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The mode of bioturbation triggers pesticide remobilization from aquatic sediments

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ABSTRACT

After their release into the aquatic environment, contaminants may – depending on the physicochemical properties – adsorb to sediments. From there these contaminants can either be buried or remobilised by abiotic factors (e.g., resuspension) as well as by the bioturbating activity of sediment dwelling invertebrates. Little is, however, know about the effects of bioturbation on the fate of pesticides. Therefore, the present study quantified the impact of the bioturbation mode of benthic invertebrate species (bio-diffusor vs. bio-irrigation), the invertebrate density (i.e. 0–8 individuals per replicate), and the substance-inherent properties (i.e. hydrophobicity, water solubility) on the remobilization of sediment-associated pesticides in a laboratory-based set-up over 13 days. We found that both the bioturbation mode (i.e., species identity) and species density, as well as pesticide properties (i.e., hydrophobicity) affected the direction and magnitude of remobilisation of sediment-bound pesticides. The oligochaeta *Lumbriculus variegatus* showed a density-dependent effect on the remobilization of lindane to the water phase, whereas those with the amphipod *Monoporeia affinis* and larvae of the midge *Chironomus riparius* did not. Although these findings show that sediments not per definition are a sink for pesticides, the rates of pesticide remobilization are limited. This observation, thus, suggests that the risk for aquatic communities posed by the remobilization of pesticides from the sediment due to bioturbation is low.

1. Introduction

Pesticides (i.e., insecticides, herbicides and fungicides) are essential components of the current agricultural practice that contribute to securing food production for a growing human population (sensu Verger and Boobis, 2013). Following their application pesticides, either dissolved or associated with soil particles, frequently enter aquatic ecosystems through diffuse sources such as drainage, spray drift and/or surface run-off (Schulz, 2004). Depending on their properties, pesticides dissolved in water are either transported further downstream (Schäfer et al., 2011) or absorbed to sediments (Cooper et al., 2003) and other submerged surfaces such as macrophytes (Stehle et al., 2011). In contrast, pesticides that enter aquatic ecosystems adsorbed to soil particles will, to a large extent, be removed from the water column during low-flow conditions and deposited in sediments (Bereswill et al., 2012). Sediment-associated pesticides can be remobilised by resuspension during high discharge events or by bioturbation (Ciarelli et al., 1999). Bioturbation, defined as the disturbance or sediment-mixing caused by the burrowing and feeding activity of sediment-dwelling organisms (Roberts, 2012), provides important ecosystem processes, such as preventing phosphate leakage from sediments by oxidizing surficial sediment layer (Palmer et al., 1997). Bioturbation by a sediment-feeding carp, for example, increased the cadmium water concentration as well as the total suspended solids in the water phase (Wall et al., 1996). Similar effects on the remobilization of cadmium (Ciutat et al., 2007), copper (Remaili et al., 2016), polychlorinated biphenyls (Hedman et al., 2009; Josefsson et al., 2010) and flame-retardants (Hedman et al., 2008) have been observed for polychaetes and amphipods.

The remobilisation potential of most sediment-associated organic contaminants largely depends on their hydrophobicity, but also on their burial depth, as the depth of bioturbation varies among invertebrate species. For example, while bio-irrigators such as the polychaete *Marenzelleria* spp. are capable of remobilizing contaminants buried in deep sediment layers (up to 10 cm) (Håkanson and Jansson, 1983), bio-diffusors such as *Monoporeia affinis* rework only the upper few centimetres of the sediment omni-directionally (Håkanson and Jansson, 1983). While these processes are relatively well described for metals and some organic contaminants, the role of bioturbation for pesticide remobilisation from sediments is largely unknown. To our

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knowledge only a single study has assessed the remobilisation of pesticides through bioturbation by benthic invertebrates. In this study, the lindane concentrations in the water overlying experimental sediments increased across a gradient in densities of the bio-irrigator midge *Chironomus riparius,* whereas sediment concentrations declined (Goedkoop and Peterson, 2003).

