The influence of aquatic vegetation on the fate and behavior of organic contaminants in small streams – Mesocosm and laboratory experiments

Der Einfluss aquatischer Makrophyten auf den Verbleib und das Verhalten organischer Schadstoffe in kleinen Fließgewässern – Untersuchungen im Mesokosmen- und Labormaßstab

von Diplom-Umweltwissenschaftler Christoph Stang
geboren am 10.07.1980 in Neunkirchen / Saar

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Universität Koblenz-Landau

Berichterstatter:
Prof. Dr. Ralf Schulz, Landau
Prof. Dr. Andreas Lorke, Landau

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1. Abstract

Aquatic macrophytes can contribute to the retention of organic contaminants in streams, whereas knowledge on the dynamics and the interaction of the determining processes is very limited. The objective of the present study was thus to assess how aquatic macrophytes influence the distribution and the fate of organic contaminants in small vegetated streams. In a first study that was performed in vegetated stream mesocosms, the peak reductions of five compounds were significantly higher in four vegetated stream mesocosms compared to a stream mesocosm without vegetation. Compound specific sorption to macrophytes was determined, the mass retention in the vegetated streams, however, did not explain the relationship between the mitigation of contaminant peaks and macrophyte coverage. A subsequent mesocosm study revealed that the mitigation of peak concentrations in the stream mesocosms was governed by two fundamentally different processes: dispersion and sorption. Again, the reductions of the peak concentrations of three different compounds were in the same order of magnitude in a sparsely and a densely vegetated stream mesocosm, respectively, but higher compared to an unvegetated stream mesocosm. The mitigation of the peak reduction in the sparsely vegetated stream mesocosm was found to be fostered by longitudinal dispersion as a result of the spatial distribution of the macrophytes in the aqueous phase. The peak reduction attributable to longitudinal dispersion was, however, reduced in the densely vegetated stream mesocosm, which was compensated by compound-specific but time-limited and reversible sorption to macrophytes. The observations on the reversibility of sorption processes were subsequently confirmed by laboratory experiments. The experiments revealed that sorption to macrophytes lead to compound specific elimination from the aqueous phase during the presence of transient contaminant peaks in streams. After all, these sorption processes were found to be fully reversible, which results in the release of the primarily adsorbed compounds, once the concentrations in the aqueous phase starts to decrease. Nevertheless, the results of the present thesis demonstrate that the processes governing the mitigation of contaminant loads in streams are fundamentally different to those already described for non-flowing systems. In addition, the present thesis provides knowledge on how the interaction of macrophyte-induced processes in streams contributes to mitigate loads of organic contaminants and the related risk for aquatic environments.

2. Zusammenfassung

Aquatische Makrophyten können auch in Fließgewässern einen wichtigen Beitrag zum Rückhalt von organischen Schadstoffen leisten, wenngleich die Kenntnisse zur Dynamik und zum Zusammenwirken der relevanten Prozesse sehr begrenzt sind. In einer ersten Studie in pflanzenbestandenen Fließgerinnemesokosmen wurde eine deutliche höhere Abnahme der Maximalkonzentrationen von fünf Substanzen in vier bewachsenen Fließgerinnen, im Vergleich zu einem unbewachsenen Kontrollgerinne beobachtet. Zwar wurde substanzspezifische Sorption an Pflanzen festgestellt, die alleinige Betrachtung dieses Prozesses erklärt jedoch nicht den Zusammenhang zwischen Abnahme der Maximalkonzentration und Bewuchsdichte. In einer zweiten Mesokosmenstudie zeigte sich, dass die Abnahme der Maximalkonzentration in den Mesokosmen im Wesentlichen vom Zusammenwirken zweier Prozesse abhängig war: Dispersion und Sorption. Die Abnahme der Maximalkonzentrationen von drei unterschiedlichen Substanzen in einem wenig und einem stark bewachsenen Fließgerinne war wiederum deutlich höher als in einem unbewachsenen Kontrollgerinne, bewegte sich jedoch in einer vergleichbaren Größenordnung. In dem wenig bewachsenen Fließgerinne war die Abnahme der Maximalkonzentration vor allem auf die räumliche Verteilung der Vegetation und der damit verbundenen Verstärkung der longitudinalen Dispersion zurückzuführen. Die longitudinale Dispersion in dem dicht bewachsenen Fließgerinne war aufgrund des Bewuchses weniger stark ausgeprägt, was wiederum durch dynamische, aber reversible Sorptionsprozesse ausgeglichen wurde. Diese Beobachtungen wurden durch weiterführende Untersuchungen im Labormaßstab bestätigt. Es zeigte sich, dass es durch aquatische Makrophyten während des vorübergehenden Auftretens von organischen Kontaminanten in Fließgewässern zunächst zu einer substanzspezifischen Elimination der Substanzen aus der Wasserphase kommt. Diese Sorptionsprozesse sind jedoch vollständig reversibel, wodurch die ursprünglich sorbierten Substanzen wieder freigesetzt werden, sobald die Konzentrationen in der Wasserphase beginnen zu sinken. Die Ergebnisse der vorliegenden Arbeit zeigen, dass sich die relevanten Prozesse, die zu einer Verringerung der Belastung mit organischen Kontaminanten in Fließgewässern beitragen, grundlegend von den bekannten Prozessen in stehenden Gewässern unterscheiden. Die Arbeit liefert zudem wichtige Erkenntnisse darüber, wie das Zusammenspiel pflanzeninduzierter Prozesse in Fließgewässern beitragen kann die Belastung mit organischen Kontaminanten und das damit verbundene Risiko für die Umwelt zu mindern.

3. General introduction

3.1 Introduction

The technological progress of the last two centuries is on the one hand associated with an increase of prosperity and progress in almost all aspects of life. On the other hand, those achievements were and still are accompanied by the occurrence of a multitude of environmental problems (Millennium Ecosystem Assessment, 2005). The intensification of land management, for example, ensured the food supply for a large proportion of the world´s population. However, as a part of the green revolution, this benefit is also associated with the intensive use of plant protection products to control a variety of different pests, such as insects, weeds or fungi, in order to minimize crop failure (Matson et al., 1997; Tilman et al., 2002). Similar developments can be observed with the prolonged advancement of hygiene standards and the related development of antimicrobial cleaning agents, personal care products (PCP) and items of daily use, respectively (Singer et al., 2002). However and irrespective of their fields of use, there is one aspect that all of these compounds have in common: they are all designed to have an action on or against harmful organisms (EC, 2012, 2009a). This means, in turn, that such compounds can pose a threat for the structure and functioning of non-target ecosystems if they are released to the environment, including surface waters (Schwarzenbach et al., 2006).

According to the envisaged use of such compounds, different pathways to the environment have been identified. Anti-microbial agents, such as triclosan or triclocarban, are commonly applied in PCPs such as toothpastes as well as in cleaning agents for surface disinfection in the medical sector (Halden and Paull, 2004; von der Ohe et al., 2012). Thus, these compounds are subsequently discharged directly into the drain system, from where they finally reach waste water treatment plants (WWTP) (Lozano et al., 2013; Waltman et al., 2006). However, many of these compounds are not completely degraded in WWTPs and hence subsequently released to surface waters (Bock et al., 2010; Halden and Paull, 2005). In addition, a variety of compounds ends up in the sewage sludge and may thus enter the environment, when sewage sludge is applied as manure to agricultural areas (McClellan and Halden, 2010; Wick et al., 2010). Plant protection products are, in turn, directly applied to agriculturally used areas. Hence, these compounds can enter non-target ecosystems, such as

surface waters, during or subsequent to the field application by a multitude of pathways like spray-drift, drainage, erosion, leaching or edge-of-field runoff (Reichenberger et al., 2007; Schulz, 2004). As a result, the detection of multiple pesticides in surface waters increased in recent years raising concerns on the integrity of aquatic ecosystems (Liess and Von Der Ohe, 2005; Schäfer et al., 2011, 2007). Hence, a variety of measures and technologies have been developed to mitigate risks for the integrity of the environment resulting from this development. In the field of waste water treatment, many technical approaches and additional treatment stages were proposed to improve the purification performance of WWTPs (EPA, 2013). Also in agriculture, numerous efforts have been made to develop on-site measures to mitigate the potential risks for non-target ecosystems as a result of the application of pesticides. These measures, generally denoted as best management practices (BMP), encompass precautionary measures, such as the observance of safety margins to non-target ecosystems during pesticide application as well as the development of improved application techniques to minimize, for instance, spray drift (EC, 2009b). Beyond that, riparian buffer strips (Ohliger and Schulz, 2010; Reichenberger et al., 2007) and constructed wetlands (CW), in particular vegetated treatment systems (VTS), have been proposed as effective BMPs to mitigate the load of organic contaminants in receiving waters (Gregoire et al., 2008; Reichenberger et al., 2007; Stehle et al., 2011; Vymazal and Březinová, 2015). Vegetated treatment systems are characterized by the targeted use of aquatic vegetation to mitigate contaminant loads in receiving waters. Historically, VTSs were predominantly used for waste water treatment (Vymazal, 2010) or to mitigate loads of nutrients, heavy metals or sediments in receiving waters, respectively (Kadlec and Wallace, 2009). As a result, the processes determining the retention of nitrogen and phosphorous as well as heavy metals are well understood (Kadlec and Wallace, 2009).

However, in the last two decades the viability of VTSs for the mitigation of organic pollutants has received increased attention (Gregoire et al., 2008; Vymazal and Březinová, 2015). Hence, a variety of different VTSs, e.g. vegetated drainage ditches, vegetated wetlands or vegetated streams, and their ability to mitigate the load of organic contaminants have been investigated. Among these systems, the efficiency of vegetated drainage ditches has been intensively studied. Several studies by Moore et al. (Moore, 2008; Moore et al., 2009, 2001) revealed that vegetated drainage ditches were effective in reducing the load of different pesticides. For instance, Bennett et al. (2005) demonstrated the suitability of vegetated

agricultural drainage ditches to mitigate concentrations of the insecticides lambda-cyhalothrin and bifenthrin after a simulated runoff event and identified ditch plants to be a major sink for these compounds. Besides vegetated drainage ditches, the efficiency of vegetated wetlands on the reduction of pesticide concentrations was also demonstrated. Schulz and Peall (2001) observed retentions of water diluted azinphos-methyl between 77% and 93% in a vegetated wetland in South Africa. In a study in two constructed wetlands in Norway (Blankenberg et al., 2006), mass retention rates of up to 67% were determined for a variety of pesticide. Beyond that, the beneficial influence of vegetated wetlands on the reduction of pesticide related effects has also been demonstrated. Schulz et al. (2003b) assessed that the toxicity of methyl parathion was reduced in vegetated compared to unvegetated wetlands, respectively. Similar observations were made by (Milam et al., 2004), who also found reduced toxic effects on the aquatic biota in vegetated constructed wetlands. However, all of these studies linked the reduction of pesticide loads to mass retention processes within the investigated systems mainly driven by sorption of the compounds to aquatic vegetation. A meta-analysis by Stehle et al. (2011) demonstrated the influence of macrophyte coverage and the HRT in combination with the compounds organic carbon-water partitioning coefficient (K_{OC}) on the retention performance of VTSs. According to Imfeld et al. (2009), sorption to macrophytes enhances the residence time of the compounds in VTSs and thus increases the available time for degradation processes (e.g., photolysis and microbial degradation) to occur. Indeed, the ability of aquatic vegetation to eliminate pesticides from the aqueous phase was shown in several studies on different experimental scales. Besides the above described studies that were performed on the field or mesocosm scale, different laboratory (Crum et al., 1999; Olette et al., 2008) and microcosm studies (Bouldin et al., 2005) also demonstrated the ability of aquatic macrophytes to mitigate loads of organic pollutants. A study by Hand et al. (2001) found, for example, rapid adsorption of the insecticide lambda-cyhalothrin to aquatic plants in a laboratory experiment. However, all of the above mentioned studies made no statements regarding the durability of sorption, or described sorption to macrophytes to be consistent over time, while there are only a few studies available that highlighted and described the persistence of sorption processes. Passeport et al. (2011) determined high coefficients for pesticides adsorption to wetland plants and forest litter followed by compound related desorption. Similar observations were made in a mesocosm experiment by Moore et al.

(2013), where the authors concluded that plant sorption is at least partially reversible, since 19 to 29% and 8 to 16% of the initially retained atrazine and diazinon loads, respectively, were released after the mesocosms were flushed with clean water six hours after a simulated runoff event.

Nevertheless, the findings on the persistence of sorption processes may hold true for rather static systems with negligible flow velocities and thus long HRTs of up to days or weeks. However, vegetated streams are in turn characterized by dynamic discharge regimes resulting in comparably short HRTs. As a result, vegetated streams are mainly characterized by the occurrence of transient exposure peaks accompanied with a dynamic increase and decrease of contaminant concentrations in the aqueous phase, as it may occur, for instance, during an edge-of-field runoff event. Thus, the temporal scale on which sorption processes occur is limited and knowledge on the dynamics as well as on the persistence of sorption processes under these conditions is scarce. There are only a few field studies that investigated the suitability of vegetated streams, whereas these studies rather aimed to assess the suitability of vegetated streams on the mitigation of pesticide concentrations than to gain a deeper understanding of the dominating processes influencing mitigation. Dabrowski et al. (2006) reported reductions of azinphos-methyl concentrations of 61 to 90% in a vegetated tributary of the Lourens River, South Africa, after a spray drift and a runoff event. A concentration reduction of 90% in a flow-through wetland in South Africa was reported also for azinphos-methyl, whereas only 10.5% of the overall mass retention of 61% was attributed to sorption to macrophytes (Schulz et al., 2003a). In addition, Elsaesser et al. (2011) found merely 5% of experimentally applied pesticides adsorbed to macrophytes in the Lier wetland, Norway, and thus concluded that the increase of peak reductions in the vegetated wetland cells may have been a result of vegetation induced dispersion processes.

The conclusions drawn by Elsaesser et al. (2011) are indeed crucial, considering the influence of aquatic macrophytes on the mitigation of contaminant concentrations in vegetated streams. As already mentioned above, vegetated streams are characterized as flow-through systems with a dynamic discharge regime. Hence, processes besides sorption to macrophytes and their influence on the mitigation of contaminant peak concentrations need to be considered. According to Sukhodolov and Sukhodolova (2012), aquatic vegetation has a significant impact on the hydraulic conditions and the related mixing or retention processes in streams, respectively. The presence of aquatic macrophytes can, on the one

hand, enhance turbulence and thus longitudinal dispersion (Nepf, 2012). On the other hand, aquatic macrophytes can promote the formation of dead zones resulting in transient storage of solutes (Choi et al., 2000). Considering the occurrence of these processes in vegetated streams, the macrophytes morphology (Albayrak et al., 2011) as well as the vegetation density and the related spatial distribution in the aqueous phase are of particular importance (Nepf et al., 2007; Shucksmith et al., 2011).

3.2 Thesis objectives

The thesis aimed to assess how aquatic vegetation influences the distribution and the fate of organic contaminants in aquatic ecosystems with special attention to vegetated streams (Figure 3.1). This is of special interest, since vegetated streams have been proposed as a suitable risk mitigation measure (RMM) to reduce the load of contaminants with environmental concern in surface waters, especially in areas where the installation of constructed VTSs is not feasible. However, the suitability of vegetated streams for this particular purpose has been demonstrated (Dabrowski et al., 2006; Elsaesser et al., 2011; Schulz et al., 2003a), whereas knowledge on the conjoint interaction of macrophyte-induced processes in vegetated streams is limited. Hence, this thesis comprises three experimental approaches to uncover and quantify the major processes that govern the mitigation of organic contaminants in such systems.

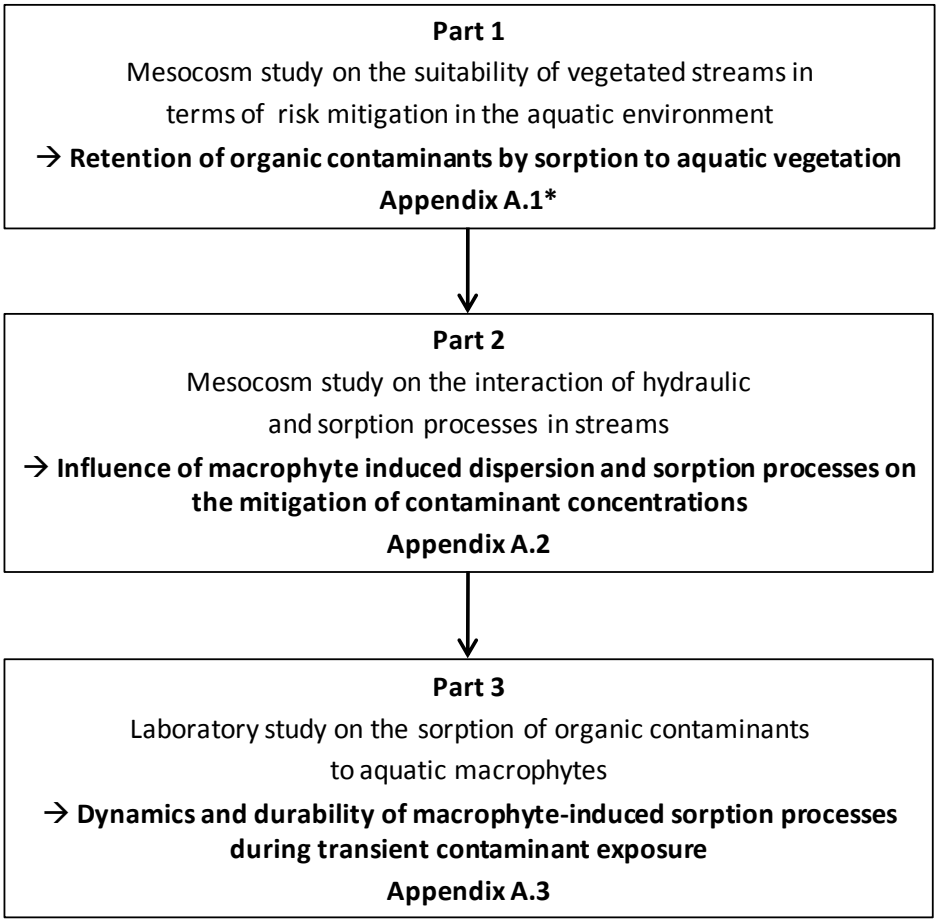

Figure 3.1 Flowchart on the coherence of the introduced publications (**Appendix A.1 – A.3**) with regard to the thesis´ objectives.

* According to §7 (6) of the doctoral degree regulations, I hereby declare that the data on the aqueous concentrations in **Appendix A.1** were already described in my diploma thesis (Stang, 2010).

3.3 Thesis outline

Part 1 – Suitability of vegetated streams for the mitigation of contaminant loads

The first part of the thesis (Appendix A.1) represents an experimental study in vegetated stream mesocosms. The study aimed to gain basic knowledge on the influence of aquatic vegetation and the magnitude of the retention of organic contaminants in small, flowing vegetated streams during peak exposure, as it may occur, for instance, during edge-of-field run-off events. For this purpose, four out of five stream mesocosms were planted with varying densities of the submerged macrophyte *Elodea nuttallii*, while one stream mesocosm remained without vegetation. After the experimental application of two biocidal and three fungicidal compounds covering a wide range of physico-chemical properties, aqueous, sediment and macrophyte samples, respectively, were taken at the inlet and the outlet of each stream. The chemical analyses of these samples aimed to reveal a compound-specific retention by sorption to macrophytes or to the sediment.

Part 2 – Identification and quantification of major retention processes in vegetated streams

A second mesocosm study (Appendix A.2) was conducted to assess the role of submerged vegetation and its density-related spatial distribution on the mitigation of pesticide peak concentrations and the mass retention of organic contaminants in such systems. The mesocosm study was conducted in three vegetated stream mesocosms of which one mesocosm remained free of vegetation, while the two remaining mesocosms were densely and sparsely covered by macrophytes, respectively (Figure 3.2).

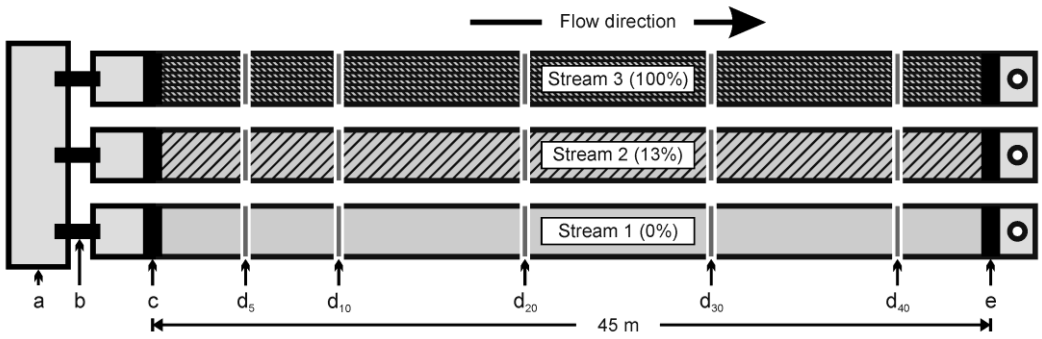

Figure 3.1 Schematic outline of the three vegetated stream mesocosms differing in vegetation density (%). (a) Water reservoir; (b) transfer pipe; (c) stream inlet with spillway and point of application; (d_5-d_{40}) sampling sites at 5, 10, 20, 30 and 40 m; (e) spillway to outlet. **[Appendix A.2]**

In a first experimental phase, the conservative tracer uranine was experimentally applied to the stream mesocosms to determine the hydraulic conditions in each stream mesocosm and to establish the appropriate sampling times for the second experimental phase. The second experimental phase entailed separate dosing events of three pesticides to the stream mesocosms at an interval of eight days. Subsequent to each pesticide application, samples of all relevant matrices (water, macrophyte and sediment) were taken according to the previously determined sampling protocol aiming on the traceability of peak curves in the aqueous phase and the concentration gradients in macrophyte as well as sediment samples.
On the basis of the measured tracer data, the longitudinal dispersion coefficient and the influence of transient storage by dead zones in each stream mesocosm were estimated using the non-linear least square fit routine OTIS-P, an extension of the one-dimensional solute transport model for streams and rivers (OTIS; Runkel and Broshears, 1998). This average dispersion coefficient was finally used to model the pesticide concentration peaks solely attributable to longitudinal dispersion at the outlet of each stream mesocosm with OTIS. The comparison of the modeled and the measured peak concentrations, respectively, facilitated to separate the effect of hydraulic and mass retention processes on the decrease of the pesticide concentrations. Furthermore, initial and overall mass recovery rates were determined on the basis of the measured pesticide concentrations in aqueous, macrophyte and sediment samples. The mass recovery rates enabled to assess the dynamics as well as the magnitude and the persistence of the compound-specific sorption processes and thus the retention of the pesticides within the stream mesocosms.

Part 3 – Assessment of pesticide sorption dynamics during peak exposure

Both mesocosm studies revealed that compound-specific sorption to macrophytes constitutes an important process in the elimination of organic contaminants from the aqueous phase and thus the retention of these compounds in vegetated streams. However, the results of the second mesocosm experiment indicated that sorption to macrophytes is a highly dynamic and reversible process during transient peak exposure.
Hence, a laboratory study in water-macrophyte systems that aimed at the investigation of the dynamics and the persistence of sorption processes during a pesticide peak exposure scenario represents the third part of this thesis. This study encompassed two experimental approaches. In a static long-term experiment, macrophytes were exposed to five different

pesticides over a period of 48 hours to gain fundamental insights on the dissipation dynamics of these compounds from the aqueous phase in the presence of aquatic macrophytes. In addition, the study entailed a semi-static short-term exposure scenario, where a peak exposure scenario was simulated to assess the consistency of sorption and the dynamics of desorption processes, respectively. A correlation analysis based on the results of the semi-static short-term exposure scenario was performed to assess the best match of the experimentally derived sorption of pesticides with one out of three coefficients proposed in the scientific literature.

4. General discussion

4.1 Influence of aquatic vegetation on the reduction of contaminant peaks

The findings of both mesocosm-studies that are part of this thesis, revealed the beneficial influence of aquatic vegetation on the mitigation of contaminant concentrations in the aqueous phase. In the first mesocosm experiment, the peak reductions were generally higher in the vegetated stream mesocosms compared to the unvegetated stream. In the unvegetated stream, the peak reductions ranged from 48 to 71%, while peak reductions of 59 to 94% were assessed in the vegetated streams. The observed peak reductions in the vegetated streams were thus higher by 20 to 25% compared to the peak reductions in the unvegetated stream. However, no correlation (R^2 = 0.101; p < 0.672; n = 20) between the peak reduction and the macrophyte density in the vegetated streams was found in this study. These observations revealed the necessity to reassess the primary assumption that the peak reduction is mainly driven by the retention of organic contaminants within these systems. Based on the described observation and the findings of a variety of studies dealing with the alteration of hydraulic conditions in streams and wetlands by aquatic vegetation (Albayrak et al., 2011; Nepf and Vivoni, 2000; Sukhodolov and Sukhodolova, 2012; Sukhodolova and Sukhodolov, 2012), it was reasoned that these processes need to be included into further considerations. Hence, the second mesocosm experiment aimed to jointly investigate sorption and hydraulic processes, such as longitudinal dispersion or transient storage by dead zones, to obtain a deeper understanding on how the reduction of contaminant peaks is promoted by these fundamentally different processes. Indeed, the tracer experiment revealed that the peak reductions of the conservative tracer uranine were generally higher in the vegetated streams, whereas the highest peak reduction was observed in the sparsely vegetated stream mesocosm (Figure 4.1 a). The parameter estimation with OTIS-P identified longitudinal dispersion to be the dominant process influencing the peak reduction of the tracer, while transient storage was found to be negligible in the stream mesocosms. The dispersion coefficient was lower in the densely vegetated stream (D = 0.20±0.03 dm^2/s) compared to the sparsely vegetated stream (D = 0.55±0.19 dm^2/s). Due to these finding, it was assumed that the spatial distribution of the macrophytes in the

water column determined the longitudinal dispersion and thus the peak reduction of the tracer in the vegetated stream mesocosms. While the macrophytes in the sparsely vegetated stream only protruded to the middle of the water column, the water column in the densely vegetated stream was abundantly covered with macrophytes (Figure 4.1).

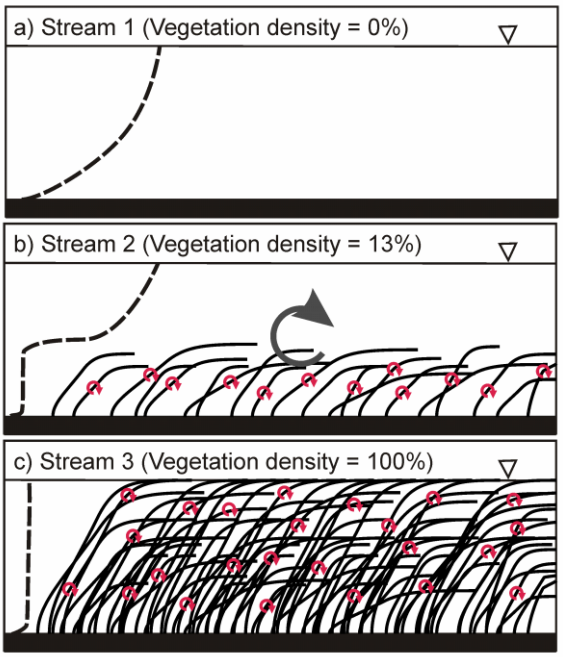

Figure 4.1 (a–c) Schematic outline of the vegetation distribution in the three stream mesocosms and the influence of the vegetation on the flow velocities and turbulence within the streams. Dashed lines represent the vertical distribution of the flow velocities, red circlets represent the stem and leaf wake turbulence in the vegetated stream 2 and 3, grey circle represents vertical exchange zone turbulence, and inverted triangles represent the water surface. The principle design of the figure was described by Nepf and Vivoni (2000). **[Appendix A.2]**

According to the findings of Nepf and Vivoni (2000), a distribution of vegetation as described for the sparsely vegetated stream amplifies dispersion by stem wake turbulence and by vertical exchange turbulence as a result of the formation of two flow zones (Figure 4.1b). However, in streams where the vegetation extends into the entire water column dispersion is merely amplified by stem wake turbulence (Figure 4.1c).

