Interactions between nutrients and toxicants in shallow freshwater model ecosystems

Promotor:
Prof. Dr. A. A. Koelmans
Persoonlijk hoogleraar bij de leerstoelgroep Aquatische Ecologie en Waterkwaliteitsbeheer, Wageningen Universiteit

Co-promotor:
Dr. T. C. M. Brock
Senior wetenschappelijk onderzoeker
Alterra, Wageningen Universiteit en Research centrum

Samenstelling promotiecommissie:

Prof. Dr. W.P. Cofino	Wageningen Universiteit
Prof. Dr. A.J. Murk	Wageningen Universiteit
Prof. Dr. W. Admiraal	Universiteit van Amsterdam
Prof. Dr.ir. A.J. Hendriks	Radboud Universiteit Nijmegen

Dit onderzoek is uitgevoerd binnen de onderzoeksschool SENSE (Socio-Economic and Natural Sciences of the Environment).

Interactions between nutrients and toxicants in shallow freshwater ecosystems

Ivo Roessink

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. M. J. Kropff,
in het openbaar te verdedigen
op woensdag 27 februari 2008
des namiddags om half twee in de Aula

CIP-data Koninklijke Bibliotheek, Den Haag
Interactions between nutrients and toxicants in shallow freshwater ecosystems / Roessink, I.
PhD-thesis Wageningen University, Wageningen, The Netherlands – with references – with summary in English and Dutch
ISBN: 978-90-8504-875-6

“Not everything that counts can be counted,
and not everything that can be counted counts.”
Albert Einstein

Voor mijn schatjes
Ivo

Summary

The structure of a freshwater community is influenced by several factors, including the trophic (*i.e.*, nutrient) status of the specific system and the background concentrations of persistent pollutants in the sediment. Since these factors are subject to spatio-temporal variation, it is likely that the response of an aquatic community to an additional stressor also varies in space and time. Additional stressors may comprise toxicants that enter the aquatic environment through agricultural or industrial use.

This thesis investigates the influence of the trophic status of a shallow freshwater system and/or the presence of persistent pollutants in the sediment on the fate and ecological effects of an insecticide and a fungicide/biocide. Additionally, this thesis aims to shed light on the influence of macrophytes and fish on the partitioning and redistribution of sediment-bound polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). PAHs and PCBs are predominantly 'historical' pollutants that accumulate in sediments and are rather persistent.

What is the effect of an insecticide on the aquatic community of a eutrophic phytoplankton-dominated or a mesotrophic macrophyte-dominated shallow freshwater ecosystem?

To answer this question, 12 cylinders were inserted in both a eutrophic phytoplankton-dominated and a mesotrophic macrophyte-dominated freshwater ecosystem. Both types of freshwater ecosystem were treated with the insecticide lambda-cyhalothrin 3 times, at weekly intervals, at concentrations of 0, 10, 25, 50, 100 and 250 ng/L (n=2). The dissipation rate of the insecticide from the water phase proved similar between the two types of test system. After 1 day, only 30% of the amount of lambda-cyhalothrin applied was still present. Direct toxic effects were predominantly observed on insects and crustaceans. These effects on sensitive taxa matched the results of short-term laboratory experiments. A remarkable result is that of the small differences in ecological threshold values of direct toxic effects between the phytoplankton-dominated and macrophyte-dominated systems. The ecological threshold value is the concentration at which few or no effects of the test compound are observed. Larger differences in the response of the two systems comprised the rate of recovery and indirect effects on less sensitive organisms (*e.g.*, through shifts in predation and competition) at concentrations higher than the threshold level. Effects were more pronounced in the plankton-dominated system, and the rate of recovery was faster there as well. This phenomenon can be explained by the presence in the plankton-dominated systems of generally smaller

organisms, which have a shorter life-cycle than those in the macrophyte-dominated systems. The results of this experiment are presented in detail in chapter 2.

What is the influence of historical pollution in the sediment on the response of the aquatic community to fungicide/biocide exposure?

To answer this question, 10 model ecosystems with polluted sediment and 10 model ecosystems with clean sediment were constructed. The sediments originated from floodplain lakes along the river Waal in the Netherlands. The quality of the sediment determined the type of aquatic community that developed in the two types of system. From the start, the macrophyte vegetation developed better on the polluted sediment, which contained not only more toxicants but also more nutrients.

The fungicide/biocide triphenyltin acetate (TPT) was applied once, at concentrations of 0, 1, 3, 10, 30 and 100 µg/L (n=2 per sediment type). Dynamics of the TPT concentration in the water phase were similar between the two system types. Nevertheless, higher concentrations of TPT were observed in the clean sediment systems, where fewer macrophytes were present. In both system types, representatives of several taxonomic groups (snails, worms, crustaceans and insects) showed a clear response to the TPT treatment. Despite the fact that TPT was very persistent in the sediment, no treatment-related effect on sediment-dwelling nematodes was observed. Although some differences in intensity and duration of effects were observed between the two system types, the presence of historical pollutants in the sediment hardly influenced the overall sensitivity of the aquatic community. The results of this experiment are presented in detail in chapter 3.

Can TPT-related effects observed in model ecosystems be predicted by short-term toxicity experiments in the laboratory?

To answer this question, representatives of taxonomic groups sensitive to TPT in the microcosms were also tested in the laboratory by means of so-called ‘single species tests’ (SST; duration 96h). In total, 32 different aquatic taxa were studied. When possible, the responses of these taxa were used to calculate the concentration at which 50% of the test organisms showed a treatment-related effect to TPT application (EC_{50}). Additionally, EC_{50} values were calculated for populations of taxa that showed a treatment-related response to TPT application in the two types of model ecosystem. These microcosm EC_{50} values were calculated 2, 4 and 8 weeks post TPT application, since this was the period in which the largest effects were observed. The EC_{50} values calculated for different taxa were used to obtain a so-called species sensitivity distribution curve

(SSD), from which a value can be derived at which 95% of the species tested are protected. This value is called the hazardous concentration to 5% of the species tested (HC_5).

The calculated HC_5 value, based on 96-h SST, was 1.3 µg/L. The calculated HC_5 values for microcosms (2–8 weeks after application) varied between 0.2 and 0.6 µg/L (based on peak concentrations of TPT in the water phase of the test systems). The taxa that were sensitive in the microcosm also showed a sensitive response in the laboratory SST, with the exception of insects, which sometimes showed a pronounced treatment-related response in the microcosms but were rather insensitive in the laboratory. In general, the taxa tested responded less sensitively in the laboratory SST than in the microcosm experiments. Possible explanations for this phenomenon include the potentially long time needed (>96h) to express effects, and additional chronic exposure via the food chain in the model ecosystems. A remarkable finding is that the HC_5 values hardly differed between the microcosms with clean and polluted sediment. This implies that ecological threshold levels for toxic effects of TPT are apparently not influenced by background pollutants present in the sediment. The results of this experiment are presented in detail in chapter 4.

Can the presence of background pollutants and recently added TPT in sediments be measured with standardized bio-assays?

The observations described above concern taxa that occur more or less ‘naturally’ in the model ecosystems. Another technique to assess the impact of toxicants under field conditions is that of using standardized bio-assays. Two aquatic species that are frequently used to test sediment quality are the midge *Chironomus riparius* and the mayfly *Ephoron virgo*, however these species were not observed in the model ecosystems. Sediment from our TPT model ecosystem experiment was used to assess the response of both species to pollutants in the sediment. Fifteen weeks after the TPT application, sediment samples were taken from each individual test system, with the exception of the 3 µg/L level in both types of test system, and transferred to the laboratory for use in the bioassays. A previous study had already determined the contaminants in the sediment (PAHs, PCBs and metals), and TPT concentrations were measured in the control and the 30 µg/L test immediately after sampling. Linear regression was used to determine the TPT concentrations in the other sediments. For each of the test systems, 25 mL mixed sediment was put in a glass jar, and 20 larvae of *E. virgo* or 5 larvae of *C. riparius* were added. After 10 days, the growth and survival of the organisms was assessed. The bio-assay experiments showed that the type of sediment (containing either small or large quantities of historical background

pollution) hardly impacted on the growth of *C. riparius*. A possible explanation is that the polluted sediment also contained more organic matter, which may have served as a suitable food source and may have compensated for the negative effects of the background pollutants. However, the survival and growth of *E. virgo* larvae was considerably lower at higher concentrations of PAHs, PCBs and metals. It is possible that the nature of the organic material also influenced the outcome in this case. In both types of sediment, it was impossible to establish a dose-response relationship for sediment-bound TPT, for either *C. riparius* or *E. virgo*. Apparently, there was no cumulative effect of the historical pollution and the recently added TPT. The results of this experiment are described in detail in chapter 5.

What is the influence of aquatic macrophytes and fish on the partitioning of sediment-bound pollutants (PCBs and PAHs) in different compartments of aquatic ecosystems?

To answer this question, a laboratory experiment was undertaken to assess the influence of the presence or absence of aquatic macrophytes and/or fish on the partitioning and concentration of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the following system compartments: sediment, seston (suspended matter), macrophytes, periphyton (algae attached to substrate), floating algae biomass (flab), zooplankton, sediment-dwelling worms, other invertebrates and fish. Additionally, a distinction was made between ‘historical’ and recently added pollutants. For this purpose, 12 model ecosystems were constructed with polluted sediment originating from floodplain lakes alongside the river Waal in the Netherlands. When inserting the sediment in the test systems, two PCB congeners and a labelled PAHSwere added. The systems were then equipped with only fish, fish and macrophytes, only macrophytes or no fish and no macrophytes (each n=3). The fish used was carp (*Cyprinus carpio*), and the aquatic macrophytes consisted of *Elodea nuttallii*. After 4 months, all of the above system compartments were sampled and analyzed for 19 different PCB and 15 PAH congeners, all originating from the sediment compartment.

The results indicate that recently added PCBs and PAHs were more mobile in the model ecosystem than the ‘historic’, more sequestered congeners. Most biomass in the model ecosystem consisted of aquatic macrophytes, whose presence depleted the fast desorbing fraction of PCBs and PAHs in the sediment by 26 and 31%, respectively. However, the presence of macrophytes did not significantly decrease the concentrations of PCBs and PAHs in the carp. A clear biological effect of the presence of carp was their predation on invertebrates. Additionally,

the foraging behaviour of the carp resulted in resuspension of the sediment, increasing the amounts of PCBs and PAHs in other compartments. The results of this experiment are described in detail in chapter 6.

The experiments presented in this thesis show that the relation between pollutants (nutrients and toxicants) and aquatic communities works two ways, in that pollutants influence the structure and functioning of the aquatic community, while organisms influence the environmental fate of pollutants.

Chapters 2 and 3 show that nutrient levels and/or background pollutants in the sediment influence the structure of the aquatic community. However, this hardly impacted the threshold values for direct toxic effects of the additional stressors lambda-cyhalothrin and TPT. However, at concentrations higher than the threshold values, larger differences were found between the different types of test system in the indirect effects and recoverability of sensitive populations (chapters 2, 3, and 4).

Predicting the observed model ecosystem response by means of short-term laboratory experiments with aquatic invertebrates and the two toxicants (the insecticide lambda-cyhalothrin and the fungicide/biocide TPT) proved more difficult for TPT than for lambda-cyhalothrin. The findings for lambda-cyhalothrin can be explained by its specific mode of action (neurotoxic to arthropods), the short time required to reach the maximum effect, and the relatively fast dissipation rate from the system. The findings for TPT can be explained by its persistent character and the relatively long time needed before effects are expressed (*e.g.*, via bioaccumulation in the food chain).

Chapter 6 shows that aquatic macrophytes and carp influence the environmental fate of PCBs and PAHs. The relatively large impact of macrophytes can be explained by the rather large biomass of aquatic vegetation in the model ecosystems. The systems containing macrophytes had considerably lower concentrations of PCBs and PAHs in the fast desorbing fraction. Chapter 3 also shows that macrophytes impact on the amount of TPT that partitions into the sediment. The relatively large impact of carp on the environmental fate of PCBs and PAHs in the model ecosystems can largely be explained by their behaviour (predation and resuspension of the sediment).

Table of Contents

Chapter *page*
Summary 7
General introduction 15
Effects of lambda-cyhalothrin in two ditch microcosm systems of different trophic status 33
Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality 63
Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field 113
The influence of sediment quality on the responses of benthic invertebrates after treatment with the fungicide triphenyltin acetate 145
Impact of macrophytes and fish on partitioning of sediment-originating PCBs and PAHs in freshwater model ecosystems 165
General discussion 209
Nederlandse samenvatting 233
Dankwoord 241
Curriculum Vitae 246
Publications 247

Chapter 1

General introduction

Introduction

Nutrients and toxic contaminants are two major environmental stressors in aquatic ecosystems. As nutrient levels impact the trophic status of a system, thus influencing the sedimentation of abiotic and biotic particles, they also impact the deposition of xenobiotic compounds sorbed to these particles [1]. Interactions between nutrients and toxicants have been reported to lead to environmental effects that cannot be predicted on the basis of the impact of each single stressor [1-4]. The effects of such interactions have hardly been studied at the ecosystem level [5], and insofar as they have been addressed, nutrient–toxicant interactions have received more attention in deep water bodies than in shallow aquatic ecosystems. It is especially in shallow systems, however, that macrophyte-mediated processes can substantially impact these interactions [6, 7]. Furthermore, compared to deeper waters, the sediment compartment in shallow systems greatly influences water quality processes and the structure and functioning of the aquatic community in the water column. In the Netherlands and many other countries, sediments have been a sink for pollutants in recent decades, and now that water quality has improved, these sediments may become a net source of pollutants to the water column [8, 9].

This thesis addresses the impact of nutrient status and background pollution in the sediment on the fate and/or effects of existing and/or additional organic micro-pollutants in microcosms mimicking shallow freshwater communities. The main research questions were:

- What is the influence of the trophic status of the system on the response of an aquatic community to a toxic shock provided by additional dosage of a non-persistent organic micro-pollutant (the insecticide lambda-cyhalothrin)?
- Does sediment quality affect the response of an aquatic community to an additional stressor in the form of a persistent organic micro-pollutant (the biocide/fungicide triphenyltin [TPT])?
- Do aquatic invertebrates studied in the laboratory (*i.e.*, excluding food-web effects) show the same concentration–response relationships to TPT as aquatic invertebrate populations in outdoor microcosms (including food-web and food-quality effects) and can responses be extrapolated to different types of communities?
- What is the effect of ecosystem structure, particularly the absence or presence of macrophytes and fish, on the distribution of sediment-derived polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) in the various ecosystem compartments?

To answer these questions, various types of experimental model ecosystems were used. Outdoor test systems were constructed that were phytoplankton-dominated or macrophyte-dominated (chapter 2), or were macrophyte-dominated and contained polluted or clean sediment (chapters 3 and 4). In addition, indoor microcosms were constructed that contained polluted sediment with different ecological structures (chapters 6). The focus of the research in the indoor PCB/PAH microcosm experiment was on the impact of biota on the fate of organic micro-pollutants, while the outdoor microcosm experiments with lambda-cyhalothrin and triphenyltin primarily focused on the impact of the toxicant on the structure and functioning of the community.

Study area

The research discussed in this thesis was part of the Stimulation Programme on System-oriented Ecotoxicological Research (SSEO), funded by the Dutch Organization for Scientific Research (NWO). One of the sites studied in this programme was the Afferdense en Deestsche Waarden (ADW) floodplain along the river Waal, in the Rhine catchment area in the Netherlands. The ADW floodplain comprises agricultural fields, extensively grazed grasslands and a number of lakes, and is flooded frequently. Historically, the ADW floodplain was used by a local brick factory, which used clay deposits to produce bricks. Nowadays, with most of its clay depleted, the ADW floodplain is being further excavated and redeveloped into a wildlife/recreational area, and to provide more water storage capacity during flooding events.

The interactions between toxicants and ecosystem types studied in this thesis are rather diverse. Besides variations in contaminant type, systems differ with respect to ecosystem structure and functioning. For instance, they can be macrophyte- or plankton-dominated and, consequently, harbour different communities. Both system types can be found in close proximity in river floodplains, one of the most dynamic aquatic environments [12]. River floodplains are governed by the river, and controlled by two major ecological processes, one longitudinal and one lateral (see Fig. 1). The longitudinal process, running from spring to estuary, corresponds to the so-called River Continuum Concept described by Vannote [10]. This process is driven by particulate organic material (food), and the type of this particulate matter determines what kind of functional groups, and thus communities, can be found in different parts of the river.

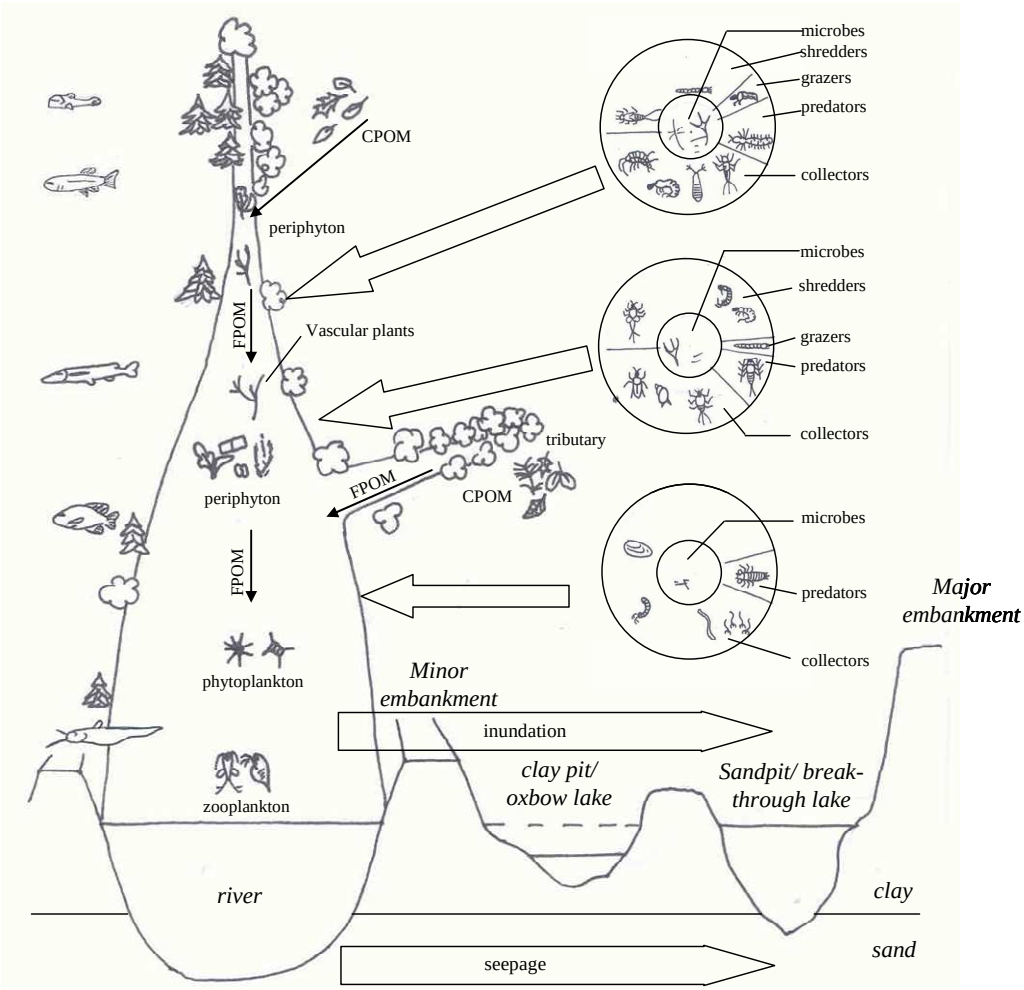


Figure 1: Schematic depiction of the River Continuum Concept and Flood Pulse Concept. CPOM=Coarse Particulate Organic Matter; FPOM=Fine Particulate Organic Matter (Illustration changed after [10] and [11])

Downstream, where the current slows down, particulate organic matter becomes finer (as it is processed several times) and sedimentation occurs. It is in this section that the second process, described by the Flood Pulse Concept, takes place [11, 13]. This process is driven by seasonal discharge peaks and allows an exchange of matter and organisms between the river and the floodplain or lakes within the floodplain, changing their ecological and chemical properties for shorter or longer periods of time. These processes essentially shape the type of aquatic communities that can occur in floodplain lakes. The occurrence of pollutants merely determines to what extent these ‘potential’ communities are actually present.

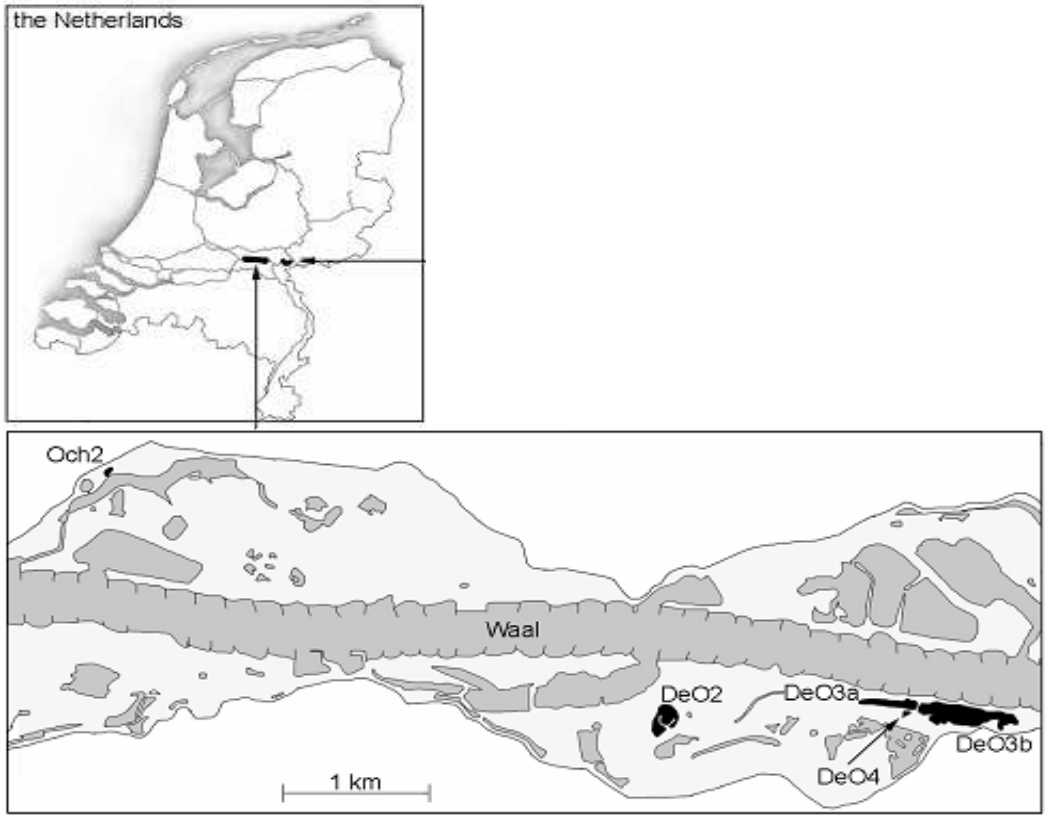


Figure 2: Study area and sampling locations for sediment (DeO2 and DeO3b) in the Afferdensche and Deestsche Waarden, a floodplain area along the river Waal (a branch of the River Rhine). Picture kindly provided by Dr. E. de Haas.

Figure 2 shows the location of the lakes coded DeO2 and DeO3b, respectively, which provided the clean and polluted sediments for some experiments described in this thesis (chapters 3-6). These sites were also part of a previous PhD project, which was designed to unravel the dominant steering mechanisms in the ecological development of floodplain lakes along the river Rhine [14, 15]. It was particularly in the 1970s that large amounts of polluted sediment were deposited in these floodplains, comprising mainly metals, PAHs and PCBs. Although the input of these compounds has decreased significantly (*e.g.*, due to legislation) they are very persistent in the environment. Consequently, these compounds are still present in sediments today.

Since rivers are an important transport medium, the adjacent floodplains can also be affected by compounds originating from ships, *e.g.*, anti-fouling paints. Components of these paints, *e.g.*, organotins, have been found to accumulate in sediments of marinas [16] but also to be transported further down the catchment [17]. Additionally, organotins may originate from agricultural sources [18], and floodplain lakes near agricultural fields may be affected by short-term exposures to other, less persistent, plant protection products (*e.g.*, pyrethroid insecticides).

Profiles of the compounds used in the experiments

Lambda-cyhalothrin is a synthetic pyrethroid insecticide and exerts its toxicity by disrupting signal transmission in the nerve axons [19]. The compound sorbs readily to organic matter due to its low water solubility (5 μg/L at 20 ºC) and high hydrophobicity (log K_{ow}= 7) [20]. In aquatic ecosystems, lambda-cyhalothrin is subject to transformation via biotic and abiotic processes, with alkaline hydrolysis near the leaf surface of macrophytes or algae being considered the most important process [21]. The half-life of the compound is typically less than one day [21, 22]. Non-arthropod invertebrates and plants are relatively insensitive to lambda-cyhalothrin [23], while arthropods are on the whole more sensitive than fish. Within the arthropods, macrocrustaceans and insects represent the most sensitive species [23, 24]. Since the compound is non-persistent and dissipates rapidly from the water phase, it is expected that organisms are subject to short-term exposures causing acute effects.

Triphenyltin is an organotin compound and is used as a fungicide in a variety of crops [25] and as a biocide in anti-fouling paints on ships [26]. TPT is highly toxic to a wide range of aquatic organisms [27-29]. The specific mode of action of the compound is not fully understood, but it is known to target a variety of fundamental processes (*e.g.*, inhibition of mitochondrial oxidative phosphorylation, protein and DNA synthesis) which are not immediately visible and may take time to produce observable effects [30, 31]. In the environment, triphenyltin degrades via biodegradation and photodegradation, with reported half-lives of 60 to 240 days [32]. However, residence times of over ten years have also been reported [26].

Polycyclic aromatic hydrocarbons are amongst the most widespread pollutants, with sources including natural (oil seeps, bitumen, forest fires) and anthropogenic processes (petroleum exploration, production and combustion) [33]. The environmental problem of PAHs lies in their long half-lives, *e.g.*, up to 38 years for fluoranthene, [34] and their mutagenic and carcinogenic potential [35]. Polychlorinated biphenyls, a collection of 209 synthetic halogenated aromatic hydrocarbons, have been used extensively in insulation or as cooling agents in electrical applications [36]. PCBs are known to bioaccumulate and biomagnify in the food chain and can induce a variety of effects, including death, birth defects, reproductive failure, liver damage and tumours [36]. The toxicological properties of PCBs are governed by two factors: the partition coefficient (K_{ow}) and steric factors, resulting from different positions of the chlorine substitutes. In general, PCB congeners with the highest K_{ow} values, and a high number of substituted chlorines in adjacent positions, cause the greatest environmental concern [36]. These

compounds are also persistent in the environment, and long half-lives, *e.g.*, up to 11 years for PCB52, have been reported in soil [34].

Model ecosystems

Causal mechanisms and interactions between chemical stressors and/or properties of ecosystems cannot easily be identified in field studies, because of confounding factors. In addition, multiple alternative mechanisms may explain the observed patterns. To test our hypotheses, elements or processes typical of floodplain lakes were mimicked in indoor and outdoor microcosms (Fig. 3).

Figure 3: Experimental set-ups used in the research for this thesis, including indoor single species tests (A & B), enclosures in experimental ditches (C), indoor glass microcosms (D) and outdoor concrete microcosms (E).

Microcosms allow experimental set-ups for hypothesis testing and replication, while on the other hand providing realism in terms of ecological processes and exposure to compounds to be tested [37]. Although the diversity in freshwater model ecosystems is large, a rough division can be made into two types, the ‘generic’ and the ‘semi-realistic’ systems. ‘Generic’ systems exhibit some basic properties, chosen by the experimenter, common to all ecosystems (*e.g.*, nutrient cycling, species interaction and decomposition), while the ‘semi-realistic’ systems mimic real ecosystems [38, 39]. Model ecosystem studies generally focus on interactions in specific test systems and it has been found that many factors can influence the outcome of the studies conducted in them. Since

the selection of test conditions and types of communities enclosed in the system have an a priori influence on the experimental results, model ecosystem studies (and any other type of study for that matter) are to some extent anecdotal. However, several of these anecdotal observations can be generalized so as to obtain rules-of-thumb that may be suitable for spatio-temporal extrapolation [40].

Analysis of complex data sets and presentation of results

In model ecosystem studies, the sampling in time of various structural (*e.g.*, abundance of invertebrate taxa) and functional (*e.g.*, dissolved oxygen production) endpoints results in a complex set of data points, which can be difficult to analyze, present and interpret. Analyzing these experimental data with traditional statistical tools used in ecotoxicology, like the Williams test (to derive NOECs) or regression analysis (used to determine EC_{50} values), only offers information on the concentration–response relationship of one endpoint. Although these univariate techniques are powerful tools, they are not always suitable, because of the variability and/or low abundance of the majority of taxa. Consequently, univariate statistics can only be used to evaluate the effect of the treatment in aquatic micro/mesocom studies for a limited number of endpoints [41]. In contrast, multivariate techniques do not suffer from this problem and may be used to describe the effects of a stressor at the community level. The advantage of methods like Principal Component Analysis (PCA) and Redundancy Analysis (RDA) is that they use and summarize all information simultaneously. Nevertheless, their output is often complex, and when time-dependency is included, it becomes too cluttered to be properly interpreted [42]. A technique called Principal Response Curve (PRC; see Fig. 4) analysis solves this problem by focusing on the difference between species compositions in the treated systems and that in the control system at a corresponding point in time. Treatment-related statistical differences within a PRC diagram can be tested by means of Monte Carlo permutation tests [42].

Another way of presenting an overview of the responses observed in a model ecosystem study is to use ‘effect classes’ for different categories of endpoints (*e.g.*, Cladocera, Copepoda and Ostracoda may be incorporated into the structural category of ‘micro-crustaceans’). Within each category, the most sensitive endpoint is decisive for the classification in an effect class (which makes this a worst-case approach). In this thesis, five effect classes are distinguished: 1: No effect; 2: Slight transient effects; 3: Clear short-term effects, with a recovery time <8 weeks; 4: Clear medium-term effects, with a recovery time >8 weeks but within the period studied; and 5: Clear long-term effects with no full

recovery observed. The 8-week limit was established to differentiate between short-term and longer-term effects (adapted after [43]).

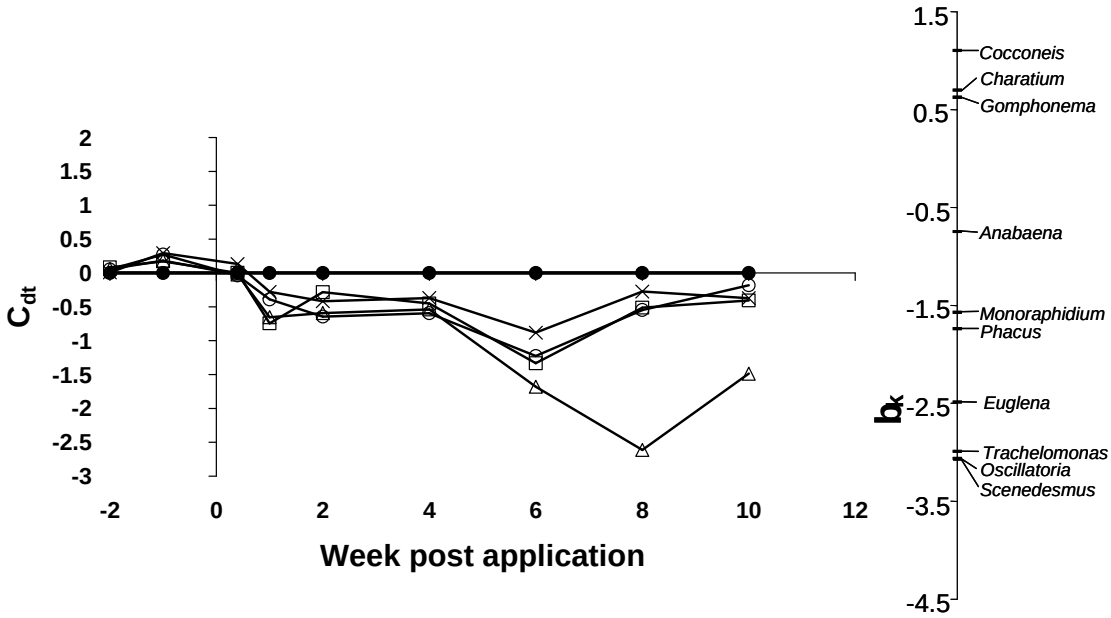


Figure 4: Example of a Principal Response Curve diagram. The horizontal axis represents the control test systems. The vertical axis represents the differences in phytoplankton community structure between the treatment and control systems, expressed as regression coefficients (C_{dt}) of the PRC model. The accompanying axis depicting taxon weights (b_k-axis) can be interpreted as the affinity of each species to the response depicted in the diagram. The higher the weight of a particular taxon, the more clearly the actual response pattern of this taxon is likely to follow the pattern in the PRC, while taxa with a more negative weight are likely to show the opposite pattern to that of the PRC.

The final technique discussed here is the species sensitivity distribution approach (SSD) (Fig. 5). Posthuma *et al.* [44] defined the SSD as a statistical distribution describing the variation in sensitivity to a particular toxic compound or mixture among a set of species. The species set may comprise species from a specific taxonomic group (*e.g.*, arthropods), a selected species assemblage or a natural community. The SSD is estimated from toxicity data (*e.g.*, EC_{50} values or NOECs) and is visualized as a cumulative distribution. It will be evident that the derivation of the SSD and the conclusions drawn from it rely heavily on the number of data points used to construct the distribution. Environmental quality criteria are derived by choosing a cut-off percentage *p* (to protect 1-*p* percent of the species tested; Y-axis). The corresponding desired 'safe' concentration (HC_p) can then be found on the X-axis (see Fig. 5). The most commonly used value of *p* is 5, establishing a 95% protection criterion. Note that this has been a political decision, and not one based on science [45].

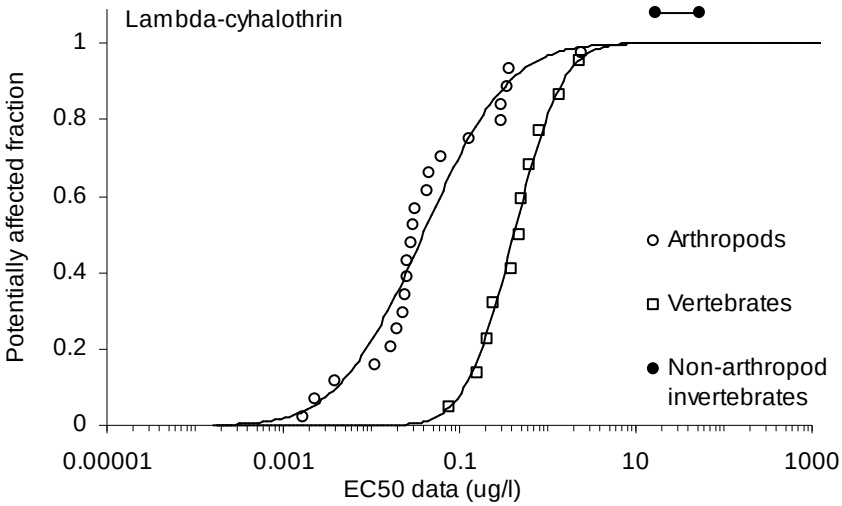


Figure 5: Species Sensitivity Distribution curve showing the potentially affected fraction (PAF) of test species based on EC_{50} values; example from [46].

This thesis does not rely on one technique, but uses a combination of all of the above methods. Some methods, like the PRC analysis, have also been applied in a different setting than that for which they were originally developed (see chapter 6). This combination of techniques ensures that a more complete overview of the responses observed in the experiments is presented.

Outline of the thesis

Chapter 2 describes an experiment assessing the influence of trophic status on the response of an aquatic community to lambda-cyhalothrin as an additional stressor. To this end, two sets of fourteen enclosures, twelve to monitor effects and two to monitor the fate of the chemical, were inserted in two experimental ditches each. The ditches were either macrophyte- or phytoplankton-dominated, that is, they had different species compositions. It was hypothesized that systems differing in trophic status (containing different species) would react differently to additional stress from the insecticide lambda-cyhalothrin. Direct and indirect effects of lambda-cyhalothrin on the aquatic communities were analyzed and related to structure differences between the systems.

In the experiments described in chapter 3, clean and polluted sediments were taken from floodplain lakes along the river Waal (in the Rhine catchment area) and used to construct twenty macrophyte-dominated outdoor mesocosms (ten of each sediment type). In addition to the organisms that entered the test systems via the sediment, all systems were seeded with an invertebrate community from a clean site near the town of Wageningen, the Netherlands. It was hypothesized that aquatic communities that developed on polluted sediment would be more

vulnerable to additional stress than communities developing on clean sediment. This was based on the assumption that coping with multiple stressors is harder than coping with a single stressor. It was assumed that the additional stress originated from bioavailable contaminants in the sediment (see chapter 6). To test this hypothesis, the additional stressor triphenyltin acetate (TPT) was applied to the test systems and responses of nematodes, phytoplankton, macrophytes, zooplankton and macroinvertebrates were related to the toxicant applied and the type of sediment (clean or polluted).

Chapter 4 focuses on the comparison of data generated in the outdoor microcoms with data generated in single-species laboratory toxicity tests. The semi-field response of phytoplankton, zooplankton and macroinvertebrates to TPT, as described in chapter 3, was compared with the response of several phytoplankton, zooplankton and macroinvertebrate taxa in acute laboratory toxicity tests. The laboratory tests were performed for two reasons: (i) to identify the taxonomic groups that are sensitive to TPT, so as to allow a distinction to be made between direct effects due to toxicity and indirect effects due to ecological interactions in the microcosm experiment and (ii) to investigate whether the results in terms of the responses of the taxa tested in the laboratory could be used to predict the response of the community tested in the outdoor microcosms.

Chapter 5 deals with the impact of historical sediment pollution and the additional chemical stressor TPT on two benthic invertebrates in whole-sediment bioassays. The invertebrates used in these experiments were the sensitive mayfly *Ephoron virgo* and the tolerant midge *Chironomus riparius*. The sediments used in the bioassays originated from the experiment described in chapter 3 and were extracted from the clean and polluted sediment-filled cosms 15 weeks after TPT application.

Chapter 6 discusses how sediment-derived pollutants (in this case PCB and PAH) behave in aquatic systems representing different trophic status (macrophyte- and phytoplankton-dominated, representing mesotrophic and hypertrophic conditions, respectively) with and without fish. Twelve indoor systems were constructed with four replicates for each treatment level. PCB and PAH contents were analyzed in the following system compartments: sediment (both Soxhlet- and TENAX-extracted), suspended solids, macrophytes, periphyton, flab, zooplankton, Oligochaeta, mix fauna (being the sum total of the remaining macroinvertebrates) and fish. The presence of macrophytes and/or fish in

particular was expected to strongly influence the distribution of sediment-derived PCBs and PAHs in the test systems.

The thesis ends with a discussion of the main implications of the results for the evaluation of multi-stress effects associated with pollution and eutrophication, and offers some concluding remarks (chapter 7).

References

[1] Koelmans, A. A., Van der Heijde, A., Knijff, L. M. and Aalderink, R. H. 2001. Integrated Modelling of Eutrophication and Organic Contaminant Fate & Effects in Aquatic Ecosystems. A Review. Water Research 35 pp. 3517-3536

[2] Breitburg, D. L., Sanders, J. G., Gilmour, C. C., Hatfield, C. A., Osman, R. W., Riedel, G. F., Seitzinger, S. P. and Sellner, K. G. 1999. Variability in responses to nutrients and trace elements, and transmissions of stressor effects through an estuarine food web. Limnology and Oceanography 44 pp. 837-863

[3] Kramer, P. R. G., Jonkers, D. A. and Van Liere, L. 1997. Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems. RIVM. Bilthoven. The Netherlands

[4] Skei, J., Larsson, P., Rosenberg, R., Jonsson, P., Olsson, M. and Broman, D. 2000. Eutrophication and contaminants in aquatic ecosystems. Ambio 29 pp. 184-194

[5] Hartgers, E. M., Aalderink, G. H., Van den Brink, P. J., Gylstra, R., Wiegman, W. J. and Brock, T. C. M. 1998. Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms. Aquatic Ecology 32 pp. 135-152

[6] Brock, T. C. M., Crum, S. J. H., Van Wijngaarden, R. P. A., Budde, B. J., Tijink, J., Zuppelli, A. and Leeuwangh, P. 1992. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: I. Fate and primary effects of the active ingredient chlorpyrifos. Archives of Environmental Contamination and Toxicology 23 pp. 69-84

[7] Schulz, R., Moore, M. T., Bennett, E. R., Milam, C. D., Bouldin, J. L., Farris, J. L., Smith, S. and Cooper, C. M. 2003. Acute toxicity of Methyl-Parathion in wetland mesocosms: Assessing the influence of aquatic plants using laboratory testing with *Hyalella azteca*. Archives of Environmental Contamination and Toxicology 45 pp. 331-336

[8] Gunnarsson, J., Bjork, M., Gilek, M., Granberg, M., Rosenberg, R. 2000. Effects of eutrophication on contaminant cycling in marine benthic systems. Ambio 29 pp. 252-259

[9] Moermond, C. T. A., Roozen, F. C. J. M., Zwolsman, J. J. G. and Koelmans, A. A. 2004. Uptake of sediment-bound bioavailable polychlorobiphenyls by benthivorous carp (*Cyprinus carpio*). Environ. Sci. Technol. 38 pp. 4503-4509

[10] Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. and Cushing, C. E. 1980. River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37 pp. 130-137

[11] Junk, W. J. 1999. The flood pulse concept of large rivers: learning from the tropics. Archiv für Hydrobiologie, supplement large rivers 115 pp. 261-280

[12] Van den Brink, F. W. B. 1993. Growth and morphology of four freshwater macrophytes under the impact of the raised salinity level of the Lower Rhine. Aquatic Botany 45 pp. 285-297

[13] Bayley, P. B. 1991. The flood pulse advantage and the restoration of river-floodplain systems. Regulated Rivers Research & Management 6 pp. 75-86

[14] Van Geest, G. J. 2005. Macrophyte succession in floodplain lakes; spatio-temporal patterns in relation to river hydrology, lake morphology and management. Wageningen University, Wageningen,

[15] Roozen, F. C. J. M. 2005. Transparency of floodplain lakes; a study of plankton and suspended matter along the lower Rhine. Wageningen University, Wageningen,

[16] Fent, K. and Hunn, J. 1995. Organotins in freshwater harbours and rivers: temporal distribution, annual trends and fate. Environmental Toxicology and Chemistry 14 pp. 1123-1132

[17] Van den Brink, P. J. and Kater, B. J. 2006. Chemical and biological evaluation of sediments from the Wadden Sea, The Netherlands. Ecotoxicology 15 pp. 451-460

[18] Stäb, J. A., Cofino, W. P., Van Hattum, B. and Brinkman, U. A. T. 1994. Assessment of transport routes of triphenyltin used in potato culture in the Netherlands. Analytica Chimica Acta 286 pp. 335-341

[19] Clark, J. M. and Brooks, G. M. 1998. Neurotoxicology of pyrethroids: Single or multiple mechanisms. Environmental Toxicology and Chemistry 8 pp. 361-372

[20] Hand, L. H., Kuet, S. F., Lane, M. C. G., Maund, S. J., Warinton, J. S. and Hill, I. R. 2001. Influences of aquatic plants on the fate of the pyrethroid insecticide lambda-cyhalothin in aquatic environments. Environmental Toxicology and Chemistry 20 pp. 1740-1745

[21] Leistra, M., Zweers, A. J., Warinton, J. S., Crum, S. J. H., Hand, L. H., Beltman, W. H. J. and Maund, S. J. 2003. Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. Pest Management Sciences 60 pp. 75-85

[22] Van Wijngaarden, R. P. A., Arts, G. H. P., Cuppen, J. G. M., Crum, S. J. H. and Brock, T. C. M. 2004. Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study. Environmental Toxicology and Chemistry 23 pp. 1479-1498

[23] Schroer, A. F. W., Belgers, D., Brock, T. C. M., Matser, A., Maund, S. J. and Van den Brink, P. J. 2004. Acute toxicity of the pyrethroid insecticide lambda-cyhalothrin to invertebrates of lenthic freshwater systems. Archives of Environmental Contamination and Toxicology 46 pp. 324-335

[24] Maund, S. J., Hamer, M. J., Warinton, J. S. and Kedwards, T. J. 1998. Aquatic Ecotoxicology of the pyrethroid insecticide Lambda-cyhalothrin: Considerations for Higher-Tier aquatic risk assessment. Pesticide Science 54 pp. 408-417

[25] Grigarick, A. A., Webster, R. K., Meyer, R. P., Zalom, F. G. and Smith, K. A. 1990. Effect of pesticide treatments on nontarget organisms in california rice paddies. Hilgardia 58 pp. 1-36

[26] Fent, K. and Hunn, J. 1991. Phenyltins in water, sediment, and biota of freshwater marinas. Environmental Science and Technology 25 pp. 956-963

[27] Fargasová, A. 1998. Comparison of effects of tributyl-, triphenyl-, and tribenzyltin compounds on freshwater benthos and alga *Scenedesmus quadricauda*. Bulletin of Environmental Contamination and Toxicology. 60 pp. 9-15

[28] Rehage, J. S., Lynn, S. G., Hammond, J. I., Palmer, B. D. and Sih, A. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile ambystoma barbouri salamanders. Environmental Toxicology and Chemistry 21 pp. 807-815

[29] De Zwart, D. 2002. Observed regularities in species sensitivity distributions for aquatic species. In: Posthuma, L., Suter II, G. W. and Traas, T. P., eds, Species sensitivity distributions in ecotoxicology, Lewis Publishers, Boca Raton, pp. 133-154

[30] Chandra, S., Polya, G. M., James, B. D. and Magee, R. J. 1989. Inhibition of oxidative phosphorylation by organotin thiocarbamates. Chemico-Biological Interactions 71 pp. 21-36

[31] Tiano, L., Fedeli, D., Santoni, G., Davies, I. and Falcioni, G. 2003. Effect of tributyltin on trout blood cells: changes in mitochondrial morphology and functionality. Biochimica et Biophysica Acta 1640 pp. 105-112

[32] Loch, J. P. G. 1990. Accumulation and leaching of the fungicide fentin acetate and intermediates in sandy soils. Water, Air, and Soil Pollution 53 pp. 119-129

[33] Yunker, M. B. and Macdonald, R. W. 1995. Composition and origins polycyclic aromatc hydrocarbons in the Mackenzie River and on the Beaufort Sea Shelf. Arctic 48 pp. 118-129

[34] Doick, D. J., Klingelmann, E., Burauel, P., Jones, K. C. and Semple, K. T. 2005. Long-term fate of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in an agricultural soil. Environmental Science and Technology 39 pp. 3663-3670

[35] Zedeck, M. S. 1980. Polycyclic aromatic compounds: A review. Journal of Environmental Pathology and Toxicology 3 pp. 537-567

[36] Eisler, R. 1986. Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Fish and Wildlife Service.

[37] Brock, T. C. M., Bos, A. R., Crum, S. J. H. and Gylstra, R. 1995. The model ecosystem approach in ecotoxicology as illustrated with a study on the fate and effects of an insecticide in stagnant freshwater microcosms. In: Koch, B. and Niesser, R., eds, Immunochemical detection of pesticides and their metabolites in the water cycle, Research report, Deutsches Forschungsgemeinschaft, VCH, Weinheim, pp. 167-185

[38] Taub, F. B. 1969. A biological model of a freshwater community: a gnotobiotic ecosystem. Limnology and Oceanography 14 pp. 136-142

[39] Kersting, K. 1984. Development and use of an aquatic micro-ecosystem as a test system for toxic substances. Properties of an aquatic ecosystem IV. Internationale Revue der Gesamten Hydrobiologie 69 pp. 567-607

[40] Van Wijngaarden, R. P. A. 2006. Interpretation and extrapolation of ecological responses in model ecosystems stressed with non-persistent insecticides. Wageningen University, Wageningen, The Netherlands

[41] Van Wijngaarden, R. P. A., Van den Brink, P. J., Crum, S. J. H., Oude Voshaar, J. H., Brock, T. C. M. and Leeuwangh, P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: I. Comparison of short-term toxicity between the laboratory and the field. Environmental Toxicology and Chemistry 15 pp. 1133-1142

[42] Van den Brink, P. J. and Ter Braak, C. J. F. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18 pp. 138-148

[43] Brock, T. C. M., Van Wijngaarden, R. P. A. and Van Geest, G. J. 2000. Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra. Wageningen. The Netherlands

[44] Posthuma, L., Suter II, G. W. and Traas, T. P. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, London

[45] Van Straalen, N. M. and Denneman, C. A. J. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotoxicology and Environmental Safety 18 pp. 241-251

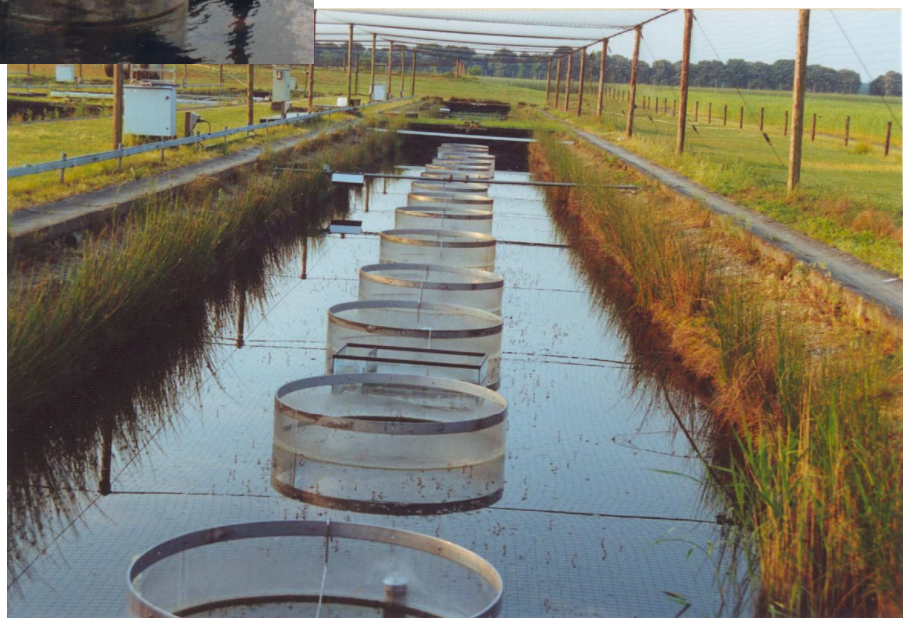
[46] Maltby, L., Blake, N. N., Brock, T. C. M. and Van den Brink, P. J. 2005. Insecticide species sensitivity distributions: the importance of test species selections and relevance to aquatic ecosystems. Environmental Toxicology and Chemistry 24 pp. 379-388

Chapter 2

Effects of lambda-cyhalothrin in two ditch microcosm systems of different trophic status

I. Roessink
G. H. P. Arts
J. D. M. Belgers
F. Bransen
S. J. Maund
T. C. M. Brock

Environmental Toxicology and Chemistry (2005) 24 (7) pp. 1684–1696



Abstract

The fate and effects of the pyrethroid insecticide lambda-cyhalothrin were compared in mesotrophic (macrophyte-dominated) and eutrophic (phytoplankton-dominated) ditch microcosms (±0.5 m^3). Lambda-cyhalothrin was applied three times at one-week intervals at concentrations of 10, 25, 50, 100, and 250 ng/L. The rate of dissipation of lambda-cyhalothrin in the water column of the two types of test systems was similar. After 1 d, only 30% of the amount applied remained in the water phase. Initial, direct effects were observed primarily on arthropod taxa. The most sensitive species was the phantom midge (*Chaoborus obscuripes*). Threshold levels for slight and transient direct toxic effects were similar (10 ng/L) between types of test systems. At treatment levels of 25 ng/L and higher, apparent population and community responses occurred. At treatments of 100 and 250 ng/L, the rate of recovery of the macroinvertebrate community was lower in the macrophyte-dominated systems, primarily because of a prolonged decline of the amphipod *Gammarus pulex*. This species occurred at high densities only in the macrophyte-dominated enclosures. Indirect effects (*e.g.*, increase of rotifers and microcrustaceans) were more pronounced in the plankton-dominated test systems, particularly at treatment levels of 25 ng/L and higher.

Introduction

Freshwater ecosystems in agricultural landscapes have the potential to be exposed to elevated levels of nutrients and plant-protection products, which may result in effects on non-target organisms. To date, however, the relationship between nutrient status of the ecosystem and the fate and potential effects of plant-protection products have received little attention [1, 2].

In the present study, we assessed the impacts of multiple applications of lambda-cyhalothrin, a pyrethroid insecticide, in meso- and eutrophic shallow freshwater ecosystems. These systems are characterized by macrophytes or phytoplankton, respectively, as the major primary producers. The present study comprised part of a larger program that examined the aquatic fate of lambda-cyhalothrin [3], its toxicity to invertebrates in the laboratory [4], and field responses of aquatic organisms in different system types and times of the year.

The pristine state of the majority of shallow freshwater ecosystems probably is characterized by relatively clear water and a diverse aquatic macrophyte vegetation. When such systems experience increased nutrient loading, a shift occurs from clear to turbid water, and submerged macrophytes often are replaced by phytoplankton [5]. The structure and function of macrophyte- and plankton-dominated aquatic communities

differ considerably. In macrophyte-dominated systems, aquatic vascular plants form the majority of the biomass and play an important role, providing the main microhabitat supporting other aquatic organisms (*e.g.*, as a substrate for periphytic organisms and as a refuge for zooplankton and macroinvertebrates). In addition, macrophytes influence several important physicochemical properties of their environment by their architecture (*e.g.*, light interception) and metabolism (*e.g.*, oxygen production). In contrast, plankton communities usually are less diverse and are characterized by a lower total biomass and shortened life cycles; consequently, higher turnover rates, higher concentrations of particulate organic particles, and dissolved organic matter may be present [5], resulting in a more turbid water column. Conversely, water columns in macrophyte-dominated systems usually are more clear. These differences in structure and function of plankton- and macrophyte-dominated systems could, potentially, result in different responses to toxicant stresses. The potential for different types of aquatic ecosystems to respond in a different manner has been identified as a key question for the interpretation of micro- and mesocosm studies in pesticide regulation [6].

Highly lipophilic, lambda-cyhalothrin binds rapidly and extensively to organic matter and other particles, resulting in rapid dissipation from the water column [7-9]. Results of single-species toxicity tests show that insect larvae and macrocrustacea are particularly sensitive (Fig. 1)[4, 10]. Extensive laboratory and field aquatic ecotoxicological studies of lambda-cyhalothrin have resulted in a well-established pattern of effects. Therefore, this compound was chosen to investigate potential impacts of plant-protection products in systems of differing trophic status. Considering the differences in ecological structure and function of meso- and eutrophic shallow freshwaters and following application of similar treatment concentrations of lambda-cyhalothrin, our main goal was to investigate possible differences in direct and indirect effects of lambda-cyhalothrin Lambda-cyhalothrin effects in cosms of unequal trophic status application macrophyte- and phytoplankton-dominated microcosms. Furthermore, we aimed to identify the ecological threshold level (the lowest observed concentration with significant, but slight and transient, effects on the aquatic community). Finally, we investigated whether the ability of affected populations and the total invertebrate community to recover from insecticide stress differed between the two types of systems.

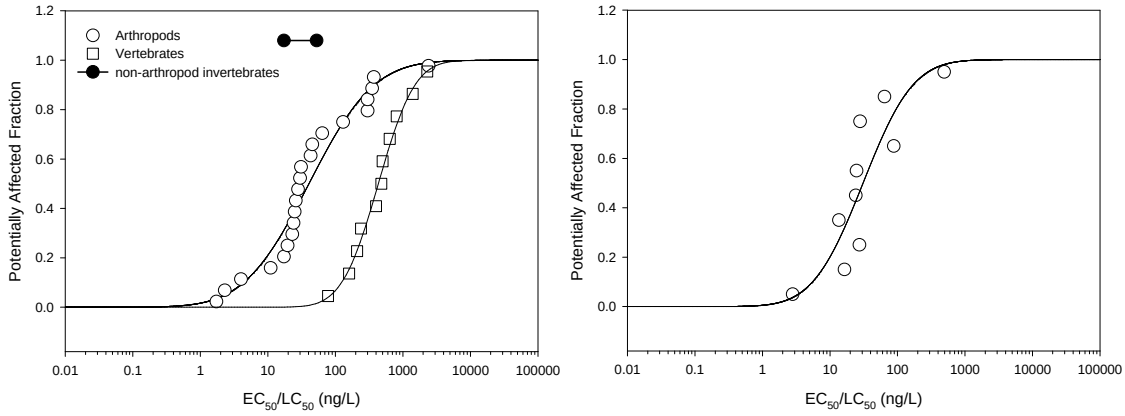


*Figure 1: (**A**) Species-sensitivity distribution curves of lambda-cyhalothrin showing the potentially affected fraction (PAF) of tested species based on acute effect/lethal concentration values at which 50% of tested population responded (EC_{50}/LC_{50}) for aquatic arthropods, aquatic vertebrates (mainly fish), and aquatic nonarthropod invertebrates available in the literature [11]. (**B**) Species-sensitivity distribution for arthropod taxa also observed in the field enclosures of the present study. Because toxicity values for nonarthropod invertebrates are scarce, it was considered to be more appropriate to plot the concentration range of their EC_{50}/LC_{50} values. (Data after Schroer et al. [4].)*

Materials and methods

Test systems

Simultaneous treatments included a macrophyte- and plankton-dominated ditch system. The macrophyte-dominated ditch represented mesotrophic conditions, and the plankton-dominated ditch was typical of eutrophic conditions. Test systems consisted of polycarbonate, light-permeable cylinders, which are referred to here as enclosures (diameter, 1.05 m; height, 0.9 m). In each ditch system, 14 enclosures were pressed into the sediment (depth, 15 cm) and had the same water level as the ditch (0.5 m). Within each ditch, 12 enclosures were used for effect studies, with the remainder used for fate studies.

The dominant top predators in the enclosures were Zygoptera in the macrophyte-dominated enclosures and the dipteran *Chaoborus obscuripes* in the plankton-dominated test systems. Average macrophyte biomass (± standard deviation) in the macrophyte-dominated enclosures was 117 ± 47 g/m^2. No macrophytes occurred in the enclosures in the phytoplankton-dominated ditch. Average dissolved organic carbon concentrations were 8.8 and 17.8 mg/L for the macrophyte- and phytoplankton-dominated systems, respectively. The corresponding mean values were 4.9 and 14 mg/L, respectively, for total suspended solids and 58.47 ± 47.5 and 157.06 ± 52.6 mg/L, respectively, for chlorophyll-*a*. So, the macrophyte-dominated systems were characterized by lower water concentrations of dissolved organic carbon, suspended solids, and phytoplankton chlorophyll-*a* compared with the plankton-dominated

systems. Enclosures in the phytoplankton-dominated ditch received weekly additions of KH_2PO_4 and NH_4NO_3 to achieve concentrations of 0.15 mg/L of P and 0.90 mg/L of N to maintain eutrophic conditions. Nutrient levels were monitored simultaneously with zooplankton and phytoplankton sampling. Further details of the characteristics of the systems have been described by Leistra *et al.* [3].

Lambda-cyhalothrin applications and analyses

The formulated product KARATE with ZEON Technology™ (100 g/L of lambda-cyhalothrin as a capsule suspension; Syngenta, Basel, Switzerland) was applied three times at weekly intervals to the enclosures to achieve nominal lambda-cyhalothrin concentrations of 10, 25, 50, 100, and 250 ng/L (n = 2 in each type of test system), and two enclosures were used as untreated controls. Randomly allocated treatments were made on May 16, 2001, and the same treatments were repeated on May 23 and 30, 2001. The treatment concentration of 250 ng/L was based on the concentration derived from The Netherlands' pesticide regulatory scenario of a 5% drift emission from a field application of 0.015 kg/ha of lambda-cyhalothrin into a ditch with a depth of 0.3 m. Laboratory toxicity data regarding standard and additional aquatic species (Fig. 1) indicated that an exposure concentration of 250 ng/L would have a significant impact on the arthropod community [4]. Application of 4 L of test solution per enclosure included gentle stirring so as not to disturb sediments or damage plants. The concentration of lambda-cyhalothrin in the well-mixed application solution was measured by taking a 2-mL sample for hexane extraction. To estimate the initial concentrations, the measured concentrations from the application solution were used. Further fate samples were taken only in the highest concentration treatment (250 ng/L) at 1, 3, 6, 9, 24, 72, and 168 h after the first application. Sampling techniques and extraction methods have been described in detail by Leistra *et al.* [3].

Macroinvertebrates

Macroinvertebrates were sampled from each enclosure on weeks -1, 1, 3, and 6 by means of litter bags and two types of artificial substrates (multiplates and pebble baskets) as described by Brock *et al.* [12]. In each system, two multiplates, two pebble baskets, and two litter bags were incubated. The multiplates and pebble baskets were gently retrieved using a net. This sampling method also collected the planktonic phantom midge (*C. obscuripes*). The macroinvertebrates present on both substrates and the litter bags were sorted by hand, identified to the lowest practical taxonomic level, counted, and then returned to the enclosures. Counted

numbers of macroinvertebrates from artificial substrates and litter bags were pooled for further analysis.

Zooplankton

Zooplankton was sampled from each enclosure on weeks -1, 1, 2, 3, 4, and 6 using Perspex tube (length, 0.4 m; volume, 0.8 L; Vink Holding BV, Zeist, The Netherlands). Several subsamples were collected from across the enclosures until a 10-L sample had been obtained, from which 5 L were used for zooplankton analysis. The 5-L sample was concentrated by means of a plankton net (mesh size, 55 mm; Hydrobios, Kiel, Germany) and was preserved with formalin (final volume, 4%). Cladocera and Rotifera were counted and identified to the lowest possible taxon. Copepods were counted and classified as calanoids and cyclopoids. Zooplankton abundances were adjusted to numbers of organisms per liter.

Bioassays

Two crustacean species (*Asellus aquaticus* and *Daphnia pulex*) and one insect species (*C. obscuripes*) were tested using in situ bioassays incubated in the enclosures. The aim of the bioassays was to measure acute effects of lambda-cyhalothrin on macroinvertebrate species and to assess the potential recovery of these species. The bioassay cages for *Asellus* and *Chaoborus* sp. were constructed from stainless-steel gauze (mesh size, 0.5 mm; length, 33 cm; diameter, 6 cm; volume, 930 cm^3). For the *Daphnia* bioassay, a glass container (water volume, 100 mL) sealed with 55-mm gauze was used.

One month before dosage, the bioassay organisms were collected from experimental ditches and in field ditches near Wageningen (The Netherlands). The organisms were then stored in aquaria in the laboratory. *Daphnia* organisms were supplied as food to *Chaoborus* larvae. *Populus x canadensis* leaves were added to the *Asellus* aquaria for substrate and food, and algae from a stock culture were added to the *Daphnia* stocks. The *Daphnia* stocks were held in a 100-L aquarium and placed in one of the experimental ditches. *Chaoborus* larvae were maintained at 5°C to slow development and prevent emergence. One week before exposure, the organisms were placed in experimental ditches to acclimate them to experimental conditions, with bioassay organisms selected from aquaria. Twenty-five adult *Asellus* organisms (average size, 6.0 mm; standard deviation, 1.3 mm), 20 adult female *Daphnia* organisms (age, 7 d), and 30 fourth-instar *Chaoborus* larvae were placed in each bioassay cage.

Bioassays were performed in both enclosure experiments immediately after the first application of lambda-cyhalothrin to study direct effects of the doses applied (type 1) and after the last application

for potential recovery (type 2). Acute exposures were used because of the rapid dissipation of lambda-cyhalothrin from the water column. *Chaoborus obscuripes* and *A. aquaticus* were introduced on the day before application in the type 1 bioassays, whereas *D. pulex* was introduced on the day of application. The type 1 bioassays with *C. obscuripes*, *A. aquaticus*, and *D. pulex* were exposed for 6 d, and affected and unaffected individuals were counted at days 1, 2, 3, and 6 (except for *D. pulex*, which were not counted on day 3). In the type 2 bioassay, new bioassay cages containing *C. obscuripes* and *A. aquaticus* were introduced into the enclosure on days 21, 4, and 8 after the last application. Cages introduced on days 21 and 4 were studied after 4 d, and those introduced at day 8 were retrieved after 6 d. Bioassay endpoints were immobility and mortality. Daily movement was used to replenish water supply to the cages.

Primary producers

Phytoplankton was sampled simultaneously with zooplankton. Of the remaining 5-L sample from the original 10-L sample, a 1-L subsample was taken for chlorophyll-*a* analysis and concentrated using a glass-fiber filter (GF52; mesh size, 1.2 mm; Schleicher & Schuell, Dassel, Germany). Filters were stored in Petri dishes, wrapped in aluminum foil, and stored at -20°C for analysis. Analysis was performed using a spectrophotometer (DU-64; Beckman Instruments, Fullerton, CA, USA) following the method described by Moed and Hallegraeff [13].

Glass slides (7.6 x 2.6 cm) were used as an artificial substrate for periphyton. The slides were positioned vertically in a frame at a fixed depth of approximately 25 cm below the water surface. The substrates were introduced 15 or 16 d before the first application. Substrates were collected on days 21, 6, 13, 20, 27, and 41. Five glass slides were retrieved for each chlorophyll-*a* analysis. The slides were brushed clean, and the removed periphyton were washed into a bottle with tap water. The chlorophyll-*a* content of the sample was analyzed following the procedures described for phytoplankton.

The macrophyte-dominated ditch was inhabited primarily by *Elodea nuttallii*. At the end of the experiment, the total macrophyte biomass of the enclosures was harvested. The plant material harvested was rinsed to remove sediment particles, periphyton, and macroinvertebrates and then dried (105 ºC, 24 h) to obtain dry weight.

Community metabolism and decomposition

Measurements of dissolved oxygen, pH, conductivity, and alkalinity were performed eight times at weekly intervals starting two weeks before application of the pesticide. The dissolved oxygen was

measured with a Wissenschaftich-Technische Werkstätte (WTW) Oxi330 oxygen meter (Weilheim, Germany) and oxygen probe at a depth of 10 cm. Conductivity and pH were measured with a WTWLF 191 conductivity meter and a WTW PH197 pH meter, respectively. Alkalinity was measured in 100-mL samples taken at a depth of 10 cm (titration with 0.05 N HCl until pH 4.2; WTW PH197 pH-meter).

Decomposition of particulate organic matter was studied by means of the litter bag technique [13] using *P. x canadensis* leaves. The *Populus* leaves were soaked three times for a period of 2 d to remove soluble humic compounds. To allow storage of this material, it was dried in an oven for 72 h at 60 ºC. In the decomposition measurements, 2 g dry weight of *Populus* leaves were enclosed in each litter bag, consisting of a glass Petri dish (diameter, 11.6 cm) closed with a cover of stainless-steel wire (mesh size, 0.7 x 0.7 mm) in which two holes (0.5 cm) were punched to allow most invertebrates to enter. In each enclosure, two litter bags were incubated at the sediment surface for a period of two weeks and replaced when sampled. At the end of each two-week incubation period, retrieved litter bags were gently washed in the overlying water of the enclosure to remove adhering sediment particles and then emptied into a white tray for separation of the particulate organic matter and the macroinvertebrates. The organic plant material was dried in aluminum foil at 105 ºC for 24 h to obtain dry weight. The decomposition over a two-week period was expressed as the percentage of remaining organic material.

