square kilometers, is situated near the village of Afferenden along the lower River Rhine (longitude 51°54′N, latitude 5°39′E) (Fig. 1). Nature and flood-

plain lakes dominate the far eastern part of the floodplain section, whereas land use comprise arable fields and pasture in its western, central and southeastern part. Inundations can occur more than twice a year (Thonon and Klok, 2007).

The soil is a typical floodplain soil consisting of large amounts of clay and moderate levels of organic matter, with high pH values (Table 1). The contamination levels of the floodplain soils and sediments generally peak between 0.5 and

Table 1 – Ranges (min–max) of soil and sediment characteristics and contaminant levels: percentage clay, percentage organic matter content (OM), pH, and total P, Chl a, Cd, Cu, Zn, Pb and sum of polycyclic aromatic hydrocarbons (ΣPAHs) in mg/kg DW

	Soil	Sediment
clay	9–30%	8–50%
OM	8–15%	1–13%
pH	7.3–7.4	6.7–7.0
Total P		434–1703
Chl a		17–55
Cd	1.6–5.7	0.05–2.37
Cu	54–126	9–134
Zn	357–980	42–1043
Pb	109–175	13–191
ΣPCBs		0.55–10.52
ΣPCBs		4.4–133

Sum of polychlorinated biphenyls (ΣPCBs) in μg/kg DW. Data from Koelmans and Moermond (2000), Moermond and Koelmans (2002), De Haas et al. (2002), Ma et al. (2004), De Haas et al. (2005b), Van Vliet et al. (2005), Zorn et al. (2005a), De Haas et al. (2006), Klok et al. (2006a), Roessink et al. (2006), Wijnhoven et al. (2006a), Boivin et al. (2007).

2 m depth (Middelkoop et al., 2001, 2002). The upper soil layer of the floodplains reflects the improved sediment quality with moderate levels of cadmium and copper, but still relatively high levels of zinc (Table 1). Both sediment characteristics and contaminant levels resemble those of the terrestrial soil. In addition, in the sediment also elevated levels of PAHs and PCBs were measured (Table 1).

3. Accumulation in organisms

Metal levels monitored in earthworms in ADW soils showed elevated concentrations of cadmium, copper, lead and zinc (Ma et al., 2004; Van Vliet et al., 2005). Accumulation of heavy metals in earthworms proved to be species specific and strongly influenced by flooding: accounting for flooding in regressions of accumulation increased the explained variation from 5 to 67% (Van Vliet et al., 2005), see also Van Gestel (2008-this issue).

Two of the five aquatic studies performed in the ADW floodplains addressed toxicant accumulation in biota exposed to floodplain lake sediments. Moermond et al. (2004) reported elevated ΣPCB levels in mixed invertebrates samples and detected 15 individual PCB congeners and 13 individual PAHs in mixed invertebrates samples and in oligochaetes originating from three polluted floodplain lakes (Moermond et al., 2005).

Accordingly, Van der Geest and Paumen (2008-this issue) measured significant copper accumulation in oligochaetes exposed to contaminated ADW sediment in whole sediment bioassays. Concentrations of ΣPCB in fish kept in enclosures above contaminated sediments increased over 10 fold at the end over the two months exposure period, but was still four times lower than the concentration found in 0+ bream (*Abramis brama*), caught in the same lake in the same period (Moermond et al., 2004).

It is concluded that in spite of the many variables that change the bioavailability of both metals and organic compounds, they do accumulate in the floodplain inhabiting organisms.

4. Effects of toxicants on single species

Recently, effects of heavy metal pollution of soils on abundance and biomass of earthworms in river floodplains have received much attention (Hobbelen et al., 2004; Ma et al., 2004; Vandecasteele et al., 2004; Hobbelen et al., 2006). These studies included floodplains of the river Scheldt (Vandecasteele et al., 2004) and the SSEO sites ADW (Ma et al., 2004) and Biesbosch (Hobbelen et al., 2004, 2006), which vary strongly in heavy metal levels. The highest levels were reported from floodplains of the Scheldt (cadmium 34.3 mg/kg; copper 332 mg/kg; zinc 2.742 mg/kg; pH 7.9) (Klok et al., 2007).

Levels at the SSEO sites were lower with higher levels for Biesbosch than for ADW (Van Gestel, 2008-this issue). Only at one site, notably the one with the lowest heavy metal levels negative relations between earthworm biomass and density and metal levels in soil were found (Ma et al., 2004). Earth-

worm biomass and density were, however, also strongly correlated with the height of the floodplain, which is indicative for inundation frequency and duration. Therefore Ma et al. (2004) concluded that the negative correlation between metals and earthworms may equally well be explained by flooding. In less dynamic environments effects on biomass and density have been reported at lower heavy metal levels (cadmium 13.2 mg/kg; copper 74.8 mg/kg; zinc 710 mg/kg; pH 7.25) by Spurgeon and Hopkin (1999), see also (Bisessar, 1982; Bengtsson et al., 1983; Hunter et al., 1987).