Nevertheless, the peak reduction patterns that were determined as a result of the subsequent application of pesticides partly differed from those observed during the tracer experiment. While the peak reduction patterns in the unvegetated and the sparsely vegetated stream, respectively, were similar to those observed during the tracer experiment, the mitigation of the maximum concentrations of the applied pesticides was considerably higher and also differed between the investigated compounds (Figure 4.2 b-d).

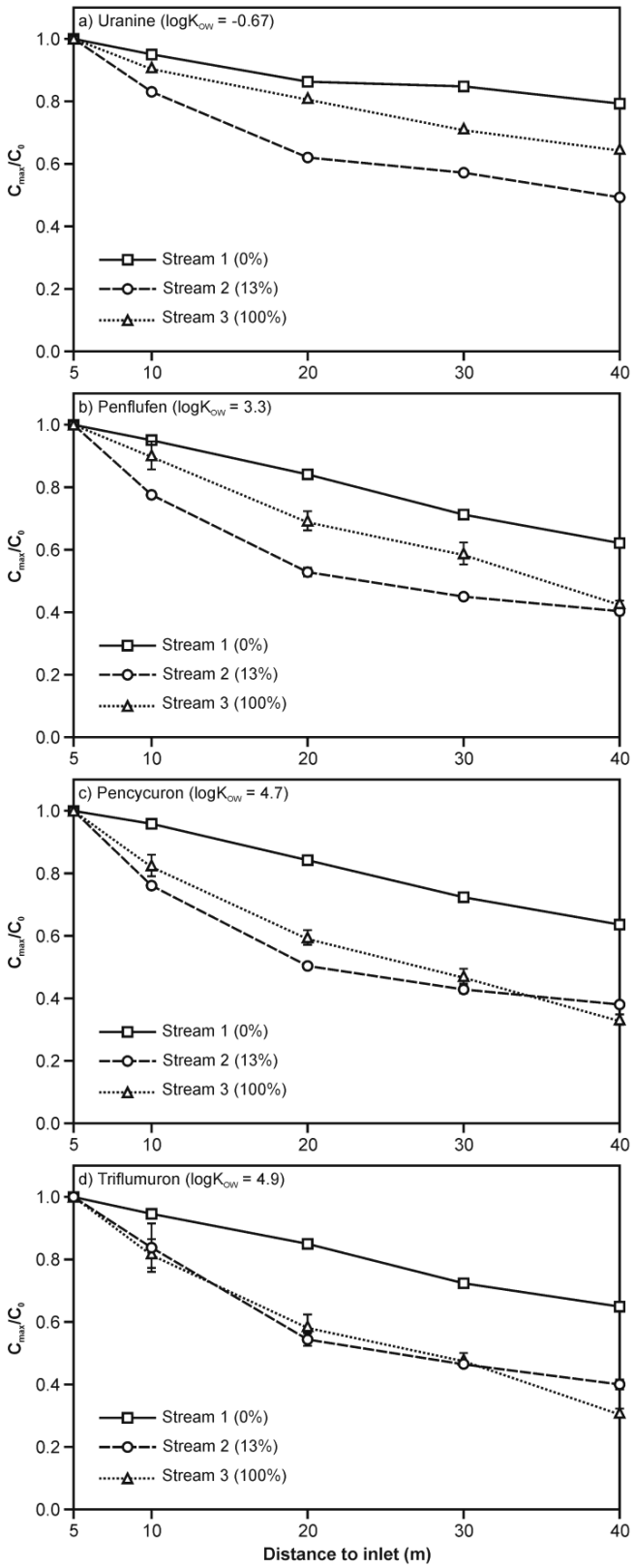

Figure 4.2 (a–d) Decline in maximum peak concentrations of the tracer and all three plant protection products in stream mesocosms differing in vegetation density (%) with increasing distance to the inlet. Error bars display the difference in maximum concentrations of both pesticide-applications (n = 2). **[Appendix A.2]**

The modeling of the maximum peak concentrations as they would have been expected at the outlet sampling sites of each stream mesocosm, if longitudinal dispersion displayed the only process governing the degree of concentration mitigation, revealed the co-occurrence of at least one additional and compound-specific process that determined the peak reduction in the vegetated streams (Figure 4.3).

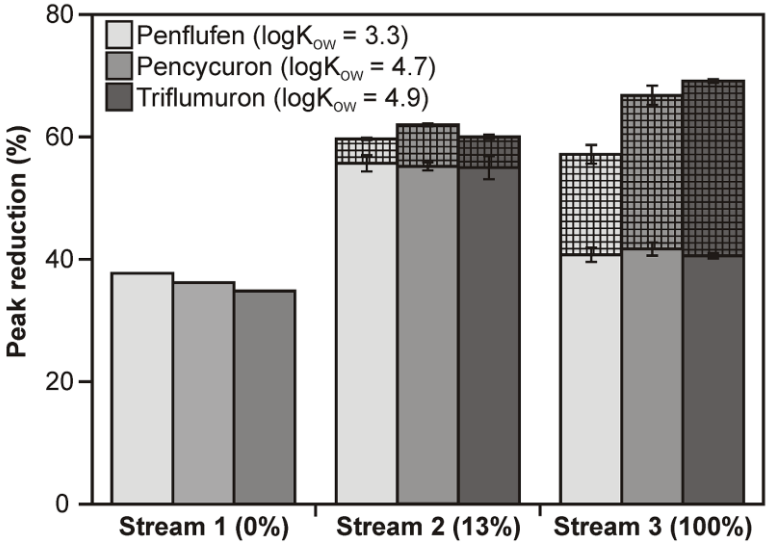

Figure 4.3 Contributions of dispersion and reversible sorption to macrophytes on the peak reduction in all stream mesocosms. Open bar sections represent dispersion and shaded bar sections represent sorption. Error bars represent the difference between the fractions of both PPP applications (n = 2). **[Appendix A.2]**

4.2 Mass retention of organic contaminants in vegetated stream mesocosms

The analyses of macrophyte and sediment samples from both mesocosm experiments revealed compound specific mass retentions by macrophytes, whereas the sediment compartment was found to be of negligible importance for mass retention processes.

In the first mesocosms study, average mass retentions of the applied compounds ranged from 6±4% to 81±1% in the vegetated streams. Mass balance calculations clearly indicated a compound-specific retention of the investigated compounds by macrophytes (Figure 4.4), which may thus have contributed to an additional increase of the peak reduction in the vegetated streams. However, due to methodological limitations, no quantitative assessment on the influence of the mass retention on the effectively measured peak reductions could be derived in the first mesocosm study.

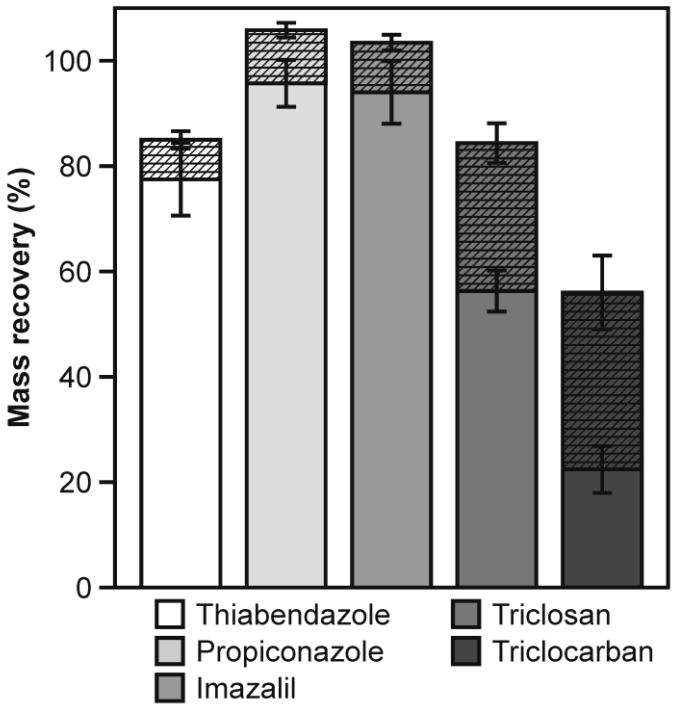

Figure 4.4 Average (±SE) mass recoveries (REC) of the applied biocides and fungicides in the vegetated stream mesocosms 2 – 5. Plain bars (including the lower error bars) represent the proportion of the applied amount of biocides that remained in the aqueous phase; shaded bars (including the upper error bars) represent the proportion that was found in the macrophytes. Error bars represent the standard error of the mass recoveries in the examined matrices of the vegetated stream mesocosms 2 – 5. **[Appendix A.1]**

Nevertheless, this knowledge gap could be closed by examining the data derived from the second mesocosm study. The results of the mass balances from this study confirmed the above drawn conclusion on the influence of a co-occurring process on the degree of the peak reduction, especially in the densely vegetated stream. In this stream, compound-specific initial mass retentions by macrophytes between 7.9±0.1% and 27.0±2.0% were determined (Table 1). These findings correspond to the amount of the compounds that were found to be remained in the aqueous phase (75.1±0.1% to 96.9±0.4%) at the stream mesocosms outlet (Table 1). Since the observed mass retentions followed the same pattern as the observed peak reductions in this stream mesocosm, it was concluded that the initial mass retention by sorption of the applied pesticides to macrophytes was the second prominent processes determining the peak reduction in the vegetated streams.

Table 4.1 Initial mass recovery rate (0.5 h after the passage of the peak) in the unvegetated stream 1 and mean initial mass recovery rates in the vegetated streams 2 and 3 (±min/max; n = 2); mean overall mass recovery rates (six hours after the start of the application) in the densely vegetated stream 3 (±min/max; n = 2). **[Appendix A.2]**

			Triflumuron	Pencycuron	Penflufen
Initial mass recovery (%)	Stream 1 (0%)	Aqueous phase	99.4	100.9	99.2
	Stream 2 (13%)	Aqueous phase	91.4 (±2.2)	92.9 (±2.8)	96.0 (±1.7)
		Macrophytes	3.1 (±0.2)	2.5 (±0.3)	1.1 (±0.1)
		Total recovery	94.5	95.3	97.1
	Stream 3 (100%)	Aqueous phase	75.1 (±0.1)	81.7 (±0.2)	96.9 (±0.4)
		Macrophytes	27.0 (2.0)	16.5 (±2.2)	7.9 (±0.1)
		Total recovery	102.2	99.0	104.4
Overall mass recovery (%)	Stream 3 (100%)	Aqueous phase	95.9 (±8.0)	108.0 (±0.4)	104.0 (±4.5)
		Macrophytes	1.2 (±0.1)	0.7 (±0.0)	0.0 (±0.0)
		Total recovery	97.2	108.7	104.0

4.3 Sorption/Desorption dynamics

The results of both mesocosm studies clearly demonstrated that sorption of the investigated compounds to macrophytes, besides longitudinal dispersion, is a crucial but compound-specific process in the retention, and thus the mitigation of contaminant peaks, in vegetated streams as a function of macrophyte density.

Nevertheless, the overall mass recovery rates in the densely vegetated stream indicated that the originally retained pesticide amounts were almost entirely desorbed from the macrophytes resulting in a simultaneous increase of the mass recovery rates in the aqueous phase (Table 1). The analyses of the macrophyte samples taken in the vegetated streams confirmed this observation and illustrated the dynamics of sorption as well as of desorption of the pesticides over time, respectively (Figure 4.5). The concentrations of each of the pesticides in the macrophyte samples increased rapidly, indicating sorption with the increase

of their concentration in the aqueous phase. However, with the decrease of the aqueous pesticide concentrations, a speedy, but slightly time-delayed decrease of the pesticides in the macrophytes was observed, which in turn indicates desorption from the macrophytes.

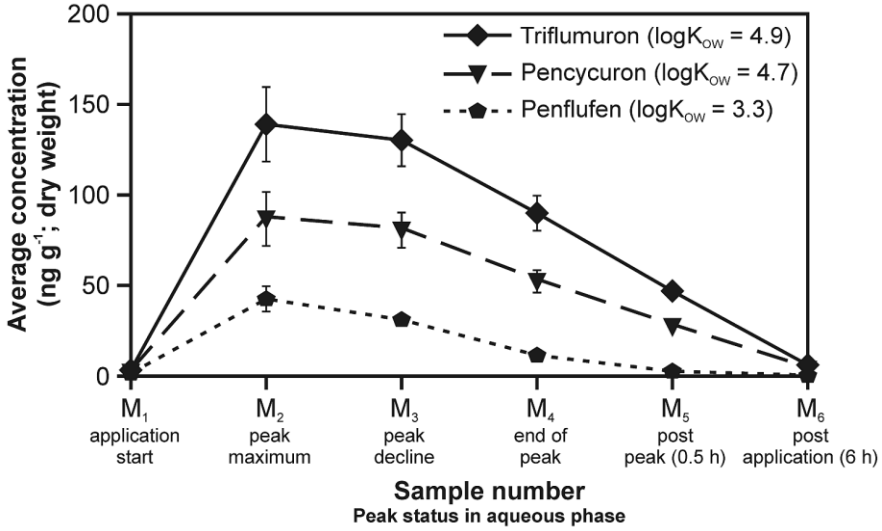

Figure 4.5 Average concentrations of penflufen, pencycuron and triflumuron in macrophyte samples taken from the densely vegetated stream 3 within six hours after the start of each application. Error bars display the standard error of the average PPP-concentrations (n = 10). **[Appendix A.2]**

Hence, a laboratory study, which constitutes the third part of this thesis, in water-macrophyte systems was conducted to reassess the sorption and desorption dynamics that were observed within the framework of the second mesocosm study. As part of this study, the semi-static short-term approach aimed on the simulation of a pesticide peak exposure to assess the compound-specific sorption dynamics of five pesticides to three aquatic macrophyte species, on the one hand, and the desorption dynamics that may occur once the macrophytes were transferred to uncontaminated medium, on the other hand. Within the exposure period of two hours, the average decrease of the investigated pesticides in the aqueous phase ranged from 4.6±3.7% to 69.4±7.0%, while the pesticide concentrations in the macrophytes increased proportionally (Figure 4.6). After the macrophytes were transferred to the uncontaminated medium, the aqueous concentration increased rapidly, accompanied by a closely coupled decrease of the pesticide concentration in the macrophytes (Figure 4.6). Thus, the results of the semi-static laboratory study are similar to the finding of the second mesocosm study. The results support the assumption, that sorption during transient peak exposure constitutes a dynamic but reversible process where a concentration decrease in the aqueous phase induces immediate desorption to obtain a proportioned equilibrium between both phases.

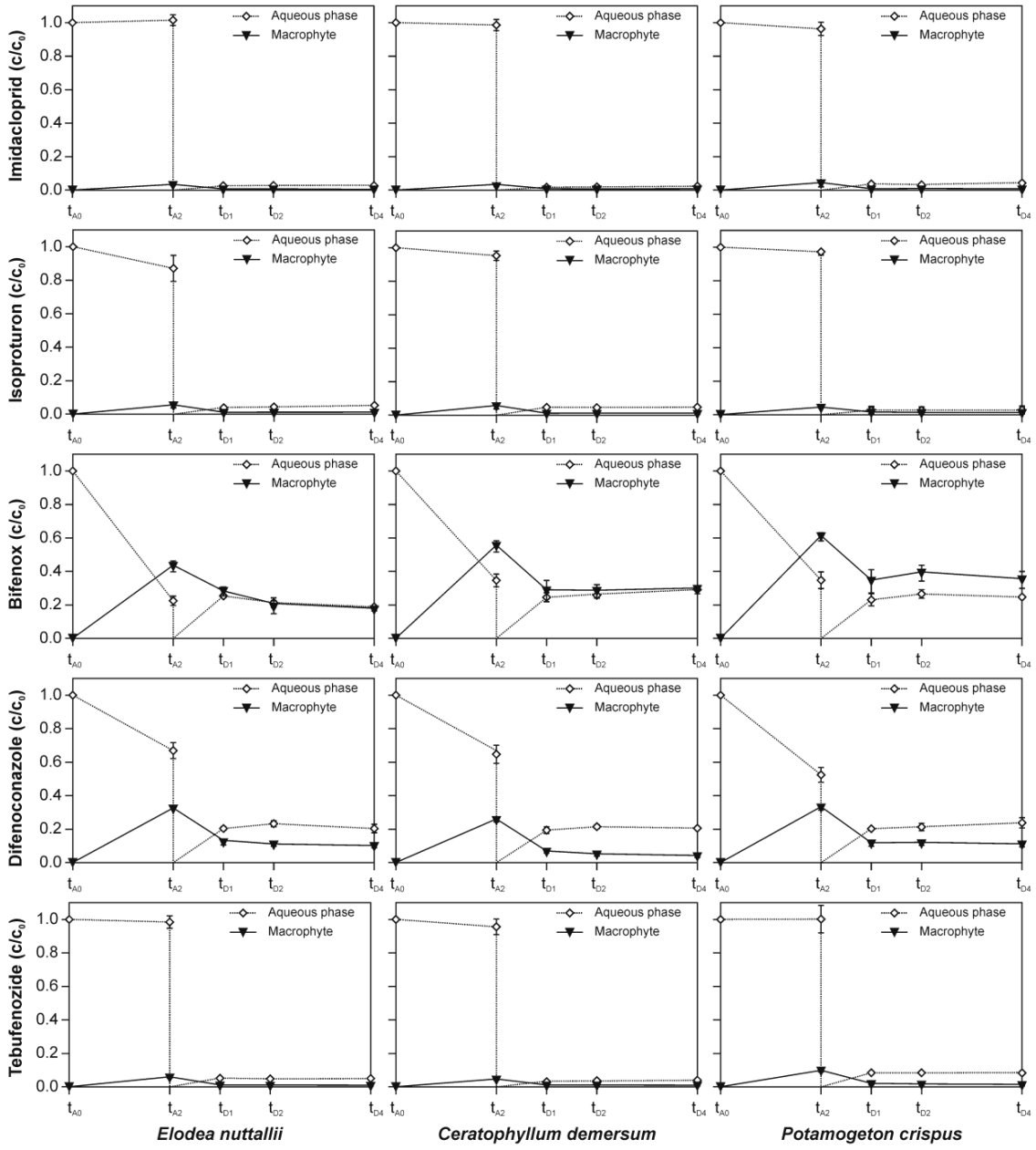

Figure 4.6 Temporal gradient of the pesticide concentration (Mean±SD) in the aqueous phase and in macrophytes. Dotted lines display the concentration in the aqueous phase, continuous lines display the concentration in macrophytes; tA0 = start of the experiment, tA2 = end of the sorption period after 2 hours and transfer of macrophytes into uncontaminated medium, tD1, D2, D4 = time (D1 = one hour, D2 = two hours, D4 = four hours) after the transfer of the macrophytes to uncontaminated medium. **[Appendix A.3]**

A correlation analysis was performed to determine the relationship between the adsorption to macrophytes and the octanol-water partitioning coefficient ($logK_{OW}$), the soil organic carbon-water partitioning coefficient ($logK_{OC}$) and a mathematically derived sorption coefficient ($logK_{D\_math}$) of the investigated compounds The correlation analysis revealed that the $logK_{OC}$ had the best predictive power (R^2 = 0.842; ρ < 0.01; n = 135) of the consulted coefficients to describe the compounds affinity to adsorb to macrophytes (Figure 4.7).

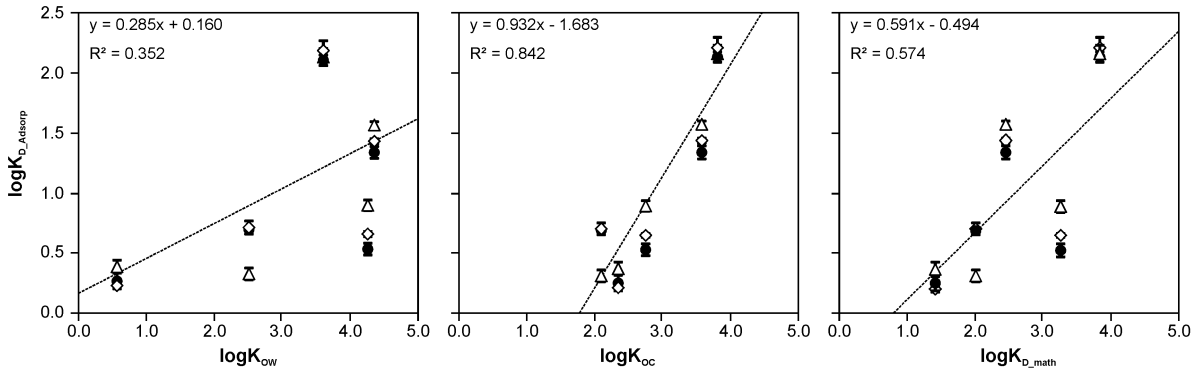

Figure 4.7 Correlation of the experimentally derived $logK_{D\_exp}$ (n = 135; ρ = 0.01) of the investigated compounds at the end of the adsorption period in treatments containing *E. nuttallii* (open diamonds), *C. demersum* (black circles) and *P. crispus* (open triangles) with different physico-chemical properties of the investigated pesticides ($logK_{OW}$, $logK_{OC}$ and $logK_{D\_math}$); for a better presentation each diamond, circle or triangle displays the logKD_exp as the mean±SD (n = 9) per pesticide. **[Appendix A.3]**

5. Conclusions and outlook

The results of the present thesis emphasize that small vegetated streams can represent suitable tools to mitigate transient peaks of organic contaminants. This is certainly not a new insight, since there were a few studies available which have already investigated the suitability of vegetated streams as risk mitigation measures. However, these studies attributed the mitigation of contaminant loads almost exclusively to sorption of the investigated compounds to aquatic macrophytes (Dabrowski et al., 2006; Elsaesser et al., 2011; Schulz et al., 2003a) and did not highlight the potential occurrence of other macrophyte induced processes. In contrast to these studies, the present thesis identified the role of aquatic vegetation in determining two fundamentally different processes whose interaction can promote the mitigation of contaminant loads in vegetated streams. On the one hand, the results of the present thesis demonstrate that aquatic vegetation amplifies dispersion processes in streams, which accelerate the decrease of maximum concentrations regardless of the compounds' physico-chemical properties. On the other hand, compound-specific retention processes by sorption to aquatic macrophytes were found to additionally contribute to the reduction of contaminant peaks in vegetated streams. This is of particular importance, since the extent of the initial mass retention by aquatic macrophytes was, however, inverted compared to the mitigation of contaminant peaks as a result of dispersion. In addition, sorption to macrophytes during short-term exposure, which is characteristic for the occurrence of organic contaminants in streams, was found to be a highly dynamic but reversible process. In other words, compound specific retention by aquatic macrophytes was shown to be immediately initiated when the concentration of organic contaminants starts to increase in the aqueous phase. The decrease of the contaminant concentrations in the aqueous phase, however, induces desorption resulting in the subsequent and also rapid release of the initially adsorbed compounds back to the aqueous phase. Hence, it would be misconceiving to compare the retention of organic contaminants in vegetated streams with the classical approach of phytoremediation in static VTSs, such as vegetated wetlands or drainage ditches, where it is assumed that sorption of organic contaminants leads to the uptake and the transformation of contaminants in aquatic macrophytes. The results of the present thesis rather demonstrate that aquatic macrophytes

can represent a temporary sink for organic contaminants in streams. Nevertheless, it can be concluded that the conjoint interaction of macrophyte-induced hydraulic and sorption processes, respectively, accelerates the decrease of organic contaminant loads in small streams and thus contributes to mitigate the risk for aquatic ecosystems to be adversely affected by such compounds.

Consequently, the present thesis contributes to improve the knowledge on how aquatic vegetation influences the fate and behavior of organic contaminants in small streams and can thus build the foundation for future research in at least two different areas of environmental sciences. First, the findings of this thesis can be used to develop improved risk mitigation strategies in areas where the installation of constructed VTSs is not feasible. Beyond that, the results of this thesis can help to refine existing modeling approaches as they are currently used to assess the fate and behavior of, for instance, plant protection or biocidal products, respectively, in the environment.

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7. Appendix

A.1 Mitigation of biocide and fungicide concentrations in flow-through vegetated stream mesocosms

Christoph Stang[1], David Elsaesser[1], Mirco Bundschuh[1,2], Thomas A. Ternes[3] and Ralf Schulz[1]

[1]Institute for Environmental Sciences, University of Koblenz-Landau, Im Fort 7, 76829 Landau, Germany

[2]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Lennart Hjelms vag 9, 750 07 Uppsala, Sweden

[3]Federal Institute of Hydrology (BfG), Am Mainzer Tor 1, 56068 Koblenz, Germany

Abstract

Organic chemicals entering surface waters may interact with aquatic macrophytes, which in turn may reduce potential negative effects on aquatic organisms. The overall objective of the present study was to determine the significance of aquatic macrophytes to the retention of organic chemicals in slow-flowing streams and thus their contribution to the mitigation of the risks that these compounds may pose to aquatic ecosystems. Hence, we conducted a study on the mitigation of the biocides triclosan and triclocarban and the fungicides imazalil, propiconazole and thiabendazole, which were experimentally spiked to five flow-through stream mesocosms (45 m length, 0.4 m width, 0.26 m water depth, discharge 1 L/s), four of which were planted with the submerged macrophyte *Elodea nuttallii* (Planch). Chemical analyses were performed using liquid chromatography-tandem mass spectrometry following solid-phase extraction for water samples and accelerated solvent extraction for macrophyte and sediment samples. The peak reductions of biocide and fungicide concentrations from the inlet to the outlet sampling sites were ≥48% in all stream mesocosms, and the peak reductions in the vegetated stream mesocosms were 20 to 25% greater than in the unvegetated mesocosm. On average, 7±3 to 10±3% and 28±8 to 34±14% of the initially applied amount of fungicides and biocides, respectively, were retained by macrophytes. There was a significant correlation between retention by macrophytes and the lipophility of the compounds.

Introduction

Detection of antimicrobial agents in aquatic ecosystems has occurred consistently over the last decade (Kolpin et al., 2002; Wick et al., 2010), primarily because of the broad application range of the identified agents. Biocides such as triclosan and triclocarban are widely used as disinfectants in personal care products, such as toothpastes and creams, as well as in other items of daily use (Singer et al., 2002). These biocides eventually enter wastewater treatment plants (WWTPs), where they are not completely degraded and are thus subsequently released to surface waters (Halden and Paull, 2005).