In the present study we assessed the effects of (i) the bioturbation mode of benthic invertebrate species (i.e., bio-diffusor, bio-irrigation) (ii) invertebrate densities, and (iii) the substance-inherent properties (i.e. hydrophobicity, water solubility) on the remobilization of sediment-associated pesticides. In our experimental design we assessed the fate of ^{14}C-labeled pesticides with common freshwater invertebrate species that differ in their mode of bioturbation, that is the amphipod *Monoporeia affinis* (Crustacea), the midge *C. riparius* (Diptera) and the oligochaete *Lumbriculus variegatus* (Annelida). The amphipod is thoroughly mixing the surface layer of sediment (Van de Bund et al., 1994) and exclusively burrows and feeds in the top 1-cm of the sediment (Lopez and Elmgren, 1989) and thus acts as a bio-diffusor. Bio-irrigating larvae of *C. riparius* instead feed on the sediment surface and build distinct tubes into the sediment which they ventilate by undulating body movements. Their tube ventilation and feeding activity should result in a net downward transport of materials (Håkanson and Jansson, 1983). Similarly, oligochaetes such as *L. variegatus* are"conveyor belt-feeders" that forage in deep sediment and defecate at the sediment surface (Robbins et al., 1979). In contrast to amphipods, oligochaetes mix sediments in a highly ordered manner, bringing deep sediments back to the surface (Håkanson and Jansson, 1983). The insecticides lindane and chlorpyrifos were used as model pesticides at concentrations that would not directly affecting the organisms (i.e., no effects could be detect during the present study). We hypothesized that the mode of bioturbation would affect the reflux of the pesticides from the sediment to the water. More specifically, we assumed that bio-diffusors would remobilize higher pesticide loads from contaminated sediment relative to bio-irrigators.

2. Material and methods

2.1. Chemicals

Lindane (International Izotope, Munich, Germany) and Chlorpyrifos (American Radiolabeled Chemicals, St Louise, MO, USA) were purchased as ^{14}C-labeled standards with a purity of $>99\%$ and a specific activity of 1.099×10^{12} Bq/mol and 1.184×10^{12} Bq/mol, respectively. These compounds were selected as they differ in their hydrophobicity and water solubility. Lindane has a log K_{ow} of 3.5 and a water solubility of 8.52 mg/L at 20 °C, while the equivalent numbers for chlorpyrifos are 4.7 and 1.05 mg/L, respectively. Stock solutions with a concentration of 30.7 µg lindane/mL and 17.0 µg chlorpyrifos/mL – concentrations have been verified by scintillation counting – were prepared with acetone (analytical grade). Concentrations of both compounds in the water phase, in the sediment, and associated with suspended particles were quantified by liquid scintillation counting using Optisafe Hisafe2 scintillation cocktail and a Tri-Carb 2100TR liquid Scintillation counter (Beckman LS6000TA, Beckman Counter AB) and Optisafe Hisafe 2 scintillation fluid (Wallac, PerkinElmer, Turku, Finland). Each sample was counted for 10 min or a minimum of 10,000 CPM. Quench corrections were done using internal ^{14}C-standards (Wallac, PerkinElmer, Turku, Finland). Scintillation counts for samples were corrected for background values using blanks with no spiking of radioactive compounds. All other chemical were purchased from VWR if not otherwise specified.

2.2. Test organisms and sediment

The chironomid *C. riparius* and the oligochaete L. *variegatus* were obtained from the in-house cultures at the Department of Aquatic Sciences and Assessment and from ECT Oekotoxikologie GmbH, respectively. *Chironomus* were cultured at 20 ± 1 °C and fed with commercial fish-food (Tetraphyll®). Three days prior to the start of the experiments approximately 200 fourth instar *Chironomus* larvae were transferred to aerated lake water at 11 ± 1 °C. *M. affinis* were collected from in the Görväln basin of Lake Mälaren near Stockholm, Sweden, and stored at 11 ± 1 °C in lake water until further use (max 10 days). Also natural fine grained sediment was sampled in the Görväln basin from a depth of 40–44 m using an Ekman sampler. The sediment is characterized by a water content of approximately 90%, an ash-free dry weight of around 13% of the dry weight and exhibits an organic carbon content of 65 mg/g sediment dry weight (Goedkoop and Johnson, 1994). In the laboratory, the sediment was sieved (250 µm) to remove ambient fauna and stored at 11 ± 1 °C for 10 days to allow sediment particles to settle. After 10 days the overlying water was discarded and replaced with fresh lake water in the experimental units (see below).