Data analysis

Before analysis, the macroinvertebrate data were $\ln(2x+1)$ transformed and the zooplankton data ($\ln 10x+1$) transformed, where x is the abundance value. This was done to down-weight high abundance values and approximate a log-normal distribution of the data (for rationale, see Van den Brink *et al.* [14]). No-observed-effect concentration (NOEC) calculations at the parameter or taxon level were derived using the Williams test (analysis of variance) [15]. This test assumes that the mean response of the variable is a monotonic function of the treatment, thus leading to the expectation of increasing effects with increasing dose. The analyses were performed with the Community Analysis computer program [16], resulting in a summary of NOECs for each sampling week for the data analyzed. The threshold level for p was 0.05 for all statistical analyses.

The effects of the lambda-cyhalothrin treatment at the community level of zooplankton and macroinvertebrates were analyzed by the principal response curves (PRC) method, which is based on the redundancy analysis ordination technique, the constrained form of

principal component analysis [17]. The PRC method yields a diagram showing the deviations in time of the treatments compared to the controls. The PRC analysis was performed using the CANOCO software package, version 4.02 [18]. The results of the PRC analysis also can be evaluated in terms of the fractions of variance explained by the factors time and treatment, and the fraction of the variance that is explained by treatment is shown in the PRC diagram. In the CANOCO computer program, redundancy analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on the species composition of the samples [19].

The significance of the PRC diagram in terms of displayed treatment variance was tested by Monte Carlo permutation of the enclosures (*i.e.*, by permuting whole time series of microcosms in the partial redundancy analysis from which PRC is obtained), using a *F*-type test statistic based on the eigenvalue of the component [17].

Monte Carlo permutation tests also were performed for each sampling date, using the ln-transformed intended doses as the explanatory variable [19], thus allowing the significance of the treatment regime to be tested for each sampling date. If a significant relation between treatment regime and species composition was found, then treatment levels differing significantly from the controls were determined to infer NOECs at the community level ($NOEC_{community}$). Calculations of the $NOEC_{community}$ were performed by applying the Williams test [17, 19].

In addition to analysis of the significance of treatment regime, Monte Carlo permutation tests were further performed to test whether the communities differed significantly between system structure (macrophyte- *vs.*phytoplankton-dominated) and interaction effects. Treatment was tested for each sampling date by introducing ln-transformed treatment levels as explanatory variables and nominal variables denoting system structure plus its interaction with treatment as covariables. Interaction was tested by entering the interaction between system structure and treatment as explanatory variables and ln-transformed treatment levels and the nominal variable denoting system structure individually as covariables. A more detailed description of permutation tests for multifactorial analysis of variance has been provided by Anderson and Ter Braak [20].

Results

Fate of lambda-cyhalothrin

Measured concentrations in the solutions applied to the test systems varied between 93 and 113% of the target concentration (Table 1). The dissipation of lambda-cyhalothrin from the water phase was very similar between ditches, as is shown in Figure 2. After 1 d, more than

70% had dissipated from the water column in both the macrophyte-dominated and the phytoplankton-dominated enclosures. None of the lambda-cyhalothrin applied in the water column was recovered from sediment samples (all samples were less than the detection limit; see Leistra *et al.* [3] for more details).

Table 1. Initial concentrations of lambda-cyhalothrin in the overlying water phase of the enclosures as a percentage of the nominal concentrations (average and range of all treatments at each application).

	% Nominal concentration		
System	Application 1	Application 2	Application 3
Macrophyte-dominated	104 (100-116)	113 (106-126)	95 (80-113)
Phytoplankton-dominated	103 (98-106)	106 (100-114)	93 (78-116)

Macroinvertebrates

A total of 64 different macroinvertebrate taxa were identified in the enclosures, with 55 occurring in the macrophytedominated systems and 49 in the plankton-dominated systems. Most of these taxa, however, were present at low abundance or only occurred periodically. The macrophyte-dominated ditch enclosures were characterized by snails (*e.g.*, *Armiger cristata*), Nematocera (*e.g.*, *C. obscuripes*), amphipods (*Gammaruspulex*), isopods (*Asellus aquaticus*), Ephemeroptera (*e.g.*, *Cloeon dipterum*), bivalves (*Pisidium* sp.), and Tubellaria (*Polycelis niger*/*tenuis* and *Mesostoma* sp.).

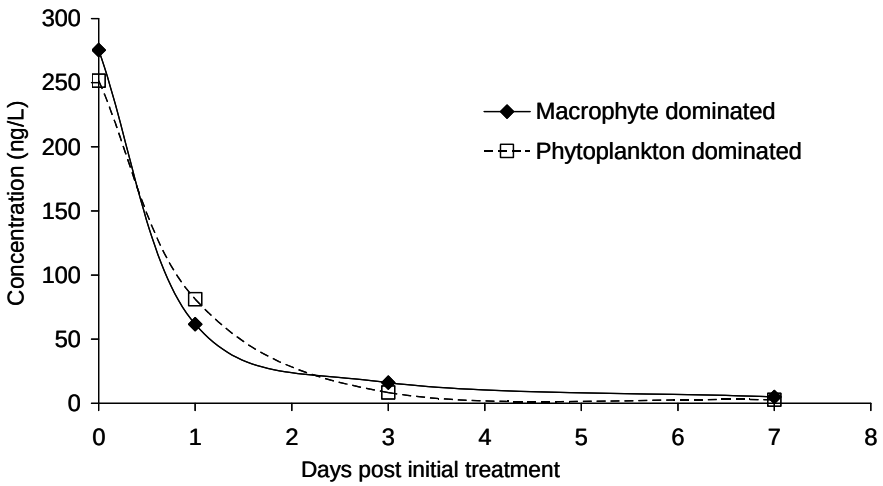


Figure 2: Dissipation of lambda-cyhalothrin in the water phase of the enclosures placed in the macrophyte- and phytoplankton-dominated microcosms after treatment with 250 ng/L. Plotted standard deviations are too small to exceed the markers.

The community in the enclosures in the phytoplanktondominated ditch was characterized by nematocerans (*Chaoborus obscuripes* and chironomids), Ephemeroptera (*e.g.*, *Cloeon dipterum* and *Caenis* sp.),

snails (*e.g.*, *Bythinia tentaculata*), and Oligochaeta (not identified to the species level apart from *Dero* sp. and *Stylaria lacustris*), whereas amphipods (*G. pulex*), isopods (*A. aquaticus* and *Proasellus coxalis*/*meridianus*), tubellarians (*Polycelis niger*/*tenuis*), and the leech (*Erpobdella octoculata*) occurred only at low numbers.

Multivariate analysis of the macroinvertebrate community of both the macrophyte- and phytoplankton-dominated enclosures revealed clear, but different, concentration–effect responses (Fig. 3). Figure 3A (macrophyte-dominated system) indicates that compared to the controls, the largest deviations in species composition occurred in the 250 ng/L treatment. Only small deviations were found in the 10 and 25 ng/L treatments. Figure 3B (phytoplankton-dominated system) indicates that compared to controls, the largest deviation occurred in the 100 and 250 ng/L treatment, whereas only small deviations were observed in the 10 ng/L treatment. The species weights (b_k) shown on the right-hand side of the diagram can be interpreted as the affinity of each species with the response in the diagrams. For example, in Figure 3A, *G. pulex* (highest positive weight) is indicated to have decreased most at the higher treatment levels, whereas the weights for *Lymnaea stagnalis* (highest negative weight) indicate that its numbers have increased in the higher treatments. In the macrophytes-dominated systems, *G. pulex* and *Chaoborus obscuripes* showed the most pronounced treatment-related responses. In the phytoplankton-dominated enclosures, this was the case for *C. obscuripes* and *Caenis horaria*. These test systems *G. pulex* had a lower b_k score because of its very low abundance, whereas its densities were high in the presence of macrophytes. Similarly, the different b_k scores for *C. horaria* in the two test systems also can be explained by differences in abundance. *Caenis horaria* occurred at higher densities in the phytoplankton-dominated test systems. In both types of test systems, treatment-related responses predominantly comprised effects on arthropods.

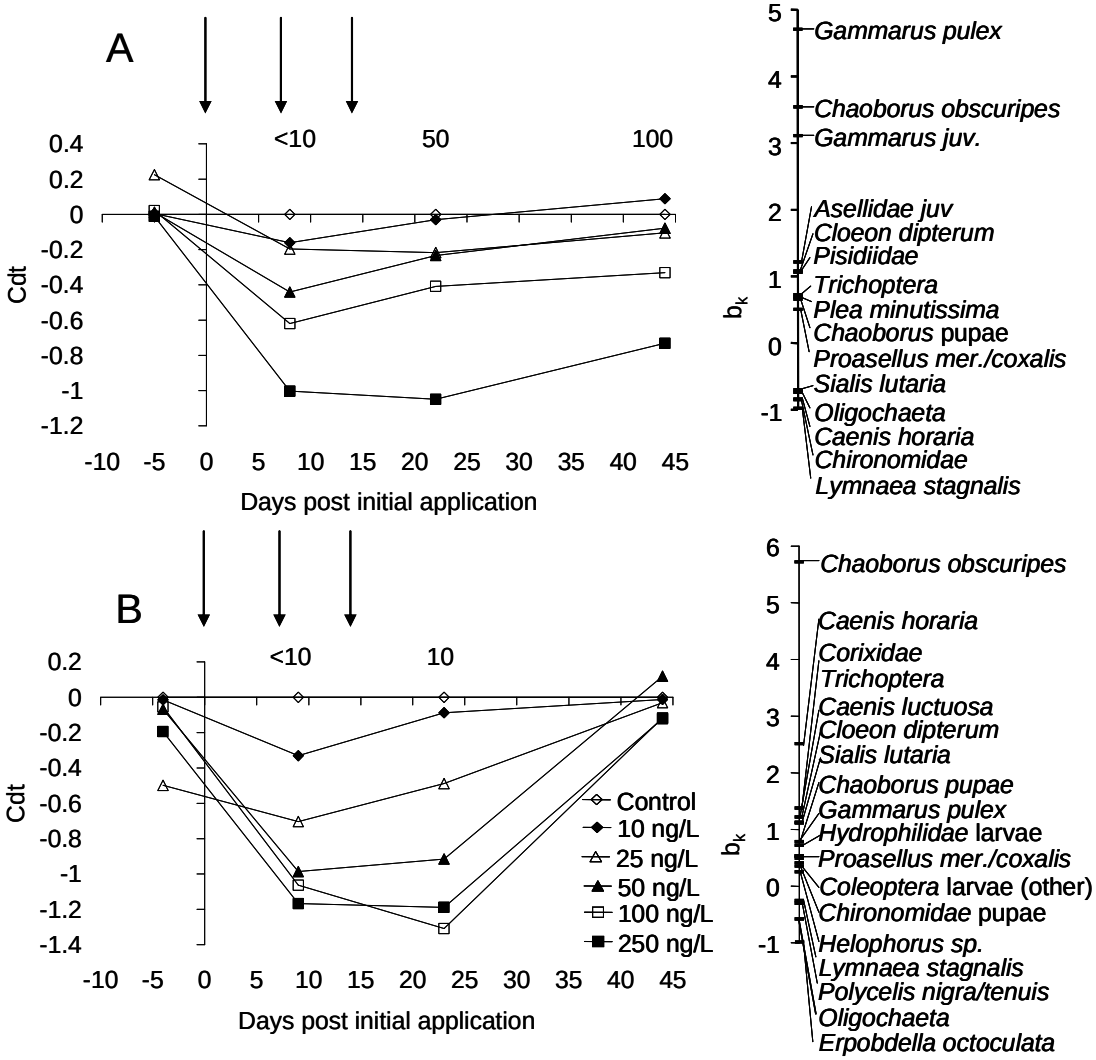


*Figure 3: Principal response curves (PRC) of the macrofauna data set from the macrophyte-dominated (**A**) and plankton-dominated (**B**) enclosures. Calculated no-observed-effect concentration values at the community level are plotted above the figures. Arrows depict lambda-cyhalothrin application dates. The vertical axis represents the difference in community structure between treatments and the control expressed as regression coefficients (Cdt) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the PRC (see Van den Brink and Ter Braak [17] for more information).*

In the macrophyte- and phytoplankton-dominated systems, 35 and 31%, respectively, of the total amount of variance was explained by time, and 35% and 40%, respectively, was explained by treatment. In both types of test system, the lowest community NOEC (<10 ng/L) was observed on the first sampling day after treatment (Table 2). It appears that the macroinvertebrate community structure differed significantly between test systems on all sampling dates (Table 3).

Table 2. Significance of the Monte Carlo permutation tests and calculated no-observed-effect concentration on community level ($NOEC_{community}$) values from the principal response curve analysis of the macroinvertebrate assemblage.

Ditch	Day	*p*	$NOEC_{community}$ (ng/L)
Macrophyte-dominated	-7	>0.05	NA*
	7	<0.001	<10
	21	<0.001	50
	42	0.016	100
Phytoplankton-dominated	-7	>0.05	NA*
	7	<0.001	<10
	21	<0.001	10
	42	>0.05	>250

*NA = not applicable

The changes in abundance of the three species with high weights in the PRC diagram are presented in Figure 4. Of these species, *Chaoborus obscuripes* was abundant in both systems, whereas *G. pulex* and *Caenis horaria* predominantly occurred in the macrophyte- and phytoplankton-dominated enclosures, respectively. In the macrophyte-dominated system, the lowest NOEC observed for *Chaoborus* sp. was 10 ng/L on days 9 and 22, whereas it was less than 10 ng/L in the plankton-dominated systems on day 9. In the macrophyte-dominated systems, *G. pulex* showed a clear decline, without recovery, in the two highest treatments. The lowest NOEC observed for *G. pulex* was 25 ng/L on day 22. In the phytoplankton- dominated enclosures, *Caenis horaria* showed a pronounced decline during the treatment period. The lowest NOEC observed for *C. horaria* was 10 ng/L on day 22.

The persistent effect on the *G. pulex* population (Fig. 4B) in the 250 ng/L enclosures of the macrophyte-dominated test systems also was reflected in the PRC diagram (Fig. 3A). In the phytoplankton-dominated systems, *G. pulex* was not abundant, and the PRC was consistent with the response of *Chaoborus obscuripes* (Fig. 4C).

Table 3. Monte Carlo permutation test (p-value) results from combined data sets of macroinvertebrate species composition in the macrophyte- and phytoplankton-dominated test systems and treatment effects of lambda-cyhalothrin.

Day relative to first application	Treatment	Ecosystem structure	Interaction between treatment and structure
-5	>0.05	0.001	>0.05
9	0.001	0.001	0.001
22	0.001	0.001	0.005
44	0.003	0.001	0.018

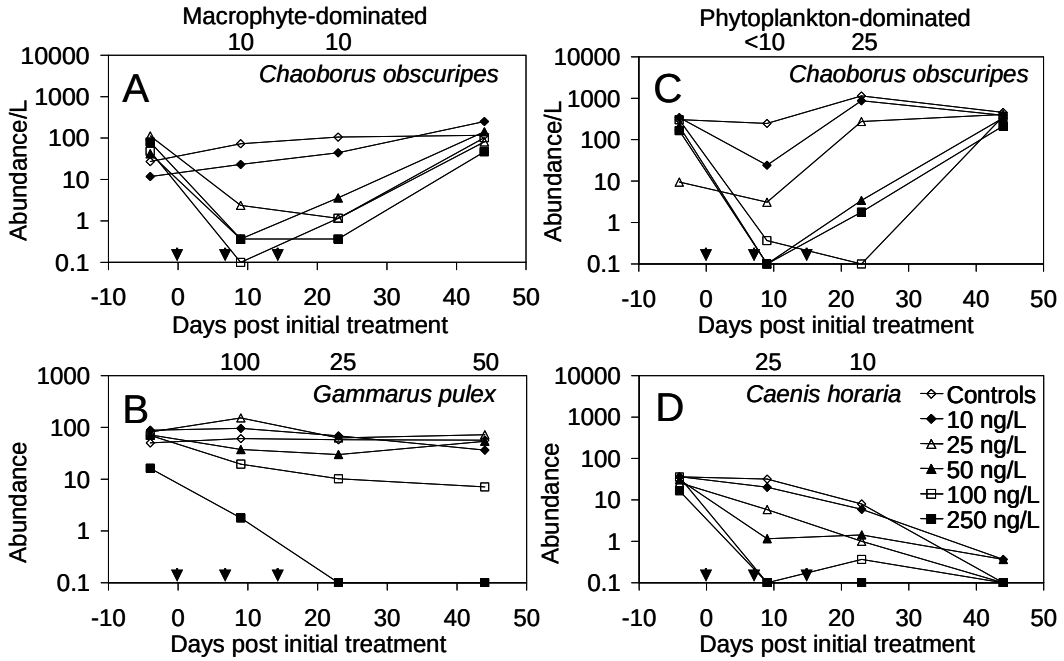


*Figure 4: Dynamics of macroinvertebrate species in the macrophyte-dominated (**A** and **B**) and phytoplankton-dominated (**C** and **D**) microcosms. Shown are the responses of the phantom midge (Chaoborus obscuripes; **A** and **C**), the amphipod Gammarus pulex (**B**) and the ephemeropteran Caenis horaria (**D**). Calculated no-observed-effect concentrations are plotted above the figures. Arrowheads depict lambda-cyhalothrin application dates.*

Zooplankton

Thirty-nine distinct zooplankton taxa were identified, with 34 occurring in the phytoplankton-dominated and 35 in the macrophyte-dominated systems. In decreasing order of abundance, the communities of both types of enclosures were dominated
by rotifers (mainly *Keratella cochlearis*, *K. quadrata*, and *Anureopsis fissa*), cyclopoid Copepoda, nematocerans (*C. obscuripes*), and Cladocera (*D. galeata*).

Multivariate analysis of the zooplankton community of both systems did not show a clear treatment-related response (Fig. 5). The percentage of the total amount of variance in the zooplankton data that is explained by treatment was 19 and 29%, respectively, for the macrophyte-dominated and phytoplankton-dominated systems, and 63 and 44%, respectively, of the total amount of variance was explained by time. In the macrophyte-dominated test systems, Monte Carlo permutation tests revealed significant differences ($p < 0.05$) in zooplankton community composition between enclosures at day 20 only. On this day, the calculated NOEC for the zooplankton community was 25 ng/L. For the phytoplankton-dominated enclosures, Monte Carlo permutation tests revealed significant differences ($p < 0.05$) in zooplankton communities on days 13, 20, and 28. A $NOEC_{community}$, however, could only be calculated at day 20 (<10 ng/L) (Table 4).

The zooplankton community differed significantly between test systems on all sampling dates (Table 5). However, both treatment regime and interaction between treatment and zooplankton community structure showed a significant effect at sampling day 20 only. On this date, a treatment-related effect to which the zooplankton communities in the macrophytes-dominated and phytoplankton-dominated test systems responded differently was observed.

Nauplii larvae (Copepoda) showed a significant treatment related decline in the macrophyte-dominated enclosures on at least two consecutive sampling days (Fig. 6A). In the phytoplankton-dominated enclosures, the response was reversed (Fig. 6D), with nauplii being least abundant in the controls. A significant increase in abundance also was observed for *Lecane lunaris* in the macrophyte-dominated systems (Fig. 6B) and for *K. quadrata* in the phytoplankton-dominated systems (Fig. 6E) on days 13 and 20. In both types of test systems, relatively low numbers of Cladocera were found in the controls (Fig. 6C and F).

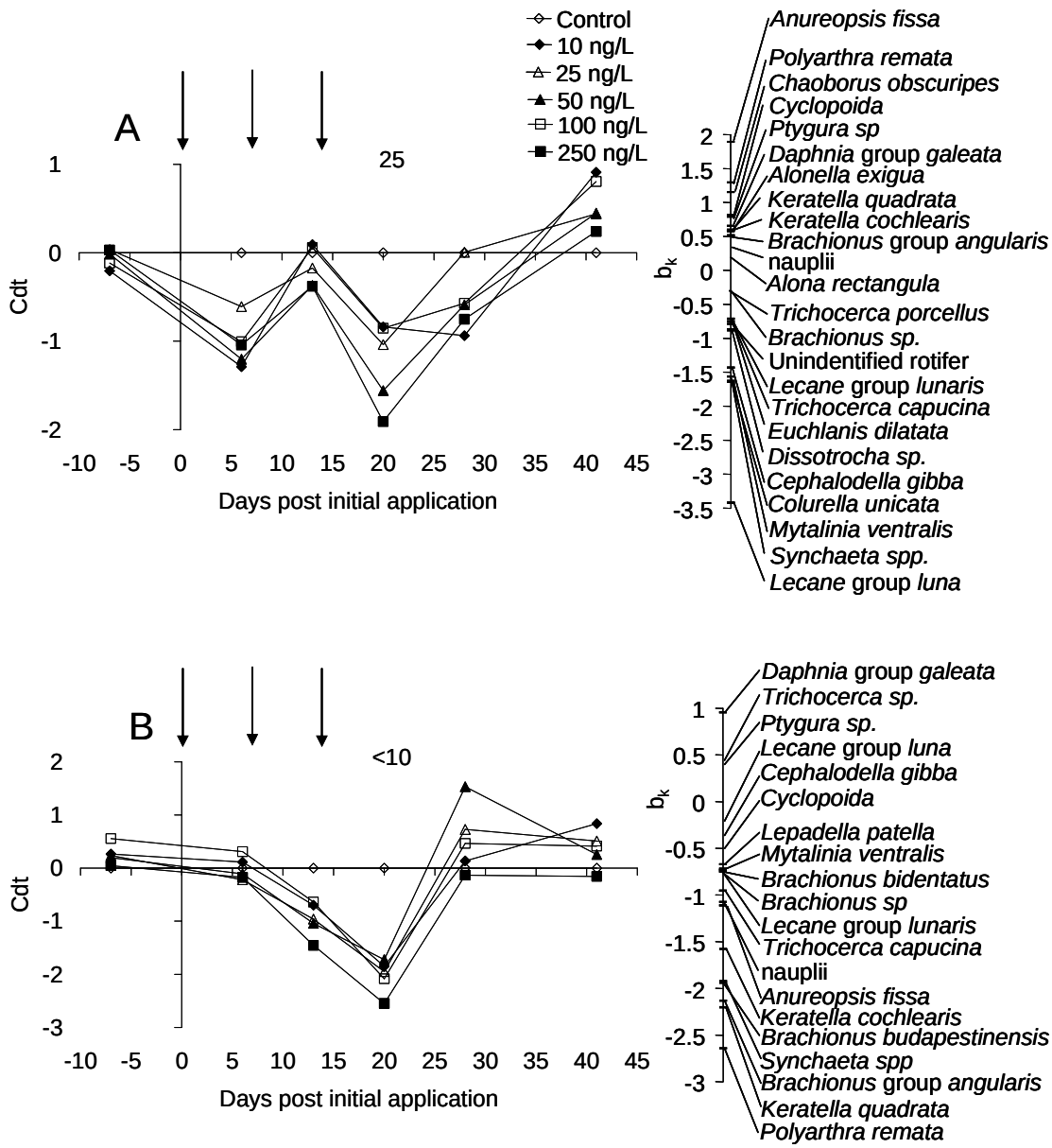


*Figure 5: Principal response curve (PRC) resulting from the analysis of the zooplankton data set of the macrophyte-dominated (**A**) and plankton-dominated (**B**) microcosms. No-observed-effect concentration values at the community level are plotted above the graph. Arrows depict lambda-cyhalothrin application dates. The vertical axis represents the difference in community structure between treatments and the control expressed as regression coefficients (Cdt) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the PRC (see Van den Brink and Ter Braak [17] for more information).*

Table 4. Significance of the Monte Carlo permutation tests and calculated no-observed-effect concentration on community level ($NOEC_{community}$) values from the principal response curve analysis of the zooplankton assemblage.

Ditch	Day	*p*	$NOEC_{community}$ (ng/L)
Macrophyte-dominated	-7	>0.100	NA*
	6	>0.100	>250
	13	>0.100	>250
	20	0.005	25
	28	>0.100	>250
	41	>0.100	>250
Phytoplankton-dominated	-7	>0.100	NA
	6	>0.100	>250
	13	0.025	>250
	20	0.010	<10
	28	0.050	>250
	41	>0.100	>250

**NA = Not Applicable since prior to application*

Table 5. Monte Carlo permutation test (p value) results from the combined data sets of zooplankton species composition in the macrophyte-dominated and phytoplankton-dominated test systems and the treatment effects of lambda-cyhalothrin.

Day relative to first application	Treatment	Ecosystem structure	Interaction between treatment and structure
-7	>0.05	0.005	>0.05
6	>0.05	0.005	>0.05
13	>0.05	0.005	>0.05
20	0.005	0.005	0.035
28	>0.05	0.005	>0.05
41	>0.05	0.005	>0.05

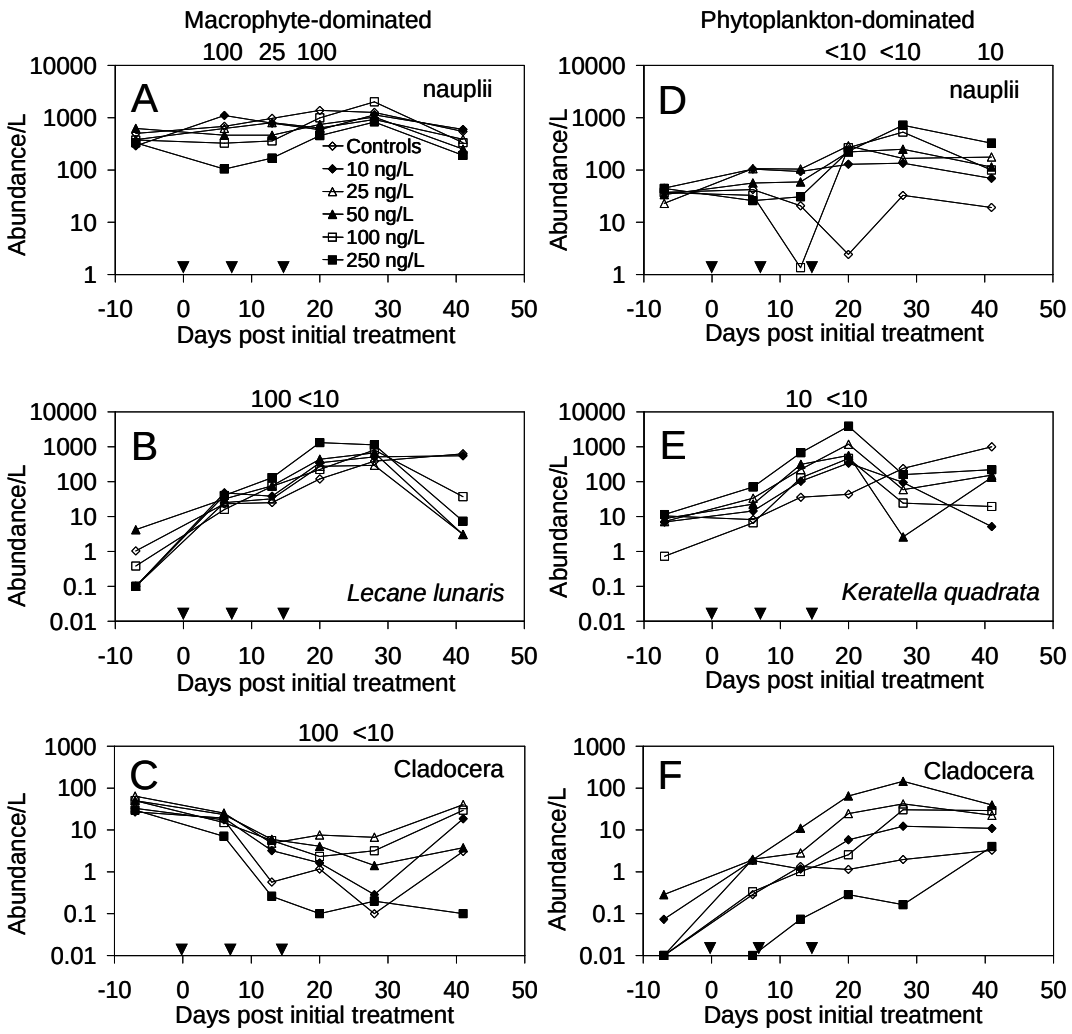


*Figure 6: Dynamics of zooplankton taxa in the macrophyte-dominated (**A–C**) and phytoplankton-dominated (**D–F**) enclosures. Shown are the geometric means of the numbers, counted per treatment concentrations, of nauplii (**A** and **D**), the rotifers Lecane lunaris (**B**) and Keratella quadrata (**E**), and Cladocera (**C** and **F**). Calculated no-observed-effect concentrations are plotted above the graphs. Arrowheads depict lambda-cyhalothrin application dates.*

Bioassays with invertebrates

Chaoborus obscuripes showed a distinct, treatment-related response in the bioassays that were incubated in both types of test systems, with the maximum response occurring on day 1 (Fig. 7A and D). In accordance with the observed decline of the population living in the enclosure (Fig. 4), the lowest NOEC was 10 ng/L or less. In the phytoplankton-dominated systems, the sensitivity of caged *Chaoborus* was somewhat less than that in the macrophyte-dominated test systems (in contrast to the observed NOEC values of the free-living population in the enclosure).

Because of the low abundance of *Asellus aquaticus* and *D. pulex* in the enclosures, comparisons of bioassay responses to population-level

effects were limited. *Asellus aquaticus* showed a clear treatment-related response in the in situ bioassays for both types of test systems (Fig. 7B and E). The maximum effect occurred on day 2, with a calculated NOEC of 25 ng/L in both systems. The results of the bioassays performed with *D. pulex* differed considerably between test systems (Fig. 7C and F). In the phytoplankton-dominated enclosures, a treatment-related response was observed, with effects gradually increasing with time and a calculated NOEC of 25 ng/L. In the macrophyte-dominated systems, numbers of *Daphnia* sp. declined in all test systems, including controls, because of aggressive predation by the microtubellarian *Mesostoma* sp. In fact, *Mesostoma* sp. predominantly occurred in the macrophyte-dominated test systems.

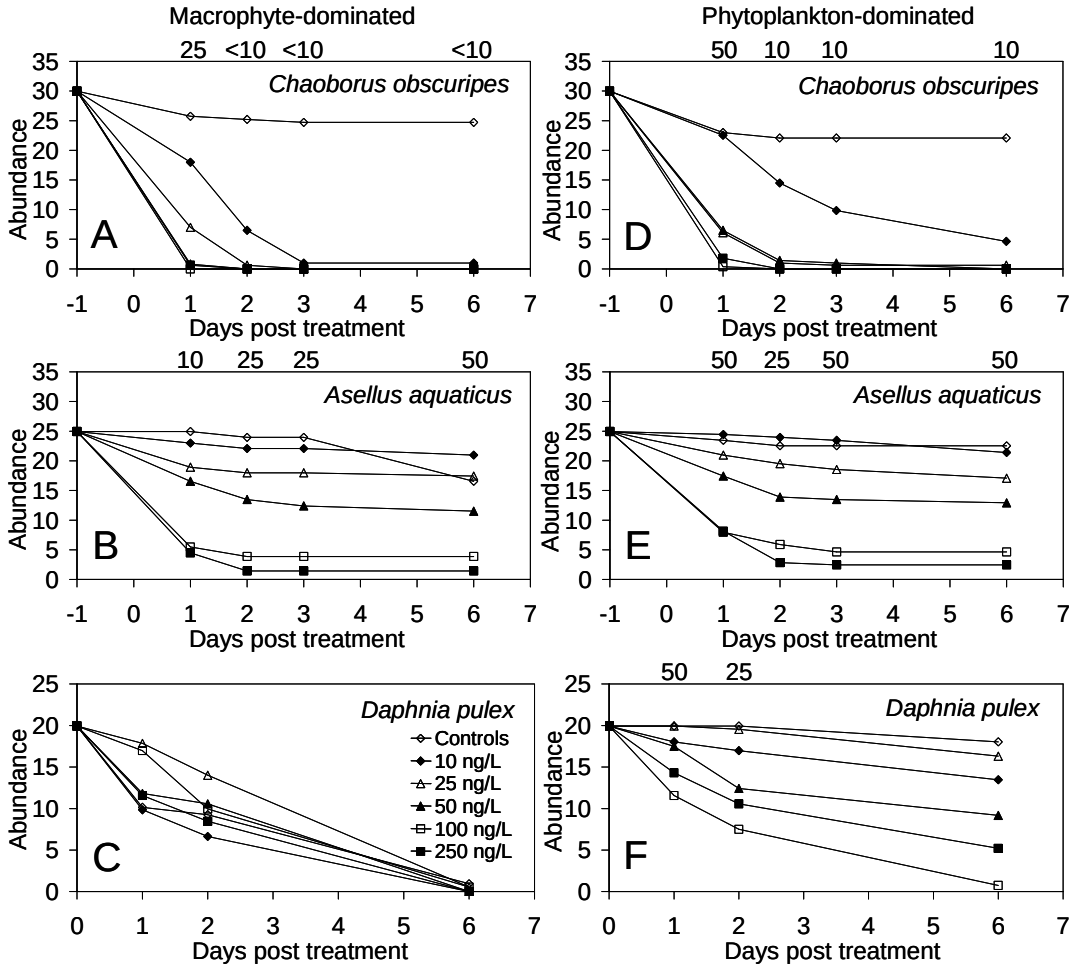


*Figure 7: Dynamics of three species in type 1 in situ bioassays incubated in the macrophyte-dominated (**A–C**) and phytoplankton-dominated microcosms (**D–F**). Shown are the response of the phantom midge (Chaoborus obscuripes; **A** and **D**), the isopod Asellus aquaticus (**B** and **E**), and the cladoceran Daphnia pulex (**C** and **F**). Calculated no-observed-effect concentrations are plotted above the figures.*

During the post-treatment period, *in situ* bioassays were performed with *C. obscuripes* and *A. aquaticus* to assess the recovery potential of invertebrates. *Chaoborus obscuripes* showed a clear, treatment-related

response (Fig. 8A and C). In a manner similar to the responses observed in the free-living population in the enclosures (Fig. 4), the rate of recovery potential increased with decreasing treatment levels. In addition, potential recovery of *Chaoborus* was faster in the phytoplankton-dominated than in the macrophyte-dominated systems (NOEC-value of 25 *vs.*10 ng/L at day 24), suggesting a faster dissipation of lambda-cyhalothrin in the cages incubated in the plankton-dominated systems. Responses of *A. aquaticus* were similar between test systems (Fig. 8B and D). Again, the rate of potential recovery increased with decreasing treatment levels, and the time to potential recovery was shorter in the phytoplankton-dominated test systems (Fig. 8).

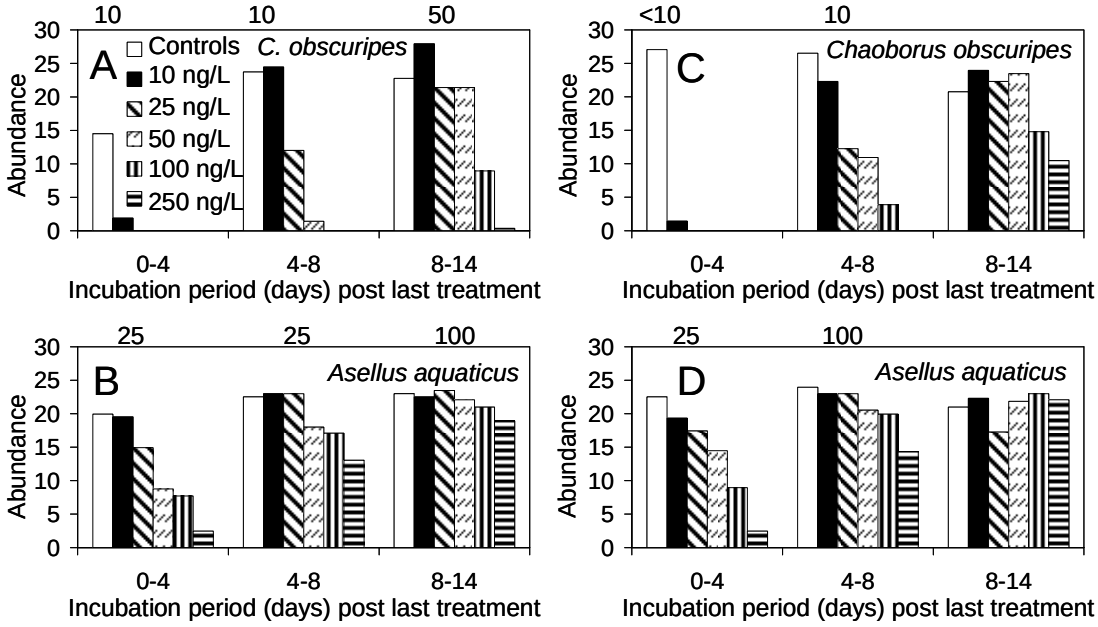


*Figure 8: Dynamics of Chaoborus obscuripes (**A** and **C**) and Asellus aquaticus (**B** and **D**) used in type 2 bioassays in the macrophyte- and phytoplankton-dominated microcosms. Calculated no-observed-effect concentrations are plotted above the graph.*

Responses of primary producers

Primary producers in the enclosures included phytoplankton (assessed as chlorophyll-*a*), macrophytes (assessed as dry weight biomass), and periphyton (assessed as chlorophyll-*a*). Phytoplankton responses in the macrophyte- and phytoplankton-dominated enclosures are presented in Figure 9A and B. The chlorophyll-*a* concentrations were lower in the macrophytedominated systems, with no apparent treatment-related effects observed. Although not significantly different, chlorophyll-*a* concentrations in the control and 250 ng/L treatment tended to be higher at the end of the experimental period than those of the intermediate treatments in the phytoplankton-dominated systems. Significant treatment-related effects on macrophytes biomass were absent, as were

treatment-related effects for periphyton chlorophyll-*a* on artificial substrates.

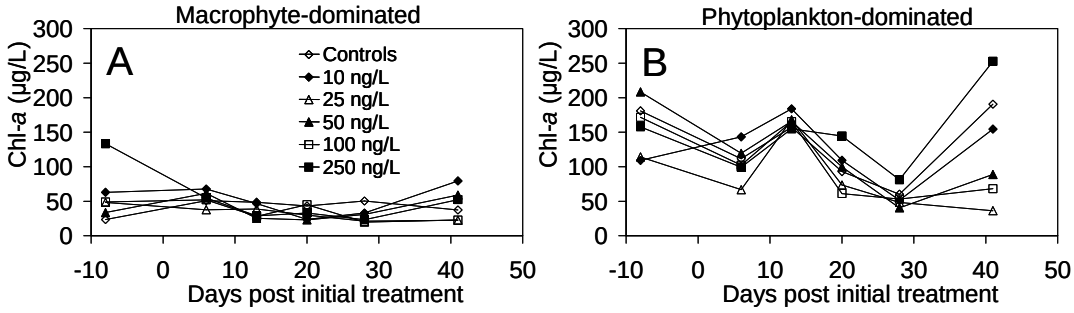


*Figure 9: Dynamics in phytoplankton chlorophyll-a (Chl-a) concentrations during the experimental period in the macrophyte-dominated (**A**) and phytoplankton-dominated (**B**) enclosures treated with lambda-cyhalothrin.*

Community metabolism and decomposition

Measured community metabolism parameters (*e.g.*, dissolved oxygen, pH, and decomposition) did not show any treatment-related effects. The residual dry weight of the *Populus* leaves in the litter bags amounted to approximately 68 and 72% in the macrophyte-dominated and phytoplankton-dominated enclosures, respectively.

Discussion

Exposure and direct effects

The community structure of the two types of test systems used in this experiment (a mesotrophic macrophyte-dominated and a eutrophic phytoplankton-dominated ecosystem) differed mainly in macrophyte biomass (117 *vs.*0 g/m2), phytoplankton densities (58 *vs.*157 mg/L of chlorophyll-*a*), and invertebrate community composition (Figs. 3 and 5 and Tables 3 and 5). Despite these differences in community structure, the direct effects of lambda-cyhalothrin application on similar arthropod taxa (*e.g.*, *Chaoborus*) were similar between test systems. This comparability is explained by the similarity of exposure regimes (Fig. 2). A priori, it was postulated that the dissipation of lambda-cyhalothrin from the water phase might be faster in the presence of macrophytes, because in other experiments increased dissipation rates were demonstrated with increasing plant densities [3]. In addition, studies with other hydrophobic insecticides have demonstrated faster dissipation in macrophyte-dominated test systems [21], and effective retention of such insecticides occurs in constructed wetlands [22]. We found that lambda-cyhalothrin dissipated at rapid and equivalent rates from both the phytoplankton-dominated enclosures (with no macrophytes) and the macrophyte-

dominated enclosures. Apparently, the higher densities of algae and suspended particles in the water column of the phytoplankton-dominated systems provided enough sorption surface to generate an equally fast dissipation rate of lambda-cyhalothrin from the water phase compared to the macrophyte-dominated systems. The sorption to algae and suspended matter would be expected to be extensive given the lipophilic and adsorptive properties of lambda-cyhalothrin [7-9].

In both types of test systems, initial direct effects were predominantly observed on arthropod populations of the macroinvertebrate assemblages. These direct effects were consistent with the laboratory toxicity data reported by Schroer *et al.* [4] for the same taxa. This indicates that similar arthropod species studied in both the laboratory and the field showed a similar short-term sensitivity at similar exposure concentrations. Other studies also have suggested that acute toxicity data of insecticides, as measured in laboratory single-species toxicity tests, can be extrapolated to populations of the same species under similar exposure conditions in the field [23-26]. Similar toxicity data between the laboratory and field for the rapidly dissipating insecticide lambda-cyhalothrin may be explained by the fact that symptoms of toxicity occur within a few hours after exposure [27].

Relationships between direct and indirect effects

In both types of test systems, indirect effects of treatment with lambda-cyhalothrin were observed. Table 6 illustrates the treatment-related responses by using the effect classes described by Brock *et al.* [28]. It appears that even at the lowest concentration tested (10 ng/L), the slight direct effects on insects (mainly *Chaoborus* sp.) also resulted in transient indirect effects on microcrustaceans and rotifers. These indirect effects on microcrustaceans and rotifers in the zooplankton were more pronounced in the phytoplankton-dominated than in the macrophyte-dominated systems. This suggests that in the phytoplankton-dominated systems, *C. obscuripes* plays a more significant role as key species in the top-down control of the zooplankton community. For example, the pronounced increase of nauplii in all treated phytoplankton-dominated enclosures (Fig. 6D) can be explained by reduction of *Chaoborus* sp. A similar response of nauplii, however, was not observed in the macrophyte-dominated systems. The reason for the differing nauplii response between test systems also might be explained by differences in species composition. Nauplii were not identified to the species level, and nauplii in the macrophyte-dominated systems possibly belonged to a more sensitive species than did those from the phytoplankton-dominated systems.

In the phytoplankton-dominated systems, a trend of higher phytoplankton densities was observed at the control and highest concentrations but not at intermediate levels, which might be explained by food-web interactions. In the control test systems, cladocerans are extensively predated by *C. obscuripes* (explaining low *Daphnia* abundance and high phytoplankton densities), whereas this very sensitive predator was temporarily eliminated by the insecticide in all other treatments. The highest treatment concentration (250 ng/L) was lethal to cladocerans, also reducing their numbers. In the intermediate treatments, cladocerans suffered neither from predation nor from insecticide stress. Consequently, grazing pressure on phytoplankton was greatest in these treatments, causing lower phytoplankton densities.

In the macrophyte-dominated enclosures, the pronounced effects on the shredder *G. pulex* did not result in apparent indirect effects on other detritivores or on breakdown of *Populus* leaves in the decomposition assay. Abundant organic material in the top layer of the sediment most probably eliminated this as a limiting factor in the competition between detritivore populations.

Recovery of affected populations and communities

One advantage of field enclosure testing is the ability to provide information regarding population and community recovery. Community effects observed in the mesotrophic macrophyte-dominated enclosures were longer-lasting than those in the enclosures of the eutrophic ditch (Fig. 3A and B). This difference can be explained by differences in recovery potential of a few affected populations (*e.g.*, *G. pulex*) between the two types of test systems. Our in situ bioassays allow a distinction to be made between potential and actual recovery. Potential recovery is defined as the decline of lambda-cyhalothrin to a concentration at which it no longer has adverse effects on sensitive arthropods. The bioassays revealed that potential recovery of even the most sensitive invertebrate in the present study (*Chaoborus* sp.) occurred 4 d after application of the lowest dose (10 ng/L) and 8 d after application of treatments up to 50 ng/L. However, the abundance of the *Chaoborus* population in 10 and 50 ng/L treatments did not differ from controls after 23 and 44 d, respectively (Fig. 4C). This indicates that even for a multivoltine and mobile insect, actual recovery takes more time than potential recovery.

Table 6. Summary of effects observed in the macrophyte-dominated and phytoplankton-dominated enclosures treated with the insecticide lambda-cyhalothrin.

	10 ng/L	25 ng/L	50 ng/L	100 ng/L	250 ng/L
Macrophyte-dominated					
Macrocrustaceans	1	2↓	2↓	4↓	4↓
Insects	2↓	3↓	3↓	3↓	3↓
Other macroinvertebrates	1	1	1	1	2↑↓
PRC macroinvertebrates	2	2	3	3	4
Microcrustaceans	1	2↓	2↓	2↓	4↓
Rotifers	2↑↓	2↑↓	2↑↓	2↑↓	2↓; 3↑
PRC zooplankton	1	1	2	2	2
Phytoplankton chlorophyll-*a*	1	1	1	1	1
Macrophyte biomass	1	1	1	1	1
Community metabolism	1	1	1	1	1
Phytoplankton-dominated					
Macrocrustaceans	- [b]	- [b]	- [b]	- [b]	- [b]
Insects	2↓	3↓	3↓	3↓	3↓
Other macroinvertebrates	1	1	1	2↑	2↑
PRC macroinvertebrates	2	3	3	3	3
Microcrustaceans	2-3↑	4↑	4↑	4↑	4↑↓
Rotifers	2↑	3↑	3↑	3↑	3↑
PRC zooplankton	2	2	2	2	2
Phytoplankton chlorophyll-*a*	1	1	1	1	2↑[c]
Macrophyte biomass	-	-	-	-	-

[a] The numbers in the table refer to preselected effect classes (Brock *et al.* [28]). 1: no effect; 2: slight effect; 3: apparent short-term effects, full recovery observed (four to eight weeks); 4: apparent effects, no full recovery observed at the end of the experiment; ↑ increase; ↓ decrease; ↑↓ increase and decrease on species and/or sampling date; PCR: principal response curve.
[b] Low abundance of free-living population.
[c] Trend of an increase.

It is evident that the time difference between potential and actual recovery will be much larger in isolated test systems for species with a low dispersal potential, such as *Gammarus* sp. In our test systems, complete recovery of *G. pulex* was not observed, whereas multivoltine insects (*e.g.*, *C. obscuripes*) recovered (Fig. 4). In previous studies, it also has been demonstrated that in isolated insecticide-stressed mesocosms, *G. pulex* showed a slow rate of recovery [19]. This species lacks insensitive aquatic life-stages (*e.g.*, resting eggs) and does not actively recolonize isolated aquatic systems via transport over and or via air. Several other

model ecosystem studies with non-persistent insecticides have shown rapid recovery of affected populations when the generation time of the population is short and a ready supply of propagules of affected populations exists [19, 29, 30].

The present study also revealed that some indirect effects (*e.g.*, increased nauplii in the plankton-dominated system) (Fig. 6D) may persist longer than the direct effects that caused these indirect effects (*e.g.*, decline in *C. obscuripes*) (Fig. 4C).

Extrapolation of responses

Despite observed differences in indirect effects and recovery of affected populations at higher concentrations between test systems, ecological impacts at the lowest treatment levels were very similar. At 10 ng/L, slight and transient deviations in the abundances of only a few species were observed in both the macrophyte- and phytoplankton-dominated systems. These results suggest a threshold effect concentration of slightly less than 10 ng/L, above which direct effects are observed. At concentrations of 25 and 50 ng/L, the overall direct effects observed in arthropod populations were similar, and recovery occurred rapidly in both types of test systems despite some differences in magnitude and duration of indirect effects (see, *e.g.*, population increases in Table 6). Concentrations greater than 50 ng/L resulted in long-term adverse effects on macrocrustaceans. Overall, the present results generally are consistent with those reported elsewhere for lambda-cyhalothrin [11, 31-33].

Conclusion

Repeated applications of lambda-cyhalothrin to mesotrophic macrophyte-dominated and eutrophic phytoplanktondominated systems resulted in a similar short-term exposure profile. Direct effects on sensitive arthropods were consistent with short-term laboratory toxicity data of the same species. No major differences were found in threshold levels for direct effects observed between the different test systems. The differences that were observed primarily concerned rate of recovery and indirect effects at higher exposure levels.

Acknowledgement—We gratefully acknowledge the assistance of the following persons: P.J. Van den Brink, R.P.A. van Wijngaarden, R. Gylstra, S.J.H. Crum, C. Udoh, J.S.Warinton, M. Leistra, H.J. Zweers, F. Gillissen, and W.H.J. Beltman. The present study received financial support from The Netherlands Organization for Scientific Research (NWO; Stimuleringsprogramma Systeemgericht Ecotoxicologisch Onderzoek, project 014.23.012), Ministry of Landbouw, Natuur en Voedselkwaliteit Research Program 359, and Syngenta.

References

[1] Van Donk, E., Prins, H., Voogd, H. M., Crum, S. J. H. and Brock, T. C. M. 1995. Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms. I. Responses of plankton and zooplanktivorous insects. Archiv für Hydrobiologie 133 pp. 417-439

[2] Kramer, P. R. G., Jonkers, D. A. and Van Liere, L. 1997. Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems. RIVM. Bilthoven. The Netherlands

[3] Leistra, M., Zweers, A. J., Warinton, J. S., Crum, S. J. H., Hand, L. H., Beltman, W. H. J. and Maund, S. J. 2003. Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. Pest Management Sciences 60 pp. 75-85

[4] Schroer, A. F. W., Belgers, D., Brock, T. C. M., Matser, A., Maund, S. J. and Van den Brink, P. J. 2004. Acute toxicity of the pyrethroid insecticide lambda-cyhalothrin to invertebrates of lenthic freshwater systems. Archives of Environmental Contamination and Toxicology 46 pp. 324-335

[5] Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London-Weinheim-New York-Tokyo-Melbourne-Madras

[6] Giddings, J. M., Brock, T. C. M., Heger, W., Heimbach, F., Maund, S. J., Norman, S. M., Ratte, H. T., Schäfers, C. and Streloke, M. 1999. Community-level aquatic system studies: interpretation criteria.

[7] Zhou, J. L., Rowland, S. and Mantoura, R. F. C. 1995. Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. Water Research 29 pp. 1023-1031

[8] Hand, L. H., Kuet, S. F., Lane, M. C. G., Maund, S. J., Warinton, J. S. and Hill, I. R. 2001. Influences of aquatic plants on the fate of the pyrethroid insecticide lambda-cyhalothrin in aquatic environments. Environmental Toxicology and Chemistry 20 pp. 1740-1745

[9] Hamer, M. J., Goggin, U. M., Muller, K. and Maund, S. J. 1999. Bioavailability of lambda-cyhalothrin to *Chironomus riparius* in sediment-water and water-only systems. Aquatic Ecosystem Health and Management 2 pp. 403-412

[10] Maund, S. J., Hamer, M. J., Warinton, J. S. and Kedwards, T. J. 1998. Aquatic Ecotoxicology of the pyrethroid insecticide Lambda-cyhalothrin: Considerations for Higher-Tier aquatic risk assessment. Pesticide Science 54 pp. 408-417

[11] Maltby, L., Blake, N. N., Brock, T. C. M. and Van den Brink, P. J. 2005. Insecticide species sensitivity distributions: the importance of test species selections and relevance to aquatic ecosystems. Environmental Toxicology and Chemistry 24 pp. 379-388

[12] Brock, T. C. M., Van den Bogaert, M., Bos, A. R., Van Breukelen, S. W. F., Reiche, R. T., J., Suykerbuyk, R. E. M. and Roijackers, R. M. M. 1992. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. Archives of Environmental Contamination and Toxicology 23 pp. 391-409

[13] Moed, J. R. and Hallegraeff, G. M. 1978. Some problems in the estimation of chlorophyll-*a* and phaeopigments from pre- and post-acidification spectrophotometric measurements. Internationale Revue der Gesamten Hydrobiologie 63 pp. 787-800

[14] Van den Brink, P. J., Van Donk, E., Gylstra, R., Crum, S. J. H. and Brock, T. C. M. 1995. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31 pp. 3181-3200

[15] Williams, D. A. 1972. The comparison of several dose levels with zero dose control. Biometrics 28 pp. 519-531

[16] Hommen, U., Veith, D. and Ratte, H. T. 1994. A computer program to evaluate plankton data from freshwater field tests. In: Hill, I. R., Heimbach, F., Leeuwangh, P., Matthiesen, P., eds, Freshwater Field Tests for Hazard Assessment of Chemicals, Lewis, Boca Raton, FL, pp. 503-513

[17] Van den Brink, P. J. and Ter Braak, C. J. F. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18 pp. 138-148

[18] Ter Braak, C. J. F. and Smilauer, P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Ithaca. New York: Microcomputer Power. USA

[19] Van den Brink, P. J., Van Wijngaarden, R. P. A., Lucassen, W. G. H., Brock, T. C. M. and Leeuwangh, P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environmental Toxicology and Chemistry 15 pp. 1143-1153

[20] Anderson, M. J. and Ter Braak, C. J. F. 2003. Permutation tests for multi-factorial analysis of variance. Journal of Statistical Computation and Simulation 73 pp. 85 - 113

[21] Crum, S. J. H. and Brock, T. C. M. 1994. Fate of Chlorpyrifos in indoor microcosms and outdoor experimental ditches. In: Hill, I. R., Heimbach, F., Leeuwangh, P., Matthiesen, P., eds, Freshwater Field Tests for Hazard Assessment of Chemicals, Lewis, Boca Raton, FL, pp. 315-322

[22] Schulz, R. and Peall, S. K. C. 2001. Effectiveness of a constructed wetland for retention of nonpoint-source pesticide pollution in the Lourens River catchment, South Africa. Environmental Science and Technology 35 pp. 422-426

[23] Van Wijngaarden, R. P. A., Van den Brink, P. J., Crum, S. J. H., Oude Voshaar, J. H., Brock, T. C. M. and Leeuwangh, P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: I. Comparison of short-term toxicity between the laboratory and the field. Environmental Toxicology and Chemistry 15 pp. 1133-1142

[24] Crossland, N. O. 1984. Fate and biological effects of methyl parathion in outdoor ponds and laboratory aquaria. II: Effects. Ecotoxicology and Environmental Safety 8 pp. 482-495

[25] Fairchild, J. F., La Point, T. W., Zajicek, J. L., Nelson, M. K., Dwyer, F. J. and Lovely, P. A. 1992. Population-, community-, and ecosystem-level responses of aquatic mesocosms to pulsed doses of a pyrethroid insecticide. Environmental Toxicology and Chemistry 11 pp. 115-129

[26] Kaushik, N. K., Stephenson, G. L., Solomon, K. R. and Day, K. E. 1985. Impact of permethrin on zooplankton communities in limnocorrals. Canadian Journal of Fisheries and Aquatic Sciences 42 pp. 77-85

[27] Giddings, J. M., Solomon, K. R. and Maund, S. J. 2001. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. Environmental Toxicology and Chemistry 20 pp. 660-668

[28] Brock, T. C. M., Van Wijngaarden, R. P. A. and Van Geest, G. J. 2000. Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra. Wageningen. The Netherlands

[29] Sherratt, T. N., Roberts, G., Williams, P., Whitfield, M., Biggs, J., Shillabeer, N. and Maund, S. J. 1999. A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemicals. Environmental Toxicology and Chemistry 18 pp. 2512-2518

[30] Hanazato, T., Yasuno, M. 1990. Influence of persistence period of an insecticide on recovery patterns of a zooplankton community in experimental ponds. Environmental Pollution 67 pp. 109-122

[31] Van Wijngaarden, R. P. A., Arts, G. H. P., Cuppen, J. G. M., Crum, S. J. H. and Brock, T. C. M. 2004. Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study. Environmental Toxicology and Chemistry 23 pp. 1479-1498

[32] Farmer, D., Hill, I. and Maund, S. J. 1995. A comparison of the fate and effects of two pyrethroid insecticides (lambda-cyhalothrin and cyphermethrin) in pond mesocosms. Ecotoxicology 4 pp. 219-244

[33] Hill, I. R., Runnalls, J. K., Kennedy, J. H. and Eloniak, P. 1994. Lambda-cyhalothrin: A mesocosm study of its effects on aquatic organisms. In: Graney, R. L., Kennedy, J. H., Rogers, J. H., eds, Aquatic mesocosm studies in ecological risk assessment, Lewis Publishers, Boca Raton, pp. 403-467

Chapter 3

Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality

I. Roessink
S. J. H. Crum
F. Bransen
E. van Leeuwen
F. van Kerkum
A. A. Koelmans
T. C. M. Brock

Ecotoxicology (2006) 15 pp. 267-293

ATLAS
Veenhuis
2001 8 23

Abstract

Floodplain lakes in the Rhine–Meuse delta of the Netherlands vary considerably in levels of sediment-bound toxicants. Microcosm experiments were done to compare the ecological impact of the fungicide triphenyltin acetate (TPT) between test systems with clean or polluted sediments (10 microcosms each). Differences in sediment quality affected the structure of the aquatic communities that developed in the microcosms. Initially, a faster growth of the macrophyte *Elodea nuttallii* was observed on the polluted sediments, which contained not only toxicants but also higher organic matter and nutrient levels. Dynamics of TPT concentrations in the overlying water were very similar between the two types of test system. Higher levels of TPT, however, were found in the sediment compartment of the clean sediment systems containing a smaller macrophytes biomass. TPT was very persistent in the sediments. In both test systems representatives of several taxonomic groups showed clear responses to a single application of TPT, although benthic Nematoda were not affected. Since few differences in the intensity and/or duration of TPT-related population responses were observed between the two types of test system, the background pollutants in the polluted sediment hardly affected the overall sensitivity of the aquatic community to the additional chemical stressor TPT.

Introduction

Populations and communities integrate the effects of environmental conditions over different spatio-temporal scales. These environmental conditions include natural and anthropogenic stress factors, and at the ecosystem level, several of them may act in concert. Although most experimental research has so far focused on the impact of individual stress factors, recent studies indicate that the effect of a particular chemical stressor may depend on the intensity of other stressors [1-3].

Multi-stress effects of pollutants are plausible in floodplain lakes of the Rhine–Meuse delta in the Netherlands. Sediments in these lakes differ in quality, with older sediments containing high concentrations of xenobiotic mixtures deposited in the 1960s and 1970s, while younger sediments are relatively clean [4]. Although a large part of the sediment-bound toxicants is not directly bioavailable, these toxicants may still cause chronic stress due to a diffuse flux from sediment to (interstitial) water. In addition, organisms in floodplain lakes have to cope with occasional flooding and short-term exposures to chemicals like pesticides [5, 6]. To date, it is not clear how the presence of sediment-bound pollutants affects the response of aquatic communities to additional stressors. It cannot be excluded that the resilience of aquatic ecosystems

against these additional chemical stressors is negatively impacted by sediment-bound 'background' pollutants. On the other hand, communities subject to 'background' pollutants may also be better adapted to cope with additional chemical stress. Our search of the open literature yielded no reports on experiments studying the impact of sediment-borne background pollutants on the sensitivity and resilience of aquatic communities subject to additional chemical stressors.

The objective of the present study was twofold: (1) to compare population and community responses (including recovery) between two types of aquatic microcosm, one containing clean and the other polluted sediment, after treatment with the additional stressor triphenyltin acetate (TPT), and (2) to experimentally investigate the ecological impact of TPT on the structure and functioning of freshwater ecosystems. We constructed microcosms containing clean or polluted sediments from river floodplain lakes, to which we added TPT as an additional stressor. The clean sediment was significantly less polluted with nutrients, metals, polyaromatic hydrocarbons (PAH), and polychlorobiphenyls (PCB) than the polluted sediment.

Ecosystem-level studies on the impact of organotin compounds in general, and of TPT in particular, are scarce. The present study is the first to be reported in the open literature that deals with the ecological impact of TPT in experimental freshwater ecosystems. The fungicide TPT is an organotin compound which is used to control a range of fungal diseases on a variety of crops [7] and was intensively used in potato crop farming in the Netherlands up to 2001. TPT compounds have also been applied as co-toxicants in some anti-fouling paints [5]. Organotins, including TPT, are highly toxic to a wide range of aquatic organisms [8-10]. The most notorious and well-known compound is probably tributyltin (TBT), which was/is primarily used as an anti-fouling biocide on ships. In terms of the relative toxicity of all organotin compounds for aquatic organisms, TPT compounds and TBT are reported to be amongst the most toxic [11, 12]. Organotins degrade in the environment via biodegradation and photodegradation with half-lifes of 60–240 days [13], but residence times for TPT and TBT in sediments of over ten years have also been reported [14].

The present paper focuses on the overall ecological impact of TPT in the two types of freshwater microcosm we constructed, with relatively clean and polluted sediments from river floodplain lakes. A second paper (Part II, [15]) will compare the concentration–response relationships of aquatic populations in the TPT-stressed microcosms with the results of acute laboratory single species tests performed with TPT and a wide variety of freshwater species, including algae, macrophytes, zooplankters, and macroinvertebrates.

Materials and methods

Outdoor microcosms and experimental design

A total of 20 concrete outdoor microcosms (with a length of 140 cm, a width of 120 cm, and a depth of 80 cm) were used in the experiment. A sediment layer of approximately 10 cm and a water layer of approximately 50 cm were introduced in the test systems in November 2000, 8 months prior to the start of the experiment. The sediments originated from two lakes situated alongside the river Waal, in the Rhine catchment area. One of the lakes was polluted, while the other lake was considered to be clean (lakes coded DeO3B and DeO2, respectively in De Haas *et al.* [16]; Moermond and Koelmans [17]). The levels of inorganic and organic contaminants, as well as organic matter and nutrient (phosphorus) levels, were higher in the polluted sediment, while the greatest difference between the two was in PCB content (by a factor of 22) (see Table 1). Ten cosms were filled with polluted sediment (PS cosms) and 10 cosms with clean sediment (CS cosms).

After the sediments had been introduced, 20 shoots of the macrophyte *Elodea nuttallii* were planted in each microcosm, evenly distributed over the sediment compartment. The macroinvertebrate community in the test systems originated partly from individuals that were introduced via the sediment, but additional macroinvertebrates were also introduced 7 and 4 months before the start of the experiment, allowing enough time for the populations to acclimatize to the conditions in the test systems. The additional macroinvertebrates used as seeding material comprised taxa that are regularly found in shallow freshwater ecosystems in the Netherlands and were suspected to be sensitive to TPT (*e.g.*, Annelida, Tricladida, Mollusca). Phytoplankton and zooplankton species were not introduced as such but entered the cosms via the collected sediments, but also via the water used to fill the microcosms, which was collected from a freshwater reservoir present at the experimental facility.

The study was intended to end approximately 12 weeks after the TPT application. In the course of the experiment, several macroinvertebrate taxa died out in the microcosms, as a direct result of TPT application. These macroinvertebrates included Mollusca, Annelida and Turbellaria, taxa with not very highly developed abilities to recolonize isolated test systems. We therefore decided to prolong the study to investigate the possible recovery of invertebrate populations. To stimulate recovery of several macroinvertebrate taxa, they were deliberately reintroduced in low numbers in all microcosms at weeks 5, 19, and 34 after the TPT application (see Table 2). The second part of the experiment focused on the responses and recovery of zooplankton

(additional sampling in week 23) and macroinvertebrates (additional sampling in weeks 23 and 40), since they comprised the most sensitive species.

Table 1. Sediment quality parameters of the two types of sediment used to construct the microcosms: Cd, Cu, Zn, ΣPAH in mg/kg dry weight, ΣPCB in µg/kg dry weight, OM (organic matter) in % of dry sediment, Total P in g/kg. Data according to De Haas et al. [16] and Moermond and Koelmans [17].

	Clean (DeO2)	Polluted (DeO3b)
Cd	0.17	2.19
Cu	12	82
Zn	42	507
ΣPAH	0.55	5.87
ΣPCB	4.37	133.22
OM	2.7	9.0
Total P	0.4	1.5

Triphenyltin acetate application and analysis

In both types of test system (represented by 10 microcosms each), triphenyltin acetate was applied once as Fentin acetate Pestanal® (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) on June 18, 2001. The treatment regime followed a regression design with nominal TPT concentrations in the water column of 0, 1, 10, 30, and 100 µg/L (n=2). Treatment levels were derived from toxicity data available [18, 19] and were randomly allocated to the cosms.

Each cosm was treated by pouring 4 L of an application solution into the water and stirring gently with an iron rod to aid mixing, whilst taking care not to disturb the sediment layer or damage plants. In all microcosms, 0.42 mL 96% ethanol (methanol-free, 4% water) per liter water was used as a carrier solvent (0.04% v/v). Control cosms were treated with an equal amount of carrier solvent without TPT. Control cosms without carrier solvent were not present. To estimate initial concentrations, TPT was analyzed in subsamples taken from the solutions applied.

In the field, triphenyltin acetate is quickly converted to triphenyltin hydroxide [13]. Since our analysis of water and sediment samples was unable to distinguish between the acetate and hydroxide form, we refer to both as TPT in presenting data on the fate of the test compound. Water samples to study the fate of TPT were only taken from the microcosms treated with 10 and 30 µg/L. This was done at 3 and 10 h and at 1, 3, 7, 14, and 28 days after TPT application. Depth-integrated water samples were taken using a Perspex tube (Ø 4 cm, length 50 cm), and on each sampling date two samples were collected from each cosm and combined. Approximately 500 mL of each combined sample was stored in a glass

bottle and taken to the laboratory for further analysis. In the laboratory, 100 mL was transferred to a 250-mL Schott bottle (Louwers-Hapert, Hapert, The Netherlands) and used for further TPT analysis.

Sediment samples were taken at weeks 2, 4, 15, 25, and 40 after TPT application. Sediment was sampled using a Perspex tube (Ø 4 cm, length 50 cm), and two samples per sampling date were collected from the controls and the 30 µg/L cosms. The water phase was removed and the cores were stored in a freezer at < -20 ºC until analysis. The top 5 cm of each sediment core was transferred to a glass centrifuge tube (Ø 4.8 cm, content 240 cm^3) and thawed overnight. The next day, the cores were mixed with a rod for 0.5 min and centrifuged at 2656 *g*. Pore water was collected in a glass bottle and the remaining sediment was stored at 5 ºC. To analyze the pore water, 5 mL sodium acetate buffer (pH=5), a known amount of hexane, and 100 µL 2% sodium tetraethylborate and ethanol were added and the mixture was shaken for 15 min at 0.52 *g*. A known amount of the hexane was evaporated to 1 mL and transferred to a GC vial. Extraction efficiency was determined by adding 0.05 or 0.25 mL of a 100 µg/mL TPT solution to 25 mL pore water and treating these solutions as samples. The extraction efficiency in pore water was tested by spiking pore water from the controls with a known amount of TPT in ethanol. The recovery was found to be 133% (*n*=6; SD=23%); no corrections were made for this efficiency.