Fungicides, in contrast, are commonly used as plant protection products and hence applied directly to agricultural areas. Thus, their main pathways to surface waters are spray drift or edge-of-field runoff (Zaring, 1996); however, trace concentrations (ng/L) of fungicides have also been detected in WWTP effluents (Kahle et al., 2008). Information on the occurrence and fate of fungicides in the aquatic environment is limited. Kreuger (1998) detected two fungicides in an agricultural catchment in Sweden, with propiconazole having the higher concentration (2.8 mg/L). A variety of other fungicides have been detected at total concentrations of up to 16.4 mg/L per sample in streams flowing through vineyard areas in Germany (Bereswill et al., 2012) and France (Rabiet et al., 2010). Nevertheless, Castillo et al. (2000) found mean concentrations of 74 mg/L for thiabendazole and 126 mg/L for imazalil in streams draining banana plantations in Costa Rica, concentrations up to one order of magnitude greater than those reported in Europe.

Vegetated treatment systems (VTSs), such as constructed wetlands or vegetated streams and ditches, have been evaluated as on-site measures to mitigate the exposure levels of potentially toxic chemicals and hence the risk for aquatic ecosystems. The majority of studies, however, have dealt with the retention of insecticides in these systems (e.g. Schulz et al., 2003b; Braskerud and Haarstad, 2003;). The retention potential of such systems for more hydrophilic fungicides seems to be much lower. Blankenberg et al. (2006) reported average mass retentions ranging from 3 to 43% for the fungicides metalaxyl, propiconazole, and fenpropimorph in the constructed wetlands of Grautholen (entirely covered with vegetation; length = 100 m; surface area = 840 m2) and Lier (eight wetland cells with different types of filters; length = 40 m; surface area = 120 m2/cell) in Norway. A study by Elsaesser et al. (2011) in the Lier wetland (Norway) found even lower mass retentions (<4%) of the applied fungicides tebuconazole and trifloxystrobin. Studies of the fate and retention

of biocides in VTSs are also limited. Waltman et al. (2006) investigated the mitigation of triclosan concentrations in an experimental constructed wetland (46 by 46 m, retention time = 4.3 d) receiving secondary treated wastewater from the Pecan Creek Water Reclamation Facility (Denton, TX) and reported a mean decrease in triclosan concentrations from 0.09 mg/L in the wetland inflow to 0.04 mg/L in the wetland outflow.

In the majority of studies dealing with aquatic vegetation and risk mitigation measures, sorption to aquatic macrophytes has been postulated as a major sink for dissolved organic contaminants, depending on their octanol-water partition coefficients (K_{OW}) (Stehle et al., 2011). In addition, sorption to macrophytes enhances residence time of the compounds in VTSs and thus increases the available time for degradation processes (e.g., photolysis and microbial degradation) to occur (Imfeld et al., 2009). It is therefore unsurprising that only limited attention has been given to date to more hydrophilic substances, such as fungicides. In addition to aquatic macrophytes, sediments have been identified as a potential sink for organic chemicals in aquatic systems, although the retention rates vary among the investigated systems. For instance, (Moore et al., 2008) reported sediment to be a major sink, with 50% of the experimentally applied cis- and trans-permethrin associated with the sediment compartment. In contrast, in a study by Bennett et al. (2005), sediment was only a minor sink for bifenthrin and lambda-cyhalothrin in a vegetated agricultural drainage ditch.

Although the majority of published studies have dealt with the retention of organic pollutants in more or less static constructed wetlands or vegetated ditches (Stehle et al., 2011), studies in flowing systems, whether designed as recirculating (Beketov and Liess, 2008) or as flow-through wetlands and streams, are quite limited (Dabrowski et al., 2006; Elsaesser et al., 2011; Schulz et al., 2003a). The flow-through stream mesocosm facility used in this study was built to simulate small, vegetated streams (Elsaesser et al., 2013); hence, as part of the European Union LIFE project ArtWET (Gregoire et al., 2008), the objective of the present study was the assessment of the retention of biocides and fungicides of environmental concern, covering a broad range of K_{OW} values, in small, flowing vegetated streams.

Materials and Methods

General Experimental Setup

The present study was performed in the vegetated flow-through stream mesocosm facility at the University Koblenz–Landau, Campus Landau, Germany, described in detail by (Elsaesser et al., 2013). Briefly, for the current experiment, 5 out of 16 vegetated stream mesocosms were used, each 45 m long and 0.4 m wide. All of the stream mesocosms contained a 0.12-m sediment layer under a 0.26-m water column, with four streams additionally planted with the submerged macrophyte *Elodea nuttallii* (Planch). The mesocosms can be run in a flow-through mode during the experimental phases, in which water is discharged at the outlet, and in a circulation mode outside of the experimental phases, in which the outflowing water is pumped back into the inlet. Throughout both the preliminary tracer and the biocide and fungicide application, water flowed from a spillway attached to a 200-m3 water reservoir with a discharge of 1 L/s, resulting in a flow velocity of approximately 0.01 m/s, which corresponds to slow-flowing natural streams, and a hydraulic retention time of approximately 78 min. Two sampling sites were established within each stream: the first was 2 m below the water inlet, and the second was 2 m above the outlet, providing a total stream length of 41 m between the two sampling sites (Figure A1-1). Each stream mesocosm was treated with a mixture of fungicides and biocides on 28 August 2009. Water and sediment samples from all stream mesocosms and additional macrophyte samples from the vegetated stream mesocosms were taken following a sampling protocol that was derived from the data of the tracer experiment. Weather conditions were favorable during the entire experimental phase (maximum temperature = 22.1°C; wind speed <2.1 m/s; no precipitation) and thus did not affect the experiment.

Sediment and Macrophytes

The stream mesocosms were filled with sediment in February 2009. The sandy loam (clay: 104 g/kg, silt: 185 g/kg, sand: 711 g/kg) used was taken from a sand quarry and not subject to any anthropogenic impact for at least 15 years before its use in the vegetated stream mesocosms. The texture, particle size distribution and total organic carbon (TOC = 0.78%) were determined according to the standards DIN ISO 11277 (DIN, 2002), DIN 18123 (DIN, 2011), DIN 19682-2 (DIN, 2007), and DIN EN 13137 (DIN, 2001). In March 2009, western waterweed, *E. nuttallii*, was taken from the Queich River (49°13´09.53´´ N; 07°53´50.76´´ E)

in the Palatinate forest, a low mountain range 15 km west of Landau. Whorls (length = 15 cm) of the waterweed were randomly inserted into the sediment of four stream mesocosms, and one stream was left unplanted. To allow for macrophyte adaptation and growth, the stream mesocosms were maintained with a moderate daily water exchange of approximately 50 L for 5 month before the addition of the treatment chemicals. During the 5-month period, the waterweed grew and propagated rapidly, ultimately covering all planted stream mesocosms at a high density. Residues of the selected biocides and fungicides were not found in the water, macrophytes, or sediment samples taken before the beginning of the application. At the end of the study, composite macrophyte samples were removed from four 0.2-m^2 areas and lyophilized to determine the macrophyte biomass of each stream mesocosm (Table 1).

Table 1 Total dry weights of composite macrophyte samples and total biomasses in stream mesocosms 14 d post-treatment.

Stream No.	Total dry weight (g/m^2)	Total biomass (g)
1	0	0
2	193	3,474
3	269	4,842
4	230	4,140
5	131	2,358

Application Procedure

For the preliminary tracer and for the biocides and fungicides experiment, 0.5 L of a stock solution was spiked to each stream mesocosm. The tracer stock solution contained sodium chloride (100 g/L), and the stock solution for the main experiment contained a mixture of analytical standards of the fungicides imazalil (1-[2-(Allyloxy)-2-(2,4-dichlorophenyl)ethyl] imidazole), propiconazole ((1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole), thiabendazole ((2-(4-Thiazolyl)benzimidazole) and the biocides triclosan (1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea) at concentrations ranging from 1.3 to 115.2 mg/L (Table 2). During the application procedure, the flow was blocked at the inlet, enclosing a total water volume of approximately 96 L. After spiking, the blocked water volume was stirred for 10 seconds using a stainless steel mixer, ensuring complete mixture of the spiked compounds into the 96-L water volume before being slowly released into the inlet areas of

the respective stream mesocosms. The biocide and fungicide concentrations applied to the stream mesocosms were calculated using a literature survey of studies dealing with biocide and fungicide concentrations in surface waters (Castillo et al., 2000; Halden and Paull, 2004; Wick et al., 2010). The amount of each biocide and fungicide spiked to the stream mesocosms was chosen to obtain inlet concentrations 10 times greater than the concentrations found in the literature to represent worst-case conditions and to ensure detectable concentrations of each compound even at the outlet of the stream mesocosms.

Water, Sediment and Macrophyte Samples

As described above, the tracer stock solutions were applied to each stream mesocosm, and changes in the specific conductivity during the passage of the tracer were continuously measured and recorded at the inlet and the outlet using a conductivity meter (Cond 340i, WTW GmbH, Weilheim, Germany). Following the tracer experiments, each stream mesocosm was run in flow-through mode until the specific conductivity reached background levels to ensure the complete removal of the tracer from each stream mesocosms. Despite the application of high amounts of sodium chloride, no effects on macrophytes were observed subsequent to the tracer experiment.

During the passage of the biocide and fungicide peaks, seven water samples were taken in total at the inlet and outlet sampling sites, with the objective of capturing accurate peak curves (Figure A1-2 – A1-6). On the basis of the flow conditions determined using the tracer data, samples were taken 0 to 17 min after spiking at the inlet and from 37 to 100 min after spiking at the outlet sampling sites. In general, water, macrophyte and sediment samples were taken simultaneously. Because of the particularly short sampling intervals of less than 1 min, only five sediment and macrophyte samples were taken at the inlet sampling sites. Water samples were collected by submerging 1-L amber glass bottles, ensuring that no sediment entered the bottles, and were then stored on-site in refrigerators at 6°C for a maximum of 48 h before extraction in the laboratory. Macrophyte samples consisted of submerged macrophyte material taken from approximately 5 cm above the sediment with stainless steel scissors. Sediment samples were taken from the upper 1 cm of sediment using 110-mL aluminum containers. All sediment and macrophyte samples were stored at -20°C in aluminum containers immediately after sampling. All devices used for sampling were rinsed with acetone and distilled water before use. Temperature (21.0 ± 0.6°C), pH (8.9 ± 0.1),

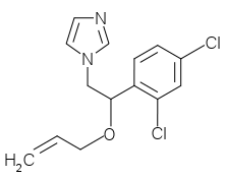
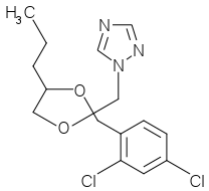
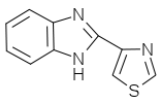
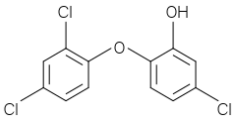
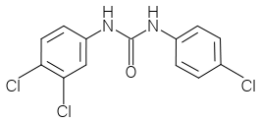
oxygen saturation (112.8 ± 1.5%), and conductivity (174 ± 7.6 mS/cm) were measured (n = 5) during the sampling period (Multi 340i, WTW GmbH, Weilheim, Germany) and varied by less than 5% among the stream mesocosms.

Analysis and Extraction of Water, Macrophyte and Sediment Samples

Water samples were processed and analyzed following an adapted method reported in Wick et al. (2010). Briefly, for all water samples, volumes of 500 mL were extracted within 48 h using solid phase extraction (SPE). For this purpose, the pH of each sample was adjusted to 6.3 to 6.5 using 3.5 M H_2SO_4. Additionally, each sample was spiked with 50 µL of a surrogate standard mixture (1 ng/µL; imazalil-d_5, propiconazole-d_5, thiabendazole-d_6, triclosan-$^{13}C_{12}$, triclocarban-$^{13}C_6$). The SPE cartridges (OASIS HLB 200 mg, 6 mL; Waters Corporation, Milford, MA) were conditioned with 2 mL heptane, 2 mL acetone, 3 × 2 mL methanol and 4 × 3 mL Millipore water and the samples were then percolated through the SPE cartridges with an average flow rate of 20 mL/min. The cartridges were air dried for 2 h and deepfrozen at -20°C until analysis. The cartridges were eluted with 4 × 2 mL methanol/acetone (60/40, v/v). The eluate was evaporated to a volume of 500 µL using nitrogen, spiked with 500 µL Milli Q water mixed with 0.1% formic acid (v/v) and being analyzed by liquid chromatography-tandem mass spectrometry (LC‑MS/MS). The high-performance liquid chromatography system was coupled to a tandem mass spectrometer (API 4000, Applied Biosystems, Foster City, CA) and consisted of an Agilent 1200 Series (Agilent Technologies, Waldbronn) liquid chromatographic system that was equipped with a Synergi Fusion-RP 80A column (150 mm by 3 mm, 4 µm) and a SecurityGuard pre-column (4 mm by 3 mm) (both Phenomenex, Aschaffenburg, Germany). For the determination of imazalil, thiabendazole, and propiconazole, the electrospray ionization source was operated in the positive ion mode, while triclosan and triclocarban were determined using the negative ion mode. Two multiple reaction monitoring transitions for the quantification and the confirmation of the analytes were monitored (Table A1-1). The mobile phases A and B for the determination of the positively charged analytes were 10 mM ammonium formate buffer (adjusted to pH 3.2 with formic acid) and acetonitrile with 0.1% formic acid (v/v), respectively. The gradient program was as follows: 0 to 1 min 0% B, 1 to 2 min increase to 30% B, 2 to 19 min increase to 80% B, 19 to 25 min 80% B, 25 to 27 min decrease to 0% B, 27 to 32 min 0% B. For the determination of the negatively charged analytes, the mobile phase A (Milli-Q water

containing 0.1% formic acid [v/v]) and B (acetonitrile) were run using the following gradient program: 0 to 1 min 0% B, 1 to 2 increase to 40% B, 2 to 19 min increase to 80% B, 19 to 26 min 80% B, 26 to 28 decrease to 0% B, 28 to 33 min 0% B. For both ionization modes, injection volumes were 25 µL and the flow rate was adjusted to 0.4 mL/min. Biocide and fungicide residues from the lyophilized sediment and macrophyte samples, respectively, were extracted by accelerated solvent extraction (ASE) and finally quantified according to the same analytical method as used for water sample analyses. Subsamples of 1 g of each sediment sample or 0.5 to 1 g of each macrophyte sample were weighed into 22 mL stainless steel extraction cells and spiked with 100 µL of a surrogate standard mixture (1 ng/µL). The extraction cells were filled with cindered sea sand (Riedel-de Hean, Seelze, Germany) after the solvent originating from the surrogate standard mixture was entirely evaporated. The extractions were performed using a Dionex ASE 200 instrument (Sunnyvale, CA) with an extraction method comprising an equilibration period of 5 min, followed by four static cycles of 10 min at a temperature of 80°C and a flush volume of 40% after each static cycle. The sediment and macrophyte extracts were finally diluted in groundwater and processed as described for the water samples. The sediment samples were extracted by ASE according to the method of Wick et al. (2010) for activated sludge samples using water and methanol (50/50, v/v) as solvents. The extractions of fungicide and biocide residues from the macrophyte material were performed using methanol and acetone (60/40, v/v) as solvents. For method validation, 1 g of unloaded macrophyte samples was spiked with 100 mL of an analytical and surrogate standard mixture (1 ng/µL) before extraction. Relative recoveries of the target compounds were determined as the ratio of the measured and the spiked amount of the analytical standard, resulting in mean relative recoveries ranging from 100 ± 2.5% for propiconazole to 121 ± 6.1% for triclosan (Table A1-2).

Table 2 Physicochemical properties of biocides and fungicides used in the present study.

	Imazalil	Propiconazole	Thiabendazole	Triclosan	Triclocarban
Type	Fungicide	Fungicide	Fungicide	Biocide	Biocides
Chemical structure					
Molecular weight	297.18†	342.22†	201.25†	289.54†	315.58†
K_{OW}	363†	5,250†	245†	57,544§	125,863§
Water solubility (mg/L)	184†	150†	30†	1.97 - 4.6¶	0.65 - 1.55¶
Aqueous hydrolysis DT50 (days)	Stable†	53.5†	203†	60¶	60¶
Aqueous photolysis DT50 (days)	6.1†	Stable†	1.2†	Stable‡	0.5 #

† PPDB (2009); ‡ NICNAS (2009); § Wick et al. (2010); ¶ Halden and Paull (2004); # USEPA (2002).

Data Analyses

The amounts of the biocides and fungicides (M_{out}) that remained in the aqueous phase and thus were not retained within the stream mesocosms were determined as the integral of the peak curves at the outlet sampling sites. Relative biocide and fungicide mass retentions (RET) were calculated using Equation 1, where M_{Spike} is the amount of the respective substance that was applied to each stream mesocosm:

$$RET(\%) = \left(\frac{M_{Spike} - M_{Out}}{M_{Spike}} \right) \times 100 \quad (1)$$

Relative amounts of the applied biocides and fungicides that were recovered in the macrophytes (REC_{Macro}) from the vegetated stream mesocosms were calculated using Equation 2, where $C_{MacroIn}$ and $C_{MacroOut}$ are the maximum concentrations of each compound in the macrophyte samples taken at the inlet and outlet sampling sites, respectively, and $m_{Biomass}$ is the dry weight of the macrophyte biomass in the vegetated stream mesocosms:

$$REC_{Macro}(\%) = \frac{\left[\left(\frac{C_{MacroIn} + C_{MacroOut}}{2} \right) \times m_{Biomass} \right]}{M_{Spike}} \quad (2)$$

The total recovery rates (REC_{Total}) for each compound in the aqueous phase and in the macrophytes of the vegetated stream mesocosms were estimated using Equation 3, where M_{Macro} is the amount of the compounds associated with the macrophytes in the vegetated stream mesocosms:

$$REC_{Total}(\%) = \left(\frac{M_{Out} + M_{Macro}}{M_{Spike}} \right) \times 100 \quad (3)$$

Correlation analyses for peak reduction, relative mass retention (RET), and the relative amounts of the applied biocides and fungicide masses that were recovered in the macrophytes (REC_{Macro}) as a function of K_{OW} were performed using Spearman´s rank correlation test in SPSS 17.0 (SPSS, Chicago, IL).

Results and Discussion

Decrease of Maximum Concentrations

The application of different amounts of biocides and fungicides to the stream mesocosms resulted in compound-specific maximum concentrations across all inlet sampling sites, with a

range from 1.8 µg/L for triclosan up to 93 µg/L for imazalil (Table 3). These maximum inlet concentrations finally resulted in maximum concentrations ranging from 0.2 to 20 µg/L at the outlet sampling sites of all stream mesocosms. Similar peak reductions were observed for the other compounds in all of the stream mesocosms, whereas the peak reductions were generally greater in the vegetated stream mesocosms 2‑5. Solely, triclosan showed the greatest peak reduction (95%) in the unvegetated stream 1, which we consider to be an artifact that may be attributed to inaccurate sample preparation leading to a loss of triclosan or the corresponding surrogate standard and thus to an overestimation of the peak reduction. For all other compounds, the peak reductions in the unvegetated stream 1 ranged from 48 to 71% and thus were less than the respective peak reductions in all of the vegetated stream mesocosms. The peak reductions in the vegetated stream mesocosms 2‑5 were between 59 and 94% and thus 20 and 25% greater than those in the unvegetated stream 1 resulting in average peak reductions of 64 to 91%. However, increasing macrophyte biomass did not consistently affect the peak reduction rate of the applied compounds in the vegetated stream mesocosms ($R2 = 0.101$; $p < 0.672$; $n = 20$). Nevertheless, our findings are in accordance with studies that compared the reduction of the maximum concentrations of organic pollutants in vegetated and unvegetated treatment systems, although some of those studies were performed in non-flowing ditches or wetlands. Gill et al. (2008), for instance, found a median concentration reduction of 38% for the insecticide chlorpyrifos at the end of a 200 m vegetated ditch compared with 1% in a conventional, unvegetated tailwater ditch. A study in vegetated and unvegetated wetland mesocosms revealed reduced transport of methyl-parathion (MeP) in the vegetated wetland (87% of the applied MeP associated with vegetation), resulting in lower MeP concentrations and hence reduced toxicity for *Hyalella azteca* exposed to water from the vegetated wetland (Schulz et al., 2003b).

However, the stream mesocosms used in the present study were designed to simulate small, slow-flowing, natural streams. For this reason, the stream mesocosms were built as flow-through systems with slow flow velocities (approximately 1 cm/s). Thus, hydraulic processes such as dispersion must be considered among the processes that enhance peak reduction in these systems. The peak reductions in the unvegetated stream 1 may hence be attributed to dispersion caused by the shear velocities between the middle of the water column and the border areas of the stream mesocosm (Rutherford, 1994).

Due to the presence of the macrophytes in the vegetated stream mesocosms, two processes may have contributed to the increased peak reduction in those stream mesocosms. On the one hand, stem wake turbulence behind the macrophytes may have amplified dispersion (Nepf, 2000); on the other hand, compound-specific sorption to the macrophytes may have led to an elimination of biocides and fungicides from the aqueous phase (Stehle et al., 2011).

Appendix

Table 3 Peak concentrations at the inlet (C_{in}) and the outlet (C_{out}) sampling sites and peak reductions (Red.) in all stream mesocosms, as well as the average peak reductions ($\overline{X}$ Red.) of the experimentally added fungicides and biocides in the vegetated stream mesocosms 2–5.

	Stream 1			Stream 2			Stream 3			Stream 4			Stream 5			Stream 2 - 5
	C_{in} (µg/L)	C_{out} (µg/L)	Red. (%)	C_{in} (µg/L)	C_{out} (µg/L)	Red. (%)	C_{in} (µg/L)	C_{out} (µg/L)	Red. (%)	C_{in} (µg/L)	C_{out} (µg/L)	Red. (%)	C_{in} (µg/L)	C_{out} (µg/L)	Red. (%)	$\overline{X}$ Red. (%; ±SE)
Imazalil	68.9	20.3	71	-††	6.0	-‡‡	42.5	6.7	84	74.4	7.7	90	92.5	6.4	93	89±3
Propiconazole	4.4	1.4	69	-††	0.5	-‡‡	3.0	0.4	85	4.5	0.6	87	4.9	0.5	90	87±2
Thiabendazole	18.4	9.5	48	-††	6.9	-‡‡	14.9	5.0	66	19.4	7.9	59	17.8	6.2	65	64±2
Triclosan	2.9	0.2	95	3.1	0.2	93	1.8	0.2	88	2.5	0.3	89	4.0	0.2	94	91±2
Triclocarban	19.7	6.1	69	-††	0.7	-‡‡	12.2	1.3	89	6.0	1.5	75	18.9	1.2	94	86±6

†† No concentrations were measured due to SPE-cartridge malfunction.

‡‡ Relative peak reduction could not be calculated due to missing inlet concentration.

Mass Retention

Although the alteration of hydraulic processes may have affected the peak reduction to the same extent for all compounds, mass retentions (RET) were found to differ greatly among compounds. Figure 1 shows that the more hydrophilic fungicides were less strongly retained within the vegetated stream mesocosms than were the hydrophobic biocides. An average of 6±4% of the applied amount of imazalil was retained in the vegetated stream mesocosms, followed by an average mass retention (RET) of 11±2% and 21±5% for propiconazole and thiabendazole, respectively. For the biocides, average mass retentions (RET) of triclosan and triclocarban of 56±7% and 81±1%, respectively, were determined in the vegetated stream mesocosms.

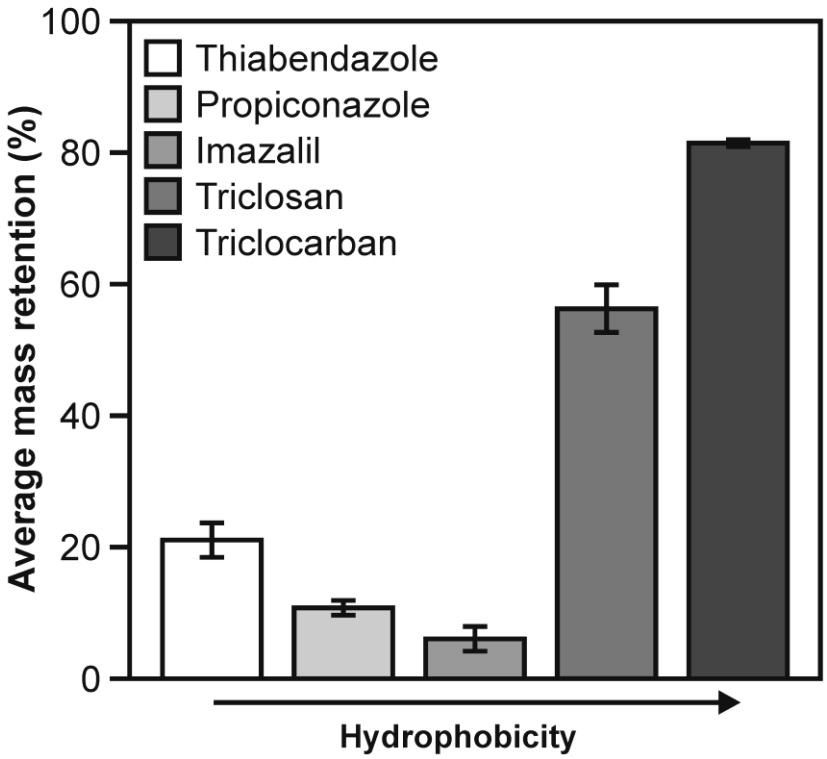


Figure 1 Average (±SE) mass retentions (RET) in the vegetated stream mesocosms 2 – 5 based on calculated outlet mass (M_{Out}) and spiked mass (M_{Spike}) at the inlet.

A correlation analysis on relative mass retention (RET) as a function of lipophility confirmed these findings and showed a significant positive correlation ($R^2 = 0.84$; $p < 0.001$; $n = 20$) between the two variables (Figure 2). The findings of the present study are in accordance with Matamoros et al. (2007), who postulated increased plant uptake of highly sorptive insecticides compared with less sorptive herbicides to be an important elimination pathway

in a horizontal subsurface flow constructed wetland. In addition, Blankenberg et al. (2006) investigated the retention of pesticides in two experimental wetlands in Norway and found mass retention rates for the fungicides metalaxyl (3 - 41%), propiconazole (13 - 25%) and fenpropimorph (10 - 36%) similar to those of the present study. Nevertheless, the majority of studies that focused on mass retention in wetlands and vegetated streams and ditches dealt with more lipophilic compounds, for which a strong partitioning to macrophytes (Hand et al., 2001) and thus a high retention in vegetated systems is likely (Stehle et al., 2011), in contrast to the present study.

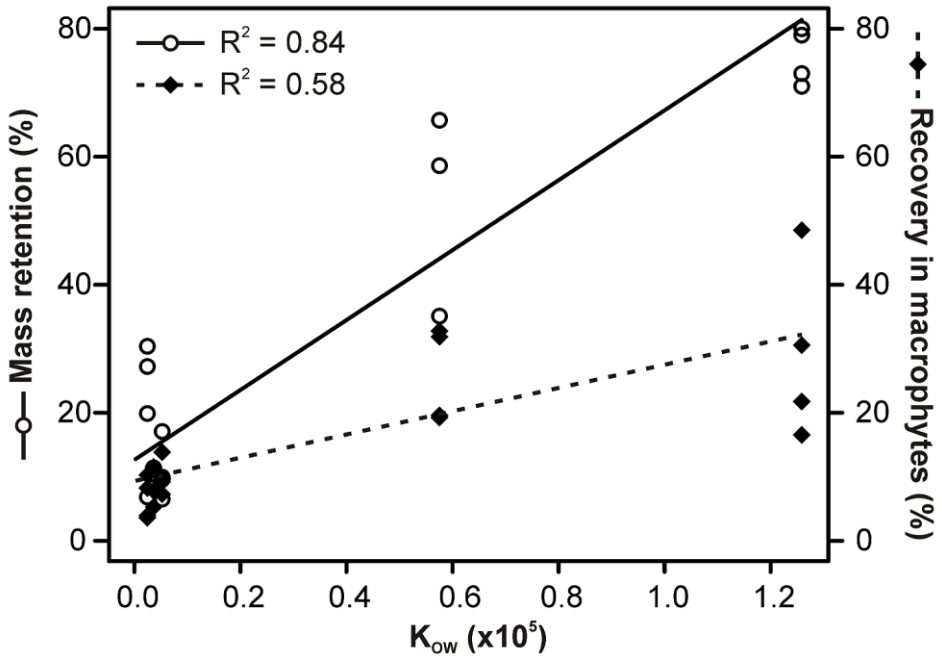


Figure 2 Correlation of relative mass retention (RET; open circles) and relative amounts of the applied compounds recovered in the macrophytes (RECMacro; black diamonds) as a function of the K_{OW} of the compounds.