Also effects on the composition of the population, segregated in cocoons, juveniles, subadults and adults, have not been reported in studies on floodplains, whereas in other environments populations consisted of more individuals in the younger development classes at sites with high levels of heavy metals compared to those with background levels (Spurgeon and Hopkin, 1999; Klok et al., 2006a). At these sites the average individual weight was also lower, and maturation delayed (Klok et al., 2006a).

Effects of exposure to floodplain lake sediments have been determined on several aquatic species. No difference in growth of the benthic diatom *Nitzschia perminuta* was observed between a reference and contaminated sediment after 4 days of exposure to intact sediment cores (Van der Geest and Paumen, 2008-this issue). De Haas et al. (2002) selected seven floodplain lakes representing a clear pollution gradient. The responses of the mayfly *Ephoron virgo* and the midge *Chironomus riparius* to these sediments were assessed in 10-day growth bioassays with both species and a 28-day emergence experiment with *C. riparius*. A decrease in both survival and growth of *E. virgo* was observed with increasing contaminant levels. In contrast, *C. riparius* responded to the food quantity and quality in the sediments in spite of the toxicants present. This was confirmed by choice experiments, demonstrating that midge larvae showed a clear preference for sediments with higher food quality, which overruled avoidance of the sediments with higher toxicant concentrations (De Haas et al., 2006). Yet when the food quality of two sediments was equal a higher proportion of the larvae choose the sediment with the lower contaminant concentrations (De Haas et al., 2006). In addition, it was also observed that *C. riparius* larvae suffered from the high contaminant levels, reflected by a higher incidence of mentum deformities at higher contaminant concentrations in the sediment (De Haas et al., 2005a). Thus from the three aquatic species tested one was strongly inhibited, one only slightly and the third not at all.

In conclusion we can state that effects on single species were ambiguous. In some species clear effects were found, in others effects were not detected, at least not at the scale of a single floodplain.

5. Effects on communities

Earthworm communities in the floodplain comprise four to six species usually dominated by *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Allolobophora chlorotica* and *L. terrestris* (Zorn et al., 2005a). At the ADW site no effect of heavy metals on species composition was found (Ma et al., 2004). In a study with a

higher number of replicates (including other floodplains), however, the number of earthworm species significantly decreased with increasing copper and zinc levels, with only *L. rubellus* present at the highest contaminated floodplain sites (Klok et al., 2007). A similar finding was reported by Spurgeon and Hopkin (1999) in the surroundings of a smelter. Aquatic community effects have been assessed both in the field and in microcosms and experimental ponds.

De Haas et al. (2005a) reported that in sediments with high contaminant levels, high densities of species classified as 'pollution-tolerant', such as *Chironomus* sp., were observed. A species-rich invertebrate community, including mayflies and caddisflies, was observed only in the sediment with high food quality and low contaminant concentrations (De Haas et al., 2005a). Roessink et al. (2006) observed that although macro-invertebrate communities that developed on clean and polluted sediments in microcosms showed a large overlap in species composition, differences existed in relative dominance of taxa.

Thus both in the field and under semi natural conditions high contaminant levels cause a shift towards more pollution-tolerant taxa (soil: *L. rubellus*, sediment: *Chironomus* sp.). Van der Geest and Paumen (2008-this issue) observed no effects of sediment quality on benthic algal community diversity, structure and functioning. This is in agreement with results obtained by Boivin et al. (2007), who demonstrated that the genetic and physiological structure of the bacterial communities correlated with the species composition of the algal community, but hardly to the level of metal pollution. Thus metals were not proven to affect either the algal or the bacterial communities. Hence it is concluded that microphytobenthic and bacterial communities were less affected than macrofauna communities by the prevailing contaminant levels in the ADW floodplains.

6. Do contaminants contribute to population and community effects?

In general effects of toxicants on biota under field conditions are difficult to verify, even when concentrations are above legislative threshold values which are considered detrimental for biota. The absence of effects does not necessarily imply, however, that local biota are not affected, since this may equally well result from limitations of the study design, such as low statistical power or ignorance of confounding factors.