2.3. Experimental design

Bioturbation effects on the fate of lindane and chlorpyrifos were investigated in a set-up with microcosms (170 mL cylindrical glass vessels, i.d. 5.5 cm) that contained a 1.5-cm sediment layer and 33 mL of overlying lake water. Either 0, 2, 4 or 8 individuals per replicate were added to quantify the effects of invertebrate density on the fate of these pesticides. These experimental densities correspond to 3360–13,400 ind/m^2, densities that well cover those found in natural sediments (see for chironomids Armitage et al., 1995). While the experiments with lindane run with all three species of invertebrates (n=4), those with chlorpyrifos were run only with *M. affinis* (n=3).

Spiking of the experimental sediment with pesticides was done in two steps, adopting the methodology described in OECD-guideline 218 (OECD, 2004). First 2.4 g quartz sand was added to each of the test microcosms and 500 µL of pesticide stock solution was amended to this sand. Over the subsequent 24 h the solvent acetone was completely evaporated under a stream of air. Second, 36.3 g of sieved (250 µm) Görväln basin sediment was added and thoroughly mixed with the spiked sand. Subsequently, 33 mL of lake water was carefully added to each replicate without re-suspending the sediment particles. Each microcosm was aerated for 15 min/h 2.0–3.0 cm above the sediment surface using capillary tubing (i.d. 0.76 mm). The test organisms were introduced after a seven-day equilibration phase (OECD, 2004).

All experiments were run for 13 days as the emergence of *C. riparius* was expected thereafter (OECD, 2004). During the study pesticide water concentrations were quantified at days 0, 1, 2, 4, 10 and 13 by subtracting 500-µL water samples 0.5 cm above the sediment-water interface. At the termination of the experiments (i.e. on day 13), pesticide concentrations in the sediment as well as those associated with suspended particles were quantified. Suspended particles were separated from the overlying water by filtering 20 mL of the overlying water (0.2 µm pore filters). All samples were mixed with 10 mL of scintillation cocktail and analyzed using liquid scintillation counting. Please note that the concentrations associated with the suspended particles contributed far less than 1% to the overall pesticide mass budget. Thus, these measurements are not further considered in this study.

2.4. Data analysis

The effects of species identity and density on the remobilization of lindane into the water phase over time and of the sediment lindane concentration remaining at the termination of the experiment were assessed using two-factorial-repeated-measures and two-way-ANOVA, respectively. Similarly, the effect of pesticides (lindane and chlorpyrifos) and *M. affinis* density on pesticide remobilization to the water phase over time and of the sediment pesticide concentration at the termination of the experiment were assessed using two-way-repeated-measures and two-way-ANOVA, respectively. Also interaction terms of both factors were evaluated. All repeated measures analyses were corrected for potential pseudoreplication over time. To assess for statistically significant effects of species identity and densities on the sediment concentration at the termination of the experiment on one-way ANOVAs were performed. Statistical analysis and figures were generated using R version 3.0.3 for Mac including the extension package "plotrix" (Team, 2015) Alpha was set at 0.05 in all statistics.

Table 1
Output of three-factorial ANOVA corrected for pseudoreplication due to repeated measures (= the factor time was considered as random factor) assessing differences in the remobilisation of lindane from the sediment by the three invertebrate species (*C. riparius*, *L. variegatus* and *M. affinis*) at four densities (i.e., 0, 2, 4 and 8 individuals per replicate) over time.