In addition, in the present study, partitioning to macrophytes was identified to be a major compound-specific sink. The analyses of the macrophyte samples revealed that biocide and fungicide concentrations in the macrophyte samples increased with the passage of the peak, resulting in a range of concentrations from 92 to 1,365 ng/g for triclosan and thiabendazole, respectively, at the inlet sampling sites. At the outlet sampling sites, the minimum and maximum concentrations for all of the macrophyte samples and compounds were found for triclosan (32 ng/g) and triclocarban (558 ng/g). Very few of the biocides and fungicides were detected in the sediment samples taken from any of the stream mesocosms, with compound-specific maximum concentrations ranging from 1 ng/g for triclosan to 16 ng/g for imazalil (Table A1-3). In a mass balance based on these maximum concentrations, the mass

retention by the sediment would account for less than 1.2±1% of the applied compounds in all of the stream mesocosms and were thus not considered further. However, we hypothesize that the weak and inconsistent sorption affinity of the applied compounds may be explained by the relatively short peak exposure times of 5 min at the inlet and 37 min at the outlet sampling site. In addition, the comparatively low TOC of 0.78% may have also contributed to the low mass retention by the sediment.

However, due to the wide range of the spiked biocide and fungicide amounts, a direct quantitative comparison with the concentrations in the macrophyte samples is hardly possible. Thus, relative recoveries in the macrophytes (REC_{Macro}) were instead considered further.

There was a positive correlation between the relative amounts of the applied biocide and fungicide masses that were found to be associated with the macrophytes (REC_{Macro}) and the lipophility (K_{OW}) of the compounds ($R^2 = 0.58$; $p < 0.001$; $n = 20$). A mass balance (illustrated in Figure 3) supported these findings. On average, 7±3 to 10±3% of the spiked fungicides were found sorbed to the macrophytes in the vegetated stream mesocosms. Moreover, the mass balance showed average mass recoveries (REC_{Macro}) of 28±8 and 34±14% for the biocides triclosan and triclocarban associated with the macrophytes. However, there was a discrepancy between the total recovery rates (REC_{Total}) and the applied amounts of some of the compounds. Despite slight overestimations, which may be attributed to methodical inaccuracies, the total recoveries (REC_{Total}) for imazalil (103%) and propiconazole (106%) illustrate that the bulk of both compounds (94±12% of imazalil and 96±9% of propiconazole) remained in the aqueous phase. The total recoveries (REC_{Total}) of the remaining compounds in both the macrophytes and the aqueous phase ranged from 85 to 56% for thiabendazole and triclocarban, respectively. Thus, we hypothesize that the missing amounts of these compounds may be due to dissipation or degradation processes that could not be encompassed by the experimental design of the present study. Nevertheless, the results of the mass balance clearly indicated that sorption to macrophytes led to compound-specific elimination from the aqueous phase and thus may have led to additionally increased peak reductions in the vegetated stream mesocosms.

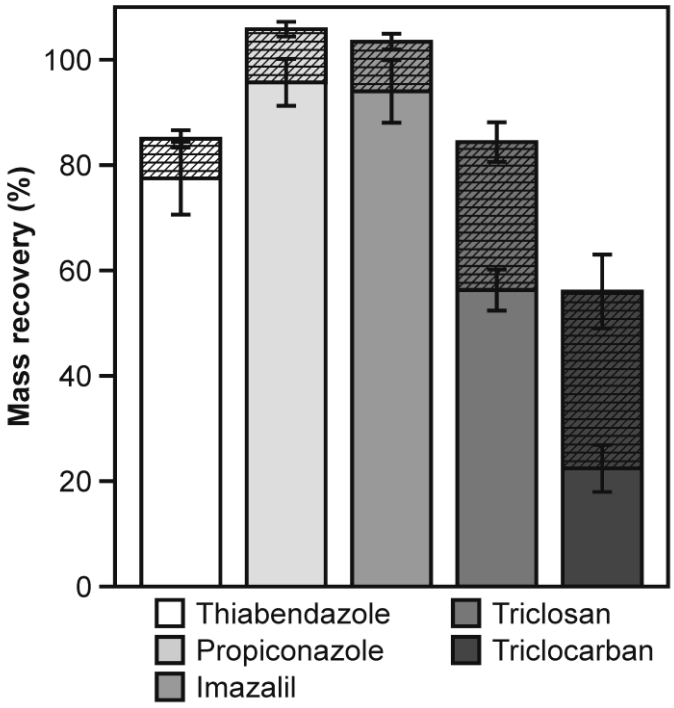


Figure 3 Average (±SE) mass recoveries (REC) of the applied biocides and fungicides in the vegetated stream mesocosms 2 – 5. Plain bars (including the lower error bars) represent the proportion of the applied amount of biocides that remained in the aqueous phase; shaded bars (including the upper error bars) represent the proportion that was found in the macrophytes. Error bars represent the standard error of the mass recoveries in the examined matrices of the vegetated stream mesocosms 2 – 5.

Conclusion

The results of the present study indicate that the presence of aquatic macrophytes may affect two processes in the stream mesocosms. On the one hand, aquatic macrophytes were a sink for lipophilic compounds, which is in accordance with available wetland studies in which the decrease in maximum concentrations is primarily attributed to sorption to macrophytes. On the other hand, the results indicate that aquatic macrophytes may amplify dispersion processes, which may accelerate the decrease in the maximum concentrations regardless of the physicochemical properties of the compounds. These findings support the assumption that slow-flowing vegetated streams or ditches as a part of risk mitigation and management strategies may help to counteract the negative effects of fungicide or biocide contaminations. As a result, slow-flowing vegetated streams and ditches may be suitable tools for the achievement of the objectives of the European Water Framework Directive (2000/60/EC). Nevertheless, further research is needed to obtain a deeper understanding, i.e., a holistic examination and quantification of the relevant processes, to derive recommendations for field applications.

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A.2 Role of submerged vegetation in the retention processes of three plant protection products in flow-through stream mesocosms

Christoph Stang[1], Matthias Valentin Wieczorek[1], Christian Noss[2], Andreas Lorke[2], Frank Scherr[3], Gerhard Goerlitz[3] and Ralf Schulz[1]

[1]Institute for Environmental Sciences, Ecotoxicology & Environment, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

[2]Institute for Environmental Sciences, Environmental Physics, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

[3]Bayer CropScience AG, Environmental Safety, Alfred-Nobel-Strasse 50, 40789 Monheim, Germany

Abstract

Quantitative information on the processes leading to the retention of plant protection products (PPPs) in surface waters is not available, particularly for flow-through systems. The influence of aquatic vegetation on the hydraulic- and sorption-mediated mitigation processes of three PPPs (triflumuron, pencycuron, and penflufen; $\log K_{OW}$ 3.3–4.9) in 45-m slow-flowing stream mesocosms was investigated. Peak reductions were 35–38% in an unvegetated stream mesocosm, 60–62% in a sparsely vegetated stream mesocosm (13% coverage with *Elodea nuttallii*), and in a similar range of 57–69% in a densely vegetated stream mesocosm (100% coverage). Between 89% and 93% of the measured total peak reductions in the sparsely vegetated stream can be explained by an increase of vegetation-induced dispersion (estimated with the one-dimensional solute transport model OTIS), while 7–11% of the peak reduction can be attributed to sorption processes. However, dispersion contributed only 59–71% of the peak reductions in the densely vegetated stream mesocosm, where 29% to 41% of the total peak reductions can be attributed to sorption processes. In the densely vegetated stream, 8–27% of the applied PPPs, depending on the $\log K_{OW}$ values of the compounds, were temporarily retained by macrophytes. Increasing PPP recoveries in the aqueous phase were accompanied by a decrease of PPP concentrations in macrophytes indicating kinetic desorption over time. This is the first study to provide quantitative data on how the interaction of dispersion and sorption, driven by aquatic macrophytes, influences the mitigation of PPP concentrations in flowing vegetated stream systems.

Introduction

The application of plant protection products (PPPs) is a common practice in conventional agriculture. As a result, PPPs can enter non-target aquatic ecosystems, either through spray-drift during application or edge-of-field runoff and drainage post application (Schulz, 2004). In addition to best management practices (BMPs), such as improved application techniques or vegetated buffer strips, vegetated treatment systems (VTSs) have been proposed and evaluated for the mitigation of PPP concentrations in receiving waters (Gregoire et al., 2008; Reichenberger et al., 2007). A meta-analysis (Stehle et al., 2011) confirmed the effectiveness of VTSs for the mitigation of PPP concentrations and identified the high lipophility of PPPs in combination with the high plant coverage in the VTSs to be the major factors influencing the mitigation performance.

In fact, the ability of aquatic vegetation to interact with PPPs has been demonstrated in studies at the laboratory (Crum et al., 1999; Olette et al., 2008), microcosm (Bouldin et al., 2006), and mesocosm scale (Moore et al., 2009). The majority of mesocosm and field studies linked the sorption of lipophilic PPPs to aquatic macrophytes or, in limited cases, to sediments with the mitigation potential of the investigated VTSs (Bennett et al., 2005; Rogers and Stringfellow, 2009). Margoum et al. (2006) and (Passeport et al., 2011) identified the ability of substrates, such as sediment, leaves, plants and soil taken from ditches, wetlands or forest buffers, respectively, to absorb and thus retain PPPs within these systems. Nevertheless, most of the studies published to date were performed in vegetated wetlands with negligible flow velocities (Gill et al., 2008; Moore et al., 2007; Schulz et al., 2003b). The few studies that investigated the mitigation potential and the fate of the PPPs in flow-through vegetated treatment systems and streams were performed either as case studies under field conditions or in vegetated wetlands with irreproducible system properties. The reductions of azinphos-methyl concentrations (61–90%) were reported from a vegetated tributary of the Lourens River, South Africa, subsequent to a spray drift and runoff event (Dabrowski et al., 2006). Schulz et al. (2003a) revealed an overall reduction in the azinphos-methyl concentrations of 90% in a flow-through wetland in South Africa with an overall mass retention of 61%, of which 10.5% were initially adsorbed to macrophytes. Elsaesser et al. (2011) found merely 5% of the PPPs applied to the Lier wetland, Norway, adsorbed to macrophytes, and thus presumed that the increase of peak reductions in the vegetated wetland cells seemed to be a result of vegetation induced dispersion processes.

However, those studies pursued relatively simplistic sampling strategies that relied on the comparison of few concentrations at the inlets and the outlets of the investigated systems and did not focus on a quantitative description of the processes leading to the mitigation of the PPP concentrations.

In addition to being a potential sink for PPPs through sorption processes, aquatic vegetation constitutes a key factor determining the hydraulic conditions in streams and wetlands (Sukhodolov and Sukhodolova, 2012). Depending on the plant density and geometry, increased velocity shear and turbulent mixing lead to enhanced longitudinal dispersion of fluid momentum (Nepf, 2000). The roughness or flow types (Nikora et al., 2007) differ significantly between emergent vegetation, which extends throughout the entire water column, and submerged vegetation, which is always superposed by a free floating water layer (Shucksmith et al., 2011). Within emergent macrophyte canopies, the relevant length scales for turbulent mixing are limited by the stem diameter and spacing. Potentially larger vortices at the canopy-scale are generated in a shear layer between the canopy and the overflow in the non-vegetated part of the water column (Nepf et al., 2007). Hydrodynamic aspects of flow-plant interactions have been studied in detail from the scales of individual stems and leaves (Albayrak et al., 2011) up to patches of vegetation (Sukhodolov and Sukhodolova, 2012; Sukhodolova and Sukhodolov, 2012). Recently, different aspects of the reactive transport of dissolved substances were investigated at the laboratory (Hansen et al., 2010) up to the field (Schuetz et al., 2012) scale. In spite of its potential importance in many engineered as well as natural aquatic ecosystems, studies that conjointly investigated the influence of aquatic vegetation on both the hydraulic and sorption processes, and thus, on the mitigation of PPPs in slow-flowing streams, are not existent so far.

The vegetated flow-through stream mesocosms in which the present study was performed facilitated the modifications of individual system inherent properties while ensuring reproducible experimental conditions. For the present study, the coverage and the spatial distribution of macrophytes were the only system properties that were modified among the stream mesocosms. Based on this experimental setup, this study aimed to quantify the role of longitudinal dispersion and sorption processes as a result of submerged vegetation and its density-related spatial distribution on the peak concentration and mass retention of three moderately lipophilic PPPs ($\log K_{OW}$ 3.3–4.9) at the mesocosm scale.

Materials and Methods

General study outline

The study phase lasted from 31st of July 2011 until 12th of August 2011 and consisted of two experimental phases. The tracer experiment was conducted in total darkness during the night from 31st of July 2011 to 1st of August 2011. The PPP experiment occurred with two separate dosing events of the stream mesocosms on the 2nd and 10th of August 2011. Generally, the tracer experiment, as well as both PPP applications were carried out during windless and rainless weather conditions, respectively. However, during the second PPP application in the unvegetated stream mesocosm the occurrence of wind gusts with wind speed over 2 on the Beaufort scale affected the application. In order to avoid any impact of precipitation on the PPP experiment, the stream mesocosms were transiently covered with tarpaulins when rain showers occurred during the entire experimental phase.

Vegetated stream mesocosms

The study was performed in three out of a total of sixteen independent stream mesocosms of the Landau stream mesocosm facility (Elsaesser et al., 2013) (Figure 1).

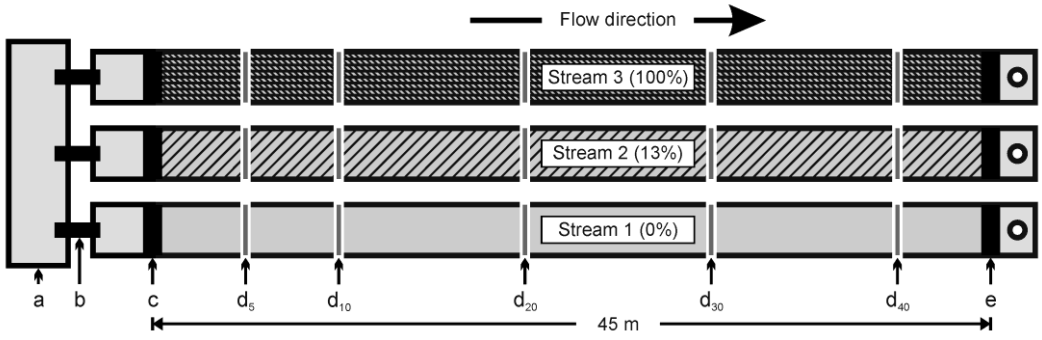


Figure 1 Schematic outline of the three vegetated stream mesocosms differing in vegetation density (%). (a) Water reservoir; (b) transfer pipe; (c) stream inlet with spillway and point of application; (d5-d40) sampling sites at 5, 10, 20, 30 and 40 m; (e) spillway to outlet.

Each of the stream mesocosms consisted of U-shaped concrete tubs (length = 45 m; width at the bottom = 0.37 m and width at the top = 0.38 m; depth = 0.5 m) and contained a 0.26-m water layer on top of a sediment layer (0.12 m) of medium loamy sand (total organic carbon = 1.0±0.4%, n = 15). Two of the stream mesocosms were planted with different densities of the submerged western waterweed (*Elodea nuttallii*). Immediately after the termination of the PPP experiment, macrophytes were removed from three sites (each 0.2 m^2) in each

vegetated stream mesocosm, lyophilized and weighed to assess the macrophytes biomass (dry weight) and relative density, respectively (Table A2-1). Stream 3 was densely covered by macrophytes, while the biomass in stream 2 was reduced to 13% relative to the densely vegetated stream 3 prior to the start of the experimental phase. Macrophytes in stream 3, however, homogeneously covered the entire cross-sectional area from the bottom boundary to the water surface. Macrophytes in stream 2 protruded only to about half of the water depth and were covered by a free flow zone in the upper part of the cross-sectional area (Figure 2 a – c).

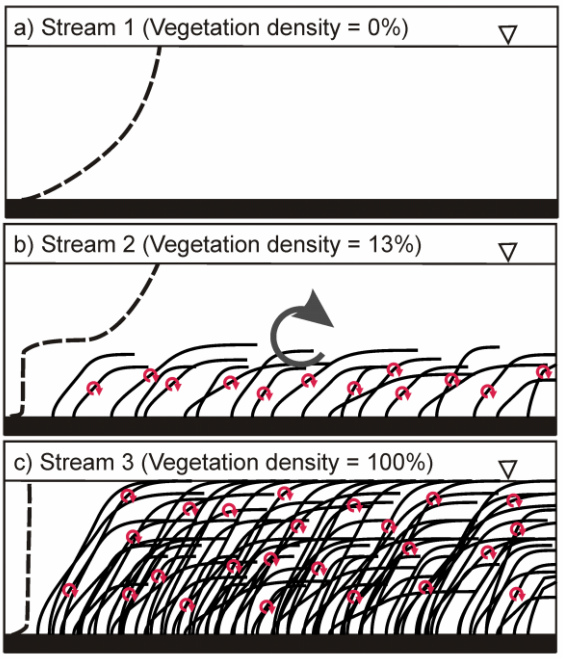


Figure 2 (a–c) Schematic outline of the vegetation distribution in the three stream mesocosms and the influence of the vegetation on the flow velocities and turbulence within the streams. Dashed lines represent the vertical distribution of the flow velocities, red circlets represent the stem and leaf wake turbulence in the vegetated stream 2 and 3, grey circle represents vertical exchange zone turbulence, and inverted triangles represent the water surface. The principle of the design was described by Nepf and Vivoni (2000).

Outside of both experimental phases, the stream mesocosms were run in a circulation mode, where the outflowing water was pumped back to the stream inlets by centrifugal pumps and the evaporation loss was compensated with tap water. However, in contrast to other studies, the stream mesocosms were run in the flow-through mode during the tracer experiment, as well as during the entire experimental phase of the PPP experiment (13 d), with water being fed to the stream mesocosms from a water reservoir at the streams head end and subsequently discharged at the stream outlets. For both the tracer and the PPP experiment, the inflow rates were adjusted to 1.07±0.02 L/s, resulting in hydraulic retention times (HRT) of 1.18±0.05 h, given as the quotient of the stream volumes and the inflow rate.

Stock solutions from a stainless steel drum (volume = 50 L) were applied to the spillway at the stream inlets for ten minutes using a 24-channel peristaltic pump (flow rate = 797.3±0.6 mL/min per stream; Ismatec IPC 24, IDEX Health & Science GmbH, Wertheim, Germany). To enhance the homogenous distribution of the applied tracer as well as the PPP solutions within the water column, stock solutions were applied via a constant horizontal line-source injection device to the entire width of the elevated spillways (Figure A2-1). Five sampling sites (d_5 – d_{40}) were established in each stream mesocosm at 5 m, 10 m, 20 m, 30 m, and 40 m below the stream inlets (Figure 1).

Tracer experiment

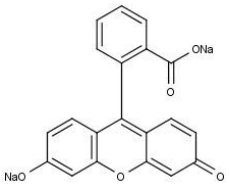
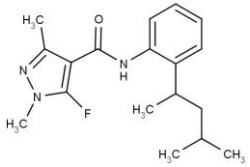
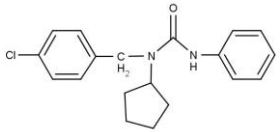
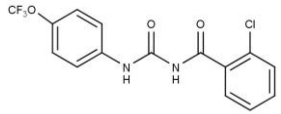
A tracer experiment was performed 2.5 d prior to the start of the PPP experiment to determine the longitudinal dispersion in each stream and to establish the appropriate sampling times for the PPP experiment. For the tracer experiment, 7,973 mL of a stock solution containing the conservative (non-sorptive) fluorescent tracer uranine (5000 µg/L; Sigma–Aldrich, Steinheim, Germany) were applied to each stream. The uranine concentrations were measured (measurement interval = 5 s) and recorded in situ using fiber-optic fluorometers (FOF, Hermess Messtechnik, Stuttgart, Germany) with fiber-optic probes placed in the middle of the water column. The recorded fluorescence intensities were subsequently converted to tracer concentrations using an external calibration.

PPP experiment

In the present study, three PPPs covering a wide range of physicochemical properties (Table 1) served as representatives for a variety of compounds that could be classified as moderately mobile or non-mobile PPPs, respectively. For stock solution preparation, analytical standards of the benzoylurea insecticide triflumuron (1-(2-chlorobenzoyl)-3-(4-trifluoromethoxyphenyl)urea), the phenylurea fungicide pencycuron (1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea) (both from Sigma–Aldrich GmbH, Steinheim, Germany), and the pyrazole fungicide penflufen (2₀-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide) (Bayer CropScience, Mohnheim, Germany) were presolved in methanol (200 ng/L; LiChrosolv_, VWR International GmbH, Darmstadt, Germany), subsequently transferred (750 µL), and mixed with MilliQ-water (Millipore, Billerica,MA, USA) in volumetric flasks (2 L), resulting in final PPP concentrations of 750 µG/L in the stock solution to obtain the target maximum concentrations of 5–10 µg/L at the first sampling site d_5. Subsequent to each

application, 15 water samples (W_1 – W_{15}) and eight sediment samples (S_1 – S_8) were collected at each sampling site in each stream mesocosm, which were supplemented by eight macrophyte samples (M_1 – M_8) from each sampling site in the vegetated stream mesocosms. To obtain accurate peak curve representations, a sampling protocol that was derived from the observations during the tracer experiment was established. According to the sampling protocol, blank samples of each compartment (W_1, M_1, S_1) were collected shortly before the arrival of the peak curve at the respective sampling site. Since the tracer experiment revealed that the period from the beginning until the end of the peak curves ranged from 23 to 41 min at the 5 m sampling sites and from 41 to 63 min at the 40 m sampling sites, nine water samples ($W_{2\text{-}10}$) were collected according to a sampling site specific sampling sequence that should enable the traceability of the respective peak curve (Figure 3). Additionally three macrophyte (M_2-M_4) and sediment (S_2-S_4) samples, respectively, were taken during the passage of the PPP peaks to illustrate potential adsorption processes of the PPPs to these compartments. Additional water samples were collected at 0.5 h post peak (W_{11}), as well as at 6, 12 and 24 h (W_{12} – W_{14}) after the start of the PPP applications. Additional water samples (W_{15}) were taken 48 h before and after the start of the second PPP application, respectively. Except for the samples collected 24 h after the start of the application, the corresponding sediment (S_5 – S_8) and macrophyte (M_5 – M_8) samples were collected simultaneously to the post peak water samples.

Table 1 Physicochemical properties of the tracer and the investigated PPPs.

	Uranine	Penflufen	Pencycuron	Triflumuron
Chemical structure				
Molecular weight (g/mol)	376.27	317.41	328.8	358.7
logKow	-0.67	3.3	4.7	4.9
K_{OC} (mL/g)	-	290 – 409	2,414 – 10,441	1,629 – 30,006
Water solubility (mg/L)	500,000	10.9	0.3	0.04
Aqueous hydrolysis (days)	-	Stable	156	Stable
Aqueous photolysis (days)	0.02	17.3	Stable	32.8

Sigma-Aldrich (2013), Kranjc (1997), European Food Safety Authority (2010-2012), Pesticide Properties Database (2013)

Water samples

Water samples were collected by immersing amber glass bottles (1 L) towards the middle of the water column. All the water samples were subsequently stored on ice until extraction in the laboratory within 48 h. Samples (1 L) were solid-phase extracted using Chromabond C18 cartridges (6 mL, 1000 mg, Macherey & Nagel, Düren, Germany) preconditioned with methanol (3 x 5 mL) and Milli-Q water (3 x 5 mL). For elution, three times 5 mL of methanol were percolated through the cartridges. Eluates were collected in 20-mL vials and evaporated until complete dryness under a gentle stream of nitrogen, before being reconstituted in 1 mL of methanol. The samples were transferred to 2-mL amber glass vials and stored at -20°C until chemical analysis was performed.

Macrophyte and sediment samples

For macrophyte sampling, stripes of *E. nuttallii* were cut just above the sediment surface. After the adherent water was drained from the macrophytes, the samples were immediately stored in aluminum bowls (Carl Roth GmbH, Karlsruhe, Germany) at -20°C. Sediment samples (approximately 28 mL) were collected by pushing aluminum cups (Carl Roth GmbH, Karlsruhe, Germany) approximately 10 mm into the sediment while aiming to preserve the sediment layer surface. The cups containing the samples were carefully lifted and transferred to aluminum bowls (Buddeberg GmbH, Mannheim, Germany), then covered with aluminum foil, and finally immediately stored at -20°C. For PPP extraction from both sample types using accelerated solvent extraction (ASE 350, Dionex Cooperation, Sunnyvale, CA, USA; Table A2-3), stainless steel extraction cells (34 mL) were loaded with 1 g of lyophilized sample and completely filled with baked out sea sand. Due to the high amount of chlorophyll as a co-extract, an additional clean up step with dispersive solid phase extraction (dSPE) was performed for the macrophyte sample extracts. The ASE extracts were evaporated under nitrogen to complete dryness and were subsequently resolved in acetonitrile (6 mL) and transferred to dSPE tubes (dSPE PSA/ENVI-Carb cleanup tube, Sigma–Aldrich, Steinheim, Germany). Cleanup tubes were shaken for 30 s and centrifuged for six minutes (3,000 rpm). The supernatant (5 mL) of each sample was finally evaporated until complete dryness and was subsequently reconstituted in methanol (1 mL). Extracts from the sediment samples were evaporated until complete dryness and also reconstituted in 1 mL of methanol. The

samples were transferred to 2-mL amber glass vials and stored at -20°C until chemical analysis was performed.

Chemical analysis

Chemical analyses were performed using an ultra high performance liquid chromatography-mass spectrometry system (UHPLC-MS; Thermo Exactive, ThermoFisherScientific, Dreieich, Germany) equipped with a Hypersil Gold C18 column (50 x 2.1 mm, particle size 1.9 µm, ThermoFisherScientific, Dreieich, Germany) according to the settings presented in Tables A2-4 and A2-5, respectively. Since adequate isotope labeled surrogate standards do not exist, matrix-matched standard solutions that were prepared out of uncontaminated blank extracts of all compartments (water, sediment, and macrophyte) were used for external calibration. For method validation of the sample preparation procedures, three replicates of uncontaminated water, macrophyte and sediment samples, respectively, were spiked with 100 µL of a solution containing 1 ng/L of each compound dissolved in methanol before being processed and finally quantified as described above. Recovery rates (±relative standard deviation) in water samples ranged from 105±5% for penflufen to 90±5% for triflumuron. Recovery rates were in a range from 75±1% for penflufen to 96±6% for triflumuron in macrophyte samples and from 84±7% for triflumuron to 96±24% for pencycuron in sediment samples. The limits of quantification (LOQ) and the limits of detection (LOD) were determined in accordance with the requirements of the german institute for standardisation (DIN) given by the DIN standard 32645 (Table 2).