After 5 days, the stored sediments were transferred to aluminum containers and mixed. A third remained in the container for dry weight analysis, a third was transferred to a Schott bottle and weighed for further TPT analysis, and a third was stored at < -10 ºC. Dry weight was analyzed by drying the samples overnight at 105 ºC. A solution of 37% HCl, 48% HBr, and deionized water (1:1:0.4 v/v/v) was added to the Schott bottle, after which the bottle was shaken (standing up) for 30 min at 0.52 *g*. A quantity of 80 mL hexane was added and the samples were extracted by shaking for 60 min at 0.52 g. The hexane layer was transferred to a 250-mL Schott bottle, after which 25 mL water, 5 mL NaAc buffer (pH=5), and 100 µL 2% sodium tetraethylborate were added, and the samples were shaken for 15 min at 175 bpm. The hexane layer was concentrated and transferred to a GC vial and analyzed. Extraction efficiency was analyzed using remaining sediment from control cosms. Amounts of 25–34 g sediment were weighed into a Schott bottle, and 250 µL of a 100 or 1 µg/mL stock solution was added. After 15 min, they were extracted and analyzed as described above. The extraction efficiency for sediments was tested by spiking blank sediment samples with a known amount of TPT in ethanol. The recovery was found to be 88% (*n*=4; SD=18%); no corrections were made for this efficiency.

Table 2. Number of individuals of each taxon introduced in each cosm to facilitate potential recovery.

Species	Week 5	Week 19	Week 34
Asellus aquaticus	10	10	20
Bythinia sp.	10		20
Erpobdella octoculata	10		
Gammarus pulex	10	10	8
Lumbriculus sp.	2		
Lymnaea stagnalis	10	5	2
Physa acuta	10	5	2
Pisidium sp.	5	10	
Planorbis contortus	10	10	
Planorbis corneus	10	5	
Planorbis planorbis	3	10	
Planorbis vortex/vorticulus		8	
Sphaerium sp.	3		
Turbellaria sp.	8	1	20
Valvata piscinalis	5		

Organotin analysis followed the methods described in the Dutch guidelines [20] and ethylated organotins were detected on GC-MSD in Selective Ion Mode (GC: HP 6890 with auto injector HP 7683; MSD: HP 5973 Network MSD, Hewlett Packard, Palo Alto, USA). 2 mL buffer solution (pH=5; 120 g HAc+272 g NaAc per liter), 100 µl 2% sodium tetraethylborate and 20 mL hexane were added. The water phase was extracted by shaking for 15 min at 0.52 *g*. Part of the hexane layer was removed and transferred to a GC vial. The limit of detection of TPT in water was 1 µg/L. The recovery of the extraction procedure was tested by spiking blank water samples with a known amount of TPT in ethanol. The recovery was found to be 92.7% (n=4; SD=12.7%). Because sediment and water recoveries fell within the measurement error of the GC–MS, no corrections were made for these recoveries.

Water quality

Dissolved oxygen (DO), pH, conductivity, and alkalinity were measured at -4, -3, -2, -1, 0.4, 1, 2, 4, 6, 8, 12, 23, and 40 weeks after TPT application. DO was measured with a WTW Oxi330 oxygen meter and oxygen probe at a depth of 10 cm. Conductivity and pH were measured with a WTW LF 191 conductivity meter and a WTW PH197 pH meter, respectively. Alkalinity was measured in 100 mL samples taken at a depth of 10 cm (titration with 0.05 N HCl until pH 4.2; WTW PH197 pH meter).

Macroinvertebrates

Macroinvertebrates were sampled from each microcosm at -4, -2, 0.4, 2, 4, 8, 12, 23, and 40 weeks after TPT application by means of artificial substrates, *viz*., multiplates and pebble baskets. In each system, two multiplates and two pebble baskets were incubated. Details of the substrates used have been described by Brock and coworkers [21].

On each sampling day, the artificial substrates were gently retrieved from the microcosm using a net to prevent organisms escaping. Pebble baskets were first washed in a container to remove invertebrates from the pebbles. Subsequently, the macroinvertebrates present on both substrates were sorted by hand, identified, counted alive, and then returned to the microcosm. Data from artificial substrates was pooled for further analysis.

Zooplankton

Zooplankton was sampled from each cosm in weeks -4, -2, -1, 0.4, 1, 2, 4, 6, 8, 12, and 23, using a Perspex tube (length: 0.4 m; volume: 0.8 L). Several subsamples were collected, evenly distributed over the cosms, until a 5-L sample had been obtained. The 5-L sample was concentrated by means of a plankton net (Hydrobios, Kiel, Germany; mesh size: 55 μm) and was preserved with formaldehyde (final concentration 4%). Cladocera and Rotifera were counted and identified to the lowest possible taxon. Copepods were counted and classified into calanoids and cyclopoids. Cladocera and copepods were counted and identified using a binocular microscope, while rotifers were counted and identified using an inverted microscope. Abundances were adjusted to numbers of organisms per liter.

Sediment dwelling Nematoda

Nematode samples were taken with the same Perspex tubes used to sample sediment for TPT analysis. On each sampling date, two sediment cores were collected from each microcosm and the top 5 cm layers of these cores were pooled for analysis. Samples were immediately preserved with formalin (final concentration approximately 4%). Sampling took place at -3, 2, and 12 weeks after TPT application. Total numbers of nematodes in the samples were determined by counting under a stereo-microscope. For identification, 100–200 specimens (in some cases the whole sample) were mounted on slides and observed under a light microscope at a magnification of 630x. Occasionally, a magnification of 1000x (oil) was used. Identification to species level was not always possible, due to the absence of adult specimens. In these cases, genus or family names are given.

Primary producers

Water samples to assess phytoplankton species composition and phytoplankton chlorophyll-*a* concentrations were taken in weeks -2, -1, 0.1, 1, 2, 4, 6, 8, and 10. In addition, phytoplankton chlorophyll-*a* samples were taken at 15 and 23 weeks after TPT application. Several depth-integrated water samples were collected using a Perspex tube (length: 0.4 m; volume: 0.8 L) until a total sample of 5 L had been obtained. From the 5-L sample, a 1-L subsample was taken for chlorophyll-*a* analysis, which was concentrated using a glass-fiber filter (Schleicher and Schuell GF52, mesh size: 1.2 μm). Filters were inserted in Petri dishes, wrapped in aluminum foil, and stored at -20 ºC prior to analysis. Pigments were analyzed using a spectrophotometer (Beckman, DU-64) following the method described by Moed and Hallegraeff [22]. Another 1-L subsample was preserved with Lugol and formalin (final concentration 4%) and used to identify the phytoplankton. In general, phytoplankton was counted and identified to genus level, using an inverted microscope.

The percentage cover of macrophytes (including filamentous algae) was estimated at -2, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 13 weeks after TPT application.

Decomposition of Populus leaves

Decomposition of particulate organic matter (POM) was studied by means of the litter bag technique [23]. The POM used consisted of *Populus* x *canadensis* leaves. The *Populus* leaves had been leached three times for 2 days to remove soluble humic compounds. To allow this material to be stored, it was dried in an oven for 72 h at 60 ºC. In the decomposition assessments, 2 g dry weight of *Populus* leaves were enclosed in each litter bag, consisting of a sieve of stainless steel wire (mesh size: 0.7x0.7 mm^2). In each microcosm, two litter bags were incubated at mid-depth in the water column for a period of 2 weeks. Whenever a set of litter bags was retrieved on a sampling day, a new set was incubated. The organic plant material was dried in aluminum foil at a temperature of 105 ºC. After 24 h, dry weight was determined. The decomposition over a 2-week period was expressed as % remaining organic material.

Data analysis

Prior to analysis, the macroinvertebrate and nematode data was ln(2x+1) transformed, where x is the abundance value. For zooplankton and phytoplankton, data was transformed by ln(10x+1) and ln(0.001x+1), respectively. This was done to down-weight high abundance values and approximates a log-normal distribution of the data [24]. No Observed

Effect Concentrations (NOECs) at parameter or taxon level were calculated using the Williams test (ANOVA) [25]. This test assumes that the mean response of the variable is a monotonic function of the treatment, thus leading to the expectation of increasing effects with increasing dose. The analyses were performed with the Community Analysis computer program [26], resulting in a summary of NOECs in each sampling week for the data analyzed. The threshold level for p was 0.05 for all statistical analyses.

The effects of the TPT treatment at the community level (macroinvertebrates, zooplankton, nematodes, phytoplankton) were analyzed by the Principal Response Curves method (PRC), which is based on the Redundancy Analysis ordination technique, the constrained form of Principal Component Analysis [27]. The PRC method yields a diagram showing the differences between treatments and controls. A full description and discussion of the PRC method has been provided by Van den Brink and Ter Braak [27-29]. The PRC analysis was performed using the CANOCO software package, version 4.02 [30]. The results of the PRC analysis can also be evaluated in terms of the fractions of the variance explained by the factors time and treatment, and the PRC diagram shows the fraction of the variance that is explained by treatment. In the CANOCO computer program, Redundancy Analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on the species composition of the samples [31, 32].

The significance of the PRC diagrams in terms of displayed treatment variance was tested by Monte Carlo permutation of the microcosms, *i.e.*, by permuting whole time series of microcosms in the partial redundancy analysis from which PRC is obtained, using a F-type test statistic based on the eigenvalue of the component [27].

Monte Carlo permutation tests were also performed for each sampling date, using the ln-transformed nominal doses as the explanatory variable [32]. This allowed the significance of the treatment regime to be tested for each sampling date. If a significant relation between treatment regime and species composition was found, we also determined which treatment levels differed significantly from the controls, so as to infer the No Observed Effect Concentration at the community level ($NOEC_{community}$). $NOEC_{community}$ was calculated by applying the Williams test [27, 32].

For the nematode community, a Maturity Index (MI) was calculated according to the following formula:

$$MI = \sum_{i=1}^{s} v_i * p_i \qquad (1)$$

where v_i is the colonizer/persister value of taxon i and p_i is the proportion of taxon i in relation to the total number of non-plant feeding nematodes. The MI is an ecological index for nematodes based on the colonizing or persisting characteristics of nematode families, and divides the nematodes over five classes on a colonizer/persister scale. Nematodes can be placed on this arbitrary c/p scale ranging from 1 (for extreme colonizers, such as Monhysteridae, which have generation times of only a few days) to 5 (for extreme persisters, such as Enoplidae, which have a generation time of one year). Plant-feeding nematodes and dauerlarvae are not taken into account [33, 34].

Results

Fate of TPT

Concentrations of TPT in the water column of the microcosms, based on measured concentrations in the solutions applied to the test systems, varied between 95 and 102% of the intended nominal concentrations (see Table 3). Figure 1 shows that 3 h after application, the measured peak concentrations in the water column were higher than the intended concentrations, *viz.*, 35 and 13 *vs.* 30 and 10 µg/L and 45 and 16 *vs.* 30 and 10 µg/L for the CS and PS systems, respectively. This can be explained by incomplete mixing during the first hours after application, particularly in the presence of higher densities of macrophytes (PS systems). Nevertheless, the overall dissipation rate of TPT in the water column was similar in both types of test system (Figs. 1 and 2).

On all sampling dates, total TPT levels in the sediment were somewhat higher in the CS cosms than in the PS cosms (Fig. 2), despite the fact that the sediment of the PS cosms had a higher organic matter content (Table 1). The reason for this might be the smaller macrophyte biomass in the CS test systems at the time of TPT application (see also Fig. 11). Most probably, a larger proportion of the TPT dose was sorbed to the larger stock of macrophytes in the PS systems.

Table 3. Percentages (mean and standard deviations) of calculated initial concentrations of triphenyltin acetate (TPT) in the overlying water of the microcosms relative to intended concentrations.

CS cosms intended TPT (µg/L)	% of intended mean	SD	PS cosms intended TPT (µg/L)	% of intended mean	SD
1	97.4	13.1	1	104.4	30.6
10	88.7	22.7	10	99.5	3.4
30	97.6	14.3	30	99.7	7.7
100	96.7	6.0	100	105.4	5.6

Concentrations of TPT in pore water remained much lower that those initially measured in the overlying water. In both types of microcosm, however, the rates of disappearance of TPT from the pore water and total sediment compartments were considerably lower than those initially measured in the overlying water (Fig. 2). TPT thus appeared to be very persistent in the sediment compartment.

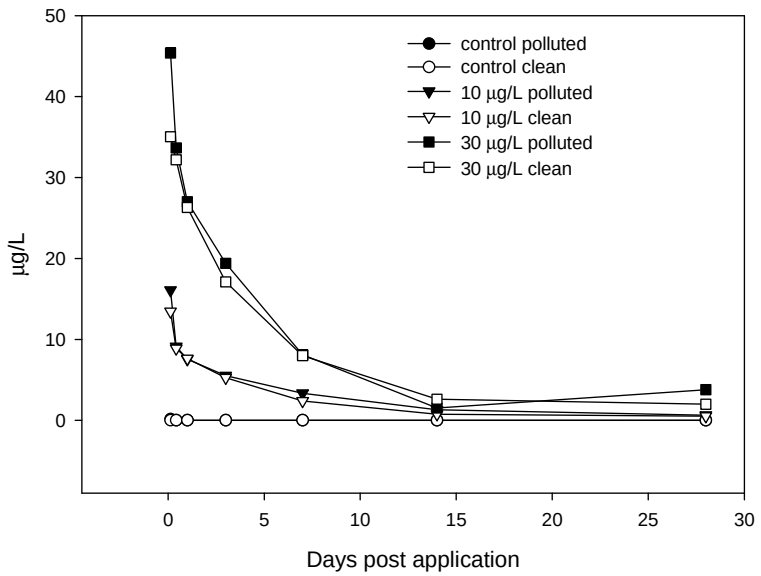


Figure 1: Dynamics of TPT concentrations (geometric mean) in the water phase for the control, 10, and 30 µg/L treatments in the CS (clean sediment) and PS (polluted sediment) cosms

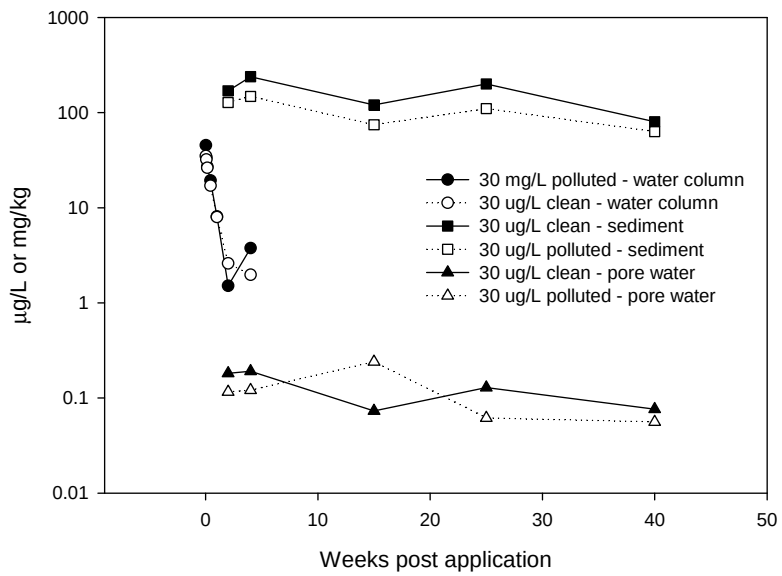


Figure 2: Dynamics of TPT concentrations (geometric mean) in the water column (µg/L), pore water (µg/L), and sediment (µg/kg dry weight) for the 30 µg/L treatment in cosms with clean (CS) and polluted (PS) sediment

Water quality

Dissolved oxygen concentration dynamics differed considerably between the CS and PS cosms (Fig. 3). In the CS cosms (Fig. 3A), DO levels were already steeply declining before TPT application. This decline in the pre-treatment period can be explained by the natural collapse (and accompanying degradation processes) of filamentous algae that initially dominated the vegetation in the CS systems (see also Fig. 11C, D). This pre-treatment decline in DO levels obscured possible treatment-related effects in terms of this endpoint (Table 4). Hardly any filamentous algae were present in the PS cosms, and DO levels here started to decline immediately after TPT application (Fig. 3B). The decline in DO concentrations in the PS cosms might be partly explained by the use of ethanol as a carrier solvent, since DO levels in the controls (which received no TPT but only the carrier solvent) dropped from approximately 12 to 6 mg/L during the first two weeks after application (Fig. 3B). The ethanol was apparently a readily available energy source for microorganisms, which most probably consumed large quantities of DO immediately after ethanol application. Nevertheless, a clear concentration–response pattern in DO concentrations was also observed in the PS cosms after TPT application (Fig. 3B). Relative to controls, statistically significant declines in DO levels were observed during the first two weeks after TPT application, with a NOEC as low as 1 µg TPT/L (Table 5). Note that all mentioned calculated NOEC values in this paper are based

on initial nominal concentrations. In all CS and PS cosms, DO concentrations increased again to levels well above 5–6 mg/L between 2 and 4 weeks after treatment.

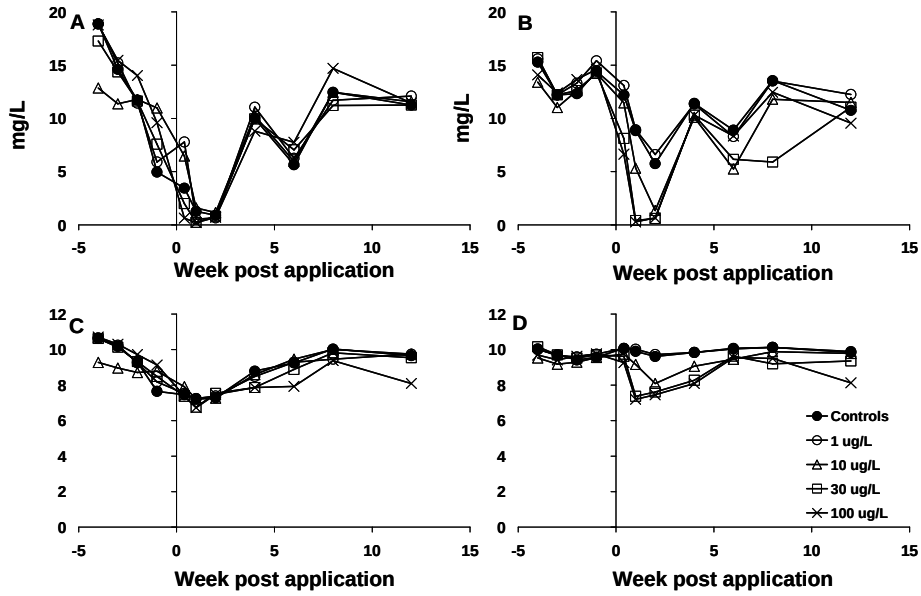


Figure 3: Dynamics of Dissolved Oxygen (A and B) and pH (C and D) in clean sediment and polluted sediment cosms treated with TPT, during the first 12 weeks after application. Calculated NOEC values are presented in Tables 4 and 5

The treatment-related decline in DO levels in the PS cosms in particular can be explained by the photosynthesis-inhibiting properties of TPT [35, 36]. This is in line with the observed treatment-related pH responses (Fig. 3D; Table 5), conductivity (Table 5), and alkalinity (Table 5) in the PS cosms during the first weeks after TPT application: conductivity and alkalinity temporarily increased, while pH and DO temporarily decreased. This response is consistent with a decrease in photosynthesis and is know as the DO-pH-Alkalinity-Conductivity Syndrome [37].

Responses of macroinvertebrates

A total of 83 different macroinvertebrate taxa were identified in the cosms, of which 82 and 77 were present in the CS and PS systems, respectively. Although there was a large overlap in species composition between system types, greater differences existed in relative dominance of taxa. The community of the CS systems showed greater abundance of Coleoptera, *Stylaria lacustris* (Oligochaeta), Ostracoda, Pisididae, and the snails *Valvata* sp. and *Planorbis planorbis*, while *Polycelis nigra/tenuis* (Tricaldida), *Cloeon dipterum* (Ephemeroptera), *Chaoborus obscuripes* (Diptera), *Asellus* sp. (Crustacea), *Erpobdella octoculata* (Hirudinea), and *Physa fontinalis* (Mollusca) were more common in the PS systems.

Multivariate analysis of the macroinvertebrate community sampled in the CS and PS microcosms revealed clear treatment-related effects in both types of test system, which can be ascribed to the treatment with TPT (Fig. 4; Table 6). For instance, Fig. 4A indicates that, compared to the controls, the largest deviations in species composition of macroinvertebrates occurred in the 100 and 30 µg/L cosms, while smaller deviations were found in the 10 µg/L cosms. The species weight (b_k) shown on the right-hand side of the diagram can be interpreted as the affinity of each species with the response in the diagram. Thus *Stylaria lacustris*, which has the highest positive species weight, had the greatest decrease at the higher treatment levels. The negative weight of Chironomidae sp. in the diagram indicates that its numbers increased at the higher treatment levels, relative to controls. Most taxa had a positive score on the b_k axis and consequently showed an overall treatment-related decrease in abundance. A limited number of taxa had a negative b_k score and thus showed an overall increase with increasing TPT concentrations.

The weaker initial response in the CS systems compared with the PS microcosms may be explained by the oxygen stress observed in all CS systems due to the natural collapse of filamentous algae around the time of TPT application (Fig. 4) and the application of ethanol as a solvent. Nevertheless, for both the CS and PS microcosms, Monte Carlo permutation tests revealed significant differences on all post-treatment sampling dates (Table 6). Both the CS and PS microcosms had a $NOEC_{community}$ <1 µg/L on one or two isolated sampling dates. On the basis of the response observed on at least two consecutive sampling dates, the overall critical threshold level was 1 µg TPT/L in both types of test system (Fig. 4, Table 6).

A comparison of the different response curves presented in Fig. 4 shows that recovery of the macroinvertebrate community seemed to be faster in the systems with polluted sediments (PS). However, at the end of the experimental period, a $NOEC_{community}$ of 10 µg/L was calculated for both the CS and PS systems (Table 6), indicating the occurrence of long-term effects after a single application of TPT. This is remarkable, since after approximately 2–3 weeks, TPT concentrations in the water column had dropped to approximately 1 µg/L in the systems treated with 30 µg/L (Fig. 1). In the 30 µg/L cosms, significant effects on the macroinvertebrate community were still observed 42 weeks after TPT application, despite the introduction of small numbers of sensitive taxa in weeks 5, 19, and 34 to facilitate potential recovery (Table 2).

Univariate analysis of the macroinvertebrates sampled in the CS and PS microcosms also revealed clear treatment-related effects in both types of test system, which can be ascribed to the treatment with TPT (Tables 4 and 5; Figs. 5, 6). Representatives of Annelida, Tricladida and

Mollusca in particular showed clear treatment-related declines in both the CS and PS systems, with overall NOECs in the range of < 1–10 µg/L. The most sensitive macroinvertebrate taxa in the CS systems included the oligochaete worm *Stylaria lacustris* and bivalve mollusks, for which NOECs < 1 µg/L were calculated on at least two consecutive sampling dates (Table 4). In the PS systems, consistent NOECs < 1 µg/L were calculated for *Stylaria lacustris* and the triclad *Polycelis nigra/tenuis*, while the snails *Planorbis contortus* and *Physa acuta* showed NOECs < 1 µg/L on two individual sampling dates (Table 5). Of these taxa bivalve mollusks and *P. contortus* were reintroduced to the cosms at week 5 and 19, while *Turbellaria* sp. were also reintroduced at week 34. And *P. acuta* was only reintroduced at week 19 and 34 (Table 2).

Compared with other taxonomic groups, the Arthropoda as a whole seemed to be less sensitive to TPT application (Figs. 5G, H). However, relatively large differences in responses were observed between taxa within the group of Arthropoda (Tables 4 and 5). For example, in both the CS and PS cosms, larvae of the phantom midge *Chaoborus obscuripes* were relatively sensitive to TPT application, without any signs of recovery (Fig. 6A, B); the CS (Table 4) and PS (Table 5) cosms showed NOECs of 10 and 1 µg/L, respectively. In contrast, Asellidae showed a short-term treatment-related decline, with complete recovery on the last two sampling dates (Fig. 6C, D). A similar response pattern was observed for larvae of the ephemeropteran *Cloeon dipterum*, but this species even showed a significant increase in densities at the highest treatment level, relative to controls, after the initial decline (Fig. 6E, F, Table 5). Taxa profiting from the TPT application were predominantly some arthropods. For example, Culicidae (Fig. 6G, H), Dytiscidae larvae (Table 4), and Chironomidae (Table 5) showed a temporary treatment-related increase in population densities, indicating the occurrence of indirect effects.

Table 4: Univariate analysis of the treatment-related responses of water quality and population endpoints in clean sediment cosms, using the Williams test (p<0.05)

	Taxon	-4	-3	-2	-1	0.4	1	2	3	4	5	6	7	8	10	12	13	16	23	42
Water quality	DO	>	>	>	1 (↑)	>	>	>		30		>		30 (↑)	>	>			1 (↑)	>
	pH	>	>	>	30 (↑)	>	10	>		>		30		>	>	30			>	10 (↑)
	Conductivity	>	>	>	30	>	>	>		10 (↑)		1 (↑)		>	>	30 (↑)			>	>
	Alkalinity	>	>	>	1	>	>	>		10 (↑)		30 (↑)		>	>	30 (↑)			>	>
Macroinvertebrates	Annelida	>		>		>		<1		1				1		10			30	10
	Glossiphonia complanata	>		30 (↑)		>		>		>				30		30			1	10
	Helobdella stagnalis	>		>		>		10		>				1		10			1	30
	Oligochaeta	>		>		1		<1		30				>		>			>	n.p.
	Stylaria lacustris	>		>		10		<1		<1				1		10			<1	>
	Tricladida	>		>		>		>		>				10		1			1	>
	Polycelis nigra/tenuis	>		>		>		>		>				10		1			1	>
	Mollusca	>		>		>		>		1				10		1			1	1
	Planorbis contortis	<1 (↑)		>		>		>		1				30		1			1	1
	Planorbis planorbis	>		>		30		10		<1				10		1			10	1
	Radix peregra	>		>		10		>		1				>		1			1	>
	Physa acuta	>		<1		>		>		>				>		<1			>	1
	Bivalvia	>		>		>		>		<1				1		1			<1	<1
	Arthropoda	>		>		30		>		>				>		>			30	30
	Asellidae	>		>		30		>		10				>		>			>	>
	Chaoborus obscuripes	>		>		>		>		>				10		>			10	10
	Chironomidae	>		>		>		30		>				1 (↑)		>			>	10 (↑)
	Cloeon dipterum	>		>		>		>		>				30		>			30 (↑)	>
	Dytiscidae larvae	>		>		>		>		>				>		>			30 (↑)	30 (↑)
Zooplankton	Cladocera	>		>	>	30	30	>		>		30		30		30			>	
	Chydorus sphaericus	>		>	>	>	<1	>		>		>		10		30			>	
	Graptoleberis testudinaria	>		>	>	>	>	>		>		>		>		10			10	
	Simocephalus vetulus	>		>	<1	>	>	>		>		>		30		>			30	
	Copepoda	>		>	>	10	10	1		1		1		1		10			10	
	Copepoida	>		>	>	1	1	1		1		>		1		>			10	
	Cyclopoida	>		>	30	>	10	1		10		1		1		10			10	
	Amoeba	10 (↑)		>	>	>	>	>		>		1 (↑)		30		>			>	
	Rotifera	>		>	>	>	>	30		10		10		>		1			>	
	Keratella quadrata	>		>	>	10	<1	<1		>		>		>		>			>	
Primary producers	Phytoplankton chl-*a*			>	>	>	30 (↑)	30 (↑)		>		>		10 (↑)	>			>	>	
	Cocconeis			>	>	>	>	>		>		10 (↑↓)		<1 (↑↓)	30 (↑↓)					
	Phacus			>	>	>	>	<1 (↑↓)		<1 (↑↓)		>		>	>					
	Trachelomonas			>	>	<1 (↑↓)	>	<1 (↑↓)		<1 (↑↓)		<1 (↑↓)		>	10 (↑↓)					
	Scenedesmus			10	>	<1	30	>		>		>		>	>					
	% cover *Elodea*			>		>	>	30	30	30	30	30	30	30	30	30	30			

The No Observed Effect Concentration (NOEC) of each endpoint is given per sampling week. Only those endpoints that showed a significant response on two consecutive dates or on three non-connected sampling dates are presented

> indicates an NOEC of >100 µg/L; empty entries indicate that no sampling occurred for these endpoints on these dates; n.p. indicates that the Williams test could not be performed due to lack of specimens in several cosms; (↑) indicates an increase instead of a decrease; (↑↓) significantly different (Williams test) but with clear non-linear response

Table 5: Univariate analysis of the treatment-related responses of water quality and population endpoints in the polluted sediment cosms, using the Williams test (p<0.05)

	Taxon	-4	-3	-2	-1	0.4	1	2	3	4	5	6	7	8	10	12	13	16	23	42
Water quality	DO	>	>	30 (↑)	>	10	1	1		>		>		10		>			>	>
	pH	>	>	>	>	10	1	1		1		1		10		10			30	>
	Conductivity	>	>	>	>	10	10 (↑)	1 (↑)		30 (↑)		1		>		30 (↑)			>	>
	Alkalinity	>	>	>	>	>	>	>		30 (↑)		1		>		10 (↑)			30 (↑)	>
Macroinvertebrates	Annelida	>		>		>		10		10				1		10			10	10
	Erpobdella sp.	n.p.		>		10		>		1				<1		>			>	>
	Erpobdella octoculata	>		>		30		<1		>				10		1			1	<1
	Glossiphonia complanata	>		>		>		>		>				10		30			1	10
	Helobdella stagnalis	>		>		>		10		10				1		10			<1	>
	Stylaria lacustris	>		>		<1		>		<1				<1		<1			>	n.p.
	Tricladida	>		>		30		<1		<1				<1		10			10	10
	Polycelis nigra/tenuis	>		>		30		<1		<1				<1		10			10	10
	Mollusca	>		30		30		1		1				1		1			>	>
	Planorbis contortus	>		>		>		<1		1				<1		>			>	>
	Physa acuta	30 (↑)		>		30		1		<1				1		<1			>	>
	Physa juvenile	>		30 (↑)		10		<1		>				>		>			>	>
	Physa fontinalis	>		>		>		1		1				>		>			>	>
	Arthropoda	>		>		30		>		<1				>		<1			1	>
	Asellidae	>		>		30		10		10				30		30			>	>
	Chaoborus obscuripes	>		>		>		10		1				>		1			1	1
	Chironomidae	>		>		30		>		>				1 (↑)		1 (↑)			>	>
	Cloeon dipterum	>		>		>		1		1				30		1 (↑)			30 (↑)	30 (↑)
	Culicidae	n.p.		>		n.p.		30 (↑)		1 (↑)				>		>			n.p.	n.p.
	Tabanidae	>		>		>		1		1				<1		>			n.p.	n.p.
	Corixidae juvenile	>		>		>		>		30				10 (↑)		30 (↑)			>	>
Zooplankton	Cladocera	>		>	30	30	10	10		10		30		>		>			10	
	Chydorus sphaericus	>		>	>	>	30	10		10		10		>		>			>	
	Graptoleberis testudinaria	>		>	>	>	>	30		1		1		10		10			>	
	Simocephalus vetulus	>		>	>	>	1	1		10		10		>		>			>	
	Copepoda	>		>	>	10	1	1		1		10		>		30			>	
	Copepoida	>		>	>	>	1	>		30		10		10		>			>	
	Cyclopoida	>		>	>	10	1	1		1		1		>		>			>	
	Rotifera	>		>	>	>	30	30		10		30		10		30			30	
	Keratella quadrata	>		>	>	10	1	1		<1		>		>		>			>	
	Lepadella patella	<1		>	>	>	>	>		1		1		30		>			<1	
Primary producers	Phytoplankton chl-*a*	>		>	>	1	<1	10 (↑)		>		10 (↑)		30 (↑)	>			1	>	
	Cosmarium	>		>	>	>	>	>		>		30 (↑)		30 (↑)	30 (↑)					
	% cover *Elodea*				>	>	>	>	30	30	30	10	10	10	30	30	10			

The No Observed Effect Concentration (NOEC) of each endpoint is given per sampling week. Only those endpoints that showed a significant response on two consecutive dates or on three non-connected sampling dates are presented

> indicates an NOEC of >100 µg/L; empty entries indicate that no sampling occurred for these endpoints on these dates; n.p. indicates that the Williams test could not be performed due to lack of specimens in the cosms; (↑) indicates an increase instead of a decrease

Responses of zooplankton

A total of 86 zooplankton taxa were identified, 57 of which occurred in the CS cosms and 76 in the PS systems. The cladocerans *Peracantha truncata*, *Disparalona rostrata*, and *Graptoleberis testudinaria*, and the rotifers *Hexarthra mira*, *Euchlanis lyra*, and *Conochilus sp* were more common in the CS cosms, while cyclopoids, the cladoceran *Simocephalus vetulus* and the rotifers *Keratella quadrata*, *Lecane lunaris* and *Lepadella patella* were more abundant in the PS cosms.

Multivariate analysis of the zooplankton community sampled in both types of test system (CS and PS) showed a clear treatment–response relationship (Fig. 7). On the b_k axis on the right side of the graphs, it can be seen again that most taxa had positive scores and thus showed an overall treatment-related decrease in abundance. A limited number of taxa had negative b_k scores and thus showed an overall increase with increasing TPT concentrations. Again, the more pronounced DO decrease in the CS systems (due to a natural decline in filamentous algae) most probably obscured the response pattern of the zooplankton community of the CS systems during the first weeks after TPT application, compared with that of the PS cosms. Nevertheless, in both types of test system, Monte-Carlo permutation tests revealed significant treatment-related effects during the whole post-treatment period, except for weeks 0.4 and 2 in the CS systems and week 12 in the PS cosms (Table 7). In both types of test system, a $NOEC_{community}$ of 1 µg TPT/L was calculated for zooplankton on at least two consecutive sampling dates (Table 7). On the last sampling date (week 23), the $NOEC_{community}$ calculated for the CS systems was 10 µg/L, while that for PS cosms was 30 µg/L. This suggests a somewhat faster recovery of the zooplankton community in the PS systems.

The three main zooplankton groups (Copepoda, Cladocera, and Rotifera) showed clear treatment-related responses (Fig. 8); overall, Copepoda were the most sensitive, with consistent NOECs as low as 1 µg TPT/L (Tables 4 and 5). The most sensitive zooplankton population in the CS microcosms, however, was that of the rotifer *Keratella quadrata*, with a NOEC < 1 µg/L on two consecutive sampling dates during the first weeks after TPT application (Table 4). This zooplankton species showed a fast recovery, in contrast to initially less sensitive taxa like Cyclopoida and *Graptoleberis testudinaria*, which still had a NOEC of 10 µg/L on the last sampling date (Table 4). The most sensitive zooplankton populations in the PS microcosms were *Graptoleberis testudinaria*, *Simocephalus vetulus*, Cyclopoida, *Keratella quadrata*, and *Lepadella patella*, all with a consistent lowest NOEC of 1 µg/L (Table 5). Of these taxa, *Simocephalus vetulus*, Cyclopoida, and *Keratella quadrata* showed a relatively fast

recovery in which significant differences could not be calculated anymore after 8 weeks post application.

Table 6. Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the macroinvertebrate communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment.

Week relative to application	CS cosms *p*-value	$NOEC_{community}$ (µg/L)	PS cosms *p*-value	$NOEC_{community}$ (µg/L)
-4	>0.05	>100	>0.05	>100
-2	>0.05	>100	>0.05	>100
0.4	0.015	>100	0.012	10
2	0.001	1	0.001	1
4	0.004	<1	0.001	1
8	0.001	1	0.001	<1
12	0.001	1	0.001	1
23	0.001	1	0.001	<1
40	0.001	10	0.010	10

Responses of sediment-dwelling nematodes

A total of 44 nematode taxa were identified in sediment samples from the cosms, 31 of which were found in the clean and 36 in the polluted sediment systems. Some characteristic taxa in the community of the CS cosms were *Tobrilus sp*., *Monhystera sp*., *Monhystera riemanni*, and *Mononchus aquaticus*. Taxa that were more abundant in the community of the PS cosms were *Eumonhystera similis*, *Daptonema dubius*, and *Etmolaimus pratensis*.

Multivariate analysis of the nematode community, followed by Monte-Carlo permutation testing, revealed that the nematode community structure differed significantly between the CS and PS microcosms. Nevertheless, no TPT-related effects on nematode community structure were found in either of the two types of test system. In addition, calculating and testing the Maturity Index of the nematode community sampled in the two types of test system did not reveal significant treatment-related effects. The calculated Maturity Index values were all in the same range (2.04–3.00; see Table 8). This relatively low range indicates that the nematode communities in both the CS and PS microcosms include mainly colonizers, characterized by high reproduction [38].

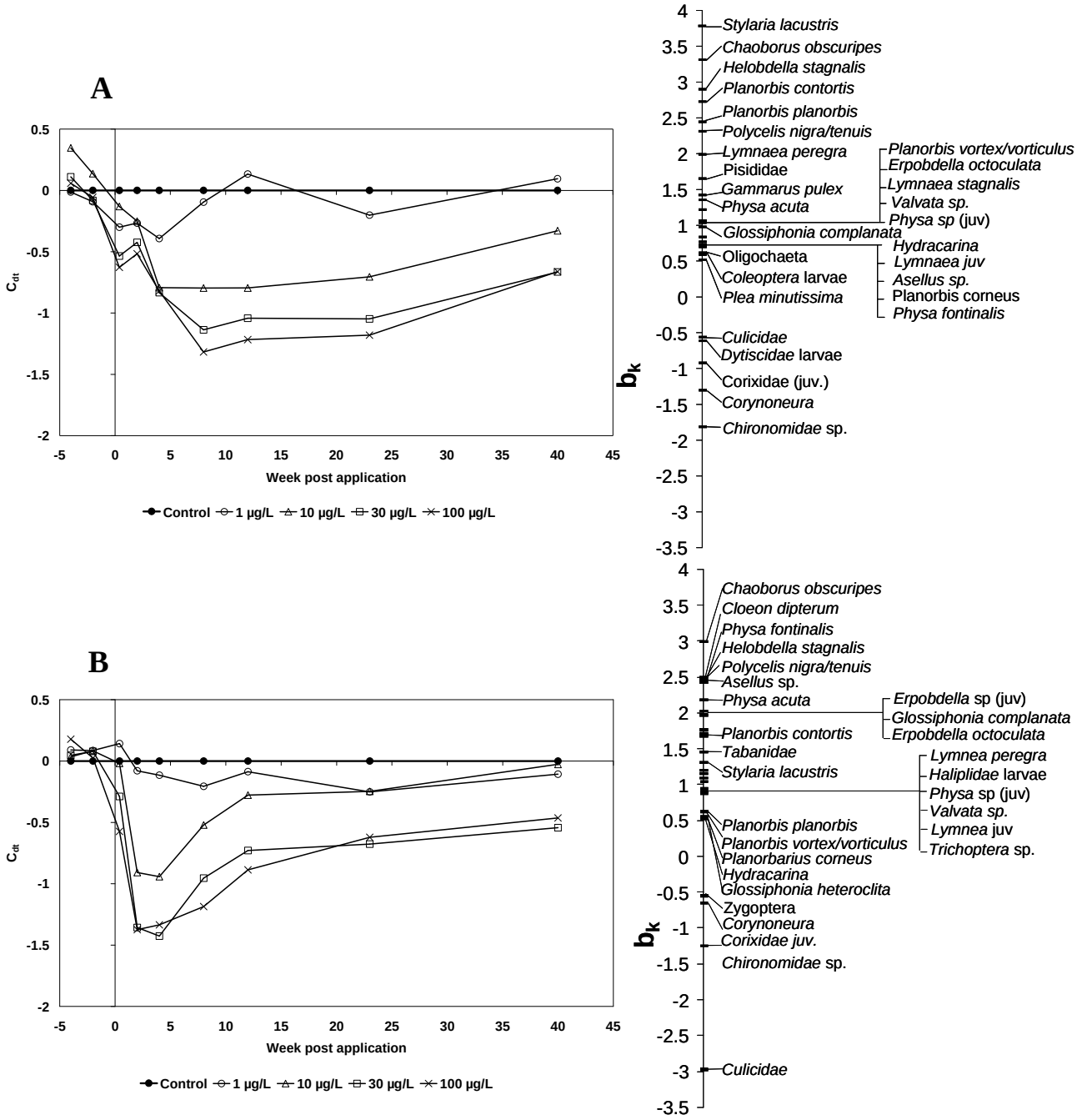


Figure 4: Principal Response Curves resulting from the analysis of the macroinvertebrate data set from the TPT treated microcosms with clean (A) and polluted (B) sediment. In the clean sediment cosms, 39% of the variance was explained by treatment and 36% by time. The corresponding percentages for the polluted sediment cosms were 41 and 38. Calculated $NOEC_{community}$ *values are presented in Table 6*

Response of primary producers

A total of 45 different phytoplankton taxa were identified, 43 of which were found in the CS microcosms and 40 in the PS test systems. Overall, the CS cosms were characterized by a higher abundance of *Cryptomonas*, *Tetraedon*, and *Monorhaphidium*, while *Anabaena*, *Epithemia*, and *Cocconeis* were more abundant in the PS cosms.

In both the CS and PS test systems, multivariate analysis of the phytoplankton community found no clear concentration–response relationship with TPT (Fig. 9). In both types of test system, the 10 µg/L treatment deviated more from the controls than the two highest treatment levels. Monte Carlo permutation testing, however, did not reveal a consistent treatment-related effect at the level of the phytoplankton community (Table 9). Only in the CS cosms were significant differences between treatments found on the first sampling date before (week -1) and after (week 0.4) the TPT treatment.

In the PRC diagrams of both the CS (Fig. 9A) and PS (Fig. 9B) microcosms, the diatom *Cocconeis* shows the highest positive b_k score, while the green alga *Scenedesmus* has the highest negative b_k score. This is in accordance with the observed population dynamics of *Cocconeis* (Fig. 10C, D) and *Scenedesmus* (Fig. 10E, F); these taxa showed an overall decline and an overall increase in densities, respectively, in the 10 µg/L microcosms relative to controls. After TPT application, no clear linear concentration–response relationship was found for *Cocconeis* or *Scenedesmus*. For this reason, the calculated NOEC values of < 1–30 µg/L for *Cocconeis* in the CS systems (Table 4) should be interpreted with caution. This is also true for the NOECs < 1 µg/L calculated for a few other phytoplankton taxa in the CS microcosms, *viz*. *Phacus* and *Trachelomonas* (Fig. 10G and Table 4). The Williams test assumes a monotonic response, and consequently may show an output that is difficult to interpret when actual responses are non-linear.

In both types of test system, significant treatment-related increases in phytoplankton chlorophyll-*a* were observed, particularly in the PS cosms with the two highest TPT concentrations (Fig. 10A and Table 4; Fig. 10B and Table 5). At the taxon level, this increase could only be confirmed by the response of Cosmarium, for which NOEC values of 30 µg/L were calculated in the PS cosms (Fig. 10H, Table 5).

The development of macrophytes in the test systems (as percentage cover) is presented in Fig. 11. The macrophytes community was dominated by *Elodea nuttallii* and developed differently in the two types of microcosm. In the CS cosms, macrophyte growth (Fig. 11A) was initially hindered by high densities of filamentous algae (Fig. 11C). Only after the filamentous algae declined was there an increase in the percentage cover of *Elodea nuttallii*, several weeks after TPT application.

In contrast, the PS cosms, in which densities of filamentous algae were low (Fig. 11D), showed an increase in the percentage cover of Elodea even in the pre-treatment period (Fig. 11B). In the CS cosms, calculated NOEC-values for the percentage cover of *Elodea nuttallii* were 30 µg/L in the period of 2–13 weeks after TPT application (Table 4). In the PS cosms, a NOEC of 10 µg/L was found in weeks 6, 7, 8, and 13, and a NOEC of 30 µg/L in weeks 3, 4, 5, 10, and 12 (Table 5). Macrophyte coverage was most affected at the highest treatment level. In the CS cosms, hardly any *Elodea* developed in the 100 µg/L cosm. In the PS cosms a pronounced decline in *Elodea* was observed 5 weeks after TPT application, without clear signs of recovery up until week 13.

Decomposition

The residual dry weight of the *Populus* leaves in the decomposition assays in the various 2-week incubation periods was approximately 45–55% in both types of test system. No treatment-related effect on litter breakdown was, however, demonstrated in either the CS or the PS cosms.

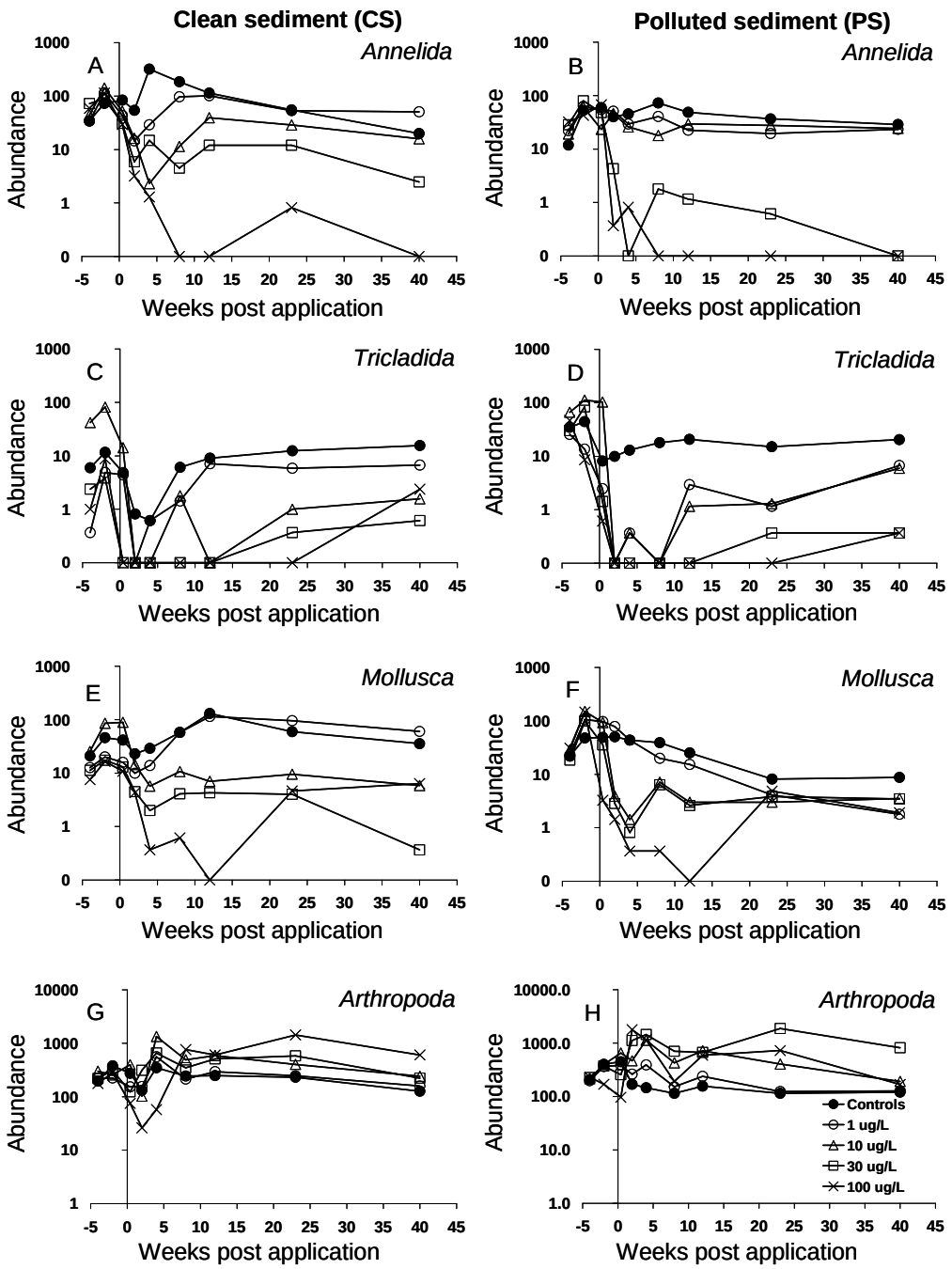


Figure 5: Dynamics of the macroinvertebrate taxonomic groups Annelida (A and B), Tricladida (C and D), Mollusca (E and F), and Arthropoda (G and H) in clean sediment and polluted sediment cosms that were treated with TPT. Calculated NOEC values are presented in Tables 4 and 5

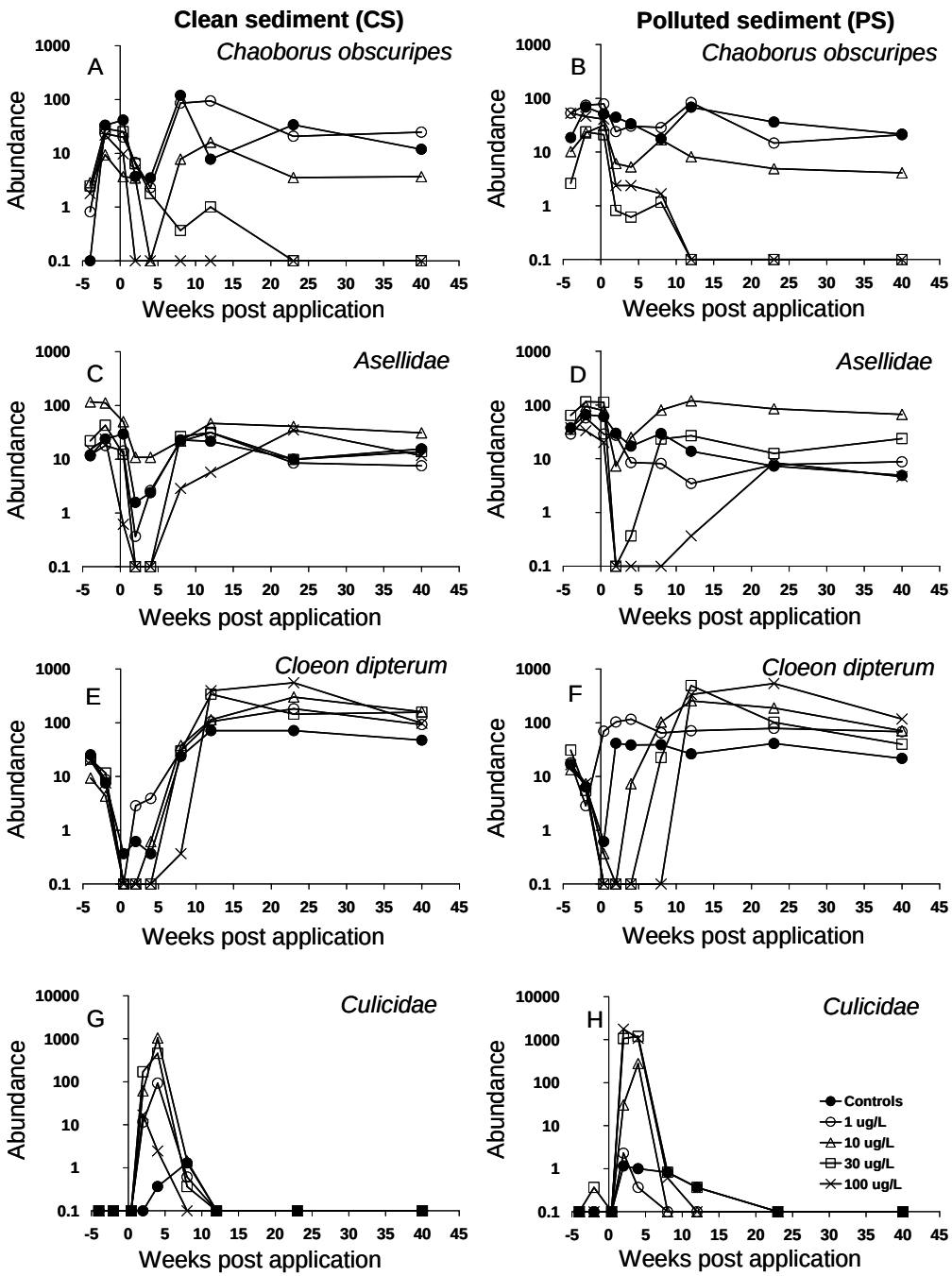


Figure 6: Dynamics of four arthropod taxa in clean sediment and polluted sediment cosms treated with TPT. The taxa represent a very sensitive species which does not recover (Chaoborus obscuripes; A and B), a sensitive taxon that recovers (Asellidae; C and D), a sensitive taxon that recovers and even increases in abundance (Cloeon dipterum; E and F), and an insensitive taxon that increases in numbers (Culicidae; G and H). Calculated NOEC values are presented in Tables 4 and 5

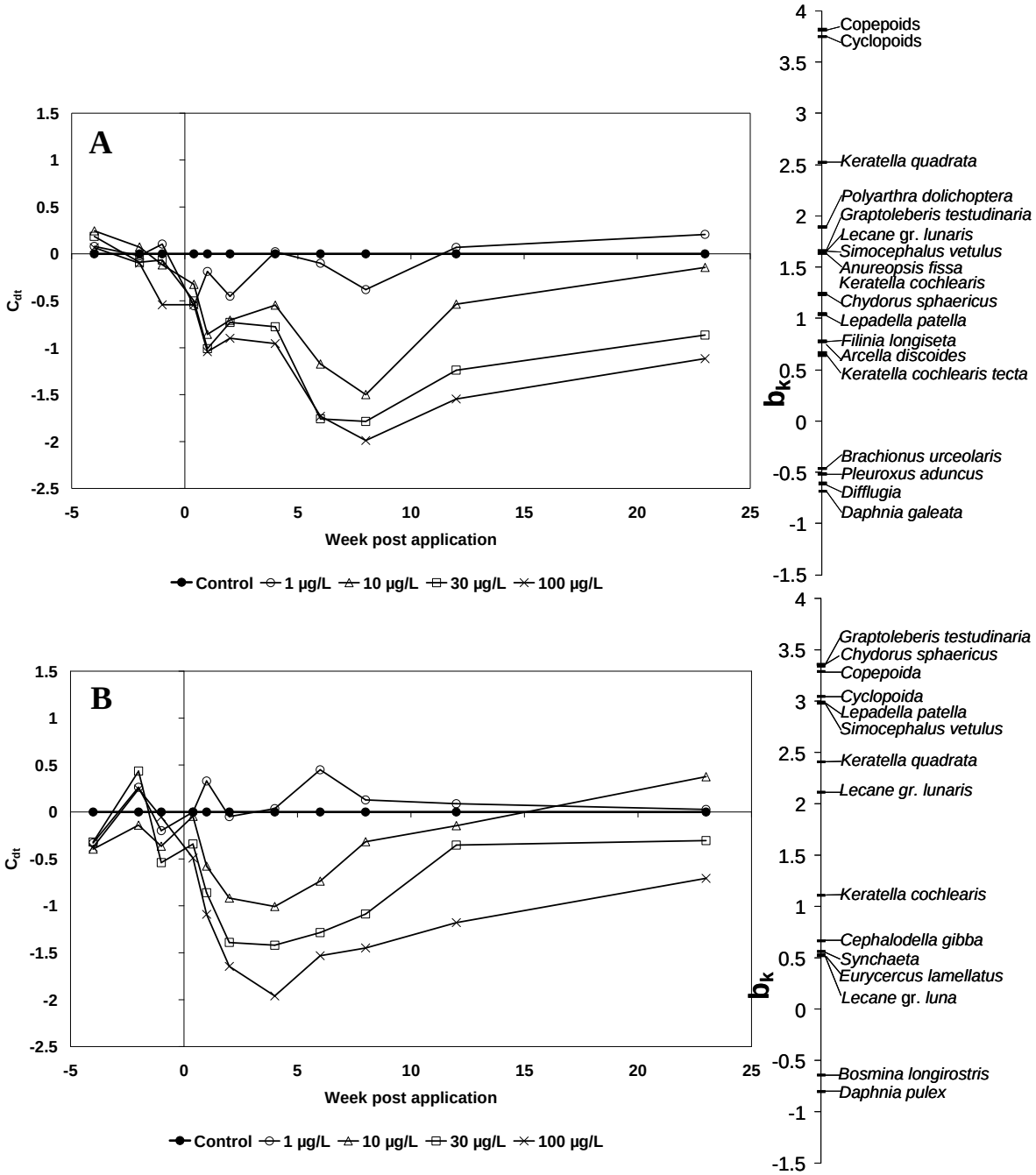


Figure 7: Principal Response Curves resulting from the analysis of the zooplankton dataset from the TPT treated microcosms with clean (A) and polluted (B) sediment. In the clean sediment cosms, 38% and 32% of the variance was explained by treatment and time, respectively. The corresponding percentages for the polluted sediment cosms were 46 and 21. Calculated $NOEC_{community}$ *values are presented in Table 7*

Table 7. Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the zooplankton communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment.

Week relative to application	CS cosms *p*-value	$NOEC_{community}$ (µg/L)	PS cosms *p*-value	$NOEC_{community}$ (µg/L)
-4	>0.05	>100	>0.05	>100
-2	>0.05	>100	>0.05	>100
-1	>0.05	>100	>0.05	>100
0.4	>0.05	>100	0.031	10
1	0.001	10	0.003	1
2	>0.05	>100	0.001	1
4	0.001	10	0.001	1
6	0.005	10	0.003	1
8	0.002	1	0.004	10
12	0.001	1	0.018	>100
23	0.006	10	0.053	30

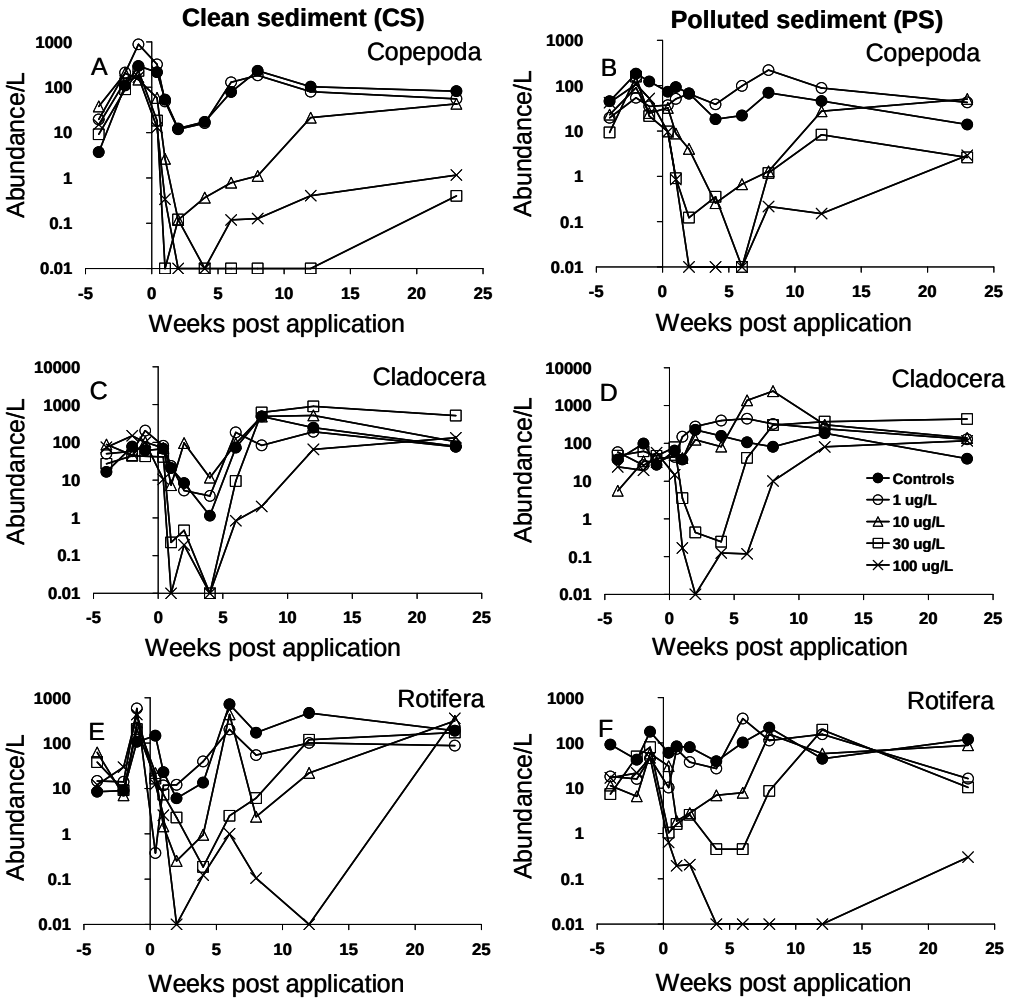


Figure 8: Dynamics of the main groups of zooplankton, Copepoda (A and B), Cladocera (C and D), and Rotifera (E and F) in clean sediment and polluted sediment cosms treated with TPT. Calculated NOEC values are presented in Tables 4 and 5.

Discussion

Ecological responses to TPT exposure

In our microcosm experiment, we observed that representatives of several taxonomic groups of freshwater invertebrates showed a clear response to a single application of TPT at treatment levels of 10 μg/L and more (Tables 4 and 5; Figs. 5, 6, 8). Soft-bodied taxa in particular seemed to be most affected. The fact that representatives of Tricladida, Annelida, Mollusca, Crustacea, Insecta and Rotifera all showed a treatment-related decline in abundance suggests that TPT is a compound with a broad biocidal mode of action. These observations in the microcosms are in line with the results of single species toxicity tests with TPT performed in the laboratory (Table 10; for further details see part II, [15]). Indeed, acute laboratory toxicity data show that several invertebrate groups are sensitive to this compound in the concentration range selected in our microcosm experiment (1–100 μg/L). In our study, the nematode community did not respond to TPT application, nor did individual populations of nematodes. This was unexpected, since several other studies have revealed significant effects of organotin compounds on benthic nematodes [39, 40]. The reason why the nematode community in our two types of microcosm did not respond to TPT application is probably twofold. First, the most likely exposure route of nematodes to TPT is via the pore water, and the concentrations of TPT measured in the pore water remained relatively low (Fig. 2). Second, the sediments we used originated from floodplain lakes, which are very dynamic systems. The nematode community originating from these systems was, not surprisingly, characterized by colonizers, which are by definition hardy organisms that can withstand dynamic, harsh, and stressful circumstances better than non-colonizing species.

The laboratory data presented in Table 10 indicate that, apart from several invertebrates, algae and vascular plants are also sensitive to TPT application. This, however, is only partly in agreement with our observations in the microcosms. In our study, the macrophyte *Elodea nuttallii* significantly declined at treatment levels of 30 and 100 μg TPT/L (Fig. 11; Tables 4 and 5) whereas its sensitivity in 21-day laboratory single species tests was lower. In the laboratory, an EC_{50} of 11.8 μg/L was recorded for the relative growth of *Elodea nuttallii* [15]. Most populations of algae in the microcosms did not show a clear linear treatment-related decline in abundance. This suggests that the extrapolation of results of laboratory single species toxicity tests to predict population-level responses in (experimental) ecosystems may be more difficult for primary producers than for invertebrates. In part, this may be explained by the fact that the measurement endpoint selected for

invertebrates in single species toxicity tests is usually mortality and/or immobility, while in algae and macrophytes this is usually growth rate. Growth rate may be more subject to environmental factors that vary in space and time (*e.g.*, light conditions, nutrient concentrations and grazing pressure) than the mortality/immobility endpoint. In addition, populations and communities of algae, which are characterized by an overall short generation time of the organisms, may develop tolerance within a relatively short period of time [28, 41-43].

In our microcosm experiment, several populations of algae increased in numbers at lower treatment levels and declined at higher exposure concentrations (see *e.g.*, Fig. 10E, F, G). The non-linear responses observed in these taxa were most probably caused by the interplay between direct and indirect effects. For example, the increase in *Scenedesmus* in the microcosms treated with 10 µg/L relative to controls in the 2–10 weeks after TPT application (Fig. 10E, F) may have been caused by a treatment-related decline in zooplankton densities in the 10 µg/L test systems (Fig. 8), resulting in a reduced grazing pressure on *Scenedesmus*. In contrast, the lower *Scenedesmus* densities relative to the 10 µg/L cosms at the two highest treatment levels might be explained by increased toxicity of TPT. This was further substantiated by single species tests performed with *Scenedesmus quadricauda*. In this species, calculated EC_{50} values at 48, 72, and 96 h after TPT application were 352.9, 29.1, and 36.0 µg/L, respectively [15], suggesting that at even longer periods after TPT application, 10 µg/L could cause toxic effects which overrule the positive effect of the release from grazing pressure.

Table 8. Maturity Index calculations for the benthic nematode community in the TPT treated microcosms with clean (CS) and polluted (PS) sediment.

	Week relative to treatment	Range of Maturity Index over treatments
CS cosms	-3	2.21-2.43*
	2	2.29-2.67*
	12	2.33-2.75*
PS cosms	-3	2.04-3.00*
	2	2.06-2.47*
	12	2.06-2.48*

**No significant treatment-related effects found*

Similar response patterns have been found for daphnids in insecticide-treated microcosms which were also inhabited by the more sensitive predator *Chaoborus obscuripes* [44]. The temporary increase in phytoplankton chlorophyll-*a* levels at the two highest treatment levels (Fig. 10A, B) might also be regarded as an indirect effect due to the reduced grazing by zooplankton. At the population level, however, this

temporary increase in phytoplankton biomass could only be confirmed for a few phytoplankton taxa (*e.g.*, *Cosmarium*, Fig. 10H). The increase in phytoplankton chlorophyll-*a* levels that we observed in our microcosms is in accordance with the results obtained by Fliedner *et al.* [40] and Jak *et al.* [45], who performed model ecosystem experiments with the organotin compounds azocyclotin and TBT, respectively. In their experiments, phytoplankton was inhibited at higher concentrations than the zooplankton, and direct toxic effects on zooplankton enabled the phytoplankton to increase at certain treatment levels.

In microcosm and mesocosm experiments, a clear distinction between direct and indirect effects can only be made on the basis of additional information. This information includes laboratory toxicity data on taxa that show a treatment-related response in the experimental ecosystems, as well as ecological information on the position of the affected organisms in the aquatic food web (see *e.g.*, [46-48]. Treatment-related increases in the abundance of populations are most likely the result of indirect effects on relatively tolerant species. Besides the indirect effects on phytoplankton, we observed temporary increases in the abundance of insect taxa in particular, *viz*., Culicidae (Table 5, Fig. 6G, H), Chironomidae (Tables 4 and 5), *Cloeon dipterum* (Tables 4 and 5; Fig. 6E, F), Corixidae (Table 5), and Dytiscidae larvae (Table 4). Literature reports show that representatives of Culicidae [49] and Chironomidae [16] are relatively tolerant to pollutants. The period of increase in Culicidae (weeks 2–4) coincided with a decrease in dissolved oxygen concentration. The air-breathing larvae of Culicidae are well-adapted to low oxygen levels in the water and feed on all types of dead and living, fine-particulate organic matter [50, 51]. During the first weeks after treatment, food in the form of fine particulate organic matter was abundant, partly as a result of the toxic effects of TPT on plants and animals, and partly because of the increase in microorganisms due to the use of ethanol as a solvent for TPT. The other insect taxa mentioned above showed treatment-related increases in abundance much later, at least 8 weeks after TPT application. Given the fact that many other populations of water organisms were negatively affected for a long time, it is difficult to explain with certainty which food web interactions played a major role in the increase in insect taxa. A possible explanation is a decreased competition for food due to a decline of other populations that utilize the same food source. For example, the increase in numbers of the periphyton grazer *Cloeon dipterum* at the highest treatment level (after its initial decline) may have been caused by the long-term decline in Mollusca and *Stylaria lacustris*, which also feed on periphyton. Another explanation might be the release from predation due to a treatment-related decline in carnivores such as Tricladida and Hirudinea.