Factors	df	SS	MS	F	P
Grouping factor					
Species	2	429	214.4	0.957	0.433
Density	3	1485	495.1	1.979	0.135
Species × Density	6	3426	570.9	2.282	0.057
Residuals	36	9006	250.2		
Time factor					
Time	4	7295	1823.8	251.753	< 0.0001
Species × Time	8	2338	292.3	40.350	< 0.0001
Density × Time	12	423	35.3	4.869	< 0.0001
Species × Density × Time	24	585	24.4	3.362	< 0.0001
Residuals	144	1043	7.2		

3. Results and discussion

3.1. The importance of the bioturbation mode for the remobilization of pesticides

Only treatments with *L. variegatus* showed a density-dependent effect ($p = 0.038$) on the remobilization of lindane to the water phase (Fig. 1). Remobilization of lindane decreased by up to 30% across the *L. variegatus* density gradient (Fig. 1A). Otherwise, the remobilization of sediment-associated lindane to the water

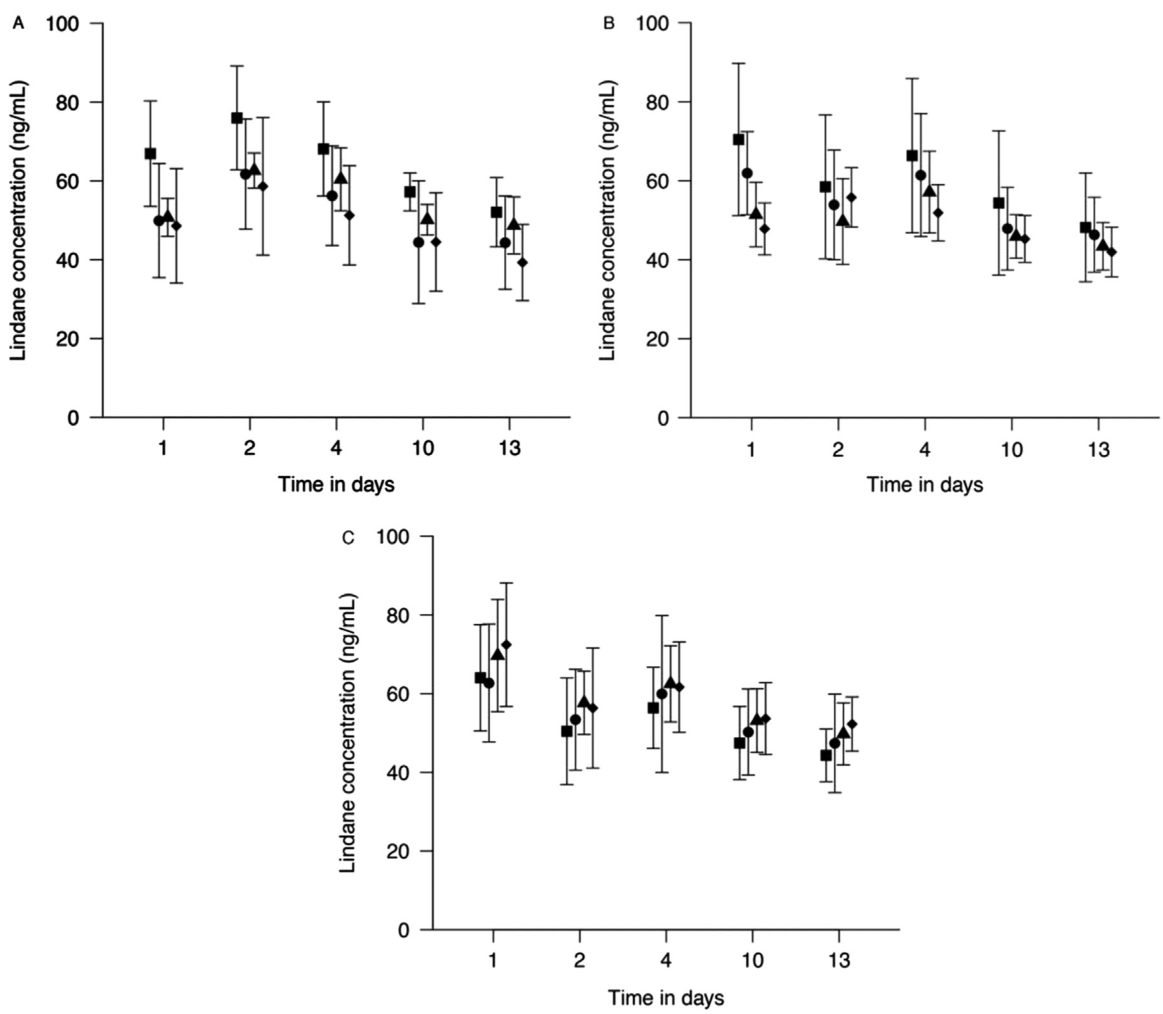

Fig. 1. Temporal changes in lindane water concentration for treatments with the oligochaete worm *Lumbriculus variegatus* (A), larvae of the midge *Chironomus riparius* (B), and the amphipod *Monoporeia affinis* (C). Symbols represent treatment with 0 (squares), 2 (circles), 4 (triangles), and 8 (diamonds) individuals per replicate. Error bars represent the 95% confidence intervals of the mean ($n = 4$).

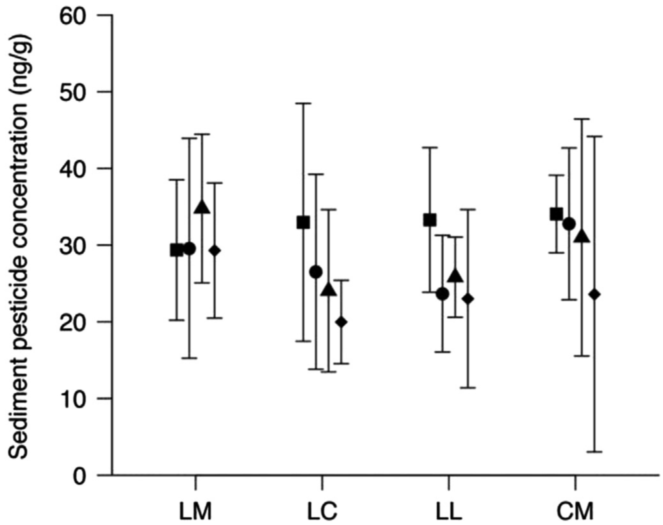