Table 2 Limits of quantification (LOQ) and limits of detection (LOD) in water, macrophyte and sediment samples.

	Penflufen		Pencycuron		Triflumuron	
	LOQ[a] (ng/L)	LOD[a] (ng/L)	LOQ[a] (ng/g)[b]	LOD[a] (ng/g)[b]	LOQ[a] (ng/g)[b]	LOD[a] (ng/g)[b]
Water	4	1	3	1	4	1
Macrophyte	1	0.3	1	0.4	2	1
Sediment	1	0.3	2	1	1	0.2

a according to DIN 3264; b given as ng/g (dry weight)

Data analysis

To separate the effect of hydraulic and sorption processes on the decrease of the PPP concentrations in the vegetated stream mesocosms 2 and 3, the concentration peaks solely attributable to hydraulic processes at sampling site d_{40} were modeled on the basis of the measured PPP peak data from the sampling site d5 using a one-dimensional solute transport model for streams and rivers (OTIS), which is freely available from the U.S. Geological Survey and described in detail by Runkel (1998). An extension of OTIS, the non-linear least square fit routine OTIS-P, was applied to estimate the longitudinal dispersion coefficient D, the mean storage zone cross-sectional area A_s and the storage zone exchange coefficient α, with the mean flow velocity u, given as the mean peak flow velocity. Although OTIS provides the possibility to take into account the lateral inflow, as well as sorption and decay processes, respectively, we focused on modeling the temporal distribution of solute concentrations given by advective transport, transient storage, and longitudinal dispersion.

$$\frac{\partial C}{\partial t} = -u\frac{\partial C}{\partial x} + \frac{1}{A}\times\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \alpha\times\left(C_S - C\right) \qquad (4)$$

at sampling sites d_{40}. In Equation 4 and Equation 5, A denotes the cross sectional area, x the longitudinal axis, C the concentration in the main flow and C_s the concentration in the storage zone of the stream mesocosms.

$$\frac{\partial C_S}{\partial t} = \alpha\times\frac{A}{A_S}\times\left(C - C_S\right) \qquad (5)$$

According to the OTIS modeling procedure (Runkel, 1998), the sections between adjacent sampling sites were defined as reaches ($r_{5\text{-}10}$, $r_{10\text{-}20}$, $r_{20\text{-}30}$, $r_{30\text{-}40}$), which in turn were subdivided into computational segments (each with a length of 0.1 m). For parameter estimation, the tracer distributions at sampling sites d_5, d_{10}, d_{20}, and d_{30} were used as input data and represented the upstream boundary condition of the respective reach, while the downstream boundary condition was implemented as a fixed zero dispersive flux between the next-to-last and the last segment downstream of the evaluated reach $r_{30\text{-}40}$. An undiscovered recording error during the tracer experiment resulted in sampling times mismatching the PPP peak concentrations at the sampling site d_{40} in the unvegetated stream 1. Hence, OTIS was applied to model the PPP peak curves at the sampling site d_{40} by setting the measured peak data from the nearest sampling site d_{30} as the upper boundary condition. Because wind gusts affected the second PPP application in the unvegetated stream

mesocosm, as noted above, the samples that were collected subsequent to this application were not used in further data analyses.

The masses of the compounds that remained in the aqueous phase were determined by the integral of the concentration distribution at the sampling sites (d_x) as a function of sampling time (t). In Equation 6, W_i is the PPP concentration (µg/) in water samples W_1 – W_{11} of all streams, as well as W_1 – W_{12} of the densely vegetated stream 3, t_{Wi} is the sampling time after the start of the respective application, and Q is the discharge rate (L/s).

$$\int_{W_1}^{W_{11/12}} f(d_x,t)dt = \sum_{i=1}^{11/12}\left(\frac{W_i + W_{i+1}}{2}\right)\times\left(t_{W_{i+1}} - t_{W_i}\right)\times Q \qquad (6)$$

The mass recovery rates ($\mathrm{REC}_{d_{40}}$) in the aqueous phase at sampling site d_{40} (Equation (7)) were determined for sampling times W_1 – W_{11}, or W_{12}.

$$REC_{d_{40}}(\%) = \left(\frac{\int_{W_1}^{W_{11/12}} f(d_{40},t)dt}{\int_{W_1}^{W_{11}} f(d_5,t)dt}\right)\times 100 \qquad (7)$$

The mass recovery rates in macrophytes (REC_{Macro}) throughout the vegetated stream mesocosms (Equation 8) were calculated on the basis of the total macrophyte biomass (M_{Macro}) and the average maximum PPP concentrations in the macrophyte samples at sampling sites d_5 – d_{40} ($C_{max_{d_i}}$; given as ng g_{-1} dry weight).

$$REC_{Macro}(\%) = \left(\frac{M_{Macro}\times\left(\frac{C_{\max_{d_5}} + \cdots + C_{\max_{d_{40}}}}{5}\right)}{\int_{W_1}^{W_{11}} f(d_5,t)dt}\right)\times 100 \qquad (8)$$

Results and Discussion

Tracer results

The tracer experiment indicated that concentration peaks were attained within 66.7, 70.4, and 65.5 min at the 40-m sampling site in streams 1, 2, and 3, respectively, corresponding to mean flow velocities of 1.15±0.13, 1.07±0.15 and 1.22±0.10 cm/s. In addition, fluorescence signals reached background noise levels within a maximum of 100 min after the start of the

application in all streams; thus the tracer can be assumed to have entirely passed the streams at this time.

Figure 4a illustrates the reduction of the maximum tracer concentrations along each of the stream mesocosms. The tracer experiment indicated the highest peak reduction (51%) in the sparsely vegetated stream 2. The maximum peak concentrations in the unvegetated stream 1 and the densely vegetated stream 3 were reduced by 21% and 35%, respectively. These findings are in accordance with the averaged dispersion coefficients (D) that were estimated for each stream using OTIS-P and indicated that the highest dispersion prevailed in the sparsely vegetated stream 2 (D = 0.55 ± 0.19 dm2 s_1), followed by the densely vegetated stream 3 (D = 0.20 ± 0.03 dm2 s_1) and the unvegetated stream 1 (D = 0.13±0.08 dm2/s). Furthermore, parameter estimation using OTIS-P indicated that according to Eqs. (1) and (2) transient storage by dead zones (As < 2.0 x 10-4 m2; α < 2.7 x 10-5/s) in all stream mesocosms was negligible during the tracer experiment. Although the current experiments, as indicated by the Reynolds number (Re = 864; Equation A1-1), were performed in the transition between laminar (Re ≤ 500) to fully turbulent flow (Re >> 500) the peak reductions in all streams were mainly attributable to longitudinal dispersion. However, the only variable parameter in the experimental setup was the vegetation density of the stream mesocosms (Table A2-1). Hence, longitudinal dispersion in the unvegetated stream 1 was predominantly caused by velocity shear and turbulent diffusion (Rutherford, 1994). As indicated by the higher dispersion coefficients, longitudinal dispersion in the vegetated streams 2 and 3 was increased in comparison to the unvegetated stream 1 due to the presence of macrophytes. However, comparing the two vegetated streams the dispersion coefficient was lower at the higher density of the macrophyte coverage, indicating that different mixing processes may have been responsible for the longitudinal dispersion. Hence, we assume that the spatial distribution of the vegetation (Figure 2 a – c) affected the turbulence characteristics and thus the dispersion processes in the vegetated stream mesocosms. The vegetation in the sparsely vegetated stream 2 merely protruded to the middle of the water column, and the upper layer of the water column was free of vegetation. According to (Nepf and Vivoni, 2000), this pattern of vegetation distribution led to the formation of two flow zones, a longitudinal exchange zone and a vertical exchange zone. Wake turbulence behind the stems and leaves of the macrophytes amplified the dispersion within the longitudinal exchange zone, whereas the formation of the turbulence at the transition between the macrophyte canopy and the

free-floating water layer increased the vertical exchange between the two flow zones and thus additionally amplified the dispersion (Figure 2b). However, in the densely vegetated stream 3, the vegetation extended into the entire water column, resulting in only the stem and leaf wake turbulence being the causes of amplified dispersion along the investigated stream mesocosm (Figure 2c).

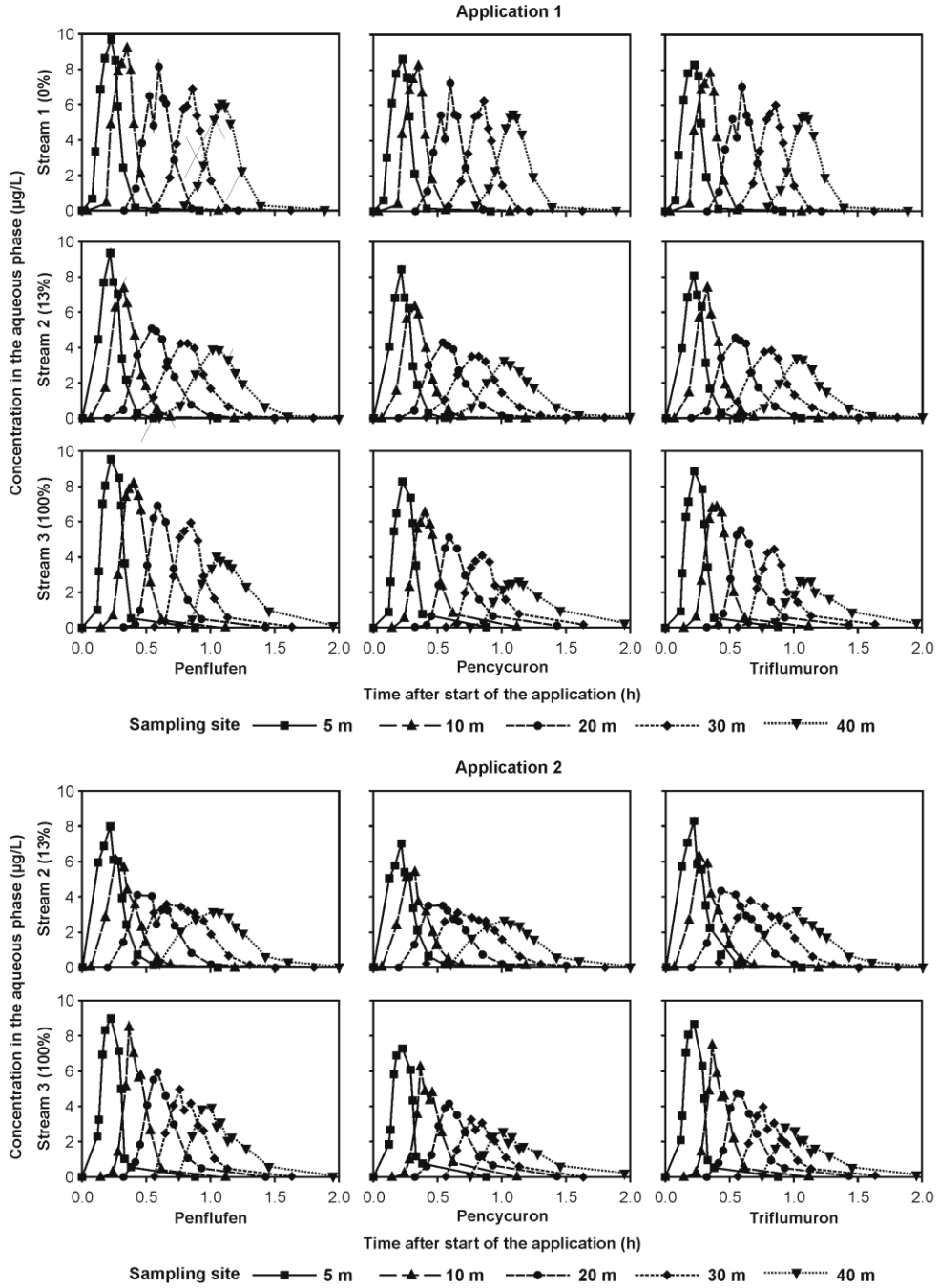


Figure 3 Peak curves of penflufen, pencycuron and triflumuron in the unvegetated stream 1, the sparsely vegetated stream 2 and the densely vegetated stream 3. Due to the disturbance of application 2 in stream 1 by wind gusts, no peak curves are presented for this application and stream.

Peak reduction of the PPPs

The average measured maximum peak concentrations at the first sampling site (d_5) were 8.5±0.8 µg/L in all stream mesocosms during both PPP applications (Figure 3). The peak reduction patterns (Figure 4b – d) in the unvegetated stream 1 and the sparsely vegetated stream 2 during both PPP applications were similar to those observed in the tracer experiment (Figure 4a). However, the peak reduction patterns in the densely vegetated stream 3 differed markedly from the results of the tracer experiment and, in addition, differed between compounds. Figure 4b – d shows the peak reduction in the densely vegetated stream 3 increases with the compounds octanol–water partitioning coefficient (K_{OW}). The peak concentration of the least lipophilic penflufen ($logK_{OW}$ = 3.3) in stream 3 at the 40-m sampling site was reduced by 57% and hence close to the peak reduction level of the same compound in stream 2. However, the peak concentrations of pencycuron ($logK_{OW}$ = 4.7) and triflumuron ($logK_{OW}$ = 4.9) were reduced by 67% and 69%, respectively, in stream 3 and thus to levels below those of both compounds in the sparsely vegetated stream 2.

Figure 5 illustrates the comparison of the peak reduction levels solely attributable to dispersion predicted with OTIS and the empirically determined peak reductions of all compounds at the sampling site d_{40} in all the stream mesocosms. In the unvegetated stream 1, the observed peak reduction is only attributable to longitudinal dispersion. However, in the vegetated streams 2 and 3, the advection–dispersion model predicted reduced peak reductions compared to those effectively measured. On the basis of the modeled maximum peak concentrations at sampling site d_{40}, 91±2% of the measured peak reductions of the PPPs in the sparsely vegetated stream 2 were attributed to dispersion (Figure 5). As already assessed in the tracer experiment, dispersion processes in the densely vegetated stream 3 were less pronounced than in the sparsely vegetated stream and accounted merely for 71%, 63% and 59% of the measured peak reduction of penflufen, pencycuron and triflumuron, respectively (Figure 5). Other studies also described the influence of dispersion on the reduction of PPPs in VTSs. Kröger et al. (2009) concluded that vegetation in the Mississippi Delta drainage ditches (USA), for example, effectively increased turbulence and thus was beneficial for pollutant mitigation.

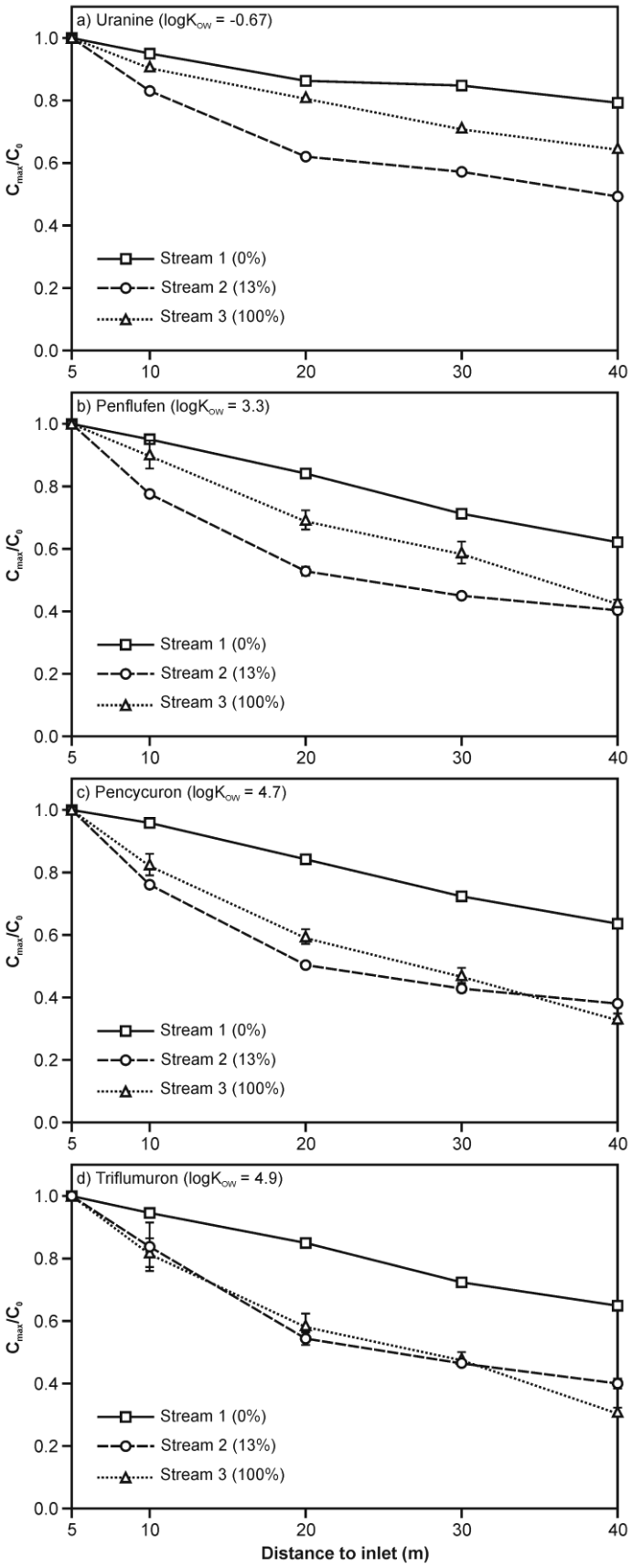


Figure 4 (a–d) Decline in maximum peak concentrations of the tracer and all three plant protection products in stream mesocosms differing in vegetation density (%) with increasing distance to the inlet. Error bars display the difference in maximum concentrations of both PPP-applications (n = 2).

A study (Elsaesser et al., 2011) in the Lier wetland (Norway) attributed the higher peak reductions in vegetated wetland cells mainly to dispersion induced by dense vegetation. Studies that highlighted and quantified the influence of the varying vegetation density on the alteration of the dispersion processes and thus on the peak reduction of the PPPs in flow-through VTSs are beyond the knowledge of the authors.

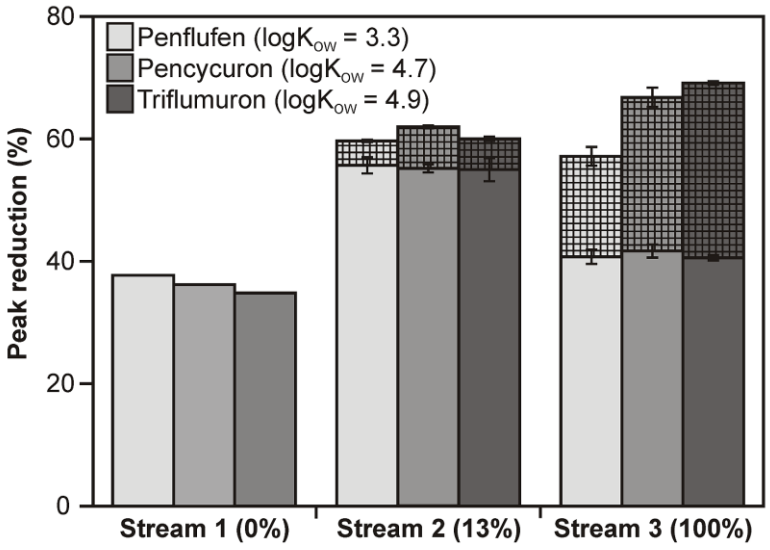


Figure 5 Contributions of dispersion and reversible sorption to macrophytes on the peak reduction in all stream mesocosms. Open bar sections represent dispersion and shaded bar sections represent sorption. Error bars represent the difference between the fractions of both PPP applications (n = 2).

However, as already discussed, the dispersion processes were identified as a major factor for the concentration reduction in the stream mesocosms, although the effect of other processes beside dispersion on the peak reduction of the applied PPPs increased with vegetation density. In other streams with higher flow velocities, flow depths and hydraulic roughness, respectively, or in streams with increased turbulence and reduced contact times between aquatic macrophytes and dissolved contaminants, longitudinal dispersion may be the predominant process influencing the reduction of contaminant peaks, whereas the influence of adsorption processes may be diminished. Nevertheless, the majority of studies in VTSs attributed the reduction of the PPP exposure levels to mass retentions through sorption processes either to macrophytes or to the sediment (Stehle et al., 2011).

Mass retention of the PPPs

In our stream mesocosm system, macrophytes and sediment were identified as the main potential sinks for PPPs. Due to a HRT of merely 1.18±0.05 h in combination with the

compounds half-life times (DT_{50}) for aqueous photolysis and hydrolysis of more than 17.3 and 156 d (Table 1), respectively, other processes that may have influenced the retention within the stream mesocosms are thus considered to be negligible.

The concentrations of all the PPPs in the macrophyte and sediment samples collected shortly before the start of both PPP applications were below the LOD (Table 2). With the passage of the PPP peaks via the aqueous phase, the PPP concentrations in the macrophyte samples from both vegetated streams increased rapidly, resulting in maximum concentrations ranging from 61 to 89, 111 to 171, and 163 to 214 ng/g (dry weight) for penflufen, pencycuron, and triflumuron, respectively, during both PPP applications. However, with the decrease of the PPP concentrations in the aqueous phase, a time-delayed decline of the PPP residues in the macrophyte samples was observed (Figure 6). Six hours after the start of both PPP applications, residues of pencycuron (3 – 5 ng/g) and triflumuron (3 – 9 ng/g) were still detected in the macrophyte samples at the 40-m sampling sites. In the simultaneously collected water samples, the concentrations of all the PPPs were below the LOQ in the unvegetated stream 1 and the sparsely vegetated stream 2. On the contrary, in the vegetated stream 3, the concentrations of pencycuron and triflumuron were still detected at levels on the order of 0.01 µg/L at the 40-m sampling site six hours after both PPP applications. However, the penflufen concentrations were below the LOQ in all of these water and macrophyte samples. Only very small amounts of PPPs (Table A2-6) were found in the sediment samples and thus were not considered further.

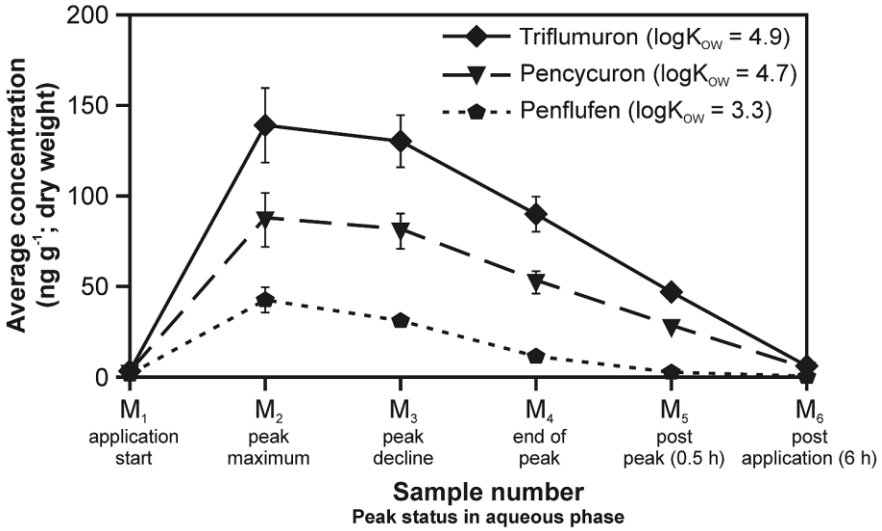


Figure 6 Average concentrations of penflufen, pencycuron and triflumuron in macrophyte samples taken from the densely vegetated stream 3 within six hours after the start of each application. Error bars display the standard error of the average PPP-concentrations (n = 10).

The initial, as well as the overall mass recovery rates (Table 3) were determined on the basis of the PPP concentrations measured in the aqueous phase (Equation 7) and in macrophyte samples (Equation 8). Relating to the initial mass recoveries, this approach aimed to quantify the amount of the applied PPPs that, on the one hand, passed the stream mesocosms in the aqueous phase within 30 min after the peak curve had passed the sampling site d40 and, on the other hand, the amount that was initially retained by the macrophytes. Beyond that, the overall mass recoveries for the densely vegetated stream 3 (Table 3) were assessed to ascertain whether the decrease of the PPP concentrations in the macrophytes at sampling timeW12 was attributable to degradation or desorption processes. A determination of the overall mass recoveries in the unvegetated stream 1 and the sparsely vegetated stream 2 could not be accomplished because the concentrations in the aqueous phase were below the LOQ at sampling time W12. For example, the initial mass recovery calculations on the basis of water samples collected at the 40-m sampling site in the densely vegetated stream 3 indicated that 75%, 82% and 97% of the applied amount of triflumuron, pencycuron, and penflufen, respectively, remained in the aqueous phase. In addition, 27%, 17%, and 8% of the applied masses of triflumuron, pencycuron, and penflufen, respectively, were measured in macrophytes, resulting in total initial recovery rates ranging from 99% to 104%. Due to the lower macrophyte biomass in the sparsely vegetated stream 2, a shift in the recovery rates between the aqueous phase and the macrophytes was observed (Table 3). In the unvegetated stream 1, 99 – 101% of the applied amounts of PPPs were recovered in the aqueous phase. However, the overall mass recovery calculations in the densely vegetated stream 3 indicated that six hours after the start of the application, the proportion of the applied amount of the PPPs in the macrophytes decreased to a maximum of 1% (Table 3). Simultaneously, the recovery rates in the aqueous phase increased to values from 96% for triflumuron to 108% for pencycuron during the same period of time.

The current findings demonstrate that sorption to macrophytes initially led to the reduction of the dissolved PPPs. As a result, the degree of sorption increased with the compounds $\log K_{OW}$ and thus followed the same pattern as already identified for the peak reduction. Thus, the sorption to macrophytes is, in addition to dispersion, the second prominent process in the vegetated stream mesocosms causing a reduction of the peak concentrations of the applied PPPs.

Table 3 Initial mass recovery rate (0.5 h after the passage of the peak) in the unvegetated stream 1 and mean initial mass recovery rates in the vegetated streams 2 and 3 (±min/max; n = 2); mean overall mass recovery rates (six hours after the start of the application) in the densely vegetated stream 3 (±min/max; n = 2).