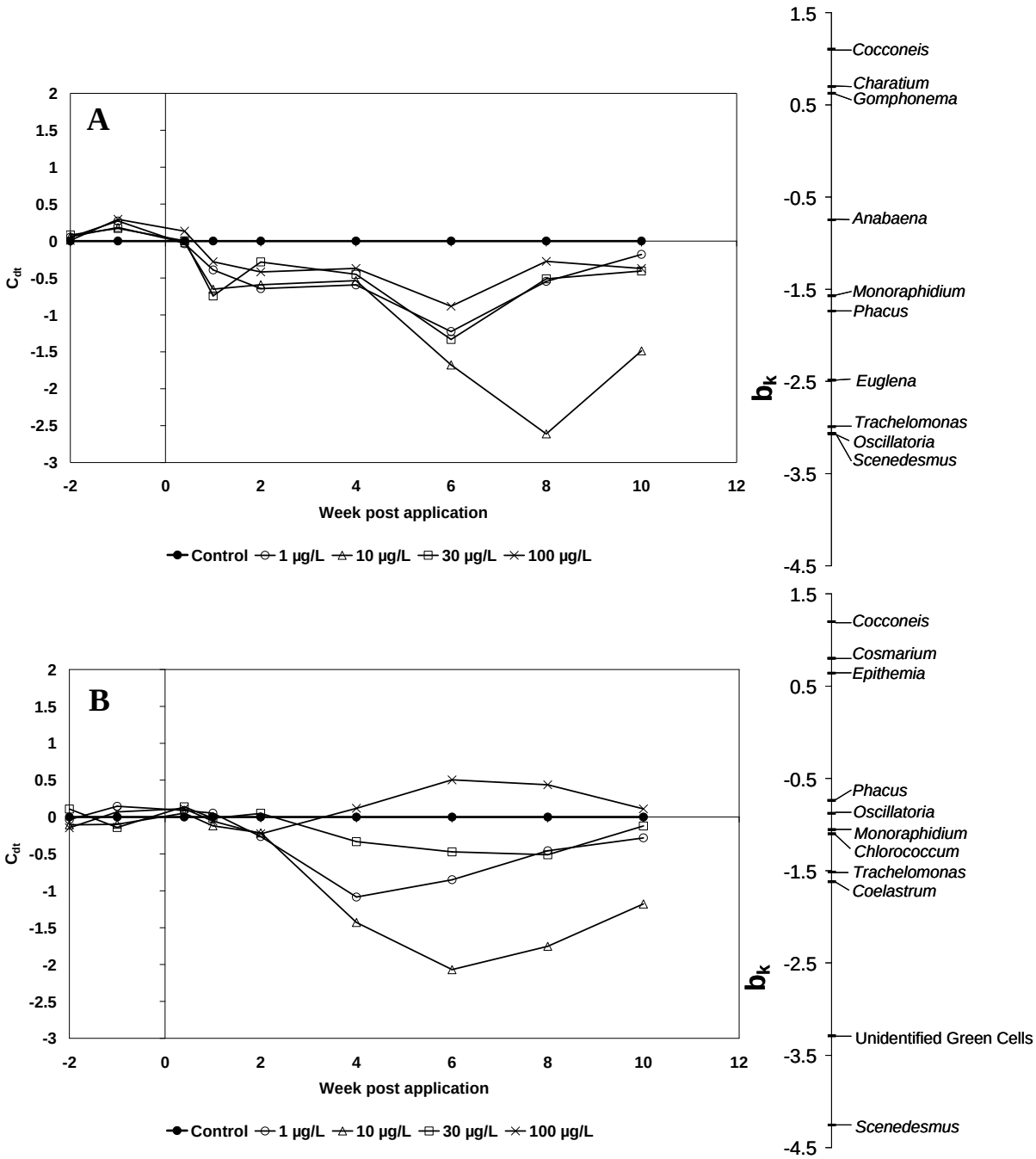


Figure 9: Principal Response Curves resulting from the analysis of the phytoplankton data set from the TPT treated microcosms with clean (A) and polluted (B) sediment. In the clean sediment cosms, 33% of the variance was explained by treatment and 38% by time. The corresponding percentages for the polluted sediment cosms were 38 and 28. Calculated $NOEC_{community}$ values are presented in Table 9

Comparing our results with those of other studies is difficult, because the only published freshwater microcosm study with an organotin compound we are aware of is that performed with the pesticide azocyclotin [40]. Most other studies have been performed in the marine environment with TBT, focusing on responses by plankton, periphyton, and benthic meiofauna [9, 39, 45, 52, 53]. Furthermore, the published

information on the ecological impact of fungicides in freshwater ecosystems is generally also very limited. The responses that we observed in our experimental freshwater ecosystems due to TPT application are more or less comparable with the effects observed in studies of macrophyte-dominated freshwater microcosms treated with the fungicide carbendazim [54, 55]. These studies also found that representatives of Tricladida, Oligochaeta, Hirudinea, Mollusca, Rotifera, and Crustacea were negatively impacted, again indicating that certain fungicides may have a broad biocidal mode of action in freshwater ecosystems.

Influence of sediment quality

The two types of microcosm used in our experiment were stocked with relatively clean or polluted sediments collected in floodplain lakes of the river Waal (in the Rhine catchment). The difference in sediment quality between these test systems related mainly to PAHs, PCBs, metals, organic matter, and nutrients (Table 1). These differences in sediment quality affected the structure of the aquatic communities that developed in the microcosms. In the microcosms with 'polluted' sediments, a dense vegetation of the macrophyte *Elodea nuttallii* had already developed at the start of the experiment, when TPT was applied. In contrast, the development of *Elodea nuttallii* in the microcosms with 'clean' sediments was retarded, partly because of lower nutrient levels and partly by a bloom of filamentous algae (Fig. 11). These differences in macrophyte growth were most probably driven by differences in nutrient content of the sediments used. Differences in the dominance of phytoplankton and invertebrate populations between microcosm types may have been caused directly by differences in sediment quality, or indirectly by differences in macrophyte biomass. Several field studies have revealed that both sediment-bound toxicants [56-58] and beds of aquatic vascular plants [59-61] have a significant effect on invertebrate communities.

That the toxicants in the sediment of the PS microcosms pose risks to benthic organisms is suggested by the sediment quality criteria that are currently used in the Netherlands [62, 63]. When concentrations of individual compounds remain below the maximum permissible concentration (MPC), the ecotoxicological hazard of the compound is considered to be tolerable. In the clean sediments that we used to construct the CS microcosms, standardized concentrations (25% lutum, 10% organic matter) of individual metals, PAHs, and PCBs were all well below MPC values [64]. In the sediment of the PS microcosms, however, the standardized concentrations of several PAHs (anthracene, phenantrene, benzanthracene) and PCBs (PCB052, PCB101, PCB118, PCB138, PCB153, PCB180) were a factor 2–5 above MPC values, while

several other compounds (including the metals Cu and Zn) were only slightly below their MPC values [64]. That the sediment of the PS microcosms was toxic to certain benthic organisms is further substantiated by the findings of 10-day whole sediment bioassays, which showed that survival of larvae of the mayfly *Ephoron virgo* was approximately 48% in tests using sediment from the controls of the PS cosms, against 78% in sediments from the controls of CS microcosms [65]. The response of this sensitive mayfly suggests that the total cocktail of background pollutants in the sediment of the PS microcosms may have affected sensitive benthic invertebrates.

Table 9. Significance of the Monte-Carlo permutation tests and $NOEC_{community}$ values calculated from the PRC analysis of the phytoplankton communities in the TPT treated microcosms with clean (CS) and polluted (PS) sediment.

Week relative to application	*p*-values CS cosms	PS cosms
-2	>0.05	>0.05
-1	0.03	>0.05
0.4	0.03	>0.05
1	>0.05	>0.05
2	>0.05	>0.05
4	>0.05	>0.05
6	>0.05	>0.05
8	>0.05	>0.05
10	>0.05	>0.05

Table 10. Acute EC_{50}/LC_{50} toxicity data of TPT for several taxonomic groups of aquatic organisms available from the literature.

Taxa	Range EC_{50}/LC_{50} (µg/L)	References
Algae (72h)	1-28	[15, 18]
Vascular plants (21 days)	5-199	[15]
Tricladida (96h)	6-7	[15]
Annelida (96h)	2-17	[18]
Mollusca (96h)	7-12	[15]
Microcrustacea (48h/96h)	1-16	[15, 19]
Macrocrustacea (96h)	9-96	[15]
Insecta (96h)	0.33-205	[15, 18]

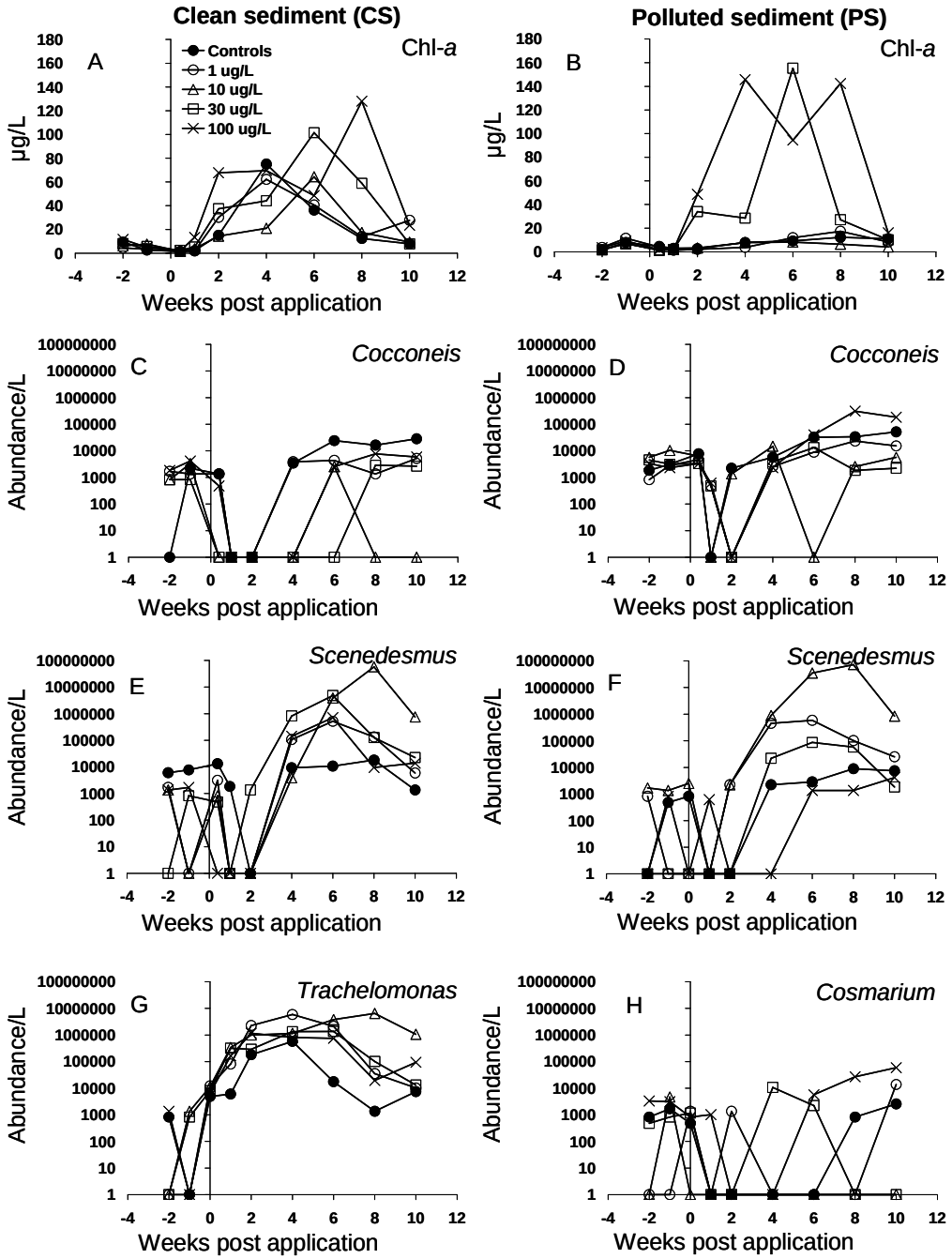


Figure 10: Dynamics of phytoplankton Chl-a (A and B) and of the four taxa found to be most important in the PRC analysis (Cocconeis; C and D, Scenedesmus; E and F, Trachelomonas; G, and Cosmarium; H) in TPT treated microcosm with clean sediment and polluted sediment. Calculated NOEC values are presented in Tables 4 and 5

The most important aim of our microcosm experiment was not to study differences in the development of the aquatic community in microcosms differing in sediment quality. The question at stake was whether sediment-bound pollutants affect the response (in terms of sensitivity and resilience) of aquatic populations and communities to additional stressors. In our experiment, this additional stressor was triphenyltin acetate (TPT), applied in a concentration range (0–100 µg/L).

Furthermore, the application of the carrier solvent ethanol can also be regarded as an additional stressor in our experiment. The carrier solvent, however, was applied to all microcosms, including the controls. In both types of test system, ethanol caused a temporary drop in dissolved oxygen levels. This drop was more pronounced in the CS cosms, since DO levels had already sharply declined in the pre-treatment period due to the senescence of the filamentous algae bloom (Fig. 3). During the first weeks after TPT application, this more pronounced drop in DO levels in the CS cosms may have obscured some direct effects of TPT. This explains why in the PRC curves for macroinvertebrates (Fig. 4), zooplankton (Fig. 7) and phytoplankton (Fig. 9), the responses were less pronounced in the CS cosms than in the PS microcosms during the first 4 weeks after TPT application. Nevertheless, in both types of test system, the single application of TPT had a long-term impact on several aquatic organisms, so that pronounced treatment-related responses were still observed when DO oxygen levels had returned to normal levels.

Following the procedures described in Van Wijngaarden *et al.* [66] and Anderson and Ter Braak [67], Monte-Carlo permutation tests can also be used to test whether communities differ significantly between types of test system, and whether there are interactions between the factors ‘‘treatment’’ and ‘‘system type’’. Results of such an analysis (Table 11) fully support the above claims that the structure of the invertebrate communities (in particular the macroinvertebrate community) differed significantly between the two types of test system and that interaction between community structure and TPT treatment regime only occurred during the first weeks after TPT application, when DO levels were much lower in the CS microcosms.

In our paper we expressed the responses of the aquatic populations and communities in terms of nominal treatment levels (= initial peak concentrations). This may complicate the extrapolation of out microcosm results to the field, since peak concentrations are often not monitored in the field. In addition, the long-term response that we observed in our test systems suggests chronic toxicity. To facilitate the extrapolation of our data to measured data from the field initial nominal concentrations used in the present study have been translated in Tables 12 and 13 into chronic twenty-one day exposure data. As a reference point concentrations at twenty-one days have been selected because the present study focusses on invertebrates and the chronic toxicity test for the standard invertebrate test species (*Daphnia magna*) last for twenty-one days. Also Fig. 2 shows that concentrations in sediment and pore-water remain rather constant indicating that in our study concentrations at 21 days have sufficient predictive value for longer term exposure concentrations in pore-water and sediment. In Tables 12 and 13 exposure via the water column is

expressed in three ways: initial nominal concentrations, concentration at 21 days, and the time-weighted average (TWA) over a twenty-one day period.

These summary tables use five effect classes (adapted after [68]) to facilitate the comparison of TPT-related responses between the two types of microcosm. TPT-related effects were observed in both CS cosms and PS cosms, at all treatment levels. At the lowest treatment level of 1 µg/L, however, effects on sensitive community endpoints in both types of test system were slight and transient (effect class 2), while observed effects on sensitive population endpoints were followed by full recovery (effect classes 3 and 4 in CS cosms and PS cosms, respectively). At the two highest treatment levels (30 and 100 µg TPT/L), class 5 effects (clear effects without full recovery) were recorded in both the CS and PS cosms, and for both community-level and population-level endpoints.

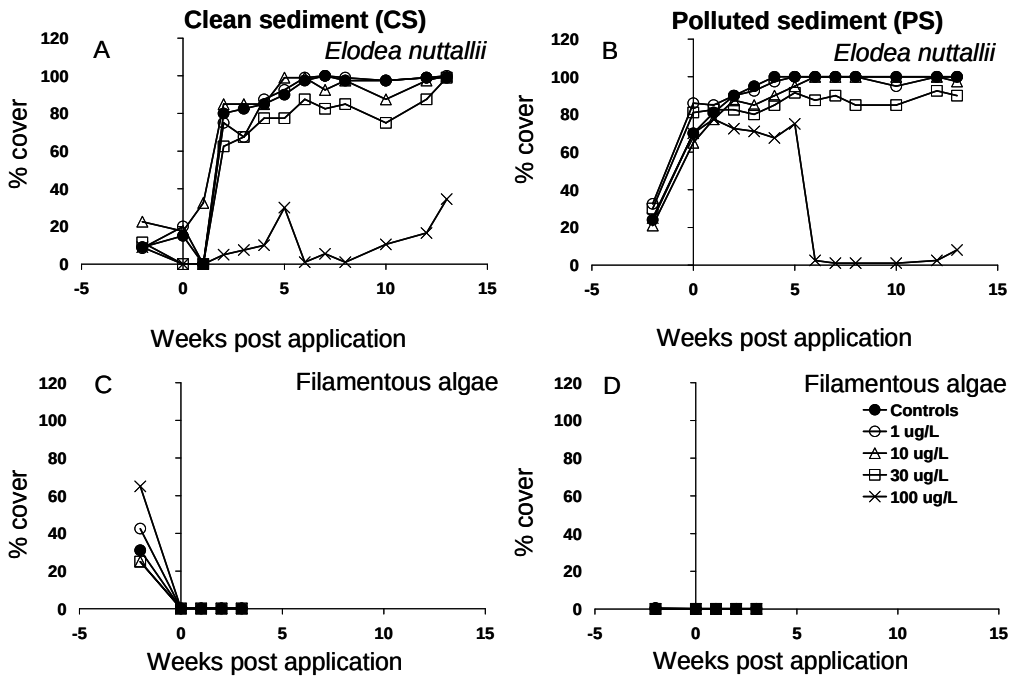


Figure 11: Dynamics in % cover of the vascular plant Elodea nuttallii (A and B) and of filamentous algae (C and D) in TPT treated microcosms with clean (CS) and polluted (PS) sediments. Calculated NOEC values are presented in Tables 4 and 5

Table 11. Results of Monte-Carlo permutation tests (p-values) on the combined macroinvertebrate and combined zooplankton datasets of the microcosms constructed with clean (CS) and polluted (PS) sediments and treated with TPT.

Week	Macroinvertebrates Treatment	System type	Interaction	Zooplankton Treatment	System type	Interaction
-4	>0.05	0.005	>0.05	>0.05	>0.05	>0.05
-2	>0.05	0.035	>0.05	>0.05	>0.05	>0.05
-1	-	-		>0.05	>0.05	>0.05
0.4	0.025	0.040	>0.05	>0.05	>0.05	>0.05
1	-	-	-	0.002	0.015	>0.05
2	0.005	0.005	0.010	>0.05	0.005	0.003
4	0.005	0.005	0.025	0.004	0.005	0.002
6	-	-	-	0.001	0.035	>0.05
8	0.005	0.015	>0.05	0.001	0.015	>0.05
12	0.005	0.005	>0.05	0.001	>0.05	>0.05
23	0.005	0.040	>0.05	0.001	>0.05	>0.05
40	0.005	>0.05	>0.05	-	-	-

The statistical significance was tested of the treatment, system type, and interaction between ‘‘treatment’’ and ‘‘system type’’ following the procedures described in Van Wijngaarden et al.[66] and Anderson and Ter Braak [69].

In agricultural area’s water concentrations of 4.3 ng/L [70] and in marinas concentrations up to 90 ng/L have been reported [71]. In the present study water concentrations are still higher on day 21 after application, although water concentrations in the 1 µg/L treatment do approximate water concentrations in marinas (Tables 12 and 13). In freshwater sediments, TPT concentrations up to 70 and 380 µg/kg have been reported for, respectively, Lake Westeinder (The Netherlands) and Lake Lucerne (Swiss) [70, 71] which is comparable with sediment concentrations found in the 30 µg/L treatment of the present study (Tables 12 and 13).

Between the two types of system tested in the present study we found a few differences in the intensity and/or duration of TPT-related responses (Tables 12 and 13). In the CS cosms, more pronounced responses occurred in the zooplankton community (PRC zooplankton) in that no recovery was observed at the 30 µg/L treatment level, while full recovery was observed at this level in the PS cosms. A few phytoplankton populations showed a more sensitive response at the three lowest treatment levels in the CS cosms than in the PS cosms. In contrast, phytoplankton chlorophyll-*a* showed a more pronounced increase in the systems treated with 10 and 30 µg/L in the PS cosms than in the CS cosms. Furthermore, at least one sensitive endpoint for Tricladida (at 1 µg/L), Annelida, and Insecta (at 10 µg/L) and for macrophytes (at 30 µg/L) appeared to respond more sensitively in the PS cosms. Overall, however, it can be concluded that the responses of the most sensitive

endpoints for each category listed in Tables 12 and 13 hardly differed between the two types of test system. This is in agreement with the very similar TPT concentration dynamics in the overlying water of the CS and PS cosms during the first weeks after application (Fig. 1).

Table 12. Summary of effects observed in the clean sediment cosms treated with the fungicide TPT CS-cosms.

Initial nominal concentration	1 µg/L	10 µg/L	30 µg/L	100 µg/L
Concentration 21-days	0.1 µg/L*	0.7 µg/L	2.1 µg/L	7.0 µg/L*
TWA 21-days	0.3 µg/L*	3.1 µg/L	9.2 µg/L	30.8 µg/L*
Pore water concentration 21-days	6.2 ng/L*	62.2 ng/L	186.7 ng/L	118.5 ng/L*
Sediment concentration 21-days	6.8 µg/kg*	68.1 µg/kg	204.4 µg/kg	681.2 µg/kg*
Community-level responses				
PRC macroinvertebrate	2	4	5	5
PRC zooplankton	1	4	5	5
PRC benthic nematodes	1	1	1	1
PRC phytoplankton	1	2	2	2
Plankton Chl-*a*	1	1	2	3
Water quality parameters	1	2	3	3
Decomposition	1	1	1	1
Population level responses				
Tricladida	1	4-5	4-5	4-5
Annelida	3	3-4	5	5
Mollusca	2	5	5	5
Insecta	1	3-4	5	5
Macrocrustacea	1	1	2	3
Microcrustacea	2	3-4	5	5
Rotifera	3	3	4	4
Benthic nematoda	1	1	1	1
Phytoplankton	2-3	2-3	5	5
Macrophytes	1	1	1	5

*The numbers in the table refer to effect classes adapted after Brock et al. [68]. 1: No effects, 2: Slight transient effects, 3: Clear short-term effects, recovery time < 8 weeks, 4: Clear medium-term effects, recovery time > 8 weeks but within the period studied, 5: Clear long-term effects, full recovery not observed. TWA= Time Weighted Average, * extrapolated from calculated concentrations in the 10 and 30 µg/L treatments.*

Nevertheless, focusing on long-term community responses, the PRC curves of the macroinvertebrate and zooplankton communities indicate that the overall rate of recovery was somewhat faster in the PS cosms than in the CS systems (Figs. 4, 7; Tables 6 and 7). A possible explanation for this might be the higher organic matter content of the sediments in the PS cosms, as well as the larger macrophyte biomass in these test systems at the time of TPT application. Due to its high affinity

with organic matter and its resistance to breakdown [72], TPT is expected to accumulate in the macrophytes and sediment compartments. Indeed, our microcosm study showed that TPT appeared to be very persistent in the sediments, but total concentrations in sediments of the PS cosms were somewhat lower than in the CS cosms (Fig. 2), which is explained by the greater biomass of macrophytes in the PS systems at the time of TPT application. Since long-term effects were observed after a single application of TPT, it is plausible that at least part of the sorbed fraction of TPT remained bioavailable. There is a relatively high potential for bioaccumulation of TPT in freshwater organisms, suggesting that uptake by ingestion of particle-bound TPT should not be ignored [72, 73]. Sequestering of TPT in organic material which is processed rapidly by benthic biota and degradation of macrophyte-associated TPT, however, may have been stronger and faster in the PS cosms, which were characterized by a higher organic matter content of the sediment and a greater initial macrophyte biomass. This may have decreased the long-term bioavailability of sorbed TPT at a somewhat faster rate in the PS systems than in the CS microcosms. This may have masked possible effects of background pollutants because the overall response would be the sum of several stress factors. A more rapidly declining TPT effect plus a background pollutant effect can be of the same magnitude as the TPT effect in the CS cosms, thus creating a similar overall response.

The observation that overall responses of sensitive populations were rather similar between test system types is further substantiated by the fact that EC_{50} values could be calculated for several invertebrate populations sampled in the microcosms, resulting in very similar species sensitivity distribution (SSD) curves between the CS and PS microcosms (for detailed results see part II; [15]). Hazardous concentrations for 5% of the species (HC_5) calculated on the basis of these microcosm–SSD curves in sampling weeks 2–8 after TPT treatment ranged from 0.3 to 0.6 μg/L for the CS cosms and from 0.2 to 0.6 μg/L for the PS cosms. These data indicate that threshold concentrations of TPT for effects on invertebrate populations, calculated by means of SSD, were also very similar for the microcosms with clean and polluted sediments. Basing SSD curves not on initial nominal concentrations but on concentrations 21 days post-application which is approximately a tenfold lower results in a similarly decreased HC_5 value ranging from 20 to 60 ng/L which is lower than measured in freshwater marinas [71].

Table 13. Summary of effects observed in the polluted sediment cosms treated with the fungicide TPT PS-cosms.

Initial nominal concentration	1 µg/L	10 µg/L	30 µg/L	100 µg/L
Concentration 21-days	0.1 µg/L*	0.9 µg/L	2.8 µg/L	9.2 µg/L*
TWA 21-days	0.4 µg/L*	3.8 µg/L	11.5 µg/L	38.2 µg/L*
Pore water concentration 21-days	3.9 ng/L*	39.5 ng/L	118.5 ng/L	394.9 ng/L*
Sediment concentration 21-days	4.6 µg/kg*	46.1 µg/kg	138.2 µg/kg	460.6 µg/kg*
Community-level responses				
PRC macroinvertebrate	2	4	5	5
PRC zooplankton	1	3	3-4	5
PRC benthic nematodes	1	1	1	1
PRC phytoplankton	1	1	1	1
Plankton Chl-*a*	2	3	3-4	4
Water quality parameters	1	3	4	4
Decomposition	1	1	1	1
Population level responses				
Tricladida	3-4	3-4	5	5
Annelida	4	5	5	5
Mollusca	2	4	4	4
Insecta	2	5	5	5
Macrocrustacea	1	1	3	4
Microcrustacea	1	3	4	4-5
Rotifera	2	3	3-4	5
Benthic nematoda	1	1	1	1
Phytoplankton	1	1	1	4
Macrophytes	1	1	3-4	5

*The numbers in the table refer to effect classes described in detail in Brock et al. [68]. 1: No effects, 2: Slight transient effects, 3: Clear short-term effects, recovery time < 8 weeks, 4: Clear medium-term effects, recovery time>8 weeks, 5: Clear long-term effects, full recovery not observed. TWA=Time Weighted Average, *extrapolated from calculated concentrations in the 10 and 30 µg/L treatments.*

In conclusion, sediment quality impacted on the structure and functioning of the aquatic community, but no effects of background pollutants were detected on the response of the aquatic community to TPT. Threshold concentrations and measurement endpoints indicative of direct toxic effects of TPT were very similar between the microcosms with clean and those with polluted sediments. Long-term recovery of the macroinvertebrate and zooplankton communities tended to be somewhat faster in the polluted sediment systems, possibly because of a slightly stronger sequestering of TPT sorbed to organic matter and the higher productivity of primary producers in these systems.

Acknowledgements - This study was subsidized by the Netherlands Organization for Scientific Research (NWO) as part of the Stimulation Program System-oriented Ecotoxicological Research (SSEO) (project no. 014.23.012). In addition, the research was supported by the UK Department of Environment, Food and Rural Affairs (Defra) and the Dutch Ministry of Agriculture, Nature and Food Safety, as part of a research program focusing on the scientific underpinning of risk assessment procedures for fungicides in the aquatic environment. The authors are indebted to G.H.P. Arts, J.D.M. Belgers, M.C. Boerwinkel, R. Gylstra, H. Keidel, C. van Rhenen-Kersten, L.J.T. van der Pas and R.P.A. van Wijngaarden for practical assistance. We owe a special debt of gratitude to P.J. Van den Brink of Alterra for his support in the statistical analysis of the microcosm data, and to Prof. M. Scheffer of Wageningen University, who commented on an earlier version of the manuscript.

References

[1] Folt, C. L., Chen, C. Y., Moore, M. V. and Burnaford, J. 1999. Synergism and antagonism among multiple stressors. Limnology and Oceanography 44 pp. 864-877

[2] Heugens, E. H. W., Hendriks, A. J., Dekker, T., Van Straalen, N. M. and Admiraal, W. 2001. A review of the effect of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31 pp. 247-284

[3] Van der Geest, H. G., Soppe, W. J., Greve, G. D., Kroon, A. and Kraak, M. H. S. 2002. Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly *Ephoron virgo*. Environmental Toxicology and Chemistry 21 pp. 431-436

[4] Beurskens, J. E. M., Mol, G. A. J., Barreveld, H. L., Van Munster, B. and Winkels, H. J. 1993. Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environmental Toxicology and Chemistry 12 pp. 1549-1566

[5] Stäb, J. A., Cofino, W. P., Van Hattum, B. and Brinkman, U. A. T. 1994. Assessment of transport routes of triphenyltin used in potato culture in the Netherlands. Analytica Chimica Acta 286 pp. 335-341

[6] Liess, M. and Schulz, R. 1999. Linking insecticide contaminantion and population response in an agricultural stream. Environmental Toxicology and Chemistry 18 pp. 1948-1955

[7] Grigarick, A. A., Webster, R. K., Meyer, R. P., Zalom, F. G. and Smith, K. A. 1990. Effect of pesticide treatments on nontarget organisms in california rice paddies. Hilgardia 58 pp. 1-36

[8] Fargasová, A. 1998. Comparison of effects of tributyl-, triphenyl-, and tribenzyltin compounds on freshwater benthos and alga *Scenedesmus quadricauda*. Bulletin of Environmental Contamination and Toxicology. 60 pp. 9-15

[9] Petersen, S. and Gustavson, K. 2000. Direct toxic effects of TBT on natural enclosed phytoplankton at ambient TBT concentrations of coastal waters. Ecotoxicology 9 pp. 273-285

[10] Rehage, J. S., Lynn, S. G., Hammond, J. I., Palmer, B. D. and Sih, A. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile ambystoma barbouri salamanders. Environmental Toxicology and Chemistry 21 pp. 807-815

[11] Laughlin, R. B. and Linden, O. 1985. Fate and effects of organotin compounds. Ambio 14 pp. 88-94

[12] Vighi, M. and Calamari, D. 1985. QSARs for organotin compounds on *Daphnia magna*. Chemosphere 14 pp. 1925-1932

[13] Loch, J. P. G. 1990. Accumulation and leaching of the fungicide fentin acetate and intermediates in sandy soils. Water, Air, and Soil Pollution 53 pp. 119-129

[14] Fent, K., Hunn, J. and Sturm, M. 1991. Organotins in lake sediment. Naturwissenschaften 78 pp. 219-221

[15] Roessink, I., Belgers, J. D. M., Crum, S. J. H., Van den Brink, P. J. and Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field. Ecotoxicology 15 pp. 411-424

[16] De Haas, E. M., Reuvers, B., Moermond, C. T. A., Koelmans, A. A. and Kraak, M. H. S. 2002. Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediment. Environmental Toxicology and Chemistry 21 pp. 2165-2171

[17] Moermond, C. T. A. and Koelmans, A. A. 2002. Stofstroom-analyse. Zware metalen, PCBs en PAKs in uiterwaardplassen. Winterbemonstering 2002 in de plassen Deest Oost3A, 3B en 4. Wageningen University. Wageningen. The Netherlands

[18] Fargasová, A. 1997. Comparative study of ecotoxicological effect of triorganotin compounds on various biological subjects. Ecotoxicology and Environmental Safety 36 pp. 38-42

[19] De Zwart, D. 2002. Observed regularities in species sensitivity distributions for aquatic species. In: Posthuma, L., Suter II, G. W. and Traas, T. P., eds, Species sensitivity distributions in ecotoxicology, Lewis Publishers, Boca Raton, pp. 133-154

[20] NVN5729:2001. 2001. Soil - Determination of organotin compounds in soil and sediment. NEN.

[21] Brock, T. C. M., Van den Bogaert, M., Bos, A. R., Van Breukelen, S. W. F., Reiche, R. T., J., Suykerbuyk, R. E. M. and Roijackers, R. M. M. 1992. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. Archives of Environmental Contamination and Toxicology 23 pp. 391-409

[22] Moed, J. R. and Hallegraeff, G. M. 1978. Some problems in the estimation of chlorophyll-a and phaeopigments from pre- and post-acidification spectrophotometric measurements. Internationale Revue der Gesamten Hydrobiologie 63 pp. 787-800

[23] Brock, T. C. M., Huijbrechts, C. A. M., Van de Steeg-Huberts, M. J. H. A. and Vlassak, M. A. 1982. In situ studies on the breakdown of *Nymphoides peltata* (Gmel.) O. kuntze (Menyanthacea); some methodological aspects of the litter bag technique. Hydrobiological Bulletin 16 pp. 35-49

[24] Van den Brink, P. J., Van Donk, E., Gylstra, R., Crum, S. J. H. and Brock, T. C. M. 1995. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31 pp. 3181-3200

[25] Williams, D. A. 1972. The comparison of several dose levels with zero dose control. Biometrics 28 pp. 519-531

[26] Hommen, U., Veith, D. and Ratte, H. T. 1994. A computer program to evaluate plankton data from freshwater field tests. In: Hill, I. R., Heimbach, F., Leeuwangh, P., Matthiesen, P., eds, Freshwater Field Tests for Hazard Assessment of Chemicals, Lewis, Boca Raton, FL, pp. 503-513

[27] Van den Brink, P. J. and Ter Braak, C. J. F. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18 pp. 138-148

[28] Van den Brink, P. J. and Ter Braak, C. J. F. 1997. Ordination of responses to toxic stress in experimental ecosystems by Principle Response Curves and similarity analysis. Toxicol. Ecotoxicol. News 4 pp. 174-178

[29] Van den Brink, P. J. and Ter Braak, C. J. F. 1998. Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. Aquatic Ecology 32 pp. 163-178

[30] Ter Braak, C. J. F. and Smilauer, P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Ithaca. New York: Microcomputer Power. USA

[31] Verdonschot, P. F. M. and Ter Braak, C. J. F. 1994. An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analyses with Monte Carlo permutation tests. Hydrobiologia 287 pp. 251-266

[32] Van den Brink, P. J., Van Wijngaarden, R. P. A., Lucassen, W. G. H., Brock, T. C. M. and Leeuwangh, P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environmental Toxicology and Chemistry 15 pp. 1143-1153

[33] Bongers, T. 1990. The maturity Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83 pp. 14-19

[34] Bongers, T. and Bongers, M. 1998. Functional diversity of nematodes. Applied Soil Ecology 10 pp. 239-251

[35] Marsot, P., Pelletier, E. and St-Louis, R. 1995. Effects of triphenyltin chloride on growth of the marine microalga *Pavlova lutheri* in continuous culture. Bulletin of Environmental Contamination and Toxicology. 53 pp. 389-395

[36] Mooney, H. M. and Patching, J. W. 1995. Triphenyltin inhibits photosynthesis and respiration in marine microalgae. Journal of Industrial Microbiology 14 pp. 265-270

[37] Brock, T. C. M., Vet, J. J. R., Kerkhofs, M. J. J., Lijzen, J., Van Zuilekom, W. J. and Gylstra, R. 1993. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte free freshwater model ecosystems: III. Aspects of ecosystem functioning. Archives of Environmental Contamination and Toxicology 25 pp. 160-169

[38] Bongers, T. 1999. The maturity index, the evolution of nematode life histrory traits, adaptive radiation and cp-scaling. Plant and Soil 212 pp. 13-22

[39] Austen, M. C. and McEvoy, A. J. 1997. Experimental effects of tributyltin (TBT) contaminated sediment on a range of meiobenthic communities. Environmental Pollution 96 pp. 435-444

[40] Fliedner, A., Remde, A., Niemann, R., Schäfers, C. and Stein, B. 1997. Effects of the organotin pesticide azocyclotin in aquatic microcosms. Chemosphere 35 pp. 209-222

[41] Kasai, F. and Hanazato, T. 1995. Effects of the triazine herbicide, Simetryn, on freshwater plankton communities in experimental ponds. Environmental Pollution 89 pp. 197-202

[42] Blanck, H. and Dahl, B. 1996. Pollution-induced community tolerance (PICT) in marine periphyton in a gradient of tri-*n*-butyl (TBT) contamination. Aquatic Toxicology 35 pp. 59-77

[43] Blanck, H. 2002. A critical review of procedures and approaches used for assessing Pollution-Induced Community Tolerance (PICT) in biotic communities. Human and Ecological Risk Assessment 8 pp. 1003-1034

[44] Roessink, I., Arts, G. H. P., Belgers, J. D. M., Bransen, F., Maund, S. J. and Brock, T. C. M. 2005. Effects of lambda-cyhalothrin in two ditch mesocosm systems of different trophic status. Environmental Toxicology and Chemistry 24 pp. 1684-1696

[45] Jak, R. G., Ceulemans, M., Scholten, M. C. T. and Van Straalen, N. M. 1998. Effects of tributyltin on a coastal north sea plankton community in enclosures. Environmental Toxicology and Chemistry 17 pp. 1840-1847

[46] Baird, D. J., Brock, T. C. M., De Ruiter, P. C., Boxall, A. B. A., Culp, J. M., Eldridge, P., Hommen, U., Jak, R. G., Kidd, K. A. and DeWitt, T. H. 2001. The food web approach in the environmental management of toxic substances. In: Baird, D. J. and Burton Jr, G. A., eds, Ecological variability: Separating natural from anthropogenic causes of ecosystem impairment, SETAC Press, Pensacola, pp. 83-122

[47] Fleeger, J. W., Carman, K. R. and Nisbet, R. M. 2003. Indirect effects of contaminants in aquatic ecosystems. The Science of the Total Environment 317 pp. 207-233

[48] Traas, T. P., Janse, J. H., Van den Brink, P. J., Brock, T. C. M. and Aldenberg, T. 2004. A freshwater food web model for the combined effects of nutrients and insecticide stress and subsequent recovery. Environmental Toxicology and Chemistry 23 pp. 521-529

[49] Hynes, H. B. N. 1963. The biology of polluted waters. Liverpool University Press, Liverpool

[50] Tun-Lin, W., Burkot, T. R. and Kay, B. H. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Medical and Veterinary Entomology 14 pp. 31-37

[51] Koenraadt, C. J. M. and Takken, W. 2003. Cannibalism and predation among larvae of the *Anopheles gambiae* complex. Medical and Veterinary Entomology 17 pp. 61-66

[52] Dahl, D. and Blanck, H. 1996. Toxic effects of the antifouling agent Irgagol 1051 on periphyton communities in coastal water microcosms. Marine Pollution Bulletin 32 pp. 342-350

[53] Evans, S. M., Kerrigan, E. and Palmer, N. 2000. Causes of imposex in the dogwhelk *Nucella lapillus* (L.) and its use as a biological indicator of Tributyltin contamination. Marine Pollution Bulletin 40 pp. 212-219

[54] Cuppen, J. G. M., Van den Brink, P. J., Camps, E., Uil, K. F. and Brock, T. C. M. 2000. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. Aquatic Toxicology 48 pp. 233-250

[55] Van den Brink, P. J., Hattink, J., Bransen, F., Van Donk, E. and Brock, T. C. M. 2000. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquatic Toxicology 48 pp. 251-264

[56] Peeters, E. T. H. M., Gardeniers, J. J. P. and Koelmans, A. A. 1999. Contribution of trace metals in structuring in situ macroinvertebrate community composition along a salinity gradient. Environmental Toxicology and Chemistry 19 pp. 1002-1010

[57] Reinhold-Dudok van Heel, H. C. and Den Besten, P. J. 1999. The relation between macroinvertebrate assemblages in the Rhine-Meuse delta (The Netherlands) and sediment quality. Aquatic Ecosystem Health and Management 2 pp. 19-38

[58] Peeters, E. T. H. M., Dewitte, A., Koelmans, A. A., Van der Velden, J. A. and Den Besten, P. J. 2001. Evaluation of bioassays versus contaminant concentration in explaining the macroinvertebrate community structure in the rihine-meuse delta, the Netherlands. Environmental Toxicology and Chemistry 20 pp. 2883-2891

[59] LaLonde, S. and Downing, J. D. 1992. Phytofauna of eleven macrophyte beds of differing trophic status, depth, and composition. Canadian Journal of Fisheries and Aquatic Sciences 49 pp. 992-1000

[60] Brock, T. C. M. and Van der Velde, G. 1996. Aquatic macroinvertebrate community structure of a *Nymphoides peltata*-dominated and macrophyte-free site in an oxbow lake. Netherlands Journal of Aquatic Ecology 30 pp. 151-163

[61] Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London-Weinheim-New York-Tokyo-Melbourne-Madras

[62] Crommentuijn, T., Sijm, D., De Bruin, J., Van Leeuwen, K. and Van de Plassche, E. 2000. Maximun permissible and negligible concentrations for some organic substances and pesticides. Journal of Environmental Management 58 pp. 297-312

[63] Van Griethuysen, C., Meijboom, E. W., Koelmans, A. A. 2003. Spatial variation of metals and acid volatile sulfide in floodplain lake sediment. Environmental Toxicology and Chemistry 22 pp. 457-465

[64] De Haas, E. M. 2004. Persistence of benthic invertebrates in polluted sediments. Universiteit van Amsterdam, Amsterdam,

[65] De Haas, E. M., Roessink, I., Verbree, B., Koelmans, A. A., Kraak, M. H. S. and Admiraal, W. 2005. Influence of sediment quality on the responses of benthic invertebrates after treatment with the fungicide triphenyltin acetate. Environmental Toxicology and Chemistry 24 pp. 1133-1139

[66] Van Wijngaarden, R. P. A., Brock, T. C. M., Van den Brink, P. J., Gylstra, R. and Maund, S. J. 2006. Ecological effects of spring and late-summer applications of Lambda-cyhalothrin in freshwater microcosms. Archives of Environmental Contamination and Toxicology 50 pp. 220-239

[67] Anderson, M. J. and Ter Braak, C. J. F. 2003. Permutation tests for multi-factorial analysis of variance. Journal of Statistical Computation and Simulation 73 pp. 85 - 113

[68] Brock, T. C. M., Van Wijngaarden, R. P. A. and Van Geest, G. J. 2000. Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra. Wageningen. The Netherlands

[69] Anderson, E. 1969. Life-cycle and growth of *Asellus aquaticus* (L.) with special reference to the effects of temperature. Rep. Inst. Freshwat. Res. Drottningholm 49 pp. 5-26

[70] Stäb, J. A. 1995. Organotin compounds in the aquatic environment. Determination, occurrence and fate. Vrije Universiteit, Amsterdam, The Netherlands

[71] Fent, K. and Hunn, J. 1995. Organotins in freshwater harbours and rivers: temporal distribution, annual trends and fate. Environmental Toxicology and Chemistry 14 pp. 1123-1132

[72] Looser, P. W., Fent, K., Berg, M., Goudsmit, G.-H. and Schwarzenbach, R. P. 2000. Uptake and elimination of triorganotin compounds by larval midge Chironomus riparius in the abscence and presence of aldrich humic acid. Environmental Science and Technology 34 pp. 5165-5171

[73] Fent, K. 1996. Ecotoxicology of organotin compounds. Critical Reviews in Toxicology 26 pp. 1-117

Chapter 4

Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field

I. Roessink
J. D. M. Belgers
S. J. H. Crum
P. J. van den Brink
T. C. M. Brock

Ecotoxicology (2006) 15 pp. 411-424

Abstract

The study objectives were to shed light on the types of freshwater organisms that are sensitive to triphenyltin acetate (TPT) and to compare the laboratory and microcosm sensitivities of the invertebrate community. The responses of a wide array of freshwater taxa (including invertebrates, phytoplankton and macrophytes) from acute laboratory Single Species Tests (SST) were compared with the concentration–response relationships of aquatic populations in two types of freshwater microcosms. Representatives of several taxonomic groups of invertebrates, and several phytoplankton and vascular plant species proved to be sensitive to TPT, illustrating its diverse modes of toxic action. Statistically calculated ecological risk thresholds (HC_5 values) based on 96 h laboratory EC_{50} values for invertebrates were 1.3 μg/L, while these values on the basis of microcosm-Species Sensitivity Distributions (SSD) for invertebrates in sampling weeks 2–8 after TPT treatment ranged from 0.2 to 0.6 μg/L based on nominal peak concentrations. Responses observed in the microcosms did not differ between system types and sampling dates, indicating that ecological threshold levels are not affected by different community structures including taxa sensitive to TPT. The laboratory-derived invertebrate SSD curve was less sensitive than the curves from the microcosms. Possible explanations for the more sensitive field response are delayed effects and/or additional chronic exposure via the food chain in the microcosms.

Introduction

Relatively few published studies have dealt with ecological risks of fungicides to freshwater communities [1]. Although several studies on the fate and effects of fungicides in aquatic ecosystems have recently been published [1-6] our knowledge of the ecological impact of fungicides is still limited.

Several of the fungicides studied to date appear to have biocidal properties, and the fact that some of these compounds may also exhibit endocrine-disrupting abilities has certainly drawn attention to this group. An example of this group are the organotins [7, 8], which are amongst the more frequently studied biocides. The present study deals with the organotin compound triphenyltin acetate (TPT), a fungicide for which little adequate freshwater laboratory toxicity data and no field or semi-field toxicity data has been published.

Organotins, including TPT, are highly toxic to all sorts of aquatic primary producers, invertebartes and vertebrates [9-12]. Organotins have been reported to inhibit mitochondrial oxidative phosphorylation and consequently energy transfer, Ca^{2+} homeostasis, protein and DNA synthesis in the cell [13-15], to cause immunosuppression and premature

apoptosis (programmed cell death) in both vertebrates and invertebrates [16, 17], and have photosynthesis inhibiting properties [18]. This variety of fundamental processes are not immediately visible and may take time before effects can be observed. Comparative studies of the relative toxicity of organotin compounds to aquatic organisms like marine crab larvae (*Rhitropanopeus harrisii*) and the freshwater microcrustacean *Daphnia magna* have shown that TPT and TBT (anti-fouling) are amongst the most toxic of these [19, 20].

The first objective of the present paper was to shed light on the types of freshwater organism that are sensitive to TPT. To this end, the acute effects of TPT on a wide array of freshwater taxa (including invertebrates, phytoplankton, and macrophytes) were tested in a laboratory setting by means of Single Species Tests (SST). Species vary markedly in their sensitivity to environmental contaminants, and this variation can be described by constructing a species sensitivity distribution (SSD). The SSD is a statistical distribution estimated from a sample of toxicity data and visualized as a cumulative distribution function [21]. Species sensitivity distributions are used to calculate the concentration at which a specified proportion of species will be affected, referred to as the hazardous concentration (HC) for *p* (%) of species (HC_p) [22]. In this way SSDs can be used to assess the potential impact of substances on aquatic ecosystems via direct toxic effects.

The second objective of the present study was to test if aquatic invertebrates measured in the laboratory show the same concentration–response relationships as aquatic invertebrate populations in outdoor microcosms and to address the difficulties involved comparing the two. Since organotin compounds are reported to exert effects via foodchain exposure, is hypothesized that only in the field situation full effects can be observed. When comparing the field with the lab response the first is hypothesized to be more sensitive.

SSDs were constructed for different endpoints (*e.g.*, macrophytes, invertebrates) and for different test systems (*e.g.*, lab, field), compared, and statistically tested for differences. Also SSDs were used to estimate the hazardous concentrations to 5% (HC_5) of the species. This procedure allowed us to evaluate the predictive value of acute laboratory toxicity tests for field effects in the assessment of ecological hazards of a single application of the fungicide TPT in freshwater ecosystems. A detailed description of the semi-field experiment, which studied the ecological impact of a single application of TPT in outdoor microcosms, has been provided in part I [23].

Materials and methods

SST in the laboratory

All tests were performed with Fentin acetate Pestanal (CAS No. 900-95-8; Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands), which was applied once at the start of each experiment. Nominal concentrations of TPT applied in the different tests are presented in Table 1. In all treatments, 0.04 % (v/v) 96% ethanol was used as a carrier solvent. Except for the algae tests, all experiments were performed with two controls, *viz*., a solvent control with an equal amount of ethanol (coded: 0+) and a 'normal' control containing only test medium (coded: 0). For logistic reasons, the algae experiments were only performed with a solvent control. To assess initial exposure concentrations, water samples were taken 1 h after TPT application. In addition, intermediate treatment concentrations were measured at 0, 1, 2, 3, and 4 days after the TPT application in tests with *Endochironomus albipennis* and *Gammarus pulex*, to study the dynamics of this substance during the test. In other invertebrate tests, water samples were only taken on day 0 (1 h after application) and on day 4 (at the end of the test). In the macrophyte tests, which lasted 21 days, TPT concentrations were measured on days 0, 2, 7, 14 and 21. The small test volumes of the algae test flasks did not allow sampling for TPT concentration assessment. Unfortunately, it was impossible to use extra 'fate' flasks, which could be sacrificed for TPT sampling, so TPT exposure concentrations in the algae tests were estimated from measurements in the stock solutions.

For the chemical analysis of TPT, depth-integrated 100-mL water samples were taken out of the test units and stored in a 250-mL flask. To these water samples was added 2 mL buffer solution (pH=5; 120 g HAc+272 g NaAc per liter), 100 µL 2% sodium tetraethyl borate and 20 mL hexane. The water was extracted by shaking for 15 min at 175 bpm. Part of the hexane layer was removed and transferred to a GC vial. Organotin analysis was performed on a GC-MSD in Selective Ion Mode (GC: HP 6890 with auto-injector HP 7683; MSD: HP 5973 Network MSD). The detection limit of TPT in water was 1 µg/L. The recovery of the extraction procedure was tested by spiking blank water samples with a known amount of TPT in ethanol. The recovery was found to be 92.7% (n=4; sd=12.7%); because this was within the measuring error of the GC-MS, no corrections were made for this recovery.

Macroinvertebrate SSTs were performed using two replicates per treatment level, and lasted 4 days (96 h). Most tests were performed in 1.8-L glass jars containing 1.5 L filtered (45 µm) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station (Table 2). Most macroinvertebrate tests involved twenty specimens per jar. The taxa *Endochironomus albipennis*, *Glyptotendipes*

sp., *Lumbriculus variegatus* and *Tubifex sp.*, however, were tested individually in 10-mL glass jars. This was necessary because affected specimens were cannibalized by less affected specimens when tested in the same jar (*Endochironomus* and *Glyptotendipes*) or because all specimens formed a tight ball which prevented accurate observation (*Lumbriculus* and *Tubifex*). The tests were done in a temperature-controlled room (20±2 ºC) with a 14 h light:10 h dark regime. The test media were not aerated during the tests. Within 4 h of dosing, dissolved oxygen concentrations (YSI model 58) and pH (WTW pH323, equipped with a Sentix pH electrode) were measured in all test units. In addition, DO and pH were measured daily at a fixed time in at least the controls and the treatments with highest concentrations (Table 2). As described in earlier experiments [29] the test medium, obtained from our experimental ditches, had an average dissolved organic carbon (DOC) concentration of 8.8 mg/L. Overnight mortality and excrements sometimes affected water quality parameters (*e.g.*, lower range of DO or pH in *Lymnaea* and *Gammarus* test). However, remaining organisms survived till the end of the test period and did not suggest increased toxicity of TPT under these conditions.

Zooplankton tests used two replicates per treatment level and lasted 4 days (96 h). We used 600-mL glass jars containing 250 mL filtered (45 µm) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station (Table 2). Other test conditions were similar to the macroinvertebrate tests.

Macrophyte tests were performed in duplicate and lasted 21 days. They were conducted in 1.8-L glass jars containing 1.5 L filtered (45 µm) nutrient-poor water originating from experimental ditches located at the Sinderhoeve field station. In these tests, the water was additionally enriched with the inorganic nutrients N (0.5 mg/L), P (0.075 mg/L) and C (0.08 mg/L), as well as with 0.1 mL/L Tropica Mastergrow (K: 0.79, Mg: 0.39, S:1.01, B: 0.004, Cu: 0.006 Fe: 0.07, Mn: 0.04 Mo: 0.002 and Zn: 0.002 (W/W%)).

Table 1. Species tested, with their concentration range. The concentration ranges presented were used in Single Species Tests (SST); when no SST was performed, the range from the Range Finding Test (RFT) is presented. Triphenyltin acetate (TPT) range presented are initial nominal concentrations. 0=control; 0+=solvent control

#	Species	RFT	SST	Concentration range (µg/L)
1	*Acanthocyclops venustus*	X	X	0, 0+, 1.7, 5, 15, 45, 135
2	*Asellus aquaticus*	X	X	0, 0+, 25, 65, 160, 400, 1000
3	*Bythinia tentaculata*	X	-[a]	0, 0+, 10, 50, 200, 1000
4	*Chaoborus obscuripes*	X	-[a]	0, 0+, 10, 50, 200, 1000
5	*Cloeon dipterum*	X	X	0, 0+, 25, 50, 100, 200, 1000
6	*Daphnia galeata*	X	X	0, 0+, 1.7, 5, 15, 45, 135
7	*Dugesia sp.*	X	X	0, 0+, 2.7, 5.5, 11, 22, 44
8	*Endochironomus albipennis*	X	X	0, 0+, 100, 200, 400, 800, 1600
9	*Erpobdella juv.*	X	X	0, 0+, 4, 10, 25, 60, 150
10	*Gammarus pulex*	X	X	0, 0+, 1.9, 4.8, 12, 30, 75
11	*Glyptotendipes sp.*	X	X	0, 0+, 100, 200, 400, 800, 1600
12	*Lumbriculus variegatus*	X	X	0, 0+, 4, 8, 16, 32, 64
13	*Lymnaea stagnalis*	X	X	0, 0+, 15, 38, 95, 237.5, 594
14	*Physa fontinalis*	X	X	0, 0+, 1.75, 4.4, 11, 28, 69
15	*Planorbis contortis*	X	X	0, 0+, 1.75, 4.4, 11, 28, 69
16	*Polycelis niger/tenuis*	X	X	0, 0+, 2.7, 5.5, 11, 22, 44
17	*Proasellus meridianus/coxalis*	X	X	0, 0+, 2.7, 5.5, 11, 22, 44
18	*Sigara sp.*	X	-[a]	0, 0+, 10, 50, 200, 1000
19	*Sphaerium sp.*	X	-[a]	0, 0+, 10, 50, 200, 1000
20	*Tubifex*	X	X	0, 0+, 4, 8, 16, 32, 64
21	*Desmodesmus subspicatus*	X	X	0+, 0.3, 1, 3, 10, 30, 100
22	*Monoraphidium minutum*	X	X	0+, 0.3, 1, 3, 10, 30, 100
23	*Scenedesmus quadricauda*	X	X	0+, 0.3, 1, 3, 10, 30, 100
24	*Selenastrum capricornutum*	X	X	0+, 0.3, 1, 3, 10, 30, 100
25	*Lemna minor*	-	X	0, 0+, 1, 10, 30, 100, 1000
26	*Lemna trisulca*	-	X	0, 0+, 1, 10, 30, 100, 1000
27	*Elodea nuttallii*	-	X	0, 0+, 1, 10, 30, 100, 1000
28	*Elodea canadensis*	-	X	0, 0+, 1, 10, 30, 100, 1000
29	*Potamogeton crispus*	-	X	0, 0+, 1, 10, 30, 100, 1000
30	*Myriophyllum spicatum*	-	X	0, 0+, 1, 10, 30, 100, 1000
31	*Ceratophyllum demersum*	-	X	0, 0+, 1, 10, 30, 100, 1000
32	*Spirodela polyrhiza*	-	X	0, 0+, 1, 10, 30, 100, 1000

[a]*=No reaction to short-term TPT exposure.*

These amounts of inorganic nutrients (N, P and K), inorganic C and trace elements were added twice a week. Other test conditions were similar to those of the invertebrate testing, with the only difference that extra illumination was provided to ensure good macrophyte growth. The

macrophytes were illuminated with Philips HPI-T, 400 W lamps at 223 μmol/m^2/s at the water surface using a 14 h light:10 h dark regime. DO and pH measurements took place in all treatments on days 1, 6, 8, 13, 15, 20 after application. Degrading biomass resulted in lower DO and pH levels in higher treatments but there was no indication that sensitivity of the macrophytes to TPT was affected.

Algae tests were performed in 100-mL cellulose-plug capped Erlenmeyer flasks with 50 mL fresh medium [24] and an initial algae density of 2*106 μm^3/ mL (on a biovolume basis using a Coulter Multisizer II electronic particle counter). Three replicates per treatment were used and the test was run for 4 days (96 h). Test units were constantly illuminated by cool-fluorescent white tubes producing 100 μmol/m^2/s at the water surface (Osram L 36W/21-840, OSRAMNederland BV, Alphen a/d Rijn, The Netherlands), at a temperature of 20±1 ºC. Phytoplankton taxa originated from algae stock cultures that have been maintained for years at the laboratory of the Department of Aquatic Ecology and Water Quality Management (Wageningen University) by regular (every 2–4 weeks) inoculation of existing stock material in fresh autoclaved medium.

Endpoints

In the invertebrate tests, sub-lethal (behavioral and immobility) and lethal effects were monitored. Since mortality is the ultimate phase of immobility, scores for mortality and immobility were summated into one logistic regression analysis of EC_x-values (Effect Concentration where x% of the population is affected). For all invertebrates, effects were scored as mortality when no response of any kind was observed for about 10 s under a stereomicroscope after repeated tactile stimulation of the organism's body. A behavioral effect was scored when invertebrate behavior in treated systems deviated from controls.

The measurement endpoints in the macrophyte tests were biomass, which was converted to relative growth (using the biomass at the start of the test), and photosystem II efficiency (Φ_{PSII}), an endpoint frequently used in phytoplankton testing [25-27] which can also be used in macrophyte testing [28]. Φ_{PSII} is a measure of the efficiency of the photosystem II electron flow, measured as chlorophyll fluorecence, and was sampled non-destructively by means of a mini-PAM photosynthesis yield analyzer (WALZ, Germany). Since the structure of the aquatic plants prevented the use of the 'leaf clip' supplied with this analyzer, the diode of the mini-PAM was fixed at 3 mm from the macrophyte by means of an adjustable stand. Every plant was sampled three times, with two-minute intervals between measurements. These three samples were pooled and the average was used for further analysis. Φ_{PSII} was sampled

on days 0, 2, 7, 9, 14, 16 and 21, while biomass was sampled at the beginning and end of the experiment. The biomass at the start of the experiment was estimated by weighing three extra portions.

The measurement endpoint for algae was Φ_{PSII}. A PHYTO-PAM phytoplankton analyzer (WALZ, Germany) was used to measure photosynthetic activity (Φ_{PSII}) every day; this was converted to chlorophyll-*a* content of the algae [27].

Field experiment

In 2001, an outdoor microcosms experiment with TPT was performed at the Sinderhoeve experimental field station at Renkum, The Netherlands, using a total of 20 concrete cosms (length 140 cm, width 120 cm, and height 80 cm) with a water column of approximately 50 cm and a sediment layer of approximately 10 cm. DOC, suspended solids, and chlorophyll-*a* concentrations in the water phase were 8.8 mg/L, 4.9 mg/L and 58.5 µg/L, respectively, as determined in earlier experiments by Roessink *et al.* [29]. The microcosm experiment aimed to compare the ecological impact of a single application of TPT between test systems with clean and systems with polluted sediments derived from river floodplain lakes. The polluted sediment contained higher levels of nutrients, metals, PAH, PCB, and organic carbon.

The experiment used a regression design with five duplicate concentrations of TPT (controls, 1, 10, 30 and 100 µg/L) per sediment type. TPT (Fentin acetate Pestanal; Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) was applied once with ethanol as the carrier solvent. Control test systems were not dosed with TPT but received an equal amount of ethanol. Responses of populations of macroinvertebrates, zooplankton, phytoplankton, and macrophytes were studied at several time intervals after TPT application. Since no major differences in community responses between systems was observed [23] only the clean sediment systems are used for the comparison with the response in the laboratory. For a detailed description of the design and results of the microcosm experiment, see Roessink *et al.* [23].

Table 2. Test conditions for selected species in laboratory toxicity experiments with the organotin compound TPT. O_2 and pH ranges are given for the control treatments only.

Species	Taxonomic group	Average size ±sd (mm; *n* ≥10)	Test unit volume (L)	O2 (mg/L) (Min–Max)	pH (Min–Max)
Acanthocyclops venustus	Microcrustacean	2.2–0.4		7.8–8.4*	8.1–8.2*
Asellus aquaticus	Macrocrustacean	5.1–1.4	1.8	6.2–8.7	7.9–8.2
Bythinia tentaculata	Mollusc	–[a]	1.8	6.3–9.3	7.4–8.0
Chaoborus obscuripes	Insect	–[a]	1.8	6.1–10.2	7.4–8.0
Cloeon dipterum	Insect	5.5–0.7	1.8	6.3–8.9	7.7–8.2
Daphnia galeata	Microcrustacean	1.8–0.3	0.6	7.1–8.9	7.7–8.2
Dugesia sp.	Turbellarian	–[b]	1.8	8.2–8.9	7.6–8.2
Endochironomus albipennis	Insect	9.2–1.2		8.0–8.7*	7.2–7.6*
Erpobdella juv.	Annelid	11.5–1.7	1.8	4.4–8.8	7.4–8.3
Gammarus pulex	Macrocrustacean	13.0–4.0	1.8	4.7–8.5	5.6–7.9
Glyptotendipes sp.	Insect	11.7–1.9		8.0–8.7*	7.2–7.6*
Lumbriculus variegatus	Annelid	31.4–5.9		8.3–8.4*	7.8–7.9*
Lymnaea stagnalis	Mollusc	26.5–6.4	1.8	0.2–8.3	7.0–7.8
Physa fontinalis	Mollusc	6.5–1.0	1.8	6.1–8.9	7.4–7.9
Planorbis contortis	Mollusc	4.3–0.7	1.8	6.1–8.8	7.8–8.1
Polycelis tenuis/niger	Turbellarian	–[b]	1.8	6.1–9.0	7.8–8.2
Proasellus meridianus/coxalis	Macrocrustacean	5.6–1.7	1.8	4.8–8.7	7.7–8.2
Sigara sp.	Insect	–[a]	1.8	5.6–9.9	7.6–8.1
Sphaerium sp.	Mollusc	8.6–1.4	1.8	6.3–8.7	7.7–8.3
Tubifex sp.	Annelid	7.3–2.4		8.4–8.5*	7.8–7.9*
Desmodesmus subspicatus	Green algae	–[c]	0.1	–[d]	–[d]
Monoraphidium minutum	Green algae	–[c]	0.1	–[d]	–[d]
Scenedesmus quadricauda	Green algae	–[c]	0.1	–[d]	–[d]
Selenastrum capricornutum	Green algae	–[c]	0.1	–[d]	–[d]
Elodea nuttallii	Vascular plant	–[c]	1.8	12.7–16.4	6.9–10.1
Elodea canadensis	Vascular plant	–[c]	1.8	9.6–13.6	7.1–9.3
Lemna minor	Vascular plant	–[c]	1.8	9.9–17.8	6.7–10.0
Lemna trisulca	Vascular plant	–[c]	1.8	9.2–12.1	6.9–9.1
Potamogeton crispus	Vascular plant	–[c]	1.8	11.7–14.8	7.1–9.7
Myriophyllum spicatum	Vascular plant	–[c]	1.8	6.9–19.9	6.6–10.4
Ceratophyllum demersum	Vascular plant	–[c]	1.8	5.1–17	6.6–10.0
Spirodela polyrhiza	Vascular plant	–[c]	1.8	7.6–10.3	6.4–8.7

*[a]=Sizes were not measured in the 'range finding' test, [b]=Tricladida could not be conserved for measurements, [c]=not selected on size, but on biovolume (2·106 μm^3/mL) in the case of algae or wet weight (approximately 2 g) in the case of macrophytes, [d]=Small size of test unit did not allow for pH and O2 measurements, *=Only measured in stock solutions, test units too small for probe.*

Data analysis

The threshold level for *p* was 0.05 for all statistical analyses. Logistic regression was used to calculate the laboratory EC_{50} values for algae and macrophytes according to the following formula after the model describing hormesis by Van Ewijk and Hoekstra [30]:

$$y = \frac{k[1 + f * \exp(\log x)]}{1 + [1 + 2 * f * \exp(a)] * \exp[b(\log x - a)]} \quad (1)$$

where *y*=expected number/biomass/relative growth, *a*=ln (EC_{50}), *b*=slope parameter, *k*=maximal growth (upper limit), *f*=hormesis, *x*=concentration.

In the case of living biomass as endpoint, the 100% effect was set at a biomass of 0 g. In the case of relative growth, the 100% effect was set at a growth of 0 g per 3 or 4 days for algae and 0 g per 21 days for macrophytes. This meant that, based on the same data, EC_{50} values for biomass and relative growth could differ substantially (for a visual representation see [31]).

Logistic regression of the invertebrate data of the laboratory SST was performed using the following general logistic model:

$$y = \frac{(1 - c)}{1 + \exp[b + \ln(x - a)]} \quad (2)$$

where *y*=expected affected fraction, *a*=ln(EC_{50}), *b*=slope parameter, *c*=fraction of affected individuals in controls.

The logistic regression of the invertebrate data obtained from the outdoor microcosms used the same general logistic model described in Eq. 1, although without the possibility of hormesis. In this case the *k* parameter stands for the expected number in the control microcosms. The models (Eq. 1 and 2) were programmed in GenStat for Windows, 6th edition [32]. A Poisson distribution of the abundance data was assumed.

SSD analyses were performed according to [33] by the ETX-2000 computer program [34]. This spreadsheet program calculates the HC_5 (Hazardous Concentration for 5% of the species) and the 90% confidence limits.

The model assumes a log-normal distribution of toxicity data, thus:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} * \exp\left(\frac{-0.5*(x-\mu)^2}{\sigma^2}\right) \quad (3)$$

where x= ln (EC_{50}), l=median EC_{50}, r=standard deviation of ln (EC_{50}).

The SSD was defined as the cumulative density function of toxicity data as follows:

$$F(x) = \int_{\infty}^{x} f(x)ds \quad (4)$$

Tests for log-normality were performed by means of the Anderson–Darling goodness-of-fit test, a standard statistic output of the ETX version 1.403 computer program. Normality of toxicity data was assumed when p was ≥0.05 [35]. In accordance with Schroer *et al.* [36], a two-sample F-test was used to assess significant differences in the variances of SSDs. T-tests were used to determine significant differences in SSDs. Both tests were performed for 'full' curve comparison.