Relatively few studies have clearly identified an influence of toxicants on population and community level at the ADW site (De Haas et al., 2002, 2005a). But even then the question remains whether subtle effects on specific biota are detrimental to the local ecosystem and would argue for sanitation of the site. In this review we used multiple lines of (independent) evidence to discuss research findings on the risk of toxicants at the ADW site.

Effects on population density and abundance in single species and composition of communities were reported. The question remains however, if the effects observed at the community level were actually caused by those single species effects and if effects on species are actually caused by the toxicants. Though Van der Geest and Paumen (20008-this issue) measured significant copper accumulation in oligochaetes, De Haas et al. (2005a) reported that the benthic community was dominated by oligochaetes in all lakes, up to about 40,000 individuals m^{-2} in the most contaminated lake (compared to 4500 in a clean lake). Likewise the high growth rate of *C. riparius* in the in situ enclosures in two of the most contaminated sediments indicated that chemical stress and mentum deformities did not prevent rapid growth, reflected by the abundance of the resident pollution-tolerant chironomids of the *Chironomus plumosus* group (De Haas et al., 2005a).

Evidence at the ADW study site was only obtained for the mayfly *Ephoron virgo*. This species responded to the sediment contamination in a dose dependent way (De Haas et al., 2002), and indeed a species-rich invertebrate community, including mayflies and caddisflies, was observed only in the sediment with high food quality and low contaminant concentrations (De Haas et al., 2005a). Hence, it is concluded that only for relatively sensitive species exposed to relatively polluted sites there is evidence for a substantial contribution of toxicants to effects at the community level. This raises the question why in the other cases effects on the community level were either absent or not supported, or even contradicted, by effects on single species.

7. Factors masking toxic effects

Under field conditions a wide variety of abiotic (e.g. physical–chemical) and biotic factors (e.g. food availability, predation, competition) jointly determine the presence and abundance of species. Below we discuss which of these factors may mask or overrule potential effects of the measured and accumulated toxicants.

7.1. Bioavailability

Low bioavailability as a consequence of high pH values and organic matter content of floodplain soils and sediments has been suggested to explain the absence of conspicuous effects of pollutants on the population and community level of biota in floodplains (Hobbelen et al., 2006; Boivin et al., 2007). Accumulation of contaminants in biota did occur and was often relatively high, which suggests that absence of effects can, at least in some cases, not be explained by low bioavailability. In addition, the soil and sediment inhabiting species themselves also increase the bioavailable toxicant fractions by their bioturbating activities. Bioturbation by earthworms (Zorn, 2004; Zorn et al., 2005b) and small mammals (Wijnhoven et al., 2006b) was studied in the ADW. Casting activities of earthworms can amount to 2 kg m^{-2} soil from the deeper layers to the surface (Zorn et al., 2005b; Van Gestel, 2008-this issue), whereas in soil surfaced by small mammals it was more than one order of magnitude less (Wijnhoven et al., 2006b). Likewise, in contaminated floodplain lake sediments, De Haas et al. (2005b) demonstrated that chironomid density strongly increased the concentrations of metals, nutrients and particles in the overlying water. Thus, though in some cases a reduced bioavailability may reduce

accumulation and effects, this certainly does not hold for all ADW studies. Hence, other factors must be responsible for the masking or absence of toxic effects.

7.2. Flooding of terrestrial soils

Floods do not only deposit layers of sediments which are rich in nutrients and contaminants in floodplains, but flooding itself also disturbs the biota in floodplains resulting in an extensive drop in biomass and number of earthworms (Zorn et al., 2005a). Some earthworm species are even virtually absent when flood waters recede (Zorn et al., 2005a). Earthworm populations probably recover from a flood by regrowth from cocoons which survive inundation. After a flood the population can grow exponentially to the level where environmental factors become limiting or the subsequent flood resets the population. In such a system where population density and biomass are strongly influenced by flooding, effects of contaminants on biomass and population density are difficult to demonstrate statistically. Since most reported studies were conducted in a single floodplain with a limited number of sample replicates the variation in population density and biomass due to flooding may statistically mask possible effects of heavy metals on these parameters. If this is the case an increase in the number of replicates would distinguish the effect of flooding from a possible toxic effect. In a study that combined data from three different floodplain studies significant negative effects ($p < 0.05$) of cadmium, copper and zinc on earthworms biomass and density (with the exception of copper for density $p = 0.053$) were found (Klok et al., 2007).