			Triflumuron	Pencycuron	Penflufen
Initial mass recovery (%)	Stream 1 (0%)	Aqueous phase	99.4	100.9	99.2
	Stream 2 (13%)	Aqueous phase	91.4 (±2.2)	92.9 (±2.8)	96.0 (±1.7)
		Macrophytes	3.1 (±0.2)	2.5 (±0.3)	1.1 (±0.1)
		Total recovery	94.5	95.3	97.1
	Stream 3 (100%)	Aqueous phase	75.1 (±0.1)	81.7 (±0.2)	96.9 (±0.4)
		Macrophytes	27.0 (2.0)	16.5 (±2.2)	7.9 (±0.1)
		Total recovery	102.2	99.0	104.4
Overall mass recovery (%)	Stream 3 (100%)	Aqueous phase	95.9 (±8.0)	108.0 (±0.4)	104.0 (±4.5)
		Macrophytes	1.2 (±0.1)	0.7 (±0.0)	0.0 (±0.0)
		Total recovery	97.2	108.7	104.0

These findings are in correspondence with a variety of studies that attributed the peak reduction in VTS with sorption of the PPPs to aquatic vegetation. A study (Dabrowski et al., 2006) using azinphos-methyl ($\log K_{OW}$ = 2.96) in a vegetated stream (average velocity = 0.08 m/s) in the Western Cape, South Africa, also indicated macrophytes to be a sink, resulting in an overall peak reduction of 69% attributed to sorption to macrophytes. In a vegetated agricultural drainage ditch in California, USA, 32% and 15% of chlorpyrifos ($\log K_{OW}$ = 4.7) and permethrin ($\log K_{OW}$ = 6.1), respectively, were found to be associated with ditch plant material, and thus contributing to the decrease of the chlorpyrifos and permethrin concentrations (Moore et al., 2011). Moore et al. (2001) investigated the transport and fate of atrazine ($\log K_{OW}$ = 2.7) and lambda-cyhalothrin ($\log K_{OW}$ = 6.9) in an agricultural drainage ditch in Mississippi, USA, and found 61% and 72% of the measured atrazine and lambda-cyhalothrin masses, respectively, to be associated with plant material one hour after the initiation of a simulated storm runoff event. In addition, 24 h after the simulated storm

runoff event, 59% and 97% of the applied atrazine and lambda-cyhalothrin, respectively, were found in plants. However, contrary to the findings of the present study, those studies made no statements regarding the durability of sorption, or described sorption to macrophytes to be consistent over time. The results of the present study indicate that the PPP concentrations initially increased rapidly in the macrophyte samples, but slowly decreased to values below the LOQ within a maximum of 12 h after the start of the applications. In conjunction with the decrease of PPP concentrations in macrophytes, increasing mass recovery rates in the aqueous phase were observed (Table 3). Thus, these observations are assumed to represent a delayed, PPP desorption kinetic from the macrophytes after the PPP concentrations in the aqueous phase had decreased subsequent to the passage of the peak. A study (Schulz et al., 2003a) of azinphos-methyl in a vegetated flow-through wetland in South Africa, assessed a mass retention of 61% within the wetland, of which 10.5% was associated with macrophytes, also decreased rather quickly within 12 h. Furthermore, the authors hypothesized that the loss of azinphos-methyl was attributed to volatilization, photolysis, hydrolysis, or metabolic degradation. Because both the initial and the overall mass recovery rates of the present study were well-balanced, these processes did not affect the fate and thus the reduction of peak concentrations for the three PPPs investigated.

Conclusion

The present study identified the role of *E. nuttallii* in determining two fundamentally different processes whose interaction promoted the PPP peak reduction in a vegetated stream mesocosm. The submerged macrophytes in the vegetated stream mesocosms amplified the dispersion and hence the peak reduction in comparison to the unvegetated stream mesocosm. Furthermore sorption processes caused an additional contribution to the peak reduction. Although in the densely vegetated stream mesocosm, where the macrophytes extended throughout the entire water column, dispersion was diminished most likely due to less shear velocity and turbulence in comparison to the sparsely vegetated stream mesocosm, the contribution of sorption processes on the peak reduction increased. Compound specific adsorption processes of the PPPs to the macrophytes were determined in all vegetated stream mesocosms. The extent of the initial PPP mass retention was, however, inverted compared to the peak reduction as a result of dispersion. In addition, PPP adsorption to the macrophytes was assessed to be a reversible process that entailed a time-

delayed kinetic desorption after the PPP peaks had passed the stream mesocosms. However, for the extrapolation of the current findings to other macrophyte species with different morphological and chemical characteristics further research on how macrophyte characteristics influence turbulence and adsorption processes is needed.

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A.3 Experiments in water-macrophyte systems to uncover the dynamics of pesticide sorption processes in vegetated surface waters/streams

Christoph Stang[1], Nikita Bakanov[1] and Ralf Schulz[1]

[1]Institute for Environmental Sciences, University Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany

Abstract

Knowledge on the dynamics and the durability of the processes governing the mitigation of pesticide loads by aquatic vegetation in vegetated streams, which are characterized by dynamic discharge regimes and short chemical residence times, is scarce. In a static long-term experiment (48 hours) the dissipation of five pesticides from the aqueous phase followed a biphasic pattern in the presence of aquatic macrophytes. A dynamic concentration decrease driven by sorption to the macrophytes ranged from 8.3% to 60.4% for isoproturon and bifenox, respectively, within the first two hours of exposure. While the aqueous concentrations of imidacloprid, isoproturon and tebufenozide remained constant thereafter, the continuous but decelerated concentration decrease of difenoconazole and bifenox in the water-macrophyte systems used here was assumed to be attributed to macrophyte induced degradation processes. In addition, a semi-static short-term experiment was conducted, where macrophytes were transferred to uncontaminated medium after two hours of exposure to simulate a transient pesticide peak. In the first part of the experiment, adsorption to macrophytes resulted in partitioning coefficients ($\log K_{D\_Adsorp}$) ranging from 0.2 for imidacloprid to 2.2 for bifenox. One hour after the macrophytes were transferred to the uncontaminated medium, desorption of the compounds from the macrophytes resulted in a new phase equilibrium and K_{D_Desorp}-values of 1.46 for difenoconazole and 1.95 for bifenox were determined. A correlation analysis revealed the best match between the compounds affinity to adsorb to macrophytes (expressed as K_{D_Adsorp}) and their soil organic carbon-water partitioning coefficient (K_{OC}) compared to their octanol-water partitioning coefficient (K_{OW}) or a mathematically derived partitioning coefficient.

Introduction

The use of pesticides is a common practice in intensive agricultural production processes and it is beyond discussion that the field application of pesticides, among others, can result in the discharge of pesticides to non-target ecosystems, such as surface waters. Hence, efforts have been made in recent years to diminish the input of pesticides into ecosystems adjacent to agricultural areas. As a result, best management practices (BMP), such as improved application techniques, field buffers or vegetated treatment systems (VTS), were developed and partially implemented in the field (Stehle et al., 2011;Bereswill et al., 2014). Especially VTS have been proposed to be highly efficient in the mitigation and retention of pesticide loads. Hence, over the years, the retention efficiency of a variety of constructed wetlands, retention ponds or vegetated streams and drainage ditches, respectively, and a variety of other VTS have been evaluated (Reichenberger et al., 2007; Gregoire et al., 2008; Stehle et al., 2011). In all of these studies, the retention of pesticides by aquatic macrophytes and partly sediments has been postulated as one of the most important processes in VTSs. Beyond that, the ability of aquatic macrophytes to eliminate pesticides from the aqueous phase has been investigated at the laboratory (Crum et al., 1999; Olette et al., 2008), microcosm (Bouldin et al., 2005), and mesocosm scale (Moore et al., 2009). Furthermore, Passeport et al. (2011) determined high coefficients for pesticides adsorption to wetland plants and forest litter followed by compound related desorption. In another study, Hand et al. (2001) observed extensive and essentially irreversible adsorption as well as a rapid degradation of lambda-cyhalothrin in two laboratory experiment and an indoor microcosm study. A meta-analysis on the retention of pesticides in VTS (Stehle et al., 2011) revealed macrophyte coverage and the hydraulic retention time (HRT) to be crucial VTS characteristics that determine the retention performance of such systems. In particular, the macrophyte coverage was found to be closely related to the physico-chemical properties of the investigated compounds, especially the soil organic carbon-water partitioning coefficient (K_{OC}). According to Stehle et al. (2011) the K_{OC} is a critical factor governing the initial retention of pesticides of these compounds in VTS. Beyond that, Crum et al. (1999) found a better correlation between sorption of six pesticides to aquatic macrophytes and the compounds solubility in water instead of the compounds octanol-water partitioning coefficient (K_{OW}). Nonetheless, the retention of three rather hydrophilic fungicides (K_{OW} = 245 – 6,607) and two lipophilic biocides (K_{OW} = 57,544 – 125,863) by aquatic macrophytes in

vegetated stream mesocosms was found to increase with the compounds K_{OW} (Stang et al., 2013). Although the ability of aquatic macrophytes to interact with pesticides is beyond discussion and several coefficients which are generally available for pesticides have been proposed to predict the fate of these compounds in the aquatic environment, knowledge on the dynamics and the durability of the underlying chemical-macrophyte interaction processes is still very limited.

This holds true especially for vegetated streams that are mainly characterized as flow-through systems with a dynamic discharge regime and comparably short chemical residence times (i.e. transient exposure peaks). A broader understanding of the underlying processes is all the more important, since vegetated streams have been promoted as a pragmatic end-of-pipe-strategy for the mitigation of pesticide loads in receiving waters. However, there are only a few studies available that highlighted the suitability of vegetated streams and the influence of aquatic macrophytes on the mitigation of pesticide concentrations in these flowing systems (Schulz et al., 2003a; Dabrowski et al., 2006; Elsaesser et al., 2011). These studies were, thus, rather designed to generally assess the suitability of vegetated streams or wetlands for the mitigation of pesticide concentrations, than to gain a deeper understanding on how the dynamics and the persistence of sorption processes to aquatic macrophytes govern the overall retention capability of these systems. After all, there is to the best of our knowledge merely one study that not only quantified the sorption processes to aquatic vegetation, but also highlighted the persistence of these processes (Stang et al., 2014). In this study, 8 to 27% of the applied pesticides were initially retained by macrophytes. However, with the passage of the contaminant peak, the concentration in the macrophyte samples decreased rapidly, while the mass recovery rates in the aqueous phase simultaneously increased. Based on the findings of this mesocosm study, the present laboratory study was designed to gain further knowledge on the interaction between pesticides and aquatic macrophytes in water-macrophyte systems. Hence, the study encompassed two experimental approaches, a static long-term and a semi-static short-term approach, respectively. The static long-term approach aimed at the determination of the general dissipation dynamics of the investigated pesticides from the aqueous phase in the presence of three aquatic macrophyte species. The semi-static short-term approach was conducted to assess the dynamics and the consistency of sorption and desorption processes, respectively, during and subsequent to a simulated peak exposure.

Material and Methods

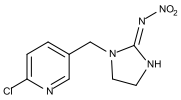
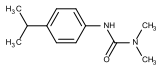
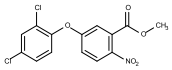
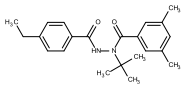
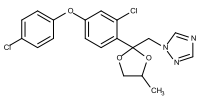
Pesticides

Five commonly used pesticides with a broad range of physicochemical properties (Table 1) were used in the present study. For stock solution preparation, analytical standards (all PESTANAL, Sigma-Aldrich GmbH, Seelze, Germany) of the insecticides imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) and tebufenozide (N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide), the herbicides isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea) and bifenox (methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate), and the fungicide difenoconazole (3-chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether) were separately dissolved in methanol (LiChrosolve, Merck KGaA, Darmstadt, Germany) resulting in concentrations of 200 ng/µL. In preparation of the experiments, 1 L of the medium (Smart and Barko, 1985) was separately spiked with 500 µL of the respective stock solution to gain a nominal pesticide concentration of 100 µg/L. The pesticide-spiked medium was subsequently stirred for 30 minutes to ensure homogenous pesticide distribution within the solution.

Macrophytes

Both experimental approaches were performed with three macrophyte species, representative for surface waters in central Europe. The western waterweed (*Elodea nuttallii*) was taken from the Queich River (49°13’09.53’’ N; 7°53’50.76’’ E) in the southwest of Germany. The rigid hornwort (*Ceratophyllum spicatum*) and the curly-leaf pondweed (*Potamogeton crispus*) were taken from ground water fed ponds in Derental (51°41’29.44’’ N; 9°25’46.24’’ E) in the north of Germany. After collection in the field, macrophytes were washed with tap water to remove deposits of sediment or particulate matter. Subsequently, macrophytes were stored in medium at 22°C and were illuminated according to a light/dark interval of 16/8 hours (6600 lx; Biolux L58/965, OSRAM GmbH, Munich, Germany) for at least one week prior to use. During collection in the field as well as during storage and the experimental phase, the macrophytes showed normal appearance and were apparently free of algae or periphyton. Chemical analyses that were performed in preparation of the experiments revealed no previous contamination with the investigated pesticides.

Table 1 Physico-chemical properties of the investigated pesticides.

	Imidacloprid	Isoproturon	Bifenox	Tebufenozide	Difenoconazole
Chemical structure					
Water solubility (mg/L)[a]	610	70.2	0.1	0.83	15
$logK_{OW}$[a]	0.57	2.5	3.6	4.25	4.36
$logK_{OC}$[a]	2.35	2.09	3.81	2.76	3.58
$logK_{D\_math}$[b]	1.39	2.0	3.03	2.42	3.17
Photolytic degradation (DT_{50}; days)[a]	Stable	1,560	265	Stable	Stable
Hydrolytic degradation (DT_{50}; days)[a]	0.2	48	2.2	Stable	Stable

a according to the Pesticides Properties Database (PPDB)

b calculated according to the formula by Crum et al. (1999)

Experimental setup

In general, all experiments were conducted in water-macrophyte systems that consisted of glass containers with a volumetric capacity of 2 L. In preparation of the experiments, the macrophytes were added to the glass jars that contained 1 L of pesticide spiked nutrient medium instantaneously before the glass jars were finally placed on a horizontal shaker (Bühler VKS 75 B control shaker, Edmund Bühler GmbH, Hechingen, Germany). The horizontal shaker was constantly operated at 55 rpm to simulate a constant water movement and thus to provide a slight circulation of the pesticide-spiked medium around the macrophytes, as it may occur in slow flowing streams. The experiments were conducted under standardized conditions (pH = 8.1±0.5) in a temperature controlled (22.5±1°C) and darkened room. The glass jars were illuminated with artificial daylight (6600 lx; Biolux L18/965, OSRAM GmbH, Munich, Germany) to provide the basis for the maintenance of photosynthetic activity to the macrophytes. While the illumination pursued a light/dark interval of 16/8 hours during the static long-term experiments, glass jars were continuously illuminated during the semi-static short-term experiments lasting six hours in total.

For the static long-term experiments, three replicates of glass jars containing 4, 8 and 16g (fresh weight) of *Elodea nuttallii*, *Ceratophyllum demersum* and *Potamogeton crispus*, respectively, were exposed to the pesticide-spiked medium and placed on the horizontal

shaker for 48 hours. Additionally, three glass jars remained free of macrophytes and served as control treatments to assess potential loss of the applied pesticides by other than macrophyte-induced dissipation or degradation processes. Throughout the experimental phase eight aqueous samples (1 mL) were taken from each glass jar at 0, 1, 2, 4, 8, 12, 24 and 48 hours ($t_{0,}$…,t_{48}) after the start of the experiment. At the end of the experimental phase the medium was decanted from the glass jars, before macrophytes were removed and immediately stored at -20°C in aluminum bowls, once remaining medium was carefully drained from the macrophytes.

The semi-static short-term experiment was designed in order to simulate a peak exposure event and thus comprised two experimental phases. During the initial sorption period, nine treatments containing 16 g (fresh weight) of *E. nuttallii*, *C. demersum* and *P. crispus*, respectively, were exposed to the pesticide-spiked medium for two hours. After this period of time, aqueous samples (t_{A2}; 1 mL) were taken from each treatment before the medium was decanted. The macrophytes were removed from glass jars and remaining medium was carefully drained. The transfer of the macrophytes to glass jars that contained 1 L of uncontaminated medium and the repositioning on the horizontal shaker, represented the start of the desorption period. To assess the desorption dynamics of the pesticides from the macrophytes, three of the total nine glass jars used per setup were removed from the horizontal shaker after one (t_{D1}), two (t_{D2}) and four hours (t_{D4}). The medium was decanted into amber glass bottles to preserve the medium for further processing. In addition, the macrophytes were immediately stored at -20°C in aluminum bowls after the drainage of remaining medium.

Chemical analyses

Aqueous samples from the static long-term experiment and the initial sorption period of the semi-static short-term experiment were immediately stored in amber glass HPLC-vials at -20°C until chemical analyses. Aqueous samples that were collected during the desorption period of the semi-static short-term experiments, however, were promptly extracted from the samples using solid phase extraction (SPE) as described in Stang et al. (2014). Briefly, samples (1L) were transferred to dropping funnels and subsequently percolated through the C18-SPE cartridges (Chromabond C18/6 mL/1000 mg, Macherey & Nagel, Düren, Germany; flow rate: 20 mL/min) previously conditioned with methanol (3 x 5 mL; LiChrosolve, Merck

KGaA, Darmstadt, Germany) and deionised water (3 x 5 mL). The eluates (3 x 5 mL of methanol) were finally evaporated until dryness, reconstituted in methanol (1 mL) and stored in amber glass vials at -20°C until chemical analyses. For the validation of the SPE procedure, three replicate samples consisting of 1 L medium were spiked with the respective pesticide and processed as described above, resulting in recovery rates ranging from 84.6±14.3% for bifenox to 106.9±4.4% for isoproturon (Table S1).

Pesticide residues in macrophyte samples from both experimental approaches were extracted by accelerated solvent extraction (ASE; ASE 350, Dionex GmbH, Idstein, Germany). For macrophyte extraction, a maximum of 1 g (dry weight) of the lyophilized samples were weighed into 34 mL extraction cells which were finally padded with cindered sea sand (Carl Roth GmbH, Karlsruhe, Germany). The extraction procedure comprised an equilibration period (5 min) and four static extraction cycles (10 min each) at 80°C with acetonitrile (LiChrosolve, Merck KGaA, Darmstadt, Germany) and acetone (SupraSolve, Merck KGaA, Darmstadt, Germany) with a ratio by volume of 50/50. Extracts were collected in amber glass vials (60 mL) and entirely evaporated under a slight stream of nitrogen, before being reconstituted in 1 mL of methanol. To eliminate potential matrix effects, samples were diluted (1/1000; v/v) with methanol prior to chemical analysis. For method validation, 1 g of lyophilized blank macrophyte samples (n = 3) were spiked with 1 mL of a solution that contained the investigated pesticide dissolved in methanol at a concentration of 100 µg/mL. After the methanol was entirely evaporated, samples were processed as described above to determine recovery rates and the repeatability of the entire extraction procedure (Table S1).

Chemical analyses were performed with an ultra high performance chromatographic system coupled to a mass spectrometer (UHPLC-MS; Exactive, Thermo Fisher Scientific, Dreieich, Germany) according to the method described in Stang et al. (2015; Table S2). The investigated pesticides were identified as the $[M+H]^+$-adducts as well as, in the case of bifenox, the $[M+NH_4]^+$-adduct, respectively (Table S3). The quantification of pesticide concentrations in all samples was performed by the use of an external calibration (1 – 100 ng/mL). Limits of quantification (LOQ) were determined according to the requirements of DIN 32645 (Table S3). The semi-quantitative analysis of bifenox-acid in aqueous samples was performed under the same chromatographic conditions, whereas the MS was operated in the negative ESI mode for the identification of the $[M-H]^-$-adduct. Bifenox-acid was identified as the ion with the exact mass of 325.9617 m/z and a retention time of 4.8 min (Figure S2).

The identity of the ion was confirmed by means of an analytical reference standard (Dr. Ehrenstorfer GmbH, Augsburg, Germany).

Data analyses

In order to identify potential degradation of the investigated compounds during the experimental phase, mass balances, expressed as the recovery rates, were compiled for both experimental approaches. The recovery rates (REC_{static}) for the static long-term approach were assessed according to Eq. 1, where M_0 is the initially applied amount of the respective pesticide, M_{48} is the corresponding fraction that remained in the aqueous phase at the end of the experimental phase and M_{Macro} is the fraction that was found in macrophytes.

$$\mathrm{REC}_{\mathrm{static}}(\%) = \left(\frac{\mathrm{M}_{48} + \mathrm{M}_{\mathrm{Macro}}}{\mathrm{M}_0} \right) \times 100 \quad (1)$$

Experimentally derived partitioning coefficients were calculated to describe adsorption ($logK_{D\_Adsorp}$) as well as desorption ($logK_{D\_Desorp}$) processes, respectively, during the semi-static short-term exposure scenario. In Eq. 2 and Eq. 3, C_{Macro} (µg/kg ww) is the pesticide concentration in macrophytes related to the biomass based on wet weight, C_{Desorp} (µg/L) is the pesticide concentration in the aqueous phase after the desorption period, and $C_{Aqueous}$ (µg/L) is the concentration that remained in the aqueous phase after the adsorption period.

$$\log K_{D\_Adsorp}(L/kg) = \log\left(\frac{C_{Macro} + C_{Desorp}}{C_{Aqueous}} \right) \quad (2)$$

$$\log K_{D\_Desorp}(L/kg) = \log\left(\frac{C_{Macro}}{C_{Desorp}} \right) \quad (3)$$

Correlation analyses for the experimentally derived $logK_{D\_Adsorp}$ as a function of the $logK_{OW}$, the $logK_{OC}$ and the mathematically derived $logK_{D\_math}$ (Crum et al., 1999), respectively, of the investigated compounds, were performed on the basis of the Pearson correlation coefficient using SPSS 21.0 software (IBM, Chicago, IL).

Results and discussion

Static long-term scenario

Generally, the degree of pesticide dissipation in the macrophyte treatments was found to be determined by two factors: the pesticide itself and the macrophyte biomass. The pesticide concentration in all control treatments remained stable (Figure S1) during the entire experimental phase, indicating that the influence of abiotic degradation processes, such as hydrolysis or photolysis, can be considered negligible. However, in the majority of the macrophyte treatments, irrespective of the macrophyte species present and the final degree of pesticide dissipation, the decrease of the investigated pesticides in the aqueous phase followed a similar pattern. Within the first two to four hours of exposure a compound specific dissipation dynamic was observed that resulted in an initial concentration equilibrium between the aqueous phase and the macrophytes. Subsequently, the pesticide concentrations in the aqueous phase remained either constant or the dissipation dynamics decelerated noticeably (Figure S1). Compared to the other investigated compounds, the decrease of the imidacloprid concentrations in all treatments was less pronounced and was, thus, hardly quantifiable during the entire experimental phase. The isoproturon concentrations in the treatments containing 4, 8 and 16 g of the respective macrophyte species decreased on average (mean±SE) by 2.0±2.6%, 6.0±2.7% and 8.3±2.9%, respectively, within the first two hours of exposure) and remained more or less stable (2.6±4.1%, 7.8±4.1% and 10.0±3.8%) until the end of the experimental phase (Figure 1). Also the dissipation rates of tebufenozide that were observed after two hours of exposure (6.5±4.0%, 3.6±3.6% and 5.6±6.7%) remained rather constant until the end of the experimental phase (3.7±3.5%, 5.9±4.6% and 9.8±6.3%). Furthermore, the recovery rates (REC_{static}) of imidacloprid, isoproturon and tebufenozide ranged from 93.6% to 106.9% in all treatments and the amount of residues that were found in the macrophytes largely corresponded with the observed dissipation of the respective pesticides in the aqueous phase (Figure 2). For instance, in the treatments with the highest biomass of the three macrophytes, the concentration of tebufenozide in the aqueous phase decreased on average by 9.8±6.3% after 48 hours. Simultaneously, the average concentration in the macrophytes increased and accounted for 4.7±2.3% of the initially applied amount of tebufenozide. It is therefore considered that the dissipation dynamics of these compounds followed a first-order kinetic,

since the decrease of the pesticide concentration in the aqueous phase can be attributed to sorption to the macrophytes.

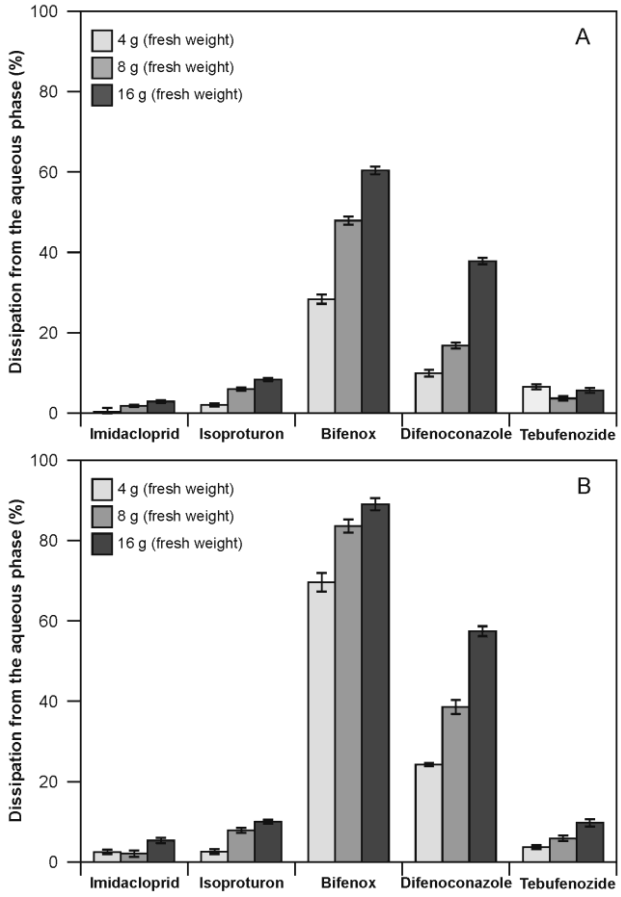


Figure 1 Average dissipation (Mean±SE) of the investigated pesticides from the aqueous phase in the presence of different biomasses of *E. nuttallii*, *C. demersum* and *P. crispus* after 2 (A) and 48 (B) hours.

However, in the difenoconazole and the bifenox treatments, the dissipation of the compounds was found to be determined by an additional process. The initial difenoconazole concentrations in the treatments containing 4, 8 and 16 g of macrophytes decreased by 9.9±5.6%, 16.8±5.1% and 37.8±5.9% (Figure 1) within two hours and resulted in biomass-related dissipation rates of 24.3±2.8%, 38.6±11.7% and 57.4±8.6% after 48 hours, respectively (Figure 1). The dissipation dynamics that were observed for bifenox were generally higher than those determined for difenoconazole and differed considerably among the three macrophyte species (Figure S1). In the treatments containing *C. demersum* and *P. crispus* the dissipation pattern was similar to the other compounds, even though resulting in higher biomass-related dissipation rates that ranged from 51.2±1.0% to 76.1±3.2% and from 70.1±1.0% to 91.2±4.0%, respectively. Furthermore, in the treatments containing *E. nuttallii*, a continuous decline of bifenox concentrations was observed. The bifenox concentration in

the treatments that contained 4 and 8 g of *E. nuttallii* decreased by 87.0±2.5% and 96.1±1.0%, respectively, after 48 hours of exposure. During the same period of time, the bifenox concentration in the treatments with the highest biomass (16 g) decreased even below the LOQ. In addition, the chemical analysis of aqueous and macrophyte samples from the difenoconazole as well as from the bifenox treatments, respectively, revealed results fundamentally different from the other investigated compounds. Indeed, there was a biomass-related concentration decrease in the aqueous phase in all macrophyte treatments, but the amount of difenoconazole and bifenox that was recovered in the aqueous phase and in the macrophytes at the end of the experimental phase did not correspond to the initially applied amount of both compounds (Figure 2).