No observed effect concentrations (NOECs) were calculated at parameter or taxon level using the Williams test (ANOVA) (Williams 1972). This test assumes that the mean response of the variable is a monotonic function of the treatment, thus leading to the expectation of increasing effects with increasing dose. The analyses were performed with the Community Analysis computer program [37], resulting in a summary of NOECs for each sampling day for the data analyzed.

Table 3. Percentages of TPT in the water phase, relative to the initial test concentrations (as tested in the stock solutions), during the SST.

Taxon	Fraction (%) of compound after				
	1h	24h	48h	72h	96h
Gammarus pulex	90.2	85.5	83.5	78.7	81.4
Endochironomus albipennis	97.2	94.8	99.8	97.8	110.2
Cloeon dipterum	88.1				101.7
*Lymnaea stagnalis**	117.0				20.9
Physa fontinalis	131.8				86.7
Planorbis contortis	137.6				105.6
*Lumbriculus variegatus**	92.5				24.1
Tubifex sp.	111.0				89.2
Polycelis niger/tenuis	88.6				104.1
Dugesia sp.	89.0				93.0
Average	104.3				81.7

**Tests are more uncertain due to loading issues*

Results

SST in the laboratory

Exposure concentrations

One hour after application, mean measured concentrations (standard deviation in parenthesis) in the water of the test systems ranged from 94% (±15), 98% (±5), to 97% (±8) of the intended nominal concentrations, for invertebrates, macrophytes and phytoplankton, respectively. Since the mean measured concentrations are well in agreement with the intended exposure concentrations, calculated toxicity values in the present paper are based on nominal concentrations.

Table 3 shows that in test systems with small taxa, exposure concentrations were rather stable (approximately 82% of initial concentration remained at the end of the test). In contrast, test systems with relatively large taxa (*Lymnaea stagnalis*, *Lumbriculus variegatus*) showed a faster decline of TPT in the water phase (down to 20.9% of initial dosage for *Lymnaea*).

In the tests with relatively small floating (*Lemna minor* and *Spirodela polyrhiza*) or submerged (*Lemna trisulca*) macrophytes, the decrease of TPT in the water phase was slower than in tests with relatively large plants. This faster decline in tests systems with relatively large plants can be explained by the relatively large macrophyte surfaces to which the substance can be sorbed. In the tests with *Ceratophyllum demersum*, *Elodea nuttallii*, *Lemna minor*, and *Myriophyllum spicatum*, no TPT could be retrieved from the water phase at 14 and 21 days after application. No periphyton growth was observed in these test systems either.

Toxicity

In total, 32 different taxa were tested in the laboratory, 27 of which were used for the estimation of an appropriate EC_x value (Tables 1, 2). The tests performed with *Bythinia tentaculata*, *Sphaerium sp*., *Sigara sp*., and *Chaoborus obscuripes* could not be used for EC_x calculations. *Bythinia tentaculata* and *Sphaerium sp*. showed behavioral avoidance to TPT exposure by closing their opercula and/or shells. When placed in clean test medium after the range finding test, *Bythinia* and *Sphaerium* resumed their normal mode of action (moving through the jar/filtering activities). For *Sigara sp*. and *Chaoborus obscuripes* no apparent treatment-related response was observed during the 96 h test period.

The use of ethanol as a carrier solvent had no adverse effects in the invertebrate tests; only in the test with *Endochironomus albipennis* was there a slight difference in behavior between control and solvent control. In the macrophyte SST, the only difference observed between control and solvent control was for *Elodea nuttallii* and *Potamogeton crispus*. In this

case, the solvent control was used in further analysis, while in all other cases controls and solvent controls were pooled into one control treatment for EC_x estimations.

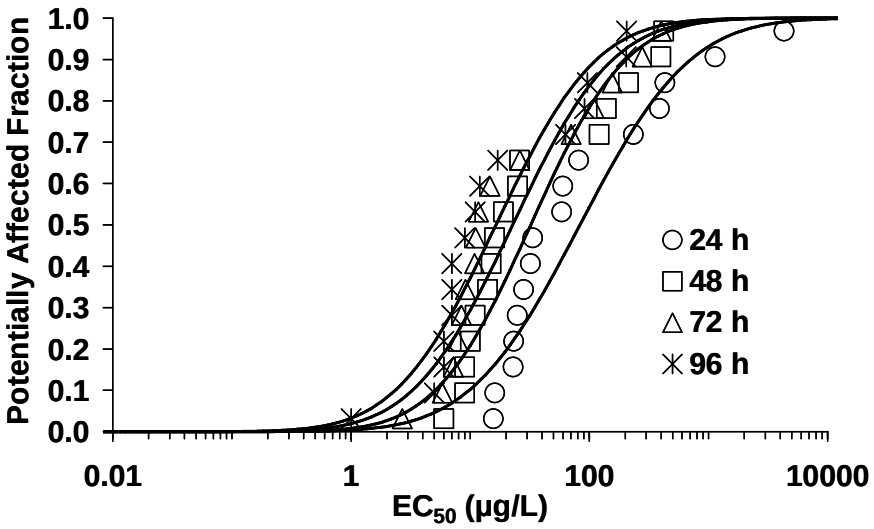


Figure 1: Species sensitivity distribution (SSD) curves calculated from the estimated EC_{50} values of the invertebrate laboratory Single Species Tests (SST) at 24, 48, 72 and 96 h after TPT application.

Looking at the range of sensitivities of the invertebrates tested; the most sensitive taxa included turbellarians, annelids, gastropods, microcrustaceans, and *Gammarus pulex*, while Insecta and Isopoda were less sensitive (Table 4). For all toxicity data presented here, survival of organisms in controls was more than 80% and all estimated EC_x values of sensitive taxa fell within the range of the tested TPT concentrations (Tables 1, 4). Of the invertebrates tested, the copepod *Acanthocyclops venustus* was the most sensitive species tested, with a 96 h EC_{50} of 1 µg/L (Table 4). The least sensitive invertebrate taxon for which an EC_{50} value was estimated was *Glyptotendipes* (96 h EC_{50}=205 µg/L). On average, the difference between EC_{10} and EC_{50} values of invertebrates was a factor of 2 to 3. The difference between EC_x and LC_x values was, on average, approximately a factor of 4. Increasing sensitivity (lower EC_{50} values) with increasing exposure time was observed for all invertebrate taxa (Table 4).

Figure 1 shows the four Species Sensitivity Distributions (SSD) constructed with the invertebrate EC_{50} values at 24, 48, 72, and 96 h. The Anderson–Darling test revealed that both of the curves were not accepted at the 0.05 but only at the 0.025 level. Acceptance at this lower level is not indicating that all the data are not log-normal but that upper values in the SSD seem to deviate from log-normality (*e.g.*, EC_{50} values of *Asellus*, *Proasellus*, *Endochironomus*, and *Glyptotendipes*).

The median EC_{50} (or location parameter l; see Eq. 3) of the species tested decreases as exposure time increases. Indicating that the average sensitivity increased over time. This phenomenon is also reflected in other percentiles of the SSD, such as the HC_5. For respectively 24, 48, 72, and 96 h median HC_5 values (with 90% lower and upper limit) of 5.0 (1.2–12.6), 2.9 (0.8–6.3), 1.8 (0.5–4.1), and 1.3 (0.4–3.0) µg/L were found. HC_5 values decreased with increasing exposure time. Statistical evaluation of the SSD curves (Fig. 1) shows that only the 24 h curve differs significantly from the other curves ($p < 0.01$).

Based on Φ_{PSII}, the algae we tested were more sensitive than the vascular plants, and with an EC_{50} of 5.6 µg/L, the green alga *Selenastrum capricornutum* was the most sensitive plant species tested (Tables 5, 6). Only for *Potamogeton crispus*, *Myriophyllum spicatum*, and *Elodea nuttallii* hormesis played a significant role in terms of relative growth based on biomass. Comparison of toxicity values based on Φ_{PSII} for days 2, 7, and 21 shows that the values were lowest on day 7 and had the smallest 95% confidence interval. *Myriophyllum spicatum* could not be analyzed by the mini-PAM because its leaf structure was too fine and delicate. The comparison for the vascular plants also shows that for most of the species tested, toxicity values based on relative growth (21 days) were lower than those based on Φ_{PSII} (7 days), except for *Potamogeton crispus* and *Lemna minor*. *Spirodela polyrhiza* (EC_{50}=4.6 µg/L based on relative growth) was the most sensitive macrophyte species tested (Table 6). The average toxicity ratio for algae tested after 72 and 96 h [EC_{50}-72 h/EC_{50}-96 h] was 1, indicating that, in contrast to the invertebrates we tested, algae did reach the incipient value within 3 days.

Comparison of sensitivities of 96 h invertebrate EC_{50} and primary producer toxicity data (Φ_{PSII} for both algae and macrophytes; relative growth for macrophytes only) showed that toxicity of TPT is in the same range (Fig. 2). Accompanying HC_5 values with lower and upper limit in parenthesis are 1.3 (0.4–3.0), 1.9 (0.4–4.9), and 4.2 (1.0–9.3), respectively. All curves partly overlap and although the lower parts of the curves seem to differentiate, 90% confidence intervals of HC_5 values overlap and statistical testing did not reveal any significant differences (p>0.05).

Table 4. Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TPT-Ac to aquatic invertebrates.

Species	x	EC_x (µg/L)		LC_x (µg/L)	
		48h	96h	48h	96h
Acanthocyclops venustus	10	2.7 (1.8-4.0)	0.1 (0.0–1.5)	2.9 (1.9–4.5)	0.1 (0.0–0.9)
	50	5.8 (4.7–7.1)	0.5 (0.1–2.2)	6.9 (5.5–8.8)	0.8 (0.3–2.0)
Lumbriculus variegates	10	4.1 (2.6–6.7)	3.5 (2.1–5.8)	21.4 (*)	13.3 (12.2–14.5)
	50	8.8 (6.7–11.5)	6.3 (4.8–8.3)	22.6 (*)	14.8 (13.7–15.9)
Physa fontinalis	10	5.8 (3.7–8.2)	4.2 (2.8–6.1)	17.2 (4.3–69.1)	10.6 (9.7–11.5)
	50	9.3 (7.6–11.5)	7.1 (5.6–9.1)	96.3 (36.3–255.0)	11.8 (10.9–12.8)
Dugesia sp.	10	2.7 (1.5–5.0)	2.9 (1.7–5.0)	24.9 (18.7–33.1)	19.0 (18.0–20.0)
	50	9.8 (7.2–13.2)	6.1 (4.6–8.0)	35.3 (29.9–41.6)	20.9 (19.9–22.0)
Polycelis niger/tenuis	10	3.1 (1.7–5.6)	3.4 (1.9–5.9)	42.4 (39.2–45.8)	20.8 (*)
	50	10.6 (7.9–14.2)	6.6 (5.0–8.8)	46.9 (43.1–51.0)	23.2 (*)
Tubifex	10	2.4 (0.6–9.2)	2.3 (0.5–9.3)	13.1 (7.7–22.1)	9.2 (5.5–15.4)
	50	14.2 (8.0–25.3)	10.7 (5.5–21.1)	27.0 (20.5–35.5)	12.9 (10.0–16.7)
Planorbis contortis	10	5.7 (3.0–10.9)	3.5 (2.2–5.5)	- [a]	- [a]
	50	14.7 (10.6–20.3)	6.6 (5.0–8.6)	- [a]	- [a]
Daphnia galeata	10	7.3 (4.9–10.8)	5.4 (3.4–8.5)	28.2 (14.1–56.5)	13.1 (*)
	50	16.1 (13.1–19.9)	8.4 (6.8–10.4)	41.9 (35.8–49.1)	16.0 (*)
Gammarus pulex	10	5.6 (2.4–13.6)	4.5 (2.1–9.8)	18.5 (4.6–74.1)	11.6 (5.7–23.7)
	50	18.5 (12.3–27.9)	8.9 (6.1–12.7)	104.4 (39.4–276.5)	12.6 (7.0–22.9)
Lymnaea stagnalis	10	10.0 (5.3–18.6)	9.7 (*)	263.5 (124.0–559.9)	85.8 (*)
	50	24.9 (18.3–34.0)	11.8 (*)	906.9 (387.0–2125.7)	92.1 (*)
Erpobdella juv.	10	15.3 (*)	9.6 (6.1–15.0)	50.5 (47.1–54.1)	23.8 (*)
	50	25.9 (*)	17.1 (13.2–22.1)	56.6 (53.2–60.3)	27.1 (*)
Cloeon dipterum	10	34.7 (19.2–63.0)	12.3 (4.9–31.2)	251.8 (173.6–365.2)	39.8 (*)
	50	120.9 (89.6–163.1)	63.0 (42.3–93.8)	442.5 (327.4–598.1)	168.9 (*)
Proasellus meridianus/coxalis	10	37.0 (19.3–71.1)	32.4 (17.1–61.2)	137.4 (74.7–253.1)	39.1 (21.4–71.6)
	50	139.0 (97.8–197.4)	90.9 (65.7–125.8)	558.5 (364.7–855.4)	138.5 (99.1–193.6)
Asellus aquaticus	10	78.3 (36.7–167.3)	26.0 (8.7–77.8)	72.8 (33.9–156.4)	72.8 (33.9–156.4)
	50	212.8 (146.3–309.5)	95.6 (56.6–161.3)	271.3 (184.1–399.7)	271.3 (184.1–399.7)
Endochironomus albipennis	10	343.0 (162.5–724.0)	181.9 (170.0–194.6)	306.8 (163.9–574.0)	179.2 (112.5–285.3)
	50	399.2 (368.2-432.9)	203.8 (191.7-216.7)	691.6 (505.2-946.8)	302.9 (232.4-394.7)
Glyptotendipes sp.	10	382.6 (*)	103.6 (57.0-188.3)	- [b]	287.7 (176.6-468.9)
	50	420.8 (*)	204.7 (149.8-279.7)	- [b]	488.6 (373.4-639.4)

All tests were performed at 20±2 ºC; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets (*)=Standard error of parameters not available due to singularity in regression model [a]=Since the response in SST did not allow clear discrimination between sublethal and lethal effects, no LC could be calculated, [b]=calculated values exceeded the tested concentration range by more than two times the internal factor

Table 5. Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TPT-Ac to several phytoplankton species.

Species		x	Time after application (h) 48	72	96
Selenastrum capricornutum	EC_x (µg/L)	10	5.5 (4.8-6.3)	2.6 (1.9-3.6)	3.2 (2.7-3.7)
		50	58.0 (51.4-65.5)	8.8 (7.0-11.0)	5.6 (4.9-6.4)
	NOEC (µg/L)		3.0	3.0	3.0
Desmodesmus subspicatus	EC_x (µg/L)	10	15.9 (8.9-28.5)	10.1 (8.4-12.3)	11.1 (9.9-12.5)
		50	101.9 (84.9-122.4)	23.0 (20.1-26.3)	18.1 (16.5-19.9)
	NOEC (µg/L)		10.0	10.0	10.0
Monoraphidium minutum	EC_x (µg/L)	10	39.2 (17.5-87.5)	14.3 (11.7-17.4)	2.5 (1.1-6.1)
		50	187.7 (104.7-336.5)	51.5 (40.5-65.4)	15.8 (10.6-23.7)
	NOEC (µg/L)		10.0	10.0	10.0
Scenedesmus quadricauda	EC_x (µg/L)	10	54.6 (35.1-84.8)	7.2 (2.9-17.9)	17.0 (12.9-22.6)
		50	352.9 (133.6-931.7)	29.1 (19.4-43.6)	36.0 (30.8-42.1)
	NOEC (µg/L)		30.0	3.0	3.0

All test were performed at 20±2 ºC; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets. The endpoint was photosystem efficiency (Φ_{PSII}) and was measured at 24, 48, 72, and 96 h. However, no EC_x values could be calculated at 24 h after application

Microcosm semi-field experiment

In the present paper we focus on the comparison of the results of the laboratory SSD with that of the microcosms constructed with clean sediment. However, we also present the summary data for the microcosms with polluted sediment. Figures 3a–c show the SSD curves constructed from the EC_{50} values (based on intended nominal concentrations) of the free-living invertebrate populations for weeks 2, 4, and 8 after application, together with the curve obtained from the 96 h invertebrate laboratory data (Table 4). We considered only the EC_x values of those taxa that had a mean abundance of 4 or higher on the artificial substrates of control microcosms. Calculated EC_x values for low-abundance taxa (≤3 per test system) were considered uncertain and therefore not representative. Statistical testing reveals significant differences ($p < 0.01$) between the curves indicating a higher sensitivity of invertebrates in the microcosms compared to the lab (Fig. 3). Overall, the HC_5 calculated from invertebrate toxicity data for microcosms (based on nominal peak concentrations) was a factor of 2–4 lower than the HC_5 calculated from laboratory invertebrate EC_{50}-96 h toxicity data. While the microcosm HC_5 values between test systems constructed with clean and polluted sediment were very similar (Table 7).

Discussion

Laboratory responses

In our laboratory experiments, we observed that representatives of several taxonomic groups of freshwater invertebrates, as well as several phytoplankton and vascular plant species, showed a clear response to a single application of TPT at treatment levels higher than 1 µg/L (Tables 4, 5). On average, EC_x values were a factor of 4 lower than LC_x values and this difference decreased (to a factor of 3) as exposure time increased, indicating that TPT is a compound with a relatively 'slow' mode of action. The average EC_{50}-48 h:EC_{50}-96 h ratio was 2, against a ratio of 4 when calculated with LC_{50} values. This ratio was considerably higher for several individual taxa. The copepod Acanthocyclops venustus had an EC_{50}-48 h/EC_{50}-96 h ratio of 6, while Physa fontinalis, Gammarus pulex and Lymnaea stagnalis had LC_{50}-48 h/ LC_{50}-96 h ratios of 8–10. This indicates that at the frequently used time interval for acute effects (48 h), the incipient value for acute toxicity of TPT may not have been reached (Fig. 1 and Table 4).

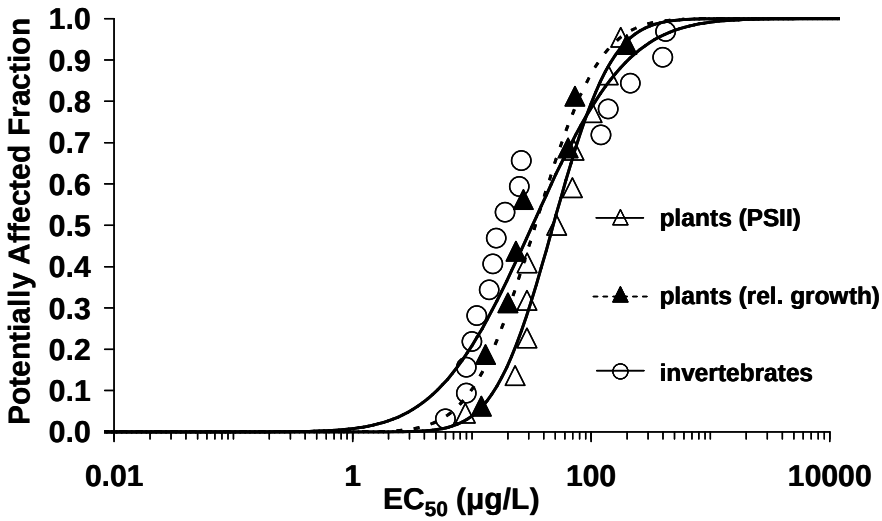


Figure 2: SSD of 28 indigenous freshwater taxa for TPT tested in the laboratory. EC_{50} values were estimated using 48 h response data for invertebrates (s) and Φ_{PSII} (D) and relative growth (▲; dotted line) responses for phytoplankton and macrophytes (Tables 4, 5)

Table 6 Results of short-term laboratory Single Species Tests (SST) of the toxicity of the fungicide TriPhenylTin-Ac to several macrophytes species

Species	x	EC_x (µg/L) Φ_{PSII} 2d	Φ_{PSII} 7d	Φ_{PSII} 21d	Relative growth 21d
Spirodela polyrhiza	10	386.2 (234.0-637.3)	5.6 (2.3-13.3)	28.9 (26.5-31.5)	0.1 (0.0-3.9)
	50	$5.6*10^3$ ($2.6*10^3$-$1.2*10^4$)	29.0 (18.3-45.8)	33.1 (30.3-36.3)	4.6 (0.7-29.5)
Potamogeton crispus [a]	10	9.0 (3.2-25.6)	5.6 (2.3-13.3)	-	23.8 (18.8-30.1)
	50	127.9 (78.5-208.2)	29.0 (18.3-45.8)	-	38.8 (31.0-48.4)
Lemna trisulca	10	21.9 (13.3-35.8)	9.9 (5.4-18.1)	11.2 (6.3-19.7)	1.8 (0.2-15.4)
	50	122.5 (93.9-159.9)	69.5 (51.1-94.6)	36.1 (27.5-47.5)	64.5 (25.6-162.6)
Ceratophyllum demersum	10	62.2 (38.8-99.7)	1.6 (0.0-82.4)	48.1 (0.9-2548.2)	0.4 (0.0-17.6)
	50	240.6 (184.9-313.1)	92.5 (18.1-473.4)	1357.3 (327.5-$5.6*10^3$)	12.9 (2.0-82.8)
Elodea nuttallii [a]	10	6.1 (1.1-34.0)	34.8 (11.0-109.9)	79.9 (x-x)	1.8 (1.1-3.0)
	50	59.4 (25.7-137.1)	101.9 (63.8-162.9)	97.7 (x-x)	11.8 (7.4-18.8)
Lemna minor	10	$9.1*10^2$ ($2.1*10^2$-$3.9*10^3$)	104.8 (93.0-118.2)	96.7 (x-x)	180.0 (x-x)
	50	$6.4*10^4$ ($1.0*10^2$-$4.0*10^7$)	138.9 (60.1-321.4)	130.4 (x-x)	198.9 (x-x)
Elodea canadensis	10	5.1 (1.9-13.8)	2.1 (0.2-23.9)	1.8 (0.0-214.9)	1.5 (0.1-29.7)
	50	197.8 (132.6-295.1)	176.6 (69.1-451.7)	44.5 (4.8-413.8)	23.4 (8.5-64.5)
Myriophyllum spicatum	10	NA	NA	NA	32.3 (18.7-55.6)
	50	NA	NA	NA	73.4 (44.9-200.0)

All tests were performed at 20±2 ºC; the test medium was filtered water from the 'Sinderhoeve' experimental field station. Calculated EC_x values are plotted with their 95% confidence limits between brackets. The endpoint was photosystem efficiency (Φ_{PSII}) and relative growth. x–x=No convergence for model, NA=not applicable, [a]=ECx-calculation not with pooled control and solvent control but with solvent controls only

Several factors seem to govern TPT toxicity in the organisms tested. In particular, organism morphology is a factor, since soft-bodied taxa (*e.g.*, triclad and annelid worms) are more susceptible to TPT than taxa with 'harder' bodies (such as *Endochironomus albipennis* and *Glyptotendipes sp.*, with more closed and chitin-based structures; see Table 4). A faster decline of TPT concentrations was indeed observed in the water phase of the test systems with relatively large soft-bodied taxa (down to 20.9% of the initial dosage for *Lymnaea*; see Table 3). The EC_x-values estimated from the *Lymnaea* and *Lumbriculus* test are more uncertain due to possible loading issues. The decline in TPT

concentrations could be test volume related and it is uncertain if the use of a larger volume would also show such a decline. Such a decline in a larger test volume would indicate that a greater amount of TPT is sorbed to the organisms, enlarging exposure and therefore likely to cause a more sensitive response (lower EC_x-values). Neither *Lymnaea stagnalis* or *Lumbriculus variegatus* are the most sensitive species and omitting them from the SSD hardly affected the HC_x-values.

Another important factor seems to be the size of the organism. Among the crustaceans, the most sensitive taxa were the zooplankters *Acanthocyclops* and *Daphnia*. These smaller organisms possess a larger surface: volume ratio for TPT uptake. It has frequently been reported in the literature that smaller and younger life stages of organisms are more susceptible to toxicants (Hutchinson *et al.* 1998). Based on Φ_{PSII}, smaller phytoplankton species are, on average, more sensitive than larger vascular plants. Establishing these 'rules of thumb' for vascular plants is more difficult because of the large differences in growth form between species (Table 6).

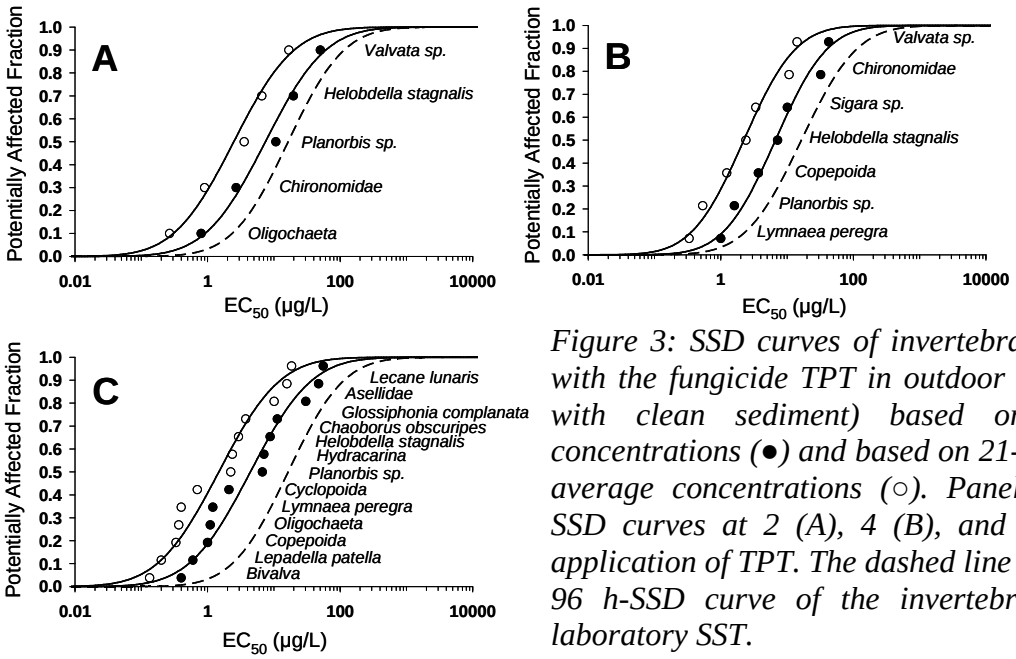


Figure 3: SSD curves of invertebrates after treatment with the fungicide TPT in outdoor cosms (constructed with clean sediment) based on initial nominal concentrations (●) and based on 21-days time weighted average concentrations (○). Panels a–c present the SSD curves at 2 (A), 4 (B), and 8 (C) weeks after application of TPT. The dashed line represents the 96 h-SSD curve of the invertebrates tested in the laboratory SST.

The 'Φ_{PSII}' endpoint shows a distinct treatment-related response from 48 h onwards, especially for submerged macrophytes, indicating that TPT did indeed inhibit photosynthesis, ultimately resulting in decreased relative growth (Table 5, 6). Except for *Ceratophyllum demersum* where periphyton growth resulted in Φ_{PSII} recovery, while the estimated EC_{50} values for relative growth (based on biomass) contradict this. When Φ_{PSII} artifacts are omitted macrophyte sensitivity does not differ from invertebrate sensitivity (p>0.05).

The use of ethanol as carrier solvent resulted in large effects on dissolved oxygen in the outdoor experiment thus posing an extra stress on

the systems [23]. However, to keep conditions similar enabling comparison between field and laboratory, ethanol was also used in the latter set-up. In contrast to the outdoor situation, due to the lack of sediment, and consequently of microbial biomass in the laboratory SST, ethanol had only minor effects on the response of the taxa tested [23].

Table 7. Calculated ecological risks thresholds (HC_5 values, with 90% lower and upper limit) in both types of outdoor microcosms based on initial peak concentrations and 21-days time weighted average concentrations (TWA).

		Week 2	Week 4	Week 8
Clean sediment	*n*	5	7	13
	Peak (µg/L)	0.4 (0.0-2.0)	0.6 (0.1-1.8)	0.3 (0.1-0.7)
	21-d TWA (µg/L)	0.1 (0.0-0.7)	0.2 (0.0-0.6)	0.1 (0.0-0.2)
Polluted sediment	*n*	11	16	9
	Peak (µg/L)	0.3 (0.1-0.6)	0.6 (0.2-1.2)	0.2 (0.0-0.6)
	21-d TWA (µg/L)	0.1 (0.0-0.2)	0.2 (0.1-0.5)	0.1 (0.0-0.2)

Comparison with literature data

Published literature data on TPT toxicity (48, 72, or 96 h) to aquatic taxa is presented in Table 8 [9, 38]. This data relates both to triphenyltin acetate and triphenyltin hydroxide. We have pooled the data for these two compounds because the acetate is rapidly hydrolyzed to hydroxide [39, 40], so we assume that triphenyltin acetate exposure can also be classified as triphenyltin hydroxide exposure.

The literature data allow acute:chronic ratios of 22 and 17 to be derived for *Daphnia magna* and *Pimephales promelas*, respectively. This indicates that effects in a long-term study of invertebrates and fish to TPT may be considerably greater than the effects in a short-term study. However, due to differences in exposure time, the slow time-to-event, and kinetics issues related with this type of compound it is hard to distinguish if the differences in effects between short and long-term studies are related to time of exposure or latency of effects.

An SSD analysis with the invertebrate data from the literature (see Table 8) resulted in an acute HC_5 value of 0.8 (0.0–4.6) µg/L. This value is somewhat lower than, but not significantly different from the acute HC_5 value of 2.9 (0.8–6.3) µg/L that we calculated from our 48 h EC_{50} values for invertebrates (Table 5). Combining the available literature data with the data from our study results in the SSD presented in Fig. 4. We used EC_{50} values based on the Φ_{PSII} response after 72 h and 7 days for green algae and vascular plants, respectively, to construct the curve for the primary producers (Tables 5, 6). The curves for plants, invertebrates, and vertebrates are located close together in the graph and sometimes partially overlap. The corresponding acute HC_5 values and 90% lower

and upper limit are 1.8 (0.7–4.0), 10.7 (3.9–19.1), and 11.9 (2.4–24.3) µg/L for the invertebrate, vertebrate, and primary producer curves, respectively. Although HC_5 values seem to differ, their confidence limits overlap and no significant differences between the curves were found. This shows once again that TPT is a compound that targets a broad spectrum of taxa in a relatively small toxicity range. Figure 4 also shows that triphenyltin acetate (transparent) and triphenyltin hydroxide (black) data points are mixed throughout the invertebrate curve, indicating that there is no great difference in toxicity and justifying the lumping of these two particular compounds. We also constructed a single SSD based on all acute toxicity data available of which the corresponding HC_5 value was 3.1 (1.5–5.3) µg/L.

Table .8 Toxicity values of triphenyltin acetate (TPT-Ac) and triphenyltin hydroxide (TPT-OH) obtained from the open literature for several taxa originating from and tested in brackish (MX), fresh (FW) and salt (SW) water [38].

Compound	Species	Taxonomic group	Water	Test duration (h)	EC_{50} (µg/L)	NOEC (µg/L)
TPT-Ac	*Skeletonema costatum*	Diatom	MX	72	0.7	
TPT-Ac	*Thalassiosira guillardii*	Diatom	SW	72	1.1	
TPT-Ac	*Thalassiosira pseudonana*	Diatom	SW	72	1.5	
TPT-Ac	*Tubifex tubifex*	Annelid	FW	96	1.9	
TPT-Ac	*Ceriodaphnia dubia*	Microcrustacean	FW	48	11.1	
TPT-Ac	*Daphnia magna**	Microcrustacean	FW	504	0.8	
TPT-OH	*Daphnia magna*	Microcrustacean	FW	48	16.7	
TPT-OH	*Daphnia pulex*	Microcrustacean	FW	48	14.7	
TPT-OH	*Gammarus fasciatus*	Macrocrustacean	FW	96	66.0	
TPT-OH	*Chironomus plumosus*	Insect	FW	96	0.3	
TPT-OH	*Chironomus riparius*	Insect	FW	48	50.0	
TPT-OH	*Cipangopaludina malleata*	Mollusc	FW	48	720.0	
TPT-OH	*Indoplanorbis exustus*	Mollusc	FW	48	840.0	
TPT-OH	*Physella acuta*	Mollusc	FW	48	300.0	
TPT-OH	*Semisulcospira libertina*	Mollusc	FW	48	550.0	
TPT-OH	*Carassius auratus*	Fish	FW	96	62.0	
TPT-OH	*Lepomis macrochirus*	Fish	MX	96	23.0	
TPT-OH	*Oncorhynchus mykiss*	Fish	FW	48	32.6	
TPT-OH	*Oryzias latipes*	Fish	MX	48	69.9	
TPT-OH	*Pimephales promelas*	Fish	FW	96	20.0	
TPT-OH	*Pimephales promelas**	Fish	FW	720		1.2
TPT-OH	*Rasbora heteromorpha*	Fish	FW	48	96.1	

**=Chronic exposure toxicity value, acute: chronic ratio>10*

Comparing laboratory and field responses

There were several sensitive populations in the microcosms, including representatives of Annelida, Mollusca, Crustacea, Insecta, and Rotifera (e.g.,*Keratella*, *Lecane*, and *Lepadella*). These observations are in line with the results of our laboratory SST with TPT (for further details on the outdoor microcosm study see part I, [23]).

The hazardous concentration for 5% of the invertebrate species (HC_5) calculated on the basis of laboratory data (EC_{50}-96 h) was 1.3 µg/L, while invertebrate HC_5 values (based on initial nominal concentrations) calculated on the basis of SSD curves derived from the clean microcosms in sampling weeks 2–8 ranged from 0.3 to 0.6 µg/L. A perhaps more realistic manner of calculating EC_{50} values is not using the initial nominal (peak) concentrations but using a more chronic exposure *e.g.*, the 21-days time weighted average (TWA) as stated in part I [23]. The TWA is approximately a factor 3 lower than the initial nominal concentration and results in a more sensitive response (Fig. 3) with HC_5 values ranging from 0.1–0.2 µg/L. In all cases, invertebrate populations in the cosms responded significantly more sensitive than invertebrate species in the laboratory (Figure 3 and Table 4). Possible explanations for the more sensitive field response immediately address the difficulties involved when comparing lab and field studies and comprise differences in sampling techniques, latency of effects, and/or additional chronic exposure via the food chain in the microcosms.

Invertebrate field sampling occurred by means of the artificial substrate technique which monitors the activity of macroinvertebrates, rather than their total numbers. A small decline in activity/movement could have a larger effect on the recolonisation of the substrate (and thus recovered numbers) than on the behavior observed in the laboratory, perhaps explaining the difference in response. Differences in sampling techniques are even larger concerning phytoplankton and vascular plants were a lab *vs.* field comparison between different endpoints (*viz.*, Φ_{PSII} and relative growth in the lab *versus* abundance and percentage coverage in the field) has to be made. Observed differences in sensitivity may reflect an effect of the different types of endpoint measured. In addition, phytoplankton responses in the outdoor test systems and the laboratory are difficult to compare, because the dose–response relationship in the outdoor test systems can easily be obscured by inter-species relationships (*e.g.*, grazing, predation and competition).

Also, TPT disappears quickly from the water phase and can sorb to other compartments (*e.g.*, sediment, macrophytes) [41]. It has been reported that TPT is transferred through the food web [42, 43] and low concentrations could mediate effects through bioaccumulation, resulting in a more sensitive response than in the laboratory.

In the outdoor cosms, invertebrates may have suffered long-term exposure to TPT via the water and/or food, causing the incipient value to be reached and maximum effects to be expressed. In contrast, exposure in the laboratory was mainly via water, and the exposure time (96 h) may also have been too short to allow this maximum effect to be expressed.

In conclusion, in the long-term the invertebrate populations in the microcosms indeed showed greater sensitivity than the invertebrate species tested in short-term lab tests. Not only was the time to event (effect expression) not reached in the lab (maximal duration 4 days) for several species in contrast to the field situation, but also differences in exposure regime (maximal 4 days in the lab *versus* minimal two weeks in the field) and measurement endpoints between the laboratory and microcosms were of influence. The populations in the microcosms suffered long-term exposure due to TPT uptake via water and food, while only short-term responses to TPT exposure (via water) were monitored in the laboratory.

Other studies comparing lab and field responses of invertebrate populations to the insecticides chlorpyrifos, ensdosulfan, and lambda-cyhalothrin found very similar lab and field SSDs [6, 36, 44, 45]. These relatively non-persistent insecticides, however, only need a short time to express their toxic effects, while exposure via the food chain plays a minor role.

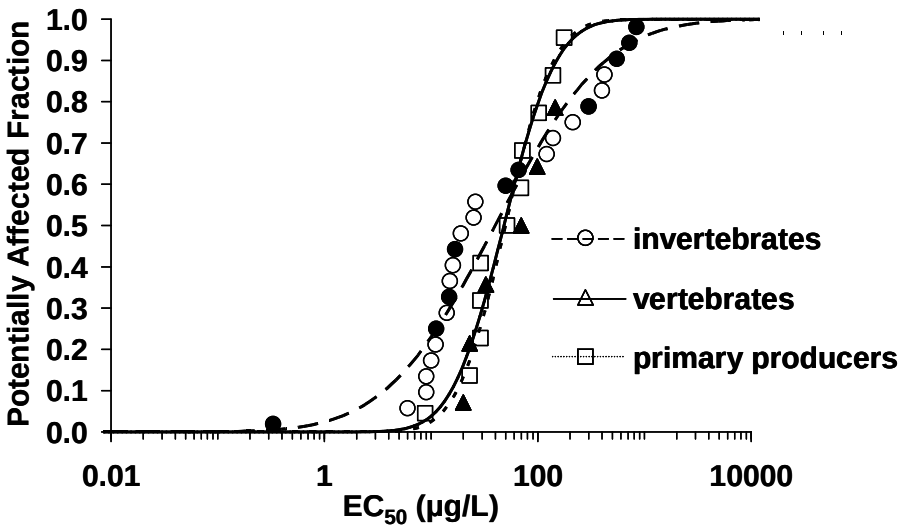


Figure 4: The analysis of species sensitivity of TPT constructed with combined lab toxicity data from the literature ([9, 38]) and the present study (Tables 4–7). The curve for primary producers (□; dotted line) was constructed with the EC_{50} values based on 72 h Φ_{PSII} for green algae and 7 days values for vascular plants. Curves for invertebrates (○; dashed line) and vertebrates (Δ; solid line) were constructed using 48 h EC_{50} values. Data points for TPT are transparent while points for TPT-OH are plotted in black

Risk assessment of TPT

HC_5 values based on concentration-response relationships observed in the outdoor microcosms did not differ between sampling dates (week 2, 4, and 8) and type of test system (constructed with clean or polluted sediment) suggesting that in the present study spatio-temporal differences in community structure did not affect the sensitivity indicated by the SSD (Table 7). The analysis of species sensitivity of TPT indicates that a very broad spectrum of aquatic taxa is affected and there does not appear to be a great difference in sensitivity between aquatic primary producers, invertebrates, and vertebrates. This suggests that every aquatic community can be expected to include taxa sensitive to TPT. The physiological processes of organisms impacted by TPT are basal and take time to get expressed in the endpoints measured, except for unicellular phytoplankton. This delay in time of onset of effects in invertebrate populations is one of the reasons why the sensitivities we observed in the laboratory were lower than those in the microcosms. In addition, the phenomenon that the compound dissipates relatively fast from the water and accumulates in organic matter and the upper sediment layers may result in a chronic long-term exposure regime in the field [23, 41]. The present study clearly shows that, for this compound that accumulates in the foodchain, data from conventional acute laboratory single species tests with invertebrates cannot be simply used to assess the risk to the aquatic community exposed to a similar concentration regime (single application) as simulated in our microcosm experiment without appropriate considerations for exposure and/or endpoints affected.

Acknowledgements - This study was subsidized by the Netherlands Organization for Scientific Research (NWO) as part of the Stimulation Program System-oriented Ecotoxicological Research (SSEO) (project no. 014.23.012). In addition, the research was supported by the UK Department of Environment, Food and Rural Affairs (Defra) and the Dutch Ministry of Agriculture, Nature and Food Safety, as part of a research program focusing on the scientific underpinning of risk assessment procedures for fungicides in the aquatic environment. The authors are indebted to L. Buijse, A. Matser, and L.J.T. van der Pas for practical assistance.

References

[1] Cuppen, J. G. M., Van den Brink, P. J., Camps, E., Uil, K. F. and Brock, T. C. M. 2000. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. Aquatic Toxicology 48 pp. 233-250

[2] Farrel, A. P., Stockner, E. and Kennedy, C. J. 1998. A study of the lethal and sublethal toxicity of polyphase P-100, an antisapatain fungicide containing 3-Iodo-2-Propynyl Butyl Carbamate (IPBC), on fish and aquatic invertebrates. Archives of Environmental Contamination and Toxicology 35 pp. 472-478

[3] Van Wijngaarden, R. P. A., Crum, S. J. H., Decraena, K., Hattink, J. and Van Kammen, A. 1998. Toxicity of Derosal (active ingredient Carbendazim) to aquatic invertebrates. Chemosphere 37 pp. 673-683

[4] Koelmans, A. A., Hubert, E., Koopman, H. W., Portielje, R. and Crum, S. J. H. 2000. Modeling the vertical distribution of carbendazim in sediments. Environmental Toxicology and Chemistry 19 pp. 793-800

[5] Van den Brink, P. J., Hattink, J., Bransen, F., Van Donk, E. and Brock, T. C. M. 2000. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquatic Toxicology 48 pp. 251-264

[6] Van den Brink, P. J., Brock, T. C. M. and Posthuma, L. 2002. The value of the species sensitivity distribution concept for predicting field effects: (Non-) confirmation of the concept using semifield experiments. In: Posthuma, L., Suter II, G. W. and Traas, T. P., eds, Species Sensitivity Distributions in Ecotoxicology, Lewis Publishers, Boca Raton, pp. 155-193

[7] Schulte-Oehlmann, U., Tillmann, M., Markert, B. and Oehlmann, J. 2000. Effects of endocrine disruptors on Prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. Ecotoxicology 9 pp. 399-412

[8] Tillmann, M., Schulte-Oehlmann, U., Duft, M., Markert, B. and Oehlmann, J. 2001. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part III: Cyproterone acetate and vinclozolin as antiandrogens. Ecotoxicology 10 pp. 373-388

[9] Fargasová, A. 1998. Comparison of effects of tributyl-, triphenyl-, and tribenzyltin compounds on freshwater benthos and alga *Scenedesmus quadricauda*. Bulletin of Environmental Contamination and Toxicology. 60 pp. 9-15

[10] Jak, R. G., Ceulemans, M., Scholten, M. C. T. and Van Straalen, N. M. 1998. Effects of tributyltin on a coastal north sea plankton community in enclosures. Environmental Toxicology and Chemistry 17 pp. 1840-1847

[11] Petersen, S. and Gustavson, K. 2000. Direct toxic effects of TBT on natural enclosed phytoplankton at ambient TBT concentrations of coastal waters. Ecotoxicology 9 pp. 273-285

[12] Rehage, J. S., Lynn, S. G., Hammond, J. I., Palmer, B. D. and Sih, A. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile ambystoma barbouri salamanders. Environmental Toxicology and Chemistry 21 pp. 807-815

[13] Chandra, S., Polya, G. M., James, B. D. and Magee, R. J. 1989. Inhibition of oxidative phosphorylation by organotin thiocarbamates. Chemico-Biological Interactions 71 pp. 21-36

[14] Girard, J.-P., Ferrua, C. and Pesando, D. 1997. Effects of tributyltin on Ca^{2+} homeostasis and mechanisms controlling cell cycling in sea urchin eggs. Aquatic Toxicology 38 pp. 225-239

[15] Tiano, L., Fedeli, D., Santoni, G., Davies, I. and Falcioni, G. 2003. Effect of tributyltin on trout blood cells: changes in mitochondrial morphology and functionality. Biochimica et Biophysica Acta 1640 pp. 105-112

[16] Stridh, H., Orrenius, S. and Hampton, M. B. 1999. Caspase involvement in the induction of apoptosis by the environmental toxicants tributyltin and triphenyltin. Toxicology and Applied Pharmacology 156 pp. 141-146

[17] Cima, F., Dominici, D., Mammi, S. and Ballarin, L. 2002. Butyltins and calmodulin: which interaction? Applied Organometallic Chemistry 16 pp. 182-186

[18] Mooney, H. M. and Patching, J. W. 1995. Triphenyltin inhibits photosynthesis and respiration in marine microalgae. Journal of Industrial Microbiology 14 pp. 265-270

[19] Laughlin, R. B. and Linden, O. 1985. Fate and effects of organotin compounds. Ambio 14 pp. 88-94

[20] Vighi, M. and Calamari, D. 1985. QSARs for organotin compounds on *Daphnia magna*. Chemosphere 14 pp. 1925-1932

[21] Posthuma, L., Suter II, G. W. and Traas, T. P. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, London

[22] Van Straalen, N. M. and Denneman, C. A. J. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotoxicology and Environmental Safety 18 pp. 241-251

[23] Roessink, I., Crum, S. J. H., Bransen, F., Van Leeuwen, E., Van Kerkum, F., Koelmans, A. A. and Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality. Ecotoxicology 15 pp. 267-293

[24] Baer, K. N. and Goulden, C. E. 1998. Evaluation of a high-hardness COMBO medium and frozen algae for *Daphnia magna*. Ecotoxicology and Environmental Safety 39 pp. 201-206

[25] Fairchild, J. F., Ruessler, D. S. and Carlson, R. 1998. Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. Environmental Toxicology and Chemistry 17 pp. 1830-1834

[26] Juneau, P., El Berdey, A. and Popovic, R. 2002. PAM fluorometry in the determination of the sensitivity of *Chlorella vulgaris*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardtii* to copper. Archives of Environmental Contamination and Toxicology 42 pp. 155-164

[27] Lürling, M. and Verschoor, A. M. 2003. F_0-spectra of chlorophyll fluorescence for the determination of zooplankton grazing. Hydrobiologia 491 pp. 145-157

[28] Snel, J. F. H., Vos, J. H., Gylstra, R. and Brock, T. C. M. 1998. Inhibition of photosystem II (PSII) electron transport as a convenient endpoint to assess stress of the herbicide linuron on freshwater plants. Aquatic Ecology 32 pp. 113-123

[29] Roessink, I., Arts, G. H. P., Belgers, J. D. M., Bransen, F., Maund, S. J. and Brock, T. C. M. 2005. Effects of lambda-cyhalothrin in two ditch mesocosm systems of different trophic status. Environmental Toxicology and Chemistry 24 pp. 1684-1696

[30] Van Ewijk, P. H. and Hoekstra, J. A. 1993. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. Ecotoxicology and Environmental Safety 25 pp. 25-32

[31] Van den Brink, P. J., Hartgers, E. M., Fettweis, U., Crum, S. J. H., Van Donk, E. and Brock, T. C. M. 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide Linuron. Ecotoxicology and Environmental Safety 38 pp. 13-24

[32] Payne, R. W. 2002. GenStat® Release 6.1 Reference Manual. Lawes Agricultural Trust, Rothamsted Experimental Station. Harpenden, Hertfordshire. UK

[33] Aldenberg, T. and Jaworska, J. S. 2000. Uncertainty of the Hazardous Concentration and Fraction Affected for Normal Species Sensitivity Distributions. Ecotoxicology and Environmental Safety 46 pp. 1-18

[34] Van Vlaardingen, P. and Traas, T. P. 2002. ETX-2000. A program to calculate risk limits and potentially affected fraction, based on normal species sensitivity distributions.

[35] Aldenberg, T., Jaworska, J. S. and Traas, T. P. 2002. Normal Species Sensitivity Distributions and probabilistic ecological risk assessment. In: Posthuma, L., Suter II, G. W. and Traas, T. P., eds, Species sensitivity distributions in ecotoxicology, Lewis Publishers, Boca Raton, pp. 49–102

[36] Schroer, A. F. W., Belgers, D., Brock, T. C. M., Matser, A., Maund, S. J. and Van den Brink, P. J. 2004. Acute toxicity of the pyrethroid insecticide lambda-cyhalothrin to invertebrates of lenthic freshwater systems. Archives of Environmental Contamination and Toxicology 46 pp. 324-335

[37] Hommen, U., Veith, D. and Ratte, H. T. 1994. A computer program to evaluate plankton data from freshwater field tests. In: Hill, I. R., Heimbach, F., Leeuwangh, P., Matthiesen, P., eds, Freshwater Field Tests for Hazard Assessment of Chemicals, Lewis, Boca Raton, FL, pp. 503-513

[38] De Zwart, D. 2002. Observed regularities in species sensitivity distributions for aquatic species. In: Posthuma, L., Suter II, G. W. and Traas, T. P., eds, Species sensitivity distributions in ecotoxicology, Lewis Publishers, Boca Raton, pp. 133-154

[39] Eng, G., Whalen, D., Kirksey, A., Otieno, M., Khoot, L. E. and James, B. D. 1996. Fungicidal activity of some organotin compounds against *Ceratocystis ulmi*. Applied Organometallic Chemistry 10 pp. 501-503

[40] Nguyen, T. T., Ogwuru, N. and Eng, G. 2000. Tolerance of *Aedes aegypti* larvae to triorganotins. Applied Organometallic Chemistry 14 pp. 345-348

[41] Looser, P. W., Fent, K., Berg, M., Goudsmit, G.-H. and Schwarzenbach, R. P. 2000. Uptake and elimination of triorganotin compounds by larval midge Chironomus riparius in the abscence and presence of aldrich humic acid. Environmental Science and Technology 34 pp. 5165-5171

[42] Stäb, J. A., Traas, T. P., Stroomberg, G., Van Kesteren, J., Leonards, P., Van Hattum, B., Brinkman, U. A. T. and Cofino, W. P. 1996. Determination of organotin compounds in the foodweb of a shallow freshwater lake in The Netherlands. Archives of Environmental Contamination and Toxicology 31 pp. 319-328

[43] Traas, T. P., Stäb, J. A., Kramer, P. R. G., Cofino, W. P. and Aldenberg, T. 1996. Modeling and risk assessment of tributyltin accumulation in the food web of a shallow freshwater lake. Environmental Science and Technology 30 pp. 1227 -1237

[44] Hose, G. C. and Van den Brink, P. J. 2004. Confirming the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. Archives of Environmental Contamination and Toxicology 47 pp. 511-520

[45] Maltby, L., Blake, N. N., Brock, T. C. M. and Van den Brink, P. J. 2005. Insecticide species sensitivity distributions: the importance of test species selections and relevance to aquatic ecosystems. Environmental Toxicology and Chemistry 24 pp. 379-388

Chapter 5

The influence of sediment quality on the responses of benthic invertebrates after treatment with the fungicide triphenyltin acetate

Elske M. de Haas
Ivo Roessink
Bets Verbree
Albert A. Koelmans
Michiel H.S. Kraak
Wim Admiraal

Environmental Toxicology (2005) 24 pp. 1133-1139

Abstract

After decades of pollution, benthic communities in flood plain lake ecosystems are likely to be exposed to a diverse assortment of sediment-bound historical toxicants and nutrients, as well as pulses of newly discharged or deposited toxicants. The aim of this study was therefore to analyze the effects of background sediment pollution on the responses of benthic invertebrates to an experimental toxic shock in a laboratory setting. Sediment from a relatively clean and a historically polluted flood plain lake located along the river Waal, a branch of the river Rhine, The Netherlands, were selected and the fungicide triphenyltin acetate (TPT) was used as the acute stressor. Juvenile stages of the mayfly *Ephoron virgo* and the midge *Chironomus riparius* were chosen as test organisms because of their different response to sediment-bound toxicants and food quantity and quality. Our results demonstrated that the type of sediment had no effect on survival and growth of *C. riparius* when exposed to sediment-associated TPT, and that *E. virgo* was more affected by sediment-associated TPT on clean sediment than on polluted sediment. For the mayfly there was no cumulative response of the historical pollution and the recent toxic shock. This observation is discussed in view of the variable content of organic matter, acting both as food and as sorbent, and leads to the hypothesis that a very strong sequestering of TPT in historically polluted sediment prevents expression of toxic effects.

Introduction

The study of the effects mixtures and multiple stressors has become recognized as a critical sub discipline within environmental toxicology [1, 2]. Yet, the time scale of pollution is also of crucial importance: Sudden toxic events may be superimposed on chronic historical pollution. This is typically the case in floodplain lake sediments of the rivers Rhine and Meuse, which contain high concentrations of xenobiotic compounds deposited in the 1960s and 1970s [3]. Since the water quality of the rivers Rhine and Meuse has improved [4], the sediments of their floodplains may act not only as a sink, but also as a source of a wide range of chemical substances, such as nutrients, metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) [3]. The benthic communities of floodplain lakes are thus exposed to a diffuse assemblage of sediment-bound toxicants and nutrients, which may alter the benthic community composition [5, 6]. In addition, the highly dynamic nature of flood plain lake sediments, due to flooding, draining, erosion, and re-deposition, may lead to pulsed exposure of benthic invertebrates to toxicants (temporarily) desorbed from sediment or transferred to suspended particles [7]. Similar pulses may originate from the occasional use of pesticides in the surrounding agricultural area [8, 9].

This study is part of a larger study which aimed to determine the capabilities of *macro*invertebrate communities equilibrated in experimental ecosystems with clean and polluted sediment to cope with a recent chemical stressor [10]. The fungicide triphenyltin acetate (TPT) was applied as a model toxicant, because TPT is highly toxic to aquatic organisms when they are exposed via the water [11-13]. However, due to its high affinity with organic matter [14-16], and its resistance to degradation [17], TPT is to be expected to accumulate in sediment, and may therefore present a risk to the benthic communities. Sediment from a relatively clean and a historically polluted floodplain lake located along the river Waal, a branch from the river Rhine, were transferred into experimental basins and spiked with a range of TPT concentrations [10]. These microcosm experiments provide information on the short- and long-term combined effects of historical sediment pollution and defined pulses of pollution on community composition, including the successional changes in the benthic community. The aim of the present study was to determine the combined effects of historical sediment pollution and a recent chemical stressor, independently from these complex community responses, on benthic invertebrate species via whole-sediment bioassays. In these bioassays the sensitive mayfly species *Ephoron virgo* and the tolerant midge species *Chironomus riparius* were used. These two species were chosen as test organisms because of their different responses in whole-sediment bioassays [18].

Materials and Methods

Triphenyltin acetate addition

Sediment for the bioassays originated from microcosms that were used to study the responses and recovery abilities of macroinvertebrate communities in clean and polluted sediments after an application of the fungicide Triphenhyltin (TPT) (Fentin acetate Pestanal®, Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) [10]. Sediments for these microcosm experiments were sampled in November 2000 from a relatively clean and a historical polluted flood plain lake located along the river Waal, The Netherlands.

A total of 20 concrete outdoor microcosms (140 ×120 × 80 cm; L x W x D) were used in the outdoor microcosm study. A sediment layer of approximately 10 cm and a water layer of approximately 50 cm were introduced in the test systems in November 2000, eight months prior to the start of the experiment. Ten microcosms were filled with polluted sediment and ten microcosms with clean sediment. The microcosms were left settling for half a year in order to restore sediment stratification and facilitate new equilibria between sediment and water. Three months before the TPT application, macroinvertebrates collected from

uncontaminated drainage ditches (Sinderhoeve Experimental Station, Renkum and Veenkampen, experimental field site of Wageningen University, The Netherlands) with an artificial substrate were introduced to develop a freshwater community. Each microcosm received an equal proportion on invertebrates, compromising several taxonomic groups. The microcosms were assigned randomly to the different treatment levels. Four microcosms were used as controls.

The microcosms were assigned randomly to the different treatment levels. Sediments in the microcosms were contaminated by adding TPT to the water column in June 2001, in the following concentrations: 0, 1, 10, 30, and 100 µg TPT/L. In all microcosms 0.5 mL 96% ethanol per L water was used as a carrier solvent. Control microcosms were treated with an equal amount of carrier solvent without TPT.

Sediment and water sampling

The sediments for the bioassays were sampled from the microcosms in October 2001, 15 weeks after TPT application using cores with an internal diameter (i.d.) of 3.8 cm. About 1 L of the top 5 cm of the cores were homogenized and stored in 600-mL polyethylene containers at -20 °C until use.

Sediment for TPT analyses was sampled the same day as the sediment for the bioassays. From the control treatments and the 30 µg TPT/L spiked microcosms sediment cores (i.d. 3.8 cm) were sampled in duplicate. The top 5 cm of the sediment was isolated and each duplicate sediment sample was transferred to a centrifuge tube and stored at -20ºC until analysis. The actual sediment-associated concentrations of TPT of the control and 30 µg TPT/L spiked treatments were used to derive the sediment-associated TPT concentrations in the 1, 10, and 100 µg TPT/L spiked treatments using linear regression.

Water was collected in January 2002 from the original sites in 25-L polyethylene jars and filtered with 1.2 µm pore sized glass microfibre filter papers (Whatman GF/C) (Whatman®, Maidstone, UK), in order to remove indigenous zooplankton, and stored at 4°C in the dark, under constant aeration.

Sediment analyses

Before analyses of TPT concentrations, the sediment was thawed and homogenized in the centrifuge tubes and centrifuged at 3500 rpm for 30 min, with a rotor diameter of 27 cm. Pore water was removed and the sediment was transferred to an aluminum tray and carefully homogenized. The sediment was weighed into a Schott bottle and 60 mL of a solution of 37% HCl, 48% HBr and deionized water (1:1:0.4, volume/volume/volume) was added to the bottles and they were shaken

on a shaking table at 175 bpm for 30 min. Next 80 mL of hexane was added to the bottles and shaken again at 175 bpm for 60 min. The hexane containing the TPT was drained into a Schott bottle and 25 mL deionized water, 5 mL Sodium acetate buffer and 100 µL Tetraethyl borate were added and shaken for 15 min at 175 rpm. The layer of hexane was then drained and evaporated. Hexane was analyzed for TPT using a gas chromatographer/mass spectrometer (HP 6890 with auto-injector HP 7683; mass selective detector (MSD): HP 5973 Network MSD) in selective ion mode.

Metals (Cd, Cu, and Zn) were measured using microwave-assisted digestion as described by De Haas *et al.* [19]. The Σ13PAHs and Σ15PCBs and grain size were measured by Koelmans and Moermond [19]. The organic matter content (OM) was measured as loss-on-ignition by combustion of 2 g dried sediment at 550°C for 6 h [20] in triplicate. Chlorophyll-*a* and phaeophytin were measured according to Lorenzen [21] in triplicate using 1 g dried sediment. Chlorophyll-*a* and phaeophytin contents were summed, because in sediments chlorophyll-*a* is already partly degraded into phaeophytin. Total phosphorous was determined using an ammoniummolybdate spectrometric method [22] in triplicate using 100 mg dried sediment.

Lipids were extracted from 0.5 g of dry sediment with 6 mL methanol containing 2.5% H_2SO_4 for 90 min at 80°C in closed test tubes. The 500 µL hexane and 1 mL 0.9% NaCl were added to the samples and placed for 1 min on a shaker and centrifuged (rotor diameter = 27 cm) at 12,000 rpm for 1 min. The 200 µL of the supernatant was transferred to a 200-µL vial. Fatty acid methyl esters (FAMEs) were measured using a gas chromatographer 8000 top (CE Instruments, Milan, Italy) by injecting a 2-µL aliquot in a polar 30-m DB-Wax column (0.25 mm i.d.; 0.5-µm film) (J&W Scientific, Folsom, CA, USA) (DB WAX means the type of column).

Whole sediment bioassays

Survival and growth of the mayfly *E. virgo* were determined in a 10-d experiment. First-instar nymphs (<48 h old) were obtained from field-collected eggs that had been maintained in artificial diapause at 4°C in our laboratory. Seven days prior to the start of the experiments, several glass slides containing *E. virgo* eggs were placed in petri dishes containing Elendt M7 medium [23] and transferred to 20°C in order to terminate the artificial diapause [24]. Sediments were thawed at 4°C four days before the start of the experiment.

One day before the start of the experiment three replicate glass jars (150-mL) with 25 mL wet homogenized sediment and 100 mL filtered site water were prepared. At the start of the experiment twenty nymphs

were transferred into each test vessel. In addition, the body length of twenty larvae was measured using an automatic image analyzer (Leica® MZ 8 Microscope equipped with a Leica® DC100 digital camera (Leica Geosystems Products, Rijswijk, The Netherlands) using the computer program Research Assistant 3 (RVC, Hilversum, The Netherlands). At the end of the test, nymphs were collected from the sediment using a magnesium sulfate solution with a density of 1.22 (503 g $MgSO_4.7H_2O$ in 1 L deionized water). First the overlying water is poured through a small (Ø 5 cm) 100-µm sieve then approximately 15 mL of magnesium sulfate is added on top of the sediment using a siphon and passed though the sieve after which the sediment is rinsed three times using tap water. Surviving nymphs were counted and body length was recorded using an automatic image analyzer.

Survival and growth of *C. riparius* larvae were determined in a 10-d bioassay. Experiments were started with first-instar *C. riparius* larvae (< 24 h old), which were obtained from a culture maintained in our laboratory. Three days prior to the start of the experiment five newly deposited egg ropes were removed from the culture and transferred to a petri dish with Elendt-M7 medium and placed at 20°C. Ten replicate 400-mL glass beakers with 75 mL homogenized wet sediment and 300 mL filtered site water were prepared one day before the start of the experiment. At the start of the experiment five larvae were transferred into each test vessel. Twenty larvae were additionally measured for body length using an automatic image analyzer. After 10 d, larvae were obtained from the sediment by sieving through a 200-µm sieve. Surviving larvae were counted and body length was recorded using an automatic image analyzer.

All experiments were conducted at 20 ± 1 °C, moderate light (~10 µmol/m^2/s) and a 16-h light regime with 30 min of twilight (~5 µmol/m^2/s) before and after each light period. During the experiments the test systems were continuously aerated. Temperature, dissolved oxygen, and pH were monitored at the beginning and the end of each test. During the experiments water quality conditions were satisfactory in all test systems.

Data analyses

Two-way analysis of variance (ANOVA) tests followed by Scheffé's post hoc test were conducted to test for significant differences in survival and growth between the two sediments and TPT treatments of both species and to assess the contribution of sediment type and TPT concentration to the observed variation in survival and growth for both species. If data were not homogenous or normal distributed the data were

log-transformed. Differences were considered significant between the test categories at the 0.05 probability level.

Median effect concentrations for survival (LC_{50}) and growth (EC_{50}) were calculated by a nonlinear curve-fitting procedure using the logistic response model after Haanstra *et al.* [25]:

$$Y = c/1+e^{b(X-a)}$$

where Y = effect (%), c = effect in control (%), a = log EC_{50} (µg/kg), b = slope, and X = log concentration (µg/kg).

The differences in effect concentrations determined for the two sediments were tested for significance by fitting the toxicity data of the treatments simultaneously to logistic models that differed in their slope parameters but had the same effect concentration parameter. A likelihood ratio test was used to test the hypothesis of similarity of effect concentrations by comparing these results to those obtained when each model had its own effect concentration parameter [26]. All statistical analyses were performed using the computer program SPSS 10.0® for Windows® (SPSS, Chicago, IL, USA).

Results

Sediment characteristics

The characteristics of the sediments used in this study are listed in Table 1. The concentrations of Cd, Cu, Zn, ΣPAHs, and ΣPCBs in the clean sediment were low, according to sediment quality criteria [28]. Toxicant concentrations, especially the organic contaminants, in the polluted sediment were much higher than in the clean sediment.

The organic matter content (OM content) was higher in the polluted sediment than in the clean sediment (4.4% and 2.0% respectively), total phosphorus concentrations were higher in the polluted sediment compared to clean sediment (1924 and 878 mg/kg dry wt, respectively) and the initial chlorophyll-*a* concentrations were higher in the clean than in the polluted sediment (14.1 and 8.3 mg/kg dry wt, respectively). Although chlorophyll-*a* concentrations were higher in the clean sediment, the sum of fatty acids (ΣFAs), the sum of polyunsaturated fatty acids (ΣPUFAs), and the sum of fatty acids of bacterial origin (ΣBacFAs) were higher in the polluted sediment (234.9, 14.3, and 127.0.4 mg/kg dry weight, respectively) than in the clean sediment (166.2, 6.07, and 101.4 mg/kg dry weight, respectively).

Table 1. Sediment characteristics. Cd, Cu, Zn, sum of polycyclic aromatic hydrocarbons (ΣPAHs,), chlorophyll-a (Chl-a), total phosphorus (P), sum of fatty acids (ΣFAs), sum of polyunsaturated fatty acids (ΣPUFAs), and sum of bacterial fatty acids (ΣBacFAs) in mg/kg dry weight, sum of polychlorinated biphenyls (ΣPCBs) in µg/kg dry weight, organic matter (OM), porosity, clay, silt, and sand in percentage.

	Clean	Polluted
Cd (mg/kg dry wt)	0.30	1.24
Cu (mg/kg dry wt)	9.0	37
Zn (mg/kg dry wt)	45	270
ΣPAHs[a] (mg/kg dry wt)	0.16	3.17
ΣPCBs[a] (µg/kg dry wt)	0.46	36.5
OM (%)	2.0	4.4
P (mg/kg dry wt)	878	1924
Chl *a* (mg/kg dry wt)	14.1	8.3
ΣFAs (mg/kg dry wt)	166.2	234.9
ΣPUFAs (mg/kg dry wt)	6.07	14.3
ΣBacFAs (mg/kg dry wt)	101.4	127.0
Porosity (%)	30.3	31.7
% Clay[a] (< 2 µm)	8.5	12.0
% Silt[a] (< 50 µm)	33.3	47.3
% Sand[a] (50-200 µm)	58.2	40.7
Texture	Sandy loam	Loam

[a] *De Haas et al.[29]*

With increasing TPT level an increasing amount of cyanobacteria on the sediment surface was observed in the polluted sediment treatments after 10 d in the mayfly bioassays, whereas no cyanobacterial mats were observed on the clean sediment. This increase of cyanobacteria resulted in increased chlorophyll-*a* concentrations in the polluted sediment, in contrast to the clean sediment, were chlorophyll-*a* concentrations were the same or slightly lower (Table 2).

Exposure concentrations

The measured and calculated concentrations of the TPT-spiked sediments are listed in Table 3. Background concentrations did not differ significantly between clean and polluted sediment. The measured concentrations of TPT were higher in the clean sediment compared to measured TPT concentrations in the polluted sediment. The sediment-associated TPT concentrations in this study are well within concentrations of TPT found in aquatic sediments (29 to 380 µg/kg dry wt in marinas) [27, 28].

Table 2. Chlorophyll-a concentrations (mg/kg dry wt) at the start and the end of the E. virgo bioassay. Standard errors are given in parenthesis.