Inundation stress even results in earlier maturation of some species as exemplified by *L. rubellus* that matures at a lower weight and a corresponding younger age in frequently inundated floodplain sites, as compared to sites which remain dry for longer periods (Klok et al., 2006b; Klok and Plum, in press). Klok et al. (2007) demonstrated with a mechanistic population model that if populations in a frequently inundated floodplain would mature at the average age found at seldom inundated sites, population viability would drastically decline. Heavy metal stress retards maturation in earthworms (Klok et al., 1997, 2006a). It can therefore be anticipated that heavy metal stress has a more drastic effect on population viability in frequent flooded sites compared to less dynamic environments.

Small mammals also showed a strong response in population abundance to floods. After a flood, recolonization takes place from refuges (un-flooded parts like heights and dikes) (Wijnhoven et al., 2006a). Recolonization of floodplains is a slow process and suggested to depend on landscape structures such as connectivity of shrubs (Wijnhoven et al., 2006a). Small mammal densities at more than 30 m from the non-flooded areas were always lower than in refuges (Wijnhoven et al., 2005), suggesting that colonization time between two successive floods (eight months) was not long enough for entire recolonization of the ADW floodplain.

It is concluded that variation induced by flooding masked possible effects of pollutants as exemplified in earthworms. Colonization by small mammals is mainly hampered by the fact that recovery depends on offspring production by the relatively low number of survivors at refuges.

7.3. Food quantity and food quality

Natural floodplains figure among the world's most fertile and productive systems (Tockner and Stanford, 2002). Species living in riparian habitat have evolved life history strategies in direct response to natural flow regimes (Bunn and Arthington, 2002). The timing of inundation events triggers breeding (e.g. in Atyidae; Bunn, 1988) resulting in synchrony of development. A spectacular example of such synchrony is the "boom or burst" dynamics found in Australian arid-zone Rivers and wetlands (Walker et al., 1995).

As a consequence population dynamics of species following a flood can be summarized by unlimited exponential growth up to the level were resources become limiting or where the system is reset by the next flood. This resetting is nicely illustrated for population abundance in earthworms living in the ADW floodplain that follow a saw-tooth pattern (Zorn et al., 2005a). In such a system it seems difficult to detect sublethal effects of contaminants in parameters such as population abundance. Only if environmental factors (e.g. food) become limiting one may expect effects of contaminates to become more prominent. This was typically the case for the sediment inhabiting chironomids. Their presence in food rich, contaminated sediments suggested a relatively low sensitivity towards the toxicant levels measured in the ADW sediments (De Haas et al., 2005a; De Haas and Kraak, 2008-this issue). Yet, on the most contaminated sediments sublethal effects on midge larvae were observed (De Haas et al., 2005a) and laboratory experiments showed that *C. riparius* is not necessarily tolerant to contaminants (De Haas et al., 2004). Addition of highly nutritive food to the sediment caused as a decrease in copper accumulation and a coinciding lower sensitivity to copper (De Haas et al., 2004). It is therefore concluded that the high food quantity and quality of the floodplain soils and sediments may mask adverse effects of the present and accumulated toxicants, resulting in an underestimation of toxicity (Ankley et al., 1994; Day et al., 1994; Harkey et al., 1994; Lacey et al., 1999).

8. Conclusions

Integrating the joint results of SSEO research efforts in the ADW floodplains made clear that species able to maintain viable populations in this highly dynamic, contaminated environment are characterized by three related characteristics: fast growth, high food demands and a short generation time (De Haas and Kraak, 2008-this issue). In fact, such species are well equipped to face any kind of (joint) stress, as far as they can complete their life cycle in between two periods of extreme stress or during prolonged periods of moderate stress (toxicants, flooding, food shortage). Their life cycle characteristics allow them to make advantage of surplus of food, partly due to the absence of slow growing species with longer generation times, which often include their predators. Moreover, after an extreme event, the same life history characteristics aid recruitment, if populations can be considered open (which is the case for most species living in floodplains). In this way effects of local available toxicants may be further

damped by emigration of affected individuals and influx of healthy specimens from elsewhere (Ares, 2003). Migration may not only damp effects on populations, but also strongly mix populations resulting in absence of genetic differences between unaffected and locally affected individuals. This may explain why effects on species with small time and spatial scales such as bacteria show no genetic response in dynamic floodplain systems (Boivin et al., 2007), whereas at even lower pollutant levels they do show effects in more stable and less well mixed systems (Boivin, 2005). It is concluded that the open, eutrophic and highly dynamic nature of the floodplains functions as a sieve, that let only pass well adapted species.

Disentangling the contribution of toxicants to observed community effects, or 'separating the signal from the noise' therefore requires 1] an increase in number of replicates (Klok et al., 2007); 2] a larger scale of investigation (Klok et al., 2007); 3] studying stable systems with comparable contamination levels (Boivin, 2005).

Acknowledgements

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