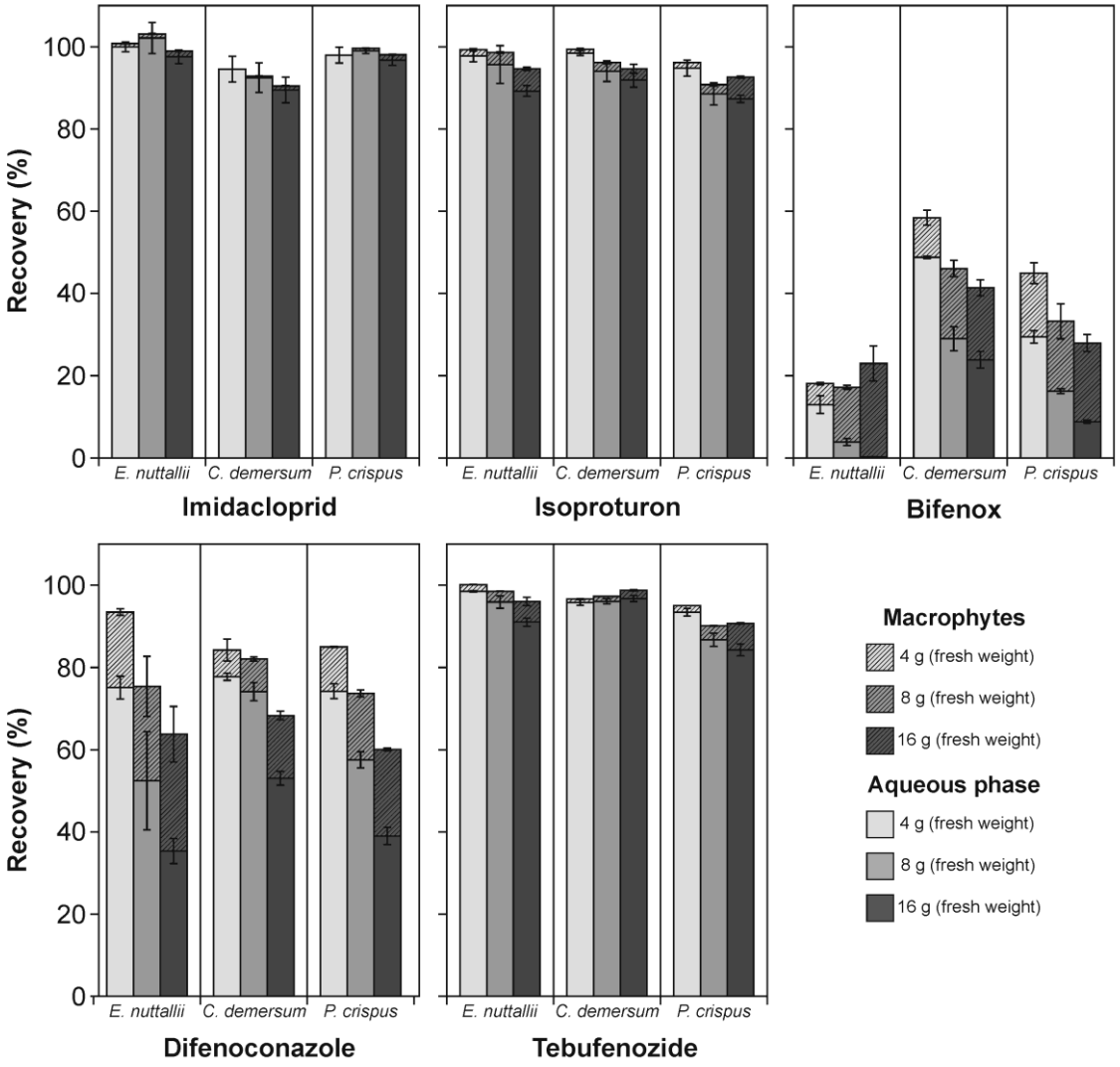


Figure 2 Cumulated recovery rates (Mean±SD) of the investigated pesticides and distribution of the remaining amount of the parent compound between the aqueous phase and the macrophytes.

In the difenoconazole treatments with the highest biomass, for instance, only 62.1% (*P. crispus*) to 70.0% (*C. demersum*) of the initially applied amount of the compound was recovered (Figure 2). Similar observations were made in the bifenox treatments, where the recovery rates (REC_{static}) in the treatments with the highest biomass merely ranged from 23.1% in the *E. nuttallii*- to 43.4% in the *C. demersum*-treatments, respectively (Figure 2). According to the observations described above, it appears that the dissipation of the difenoconazole and bifenox in the water-macrophyte systems used here is mainly dominated by two macrophyte-induced processes: sorption to macrophytes and the subsequent and continuous degradation of the parent compound. For bifenox, this assumption is in line with the rapid degradation of the compound in water-sediment systems for which a DT_{50} of 0.11 days was reported (European Food Safety Authority (EFSA), 2007) and was confirmed, since the formation of bifenox-acid was detected in all macrophyte treatments (Figure S3). Beyond that, the signal intensity of the ion that was identified as bifenox-acid increased with time in aqueous samples and was, in addition, linked to the macrophytes biomass (Figure S3). Bifenox-acid is formed in a variety of environmental matrices (EFSA, 2013) as a result of hydroxylation, a process that was also described in a study by Dosnon-Olette et al. (2011), in which the authors linked the increase of the cytochrome P450 activity with the detoxification of the fungicide dimetomorph via hydroxylation in the presence of *Elodea canadensis*. For difenoconazole no degradation products could be identified due to analytical limitations and it could thus not be clarified whether the low recovery rates (REC_{static}) in the water-macrophyte systems were attributed to macrophyte-induced degradation of the compound or if difenoconazole was bound to the macrophytes in a non-extractable manner. However, the decelerated but continuous decrease of the difenoconazole concentrations that was observed in the macrophyte treatments (Figure S1) may be regarded as an indication for the degradation of the compound. Consequently, a second-order kinetic attributed to the initial sorption and the subsequent degradation of bifenox and difenoconazole can be assumed. Similar observations were also made by Garcinuno et al. (2006), who reported mass recoveries of 57, 53 and 55% of carbaryl, linuron and permethrin, respectively, and stated that the compounds were degraded and/or bound in an irreversible manner to *Lupinus angustifolius* in a hydroponic system. Beyond that, Schulz et al. (2003a) concluded that besides sorption to living plant biomass that accounted for 10.5% of the initially retained azinphos-methyl mass,

a variety of additional degradation processes were of importance for the loss of the compound in a vegetated flow-through wetland in South-Africa.

Semi-static short-term exposure scenario

The concentration decrease until the end of the exposure period of two hours (t_{A2}) was similar to the observations that were made in the same period of time in the static long-term scenario. Whereas the average concentration decrease of imidacloprid (not quantifiable), isoproturon (7.0±6.4%) and tebufenozide (4.6±3.7%) was less pronounced, the difenoconazole and the bifenox concentrations in the aqueous phase decreased significantly, resulting in an average rate of decrease ranging from 38.7±8.0% to 69.4±7.0%, respectively. Simultaneously, the concentration of the investigated compounds in the macrophytes increased proportionally. However, after the macrophytes were transferred to the uncontaminated medium (t_{A2}), the concentration of the investigated pesticides in the aqueous phase increased rapidly accompanied by a closely coupled decrease of the pesticide concentration in the macrophytes (Figure 3). For instance, the average concentration of isoproturon in the aqueous phase at t_{D1} corresponded to 6.9±1.2% of the initially applied amount of the pesticide and thus to the concentration decrease measured at t_{A2}. Similar observations were made for imidacloprid and tebufenozide, of which 2.6±0.2% and 7.9±4.9%, respectively, of the initially applied amounts of the compounds were recovered in the aqueous phase at t_{D1}, while the concentrations in the macrophyte samples simultaneously decreased below the LOQ. These observations illustrate that a new phase equilibrium between the macrophytes and the aqueous phase was already established after this period of time. The transfer of the macrophytes from the difenoconazole and the bifenox treatments, respectively, to the uncontaminated medium resulted in a different water/macrophyte distribution of the compounds. Indeed, the concentration in the aqueous phase increased also rapidly within the first hour of the desorption period, but there was also a considerable amount of both pesticides that remained in the macrophytes (Figure 3). At the end of the adsorption period (t_{A2}), a distribution ratio of 2.1±0.5 between the aqueous phase and the macrophytes was assessed for difenoconazole, which resulted in an experimentally derived partitioning coefficient ($logK_{D\_Adsorp}$) of 1.45±0.10 (Table S4).

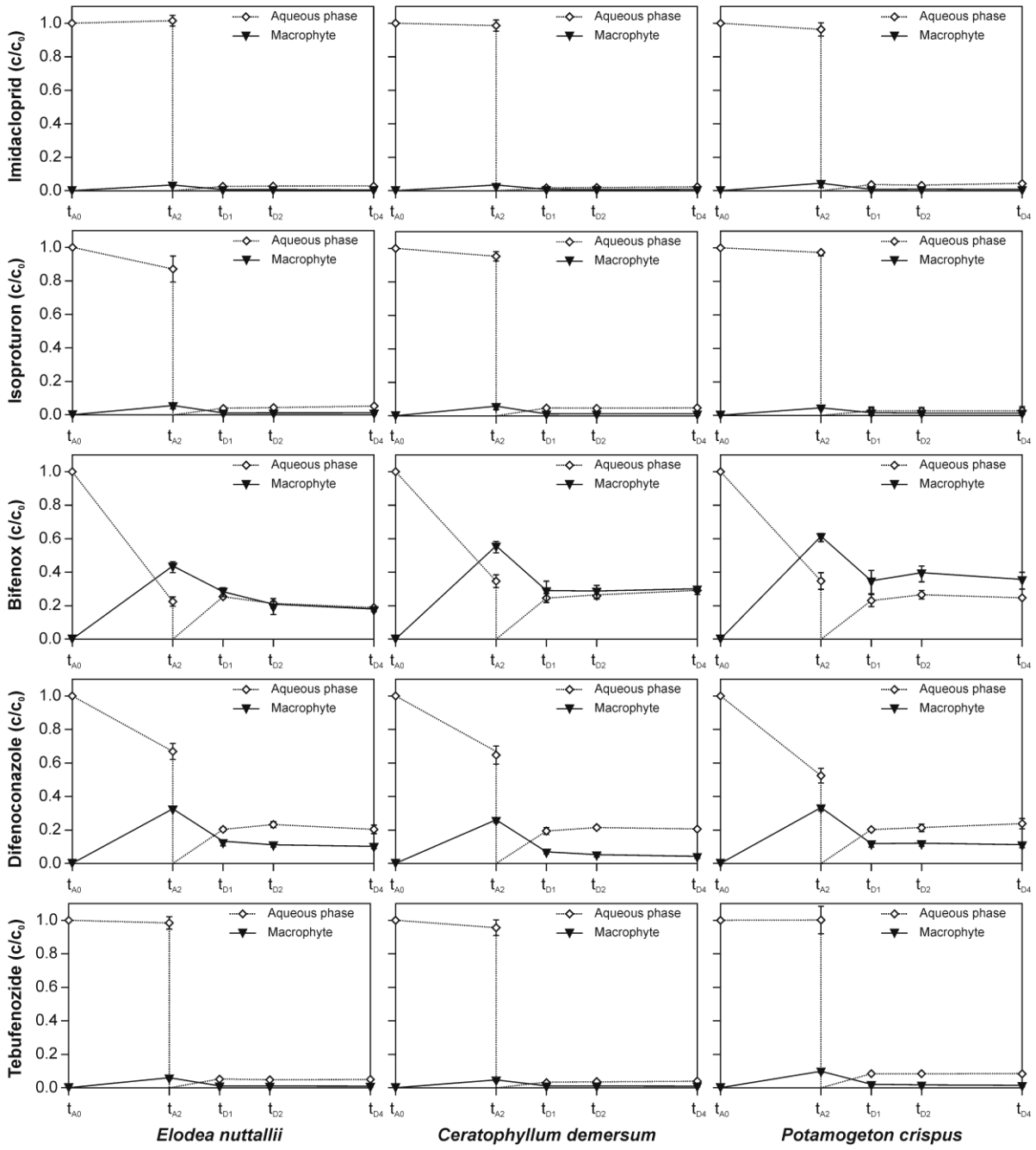


Figure 3 Temporal gradient of the pesticide concentration (Mean±SD) in the aqueous phase and in macrophytes. Dotted lines display the concentration in the aqueous phase, continuous lines display the concentration in macrophytes; tA0 = start of the experiment, tA2 = end of the sorption period after 2 hours and transfer of macrophytes into uncontaminated medium, tD1, D2, D4 = time (D1 = one hour, D2 = two hours, D4 = four hours) after the transfer of the macrophytes to uncontaminated medium.

After the macrophytes were transferred to uncontaminated medium, a similar average water/macrophyte ratio of 2.2±0.6 as well as a similar $\log K_{D\_Desorp}$ of 1.46±0.11 were determined already at t_{D1} and remained stable until the end of the desorption period. In the bifenox treatments, the transfer of the compound from the macrophytes into the aqueous phase occurred just as fast as in the difenoconazole treatments, even though the

distribution patterns between both matrices differed markedly. In the treatments containing *E. nuttallii*, a water/macrophyte distribution ratio of 0.4±0.1 and a $logK_{D\_Adsorp}$ of 2.20±0.08 at the end of the sorption period (t_{A2}) were determined (Table S4). As already found during the static long-term experiment, a considerable decrease of bifenox was observed in both matrices indicating a rapid degradation of the parent compound. Hence, the direct comparison of the compounds´ distribution between the aqueous phase and the macrophytes during the sorption and desorption period, respectively, is hardly possible. However, the observations that were made in the bifenox treatments that contained *C. demersum* and *P. crispus* allow for drawing comparative conclusions regarding the distribution of the compound between both phases. In these treatments, the pesticide showed a stronger tendency to adsorb to the macrophytes instead of remaining in the aqueous phase. Hence, an average water/macrophyte ratio of 0.7±0.1 as well as an average $logK_{D\_Adsorp}$ of 2.13±0.06 was assessed at the end of the sorption period (t_{A2}). Although the observations from the sorption period underline that bifenox is the compound with the highest affinity to adsorb to the macrophytes, the concentration in the aqueous phase increased also rapidly after the macrophytes were transferred to the uncontaminated medium, resulting in a water/macrophyte ratio of 0.8±0.2 and a $logK_{D\_Desorp}$ of 1.92±0.08 at t_{D1}. Thus, the results indicate that adsorption/desorption processes in the water-macrophyte systems followed basic equilibrium equations, as they are already described for adsorption/desorption processes in soil.

With the currently presented results in mind and considering the conditions in the field, for instance in running surface waters where an edge-of-field runoff would lead to a pesticide peak that comes along with a dynamic concentration increase and decrease in the aqueous phase, respectively, the findings of the present study provide insights in the processes that determine the macrophyte induced mitigation of pesticide concentrations in such systems. The experiments provide knowledge on the dynamics that determine the temporal frame and the persistence of sorption and desorption processes during a short term pesticide exposure. The experiments revealed, on the one hand, that sorption is a dynamic and rapid process that is implemented, once the concentration in the aqueous phase increases and thus supports the findings of (Hand et al., 2001) who also observed rapid adsorption of lambda-cyhalothrin to aquatic plants. On the other hand, the observations confirm that sorption to aquatic macrophytes constitutes a reversible process where a concentration

decrease in the aqueous phase induces desorption to obtain a equilibrium between both phases. Hence, the present study supports the findings of a study in vegetated stream mesocosms (Stang et al., 2014), where the concentration of experimentally applied pesticides in macrophyte samples increased also rapidly and continuously with the rising of the pesticide concentration in the aqueous phase, which led to an initial mass retention of the applied pesticides ranging from 7.9 to 27.0%. In addition, the pesticide concentration in the macrophytes decreased also rapidly after the concentration maximum of the contaminant peak was reached and the pesticide concentration in the aqueous phase started to decline, resulting in pesticide concentrations below the LOQ within six hours and a well balanced recovery rate at the mesocosms outlet.

The potential impact of aquatic macrophytes on the fate and the distribution of pesticides in the aquatic environment is an undisputed fact, since a variety of authors have described the beneficial contribution of aquatic macrophytes on the retention of pesticides in surface waters or vegetated wetlands (Vymazal and Březinová, 2015). However, it is also generally accepted that the compounds affinity to adsorb to macrophytes and thus the degree of elimination from the aqueous phase is primarily governed by compound specific properties. Hence, a variety of coefficients were proposed or examined concerning the predictability of a compounds affinity to interact with aquatic macrophytes. Based on the findings of a batch equilibrium study with six pesticides, Crum et al. (1999) derived a mathematical formula that described the sorption coefficient (here K_{D_math}) as a function of the compounds solubility in water. In turn, a study by Stehle et al. (2011) identified the compounds K_{OC} as one of two pesticide specific properties that determines the best retention of the investigated pesticides in vegetated treatment systems. Besides this, mass retention of three fungicides and two biocides as a function of the compounds lipophility was observed in a study in vegetated stream mesocosms (Stang et al., 2013). Indeed, the suitability of the proposed coefficients to describe a compound´s tendency to adsorb to macrophytes is certainly not unexpected, especially since a relationship between solubility in water, lipophility and the soil adsorption coefficient is generally assumed. Also in the present study, the correlation analyses on the basis on the pesticides $\log K_{D\_Adsorp}$, derived from the findings of the semi-static short-term exposure scenario, as a function of the physico-chemical substance properties $\log K_{OW}$, $\log K_{OC}$ and $\log K_{D\_math}$ revealed that sorption of the investigated pesticides to the macrophytes was significantly correlated with all of the considered coefficients,

whereas the degree of correlation varied markedly (Figure 4). The lowest correlation of the $logK_{D\_Adsorp}$ was found for the $logK_{OW}$ (R^2 = 0.352; ρ = 0.01; n = 135), followed by the mathematically derived $logK_{D\_math}$ with a (R^2 = 0.574; ρ = 0.01; n = 135). The analyses revealed that both coefficients tended either to overestimate or to underestimate the sorption to macrophytes, especially in the upper ranges of values. For instance, for the rather lipophilic compound tebufenozide ($logK_{OW}$ = 4.25) a low $logK_{D\_Adsorp}$ of 0.72 was determined, while the less lipophilic bifenox ($logK_{OW}$ = 3.6) showed an obviously stronger tendency to adsorb to the macrophytes ($logK_{D\_Adsorp}$ = 2.15). However, the compounds $logK_{OC}$ was found to have the best predictive power (R^2 = 0.842; ρ = 0.01; n = 135) of the consulted coefficients to describe the compounds affinity to adsorb to macrophytes.

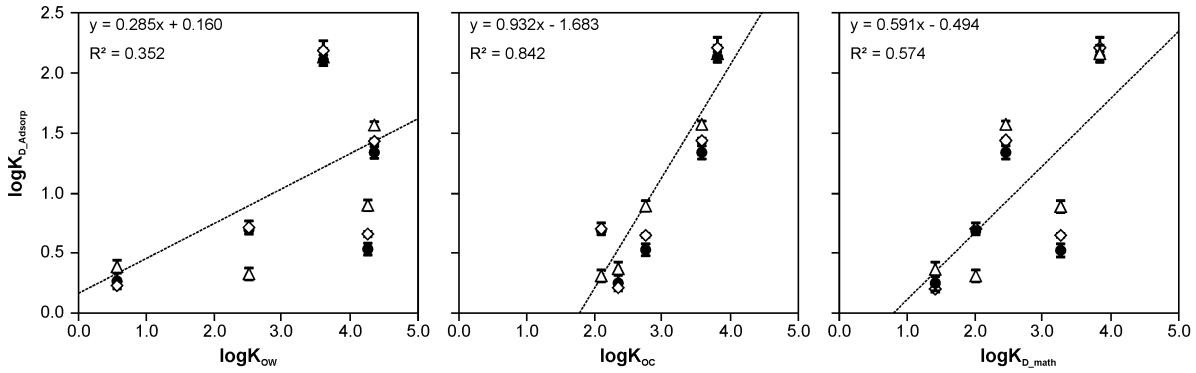


Figure 4 Correlation of the experimentally derived logKD_exp (n = 135; ρ = 0.01) of the investigated compounds at the end of the adsorption period in treatments containing E. nuttallii (open diamonds), C. demersum (black circles) and P. crispus (open triangles) with different physico-chemical properties of the investigated pesticides (logKOW, logKOC and logKD_math); for a better presentation each diamond, circle or triangle displays the logKD_exp as the mean±SD (n = 9) per pesticide.

This appears plausible when considering the function of the particular coefficients and how they are derived. While the $logK_{OW}$ displays the compounds solubility in fat, the $logK_{D\_math}$ is determined by the compounds solubility in the water. However, sorption to organic matter is, beyond others, governed by a variety of molecular as well as structural properties of a compound (Schwarzenbach et al., 2005). Hence, the sole consideration of a single property of a substance regarding the sorption to macrophytes may be misleading. In contrast, the $logK_{OC}$ is derived as a measure for the sorption of a compound to organic matter and thus its mobility in the environment (Schwarzenbach et al., 2005). Hence, the results of the present study indicate that a compounds $logK_{OC}$ is the most reliable coefficient to estimate the sorption of a pesticide to aquatic macrophytes and thus support the findings of Stehle et al. (2011). Summing up, the results of the present study are considered valuable to improve the general understanding of the interaction between aquatic macrophytes and pesticides,

especially with regard to enhance the targeted use of aquatic macrophytes within the scope of best management practices. In addition, the data presented above may be utilized to refine aquatic exposure models that are commonly used to assess the fate of pesticides in the environment.

Conclusions

The present study was performed to gain knowledge on the dynamics that govern the interaction between aquatic macrophytes and pesticides in the aqueous environment. The results of the present study demonstrate that sorption as well as desorption of pesticides to and from aquatic macrophytes, respectively, are dynamic processes that are governed by principle physico-chemical properties of the compounds. Nevertheless, it can be concluded that aquatic macrophytes can represent a temporary sink for pesticides and thus can help to mitigate pesticide loads in surface waters.

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A.4 Supplementary information for Mitigation of biocide and fungicide concentrations in flow-through vegetated stream mesocosms

Table A1-1 Transitions for the quantification (t1) and the confirmation (t2) of the investigated compounds (precursor ion/product ion).

	Transition†	
	t1 (m/z)	t2 (m/z)
Imazalil	297.1/159.0	297.1/201.0
Thiabendazole	202.1/175.1	202.1/131.1
Propiconazole	342.1/159.1	344.1/161.1
Triclosan	287.0/35.0	289.0/35.0
Triclocarban	313.0/159.9	315.0/161.9

† According to Wick et al. 2010

Table A1-2 Method recovery rates (± 95% confidence interval, n = 4) of the entire analytical procedure for different matrices

	Recovery rate (%)‡		
	Water§	Sediment¶	Macrophyte
Imazalil	101±3	106±14	102±9
Thiabendazole	96±3	105±12	108±12
Propiconazole	101±4	118±19	100±3
Triclosan	102±2	101±57	121±6
Triclocarban	105±5	108±11	105±6

‡ Recovery rates are given as relative recovery rates according to Wick et al. (2010)

§ Values correspond to recovery rates for surface water in Wick et al. (2010)

¶ Values correspond to recovery rates for activated sludge in Wick et al. (2010)

Table A1-3 Maximum concentrations measured in sediment samples from all stream mesocosms.

	Concentration (ng/g)	
	Inlet	Outlet
Imazalil	16	2
Thiabendazole	13	2
Propiconazole	1	< LOQ
Triclosan	1	1
Triclocarban	8	2

Table A1-4 Limits of quantification in water, sediment and macrophyte samples for all compounds

	Limits of quantification (LOQ) #		
	Water (ng/L)	Sediment (ng/g)	Macrophyte (ng/g)
Imazalil	2	0.5	20
Thiabendazole	2	0.5	5
Propiconazole	2	0.5	10
Triclosan	2	0.5	20
Triclocarban	2	0.5	5

LOQs were determined according to Wick et al. (2010)

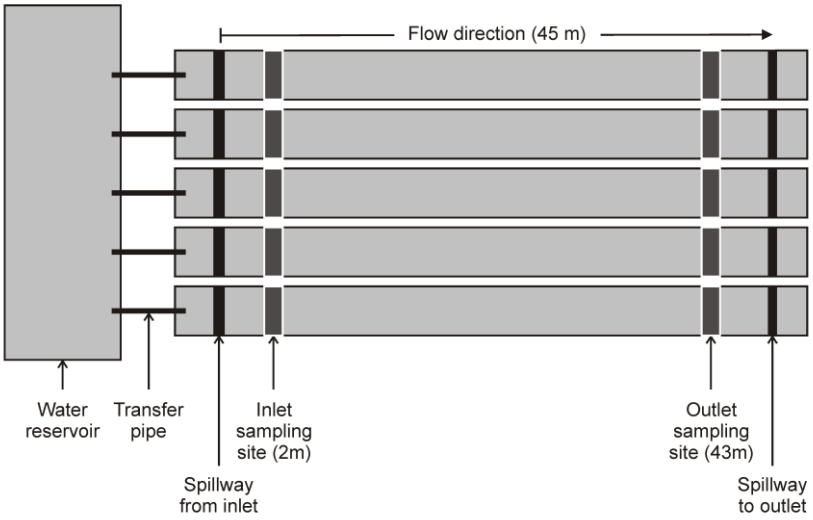

Figure A1-1 Schematic depiction of the five stream mesocosms at the Landau stream mesocosm facility used in the current experiment