	Water spiked concentrations of triphenyltin (µg/L)	Start	End
Clean	Control	14.1 (0.19)	13.9 (0.74)
	1	12.9 (0.41)	12.4 (0.53)
	10	11.1 (0.11)	11.9 (0.78)
	30	12.5 (0.07)	11.2 (0.17)
	100	12.0 (0.17)	10.8 (0.37)
Polluted	Control	8.3 (0.14)	9.9 (0.56)
	1	8.5 (0.05)	9.5 (1.00)
	10	8.0 (0.22)	10.8 (0.82)
	30	7.5 (0.29)	12.0 (0.57)
	100	6.5 (0.30)	15.1 (0.59)

Ephoron virgo

Survival in the clean sediment control was significantly higher than in the polluted sediment control (77.5% and 48.3% respectively, $p < 0.05$) (Fig. 1). With increasing TPT concentration a decrease in survival in the clean sediment was observed with significantly lower survival at the three highest TPT concentrations compared to control survival. In contrast, survival on the polluted sediment increased slightly but not significantly with increasing TPT concentration. Survival on the polluted sediment was higher at the three highest TPT concentrations than on clean sediment. The calculated 10-d LC_{50} value for the clean sediment was 41.06 µg TPT/kg, based on organic matter was 2.06 mg TPT/kg-OM. Since no significant effect of TPT was observed in the polluted sediment the LC_{50} value could not be calculated (Table 4). The two-way ANOVA showed that both sediment type and TPT were significant in explaining the observed variation in survival ($p < 0.001$) and also that the statistical interaction term between sediment type and TPT was significant ($p < 0.001$).

Growth of the nymphs in the clean sediment control was higher than growth in the polluted sediment control (267 and 238 µm, respectively), but not significantly different (Fig. 1). With increasing TPT concentration a decrease in growth of the nymphs was observed in both clean and polluted sediment. Significantly lower growth ($p < 0.05$) compared to the control was observed at the two highest TPT concentrations for both clean and polluted sediment. The two-way ANOVA verified that both sediment type and TPT were significant in explaining the observed variation of growth ($p < 0.05$). The effect of TPT on growth of *E. virgo* nymphs was higher in the clean sediment, which is reflected by the significantly lower 10-d EC_{50} value for growth of *E.*

virgo for the clean sediment ($p < 0.05$) compared to the 10-d EC_{50} value for the polluted sediment (146.2 and 771.8 µg TPT/kg, respectively) (Table 4). Smaller differences in toxicity were observed when the EC_{50} values were based on organic matter. Again the EC_{50} value for the clean sediment was lower (7.33 mg TPT/kg), but not significantly different from the EC_{50} value for the polluted sediment (17.73 mg TPT/kg) (Table 4).

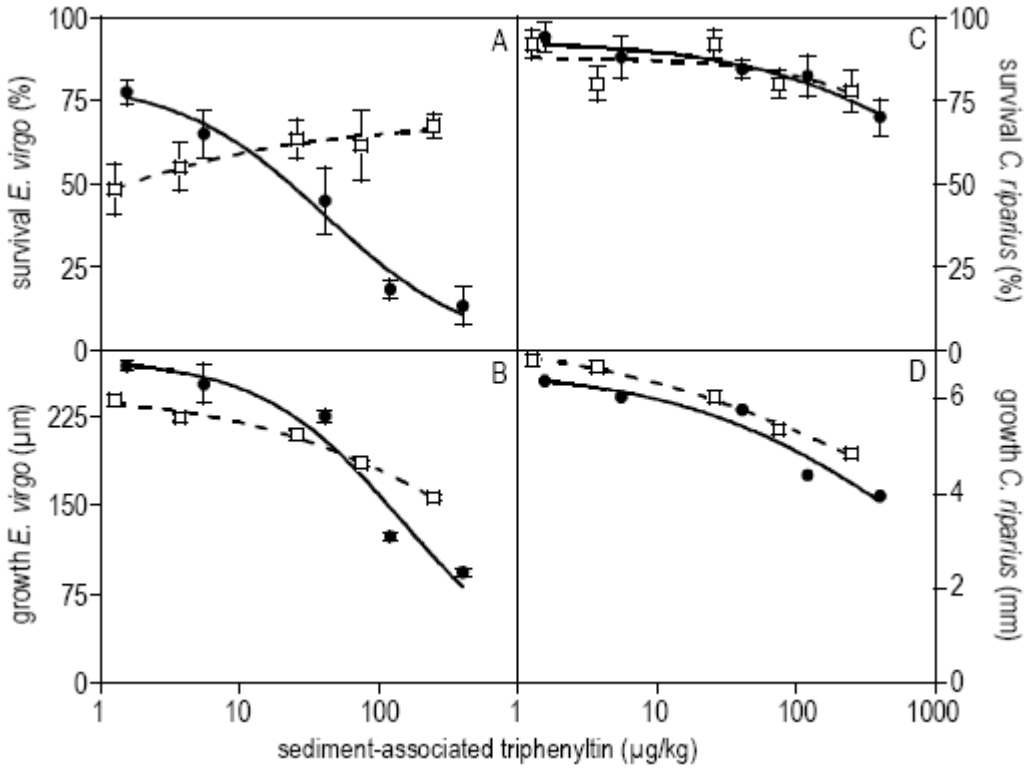


Figure 1: Survival and growth of Ephoron virgo nymphs and Chironomus riparius larvae after 10 days of exposure to clean and polluted sediment contaminated with triphenyltin (TPT). –●– = clean sediment, --□-- = polluted sediment. Lines represent the curve fit following the logistic response model after Haanstra et al. [25). Error bars = standard error.

Chironomus riparius

Survival in the clean and polluted sediment control was good (94% and 92% respectively) (Fig. 1). Survival in clean and polluted sediment decreased slightly, but not significantly, with increasing TPT concentration. No significant differences between survival on clean and polluted sediment were observed. The two-way ANOVA, however, showed that TPT was significant in explaining the observed variation in survival ($p < 0.01$), but both sediment and the statistical interaction term between sediment and TPT were not significant in explaining the observed variation of survival. This indicates that TPT had a slight effect on survival of *C. riparius* larvae, independent of the type of sediment

used. The 10-d LC_{50} values for TPT exposure to *C. riparius* could not be calculated for both clean and polluted sediment.

Table 3. Sediment triphenyltin (TPT) concentrations (µg/kg) in clean and polluted sediment at the start of the experiment.

Water spiked TPT (µg/L)	TPT (µg/kg) Clean	Polluted
Control	1.54[a]	1.25 [a]
1	5.50[b]	3.70 [b]
10	41.16[b]	25.76 [b]
30	120.39 (34.8)[a]	74.77 (14.8)[a]
100	397.72[b]	246.31 [b]

[a]*measured TPT concentrations*
[b]*calculated from linear regression with measured sediment-associated TPT concentrations in control and 30 µg TPT/L treatments.*

Growth of the larvae in the polluted sediment control was higher than growth in the clean sediment control (6.95 and 6.44 mm, respectively), but not significantly different (Fig. 1). With increasing TPT concentration a decrease in larval growth was observed in both clean and polluted sediment. Significant differences ($p < 0.05$) compared to control growth were observed at the two highest TPT concentrations on clean and at the highest TPT concentration on polluted sediment. The two-way ANOVA demonstrated that both TPT and sediment were significant in explaining the observed variation in growth ($p < 0.0001$). The 10-d EC_{50} value for growth of *C. riparius* for the clean sediment was lower than for the polluted sediment (724.6 and 1279 µg TPT/kg, respectively), but not significantly different (Table 4). However, when the 10-d EC_{50} values were based on organic matter (Table 4) the EC_{50} values were lower on the polluted sediment than on the clean sediment (36.32 and 29.94 mg TPT/kg, respectively), but not significantly different.

Discussion

The present results showed that on clean sediment, sediment-associated TPT had a clear negative effect on survival and growth of the mayfly *E. virgo* and a moderate inhibitory effect on survival and growth larvae of the midge *C. riparius*, indicating that the nymphs of the mayfly were more sensitive to TPT than the larvae of the midge, as has been observed in other studies [1, 18].

In the midge bioassays the presence of historical pollution did not affect the response curve of TPT. The midge is coping well with historical pollution, as has been observed in a previous study [18]. This indifference may relate to the high food levels that allow this species to thrive under chemical stressors [29].

Table 4. Median effect concentrations for survival (LC_{50}) and growth (EC_{50}) of Ephoron virgo nymphs and Chironomus riparius larvae after 10 d of exposure to sediment-associated triphenyltin (TPT) in a clean and a polluted sediment based on whole-sediment and organic matter (OM); n = 3; 95 % confidence limits are given in parentheses; effect concentrations per species sharing the same letter are not significantly different ($p < 0.05$); NE = no effect at highest concentration of sediment-associated TPT tested.

	LC_{50} (µg/kg dry wt)	EC_{50} (µg/kg dry wt)	OM-LC_{50} (mg/kg OM)	OM-EC_{50} (mg/kg OM)
Ephoron virgo				
Clean	41.06 (14.97-112.58)	146.2A (68.17-313.6)	2.06 (0.75-5.64)	7.33A (3.42-15.72)
Polluted	NE[a]	771.8B (472.9-1260)	NE	17.73A (10.77-29.18)
Chironomus riparius				
Clean	NE	724.6A (265.9-1975)	NE	36.32A (13.33-98.98)
Polluted	NE	1279A (757.5-2219)	NE	29.94A (17.46-51.36)

In contrast, the presence of historical pollution had a high impact on control survival of *E. virgo*, while growth was slightly retarded compared to the clean sediment control. This indicates that the historical sediment pollution is already toxic to *E. virgo*, which is in agreement with the findings of De Haas *et al.* [18].

Since *E. virgo* was more sensitive to both TPT and historical pollution than *C. riparius*, severe effects were expected after TPT exposure on the historically polluted sediment. In contrast, no additional effect on survival of *E. virgo* was observed with increasing TPT contamination, and growth of the nymphs was only slightly inhibited. The two types of sediment pollution had no additive effects, but seem to have even antagonistic effects.

The suppression of the effects of TPT on the polluted sediment may either be caused by a decrease in bioavailability, by counteracting the adverse effects, or both. Several arguments for a decreased bioavailability can be given. First, the organic matter content in the polluted sediment was 2.2 times higher than in the clean sediment. The sorption of triorganotins to sediments is a fast process involving primarily particulate organic matter constituents as sorbents [30], sorption of toxicants to particulate organic matter may indirectly influence toxicity, by changing several physical and physiological processes, which determine the bioavailability of the toxicant [2]. Indeed many other studies pointed to lower toxicity of organotin compounds when the amount of organic matter increased [11, 15, 16, 31]. There is evidence

that increasing humic and fulvic acid concentrations cause a decrease in bioconcentration of organotin compounds in aquatic organisms [16, 32]. This observed behavior is similar to the general finding that the binding of an organic chemical to the organic carbon phase depends mainly on its hydrophobicity [32].

Bioavailability may also have been reduced due to the aging of the sediments with TPT for a period of 15 weeks. Landrum *et al.* [33] observed a lower bioavailability of organic contaminants in aged sediment compared to freshly spiked sediment. Sequestering of TPT in the more sandy clean sediment may be less strong so that at a similar concentration of sorbed TPT a greater biological effect is provoked.

Second, it was observed that with increasing TPT concentrations an increasing area of the sediment surface in the polluted sediment was covered with cyanobacteria, which was not observed in the clean sediment. As a result, the contact of *E. virgo* to the contaminated sediment may have decreased, because the nymphs were able to avoid direct contact with the contaminated sediment [34, 35], because early instars live on the sediment surface and consequently the likelihood of survival may have increased [36]. Besides the increase in cyanobacteria resulted in an increase in organic matter in the top layer of the sediment, which may have further reduced the bioavailability and thereby the toxic effects of TPT in the polluted sediment. Furthermore, the cyanobacterial mat may have served as an alternative food source to the mayflies increasing their fitness and thereby reducing their susceptibility to the toxicants [2, 37]. Moreover, bacteria, algae, and fungi are capable of degrading organotin compounds into less toxic derivates, *e.g.*, debutylation of tributyltin to di- and monobutyltins. In addition, they are capable of accumulating organotin compounds [38, 39].

The results of the whole-sediment bioassays match well with the results recorded at the community level in the complementary microcosm study [10]. This microcosm study showed that the benthic invertebrate community on the polluted sediment recovered faster (12 weeks after the TPT application) from TPT addition than the benthic invertebrate community exposed via the clean sediment. Moreover, in the microcosm study, the midges were the least affected organisms in both clean and historically polluted sediment and in our study the midge *C. riparius* was only slightly affected with increasing TPT concentration. Thus, the present observations on single species are consistent with those on the development of communities.

These observations show that a sorption of a recent pollutant on historically polluted sediments does not lead to addition of effects. In contrast, the strong binding responsible for the prolonged residence of

historical loads of organic pollutants and metals appeared to be also very effective in sequestering recent toxicants.

Acknowledgement–This investigation was subsidized by The Netherlands Organization for Scientific Research (SSEO Project: 014.23.013 and 014.23.012).

References

[1] Van der Geest, H. G., Greve, G. D., Boivin, M., Kraak, M. H. S. and Van Gestel, C. A. M. 2000. Mixture toxicity of copper and diazinon to larvae of the mayfly (*Ephoron virgo*) judging additivity at different effect levels. Environmental Toxicology and Chemistry 19 pp. 2900-2905

[2] Heugens, E. H. W., Jager, T., Creyghton, R., Hendriks, A. J., Van Straalen, N. M. and Admiraal, W. 2003. Temperature-dependent effects of cadmium on Daphnia magna: Accumulation versus sensitivity. Environmental Science and Technology 37 pp. 2145-2151

[3] Beurskens, J. E. M., Mol, G. A. J., Barreveld, H. L., Van Munster, B. and Winkels, H. J. 1993. Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environmental Toxicology and Chemistry 12 pp. 1549-1566

[4] Admiraal, W., Van der Velde, G., Smit, H. and Cazemier, W. G. 1993. The rivers Rhine and Meuse in The Netherlands: Present state and signs of ecological recovery. Hydrobiologia 265 pp. 97-128

[5] Pinel-Alloul, B., Méthot, G., Lapierre, L. and Willsie, A. 1996. Macroinvertebrate community as a biological indicator of ecological and toxicological factors in Lake Saint-Francois (Quebec). Environmental Pollution 91 pp. 65-87

[6] Canfield, T. J., Brunson, E. L., Dwyer, F. J., Ingersoll, C. G. and Kemble, N. E. 1998. Assessing sediments from upper Mississippi River navigational pools using a benthic invertebrate community evaluation and the sediment quality Triad approach. Archives of Environmental Contamination and Toxicology 35 pp. 202-212

[7] Zoumis, T., Schmidt, A., Grigorova, L. and Calmano, W. 2001. Contaminants in sediments: Remobilization and demobilization. Science of The Total Environment 266 pp. 195-202

[8] Stäb, J. A., Cofino, W. P., Van Hattum, B. and Brinkman, U. A. T. 1994. Assessment of transport routes of triphenyltin used in potato culture in the Netherlands. Analytica Chimica Acta 286 pp. 335-341

[9] Liess, M. and Schulz, R. 1999. Linking insecticide contaminantion and population response in an agricultural stream. Environmental Toxicology and Chemistry 18 pp. 1948-1955

[10] Roessink, I., Crum, S. J. H., Bransen, F., Van Leeuwen, E., Van Kerkum, F., Koelmans, A. A. and Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality. Ecotoxicology 15 pp. 267-293

[11] Bao, M. L., Dai, G. and Pantani, F. 1997. Effect of dissolved humic material on the toxicity of tributyltin chloride and triphenyltin chloride to *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 59 pp. 671-676

[12] Fargasová, A. 1998. Comparison of effects of tributyl-, triphenyl-, and tribenzyltin compounds on freshwater benthos and alga *Scenedesmus quadricauda*. Bulletin of Environmental Contamination and Toxicology. 60 pp. 9-15

[13] Rehage, J. S., Lynn, S. G., Hammond, J. I., Palmer, B. D. and Sih, A. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile ambystoma barbouri salamanders. Environmental Toxicology and Chemistry 21 pp. 807-815

[14] Fent, K. and Hunn, J. 1991. Phenyltins in water, sediment, and biota of freshwater marinas. Environmental Science and Technology 25 pp. 956-963

[15] Looser, P. W., Fent, K., Berg, M., Goudsmit, G.-H. and Schwarzenbach, R. P. 2000. Uptake and elimination of triorganotin compounds by larval midge Chironomus riparius in the abscence and presence of aldrich humic acid. Environmental Science and Technology 34 pp. 5165-5171

[16] Looser, P. W., Bertschi, S. and Fent, K. 1998. Bioconcentration and bioavailability of organotin compounds: Influence of pH and humic substances. Appl Organomet Chem 12:601-611. Applied Organometallic Chemistry 12 pp. 601-611

[17] Sarradin, P.-M., Lapaquellerie, Y., Astruc, A., Latouche, C. and Astruc, M. 1995. Long term behaviour and degradation kinetics of tributyltin in a marina sediment. The Science of the Total Environment 170 pp. 59-70

[18] De Haas, E. M., Reuvers, B., Moermond, C. T. A., Koelmans, A. A. and Kraak, M. H. S. 2002. Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediment. Environmental Toxicology and Chemistry 21 pp. 2165-2171

[19] Koelmans, A. A. and Moermond, C. T. A. 2000. Stofstroom-analyse: zware metalen, PCB's en PAKs in uiterwaardplassen. T_0 vastlegging nulsituatie. Wageningen Universiteit. Wageningen. The Netherlands

[20] Luczak, C., Janquin, M. A. and Kupka, A. 1997. Simple standard procedure for the routine determination of organic matter in marine sediment. Hydrobiologia 345 pp. 87-94

[21] Lorenzen, C. J. 1967. Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. Limnology and Oceanography 12 pp. 343-346

[22] Murphy, J. and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27 pp. 31-36

[23] OECD. 2001. Guideline for the testing of chemicals, sediment-water chironomid toxicity test using spiked sediment. Guidline 218. Paris, France.

[24] Greve, G. D., Van der Geest, H. G., Stuijfzand, S. C., Kureck, A. and Kraak, M. H. S. 1999. Development and validation of an ecotoxicity test using field collected eggs of the riverine mayfly *Ephoron virgo*. Proc Sec Exp Appl Entomol Neth Entomol Soc 10 pp. 105-110

[25] Haanstra, L., Doelman, P. and Oude Voshaar, J. H. 1985. The use of sigmoidal dose response curves in soil ecotoxicological research. Plant Soil 84 pp. 293-297

[26] Van Gestel, C. A. M. and Hensbergen, P. J. 1997. Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. environmental Toxicology and Chemistry 16 pp. 1177-1186

[27] Smith, S. L., MacDonald, D. D., Keenleyside, K. A., Ingersoll, C. G. and Field, L. J. 1996. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. J GT Lakes Res 22 pp. 624-638

[28] Fent, K. and Hunn, J. 1995. Organotins in freshwater harbours and rivers: temporal distribution, annual trends and fate. Environmental Toxicology and Chemistry 14 pp. 1123-1132

[29] De Haas, E. M., Paumen, L. M., Koelmans, A. A. and Kraak, M. H. S. 2004. Combined effects of copper and food on the midge *Chironomus riparius* in whole-sediment bioassays. Environmental Pollution 127 pp. 99-107

[30] Berg, M., Arnold, C. G., Müller, S. R., Mühlemann, J. and Schwarzenbach, R. P. 2001. Sorption and desorption behavior of organotin compounds in sediment-pore water systems. Environmental Science and Technology 35 pp. 3151

[31] Meador, J. P., Krone, C. A., Dyer, D. W. and Varanasi, U. 1997. Toxicity of sediment-associated tributyltin to infaunal invertebrates: Species comparison and the role of organic carbon. Mar. Environ. Res. 43 pp. 219-241

[32] Fent, K. 1996. Ecotoxicology of organotin compounds. Critical Reviews in Toxicology 26 pp. 1-117

[33] Landrum, P. F., Eadie, B. J. and Faust, W. R. 1992. Variation in the bioavailability of the polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. . Environmental Toxicology and Chemistry 11 pp. 1197-1208

[34] West, C. W. and Ankley, G. T. 1998. A laboratory assay to assess avoidance of contaminated sediments by the freshwater oligochaete *Lumbriculus variegatus*. Archives of Environmental Contamination and Toxicology 35 pp. 20-24

[35] Kravitz, M. J., Lamberson, J. O., Ferraro, S. P., Swartz, R. C., Boese, B. L. and Specht, D. T. 1999. Avoidance response of the estruarine amphipod *Eohaustorius estruarius* to polycyclic aromatic hydrocarbon-contaminated field-collected sediments. Environmental Toxicology and Chemistry 18 pp. 1232-1235

[36] Ort, M. P. 1995. Toxicity of crude oil to the mayfly, *Hexagenia bilineata* (Ephemeroptera: Ephemeridae). Environmental Pollution 90 pp. 105-110

[37] Hall, T. J., Haley RK and LE., L. 1991. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental stream channels. Environmental Toxicology and Chemistry 10 pp. 1051

[38] White, J. S., Tobin, J. M. and Cooney, J. J. 1999. Organotin compounds and their interactions with microorganisms. Can J Microbiol 45 pp. 1-14

[39] Gadd, G. M. 2000. Microbial interactions with tributyltin compounds: Detoxification, accumulation, and environmental fate. Science of The Total Environment 258 pp. 119-257

Chapter 6

Impact of macrophytes and fish on partitioning of sediment-originating PCBs and PAHs in freshwater model ecosystems

I. Roessink
C. T. A. Moermond
F. Gillissen
A. A. Koelmans

Abstract

Ecosystem structure may significantly affect partitioning of sediment-originating hydrophobic organic compounds (HOCs). We studied effects of macrophyte and benthivorous carp additions on polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) (native *versus* freshly spiked**)** mass distribution and lipid-normalized concentrations in sediment (Soxhlet- and 6-h Tenax-extractable), suspended solids, macrophytes, periphyton, floating algae biomass, zooplankton, oligochaetes, other invertebrates and carp in replicated model ecosystems. Freshly spiked compounds were more mobile in the system than their more sequestered native counterparts. Macrophytes represented the largest amount of non-sediment (bio)mass in the systems and were capable of depleting up to 26 (PCB) and 31 (PAH) percent of the mobile, fast desorbing HOC sediment fraction in the 7 cm of sediment. However, the presence of macrophytes did not have a significant diluting effect on lipid-normalized HOC concentrations in fish. The major biological impact of carp on the test systems was their structuring of invertebrate communities through predation. The chemical impact of carp was increased partitioning of HOCs to other system compartments caused by resuspension of the sediment. For less hydrophobic HOCs, no increased partitioning occurred to floating algae biomass and periphyton.

Introduction

At present, many sediments still contain high concentrations of xenobiotic compounds [1]. These sediments may not only act as sink but also as a source of a wide range of chemical substances, for instance hydrophobic organic compounds (HOCs) such as polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [2]. As such, they can pose a threat to the ecological status of aquatic systems [3]. Numerous studies address behavior and effects of HOCs in the marine environment [4, 5], rivers [3, 6], or US great lakes [7, 8]. Relatively few studies focus on smaller, shallow floodplain lakes, which are abundant in deltas around the world, including the Rhine-Meuse delta in The Netherlands [9]. Due to their limited depth, shallow lakes have an intense water-sediment interaction as well as a potentially large impact of aquatic vegetation on its abiotic and biotic properties. This causes their functioning to be different from deep lakes [10]. Several factors may affect the ecological structure of shallow lakes, such as climate, nutrient loadings, hydrology, anthropogenic stressors and invasive species.

Besides experiencing gradual changes in ecosystem structure, shallow lakes can switch abruptly from a vegetated state with clear water to a turbid situation with high concentrations of phytoplankton and other

suspended solids, and *vice versa*. Such catastrophic regime shifts can be triggered by seasonality, grazing on macrophytes, inundation, eutrophication, benthivorous fish, toxic shocks and temperature [11]. Such changes in ecological structure, and its accompanying food web, can influence transport, partitioning, bioavailability and effects of HOCs in these systems [7, 12-17].

Unfortunately, the precise nature and mechanisms of these interactions are hard to disentangle. For instance, in several water bodies a negative relationship between lake trophic status and contaminant concentrations in biota has been observed [16-18], while in others positive relationships between bioaccumulation and lake trophy for benthic invertebrates was reported [13]. Part of this discrepancy may be explained by different volumes, areas, geography, food webs, sampling times, or species compositions between the lakes that are compared. The present study aims at systematically investigating how system trophic status and ecological structure affect partitioning, bioaccumulation and implications for risks of sediment-bound contaminants in model ecosystems mimicking floodplain lakes.

The presence of macrophytes can be an important structuring factor in shallow aquatic systems. They can reach biomass densities up to 1 kg dry weight/m^2 [19], thus representing a significant part of the total system biomass [20]. We hypothesize that macrophytes will act as a sink for HOCs and consequently may decrease the amount of HOCs in other compartments of the system. Fish are another important part of the aquatic food web. Shallow lakes are often dominated by benthivorous fish (*e.g.*, bream - *Abramis brama*) which rework sediments while foraging [21, 22]. Since 100 g bream/m^2 may resuspend 46 g sediment/m^2/day [23] it is hypothesized that the presence of benthivorous fish will mobilize more HOCs from the sediment and results in a higher amount of HOCs in other system compartments. Additionally, predation by fish will structure the invertebrate community hence influencing HOC distributions in zooplankton and other invertebrate fauna compartments.

To test these hypotheses, historically polluted lake sediment was transferred to indoor model ecosystems. Impacts of ecology on partitioning of HOCs may differ for historically sequestered *versus* recently spiked chemicals, because the latter can be assumed to be more mobile [24, 25]. Therefore, sequestered as well as freshly spiked HOCs were studied. Trophic status of the systems was characterized by presence of macrophytes (representing a macrophyte-dominated mesotrophic state) and absence of macrophytes (representing a phytoplankton-dominated eutrophic state). Addition of fish resulting in a 2x2 factorial design of four different ecological structures: with fish, with macrophytes, with fish and macrophytes, and without fish and macrophytes (Fig. 1). General

characteristics, macrophyte and invertebrate numbers and biomasses were monitored over a four-month period. To be able to distinguish between total and bioavailable (fast desorbing) HOC fractions in the sediment, Soxhlet-extractable (referred to as Total Sediment) and 6-h Tenax-extractable (referred to as TENAX) PCB and PAH concentrations [26-28] were monitored. In addition HOC fractions were monitored in biota. Results will be discussed along two angles: HOC mass distribution to address transport and partitioning issues and HOC lipid normalized concentrations in biota to address bioaccumulation.

Materials and methods

Experimental set-up

The model ecosystems consisted of glass aquaria (110 cm length, 110 cm width, 70 cm height, depth sediment layer 7 cm, height water column 50 cm) and were designed to contain four different ecosystem structures (each in triplicate): systems with fish and macrophytes (MF), systems with fish without macrophytes (PF), systems with macrophytes without fish (M), and systems with neither (P). When no macrophytes were present (P & PF), systems became phytoplankton-dominated.

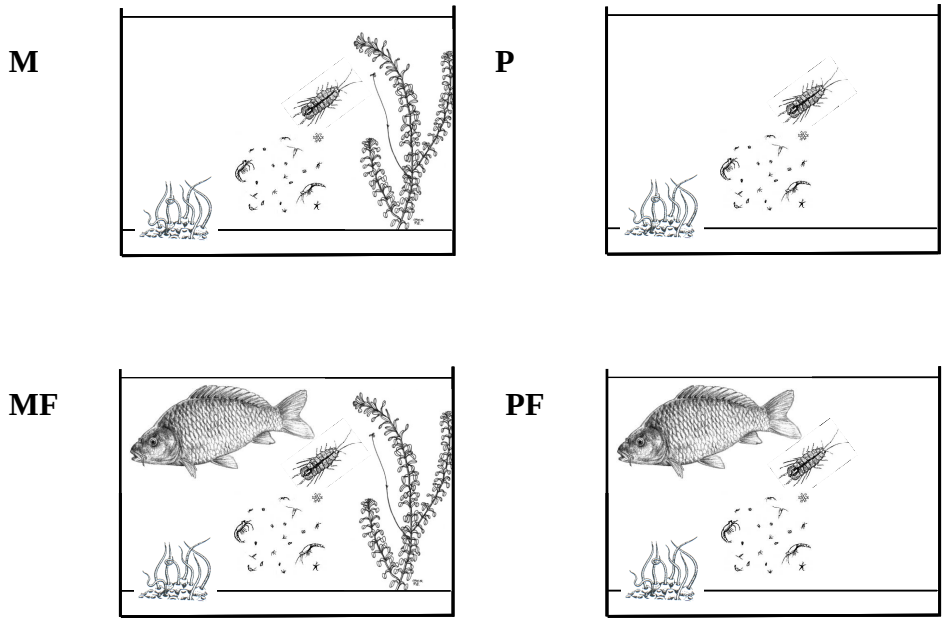


Figure 1: Schematic overview of the ecological structure of the four different treatments. With fish (PF), with macrophytes (M), with fish and macrophytes (MF), and without fish and macrophytes (P).

Sediments in the model ecosystems were spiked with a cocktail of Fluoranthene-d_{10} (Flu-d10), PCB 29, and PCB 155 in acetone. These chemicals could be analytically distinguished from their respective ‘native’ counterparts fluoranthene (Flu), PCB 28 and PCB 149, respectively. These chemical couples are assumed to differ only in time

of accumulation in the sediment (years to decades for native compounds *versus* four months for spiked compounds), not in other properties. Concentrations of spiked compounds were also similar in magnitude to concentrations measured in the sediment for their native counterparts. Spiking was performed in approximately 20 cm of water with 7 cm of sediment under dark conditions preventing photolytic PAH breakdown. During spiking, the sediment was kept in suspension by using a large mechanical stirrer [21], while sediment in corners of the model ecosystem was suspended manually. In twenty-five minutes one-third of the spike solution (total volume of 0.5 L) was slowly injected in the slurry using a continuous dosage system, after which stirring proceeded for another fifteen minutes. Over the next two days, the remaining two-thirds of the spike solution were added in the same way, after which the sediment was allowed to settle for three days. After spiking, the systems were filled to a volume of approximately 600 L using clean water from a deep well located at the 'Dreijen' area, Wageningen, The Netherlands.

Six systems were seeded with twenty 10 cm long shoots of *Elodea nuttallii*, obtained from a non-polluted site near Wageningen, The Netherlands. One week later, all model ecosystems were stocked with an invertebrate and zooplankton community from another unpolluted reference site near Wageningen. The added macroinvertebrate community (approximately 75 g wet weight) consisted mainly of *Lymnaea*, *Planorbidae*, *Asellidae*, *Gammaridae*, and *Hirudinea*. Five weeks after macrophyte insertion, three 3 cm-sized carp (*Cyprinus carpio*) were added to the model ecosystems which were designed to contain fish. Carp (*Cyprinus carpio*) was chosen as model species for benthivorous fish typical for shallow lakes. This was also a pragmatic choice since uncontaminated carp could easily be obtained from laboratory cultures from the department of Fish Culture and Fisheries at Wageningen University, The Netherlands. During the experiment no additional fish food was added.

To enable phytoplankton growth each system received nutrient dosages of 0.9 mg/L N and 0.15 mg/L P on a weekly basis. Also weekly, water levels in the model ecosystems were restored to their initial level to compensate losses due to evaporation and sampling. The experiment lasted for 15 weeks after macrophyte insertion, which is sufficiently long to reach maturity in the systems, but not so long that macrophytes start to decay [29]. Lamps were suspended over each model ecosystem, producing an average irradiance at the water surface of 140 µE/m/s and a 14:10 h light:dark photoperiod.

Sampling

Sampling occurred four months after start of the experiment. Suspended solids samples were obtained by filtering depth-integrated water samples over 0.8 µm filters (NC45, Schleicher and Schuell). Sediment was sampled randomly using five Perspex tubes (Ø 2.5 cm). The upper 5 cm of these samples were pooled and homogenized per system for analysis.

When present, floating algae biomass (FAB) was collected by scooping it from the water surface using a 500 µm sieve. All present macrophyte biomass was harvested, weighed and a representative portion was used for PCB/PAH analysis. Periphyton was mainly present on the walls of the model ecosystems. To collect periphyton, about 2000 cm^2 of wall area was scraped off with an aquarium net, after which periphyton was immediately transferred into glass containers. Fish were caught using a small net and killed using metacaine. Macroinvertebrates were sampled by means of core and net samples. Core sampling occurred with a plastic core (29 cm length, 29 cm width, 100 cm height), which was pressed into the sediment after which the enclosed sediment layer was removed and sieved (mesh size: 500 µm) to extract sediment-burrowing taxa, mainly oligochaetes. Two core samples per model ecosystem were taken and numbers and biomass of invertebrates were corrected for the total sediment surface. After sampling, the macroinvertebrates were counted and identified to the highest possible level of taxonomic resolution. Subsequently, macroinvertebrates were divided into oligochaetes and remaining invertebrates (further referred to as mixed fauna). For gut clearance they were kept overnight in aerated glass containers with filtered water from the model ecosystems in a 6 °C dark climate room. Spilled gut contents were removed and macroinvertebrates were rinsed with Barnstead Nanopure® water (Sybron-Barnstead, Dubuque, IA, USA). Zooplankton was sampled using a Perspex tube (length: 0.4 m; volume: 0.8 L). Sub samples were collected until a representative 5-L sample had been obtained. The 5-L sample was concentrated by means of a plankton net (Hydrobios, Kiel, Germany; mesh size: 55 µm) and was preserved with formalin (final volume: 4%). Cladocera and copepods were counted and identified using a binocular microscope, while rotifers were counted and identified using an inverted microscope. Abundances were adjusted to numbers of organisms per liter. For zooplankton PCB and PAH analysis the remaining model ecosystem volume (about 400 L) was filtered over the same net. All storage materials for PCB/PAH samples were pre-rinsed with picograde acetone. All samples for PCB/PAH analysis were freeze-dried and stored in brown glass jars at –20 °C until analysis.

Analytical procedures

NH_4NO_3 and KH_2PO_4 (both >99% purity) were obtained from Merck (Darmstadt, Germany). PAHs (anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(k)-fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(ah)anthracene, indeno(123)pyrene, phenantrene, fluoranthene, pyrene and 2-methyl-chrysene) were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). PCBs (IUPAC numbers 18, 20, 28, 31, 44, 52, 101, 105, 118, 138, 143, 149, 153, 170, 180, 194, and HCB) were purchased from Promochem (Wesel, Germany). Acetone used for spiking was picograde quality (Promochem). Other chemicals used for extraction and cleanup are described in [28].

Samples used for quantitative analyses (in triplicate) were dried at 105 °C and ashed at 550 °C until constant weight to obtain dry weight and organic matter content. Total PCB and PAH concentrations were determined through a 16-h Soxhlet-extraction with hexane:aceton (3:1 v:v) followed by detection with GC-ECD, as described in [26, 30]. 6-h Tenax-extractable concentrations in sediment were determined according to [26]. With every ten samples, one sample to determine clean up recoveries was included. Cleanup recoveries for 48 Soxhlet-extracted samples averaged 94 ± 4 (s.d.) % for PAHs and 97 ± 9 % for PCBs and analytes in reference samples were typically within error limits of reported values. All samples were corrected for cleanup recoveries and blanks.

Data analysis

Data analysis addressed sum parameters ΣPCB and ΣPAH and six key parameters. These parameters were PCB 28, PCB 149 and Flu (native), and PCB 29, PCB 155, and Flu-d10 (spiked). They were selected to represent lower chlorinated PCBs (PCB28/29), higher chlorinated PCBs (PCB149/155), and PAHs. Since PCBs have a broad range of K_{ow} values, which determines their behavior in the environment, ΣPCB is also divided into lower and higher chlorinated congeners (in our study ΣPCB_{low} comprised HCB, PCB 18 till PCB 52 and ΣPCB_{high} PCB 101, 105, 118, 138, 149, 153, 155, 170, 180, and 194).

Threshold levels for *p* were 0.05 for all statistical analysis. All PCB and PAH data were log-transformed before analysis. Complex parameters, *e.g.*, sumPCB (ΣPCB), were calculated before being log-transformed. PCB and PAH data below detection limits were replaced by half of the detection limit for that specific congener in that specific sample. The influence of fish and macrophyte presence on PCB and PAH levels in the system compartments (sediment, suspended solids, macrophytes, periphyton, FAB, zooplankton, oligochaetes, mix fauna,

and fish) was analyzed using ANOVA (both two-way and one-way analysis) within the statistical software package SAS, version 9.1. Using this ANOVA approach the effect of the presence of fish and/or macrophytes on the HOC levels in the several compartments was analyzed on the congener level. Detailed results of the ANOVA calculations will not be included in this paper, but are available on request.

Statistical tests were applied to water quality variables, species abundance, and HOC data. The distribution of invertebrates over the different types of model ecosystems was analyzed using redundancy analysis ordination technique (RDA), the constrained form of principal component analysis [31]. Prior to RDA analysis, macroinvertebrate data were $\ln(2x+1)$ and zooplankton data $\ln(10x+1)$ transformed, where x is the abundance data (for rationale, see [32]), and then grouped together in a new invertebrate data set. RDA was performed using the CANOCO computer program, version 4.02 [33]. A Principal Response Curve (PRC) analysis of the HOC data was also performed. For more details on this type of analysis see also [31, 34]. In the present PRC analysis the treatment containing macrophytes and fish (MF) was regarded as control treatment. In this treatment all compartments, including fish and macrophytes, were present which enables comparison with other treatments. Instead of sampling points in time the present experimental set-up yielded sampling points in different system compartments (*e.g.*, sediment, suspended solids, macrophytes, periphyton, FAB, zooplankton, oligochaetes, mix fauna, and fish), which can be plotted on the x-axis. However, this resulted in an unbalanced design since not all compartments were present in the analysis. Thus, the compartment fish could only be analyzed in the treatments MF and PF, which contained fish, and logically not in treatments M and P, in contrast to compartment zooplankton, which was present in all four treatment types.

Results and Discussion

General characteristics and ecology

Water quality variables (dissolved oxygen (DO), pH, and conductivity) are presented in Table 1. Of these variables, only pH differed significantly between macrophyte and phytoplankton-dominated systems at the end of the experiment ($p < 0.01$; average levels 10.0 and 9.0 respectively). DO was low in the two weeks after spiking but increased rapidly before invertebrates were introduced to the systems. DO levels in phytoplankton-dominated systems (8.0 ±2.0 mg/L) were lower than in macrophyte-dominated systems (8.8 ±1.9 mg/L), but the difference was not significant. This indicates that conditions in all systems were equal, apart from the presence of plants and/or fish and

consequently that any differences observed in invertebrate communities between systems were caused by the presence of plants and/or fish. These variables are within normal range for the species present [35, 36], which suggests sufficient agreement between model ecosystems and natural systems.

Figure 2 shows results of the RDA analysis of the invertebrate abundance data. The snail *Lymnaea stagnalis* is plotted in the upper left quadrant of the figure near the treatment code M indicating that in this treatment relatively more *Lymnaea* are present. Taxa like the bivalve *Pisidiidae* in the opposite quadrant (lower right quadrant) are relatively rare in the M treatment. Figure 2 therefore indicates that most macroinvertebrate species are found when macrophytes are present but fish are not (M). When fish is present (PF and MF) only robust macroinvertebrate species with shells (*Planorbidae and Pisidiidae*) or hard exoskeletons (*Agabus sp.*), which can withstand predation by these fish, are found. In systems containing fish relatively more zooplankton is found, especially rotifers and copepods. These taxa are small, reproduce relatively fast, and are agile which makes them harder to predate and thus enables them to coexist with fish. Without fish (M and P), these taxa are relatively less abundant because in these systems they are out-competed and predated by larger zooplankton and macroinvertebrates. Since these responses are reported earlier [22, 37] in field systems this illustrates that test systems used in this experiment comprise realistic food webs.

Table 1. Water quality variables presented as geometric means per treatment

	Dissolved Oxygen				pH				Conductivity			
Day	M	MF	P	PF	M	MF	P	PF	M	MF	P	PF
1	2.3	3.1	2.5	2.7	7.45	7.5	7.5	7.5	413	414	406	410
8	0.5	0.4	0.8	0.8	7.4	7.4	7.4	7.5	407	409	401	405
22	1.0	1.1	0.9	0.9	7.4	7.4	7.5	7.4	389	390	390	452
29	3.8	3.6	3.7	3.9	7.5	7.5	7.5	7.4	369	370	369	370
36	6.2	5.9	4.7	5.4	7.8	7.8	7.7	7.7	379	380	380	379
43	9.8	9.7	9.9	9.7	8.6	8.5	8.6	8.6	376	376	376	380
50	7.2	7	6.9	7.12	8.5	8.4	8.5	8.5	381	378	388	388
57	9.4	9.8	8.4	8.6	8.8	8.6	8.6	8.7	354	354	392	387
64	9.4	9.6	8.7	8.8	8.6	8.7	8.4	8.5	293	282	339	335
71	14.0	12.7	13.0	12.6	9.2	9.2	8.7	8.8	224	222	277	276
78	11.0	8.3	9.7	8.4	9.9	8.7	9.2	8.7	205	229	239	267
85	11.4	9.3	11	9.0	10.3	9.2	9.5	8.8	214	164	229	274
92	9.3	7.9	8	7.5	10.2	9.3	9.4	8.8	207	219	224	262
100	8.4	7.7	7	7.1	10.3	9.5	9.3	8.8	203	216	228	262
106	8.6	7.8	7.3	7.0	10.3	9.7	9.4	8.9	204	209	228	256
113	7.8	7.5	6.5	6.1	10.2	9.8	9.3	8.6	204	205	237	262
120	8.1	7.8	7.0	6.4	10.2	9.8	9.1	8.7	201	196	239	267
127	7.6	6.6	6.2	5.9	10.3	9.8	9.3	8.7	197	201	242	270

Combined with the observed growth (from 3 ± 0.3 cm and 0.5 ± 0.13 grams to 6.7 ± 0.7 cm and 4.2 ± 1.4 grams) of the fish, it can be concluded that the systems were able to sustain small fish for at least a four-month period. Grift *et al.* [38] reported approximately 2.1 YOY (young-of-the-year) fish.m^{-2} in 3 shallow floodplain lakes alongside the river Waal, The Netherlands. Copp [39] estimates a YOY biomass of 11.7 g.m^{-2} in the Upper Rhône River catchment (in systems that were comparable to our experimental systems). Our test systems were stocked with 2.5 fish.m^{-2} and contained a fish biomass of 10.9 g.m^{-2} (based on wet weight) illustrating a good resemblance between our experiments and field conditions [38, 39].

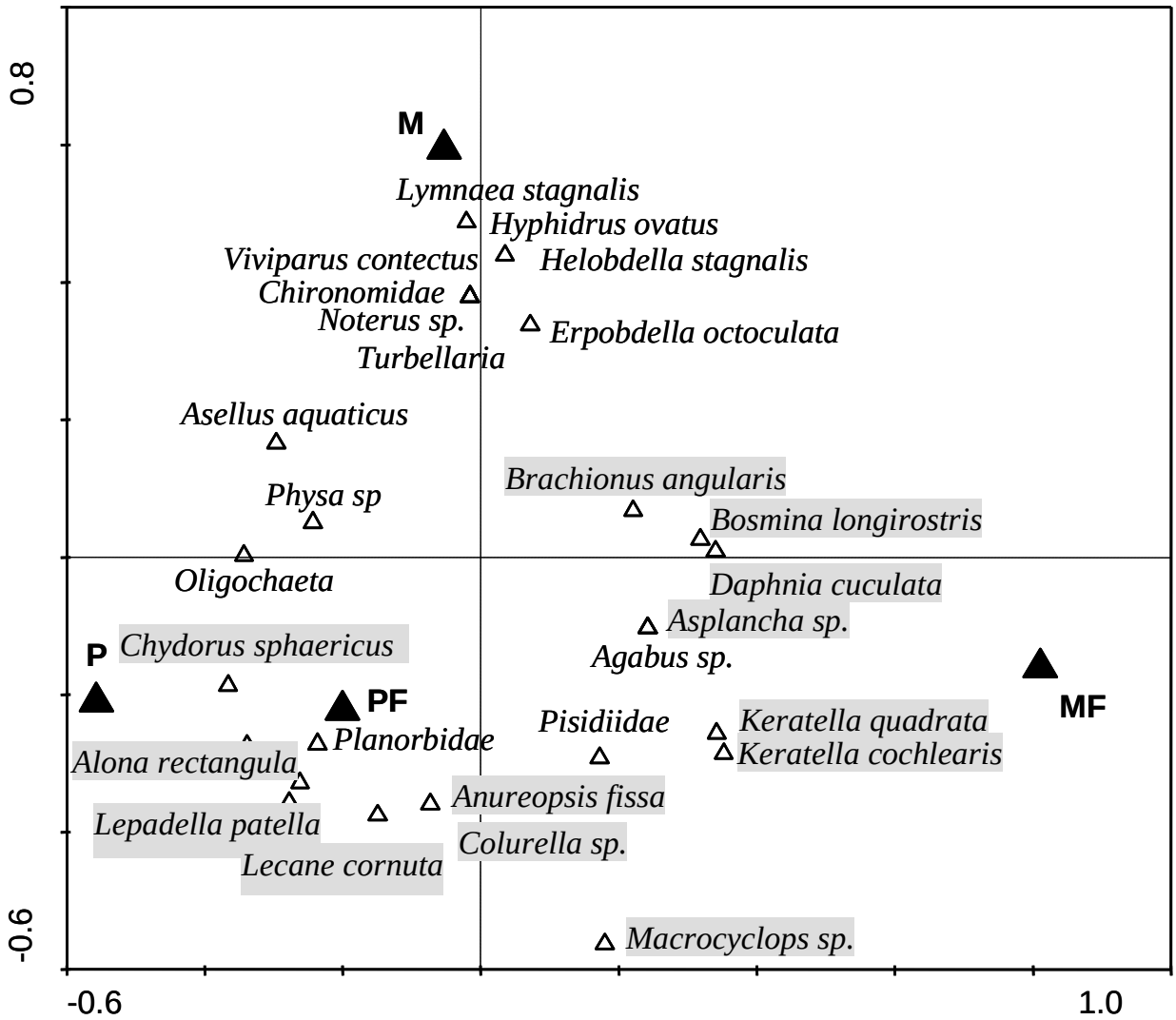


Figure 2: Results of RDA analysis of the invertebrate abundance data. Zooplankton taxa are highlighted in the graph. M = macrophyte-dominated without fish, MF = macrophyte-dominated with fish, P = phytoplankton-dominated without fish, PF = phytoplankton-dominated with fish. Species plotted close to a treatment type data point, are relatively much present in that treatment.

HOC mass distribution

On average, sediment pools contained 99.6 (±0.4) % of the total HOC mass in all four system types. The mass distribution of biota, ΣPCBs and ΣPAHs is presented in Table 2 and Figure 3. In macrophyte-dominated systems, macrophytes represent the main part of biomass (Fig. 3A). Less macrophyte and more periphyton (algae attached to the model ecosystem wall) biomass are present when fish is present in the macrophyte-dominated systems (M and MF). When no macrophytes are present (P and PF) total biomass in the systems is mainly composed of periphyton. Figures 3B and C show how the mass of ΣPAHs and ΣPCBs, which was originally for 100% bound to the sediment, is redistributed amongst different system compartments at the end of the experiment. These figures also indicate that ecological structure strongly affects the amounts of ΣPAHs and ΣPCBs which are mobilized from the sediment. When sediment is not taken into account, macrophytes (when present) dominate the mass distribution followed by periphyton and suspended solids (Fig. 3B and C). In general, biological compartments that have a large total biomass also contain a large amount of ΣPAHs and ΣPCBs (Fig. 3A-C).

Nevertheless, although macrophyte biomass is larger without fish (M) than with fish (MF), the total amount of ΣPAHs and ΣPCBs contained in the systems without fish was smaller (Table 2), although not significant due to within-treatment variation (ANOVA, $p > 0.05$; see Appendix Table 4 and 6). This suggests that bioturbation of the sediment by fish results in a higher amount of ΣPAHs and ΣPCBs available for the macrophytes. It is unclear if these ΣPAHs and ΣPCBs are indeed bioaccumulated by or sorbed to the macrophytes. Although macrophytes were washed carefully before analyses, it cannot be excluded that in the presence of fish a higher amount of suspended solids is trapped in the macrophyte/periphyton complex, which was not washed out, thus resulting in a higher amount of ΣPAHs and ΣPCBs in this macrophyte compartment.

For periphyton, exposure via the water phase seems to be strongly influenced by the presence of macrophytes, which seem to act as a sink for the contaminants and consequently lower the amount of ΣPAHs and ΣPCBs available for uptake through the water phase. PCB and PAH uptake in the mix fauna is not governed by presence of such a possible sink but primarily by fish predation. Without fish more invertebrates are present (Fig. 2), and consequently more ΣPAHs and ΣPCBs were found in this compartment. In fish (which was stocked in equal numbers) more PCBs were found when macrophytes were absent (PF). The latter was not observed for PAHs, most likely because fish are known to metabolize these compounds [40, 41].

Table 2: Average and standard deviations of amounts of biomass, ΣPCB, and ΣPAH measured at the end of the experimental period in the test systems.

		M[a]		MF		P		PF	
		average	stdev	average	stdev	average	stdev	average	stdev
Biomass (gr)	Sediment	6.4×10^4	3.2×10^3	6.4×10^4	4.4×10^3	6.2×10^4	8.7×10^3	6.3×10^4	2.3×10^3
	Susp. solids	4.3	0.9	6.9	3.7	2.1	0.9	20	14
	Macrophyte	645	52	486	210	n.p.	n.p.	n.p.	n.p.
	Periphyton	n.p.	n.p.	27	44	167	55	33	32
	FAB	2.3	0.9	3.6	0.5	2.2	0.05	1.7	1.8
	Zooplankton	0.32	0.16	0.31	0.01	0.82	0.40	0.61	0.29
	Oligochaetes	8.0	0.6	3.2	2.9	6.4	3.2	3.4	1.5
	Mix fauna	2.1	0.5	1.0	0.01	1.3	1.0	0.65	0.17
	Fish	n.p.[b]	n.p.	2.3	0.3	n.p.	n.p.	3.4	0.84
ΣPCB (μg)	Sediment	1.4×10^4	3.5×10^3	1.3×10^4	2.6×10^3	1.5×10^4	1.3×10^3	1.4×10^4	3.8×10^3
	TENAX	2.1×10^3	1.3×10^2	2.2×10^3	640×10^2	2.8×10^3	4.1×10^2	2.8×10^3	1.5×10^2
	Susp. solids	1.9	0.99	3.3	0.8	1.7	0.68	13	9.7
	Macrophyte	52	7.0	67	59	n.p.	n.p.	n.p.	n.p.
	Periphyton	n.p.	n.p.	3.0	5.1	31	10	7.1	6.2
	FAB	0.37	0.15	0.60	0.17	0.37	0.02	0.48	0.44
	Zooplankton	0.28	0.12	0.28	0.08	0.62	0.34	0.99	0.75
	Oligochaetes	2.1	0.80	0.53	0.45	2.5	0.98	1.2	0.75
	Mix fauna	0.77	0.51	0.30	0.20	0.54	0.11	0.13	0.06
	Fish	n.p.	n.p.	3.1	0.52	n.p.	n.p.	5.5	1.3
ΣPAH (μg)	Sediment	3.5×10^5	7.6×10^4	4.1×10^5	7.3×10^4	4.9×10^5	1.3×10^5	4.7×10^5	1.8×10^5
	TENAX	1.9×10^4	7.1×10^2	1.9×10^4	3.8×10^3	3.5×10^4	1.9×10^4	2.8×10^4	1.2×10^4
	Susp. solids	21	19	61	29	27	24	221	188
	Macrophyte	394	157	582	616	n.p.	n.p.	n.p.	n.p.
	Periphyton	n.p.	n.p.	34	57	236	90	104	90
	FAB	2.3	0.33	3.0	0.64	1.8	0.36	3.7	3.3
	Zooplankton	1.8	1.5	1.8	0.14	1.2	0.69	1.2	1.1
	Oligochaetes	6.7	0.62	2.1	1.9	7.2	3.8	3.2	0.26
	Mix fauna	3.3	2.0	0.26	0.14	1.5	0.68	0.30	0.30
	Fish	n.p.	n.p.	0.33	0.15	n.p.	n.p.	0.46	0.06

[a]M = macrophytes, no fish; MF = macrophytes, fish; P=phytoplankton, no-fish; PF=phytoplankton, fish;
[b]n.p. = not present

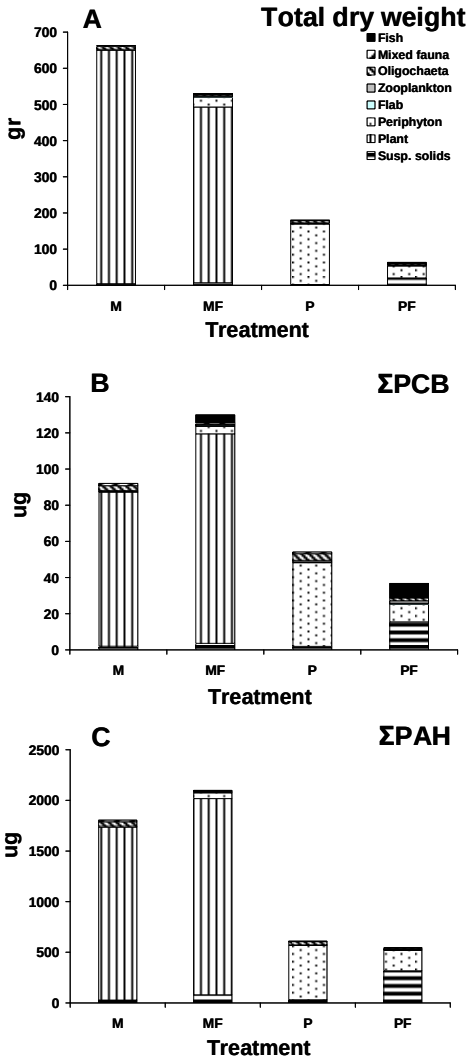


Figure 3: Panel A shows the total amount of biomass (as gram dry weight) per compartment (present in each type of test system (n = 3). Panel B and C present the same picture for the total amount of ΣPCBs and ΣPAHs, respectively. Sediment mass (about 63.2 kg dry weight per model ecosystem) is excluded from the graph.

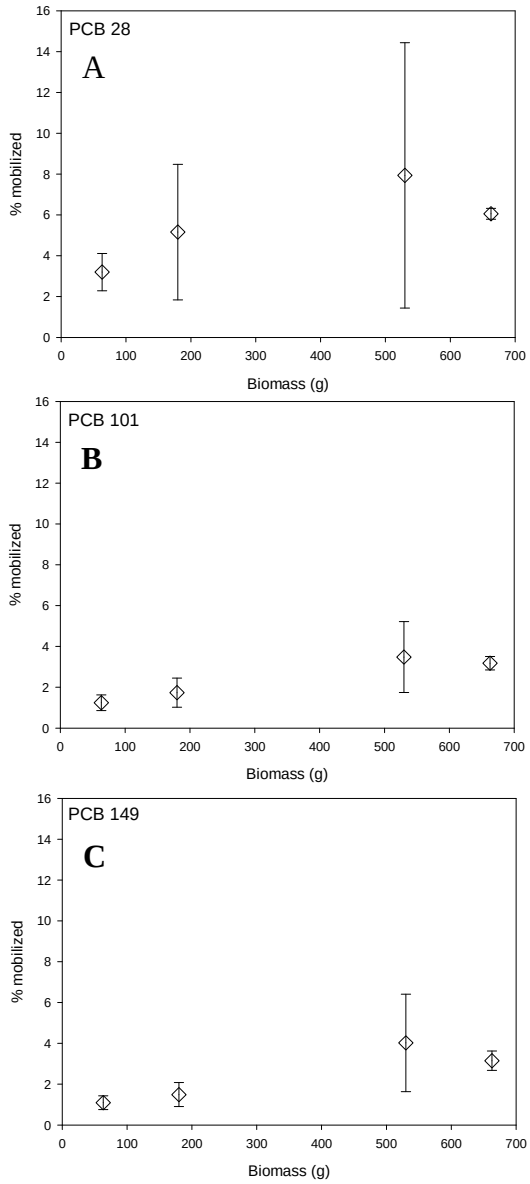


Figure 4: Percentage of PCBs mobilized from the sediment (6-h Tenax-extractable) as function of total biomass in the test system. Systems are ranked in increasing biomass: P, PF, MF, M. Error bars show standard deviations.

Mobilization by biomass

It appears that macrophytes mobilize substantial amounts of HOCs out of the sediment, and fish even further increase this mobilization. This is illustrated in Figure 4 where the relation between amounts of ΣPAHs and ΣPCBs mobilized from the sediment and total system biomass is shown. We assume that mobilization is primarily from the fast desorbing fraction, as quantified through the 6-h Tenax-extractable concentration [42]. Mobilization is calculated as:

$$\%mobilized = \frac{mass_{HOC,compartment,end}}{mass_{HOC,compartment,end} + mass_{HOC,tenax,end}} \times 100\% \qquad \text{(Equation 1)}$$

with $mass_{HOC,compartment,end}$ = the amount of HOC in a certain compartment at end of the experimental period; and $mass_{HOC,tenax,end}$ = the amount of HOC present in the 6-h Tenax-extractable fraction at end of the experimental period.

In general, Figure 4A-C suggest that more PCBs are mobilized when total system biomass is higher. This trend can be observed for both lower (PCB 28), intermediate (PCB101), and higher (PCB149) chlorinated congeners. However, due to large variations between replicate treatments no significant differences between treatments were observed. Partitioning of individual PCBs and PAHs from 6-h Tenax-extractable fractions to other compartments is further illustrated in Figure 5 and Table 3, where concentrations in plants, oligochaetes, rest fauna, and fish (at t = 4 months) are plotted against the 6-h Tenax-extractable concentrations in the sediment at t = 4 months, according to:

$$\frac{\left(\dfrac{mass_{HOC,compartment,end}}{mass_{lipids,compartment,end}}\right)}{\left(\dfrac{mass_{HOC,tenax,end}}{mass_{OC,end}}\right)} = \frac{y}{x} = a \qquad \text{(Equation 2)}$$

with a = slope of the regression; $mass_{lipids,compartment,end}$ = the amount of lipids in that specific compartment at end of the experimental period; and $mass_{OC,end}$ = the amount of organic carbon present in sediment at end of the experimental period.

Since the experiment was performed in a closed laboratory environment, external PAH or PCB influxes were assumed to be not significant. Consequently, when no PAHs or PCBs are present in the 6-h Tenax-extractable fraction, then also none will be found in macrophytes and the y-x intercept can be forced through zero ($y = ax$). The slopes of the y-x regressions (Table 3) can be considered as ecosystem based, semi-field derived lipid and organic carbon normalized biota to sediment accumulation factors (BSAF) for PCBs after 4 months of exposure.

Table 3: Regression results of the distribution of PCB and PAH congeners in the 6-h Tenax-extractable fraction versus macrophytes, oligochaetes, mix fauna, and fish at the end of the experimental period. Since the regression is assumed to start in the origin of the graph this results in y = ax.

		PCB		PAH	
		a	R^2	a	R^2
Plants	M	1.51	0.86	0.88	0.72
	MF	1.73	0.83	2.02	0.64
Oligochaetes	M	5.20	0.82	0.016	0.76
	MF	2.40	0.84	0.004	0.53
	P	3.90	0.84	0.63	0.48
	PF	5.24	0.70	0.96	0.49
Rest fauna	M	7.42	0.96	0.007	0.74
	MF	9.79	0.60	0.001	-0.04
	P	5.50	0.92	0.79	0.45
	PF	3.45	0.69	0.39	0.41
Fish	MF	9.21	0.71	0.0003	0.41
	PF	8.44	0.77	0.046	0.44

Figure 5 and Table 3 show that for macrophytes this slope of the regression lies between 1 and 2 which falls within the range of 1-2 suggested by the equilibrium partitioning theory (EPT)[43-45]. For oligochaetes, mix fauna, and fish the slope of the regression increases to 2.4-5.2, 3.5-9.8, and 8.4-9.2, respectively (Figure 5 and Table 3). These values exceed the ones being predicted by EPT and increase with trophic level, which suggests that biomagnification is relevant for these compartments. Note that data points in Figure 5 relate to different PCB congeners and are randomly distributed around the regression line. This suggests that BSAFs in this case are not significantly different for different congeners. Comparison of 95% confidence intervals of each regression revealed that for macrophytes all intervals overlapped and consequently M and MF did not differ statistically. For oligochaetes the MF treatment differed from the other treatments (M, P, and PF). This difference may relate to the lower numbers of worms in treatments containing fish plus the presence of macrophytes (acting as competing ‘sink’ for PCB). For mix fauna the PF treatment differed from the others, fish predation and lack of refugia (*e.g.*, macrophytes) resulted in a different mix fauna community in this treatment (Fig. 2), with probably different uptake and depuration mechanisms and consequently different partitioning of PCBs. No difference was observed for fish. PAH data

were not used for these analyses, because of possible PAH degradation and larger uncertainties between PAH fast desorbing fractions and 6-h Tenax extractable concentrations [40, 46].

Effects of macrophytes and fish on mass distribution of HOCs

The presence of fish reduced the amount of HOCs in oligochaetes and mix fauna, by a factor 2.7 (PCBs) and 3.4 (PAHs). Due to fish predation they comprise less biomass in the MF and PF systems and consequently also contain a lesser total amount of PCBs and PAHs. The presence of fish also resulted in a significant increase of higher chlorinated, less mobile PCBs, with exception of spiked PCB155, in suspended solids. Probably, due to intense interaction with the water phase, less hydrophobic (low chlorinated) or less sequestered PCBs (like PCB 155) can partition to the water phase faster and be scavenged by other compartments resulting in this discrepancy.

Figure 6 shows the results of PRC analysis of the PCB and PAH data. In the PRC graph deviations from the control (in this case the MF treatment) are presented on the C_{dt}-axis while on the x-axis the different system compartments are plotted. The weight on the b_k-axis on the right-side of the diagram can be interpreted as the affinity of each congener with the response in the diagram [31]. In this type of analyses, deviations less than 0.5 are not very informative, and as a consequence for PCBs (Fig. 6A) only the deviations of the compartments periphyton and mix fauna are relevant. Since all b_k-scores are positive no congeners decrease in the samples. PCB 031 (with the highest positive weight on the b_k-axis) is indicated to have increased the most in samples with the highest C_{dt} score; whereas the weight of PCB 170 indicates that its increase is smallest of all congeners. Thus, especially lower chlorinated and more mobile congeners are found in treatments deviating from the control (MF) (Fig. 6A). The corresponding eigen-values of the PRC analysis are 0.068 and 0.009 for the first and second canonical axis, respectively. The fact that the first eigen-value is more than 7 times bigger than the second value indicates that congener response is dominated by one factor. Since the distribution of PCBs on the b_k-axis shows that lower chlorinated congeners have higher scores than higher chlorinated congeners this factor is therefore most likely $\log K_{ow}$. Deviations in PAHs between systems types are found in compartments periphyton, mix fauna, and also in suspended solids (Fig. 6B). Since in fish-free systems the suspended solids compartment contained lower amounts of Flu-d_{10} and benzo(e)pyrene (BeP) it appears that the concentrations of PAHs and PCBs in suspended solids in these systems is altered by fish-mediated resuspension of sediment. Unlike in the PCB response, in the PAH response no overall governing factor could be observed.

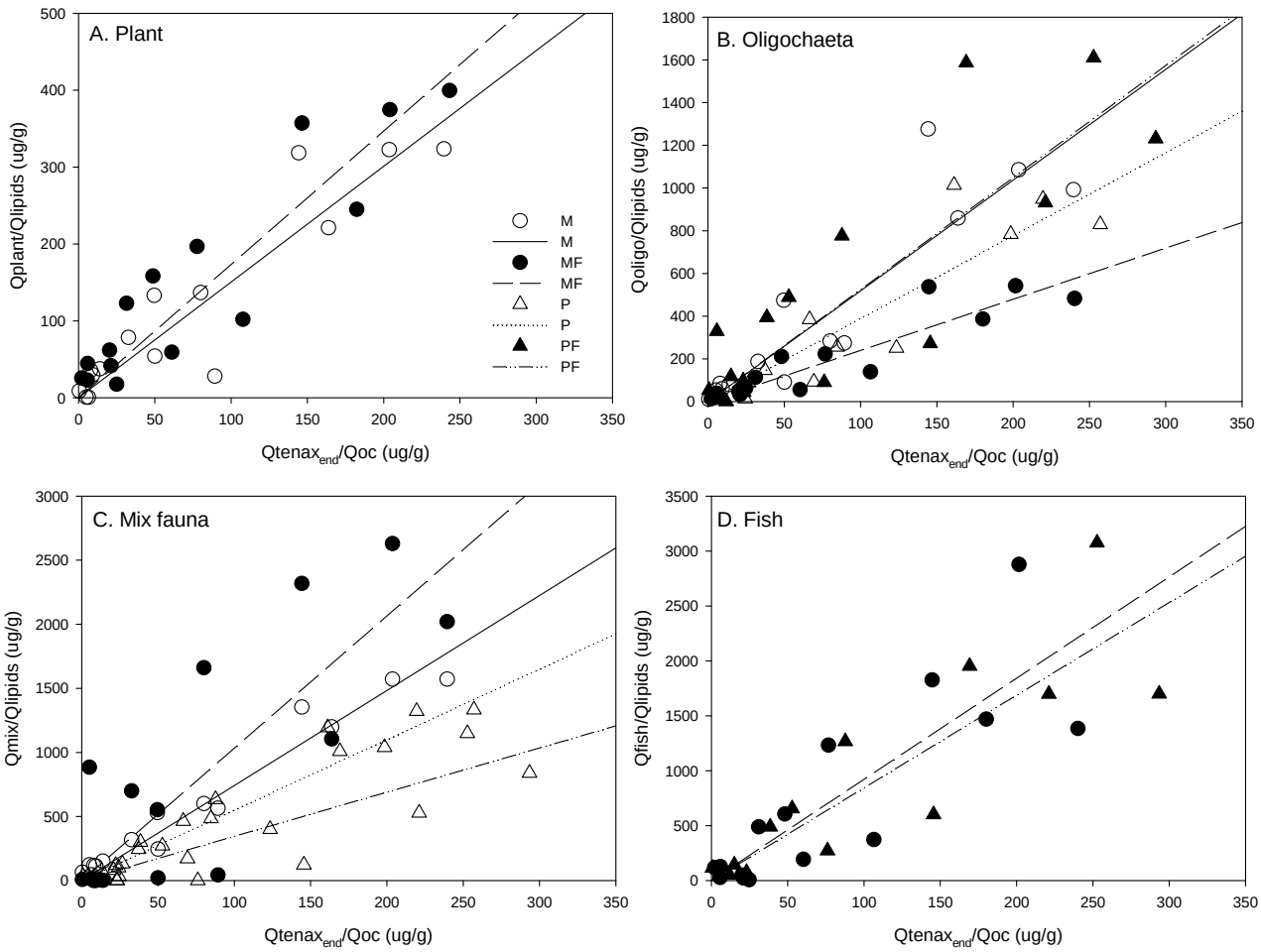


Figure 5: Distribution of PCB congeners in the 6-h Tenax-extractable fraction versus macrophytes (A), oligochaetes (B), mix fauna (C), and fish (D) at the end of the experimental period.

While the presence of fish does not alter FAB biomass it significantly lowers total amounts of all PAHs except Flu-d_{10} measured in the FAB compartment (see Appendix Table 6, data other congeners not shown). Because of the increase in Flu-d_{10}, the decrease in all other PAHs is not reflected in ΣPAH (see Appendix Table 6). Since FAB floats on the water surface its only exposure route is via the water phase meaning that it competes for PAHs and PCBs with *e.g.*, suspended solids, which are more abundant in fish containing systems. We hypothesize that this lowers the amount of PAHs and PCBs in FAB. Flu-d_{10} is less sequestered in the sediment and thus more available and abundant in the aqueous phase.