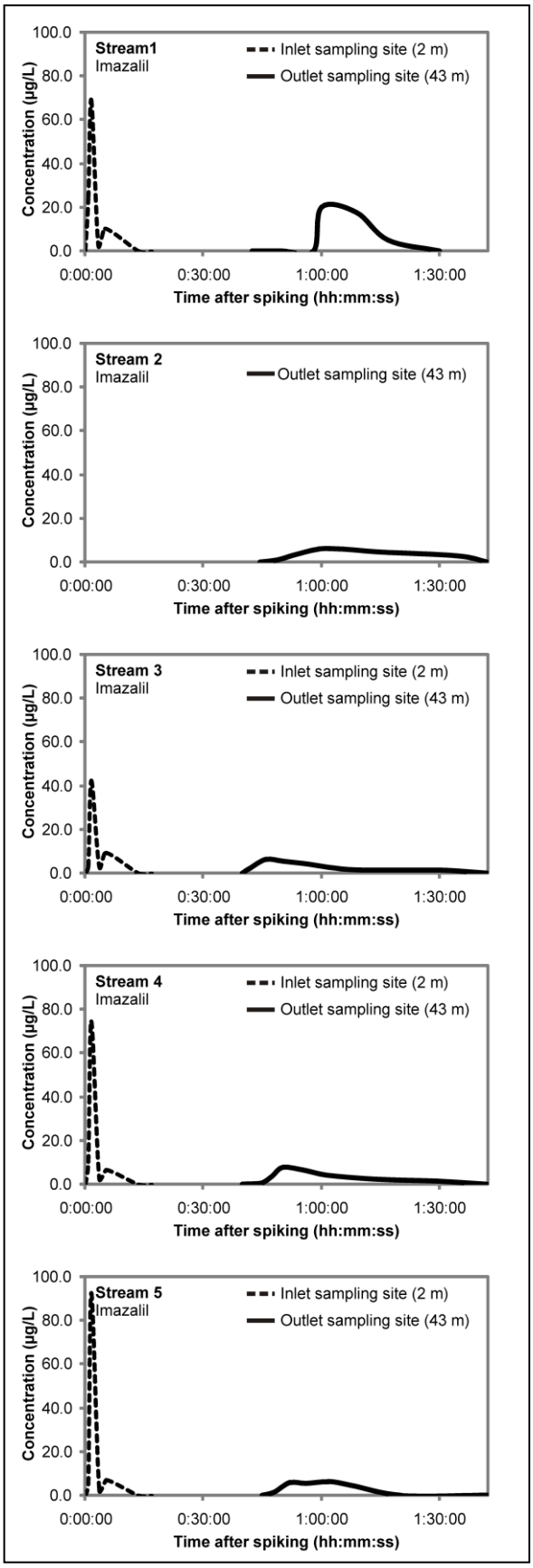

Figure A1-2 Peak curves of imazalil in all stream mesocosms

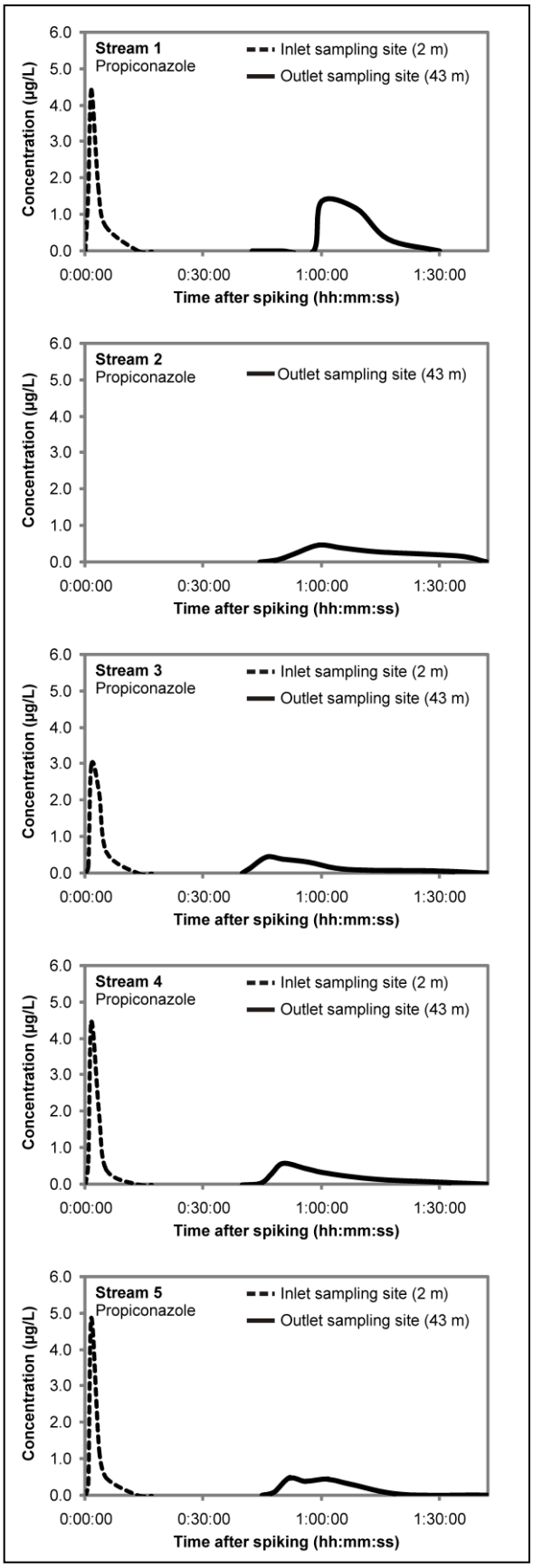

Figure A1-3 Peak curves of propiconazole in all stream mesocosms

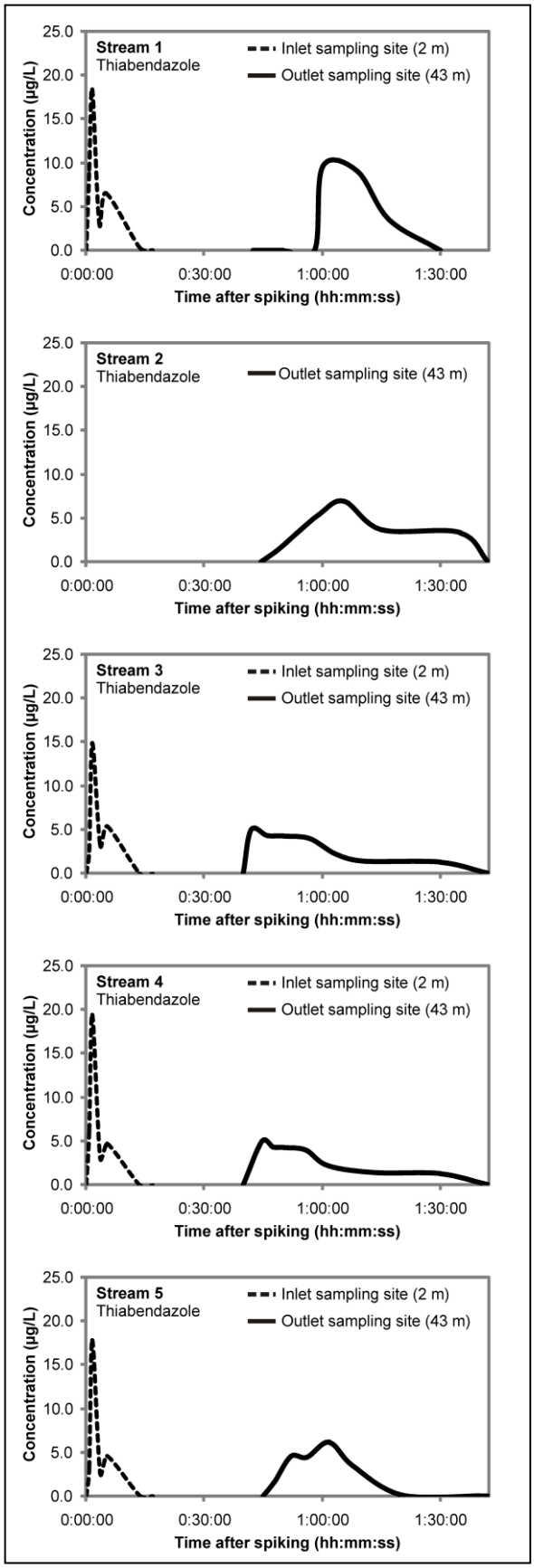

Figure A1-4 Peak curves of thiabendazole in all stream mesocosms

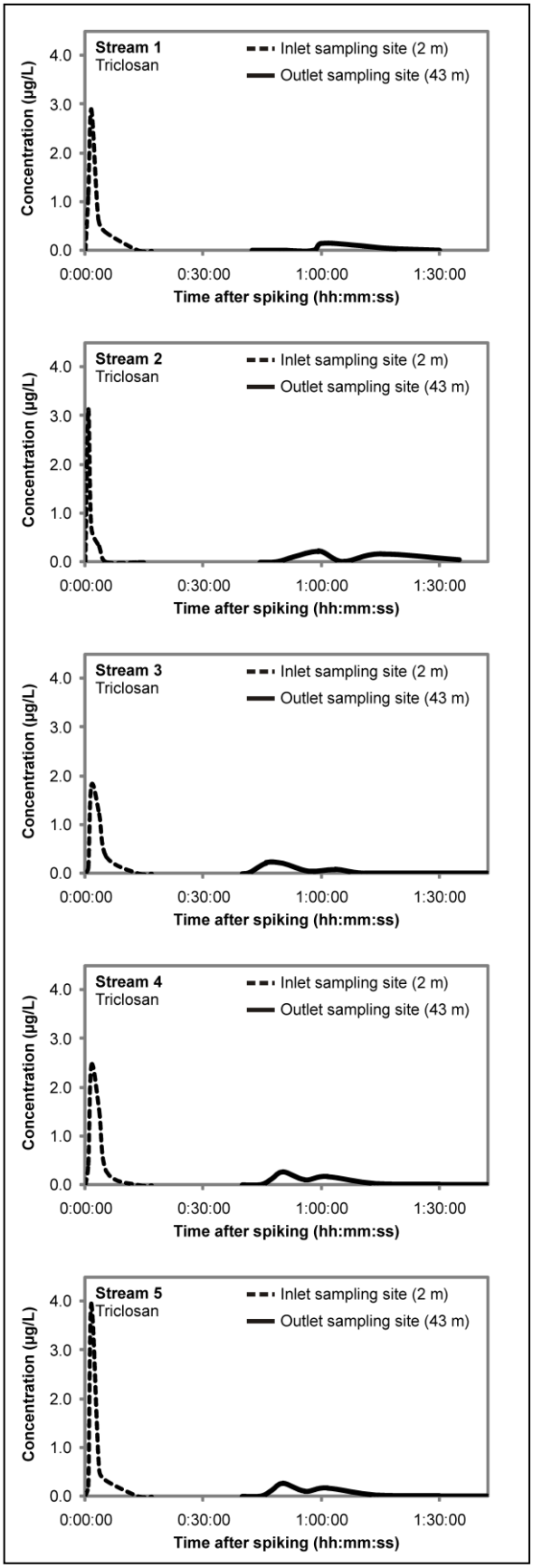

Figure A1-5 Peak curves of triclosan in all stream mesocosms

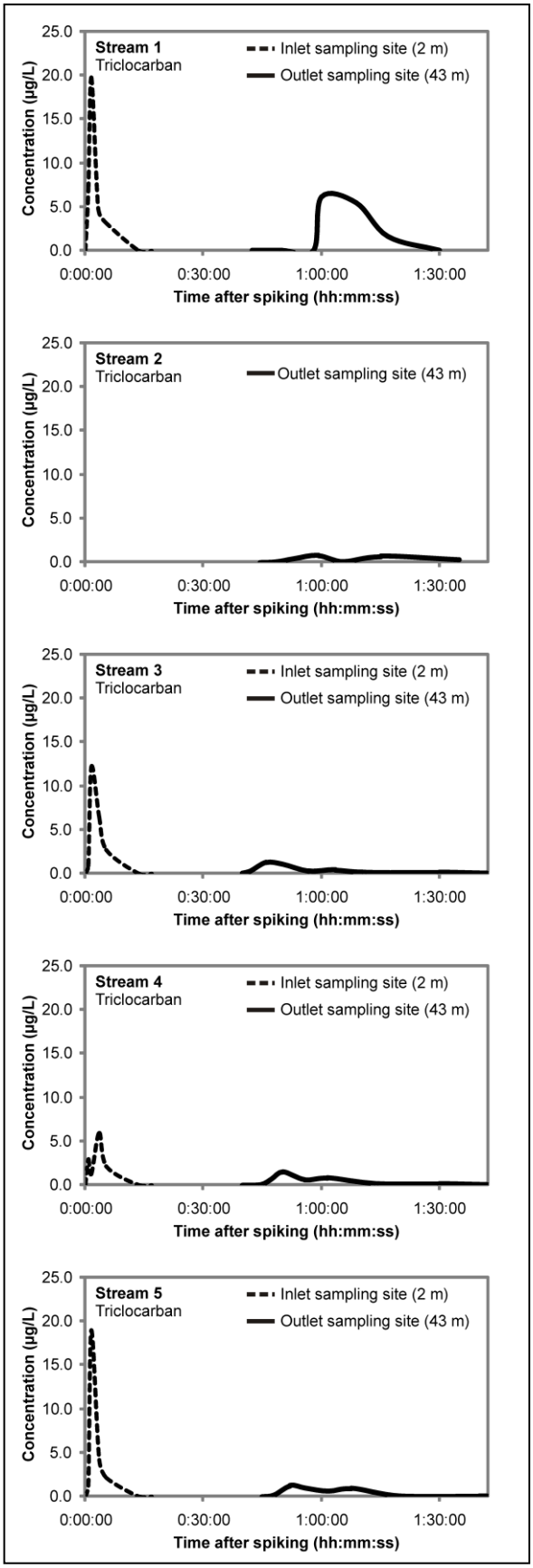

Figure A1-6 Peak curves of triclocarban in all stream mesocosms

A.5 Supplementary information for Role of hydraulic and sorption processes in the retention of three plant protection products in vegetated stream mesocosms

Table A2-1 Biomass per area, total biomass and vegetation density of the three stream mesocosms.

	Biomass (g/m^2 ±SD; dry weight; n = 3)	Total biomass (g; dry weight)	Vegetation density (%)
Stream 1	No vegetation	0	0
Stream 2	79.8±8.5	1,437	13
Stream 3	591.8±57.9	10,653	100

Table A2-2 Physical and chemical water quality parameters of the three stream mesocosms measured during both application periods in August 2011 (n = 13).

	Temperature (°C)	pH	Oxygen saturation (%)	Conductivity (µS/cm)
Stream 1 (0%)	18.5±1.5	8.6±0.2	101.0±13.7	136.2±2.3
Stream 2 (13%)	18.4±1.5	8.7±0.3	105.0±14.4	136.2±2.4
Stream 3 (100%)	18.7±1.7	8.9±0.5	117.3±19.1	134.2±3.8

Table A2-3 ASE settings for the extraction of residues of penflufen, pencycuron and triflumuron from macrophytes and sediment samples.

		ASE settings
Eluents (v/v)		Acetonitril/Acetone (50/50)
Temperature (°C)		60
Quilibration period (min)		5
Extraction cycles	Number	4
	Duration (min)	10
Rinse volume (%)		40
Purge time (s)		30

Table A2-4 UHPLC-gradient for the determination of penflufen, pencycuron and triflumuron in SPE eluates and ASE extracts. Both eluents contained 0.1% formic acid and 4 mM ammonium format.

Time	Eluents		Flow rate (µL/min)
	A (MilliQ)	B (Methanol)	
0 – 2	95	5	200
2 – 10	0	100	200
10 – 12	95	5	200

Table A2-5 MS parameters for the determination of penflufen, pencycuron and triflumuron in water, macrophyte and sediment samples.

	Penflufen	Pencycuron	Triflumuron
Ionisation	ESI	ESI	ESI
Polarity	+	+	-
Spray voltage (kV)	3.5	3.5	4.0
Capillary temperature (°C)	275	275	275
Scan range (m/z)	100 – 2000	100 – 2000	100 – 2000
Resolution (@ 2 Hz)	50.000	50.000	50.000
Atomic mass	317.1898	328.1337	358.0327
Adduct	M+H	M+H	M-H
Exact mass	318.1976	329.1415	357.0259

Table A2-6 Maximum PPP residues in sediment samples measured during both applications. Concentrations are given as ng/g (sediment dry weight).

		Penflufen (ng/g)	Pencycuron (ng/g)	Triflumuron (ng/g)
Application 1	Stream 1	2	5	3
	Stream 2	1	2	4
	Stream 3	6	2	1
Application 2	Stream 2	4	6	4
	Stream 3	2	6	2

Figure A2-1 Photograph of a tracer application conducted during darkness in a vegetated stream mesocosm.

Equation A2-1 Calculation of the Reynolds number (Re) where u is the mean flow velocity, A is the cross sectional area, b is the width of the stream mesocosms and h is the water level.

$$\mathrm{Re} = \frac{u \times \frac{A}{b + 2h}}{\nu} \qquad \text{(A2-1)}$$

A.6 Supplementary information for Experiments in water-macrophyte systems to uncover the dynamics of pesticide mitigation processes in vegetated surface waters/streams

Table A3-1 Recovery rates (±relative standard deviation; RSD) for the extraction of the investigated pesticides by Solid Phase Extraction (SPE) and Accelerated Solvent Extraction (ASE) from the aqueous and macrophyte samples, respectively.

	Recovery (%±RSD)			
	Aqueous phase	*Elodea nuttallii*	*Ceratophyllum demersum*	*Potamogeton crispus*
Imidacloprid	90.6±6.2	79.2±3.7	69.7±8.8	82.3±3.2
Isoproturon	106.9±4.4	76.2±3.9	76.7±8.1	83.1±2.2
Bifenox	84.6±14.3	79.0±4.0	84.3±9.4	91.5±3.8
Tebufenozide	98.0±3.2	93.4±3.5	93.9±8.0	99.4±1.7
Difenoconazole	95.2±9.7	77.8±3.0	72.4±6.3	78.1±1.2

Table A3-2 UHPLC1-gradient for the determination of the investigated pesticides. Both eluents contained 0.1% formic acid and 4 mM ammonium format.

Time	Eluents		Flow rate (µL/min)
	A (MilliQ)	B (Methanol)	
0 – 2	95	5	200
2 – 10	0	100	200
10 – 12	95	5	200

[1] The UHPLC-System consisted of UHPLC-Pump (Accela; Thermo Fisher Scientific, Dreieich, Germany) and a Hypersil Gold C18 separating column (50 x 2.1 mm, 1.9 µm particle size; Thermo Fisher Scientific, Dreieich, Germany).

Table A3-3 Parameters for the identification and quantification of the investigated pesticides in aqueous and macrophyte samples.

Pesticide	Molecular mass (g/mol)	Adduct	m/z	Electrospray Ionisation	Retention time (min)	LOQ (µg/L; µg/kg)
Imidacloprid	255.0518	[M+H]	256.0596	+	4.2	1
Isoproturon	206.1414	[M+H]	207.1492	+	4.6	1
Bifenox	340.9852	$[M+NH_4]$	359.0196	+	5.0	3
Tebufenozide	352.2145	[M+H]	353.2223	+	4.8	1
Difenoconazole	405.0641	[M+H]	406.0720	+	4.5	1
Bifenox-acid	326.9696	[M-H]	325.9617	-	4.5	-

Table S4 Partitioning coefficients calculated to describe adsorption ($logK_{D\_Adsorp}$) as well as desorption ($logK_{D\_Desorp}$) processes on the basis of the results of the semi-static short-term exposure experiments.

Macrophyte	K_{D_Adsorp}					K_{D_Desorp}	
	Bifenox	Difenoconazole	Tebufenozide	Isoproturon	Imidacloprid	Bifenox	Difenoconazole
E. nuttallii	2.19±0.08	1.43±0.02	0.67±0.01	0.71±0.05	0.22±0.02	2.03±0.07	1.56±0.07
C. demersum	2.11±0.04	1.34±0.05	0.54±0.05	0.70±0.02	0.27±0.06	1.98±0.06	1.22±0.72
P. crispus	2.14±0.07	1.57±0.03	0.90±0.05	0.33±0.05	0.39±0.06	1.85±0.09	1.56±0.07

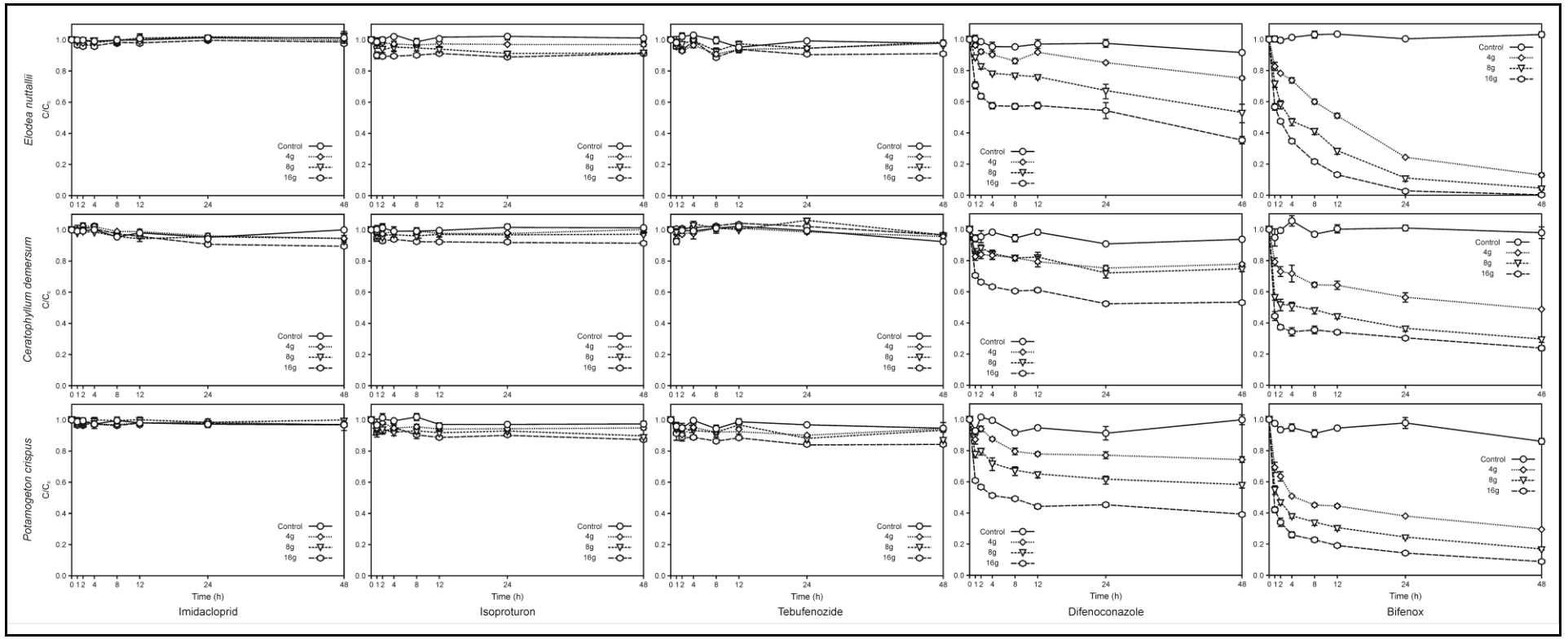

Figure A3-1 Concentration gradients of the investigated pesticides during the experimental phase (48 hours) of the static long-term scenario.

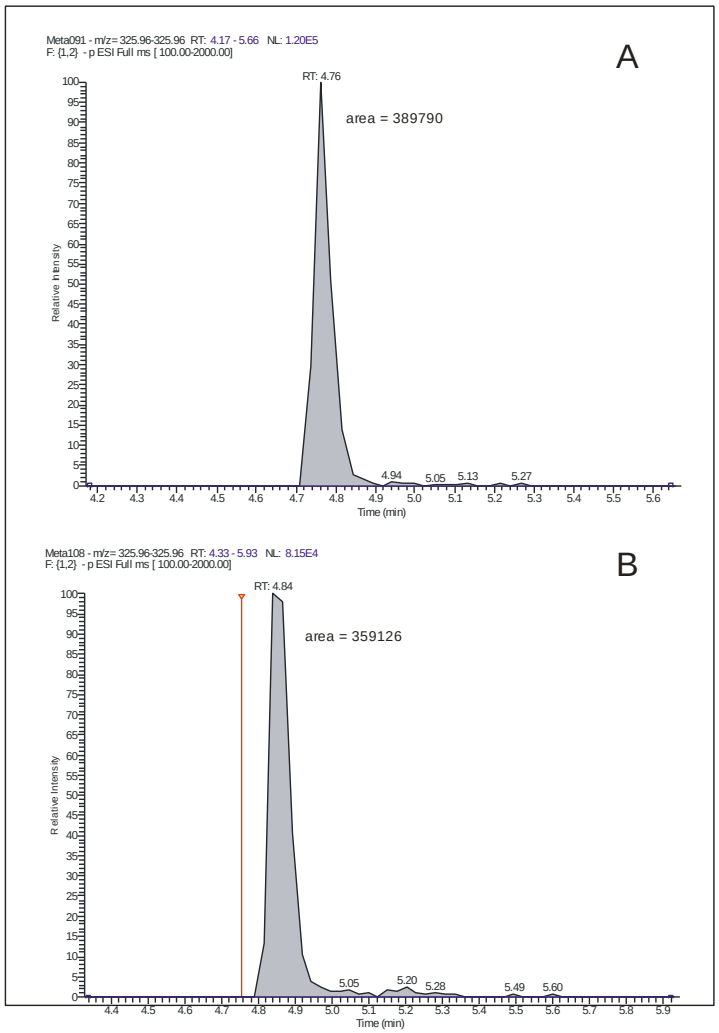

Figure A3-2 Chromatogram of the reference standard for bifenox-acid (A) and an aqueous sample from a bifenox treatment containing *E. nuttallii* (B).

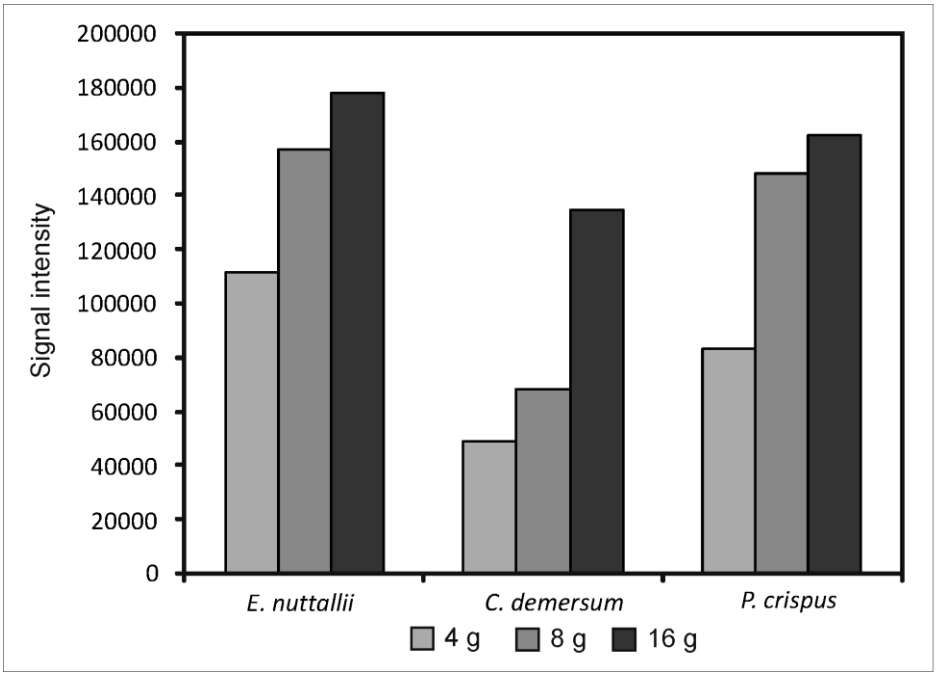

Figure A3-3 Signal intensity of the ion with the mass-to-charge ratio of 325.9617 m/z identified as the [M-H]--adduct of bifenox-acid in aqueous samples that were taken from bifenox treatments 48 hours after the start of the static long-term experiments.

A.7 Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

(Christoph Stang)

A.8 Curiculum vitae

Werdegang

Seit 03/2014	Wissenschaftlicher Mitarbeiter im Fachgebiet Biozide des Umweltbundesamtes in Dessau-Roßlau
Seit 08/2010	Doktorand in der Arbeitsgruppe Ecotoxicology & Environment am Institut für Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau
04/2011 – 04/2012	Kooperation mit Bayer CropScience zum Rückhalt von Pflanzenschutzmitteln in pflanzenbestandenen Fließgerinne-mesokosmen
09/2010 – 11/2010	Arbeitsaufenthalt bei der Bundesanstalt für Gewässerkunde in Koblenz, Referat G2: Gewässerchemie
06/2010 – 09/2010	Leitung der Freilandpraktika Aquatische Systeme und Naturräume für Studierende der Umweltwissenschaften der Universität Koblenz-Landau
04/2002 – 08/2010	Studium der Diplom-Umweltwissenschaften an der Universität Koblenz-Landau, Campus Landau
08/2007 – 10/2007	Praktikum im Sachgebiet Umweltmesstechnik (Analytische Task Force) des Amtes 37 der Stadt Mannheim (Feuerwehr und Katastrophenschutz)

06/2007 – 07/2007	Teilnahme am Forschungsprojekt SOILMISS in Mississippi, USA, in Kooperation mit dem National Sedimentation Laboratory des United States Department of Agriculture, Oxford, Mississippi, USA
08/1991 – 06/2001	Allgemeine Hochschulreife am Leibniz-Gymnasium in St. Ingbert

Veröffentlichungen

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