As discussed before, presence of macrophytes results in significant differences in masses of PCBs and PAHs in the sediment, TENAX, periphyton, and fish compartments. The effect is most significant (ANOVA; $p < 0.05$; see Appendix Tables 5 and 6) for added compounds PCB 29, 155, and Flu-d_{10} but was also observed for PCBs 18, 20, 28, and 31 (total concentrations in sediment) and PCB 44, 52, 101, and 105 (6-h

Tenax-extractable concentrations). Macrophyte presence decreases amounts of periphyton and consequently has a significant influence on the total periphyton HOC content (ANOVA; $p < 0.05$; see Appendix Table 5 and 6). However, macrophyte presence also significantly influences other compartments like the sediment, which does not differ in size among treatments. This indicates that macrophytes, when present, provide a sink and can consequently 'drain' the sediment.

There is a very consistent difference between amounts of spiked and native HOCs observed in different system compartments (results available on request, see also Moermond *et al.* [46]). Especially spiked compounds are bioaccumulated in the biotic compartments, with a larger difference for the less hydrophobic PCB29 than for the more hydrophobic PCB155. For PCB 29 and its native counterpart PCB 28, the difference was statistically significant in the Sediment, TENAX, zooplankton, oligochaetes, and mix fauna compartments, while for PCB 155 and its native counterpart PCB 149 this was only the case for the TENAX compartment. A statistical significant difference between amounts of Flu and Flu-d_{10} could be observed in sediment, TENAX, FAB, oligochaetes, and mix fauna. In all cases differences were more pronounced when fish or macrophytes were present.

Lipid normalized HOC concentrations

Toxicological effects are related to actual concentrations in biota [47], rather than total mass distributions. Therefore, also effects of ecological structure (*e.g.*, the influence of fish and macrophytes) were evaluated on concentrations of PAHs and PCBs in biota (see Appendix Tables 7-9). Note that because all model ecosystems used the same sediments, statistical tests among treatments by using biota concentrations yield the same results as would have been obtained if biota sediment accumulation factors (BSAFs) were used.

The presence of fish seems to have the largest influence on PCB and PAH concentrations in macrophyte and mix fauna compartments (see Appendix Table 7 and 9). When fish is present, macrophytes contain a significantly higher concentration of spiked PCBs. Although less significant ($p = 0.08$), this also seems to be the case for some of the higher chlorinated native congeners (PCB 105, 118, 149, 153, and 155). It is plausible that bioturbation by fish mobilized these PCBs, resulting in higher water concentrations and consequently a higher exposure to PCBs for the macrophytes. However, it is unlikely that only higher chlorinated PCBs are mobilized. Probably, lower PCBs are also mobilized from the sediment but may be accumulated less in the macrophyte compartment due to increased desorption from the plant material and scavenging by other compartments. Besides, in the absence of fish macrophytes have a

significantly higher percentage of organic carbon and a higher lipid fraction (both $p = 0.04$, data not shown), consequently diluting their lipid-normalized PCB concentrations. Probably, both processes amplify possible differences between treatments. The same response, although not statistically significant, can also be observed for PAH concentrations.

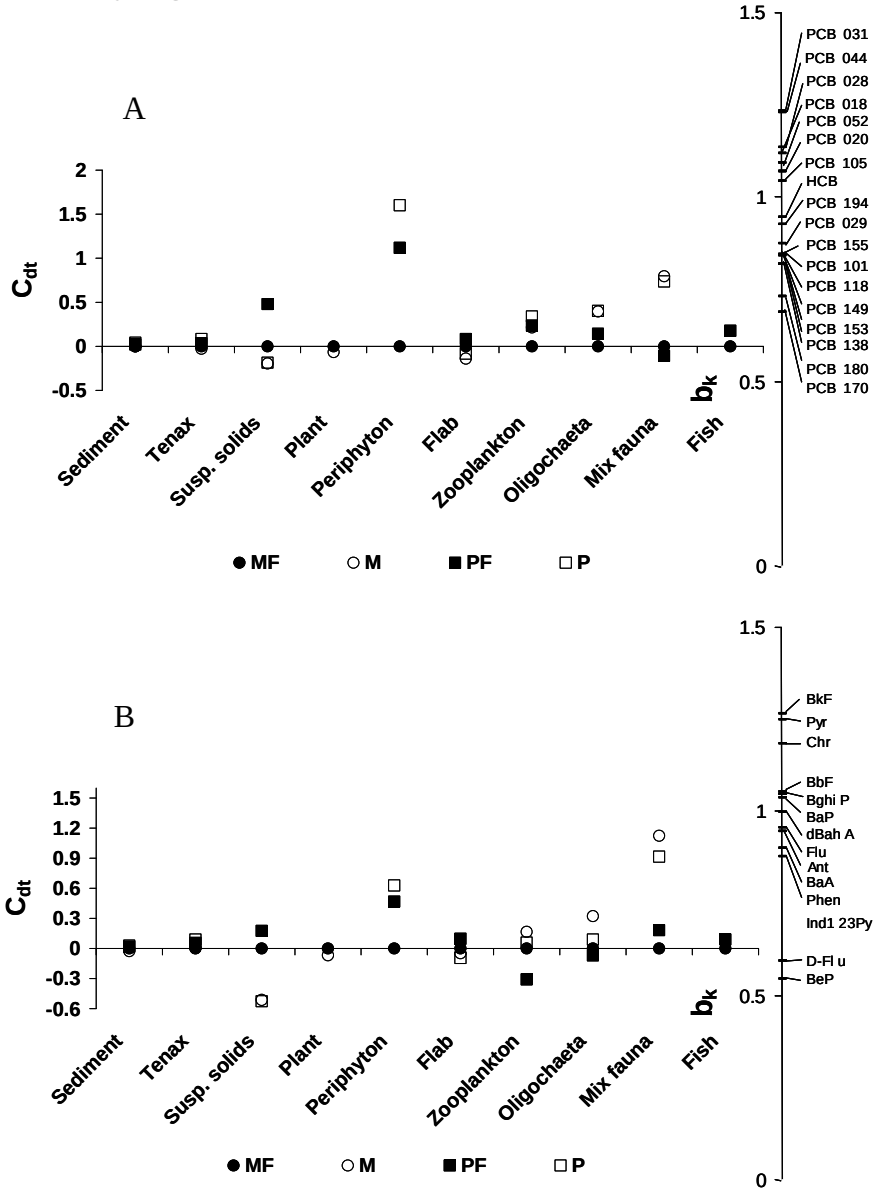


Figure 6: Results of the Principal Response Curve (PRC) analysis of PCB (A) and PAH (B) data based on the mass distribution. On the x-axis the different system compartments are plotted and on the y-axis (C_{dt}) the deviation of the M, P, and PF systems from the MF systems (which are considered controls) is presented. On the b_k-axis on the right side of the diagram the congeners are plotted. MF= macrophyte-dominated with fish, M= macrophyte-dominated, PF= phytoplankton-dominated with fish, P= phytoplankton-dominated.

For the mix fauna compartment, the presence of fish did not only result in a lower PCB 28 concentration (Appendix Table 7) but also in significantly lower HCB, PCB 18, 31, 44, and 52 concentration (for all: $p < 0.05$, data not shown) compared with the no-fish situation. On average ΣPCB_{low} (HCB, PCB 18, 20, 28, 31, 44, and 52) was a factor 3.2 lower when fish was present than when fish was not present. Since the percentage lipids of the compartment was a factor of 1.4 higher and significantly different ($p = 0.01$) when fish were not present, this difference is due to a concentration effect rather than a dilution effect. Thus, the difference is most probably caused by the difference in species composition of the mix fauna compartment between non-fish and fish containing systems (Figure 2).

Appendix Table 8 and 9 shows that in the presence of macrophytes both sediment and TENAX compartments contain lower concentrations of ΣPCBs and ΣPAHs although the treatment effect is not always statistically significant. Regarding individual compounds, differences are most clear with spiked (relatively mobile) compounds (results not shown) but are also observed in native PAHs. In systems with macrophytes less periphyton is present, and consequently a lower total amount of HOCs is found in this compartment (Fig. 3). Additionally, Appendix Table 8 and 9 shows that not only total amounts, but also concentrations of PCBs and PAHs decrease when macrophytes are present, although this is only significant for PCBs. Concentrations of PCBs in oligochaetes are considerably lower (factor 1.3 to 1.6) when macrophytes are present, but this is not statistically significant. This lack of statistical significance is due to a high variation in numbers of oligochaetes, biomass, and lipid content within MF and P treatments.

The presence of macrophytes did not result in a significant difference in individual and total PCB concentrations in fish while it did for total amount of higher PCBs (ΣPCB-high) in this compartment (Appendix Table 8). Fish in non-macrophyte systems have a slightly higher, moderately significant ($p = 0.06$), biomass (Fig. 3, Table 2) and also a higher amount of lipids. Although this increased biomass is sufficient to contain a significantly higher total amount of PCB mass ($p < 0.05$), PCB concentrations in the fish did not differ between macrophyte and non-macrophyte treatments (Appendix Table 8).

While the presence of macrophytes generally resulted in lower concentrations of PCBs in suspended solids, periphyton, FAB, zooplankton, oligochaetes, mix fauna, and fish compartments, Appendix Table 9 shows that this is not the case for PAHs. When macrophytes are present, lower PAH concentrations in Soxhlet and TENAX compartment are measured but PAH concentrations seem to increase in suspended solids, zooplankton, and macroinvertebrates. Possibly, PAHs mobilized

from the sediment by macrophytes are also available for compartments which are in close contact to the sediment, such as macroinvertebrates, which reside at or close to the sediment-water interface [48], and zooplankton, which is linked to the sediment due to its ability to scavenge organic particles from its top layer [49]. These interactions close to the sediment could enable invertebrates to incorporate plant-mobilized PAHs from the sediment and attain a higher concentration of PAHs. This process is also assumed to occur in the fish compartment but since PAHs can be metabolized by fish [40] this process is not reflected in final concentrations in this compartment. However, compartments like periphyton and FAB, which are solely exposed to PAHs via the water phase, do not show an increase but even so a decrease in PAH concentration (with exception of Flu-d_{10} and benzo(a)pyrene in the FAB compartment) suggesting that PAHs are less available in the water column.

The differences in ecological structure or trophic status of our test systems, representing meso- and eutrophic conditions, did alter concentrations of PCBs and PAHs in biota. However these effects were not as explicit as in reported field studies, which investigated effects of eutrophication on contaminant cycling in marine benthic systems [12] and the effect of lake trophic status on the uptake of persistent pollutants in northern pike (*Esox lucius*) [13, 17]. A main difference with our study, is that in these studies, the atmosphere was the primary source of HOCs. In contrast, we investigated systems in which the main source of PCBs and PAHs was the sediment. In systems where the main source of HOCs is the inflow via water or atmosphere ([13, 17]), phytoplankton has an important role as scavenger of water- or atmosphere-originating HOCs. An increase in phytoplankton density results in a dilution of its HOC concentration [17]. However, this process may increase concentrations in other compartments. An increase in phytoplankton density may result in a higher intake of organic material (phytoplankton or detritus) by invertebrates and thus increase their net HOC uptake [13]. In our test systems phytoplankton does not have this crucial role. Since all test systems originally contained the same sediment and concentrations in invertebrates did not differ between systems, it seems that exposure of (benthic) invertebrates to sediment-originating PCBs and PAHs did not differ among system types. Also PCB and PAH concentrations in free-swimming zooplankton were similar among types of test systems, which indicates that exposure via the water phase or phytoplankton food did not differ.

In summary, the presence of fish increases concentrations in macrophytes, suspended solids and mix fauna (only significant for PCB concentrations) through bioturbation of the sediment and active

structuring through predation (Fig. 2, Tables 3, and Appendix 7 and 9). The hypothesis that macrophytes act as a sink and lower amounts and concentrations of PCBs and PAHs in other compartments is also confirmed. Total amounts and concentrations in sediment and TENAX compartments are lowered when macrophytes are present, thus potentially reducing risks of PCBs and PAHs to benthic and pelagic biota.

Since structure and behavior of tests systems in this study (*e.g.*, species interactions) were similar to what is reported in (semi-)field situations [22, 37] it is assumed that processes in our systems regarding fate of PCBs and PAHs also resemble those occurring in the field. In our test systems, the presence of young carp greatly impacted the presence of invertebrates and resulted in different species compositions in mix fauna and zooplankton compartments. This structuring influence of benthivorous fish was very important for the invertebrate PCB and PAH content. Additionally, macrophytes were able to mobilize HOCs from the sediment, which has important implications for transport of these compounds. On average, macrophyte-dominated systems yielded 565,9 ± 162.9 (s.d.) gram (dry weight) of plants at a system surface of 1.21 m^2. This plant biomass contained on average 55.1 ± 34.8 (s.d.) µg ΣPCB and 0.488 ± 0.415 (s.d.) mg ΣPAH. Recalculating this to field proportions, results in a mobilization from the sediment pool to macrophytes of 0.46 gram ΣPCB and 4.0 gram ΣPAH per hectare during the growth season. Extrapolating from the model ecosystem experiment this means that the macrophytes mobilize approximately 50 and 24 percent of PCB and PAH from the top centimeter of the mobile 6-h Tenax-extractable fraction. In flood plain lake systems, macrophyte biomass degradation followed by inundation by the river thus can induce PCB and PAH fluxes into the floodplain or downstream. This means that a clear macrophyte-dominated status, which is a preferred state in water management (*e.g.*, Water Framework Directive [50-52]) can result in increased HOC mobilization and transport.

Acknowledgements - This research was financed by the Dutch Organization for Scientific Research (NWO; grant SSEO 14.023.12 and SSEO 14.23.011). We thank Fred Bransen, Gerdine Gaikhorst, John Deneer, Dennis Lammertsma, Thijs Meijer, and Ronald Gylstra for help with the experiment and subsequent HOC analyses. Also we gratefully acknowledge the assistance of Menno Thomas, Cajo ter Braak, and Paul van den Brink with the data-analysis.

References

[1] Koelmans, A. A. and Moermond, C. T. A. 2000. Stofstroom-analyse: zware metalen, PCB's en PAKs in uiterwaardplassen. T_0 vastlegging nulsituatie. Wageningen Universiteit. Wageningen. The Netherlands

[2] Beurskens, J. E. M., Mol, G. A. J., Barreveld, H. L., Van Munster, B. and Winkels, H. J. 1993. Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environmental Toxicology and Chemistry 12 pp. 1549-1566

[3] Peeters, E. T. H. M., Dewitte, A., Koelmans, A. A., Van der Velden, J. A. and Den Besten, P. J. 2001. Evaluation of bioassays versus contaminant concentration in explaining the macroinvertebrate community structure in the rihine-meuse delta, the Netherlands. Environmental Toxicology and Chemistry 20 pp. 2883-2891

[4] Dachs, J., Bayona, J. M. and Albaiges, J. 1997. Spatial distribution, vertical profiles and budget of organochlorine compounds in Western Mediterranean seawater. Marine Chemistry 57 pp. 313-324

[5] Lipiatou, E., Tolosa, I., Simo, R., Bouloubassi, I., Dachs, J., Marti, S., Sicre, M.-A., Bayona, J. M., Grimalt, J. O., Saliott, A. and Albaiges, J. 1997. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep Sea Research Part II: Topical Studies in Oceanography 44 pp. 881-905

[6] De Lange, M., De Jonge, J., Den Besten, P. J., Oosterbaan, J. and Peeters, E. T. H. M. 2003. Sediment pollution and predation affect structure and production of benthic macroinvertebrate communities in the Rhine–Meuse delta, The Netherlands. Journal of the North American Benthological Society 23 pp. 557-579

[7] Gobas, F. A. P. C. and MacLean, L. G. 2003. Sediment-Water Distribution of Organic Contaminants in Aquatic Ecosystems: The Role of Organic Carbon Mineralization. Environ. Sci. Technol. 37 pp. 735-741

[8] Schneider, A. R., Eadie, B. J. and Baker, J. E. 2002. Episodic particle transport events controlling PAH and PCB cycling in Grand Traverse Bay, Lake Michigan. Environ. Sci. Technol. 36 pp. 1181-1190

[9] Van Geest, G. J., Roozen, F. C. J. M., Coops, H., Roijackers, R. M. M., Buijse, A. D., Peeters, E. T. H. M. and Scheffer, M. 2003. Vegetation abundance in lowland flood plan lakes determined by surface area, age and connectivity. Freshwater Biology 48 pp. 440-454

[10] Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London-Weinheim-New York-Tokyo-Melbourne-Madras

[11] Scheffer, M., Carpenter, S. R., Foley, J. A., Folke, C. and Walker, B. 2001. Catastrophic shifts in ecosystems. Nature 413 pp. 591-596

[12] Larsson, P., Andersson, A., Broman, D., Nordback, J. and Lundberg, E. 2000. Persistent Organic Pollutants (POPs) in Pelagic Systems. AMBIO: A Journal of the Human Environment 29 pp. 202-209

[13] Gunnarsson, J., Bjork, M., Gilek, M., Granberg, M., Rosenberg, R. 2000. Effects of eutrophication on contaminant cycling in marine benthic systems. Ambio 29 pp. 252-259

[14] Berglund, O., Larsson, P., Ewald, G. and Okla, L. 2001. Influence of trophic status on PCB distribution in lake sediments and biota. Environmental Pollution 113 pp. 199-210

[15] Koelmans, A. A., Van der Heijde, A., Knijff, L. M. and Aalderink, R. H. 2001. Integrated Modelling of Eutrophication and Organic Contaminant Fate & Effects in Aquatic Ecosystems. A Review. Water Research 35 pp. 3517-3536

[16] Holmqvist, N., Stenroth, P., Berglund, O., Nyström, P., Graneli, W. and Larsson, P. 2005. Lake trophic status and bioaccumulation of polychlorinated biphenyls in benthic organisms: a comparison between littoral and profundal invertebrates. Canadian Journal of Fisheries and Aquatic Sciences 62 pp. 1201-1209

[17] Larsson, P., Collvin, L., Okla, L. and Meyer, G. 1992. Lake productivity and water chemistry as governors of the uptake of persistent pollutants in fish. Environmental Science and Technology 26 pp. 346-352

[18] Berglund, O., Larsson, P., Ewald, G. and Oklaa, L. 2001. The effect of lake trophy on lipid content and PCB concentrations in planktonic foodwebs. Ecology 82 pp. 1078-1088

[19] Brock, T. C. M. 1988. De invloed van waterplanten op hun omgeving. In: Bloemendaal, F. H. J. L. and Roelofs, J. G. M., eds, Waterplanten en waterkwaliteit, Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging, pp. 27-41

[20] Den Hartog, C. and Van der Velde, G. 1988. Structural aspects of aquatic plant communities. In: Symoens, J. J., eds, Vegetation of inland waters, Kluwer Academic Publishers, pp. 113-153

[21] Scheffer, M., Portielje, R. and Zambrano, L. 2003. Fish facilitate wave resuspension of sediment. Limnology and Oceanography 48 pp. 1920-1926

[22] Zambrano, L., Scheffer, M. and Martínez-Ramos, M. 2001. Catastrophic response of lakes to benthivorous fish introduction. Oikos 94 pp. 344-350

[23] Breukelaar, A. W., Lammens, E. H. R. R., Klein Breteler, J. G. P. and Tátrai, I. 1994. Effects of benthivorous bream (*Abramis brama*) and carp (*Cyprinus carpio*) on sediment resuspension and concentrations of nutrients and chlorophyll *a*. Freshwater Biology 32 pp. 113-121

[24] Kraaij, R. H., Tolls, J., Sijm, D., Cornelissen, G., Heikens, A. and Belfroid, A. 2002. Effects of contact time on the sequestration and bioavailability of different classes of hydrophobic chemicals to benthic oligochaetes (Tubificidae). Environmental Toxicology and Chemistry 21 pp. 752-759

[25] Landrum, P. F., Eadie, B. J. and Faust, W. R. 1992. Variation in the bioavailability of the polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. Environmental Toxicology and Chemistry 11 pp. 1197-1208

[26] Cornelissen, G., Rigterink, H., ten Hulscher, D. E. M., Vrind, B. A. and van Noort, P. C. M. 2001. A simple TENAX extraction method to determine the availability of sediment-sorbed organic compounds. Environmental Toxicology and Chemistry 20 pp. 706-711

[27] Moermond, C. T. A., Roozen, F. C. J. M., Zwolsman, J. J. G. and Koelmans, A. A. 2004. Uptake of sediment-bound bioavailable polychlorobiphenyls by benthivorous carp (*Cyprinus carpio*). Environ. Sci. Technol. 38 pp. 4503-4509

[28] Moermond, C. T. A., Zwolsman, J. J. G. and Koelmans, A. A. 2005. Black carbon and ecological factors affect *in situ* Biota to Sediment Accumulation Factors for hydrophobic organic compounds in flood plain lakes. Environ. Sci. Technol. 39 pp. 3101-3109

[29] Van den Brink, P. J., Hartgers, E. M., Fettweis, U., Crum, S. J. H., Van Donk, E. and Brock, T. C. M. 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide Linuron. Ecotoxicology and Environmental Safety 38 pp. 13-24

[30] Jonker, M. T. O. and Koelmans, A. A. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment: Mechanistic considerations. Environ. Sci. Technol. 36 pp. 3725-3734

[31] Van den Brink, P. J. and Ter Braak, C. J. F. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18 pp. 138-148

[32] Van den Brink, P. J., Van Donk, E., Gylstra, R., Crum, S. J. H. and Brock, T. C. M. 1995. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31 pp. 3181-3200

[33] Ter Braak, C. J. F. and Smilauer, P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Ithaca. New York: Microcomputer Power. USA

[34] Van den Brink, P. J., Van Wijngaarden, R. P. A., Lucassen, W. G. H., Brock, T. C. M. and Leeuwangh, P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environmental Toxicology and Chemistry 15 pp. 1143-1153

[35] Roessink, I., Arts, G. H. P., Belgers, J. D. M., Bransen, F., Maund, S. J. and Brock, T. C. M. 2005. Effects of lambda-cyhalothrin in two ditch mesocosm systems of different trophic status. Environmental Toxicology and Chemistry 24 pp. 1684-1696

[36] Arts, G. H. P., Buijse-Bogdan, L. L., Belgers, J. D. M., van Rhenen-Kersten, C. H., van Wijngaarden, R. P. A., Roessink, I., Maund, S. J., van den Brink, P. J. and Brock, T. C. M. 2006. Ecological impact in ditch mesocosms of simulated spray drift from a crop protection program for potatoes. Integrated Environmental Assessment and Management 2 pp. 105-125

[37] Diehl, S. 1992. Fish predation and benthic community structure: The role of omnivory and habitat complexity. Ecology 73 pp. 1646-1661

[38] Grift, R. E., Buijse, A. D., Van Densen, W. L. T., Machiels, M. A. M., Kranenbarg, J., Klein Breteler, J. G. P. and Backx, J. J. G. M. 2003. Suitable habitats for 0-group fish in rehabilitated floodplains along the lower River Rhine. River Research and Applications 19 pp. 353-374

[39] Copp, G. 1989. The habitat diversity and fish reproductive function of floodplain ecosystems. Environmental Biology of Fishes 26 pp. 1-27

[40] Tuvikene, T. 1995. Responses of fish to Polycyclic Aromatic Hydrocarbons (PAHs). Ann. Zool. Fennici 32 pp. 295-307

[41] Hawkins, S. A., Billiard, S. M., Tabash, S. P., Brown, R. S. and Hodson, P. V. 2002. Altering cytochrome P4501A activity affects polycyclic aromatic hydrocarbon metabolism and toxicity in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 21 pp. 1845-1853

[42] Baker, J. E., Eisenreich, S. J. and Eadie, B. J. 1991. Sediment trap fluxes and benthic recycling of organic carbon, polycyclic aromatic hydrocarbons, and polychlorobiphenyl congeners in Lake Superior. Environmental Science and Technology 25 pp. 500-509

[43] Vanier, C., Planas, D. and Sylvestre, M. 1999. Empirical relationships between polychlorinated biphenyls in sediments and submerged rooted macrophytes. Canadian Journal of Fisheries and Aquatic Sciences 56 pp. 1792-1800

[44] Bierman Jr., V. J. 1990. Equillibrium partitioning and biomagnification of organic chemicals in benthic animals. Environmental Science and Technology 24 pp. 1407-1412

[45] Ferraro, S. P., Lee II, H., Smith, L. M., Ozretich, R. J. and Specht, D. T. 1991. Accumulation factors for eleven polychlorinated biphenyl congeners. Bulletin of Environmental Contamination and Toxicology 46 pp. 276-283

[46] Moermond, C. T. A., Roessink, I., Jonker, M. T. O., Meijer, T. and Koelmans, A. A. 2007. Impact of polychlorinated biphenyl and polycyclic aromatic hydrocarbon sequestration in sediment on bioaccumulation in aquatic food webs. Environmental Toxicology and Chemistry 26 pp. 607-615

[47] Friant, S. L. and Henry, L. 1985. Relationship between toxicity of certain organic compounds and their concentrations in tissues of aquatic organisms: A perspective. Chemosphere 14 pp. 1897-1907

[48] Forbes, V. E. and Forbes, T. L. 1997. Dietary absorption of sediment-bound fluoranthene by a deposit-feeding gastropod using the 14C:51Cr dual-labeling method. Environmental Toxicology and Chemistry 16 pp. 1002-1009

[49] Palmer, M. A., Covich, A. P., Lake, S. A. M., Biro, P., Brooks, J. J., Cole, J., Dahm, C., Gibert, J., Goedkoop, W., Martens, K., Verhoeven, J. O. S. and Van De Bund, W. J. 2000. Linkages between aquatic sediment biota and life above sediments as potential drivers of biodiversity and ecological processes. BioScience 50 pp. 1062-1075

[50] Wallin, M., Wiederholm, T. and Johnson, R. K. 2003. Guidance of establishing reference conditions and ecological status class boundaries for inland surface waters. CIS Working Group 2.3. Luxembourg. Luxembourg

[51] Altenburg, W., Arts, A., Baretta-Bekker, J. G., Van den Berg, M. S., Van den Broek, T., Buskens, R., Bijkerk, R., Coops, H. C., Van Dam, H., Van Ee, G., Franken, R., Higler, B., Ietswaart, T., Jaarsma, N., De Jong, D. J., Joosten, A. T. M., Klinge, M., Knoben, R. A. E., Kranenbarg, J., Noordhuis, R., Pot, R., Twisk, F., Verdonschot, P. F. M., Vlek, H. and Wolfstein, K. 2004. Referenties en concept-maatlatten voor meren voor de Kader richtijn Water. STOWA. Utrecht. The Netherlands

[52] Beers, M., Van den Berg, M. S., Van den Broek, T., Buskens, R., Coops, H. C., Van Dam, H., Duursema, G., Fagel, M., Ietswaart, T., Klinge, M., Knoben, R. A. E., Kranenbarg, J., De Leeuw, J., Van der Molen, J., Noordhuis, R., Nijboer, R. C., Pot, R., Verdonschot, P. F. M., Vlek, H. and Vriese, T. 2004. Referenties en concept-maatlatten voor rivieren voor de KaderRichtlijn Water. STOWA. Utrecht. The Netherlands

Table 4: Results of the ANOVA analysis for the presence of fish of mass balance based data for some selected PCBs. p-values are based on two-way ANOVA results of log-transformed data. PCB_{low} contains HCB, PCB 18, 20, 28, 31, 44, and 52. PCB_{high} contains PCB 101, 105, 118, 138, 149, 153, 155, 170, 180, and 194.

			PCB 28			PCB 29			PCB 149			PCB 155		
Effect	Compartment		mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value
Fish	Sediment	0	543628.86	138086.99	0.9244	1194764.12**	249888.73	0.2535	1398720.20	360718.84	0.6283	1290369.54	334191.55	0.6522
		1	548939.14	95848.64		1324556.77**	328098.95		1291733.71	310727.28		1346144.23	351757.12	
	Tenax	0	23358.81	15113.91	0.8201	425594.89**	87916.92	0.3004	320555.21	32650.02	0.526	506233.45	99204.16	0.6314
		1	24588.11	5157.00		462992.33**	124864.16		306187.92	40216.06		528249.26**	125021.51	
	Susp. solids	0	10.44	44.82	0.2026	2.51	34.91	0.2876	240.70	115.03	0.0292	119.28	63.72	0.9798
		1	147.23	360.45		55.60	774.91		750.73	956.21		123.96	1322.60	
	Macrophytes*	0	1040.97	146.16	0.6065	11795.15	4468.40	0.7761	8979.95	1004.96	0.9781	21112.17	4120.58	0.6269
		1	1360.30	1504.89		9853.78	13843.66		8857.94	9472.32		27611.73	28443.45	
	Periphyton	0	1173.79	949.14	0.4443	4429.40	2518.09	0.4855	3858.35	1567.60	0.511	9610.38	6225.07	0.4818
		1	18.82	191.62		42.47	750.08		50.63	619.27		67.22	1204.05	
	FAB	0	2.44	2.44	0.1897	20.29	11.32	0.9671	60.07	15.82	0.7259	37.87	20.00	0.789
		1	11.89	5.83		35.68	18.39		97.01	27.42		60.32	27.78	
	Zooplankton	0	5.57	3.29	0.4505	37.61	23.70	0.9523	58.33	48.58	0.9144	118.79	121.85	0.7621
		1	1.54	5.58		38.84**	65.54		61.78	110.94		99.87	142.70	
	Oligochaeta	0	32.50	19.93	0.0075	205.66**	105.90	0.0187	354.75	137.29	0.0074	606.32	282.03	0.0191
		1	9.01	7.23		57.68**	62.28		103.05	101.04		196.98	188.92	
	Mix fauna	0	9.64	6.55	<0.0001	97.16**	99.42	0.0024	103.47	62.12	0.0061	223.49	144.43	0.0078
		1	0.00	0.01		18.42**	8.58		31.40	23.64		56.76	45.44	
	Fish	0												
		1												

0 = fish not present; 1 = fish present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*
*** Statistically significant difference (p<0.05) between the amounts of spiked and native compound.*

Table 4: Continued

			ΣPCB			ΣPCB-low			ΣPCB-high		
Effect	Compartment		mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value
Fish	Sediment	0	14414041.83	2527322.34	0.6343	5709763.65	1333359.70	0.8766	8487116.94	2556757.76	0.5522
		1	13282784.83	2984303.89		5681658.47	1255191.43		7576277.86	1861826.88	
	Tenax	0	2371367.93	472131.68	0.9036	1056799.76	404864.86	0.8947	1271529.15	191254.27	0.5685
		1	2351397.39	1088316.18		1037714.23	1081410.35		1184915.62	143025.73	
	Susp. solids	0	1683.90	762.62	0.033	171.34	331.78	0.0627	1362.79	561.14	0.0315
		1	5198.72	8063.33		1141.88	2831.73		3967.52	5280.25	
	Macrophytes*	0	51217.68	7018.42	0.8888	7425.31	1619.11	0.9614	43702.81	6267.12	0.8664
		1	52929.33	58811.84		9933.30	13351.70		42740.35	45570.72	
	Periphyton	0	29502.15	10098.59	0.6731	9776.96	3524.08	0.6035	19711.05	6663.91	0.6423
		1	473.19	5134.53		156.31	1639.35		312.52	3541.35	
	FAB	0	360.75	92.64	0.6382	55.15	16.29	0.8787	304.54	82.82	0.621
		1	628.35	173.89		116.74	46.28		506.20	151.40	
	Zooplankton	0	372.78	291.69	0.9306	51.06	36.66	0.9682	320.59	256.70	0.9366
		1	410.68	616.37		48.23	62.68		356.03	554.61	
	Oligochaeta	0	2181.69	828.90	0.0081	278.96	111.06	0.014	1872.18	839.11	0.0102
		1	661.98	661.63		78.82	82.89		567.55	624.72	
	Mix fauna	0	580.83	354.06	0.0075	86.96	80.04	0.0022	483.91	284.57	0.0115
		1	179.13	158.35		4.26	17.02		169.23	141.95	
	Fish	0									
		1									

0 = fish not present; 1 = fish present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 5: Results of the ANOVA analysis for the presence of macrophytes of mass balance based data for some selected PCBs. p-values are based on two-way ANOVA results of log-transformed data. PCB_{low} contains HCB, PCB 18, 20, 28, 31, 44, and 52. PCB_{high} contains PCB 101, 105, 118, 138, 149, 153, 155, 170, 180, and 194.

		PCB 28			PCB 29			PCB 149			PCB 155		
Compartment		mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value
Sediment	0	608585.61	120180.35	0.0611	1481369.19**	239149.56	0.0045	1371885.57	268200.12	0.8027	1593205.03	247857.68	0.003
	1	490348.70	66773.11		1068290.68**	131674.50		1317000.54	401874.02		1090269.92	134128.48	
Tenax	0	27823.32	13164.18	0.2086	532616.28**	80656.75	0.0014	328932.08	31968.26	0.1965	600102.15**	91105.30	0.0082
	1	20642.71	5801.55		369960.84**	37594.69		298390.27	34996.05		445619.87**	51462.19	
Susp. solids	0	26.80	367.76	0.7004	23.87	783.43	0.6189	486.16	1057.42	0.5489	342.47	1332.28	0.1986
	1	57.35	38.35		5.84	44.69		371.70	208.59		43.18	102.80	
Macrophytes	0												
	1												
Periphyton	0	767.53	857.68	0.0216	2874.92	2541.41	0.0221	2426.47	1923.81	0.0218	5447.66	6329.83	0.0205
	1	0.87	203.15		1.20	785.18		2.12	893.84		1.94	1547.21	
FAB	0	9.65	7.16	0.3434	39.15	16.22	0.8706	73.28	27.89	0.3639	67.33	23.10	0.9661
	1	2.91	3.90		18.78	9.69		75.89	31.72		34.55	17.29	
Zooplankton	0	8.46	4.42	0.2265	57.61	59.41	0.1535	93.15	99.12	0.1285	163.21	144.93	0.1823
	1	1.01	2.42		25.35**	12.89		38.69	13.70		72.69	37.52	
Oligochaeta	0	22.89	24.12	0.1463	152.35**	129.21	0.1592	245.17	174.19	0.1904	518.28	327.46	0.068
	1	12.79	12.49		77.86**	88.99		149.12	178.17		230.44	256.45	
Mix fauna	0	0.16	6.53	0.6348	33.04**	47.21	0.2308	44.86	43.79	0.1757	95.55	113.28	0.4218
	1	0.22	8.69		54.17**	114.53		72.42	75.27		132.76	174.13	
Fish*	0	29.54	16.81	0.14	294.17	244.95	0.1671	731.02	134.76	0.0216	1920.95	360.42	0.0455
	1	12.97	6.65		87.65	88.36		380.40	98.71		973.99	135.52	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*
*** Statistically significant difference (p<0.05) between the amounts of spiked and native compound.*

Table 5: Continued

			ΣPCB			ΣPCB-low			ΣPCB-high		
Effect	Compartment		mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value	mean (ng)	stdev	*p*-value
Macrophyte	Sediment	0	14553783.88	2648763.17	0.265	6321720.35	1310918.71	0.0588	8212631.47	1468505.68	0.5691
		1	13155246.61	2790176.61		5131661.20	828355.93		7829494.89	2903389.77	
	Tenax	0	2663726.12	983382.38	0.0855	1269535.90	954759.67	0.1809	1308804.19	157210.21	0.0176
		1	2093318.95	420353.86		863824.45	535381.53		1151168.96	149670.24	
	Susp. solids	0	3712.29	8642.45	0.3001	611.45	2922.23	0.4854	2741.64	5762.41	0.3937
		1	2358.15	1125.84		319.97	316.16		1972.13	897.60	
	Macrophytes	0									
		1									
	Periphyton	0	19651.99	13128.07	0.0161	6465.92	4466.08	0.0158	13175.85	8699.48	0.017
		1	20.96	6284.26		7.03	1621.93		13.56	4662.33	
	FAB	0	481.52	217.43	0.4473	95.75	59.19	0.7163	383.06	163.52	0.4102
		1	450.33	189.11		65.06	28.08		384.17	166.05	
	Zooplankton	0	573.61	560.85	0.158	73.55	56.37	0.1371	494.17	505.64	0.1751
		1	266.89	89.81		33.48	20.74		230.97	71.41	
	Oligochaeta	0	1588.34	1064.35	0.1065	202.89	148.07	0.1269	1334.62	1034.28	0.1331
		1	909.27	1044.02		108.37	119.76		796.15	930.51	
	Mix fauna	0	256.95	236.31	0.2685	8.65	56.36	0.1855	230.20	195.92	0.2958
		1	404.91	431.00		42.84	91.83		355.74	341.02	
	Fish*	0	5347.88	1304.67	0.0247	588.98	258.15	0.0951	4746.93	1051.26	0.0195
		1	3054.76	515.63		226.96	110.34		2816.86	452.91	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*
*** Statistically significant difference (p<0.05) between the amounts of spiked and native compound.*

Table 6: Combined results of the ANOVA analysis for the presence of fish or macrophytes of mass balance based data for some selected PAHs. p-values are based on two-way ANOVA results of log-transformed data.

			Flu-d10			Flu			ΣPAH		
Effect	Compartment		mean (µg)	stdev	*p*-value	mean (µg)	stdev	*p*-value	mean (µg)	stdev	*p*-value
Fish	Sediment	0	155075.61**	32068.17	0.5255	62360.16	19422.55	0.5984	409447.84	112820.52	0.6876
		1	161332.70**	21446.53		67941.41	23200.52		429272.70	128280.61	
	Tenax	0	74523.23**	14906.65	0.1867	6374.05	3142.48	0.7956	24491.55	14579.27	0.5065
		1	83704.19**	20252.79		6070.06	1910.99		22135.56	9447.50	
	Susp. solids	0	1.08	3.76	0.1272	0.62	2.01	0.1656	4.48	19.65	0.156
		1	26.43	66.70		10.04	13.94		77.79	148.96	
	Macrophytes*	0	1261.76	420.69	0.6053	71.28	17.80	0.9831	374.18	156.93	0.6674
		1	837.56	1542.81		70.36	100.06		395.59	615.72	
	Periphyton	0	295.23	64.49	0.8452	33.75	13.13	0.5577	224.91	89.87	0.9894
		1	52.33	83.07		7.26	9.73		48.11	72.87	
	FAB	0	2.98**	2.06	0.7286	0.37	0.11	0.0016	2.27	0.67	0.8015
		1	4.24**	2.06		0.58	0.19		3.79	1.55	
	Zooplankton	0	0.96	0.55	0.6981	0.17	0.14	0.5442	1.17	1.11	0.666
		1	0.39	3.56		0.05	0.14		0.35	0.74	
	Oligochaeta	0	28.54**	9.98	0.0003	1.33	0.46	0.0019	6.66	2.43	0.0002
		1	6.20**	3.24		0.40	0.21		2.29	1.33	
	Mix fauna	0	5.85	7.07	0.0235	0.34	0.33	0.0115	1.99	1.67	0.0129
		1	1.62**	2.29		0.00	0.04		0.22	0.21	
	Fish	0									
		1									

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*
*** Statistically significant difference (p<0.05) between the amounts of spiked and native compound.*

Table 6: Continued

			Flu-d10			Flu			ΣPAH		
Effect	Compartment		mean (µg)	stdev	p-value	mean (µg)	stdev	p-value	mean (µg)	stdev	p-value
Macrophyte	Sediment	0	179989.49**	13775.62	0.0025	73523.47	24946.89	0.1577	466651.83	140781.44	0.0629
		1	139001.26**	18141.53		57625.64	10789.13		376650.79	57891.87	
	Tenax	0	92160.59**	15890.53	0.005	7348.69	3084.90	0.105	28269.57	14851.01	0.0067
		1	67685.19**	7452.18		5265.00	605.47		19177.30	2438.53	
	Susp. solids	0	2.81	71.65	0.5152	1.27	15.77	0.4829	11.50	160.18	0.5896
		1	10.12	11.68		4.88	3.56		30.26	30.84	
	Macrophytes	0									
		1									
	Periphyton	0	214.27	105.00	0.0094	27.88	12.19	0.0088	194.60	76.96	0.0134
		1	20.67	38.87		2.52	8.72		14.78	69.01	
	FAB	0	3.71**	2.18	0.9164	0.45	0.27	0.1402	2.77	2.15	0.6049
		1	3.14**	2.47		0.41	0.11		2.58	0.61	
	Zooplankton	0	0.26	3.88	0.4651	0.04	0.15	0.3551	0.27	0.80	0.4152
		1	1.44	1.08		0.23	0.13		1.55	0.98	
	Oligochaeta	0	12.04**	9.59	0.4527	0.82	0.61	0.4335	4.57	3.25	0.803
		1	14.71**	17.57		0.66	0.64		3.34	2.82	
	Mix fauna	0	1.86	1.89	0.0602	0.04	0.12	0.4837	0.53	0.81	0.0766
		1	5.09**	7.20		0.01	0.42		0.82	2.10	
	Fish*	0	0.03	0.05	0.9725	0.07	0.01	0.2586	0.45	0.06	0.2349
		1	0.03	0.02		0.04	0.03		0.31	0.15	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*
*** Statistically significant difference (p<0.05) between the amounts of spiked and native compound.*

Table 7: Results of the ANOVA analysis for the presence of fish of concentration based data for some selected PCBs. p-values are based on two-way ANOVA results of log-transformed data. PCB_{low} contains HCB, PCB 18, 20, 28, 31, 44, and 52. PCB_{high} contains PCB 101, 105, 118, 138, 149, 153, 155, 170, 180, and 194.

			PCB 28			PCB 29			PCB 149			PCB 155		
Effect	compartment		mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value
Fish	Sediment	0	423.48	88.20	0.3643	930.71	206.08	0.19	1089.59	170.30	0.7624	1005.19	329.30	0.3723
		1	484.99	172.84		1170.25	497.31		1141.25	408.68		1189.32	504.11	
	Tenax	0	18.20	10.11	0.4933	331.53	73.43	0.2062	249.71	23.22	0.4687	394.35	104.77	0.3423
		1	21.72	9.78		409.06	172.84		270.52	76.87		466.71	169.42	
	Susp. solids	0	17.61	304.21	0.46	3.31	149.24	0.5602	489.95	513.82	0.9617	242.79	373.82	0.4409
		1	98.25	222.88		24.18	387.84		501.00	507.43		53.92	486.95	
	Macrophytes*	0	37.68	11.29	0.0402	426.93	279.23	0.3723	325.03	78.64	0.0825	764.17	287.19	0.0469
		1	92.65	43.80		671.12	453.72		603.29	267.70		1880.57	799.72	
	Periphyton	0	159.15	121.92	0.5392	600.58	174.21	0.5694	523.15	86.56	0.6016	1303.06	481.21	0.5593
		1	7.35	111.52		16.59	513.14		19.78	323.72		26.26	547.19	
	FAB	0	14.62	25.19	0.5307	193.45	138.75	0.5712	572.72	115.01	0.4558	361.05	242.03	0.4907
		1	84.12	125.29		252.41	407.47		686.23	364.30		426.67	527.59	
	Zooplankton	0	1.12	107.79	0.4956	1.96	628.51	0.5407	4.35	1129.84	0.5348	4.50	2515.70	0.3608
		1	0.12	122.90		0.16	1325.21		0.38	2171.90		0.26	2885.81	
	Oligochaeta	0	88.28	21.67	0.3099	558.64	139.74	0.3129	963.63	513.36	0.3257	1646.99	751.88	0.3608
		1	20.74	64.59		85.46	484.32		165.25	651.71		258.02	1411.42	
	Mix fauna	0	135.77	91.09	<0.0001	1368.18	925.94	0.1228	1457.08	524.47	0.4671	3147.23	1993.46	0.3653
		1	0.00	0.01		715.00	295.91		1218.81	848.06		2202.99	1607.99	
	Fish	0												
		1												

0 = fish not present; 1 = fish present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 7: Continued

			ΣPCB			ΣPCB-low			ΣPCB-high		
Effect	Compartment		mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value
Fish	Sediment	0	11228.42	1467.78	0.6413	4447.86	1199.92	0.4412	6611.39	1329.71	0.8291
		1	11735.39	4437.44		5019.77	2160.02		6693.67	2332.26	
	Tenax	0	1841.04	351.20	0.4609	817.32	305.59	0.6112	989.88	98.72	0.4451
		1	2070.43	1008.68		909.79	925.84		1045.87	297.66	
	Susp. solids	0	3427.44	4105.42	0.9393	348.47	1705.38	0.4533	2773.94	2851.45	0.9663
		1	3458.88	4540.25		749.92	1781.26		2647.68	2824.50	
	Macrophytes*	0	1852.20	497.09	0.0801	267.22	119.86	0.2043	1581.73	385.08	0.0591
		1	3603.15	1727.52		674.67	450.53		2910.76	1289.46	
	Periphyton	0	4000.16	772.73	0.7688	1325.64	326.08	0.7005	2672.60	451.79	0.7363
		1	184.80	2849.57		61.02	976.89		122.07	1887.63	
	FAB	0	3438.60	742.10	0.4606	524.90	207.56	0.5746	2903.59	554.49	0.4438
		1	4443.89	3187.80		824.77	1042.82		3580.70	2165.15	
	Zooplankton	0	40.82	7158.95	0.5224	10.15	1100.70	0.5165	28.36	6099.84	0.5243
		1	3.92	12464.15		1.06	1327.89		2.58	11138.53	
	Oligochaeta	0	5925.92	3243.96	0.3386	757.18	144.81	0.3284	5085.44	3275.43	0.3412
		1	1221.81	4210.36		184.37	832.80		970.07	3736.78	
	Mix fauna	0	8178.21	3168.35	0.4218	1222.51	946.82	0.0837	6814.43	2274.06	0.6482
		1	6949.95	5756.06		95.58	619.51		6568.38	5150.37	
	Fish	0									
		1									

0 = fish not present; 1 = fish present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 8: Results of the ANOVA analysis for the presence of macrophytes of concentration based data for some selected PCBs. p-values are based on two-way ANOVA results of log-transformed data. PCB_{low} contains HCB, PCB 18, 20, 28, 31, 44, and 52. PCB_{high} contains PCB 101, 105, 118, 138, 149, 153, 155, 170, 180, and 194.

			PCB 28			PCB 29			PCB 149			PCB 155		
Effect	Compartment		mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value
Macrophyte	Sediment	0	513.85	160.09	0.1126	1250.78	432.37	0.0533	1158.34	402.30	0.6214	1345.21	433.86	0.0483
		1	399.69	77.04		870.79	240.42		1073.52	173.94		888.70	257.08	
	Tenax	0	23.49	9.91	0.2134	449.71	142.16	0.0308	277.73	74.57	0.243	506.69	141.95	0.0812
		1	16.83	8.74		301.56	80.17		243.22	18.27		363.24	105.19	
	Susp. solids	0	30.27	320.75	0.7815	26.96	362.84	0.5192	662.64	576.25	0.2322	466.79	452.94	0.1682
		1	57.15	30.43		2.97	43.53		370.44	255.14		28.04	98.99	
	Macrophytes	0												
		1												
	Periphyton	0	154.75	108.13	0.0421	579.66	358.74	0.0384	489.24	186.39	0.0382	1098.39	490.48	0.0339
		1	0.36	80.13		0.50	309.69		0.88	352.54		0.81	610.24	
	FAB	0	98.02	119.68	0.4357	397.58	351.02	0.9295	744.21	327.50	0.5235	683.71	423.19	0.9626
		1	12.87	21.42		132.48	77.98		535.28	129.73		243.73	107.57	
	Zooplankton	0	5.32	108.32	0.1254	17.57	1160.56	0.1158	35.80	1889.95	0.1153	41.21	2707.29	0.1171
		1	0.03	101.59		0.02	496.15		0.05	858.15		0.03	1710.45	
	Oligochaeta	0	100.92	17.13	0.2349	671.75	157.40	0.2336	1080.98	507.91	0.2706	2285.20	438.17	0.226
		1	18.14	61.83		71.07	450.44		147.31	599.60		185.96	1350.74	
	Mix fauna	0	0.80	129.29	0.8915	833.03	854.16	0.388	1131.21	568.06	0.1987	2409.27	2180.80	0.6454
		1	0.73	82.71		1174.32	816.26		1569.91	745.81		2877.77	1592.96	
	Fish*	0	72.79	34.99	0.2374	722.88	559.84	0.239	1764.80	592.81	0.5168	4691.07	440.02	0.1039
		1	44.41	17.84		308.97	254.94		1420.72	639.65		3571.21	770.13	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 8: Continued

			ΣPCB			ΣPCB-low			ΣPCB-high		
Effect	Compartment		mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value	mean (µg/kg)	stdev	*p*-value
Macrophyte	Sediment	0	12288.36	4309.10	0.2583	5337.69	2094.78	0.17	6934.26	2255.29	0.4176
		1	10723.15	1170.68		4182.94	981.85		6382.00	1366.40	
	Tenax	0	2243.27	734.05	0.1365	1066.23	704.54	0.2064	1104.67	273.68	0.0945
		1	1699.19	718.30		697.40	660.28		937.19	77.81	
	Susp. solids	0	5044.77	4875.41	0.1469	820.22	2085.20	0.3732	3736.89	3186.88	0.1602
		1	2349.98	1249.97		318.60	264.71		1965.41	1083.00	
	Macrophytes	0									
		1									
	Periphyton	0	3962.21	1730.78	0.024	1303.56	627.86	0.0252	2656.58	1105.48	0.0259
		1	8.74	2477.41		2.93	638.51		5.66	1838.90	
	FAB	0	4889.03	2942.23	0.558	971.40	979.98	0.7867	3890.05	1963.38	0.5291
		1	3175.64	814.87		457.99	153.29		2709.85	699.53	
	Zooplankton	0	282.82	10838.08	0.1167	50.62	1171.64	0.121	215.39	9679.17	0.116
		1	0.57	5729.44		0.21	1037.78		0.34	4691.66	
	Oligochaeta	0	7002.63	3256.08	0.2412	893.45	555.17	0.2217	5884.52	3445.54	0.2507
		1	1033.94	3648.13		156.25	591.98		838.34	3093.88	
	Mix fauna	0	6478.18	3359.22	0.3982	126.82	1137.08	0.3222	5804.52	2322.96	0.4267
		1	8773.79	5238.87		921.39	748.99		7711.20	4770.72	
	Fish*	0	13092.98	2682.95	0.3244	1444.07	565.55	0.162	11620.74	2174.74	0.4534
		1	11396.93	3372.77		799.96	312.08		10547.30	3303.49	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 9: Combined results of the ANOVA analysis for the presence of fish or macrophytes of concentration based data for some selected PAHs. p-values are based on two-way ANOVA results of log-transformed data.

			Flu-d10			Flu			ΣPAH		
Effect	Compartment		mean (mg/kg)	stdev	*p*-value	mean (mg/kg)	stdev	*p*-value	mean (mg/kg)	stdev	*p*-value
Fish	Sediment	0	120.80	30.99	0.2502	48.58	11.02	0.3047	318.96	62.89	0.3107
		1	142.54	44.94		60.03	35.68		379.26	205.01	
	Tenax	0	58.05	12.95	0.1432	4.97	2.19	0.7181	19.08	10.11	0.2444
		1	73.95	27.66		5.36	3.03		19.56	14.05	
	Susp. solids	0	1.82	15.19	0.3293	1.04	11.97	0.4095	7.55	107.50	0.3861
		1	17.64	41.29		6.70	9.29		51.91	78.32	
	Macrophytes*	0	45.67	21.60	0.6887	2.58	0.33	0.1417	13.54	4.45	0.5148
		1	57.04	55.85		4.79	3.30		26.91	21.05	
	Periphyton	0	40.03	7.86	0.4775	4.58	1.67	0.347	30.49	11.19	0.4319
		1	47.44	56.54		6.58	4.98		43.61	36.87	
	FAB	0	26.75	13.44	0.581	3.14	0.88	0.9447	18.93	7.72	0.5614
		1	30.02	52.43		4.09	4.45		26.82	31.31	
	Zooplankton	0	38.18	41.24	0.5841	6.63	5.62	0.439	46.46	41.45	0.5413
		1	9.30	70.09		1.29	7.52		8.43	54.05	
	Oligochaeta	0	77.52	28.17	0.1647	3.62	0.63	0.8533	18.08	2.76	0.2884
		1	53.09	25.25		3.44	1.73		19.59	11.69	
	Mix fauna	0	82.41	57.01	0.5621	4.82	2.66	0.0387	28.04	13.90	0.3841
		1	63.00	90.24		0.01	1.61		8.34	8.10	
	Fish	0									
		1									

0 = fish not present; 1 = fish present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Table 9: Continued

			Flu-d10			Flu			ΣPAH		
Effect	Compartment		mean (mg/kg)	stdev	*p*-value	mean (mg/kg)	stdev	*p*-value	mean (mg/kg)	stdev	*p*-value
Macrophyte	Sediment	0	151.97	41.93	0.059	62.08	35.17	0.1864	394.01	200.60	0.1297
		1	113.30	23.62		46.97	8.61		307.02	50.19	
	Tenax	0	77.81	23.99	0.05	6.20	3.22	0.1112	23.87	14.94	0.039
		1	55.17	15.12		4.29	0.55		15.63	1.71	
	Susp. solids	0	3.18	41.15	0.612	1.44	13.19	0.5842	12.99	109.59	0.6818
		1	10.08	14.26		4.86	4.19		30.16	36.81	
	Macrophytes	0									
		1									
	Periphyton	0	43.20	46.97	0.0937	5.62	4.77	0.1207	39.24	34.18	0.0819
		1	46.47	45.31		5.66	0.90		33.21	8.01	
	FAB	0	37.65	49.49	0.9272	4.55	4.25	0.623	28.12	30.76	0.757
		1	22.15	16.00		2.88	0.97		18.20	8.25	
	Zooplankton	0	4.00	65.27	0.2459	0.61	2.37	0.1585	4.10	11.58	0.196
		1	88.78	48.95		13.95	4.06		95.46	30.89	
	Oligochaeta	0	53.08	22.26	0.1644	3.60	0.84	0.888	20.14	6.96	0.3231
		1	77.53	29.32		3.46	1.64		17.60	10.35	
	Mix fauna	0	47.02	32.69	0.0908	0.61	2.32	0.4347	13.26	15.17	0.1218
		1	110.42	79.24		0.08	3.83		17.64	16.51	
	Fish*	0	0.04	0.11	0.6089	0.17	0.02	0.8204	1.12	0.17	0.8299
		1	0.09	0.05		0.15	0.10		1.06	0.56	

0 = macrophytes not present; 1 = macrophytes present.
** No two-way but one-way ANOVA has been used because only two system types could be compared.*

Chapter 7

General discussion

I. Roessink
A.A. Koelmans
T.C.M. Brock

Will be published in modified form in special SSEO issue of
Science of the Total Environment

Introduction

Pollution by nutrients and pollution by toxic contaminants are two major environmental stressors in aquatic ecosystems. As nutrient levels impact on the trophic status of a system, thus influencing the sedimentation of abiotic and biotic particles, they also impact on the deposition of xenobiotic compounds sorbed to these particles [1]. As a consequence, increased levels of nutrients and toxicants may co-occur, and interactions between them have been reported to lead to environmental effects that cannot be predicted on the basis of the impact of each individual stressor [1-4]. Compared to deeper waters, shallow waters have a relatively large interaction with the sediment compartment. In the Netherlands, and many other countries, sediments have been a sink of pollutants in recent decades, and now that water quality has improved, these sediments may become a net source of pollutants to the water column [5, 6].

Table 1. Differences between deep and shallow water bodies [7-9].

Shallow	Deep
Fully mixed water column	Stratified water column for most of the year
Large area above wave base	Small area above wave base
Resuspension	Sedimentation
Macrophyte-dominated	Phytoplankton-dominated; only macrophyte-dominated littoral zone

Apart from the properties of a toxicant itself, the characteristics of the water body, in particular its morphology, determine which nutrient–toxicant interactions are most important (Table 1). To date, nutrient–toxicant interactions have received more attention in deep water bodies than in shallow aquatic ecosystems. In mesotrophic surface waters, nutrient levels in the water compartment are relatively low, but rooted macrophytes may efficiently use nutrients from the sediment.

Eutrophication often decreases the total biomass of macrophytes in shallow waters. High nutrient levels may result in algae blooms, or the formation of a closed layer of floating macrophytes (*e.g.*, *Lemna*). In turn, this may decrease the submerged vegetation by competition for light and nutrients. Although the total biomass of primary producers often decreases in eutrophied shallow waters, the amount of POC (particulate organic matter) and DOC (dissolved organic matter) in the water column may increase (*e.g.*, due to an increase in primary producers with a higher turnover rate) [10]. Depending on the nutrient input, stagnant shallow water bodies can be either plankton-dominated (having a turbid water column) or macrophyte-dominated (having a relatively clear water

column). Macrophytes influence several important properties of their environment by their architecture (*e.g.*, by creating microhabitats or providing substrate) and metabolism (*e.g.*, by producing oxygen). This is why macrophyte habitats usually support diverse invertebrate and periphyton communities. In contrast, plankton communities are less diverse and characterized by a lower total biomass [9]. These differences between macrophyte-dominated and plankton-dominated freshwater habitats could potentially result in different vulnerability to toxic stress.

This thesis aims to elucidate the impact of nutrient status and/or historic sediment pollution on the fate and/or effects of selected organic micro-pollutants in microcosms mimicking shallow freshwater communities. Within this chapter experiments within the project 'Feedbacks between nutrients and toxicants in model ecosystems and field enclosures' (see [11-16]) results found in the open literature are discussed. Of the test systems used in the research for the present thesis, the macrophyte-dominated and phytoplankton-dominated systems represented two different situations in terms of nutrient status (mesotrophic and eutrophic conditions, respectively). The toxicants we selected were sediment-associated PCBs and PAHs, a non-persistent insecticide (lambda-cyhalothrin) and a persistent organotin (triphenyltin).

The specific research questions were:

- Which mechanisms affecting nutrient–toxicant interactions in aquatic ecosystems have been reported in the open literature?
- What is the influence of the presence of macrophytes and/or fish on the redistribution and partitioning of sediment-associated PCBs and PAHs in shallow freshwater systems?
- What is the influence of the presence of macrophytes on water dissipation, and on the direct and indirect effects of the insecticide lambda-cyhalothrin in freshwater ecosystems?
- Do sediment-associated micro-pollutants influence the ecological impact of the additional chemical stressor TPT?

Mechanisms reported to affect nutrient–toxicant interactions in aquatic ecosystems

An important link between nutrients and toxicants is the carbon cycle [1, 17]. Trophic status (*i.e.*, whether a system is oligotrophic, mesotrophic or eutrophic) can influence the bioavailability and cycling of toxicants [3, 18, 19]. Conversely, the level of toxicant pollution can directly (via toxic effects on primary producers) or indirectly (via toxic effects on invertebrate grazers) influence primary production and facilitate expressions of eutrophication [4, 20, 21].

When considering the interaction between nutrients and organic micro-pollutants in freshwater ecosystems, a distinction can be made between [1, 3]:

1. the impact of biota on the partitioning, dissipation and degradation of the organic micro-pollutants; and
2. the impact of the organic micro-pollutants on the structure and functioning of the biota.

Using this distinction, several mechanisms have been reported in the literature that could play a role in the interaction between nutrients and organic micro-pollutants in aquatic ecosystems, *viz*;

1A. Dilution of toxicants through biomass increase. A higher biomass as a consequence of shifts in nutrient levels results in a dilution of toxicants within this larger biomass [4, 22].

1B. Lower bioavailability through increased levels of DOC and POC. An increased production increases dissolved organic carbon (DOC) and living and dead particulate organic carbon (POC) levels in the water phase. Increased partitioning of organic micro-pollutants to DOC and POC results in a lower concentration of these toxicants in the water phase and, consequently, a lower exposure of water organisms in the water column [4, 17].

1C. Sedimentation. By increasing production, eutrophication leads to an increased flow of dead organic material to the sediment, also increasing the sedimentation of toxicants [4].

1D. Sediment–water interactions. Microbial degradation of POM and benthos activity in sediments can reintroduce organic micro-pollutants into the water column [4]. Under highly eutrophic conditions, however, oxygen depletion and anoxic conditions may hamper microbial activity and bioturbation by macroinvertebrates, and, consequently, the release of organic micro-pollutants from the sediment to the water.

1E. Plant–water interactions. Nutrient-induced changes in biomass and productivity of primary producers can significantly influence the fate of toxicants. In mesotrophic to slightly eutrophic shallow waters, for example, macrophytes may be the dominant primary producers. These macrophytes have a low turnover rate compared with that of algae. Because of their high standing stock and large plant surface area, macrophyte vegetation can scavenge organic micro-pollutants such as pesticides from the water column [23-25] and provide favourable circumstances for the biological and physical breakdown of these compounds. Macrophyte photosynthesis has been reported to increase pH levels near plant leaves, increasing the hydrolysis of organic micro-pollutants like

pesticides [26, 27]. When the macrophyte biomass decomposes at the end of the season, the 'captured' non-persistent organic micro-pollutants have been degraded and no longer impact on the aquatic community. In contrast, persistent pollutants, which can also sorb to macrophytes [28], are merely transported to another compartment of the aquatic ecosystem (*e.g.*, sediment) where they might still influence aquatic communities. In addition, the uptake of persistent organic pollutants (POP) in the food web may be higher when algae with higher lipid content are present [4].

2A. Nutrition-mediated sensitivity to toxicants. Depending on the availability of nutrients, algae can be more or less susceptible to certain toxicants [29]. In invertebrates, a larger pool of high-quality food is reported to result in better organism physiology and thus a higher tolerance to toxic stress. For example, Pieters *et al.* [30] showed that under low-food conditions, a *Daphnia magna* population was a factor 2–3 more sensitive to an insecticide than under high-food conditions. A high food supply to *Daphnia magna*, however, may result in a more sensitive next generation: under high-food conditions, *D. magna* produces more numerous but smaller offspring, and smaller individuals are usually more susceptible to toxicant stress [31-34].

2B. Shifts in species interactions. In plankton-dominated communities, eutrophication effects can be triggered at lower nutrient levels in the presence of organic micro-pollutants that reduce plankton grazers [35]. In macrophyte-dominated communities, toxicants can target important grazers of periphytic algae or phytoplankton and thus trigger eutrophication effects [36-38]. In systems under stress, overall species diversity decreases, while the relative abundance of *r*-strategists (short life-cycle) increases [39]. For instance, herbicides may cause symptoms of eutrophication by reducing macrophyte biomass and stimulating phytoplankton biomass via increased nutrient levels due to the decay of macrophytes. Furthermore, planktonic algae may adapt faster to toxicants than macrophytes, due to their short life-cycle [40, 41]. Insecticide stress may cause a decline of relatively large cladocerans in favour of relatively small zooplankton (*e.g.*, microcrustaceans and rotifers) [42].

Fate of PAHs and PCBs in macrophyte- *versus* phytoplankton-dominated systems

The focus of our research in the PCB/PAH microcosm experiment was on the impact of biota on the fate of organic micro-pollutants (chapter 6). This experiment used 600-L macrophyte- and phytoplankton-dominated cosms with and without fish, constructed with sediment containing historical and added PCBs and PAHs. The macrophyte-dominated systems had higher total living biomass, and generally lower concentrations of PCBs were observed in suspended solids, periphyton, filamentous algae, zooplankton, invertebrates and fish. Only for periphyton, however, were PCB levels statistically different between macrophyte-dominated and phytoplankton-dominated systems. No similar relationship could be demonstrated for PAHs in the plankton- and macrophyte-dominated test systems (chapter 6).

Macrophytes can mobilize considerable amounts of persistent organic pollutants from the sediment (*e.g.*, via uptake by the roots of macrophytes, subsequent transport to above-ground plant parts and senescence of these plant parts). Compared to the sediment in the phytoplankton-dominated systems, the presence of macrophytes did indeed result in considerably lower concentrations of the PAHs and PCBs of the sediment, especially the labile (6h TENAX-extractable) fractions (chapter 6 and [15]).

The PAH/PCB microcosm experiment also studied the impact of fish on the fate of the organic micro-pollutants. In the presence of fish, concentrations of less highly chlorinated PCBs in invertebrates were on average a factor of 3.2 lower than in microcosms without fish. A plausible explanation is the selective predation pressure of fish on certain macroinvertebrate taxa (chapter 6). Although not statistically significant, this trend was also observed when concentrations of some selected congeners (PCBs 28 and 29) were expressed as biota-to-sediment accumulation factors (BSAFs). This trend of lower BSAFs in the presence of fish was otherwise only observed in more highly chlorinated PCBs (PCBs 149 and 155) or PAHs (Flu and Flu-*d*10) in the plankton-dominated system, and not in the macrophyte-dominated-system, indicating that the impact of selective fish predation varied between the two system types [15]. This is in line with the findings of Moermond *et al.* [43], who observed lower invertebrate BSAFs in a lake with high fish density than in lakes with lower fish densities. However, quite a few fate measurement endpoints in the biota of the indoor food web accumulation experiment yielded rather high intra-treatment variability, and although trends were clearly demonstrated, statistically significant differences were not always found (chapter 6 and [15]).

In conclusion, the macrophyte- or phytoplankton-dominated status of the test systems used (representing mesotrophic and eutrophic conditions) did alter the concentrations of especially persistent PCBs in other system compartments (including fish and invertebrates) particularly through mechanisms 1A and 1D in the above classification. However, these alterations were not as clear-cut as those in other studies in the field, which investigated the effects of eutrophication on contaminant cycling in marine benthic systems [5] and the effect of lake trophic status on the uptake of persistent pollutants in northern pike (*Esox lucius*) [44].

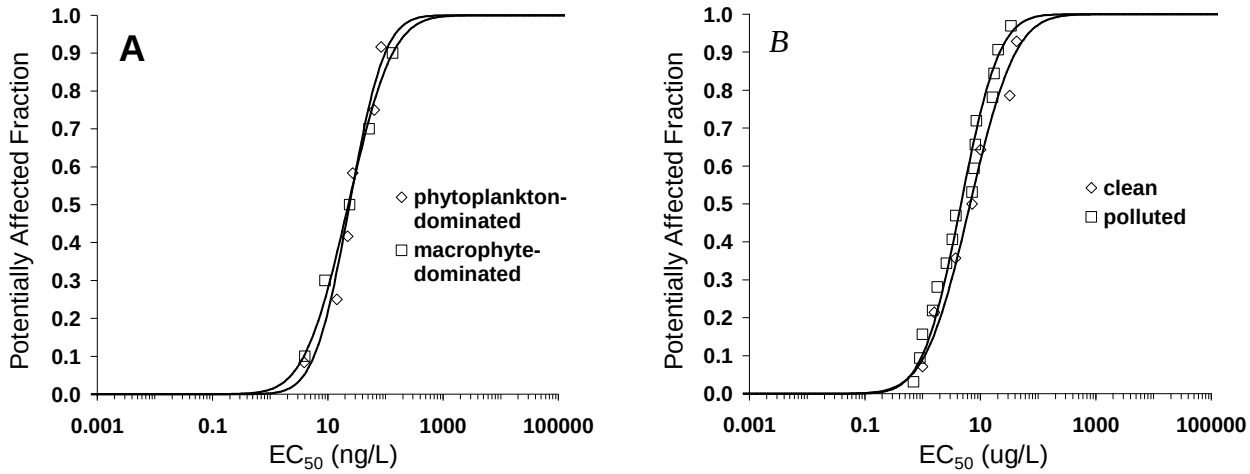


Figure 1: Panel A shows the species sensitivity distribution (SSD) of estimated EC_{50} values (effect concentrations at which 50% of the test organisms show an effect) for arthropods, two weeks after the initial application of the additional stressor lambda-cyhalothrin in the phytoplankton- (◊) and macrophyte-dominated (□) test systems [12]. Panel B shows the SSD of estimated EC_{50} values of invertebrates, four weeks after the application of the additional stressor triphenyltin in the test systems constructed with clean (◊) and polluted (□) sediments [14]. (For more detailed information on the species sensitivity distribution concept, see [45])

Impact of a non-persistent insecticide in shallow aquatic ecosystems

The outdoor enclosure experiments with lambda-cyhalothrin primarily focussed on the impact of the toxicant on the structure and functioning of the community. These studies in plankton- and macrophyte-dominated freshwater model ecosystems revealed rather similar exposure regimes in the water columns of both systems after similar doses of this pyrethroid insecticide had been applied (chapter 2). After 1 day, more than 70% of the toxicant had dissipated from the water column in both the macrophyte-dominated and the phytoplankton-dominated enclosures, most probably due to sorption to DOC or POC (plankton-dominated system) or vascular aquatic plants (macrophyte-dominated system). This corresponds to mechanisms 1B and 1E discussed above.

Figure 1A shows a comparison of the direct toxic effects on arthropods in both types of system two weeks after the initial application of lambda-cyhalothrin, using model ecosystem Species Sensitivity Distributions (SSD). Lambda-cyhalothrin was applied three times, at weekly intervals. The time-to-effect after exposure of arthropods to lambda-cyhalothrin is very short [46]. Furthermore, lambda-cyhalothrin is a highly lipophilic compound ($\log K_{ow}$= 7.0), which therefore binds rapidly to organic matter and other particles [23, 47]. Hence, the best time for a relevant characterization of the direct toxic effects is in the application period. The SSD curves derived from the plankton- and macrophyte-dominated systems are remarkably similar (Figure 1A), and this is also the case for the calculated HC_5 values (the hazardous concentration at which 5% of the tested population has a toxicity value lower than this concentration) (Table 2). Nevertheless, the duration and magnitude of the effects did differ between systems at exposure concentrations higher than the threshold level for direct toxic effects. In the plankton-dominated system, a more pronounced response to lambda-cyhalothrin application was observed at the 25, 50, and 100 ng/L treatment levels than at the same treatment levels in the macrophyte-dominated systems. In particular, indirect effects on microcrustaceans and rotifers were more pronounced in the plankton-dominated systems (Table 3). In contrast, recovery of affected populations was faster in the plankton-dominated systems. These observations correspond to mechanism 2B discussed above.

Table 2. Hazardous concentrations for five percent of the test species (HC_5), with 95% confidence intervals in parentheses, calculated on the basis of semi-field EC_{50} values for the invertebrate communities of the lambda-cyhalothrin and triphenyltin microcosm experiments.

Additional stressor	Test system	HC_5
Insecticide: lambda-cyhalothrin	Phytoplankton-dominated	3.42 (0.39-9.03) ng/L
	Macrophyte-dominated	1.92 (0.07-7.29) ng/L
Organotin: triphenyltin acetate	Clean sediment	0.57 (0.05-1.81) µg/L
	Polluted sediment	0.61 (0.22-1.18) µg/L

Chaoborus obscuripes is a species occurring in both types of systems. However, in the less diverse plankton-dominated system, the effects on this species apparently had a greater influence on the top-down control of zooplankton than in the more species-rich macrophyte-dominated systems, where other species can wholly or partly take over control. The overall shorter life-cycle of the species present in the plankton-dominated systems enabled the community to recover more

quickly from the insecticide application than the community in the macrophyte-dominated systems, which included more taxa with a relatively long life-cycle (*e.g.*, *Gammarus pulex*) (chapter 2).

In the lambda-cyhalothrin enclosure experiments, food-web interactions sometimes masked the responses of organisms to insecticide exposure. For instance, in a bioassay with *Daphnia pulex* in the water columns of the two types of test systems, a clear dose–response relationship was observed in the phytoplankton-dominated systems (Figure 2A), whereas such a dose–response relationship was not observed in the bioassays in the macrophyte-dominated systems (Figure 2B). The bioassays in the macrophyte-dominated systems were invaded by the Turbellarian *Mesostoma sp.*, which preyed on the daphnids. This predation in all macrophyte-dominated test systems (including controls) obscured any treatment-related response of *D. pulex*.

Since the difference in trophic status between the test systems did not result in different model ecosystem SSDs, it can be argued that neither clear mitigating nor amplifying interactions were observed between nutrients and species sensitivity distributions due to direct toxic effects of lambda-cyhalothrin. Apparently, the bioavailability of lambda-cyhalothrin in the two types of test system was virtually the same. Phytoplankton Chl-*a* was five times higher in the plankton-dominated systems than in the macrophyte-dominated systems (chapter 2). Due to the lipophilic nature of the toxicant, sorption to phytoplankton and dead DOC and POC played an important role [23, 47]. However, the vascular plants in the macrophyte-dominated systems also represent a large amount of organic matter, acting as sorbing surface for the toxicant.

Table 3. Indirect effects on structural endpoints in macrophyte- and phytoplankton-dominated test systems exposed to lambda-cyhalothrin and in macrophyte-dominated systems constructed with clean and polluted sediment exposed to triphenyltin. A zero (0) indicates no indirect effects observed; + and ++ indicate slight (e.g., occurring only once) and more consistent (occurring several times and/or on consecutive sampling dates) indirect effects, respectively; – indicates that the specific group or taxon is also subject to some direct toxic effects. n.p. = not present ([12, 13]).

	Lambda-cyhalothrin experiment		Triphenyltin experiment	
	Macrophyte-dominated	Phytoplankton-dominated	Clean sediment	Polluted sediment
Macroinvertebrates				
Erpobdella octoculata	0	+	0	0
Cloeon dipterum	0	0	+	++
Chironomidae	0	0	+	++
Culicidae	0	0	0	++
Corixidae juv.	0	0	0	++
Dytiscidae larvae	0	0	++	0
Lymnaea stagnalis	+	+	0	0
Physa sp.	0	0	0	+
Zooplankton				
Microcrustaceans	+	++	0	0
Rotifers	+/-	++	0	0
Phytoplankton				
Chl-*a*	0	+	++	++
Cocconeis	0	0	++	0
Cosmarium	0	0	0	++
Trachelomonas	0	0	++	0
Phacus	0	0	++	0
Macrophytes	0	n.p.	0	0

Van Wijngaarden *et al.* [48] compared several studies performed under various conditions with neurotoxic insecticides in various parts of the world. In line with the results found by Roessink *et al.* [12], they concluded that different studies with the same non-persistent insecticide (*e.g.*, chlorpyrifos, esfenvalerate, lambda-cyhalothrin) found highly consistent effect threshold levels, regardless of study location, at least when similar exposure regimes were considered. The robustness of these studies can be explained by the fact that it is particularly the peak concentrations of these very toxic non-persistent insecticides which are responsible for the effects, as well as from the fact that arthropods usually form a predominant part of the aquatic communities [48].

In conclusion, an increase in nutrient levels of shallow freshwater ecosystems does not necessarily lead to an increase in total organic matter, but rather to a shift in the form in which this matter is present (*viz.* as DOC, phytoplankton POC or vascular plants). Since insecticides like lambda-cyhalothrin are non-persistent, do not bio-accumulate in the food

web and exert their toxicity via the water phase, and given the fact that macrophytes provide a relatively large pool of organic matter in mesotrophic shallow waters and phytoplankton does the same in eutrophic systems, combinations of the interactions discussed above (1A-1E) played more or less equal roles in mitigating bioavailability in the two types of test system. However, indirect effects and rate of recovery of affected populations may differ considerably in systems that differ in terms of their nutrient-mediated food web structure.

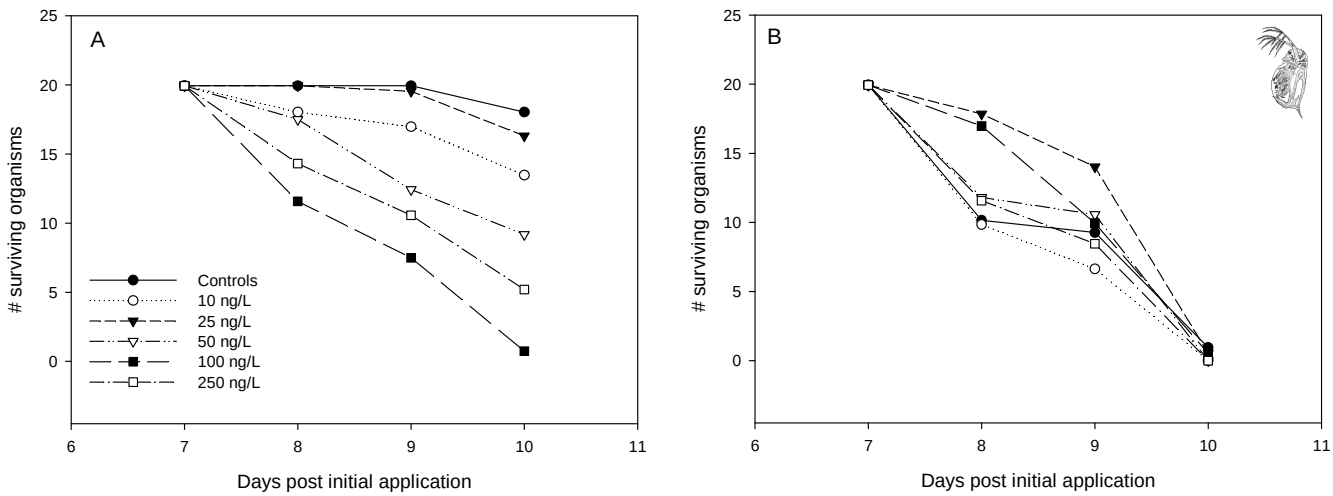


Figure 2: Results of the Daphnia pulex bioassay in the plankton-dominated (A) and macrophyte-dominated (B) test systems treated with the non-persistent insecticide lambda-cyhalothrin [12].

Impact of triphenyltin in different types of aquatic ecosystem

Eutrophication and pollution by persistent contaminants are two major long-term problems of aquatic ecosystems. The effects of nutrient loading can affect an aquatic system for years. Eutrophication and the presence of persistent micro-pollutants in sediments rich in organic matter tend to co-occur. This means that in historically polluted systems, organisms have to cope with higher nutrient and toxicant levels in the sediment, as well as with pulse exposures to non-persistent and persistent toxicants in recent pollution events. Organotin compounds are organic toxicants that frequently enter aquatic systems [49]. Residence times of organotin compounds in sediments of over ten years have been reported [50].

The outdoor microcosm experiments with triphenyltin primarily focussed on the impact of the toxicant on the structure and functioning of the community. These studies were conducted in macrophyte-dominated cosms constructed with clean or historically polluted sediment. They revealed that after a single application to the water phase, exposure in the water column was similar, although macrophyte biomass was lower in systems constructed with clean sediment. However, the somewhat lower

macrophyte biomass resulted in slightly elevated TPT concentrations in the sediment of the systems constructed with clean sediment, compared with polluted sediment systems (chapter 3). This is in line with mechanism 1E discussed above.

Figure 1B shows the species sensitivity distributions (SSD) of macroinvertebrate populations for both types of system four weeks after the application of TPT. For the combination of TPT and invertebrates, the time-to-effect after water exposure was rather long (chapter 4). Furthermore, TPT is a bio-accumulating compound and is also taken up via the food chain [51]. This indicates that it will take some time for organisms to express direct toxic effects. Hence, the best time for a relevant characterization of the toxic effects is some time after the application, and we considered four weeks after the application an acceptable period for the comparison. The SSD curves constructed for the two types of test system four weeks after TPT application are remarkably similar (Figure 1B) and this is also true for the calculated HC_5 values (Table 2) and SSD curves constructed eight weeks after the application (chapter 4). Although no obvious differences in direct effect threshold levels were observed between the two test systems, there were more pronounced differences in indirect effects (Table 3). Indirect effects on macroinvertebrates were observed in the systems constructed with polluted sediment, while indirect effects on phytoplankton taxa were more pronounced in the systems using clean sediment. This is probably a direct result of the difference in macroinvertebrate species composition between the two systems, and the lower macrophyte biomass in the systems constructed with clean sediment. The latter enabled a somewhat higher abundance of certain phytoplankton populations in these systems, which facilitated a more pronounced expression of indirect effects on these plankton populations (Table 3). These observations correspond with mechanism 2B discussed above.

Food web interaction can amplify the treatment-related response of organisms, as was demonstrated in bioassays in the water column of both test systems with *Proasellus sp*. These bioassays showed that 28 days after application, direct water toxicity was no longer present, and almost all of the test organisms at all treatment levels survived in the systems constructed with clean sediment (Figure 3A). In the systems constructed with polluted sediment, by contrast, *Proasellus* only survived at concentration levels of 10 μg/L and higher (Figure 3B). These systems also featured the predatory Turbellarian *Mesostoma sp.*, which invaded the bioassay cages and reduced *Proasellus* numbers. Since the results reported in chapter 4 showed that two other Turbellarians (*Dugesia sp.* and *Polycelis tenuis/niger*) have EC_{50}-96h values of 6.1 and 6.6 μg/L, respectively, it is likely that *Mesostoma sp.* was also affected by TPT at

concentrations of 10 µg/L and higher. Consequently, the newly incubated *Proasellus* did not suffer a negative influence on day 28 and later and survived here, whereas in the controls and at the 1 µg/L treatment levels, *Mesostoma sp.* survived and preyed on *Proasellus*. This example (Figure 3) and that presented in Figure 2 illustrate that food web interactions may cause contrasting indirect effects.

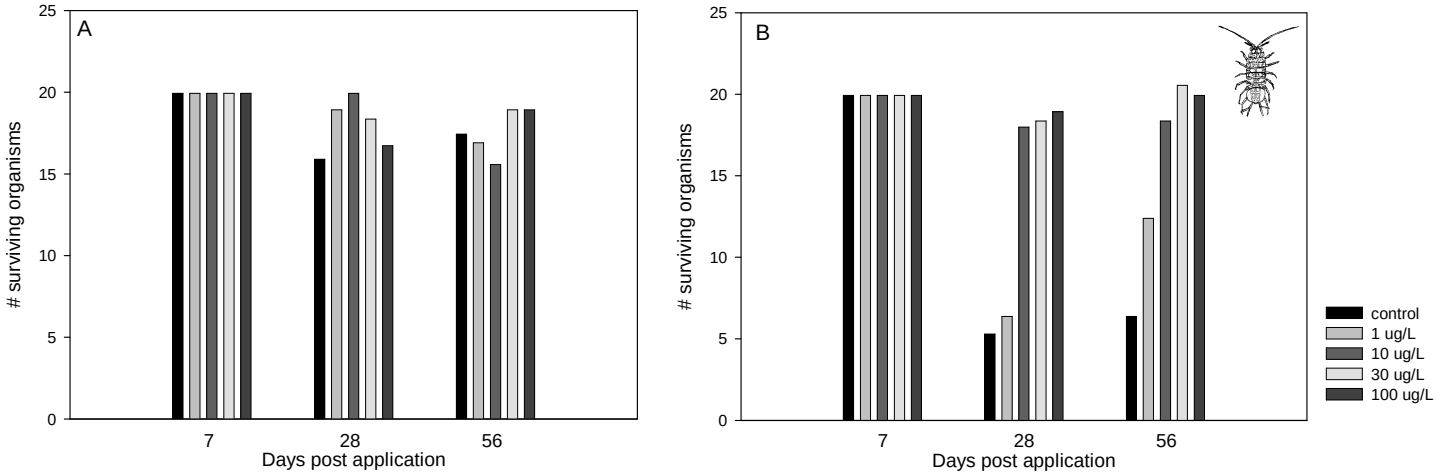


Figure 1: Results of the Proasellus sp. recovery bioassay in systems constructed with clean (A) and polluted (B) sediments. The bioassay consisted of a steel mesh cage in which 20 animals were incubated for a seven-day period, after which survivors were counted and the test population was replaced by twenty fresh animals. Bioassay cages were first inserted at day 7 post application.

In the cosm study, severe and long-lasting effects were observed on invertebrate populations (chapter 3). Since TPT concentrations in water and sediment in agricultural areas and marinas may be within the higher range of the concentrations used in our tests, [52, 53] the effects observed represent a realistic scenario for other water bodies. However, differences in sediment quality did not result in different SSDs. It can be argued that although the historical persistent pollution had affected the species composition of the community, it had no effect on the threshold level for direct toxic effects of the additional stressor TPT.

The fact that the SSDs were very similar for the two test systems does not exclude that the nutrient status of the system may affect the sensitivity of certain populations to the toxicant. Laboratory bio-assays with *Ephoron virgo* and *Chironomus riparius* on sediments from this same experiment (extracted from the cosms 15 weeks after TPT application) showed that these interactions may indeed play a role (chapter 5). In the clean-sediment assay, sediment-associated TPT had a clear negative impact on the survival and growth of *E. virgo*, while it did not have such an impact on *C. riparius*. Apparently, compensation of the negative effects of toxicants occurs when enough food is present, which

corresponds to mechanism 2A discussed above. Survival of *C. riparius* was not impacted in the historically polluted sediments that were rich in organic matter, but historic pollution did have a high impact on the survival of *E. virgo*. However, the more recently added TPT did not trigger an increased response of *E. virgo* in the polluted sediment bioassays. This system also featured a food web interaction, as during the assay the polluted sediment became covered with bacteria and cyanobacteria, and coverage increased with increasing TPT levels. This increase in organic matter may have reduced TPT availability to the mayflies, and the bacterial biomass may have served as an alternative food source, increasing organism fitness and thus decreasing susceptibility to toxicants [54, 55]. It should be noted that *Ephoron virgo* did not occur as a free-living population in our outdoor microcosms.

Conclusions

The results of the microcosm experiments described in this thesis reveal that the fate of organic micro-pollutants in shallow freshwater ecosystems seems to be affected by the presence of macrophytes. For example, concentrations of PCBs in other system compartments (suspended solids, periphyton, floating filamentous algae, zooplankton, Olichochaeta and fish) were lower in macrophyte-dominated systems, and less TPT reached the sediment. However, the lambda-cyhalothrin experiment resulted in similar water dissipation rates in the macrophyte- and plankton-dominated test systems. This allows the conclusion that although an increase in nutrients may change the form in which the bulk of the organic matter in the water column is present (either as vascular plants or phytoplankton POC), the effect thresholds for non-persistent toxicants with a short time-to-effect do not necessarily change accordingly. Consequently, the various interactions influencing the fate of micro-pollutants, as discussed above, caused smaller differences than expected between the two types of test systems in terms of mitigating the bioavailability and hence the effects of the pollutants.

At concentration levels well above the threshold level for direct toxic effects, however, a change in nutrient status does result in different interactions between toxicant- and nutrient-mediated shifts in food web interactions (Table 4). In addition, although the same taxonomic groups were impacted in the different systems in both the lambda-cyhalothrin and TPT experiments, there were nevertheless differences in the response and recoverability of individual populations (Table 4). This indicates that risks of organic micro-pollutants in shallow freshwaters cannot be exclusively assessed on the basis of toxicity data observed in the laboratory, because of spatio-temporal variation in indirect effects and rates of recovery.

Table 4. Interactions between nutrients and organic micro-pollutants in our microcosm experiments. A zero (0) indicates that no interaction was found, while + indicates there were interactions and – indicates that the experiment was not designed to study these interactions [12, 13, 16].

	Partitioning in system compartments	Species composition	Threshold level for direct toxic effects	Response of community above threshold level	Response of individual populations above threshold level	Recoverability of the community	Indirect effects due to food web interactions
Fate							
Persistent PCB/PAH	+	+	-	-	-	-	-
Impact							
Non-persistent lambda-cyhalothrin	-	+	0	+	+	+	+
Persistent TPT	-	+	0	+	+	+	+

References

[1] Koelmans, A. A., Van der Heijde, A., Knijff, L. M. and Aalderink, R. H. 2001. Integrated Modelling of Eutrophication and Organic Contaminant Fate & Effects in Aquatic Ecosystems. A Review. Water Research 35 pp. 3517-3536

[2] Breitburg, D. L., Sanders, J. G., Gilmour, C. C., Hatfield, C. A., Osman, R. W., Riedel, G. F., Seitzinger, S. P. and Sellner, K. G. 1999. Variability in responses to nutrients and trace elements, and transmissions of stressor effects through an estuarine food web. Limnology and Oceanography 44 pp. 837-863

[3] Kramer, P. R. G., Jonkers, D. A. and Van Liere, L. 1997. Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems. RIVM. Bilthoven. The Netherlands

[4] Skei, J., Larsson, P., Rosenberg, R., Jonsson, P., Olsson, M. and Broman, D. 2000. Eutrophication and contaminants in aquatic ecosystems. Ambio 29 pp. 184-194

[5] Gunnarsson, J., Bjork, M., Gilek, M., Granberg, M., Rosenberg, R. 2000. Effects of eutrophication on contaminant cycling in marine benthic systems. Ambio 29 pp. 252-259

[6] Moermond, C. T. A., Roozen, F. C. J. M., Zwolsman, J. J. G. and Koelmans, A. A. 2004. Uptake of sediment-bound bioavailable polychlorobiphenyls by benthivorous carp (*Cyprinus carpio*). Environ. Sci. Technol. 38 pp. 4503-4509

[7] Håkanson, L. 2005. The importance of lake morphometry for the structure and function of lakes. International Review of Hydrobiology 90 pp. 433-461

[8] Moss, B., Beklioglu, M., Carvalho, L., Kilinc, S., McGowan, S. and Stephen, D. 1997. Vertically-challenged limnology; contrasts between deep and shallow lakes. Hydrobiologia 342-343 pp. 257-267

[9] Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London-Weinheim-New York-Tokyo-Melbourne-Madras

[10] Roelofs, J. G. M. and Bloemendaal, F. H. J. L. 1988. Trofie. In: Bloemendaal, F. H. J. L. and Roelofs, J. G. M., eds, Waterplanten en waterkwaliteit, Stichting uitgeverij KNNV, Utrecht, pp. 113-125

[11] De Haas, E. M., Roessink, I., Verbree, B., Koelmans, A. A., Kraak, M. H. S. and Admiraal, W. 2005. Influence of sediment quality on the responses of benthic invertebrates after treatment with the fungicide triphenyltin acetate. Environmental Toxicology and Chemistry 24 pp. 1133-1139

[12] Roessink, I., Arts, G. H. P., Belgers, J. D. M., Bransen, F., Maund, S. J. and Brock, T. C. M. 2005. Effects of lambda-cyhalothrin in two ditch mesocosm systems of different trophic status. Environmental Toxicology and Chemistry 24 pp. 1684-1696

[13] Roessink, I., Crum, S. J. H., Bransen, F., Van Leeuwen, E., Van Kerkum, F., Koelmans, A. A. and Brock, T. C. M. 2006. Impact of

triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality. Ecotoxicology 15 pp. 267-293

[14] Roessink, I., Belgers, J. D. M., Crum, S. J. H., Van den Brink, P. J. and Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field. Ecotoxicology 15 pp. 411-424

[15] Moermond, C. T. A., Roessink, I., Jonker, M. T. O., Meijer, T. and Koelmans, A. A. 2007. Impact of polychlorinated biphenyl and polycyclic aromatic hydrocarbon sequestration in sediment on bioaccumulation in aquatic food webs. Environmental Toxicology and Chemistry 26 pp. 607-615

[16] Roessink, I., Moermond, C. T. A., Gillissen, F. and Koelmans, A. A. Impact of macrophytes and fish on partitioning of sediment-originating PCBs and PAHs in freshwater model ecosystems. in prep.

[17] Koelmans, A. A., Gillissen, F., Makatita, W. and Van Den Berg, M. 1997. Organic carbon normalisation of PCB, PAH and pesticide concentrations in suspended solids. Water Research 31 pp. 461-470

[18] Gunnarsson, J. S., Schaanning, M. T., Hylland, K., Skold, M., Eriksen, D. O., Berge, J. A. and Skei, J. 1996. Interactions between eutrophication and contaminants. III. Mobilization and bioaccumulation of benzo(a)pyrene from marine sediments. Marine Pollution Bulletin 33 pp. 80-89

[19] Schaanning, M. T., Hylland, K., Eriksen, D. O., Bergan, T. D., Gunnarson, J. S. and Skei, J. 1996. Interactions between eutrophication and contaminants. II. Mobilization and bioaccumulation of Hg and Cd from marine sediments. Marine Pollution Bulletin 33 pp. 71-79

[20] Fleeger, J. W., Carman, K. R. and Nisbet, R. M. 2003. Indirect effects of contaminants in aquatic ecosystems. The Science of the Total Environment 317 pp. 207-233

[21] Mosser, J. L., Fisher, N. S. and Wurster, C. F. 1972. Polychlorinated Biphenyls and DDT alter species composition in mixed cultures of algae. Science 176 pp. 533-535

[22] Gunnarsson, J., Broman, D., Jonson, P., Olsson, M. and Rosenberg, R. 1995. Interactions between eutrophication and contaminants: Towards a new research concept for the European aquatic environment. Ambio 24 pp. 383-385

[23] Hand, L. H., Kuet, S. F., Lane, M. C. G., Maund, S. J., Warinton, J. S. and Hill, I. R. 2001. Influences of aquatic plants on the fate of the pyrethroid insecticide lambda-cyhalothin in aquatic environments. Environmental Toxicology and Chemistry 20 pp. 1740-1745

[24] Karen, D. J., Joab, B. M., Wallin, J. M. and Johnson, K. A. 1998. Partitioning of chlorpyrifos between water and an aquatic macrophyte (*Elodea densa*). Chemosphere 37 pp. 1579-1586

[25] Schulz, R., Moore, M. T., Bennett, E. R., Milam, C. D., Bouldin, J. L., Farris, J. L., Smith, S. and Cooper, C. M. 2003. Acute toxicity of Methyl-Parathion in wetland mesocosms: Assessing the influence of aquatic plants using laboratory testing with *Hyalella azteca*. Archives of Environmental Contamination and Toxicology V45 pp. 331-336

[26] Brock, T. C. M., Crum, S. J. H., Van Wijngaarden, R. P. A., Budde, B. J., Tijink, J., Zuppelli, A. and Leeuwangh, P. 1992. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: I. Fate and primary effects of the active ingredient chlorpyrifos. Archives of Environmental Contamination and Toxicology 23 pp. 69-84

[27] Leistra, M., Zweers, A. J., Warinton, J. S., Crum, S. J. H., Hand, L. H., Beltman, W. H. J. and Maund, S. J. 2003. Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. Pest Management Sciences 60 pp. 75-85

[28] Särkkä, J., Hattula, M. L., Janatuinen, J. and Paasivirta, J. 1978. Chlorinated hydrocarbons and mercury in aquatic vascular plants of lake Päijänne, Finland. Bulletin of Environmental Contamination and Toxicology 20 pp. 361-368

[29] Interlandi, S. J. 2002. Nutrient-toxicant interactions in natural and constructed phytoplankton communities: results of experiments in semi-continuuous and batch culture. Aquatic Toxicology 61 pp. 35-51

[30] Pieters, B. J., Paschke, A., Reynaldi, S., Kraak, M. H. S., Admiraal, W. and Liess, M. 2005. Influence of food limitation on the effect of fenvalerate pulse exposure on the life history and population growth rate of *Daphnia magna*. Environmental Toxicology and Chemistry 24 pp. 2254-2259

[31] Enserink, L. 1995. Food mediated life history strategies in *Daphnia magna*: Their relevance to ecotoxicological evaluations. Wageningen Agricultural University, Wageningen,

[32] Enserink, L., Luttmer, W. and Maas-Diepeveen, H. 1990. Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquatic Toxicology 17 pp. 15-25

[33] Pieters, B. J. and Liess, M. 2006. Maternal nutritional state determines the sensitivity of *Daphnia magna* offspring to short-term Fenvalerate exposure. Aquatic Toxicology 76 pp. 268-277

[34] Antunes, S. C., Castro, B. B. and Goncalves, F. 2003. Chronic responses of different clones of *Daphnia longispina* (field and ephippia) to different food levels. Acta Oecologica 24 pp. S325-S332

[35] Jak, R. G., Maas, J. L. and Scholten, M. C. T. 1998. Ecotoxicicity of 3,4-dichloroaniline in enclosed freshwater plankton communities at different nutrient levels. Ecotoxicology 7 pp. 49-60

[36] Brock, T. C. M., Roijackers, R. M. M., Rollon, R., Bransen, F. and Van der Heyden, L. 1995. Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms. II. Responses of macrophytes, periphyton and macroinvertebrate grazers. Archiv für Hydrobiologie 134 pp. 53-74

[37] Brock, T. C. M., Van den Bogaert, M., Bos, A. R., Van Breukelen, S. W. F., Reiche, R. T., J., Suykerbuyk, R. E. M. and Roijackers, R. M. M. 1992. Fate and effects of the insecticide Dursban 4E in indoor Elodea-dominated and macrophyte-free freshwater model ecosystems: II. Secondary effects on community structure. Archives of Environmental Contamination and Toxicology 23 pp. 391-409

[38] Van Donk, E., Prins, H., Voogd, H. M., Crum, S. J. H. and Brock, T. C. M. 1995. Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms. I. Responses of plankton and zooplanktivorous insects. Archiv für Hydrobiologie 133 pp. 417-439

[39] Odum, E. P. 1985. Trends expected in stressed ecosystems. BioScience 35 pp. 419-422

[40] Kasai, F. and Hanazato, T. 1995. Effects of the triazine herbicide, Simetryn, on freshwater plankton communities in experimental ponds. Environmental Pollution 89 pp. 197-202

[41] Van den Brink, P. J., Hartgers, E. M., Fettweis, U., Crum, S. J. H., Van Donk, E. and Brock, T. C. M. 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide Linuron. Ecotoxicology and Environmental Safety 38 pp. 13-24

[42] Hanazato, T. 2001. Pesticide effects on freshwater zooplankton: an ecological perspective. Environmental pollution 112 pp. 1-10

[43] Moermond, C. T. A., Zwolsman, J. J. G. and Koelmans, A. A. 2005. Black carbon and ecological factors affect *in situ* Biota to Sediment Accumulation Factors for hydrophobic organic compounds in flood plain lakes. Environmental Science and Technology 39 pp. 3101-3109

[44] Larsson, P., Collvin, L., Okla, L. and Meyer, G. 1992. Lake productivity and water chemistry as governors of the uptake of persistent pollutants in fish. Environmental Science and Technology 26 pp. 346-352

[45] Posthuma, L., Suter II, G. W. and Traas, T. P. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, London

[46] Schroer, A. F. W., Belgers, D., Brock, T. C. M., Matser, A., Maund, S. J. and Van den Brink, P. J. 2004. Acute toxicity of the pyrethroid insecticide lambda-cyhalothrin to invertebrates of lenthic freshwater systems. Archives of Environmental Contamination and Toxicology 46 pp. 324-335

[47] Zhou, J. L., Rowland, S. and Mantoura, R. F. C. 1995. Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. Water Research 29 pp. 1023-1031

[48] Van Wijngaarden, R. P. A., Brock, T. C. M. and Van Den Brink, P. J. 2005. Threshold levels for effects of insecticides in freshwater ecosystems: A review. Ecotoxicology 14 pp. 355-380

[49] Loch, J. P. G. 1990. Accumulation and leaching of the fungicide fentin acetate and intermediates in sandy soils. Water, Air, and Soil Pollution 53 pp. 119-129

[50] Fent, K. and Hunn, J. 1991. Phenyltins in water, sediment, and biota of freshwater marinas. Environmental Science and Technology 25 pp. 956-963

[51] Traas, T. P., Stäb, J. A., Kramer, P. R. G., Cofino, W. P. and Aldenberg, T. 1996. Modeling and risk assessment of tributyltin accumulation in the food web of a shallow freshwater lake. Environmental Science and Technology 30 pp. 1227 -1237

[52] Fent, K. and Hunn, J. 1995. Organotins in freshwater harbours and rivers: temporal distribution, annual trends and fate. Environmental Toxicology and Chemistry 14 pp. 1123-1132

[53] Stäb, J. A. 1995. Organotin compounds in the aquatic environment. Determination, occurrence and fate. Vrije Universiteit, Amsterdam, The Netherlands

[54] De Haas, E. M., Paumen, L. M., Koelmans, A. A. and Kraak, M. H. S. 2004. Combined effects of copper and food on the midge *Chironomus riparius* in whole-sediment bioassays. Environmental Pollution 127 pp. 99-107

[55] Heugens, E. H. W., Hendriks, A. J., Dekker, T., Van Straalen, N. M. and Admiraal, W. 2001. A review of the effect of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31 pp. 247-284

Chapter 8

Nederlandse samenvatting

Samenvatting

De structuur van een zoetwater ecosysteem, en de daarin voorkomende levensgemeenschap, wordt beïnvloed door factoren zoals de trofische (nutriënten) toestand van het betreffende systeem en de achtergrondwaarden van persistente verontreinigingen in het sediment. Aangezien deze factoren variëren in ruimte en tijd kan het zijn dat de respons van de levensgemeenschap op een additionele stressfactor ook variabel is. Dergelijke additionele stressfactoren kunnen gifstoffen zijn die in oppervlaktewater terecht komen door o.a. landbouwkundig en industrieel gebruik.

Dit proefschrift heeft als doel meer inzicht te verwerven in de invloed van trofische (nutriënten)status van een zoetwater ecosysteem en/of van persistente verontreinigingen in het sediment, op het gedrag en de ecologische effecten van een insecticide en een fungicide/biocide. Tevens poogt dit proefschrift inzicht te verschaffen in de invloed van waterplanten en vis op de herverdeling van aan sediment gebonden polycyclische koolwaterstoffen (PAKs) en meervoudig gechloreerde bifenylen (PCBs). PAKs en PCBs zijn voornamelijk “historische” verontreinigingen die zich ophopen in het sediment en daar langdurig aanwezig blijven.

Wat is het effect van een insecticide op de levensgemeenschap van (i) een door algen (eutroof) gedomineerd of (ii) door waterplanten (mesotroof) gedomineerd zoetwater systeem?

Om deze vraag te beantwoorden werden 12 cilinders in een eutroof fytoplankton gedomineerd zoetwater ecosysteem en 12 cilinders in een mesotroof waterplantenrijk zoetwater ecosysteem geplaatst. In beide typen systemen werd het insecticide lambda-cyhalothrin 3 keer toegediend (interval 7 dagen) in concentraties van 0, 10, 25, 50, 100 en 250 ng/L (n=2). De verdwijnsnelheid van het insecticide uit de waterfase was gelijk tussen de twee systeemtypen. Na 1 dag was hierin nog maar 30% van de toegediende hoeveelheid aanwezig. Directe toxische effecten werden voornamelijk bij insecten en kreeftachtigen waargenomen. De directe toxische effecten op gevoelige organismen kwamen overeen met resultaten van kortdurende laboratoriumexperimenten. Een opvallende uitkomst is dat de verschillen in drempelwaarden van directe toxische effecten tussen de beide systemen klein waren. De drempelwaarde is de concentratie waarbij in het testsysteem geen of nauwelijks effecten van de gifstof waargenomen worden. Grotere verschillen in respons van de testsystemen betroffen de mate van herstel en indirecte effecten bij hogere blootstellingconcentraties. Indirecte effecten zijn effecten op voor het insecticide minder gevoelige organismen die veroorzaakt worden door het wegvallen van gevoelige organismen (o.a. verschuivingen in predatie

en competitie). In het plankton gedomineerd systeem waren de waargenomen indirecte effecten meer uitgesproken maar was het herstel van gevoelige populaties sneller. Sneller herstel kan verklaard worden door het voorkomen van gemiddeld kleinere organismen met een kortere levenscyclus in het plankton gedomineerd systeem ten opzichte van het door waterplanten gedomineerd systeem. De resultaten van dit experiment zijn beschreven in hoofdstuk 2.

Wat is de invloed van historische vervuiling in het sediment op de respons van de levensgemeenschap ten gevolge van blootstelling aan een fungicide/biocide?

Om deze vraag te beantwoorden werden 10 experimentele ecosystemen (microcosms) ingericht met vervuild en 10 microcosms met schoon sediment afkomstig uit uiterwaardplassen van de Waal. De kwaliteit van het sediment had grote invloed op de levensgemeenschappen die zich in de twee typen systemen ontwikkelden. Vanaf de start ontwikkelde de plantenvegetatie (dominantie van waterpest) zich veel beter op het vervuilde sediment, dat naast meer toxische stoffen ook meer nutriënten bevatte.

Het fungicide/biocide trifenyltin-acetaat (TFT) werd eenmalig toegediend in concentraties van 0, 1, 3, 10, 30 en 100 µg/L (n=2 per sedimenttype). Het concentratieverloop van TFT in de waterfase was vrijwel gelijk tussen de twee systeemtypen. Desalniettemin werden er hogere concentraties TFT teruggevonden in het schone sediment, waar minder waterplanten aanwezig waren. In beide systeemtypen lieten vertegenwoordigers van verschillende taxonomische groepen (o.a. slakken, wormen, kreeftachtigen en insecten) een duidelijke respons zien op de behandeling met TFT. Hoewel TFT erg persistent was in het sediment werd hierin geen behandelingseffect op nematoden waargenomen. Alhoewel er tussen de twee testsystemen enige verschillen werden waargenomen in de intensiteit en duur van effecten ten gevolge van TFT toediening kan gesteld worden dat de aanwezigheid van historische vervuiling in het sediment de algemene gevoeligheid van de aquatische gemeenschap nauwelijks beïnvloedde. De resultaten van dit experiment zijn beschreven in hoofdstuk 3.

Kunnen de waargenomen effecten van TFT in de experimentele ecosystemen voorspeld worden met kortdurende laboratorium toxiciteitexperimenten?

Om deze vraag te beantwoorden werden vertegenwoordigers van taxonomische groepen die gevoelig waren voor TFT in de microcosms ook getest in het laboratorium door middel van zogenaamde acute toxiciteittesten (toetsduur 96 uur). In totaal werden in het laboratorium 32

verschillende soorten waterorganismen bestudeerd. Voor iedere soort werd, waar mogelijk, de concentratie berekend waarbij 50% van de geteste individuen een behandelinggerelateerd effect van TFT vertoond (EC_{50}). EC_{50}-waarden werden ook berekend voor populaties van soorten die een behandelinggerelateerd effect vertoonden in de twee typen microcosms. Deze microcosm EC_{50}-waarden werden berekend op 2, 4 en 8 weken na toediening van TFT omdat in deze periode de grootste effecten waargenomen werden. Met de verkregen EC_{50}-waarden voor verschillende soorten werd een zogenaamde gevoeligheidscurve (Species Sensitivity Distribution curve = SSD) geconstrueerd. Uit deze curve kan een concentratie berekend worden die tenminste 95% van de geteste soorten min of meer beschermd. Deze concentratie wordt de HC_5 genoemd.

De berekende laboratorium HC_5-waarde, gebaseerd op 96 uur laboratoriumtesten, was 1.3 µg/L. De berekende microcosm HC_5-waarden (2-8 weken na toediening) varieerden tussen de 0.2 en 0.6 µg/L (gebaseerd op piekconcentraties van TFT in water van de testsystemen). De gevoelige soorten in de microcosm kwamen overeen met die in het laboratorium met uitzondering van insecten waarvan sommige alleen in de experimentele ecosystemen gevoelig reageerden. Alle geteste soorten reageerden overigens minder gevoelig in de korte termijn laboratorium-experimenten dan in de microcosm-experimenten. Mogelijke verklaringen voor dit fenomeen zijn dat de laboratorium testen te kort duren voor het volledig tot uiting brengen van effecten en/of additionele chronische blootstelling via de voedselketen in de modelecosystemen. Een opvallend resultaat is dat de berekende microcosm HC_5-waarden voor de systemen met schoon en vervuild sediment nauwelijks verschilden. Dit duidt erop dat de ecologische drempelwaarde voor toxische effecten van TFT blijkbaar niet beïnvloed wordt door achtergrond verontreinigingen aanwezig in het sediment. De resultaten van dit experiment zijn beschreven in hoofdstuk 4.

Is de aanwezigheid van achtergrondverontreiniging en recent toegevoegde TFT aan te tonen met gestandaardiseerde bio-assays?

De hierboven beschreven waarnemingen betreffen organismen die min of meer natuurlijk in de modelecosystemen voorkomen. Om de invloed van toxische stoffen onder veldomstandigheden in kaart te brengen kunnen ook min of meer gestandaardiseerde bio-assays worden uitgevoerd. Twee soorten waterorganismen die regelmatig gebruikt worden om de kwaliteit van sediment te toetsen zijn de haft *Ephoron virgo* en de dansmug *Chironomus riparius*. Deze soorten zijn overigens niet waargenomen in de microcosms. Sediment uit de hierboven beschreven microcosms werd gebruikt om de respons van deze

organismen op verontreinigingen in het sediment nader te bestuderen. 15 weken na TFT toediening werd uit elk afzonderlijk testsysteem van beide microcosm-typen, met uitzondering van de 3 µg/L behandeling, sediment naar het laboratorium gehaald om bio-assays mee uit te voeren. De concentratie van toxische stoffen in het sediment (metalen, PCBs en PAKs) was in een eerdere studie al bepaald en TFT concentraties in de controle en 30 µg/L behandeling werden gemeten direct na bemonstering uit de microcosms. Middels lineaire regressie werd de TFT concentratie in de overige sedimenten afgeleid. 25 mL gemengd sediment van ieder test systeem werd in glazen potten gebracht waaraan 20 larven van *E. virgo* of 5 larven van *C. riparius* werden toegevoegd. Na 10 dagen werd de overleving en groei van de organismen bepaald. De bio-assay experimenten toonden aan dat het type sediment (met weinig en veel historische achtergrondvervuiling) nauwelijks invloed had op de groei van *C. riparius.* Een mogelijke verklaring hiervoor is dat het meer vervuilde sediment ook meer organisch materiaal bevatte, dat als geschikte voedselbron fungeerde. Negatieve effecten van de aanwezige gifstoffen werden mogelijk gecompenseerd door de hogere voedingswaarde van het sediment. Echter de overleving en groei van larven van *E. virgo* was beduidend lager bij hogere achtergrondconcentraties van PAKs, PCBs, en metalen. Mogelijk speelt ook de aard van het aanwezige organisch materiaal hier een rol. Voor beide sedimenttypen kon een dosis-respons relatie van sedimentgebonden TFT niet aangetoond worden bij zowel *C. riparius* als *E. virgo*. Er kon geen cumulatief effect aangetoond worden van historische vervuiling en de recent toegevoegde TFT. De resultaten van dit experiment zijn beschreven in hoofdstuk 5.

Wat is de invloed van waterplanten en vis op de herverdeling van sediment-gebonden verontreinigingen (PCBs en PAKs) over verschillende systeemcompartimenten?

Om deze vraag te beantwoorden, is in laboratorium modelecosystemen het effect van het al dan niet voorkomen van waterplanten en/of karper bestudeerd op de distributie en concentratie van meervoudig gechloreerde bifenylen (PCBs) en polycyclische aromatische koolwaterstoffen (PAKs) in de compartimenten sediment, seston (zwevend materiaal in water), waterplanten, perifyton (algen vastgehecht aan substraten), drijvende algen (flab), zooplankton, sedimentbewonende wormen, overige evertebraten en karper. Tevens is er hierbij onderscheid gemaakt tussen historische verontreiniging en recent toegevoegde stoffen. Hiervoor werden 12 modelecosystemen met o.a. PAKs en PCBs vervuild sediment, afkomstig uit uiterwaardplassen van de Waal, ingericht. Aan dit sediment werden bij het inrichten van de systemen een tweetal PCBs en

een gelabelde PAK toegevoegd waarna de systemen werden ingericht met: alleen vis, vis plus planten, alleen planten, geen vis en geen planten (n=3). Na 4 maanden werden de verschillende compartimenten van het ecosysteem geanalyseerd op 19 verschillende PCBs en 15 verschillende PAKs, welke allen van origine afkomstig waren uit het sediment.

De resultaten geven aan dat recent aan het sediment toegevoegde PCBs en PAKs mobieler zijn dan 'oude', sterker vastgelegde PCBs en PAKs. De meeste biomassa in de modelecosystemen bestond uit waterplanten en deze waren in staat om respectievelijk tot 26 en 31 procent van de snel-desorberende fractie van PCBs en PAKs uit het sediment te onttrekken. Desalniettemin had de aanwezigheid van waterplanten geen significant verlagend effect op de concentraties PCBs en PAKs in karper. Een duidelijk biologisch effect van karper was de predatie op evertebraten. Het foerageergedrag van karper had tot gevolg dat sediment werd opgewerveld waardoor meer PCBs en PAKs in andere systeemcompartimenten terecht kwamen.

De in dit proefschrift beschreven experimenten laten zien dat de relatie tussen verontreinigingen (nutriënten en gifstoffen) en aquatische levensgemeenschappen twee kanten op kan werken: (1) de verontreinigingen hebben invloed op de structuur en het functioneren van de levensgemeenschap en (2) organismen beïnvloeden de lotgevallen van de verontreinigingen.

Uit de resultaten beschreven in de hoofdstukken 2 en 3 blijkt dat het nutriëntenniveau en/of de achtergrondgehaltes van verontreinigingen in het sediment van invloed zijn op de samenstelling van de aquatische levensgemeenschap. Dit had weinig invloed op de drempelwaarden voor directe toxische effecten van de additionele chemische stressoren lambda-cyhalothrin en TFT. Echter bij blootstellingconcentraties boven de drempelwaarden waren de verschillen in waargenomen indirecte effecten en herstel van gevoelige populaties groter tussen verschillende typen testsystemen (zie hoofdstukken 2, 3 en 4).

Kortdurende laboratoriumexperimenten naar de toxiciteit van lambda-cyhalothrin en TFT op waterorganismen tonen aan dat deze experimenten een grotere voorspellende waarde hebben voor het insecticide lambda-cyhalothrin dan voor het fungicide/biocide TFT. Voor wat betreft lambda-cyhalothrin kan dit verklaard worden door het specifieke werkingsmechanisme (neurotoxine voor vooral arthropoden), de korte tijd die nodig is om het effect te bewerkstelligen en de relatief grote verdwijnsnelheid uit het systeem. Voor wat betreft TFT kan dit verklaard worden door het persistente karakter van de stof en de langere tijd die nodig is om effect te bewerkstelligen (o.a. via accumulatie in de voedselketen).

Uit hoofdstuk 6 blijkt dat waterplanten en karper het milieugedrag van PCBs en PAKs beïnvloeden. De relatief grote rol van waterplanten kan verklaard worden door de relatief grote biomassa van waterplantenvegetatie in de microcosms. In systemen met waterplanten was de concentratie van de snel-desorberende fractie PCBs en PAKs in het sediment beduidend lager. Ook in hoofdstuk 3 is aangetoond dat waterplanten van invloed zijn op de hoeveelheid TFT dat in het sediment terecht komt. De relatief grote rol van karper op het milieugedrag van PCBs en PAKs in de microcosms kan vooral verklaard worden door het gedrag van de vis (predatie, opwerveling sediment).

Dankwoord

Laten we maar meteen openen met de stelling die het niet gehaald heeft maar die zeker van toepassing is, namelijk: het voltooien van een proefschrift is net als goede wijn of een fijne vrouw, als er meer jaren verstrijken wordt het alleen maar beter….
…en natuurlijk ontwijk ik zo ook mooi de vraag waarom het nu een krappe 8 jaar moest duren voordat dit proefschrift eindelijk voltooid werd.

Alhoewel dit specifieke project officieel startte in oktober 2000 was het eigenlijke begin al veel eerder in gang gezet. Zo weet ik zelf niet meer of ik eerder liep dan zwom, maar is het wel zeker dat mijn zwemdiploma's de eerste oorkondes waren die ik binnen sleepte. Die fascinatie met water is, soms tot verdriet van mijn ouders, altijd gebleven. Niet dat mijn ouders iets tegen water 'an sich' hadden, het was meer de manier waarop ik ermee omging. Dus als ik weer eens met een nat pak thuis kwam, een eigen kikkerpopulatie in een emmer in de achtertuin startte, door het ijs zakte, door een boze agrariër ondersteboven in de sloot gehangen was of voor de honderdduizendste keer om een vijver zeurde, wisten jullie hier altijd een positieve draai aan te geven en bleven jullie me altijd stimuleren om mijn wensen en ideeën na te streven. Ma en pa het mag duidelijk zijn dat het zonder jullie goede zorgen en inzet een heel ander plaatje geweest zou zijn en enorm bedankt hiervoor.

Tijdens mijn studie in Wageningen was het weer het water dat de boventoon voerde en niet verwonderlijk kwam ik terecht bij de club die zich nu de leerstoelgroep Aquatische Ecologie en Waterkwaliteitsbeheer (AEW) mag noemen. Ik had het hier snel prima naar mijn zin en werd door een medestudent gevraagd of ik niet mee wilde naar Nicaragua voor een stage. Natuurlijk had ik hier wel oren naar, maar mijn potentieel begeleider Rudi Roijackers dacht daar toch net iets anders over. Het heeft erin geresulteerd dat ik twee afstudeervakken op de leerstoelgroep heb gedaan en uiteindelijk naar het NERI in Denemarken ben vertrokken voor een fantastische stage. En Rudi, ik ben je nog steeds dankbaar voor die ene beslissing.
Mijn afstudeerperiode op AEW en op het NERI samen met o.a. Ronald Gylstra, John Beijer, Rudi Roijackers, Edwin Peeters, Frank Roozen, Gerben van Geest, Fred Bransen, Frits Gillissen, Bart Koelmans, Marijke Kuipers, Torben Lauridsen, Martin Søndergård en Erik Jeppesen is het begin geweest van mijn specialisatie tot aquatisch ecotoxicoloog en

vooral ook als experimentalist. En jullie allemaal bedankt voor alle gastvrijheid, behulpzaamheid en de leuke tijd die ik steeds gehad heb.

Het was daarna ook een makkelijke keuze om te gaan promoveren. En wat ook niet slecht uitkwam was dat er een leuk project startte waarbij promovendi nodig waren. Het Stimuleringsprogramma Systeemgericht Ecotoxicologisch Onderzoek (SSEO) project was derhalve mijn eerste kennismaking met de heren en dames van het huidige team Environmental Risk Assessment (ERA). Dit werd namelijk mijn nieuwe uitvalsbasis en samen met Caroline Moermond bij AEW en Elske de Haas op de UvA gingen wij dit varkentje wassen. En ik zou liegen als ik zei dat het saai was…
Met aardig wat ambitie ging ik mijn deel-project in en het waren niet bepaald kleine klussen die er gepland stonden. Veld, semi-veld, lab experimenten het kon niet op en met alleen het 'MetalFate' experiment niet uitgevoerd, vind ik persoonlijk dat er een puike reeks staat. De onderlinge co-auteurschappen geven ook aan dat we binnen de projecten elkaar met goed gevolg wisten te vinden. Caroline en Elske bedankt voor de samenwerking.

Voor degenen die niet bekend zijn met het type onderzoek dat in dit proefschrift beschreven staat, moet ik even zeggen dat dergelijk werk niet door één iemand gedaan kan worden. Dit is puur 'teamwork' en daarvoor ben ik de veel mensen dank verschuldigd.
Ronald Gylstra jij was naast Caroline en Fred mijn bemonsteringsbuddy in de kelder van Trans. Je experimentele ervaring en parate kennis waren van enorme waarde en was je kritische houding in onze discussies soms strontvervelend. Dit laatste niet in de laatste plaats omdat je vaak gelijk had en je me dwong na te denken over dingen die ik als vanzelfsprekend aannam. Fred Bransen, Frits Gillissen en Thijs Meijer, jullie hebben die enorme bulk aan monsters die we uit die kelder wisten te slepen, verwerkt; een pittige klusje al zeg ik het zelf. Ook wil ik de rest van AEW hier nog bedanken omdat ze altijd bereid waren mee te denken over punten waar ik mee zat, zowel vakinhoudelijke, procedurele en zaken die eigenlijk nergens mee te maken hadden.

Ook had dit proefschrift er niet gelegen zonder de mannen en vrouwen van het ERA team. Caroline van Rhenen-Kersten, Gertie Arts, René van Wijngaarden, Arriënne Matser, Laura Buijse, Leo van de Pas, René Aalderink, bedankt voor jullie ondersteuning bij al het experimentele werk. Paul van den Brink enorm bedankt voor het mij wegwijs maken in de multivariate analyse technieken. Het lukt me nu al aardig goed om intelligent naar die data-brij te kijken zodat anderen denken dat ik er echt

verstand van heb. Steven Crum bedankt voor al je inzet en uitpluiswerk op het chemisch analytisch lab, we hebben die organotin analyses toch mooi draaiend gekregen. Dick Belgers jij bent toch wel bij alles betrokken geweest. Samen in de kou hebben we sloten staan enten met waterpest, beestjes lopen vangen en, al zeg ik het zelf, met zijn tweeën op de brug liep het altijd als een geoliede bemonsteringsmachine. Enorm bedankt voor al je inzet en de fijne samenwerking. Ik ben erg blij dat je er op de grote dag ook weer bij bent.
Rob Merkelbach, als toenmalig teamleider durfde je het aan om een 'juveniel' zoals ik binnen het team te halen en bedankt voor alle mogelijkheden die er waren of die we samen gecreëerd hebben. Wim Beltman ik begon bij jou op de kamer en nu ik het proefschrift afrond zijn we weer samen op een kamer aanbeland. Bedankt voor je lotgevalleninzichten en het me wegwijs maken in 'het systeem'.
De rest van het team bedankt voor de leuke uitjes, de discussies en alle kennis waaruit ik mocht putten.
As for my fellow PhD-students at ERA Mascha, Nika, and Mazaar I wish you all a good promotion. I know I did not find and certainly did not solve all the bugs in the system but I guess that would have made it a bit boring for you guys, wouldn't it?

Reijer Hoijtink, Edwin van Leeuwen, Minke Huurnink, Sonja Vernooij, Harmen Hendriksma, Elizabeth Sargant, Noud Koomen, Ellen Weerman, Tessa Siderius en Marnix Oostland bedankt voor de samenwerking. Als jullie afstudeervakbegeleider heb ik in ieder geval een hoop geleerd en een zeer interessante tijd gehad. Miquel Lürling bedankt voor het medebegeleiden van een groot deel van deze studenten.

Wie ik ook zeker niet wil vergeten zijn de mannen van de mechanische werkplaats, in het bijzonder Jan Theunissen en André Zanders, Gert Nieuwboer van de Glasinstrumentmakerij en Dick Verhagen en Kees Deijs van de Technische Dienst. Zonder jullie waren er geen experimentele opstellingen geweest, geen reparaties mogelijk en was er geen stroom als ik de stoppen weer eens opgeblazen had door teveel lampen aan te sluiten. Antonie van den Toorn jij ook bedankt voor je onderwijs in de basale tractortechniek en hoe je de tank leeg maakt als je er in plaats van diesel benzine in doet.

Echter twee sleutelfiguren heb ik nog niet genoemd en dat zijn mijn beide begeleiders. Bart Koelmans om bij jou te beginnen bedankt dat je deze uitdaging met mij aan wilde gaan en voor het geduld waarmee je mij wegwijs hebt gemaakt in de wereld van de milieuchemie en met name het PCB en PAK gebied. Het POPFate verhaal was niet het makkelijkste

verhaal om te analyseren en zeker niet om op te schrijven. We hebben heel wat gebabbeld over hoe we het konden doen en veel van deze gesprekken volgde (althans in mijn herinnering) de lijn van: “Als we dit nu ‘gewoon’ zo doen en dat dan ‘even’ zo, dan wordt het vanzelf duidelijk”. Het kwam er op neer dat er voor deze data echter aardig wat ‘gewoons’ en ‘evens’ nodig waren, maar met de nodige iteraties en heel wat rode pen van jouw kant zijn we er toch uitgekomen. Bart, bedankt voor al je inzet en inhoudelijke bijdrage.
Dan blijft over mijn dagelijks begeleider Theo Brock. Theo je bent een fenomeen op zich, ik ken niemand die zo nauwgezet en genuanceerd is als jij. Met engelengeduld heb je me de eerste beginselen van het wetenschappelijk publiceren bijgebracht en bleef je schaven aan het eerste artikel tot het goed was ook al vond ik dat het al veel eerder ‘klaar’ was. Ik heb bemerkt dat de tijd die we later aan artikelen spendeerde vele malen korter was en mijn uitleg in deze is dat je moeite dus niet voor niets geweest is. Ook nam je me meteen mee naar congressen en kreeg ik de mogelijkheid om via jou in te pluggen in het ecotoxicologische netwerk. Je vermogen om de ‘ecotox’ agenda 3 jaar vooruit te voorspellen en je waarschuwing om vooral te blijven vernieuwen zullen me zeker bijblijven. En wat ik persoonlijk ook een prestatie van formaat vind, is dat ik nu eindelijk na al die jaren je handschrift kan lezen. Theo bedankt voor al je inzet, je bevlogenheid, je wijze raad en de fijne samenwerking.

Als dit een werk was waar zoveel mensen aan bijgedragen hebben waarom staat dan mijn naam boven het geheel? Nou heel simpel, het zijn mijn slapeloze nachten, doordeweekse avonden, weekenden, vakanties en grijze haren geweest.
Maar zelfs in deze periodes ben ik niet helemaal alleen geweest.
Renske, Jos, Linda, Hans, Lars, Benno en Benno bedankt voor de muzikale uitlaatklep en de uren in de Bunker.
Heiduikers en mede-onderwaterhockeyers bedankt voor de trainingen en wedstrijden waar ik me lekker kon uitleven en alle frustratie van me af kon spelen. En Stefan Frederiks jij met name bedank voor je hulp met de kaft.
Menno Thomas als goede vriend die dit traject al eerder afgelegd had, stond je altijd klaar met goede adviezen, statistische ondersteuning en niet geheel onbelangrijk, een goede fles (was het geen duikfles dan was het wel whisky). Daarom ben ik ook blij dat je me op de dag zelf ook weer met raad en daad bij zult staan (en dat ik zo geen lastige vragen uit de zaal hoef te beantwoorden).

Jelle, kleine boef, jij was een bron van energie in de afrondingsfase en je zorgde ervoor dat de spreekwoordelijke laatste loodjes niet zo zwaar wogen.
Cindy, je was er altijd bij, of je het nu leuk vond of niet. Jij hebt mijn gevloek aangehoord als ik weer eens vast zat in een artikel. Jij warmde het eten op als ik toch nog even op de Sinderhoeve nodig was of nog even een experiment op het lab moest checken. Jij hebt veel te lang aan moeten horen:”Ja schat, gaan we doen als het boekje af is…” en wat dat betreft hebben onze oosterburen het helemaal bij het rechte eind dat de vrouw van de promovendus zich uiteindelijk “Frau Doktor” mag noemen. Cindy bedankt voor het mij zo nu en dan achter de vodden aan zitten en alle liefde en warmte.

Curriculum Vitae

Ivo Roessink is op 8 december 1976 geboren te Heerhugowaard. In 1995 behaalde hij zijn VWO diploma aan het Han Fortmann College in Heerhugowaard. Datzelfde jaar is hij begonnen aan de toenmalige opleiding Milieuhygiëne aan de (eveneens toenmalige) Landbouw Universiteit Wageningen. Hier werd na het eerste jaar gekozen voor de specialisatie Waterkwaliteit, waarbinnen afstudeervakken zijn uitgevoerd in de richtingen Aquatische Ecologie en Waterkwaliteitsbeheer. In 2000 volgde een afrondende stage bij het National Environmental Research Institute (NERI) te Silkeborg in Denemarken alwaar met twee Deense medestagiaires overdag en ’s nachts de soortensamenstelling, abundantie (en in het geval van vis ook het dieet) van zooplankton, macro-evertebraten en jonge vis werd vastgesteld in twee ondiepe meren.
Na zijn afstuderen is hij in 2000 begonnen aan een promotiebaan bij de vakgroep Aquatische Ecologie en Waterkwaliteitsbeheer (AEW) van Wageningen Universiteit binnen het Stimuleringsprogramma Systeemgericht Ecotoxicologisch Onderzoek (SSEO) van NWO. Deze promotiebaan binnen dit project was een samenwerking tussen AEW en het team Environmental Risk Assessment van het onderzoeksinstituut Alterra. Wat er uiteindelijk in resulteerde dat de werkplek voornamelijk bij Alterra gesitueerd was. Dit onderzoek richtte zich op de invloed van en interacties tussen toxicanten en nutriënten op aquatische evertebratengemeenschappen. Het onderzoek uit deze periode is gebundeld in dit proefschrift.
De promotiebaan eindigde in 2005 waarna hij bij AEW als toegevoegd onderzoeker op projectbasis in dienst trad. Binnen deze aanstelling was hij werkzaam op een project van Alterra dat de invloed van refugia onderzocht op de herstelbaarheid van macro-evertebraten in door insecticiden behandelde proefsloten. Hierna is hij, na zich een korte periode bekwaamd te hebben in het schrijven van sollicitaties, in mei 2006 in dienst getreden bij Alterra (team ERA) als wetenschappelijk onderzoeker, alwaar hij nog steeds werkzaam is.

Publications

Peer reviewed

Roessink, I, Koelmans, A. A., Brock, T.C.M. 2007. Interactions between nutrients and toxicants in shallow freshwater model ecosystems. Sciences of the Total Environment *accepted*.

Moermond, C. T. A., Roessink, I., Jonker, M. T. O., Meijer, T., Koelmans, A. A. 2007. Impact of PCB and PAH sequestration in sediment on bioaccumulation in aquatic food webs. *Environmental Toxicology and Chemistry* 26 pp. 607-615.

Moermond, C. T. A., Hendriks, J., Traas, T. P., Veltman, K., Roessink, I., Koelmans, A. A. 2007. Modelling decreased food chain accumulation of PAHs due to strong sorption to carbonaceous materials and metabolic transformation. Environmental Science and Technology 17 pp. 6185-6191

Roessink, I., Moermond, C. T. A., Gillissen, F., Koelmans, A. A. Impact of macrophyte and fish on partitioning of sediment-originating PCBs and PAHs in freshwater model ecosystems. *In prep.*

Lürling, M., Roessink, I. 2006. On the way to cyanobacterial blooms: Impact of the herbicide metribuzin on the competition between a green alga (*Scenedesmus*) and a cyanobacterium (*Microcystis*). *Chemosphere* 65 pp. 618-626.

Lürling, M., Sargant, E., Roessink, I. 2006. Life-history consequences for Daphnia pulex exposed to pharmaceutical carbamazepine. *Environmental Toxicology* 21 pp. 172-180.

Roessink, I., Crum, S. J. H., Bransen, F., Van Leeuwen, E., Van Kerkum, F., Koelmans, A. A., Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. I. Influence of sediment quality. *Ecotoxicology* 15 pp. 267-293.

Roessink, I., Belgers, J. D. M., Crum, S. J. H., Van den Brink, P. J., Brock, T. C. M. 2006. Impact of triphenyltin acetate in microcosms simulating floodplain lakes. II. Comparison of species sensitivity distributions between laboratory and semi-field. *Ecotoxicology* 15 pp. 411-424.

Roessink, I., Arts, G. H. P., Belgers, J. D. M., Bransen, F., Maund, S. J., Brock, T. C. M. 2005. Effects of lambda-cyhalothrin in two ditch microcosm systems differing in trophic status. *Environmental Toxicology and Chemistry* 24 pp. 1684-1696.

Arts, G.H.P., Buijse-Bogdan, L.L., Belgers, J.D.M., Van Rhenen-Kersten, C.H., Van Wijngaarden, R.P.A., Roessink, I., Maund, S.J., Van den Brink, P. J., Brock, T.C.M. 2006. Ecological impact of pesticide drift emission from potato fields in ditch mesocosms. *Integrated Environmental Assessment and Management* 2 pp. 105-125.

De Haas E.M. I. Roessink, B. Verbree, A.A. Koelmans, M.H.S Kraak, and W. Admiraal. 2005. Influence of sediment quality on the responses of benthic invertebrates after treatment with the fungicide triphenyltin acetate. *Environmental Toxicology and Chemistry* 24 pp.1133-1139.

Not peer reviewed

Roessink I., T. C. M. Brock. Influence of sediment quality in the 'Afferdensche en Deestsche Waarden' on the response of aquatic invertebrate communities to additional stress. *SSEO Newsletter issue 8 May 2004.*

De Haas E., M. Kraak, C. T. A. Moermond, A. A. Koelmans, I. Roessink, T. C. M. Brock, G. Zwolsman. Interactions between nutrients and contaminants in floodplain lake ecosystems. *SSEO Newsletter issue 5, February 2002.*

Conference proceedings

Roessink, I., Brock, T.C.M. 2007. Multi-stress in the aquatic environment is it predictable? In: Abstract book. SETAC Europe 17th Annual Meeting, Porto, Portugal.

Brock, T. C. M., Roessink, I. 2006. Problems in interpreting in-situ bioassays with aquatic organisms to predict pesticide stress. In: Abstract book, SETAC Europe 16th Annual Meeting, The Hague, The Netherlands.

Moermond, C. T. A., Traas, T. P., Veltman, K., Roessink, I., Hendriks, A. J., Koelmans, A. A. 2006. Modelling food web bioaccumulation of sediment-bound PAHs and PCBs. In: Abstract book, SETAC Europe 16th Annual Meeting, The Hague, The Netherlands.

Roessink, I., Koelmans, A. A., Moermond, C. T. A. 2006. System structure affects PAH and PCB cycling in floodplain lakes. In: Abstract book, SETAC Europe 16th Annual Meeting, The Hague, The Netherlands.

Moermond, C. T. A., Roessink, I., Jonker, M. T. O., Meijer, T., Koelmans, A. A. 2005. Ageing affects PAH and PCB bioaccumulation in ecologically different model ecosystems. In: Abstract book, SETAC Europe 15th Annual Meeting, Lille, France.

Roessink, I., Moermond, C. T. A., Gillissen, F., Koelmans, A. A. 2005. System structure affects fate and cycling of PCBs and PAHs in model ecosystems. In: Abstract book, SETAC Europe 15th Annual Meeting, Lille, France.

Roessink I., Van Leeuwen, E., Crum, S. J. H., Belgers, J. D. M., Bransen, F., Gylstra, R., Van Wijngaarden, R. P. A., Van den Brink, P. J., Brock, T. C. M. 2004. Influence of sediment quality on the response and recovery of aquatic communities after treatment with the fungicide TriPhenylTin (TPT). In: Abstract book, SETAC Europe 14th Annual Meeting, Prague, Czechia.

Roessink I., Brock, T.C.M., Crum, S.J.H., Belgers, J.D.M., Van Leeuwen, E., Bransen, F. 2003. Impact of sediment quality on the response and recovery of zooplankton communities in microcosms after treatment with the fungicide TriPhenylTin (TPT). In: Abstract book, SETAC Europe 13th Annual Meeting, Hamburg, Germany.

Roessink, I., Arts, G. H. P., Maund, S. J. 2002. Impact of Lambda-cyhalothrin on the aquatic community of two ditches differing in trophic status. In: Abstract book, SETAC Europe 12th Annual Meeting, Vienna, Austria.

Roessink, I. 2001. Influence of sediment quality on the response and recovery of aquatic macroinvertebrates after treatment with the fungicide TriphenylTin. 13de Entomologendag, Amsterdam, The Netherlands.

Roessink, I., Van Wijngaarden R. P. A., Udoh, C. S., Maund, S. J. 2001. Impact of Lambda-cyhalothrin on the zooplankton in two ditches that differ in trophic status. In: Abstract book, SETAC Europe 11th Annual Meeting, Madrid, Spain.