Fig. 2. Sediment concentrations of lindane in treatments larvae of amphipod *Monoporeia affinis* (LM), the midge *Chironomus riparius* (LC), the worm *Lumbriculus variegatus* (LL), and the as well as sediment concentrations of chlorpyrifos in the treatment with *Monoporeia affinis* (CM) at densities of 0 (squares), 2 (circles), 4 (triangles), and 8 (diamonds) individuals per replicate after 13 days of incubation. Error bars represent the 95% confidence intervals of the mean (n=3 or 4).

phase was affected neither by invertebrate identity nor by their density (two-factorial repeated measures ANOVA, Table 1). However, the interaction of both factors was close to significant ($p=0.057$), which is also underpinned by the declining trend in lindane water concentrations for both bio-irrigating species (*C. riparius* and L. *variegatus* (Fig. 1 A and B)) and the observed opposite trends across density gradients for the bio-diffusing amphipod *M. affinis* (Fig. 1C).

Conversely, the concentrations of lindane that remained associated with the sediment at the termination of the experiment (i.e. after 13 days), were density-dependent ($p=0.046$). However, neither species identity nor the interaction term contributed significantly to the model (Fig. 2, Table 2). This density-dependent reduction in the sediment lindane concentrations is in accordance with Goedkoop and Peterson (2003). However, these authors also reported a density-dependent increase of lindane in the overlying water in presence of *C. riparius*, which was not corroborated by the observations in the present study (Fig. 1B). This discrepancy is likely due to differences in the spiking procedure between both studies. The present study applied sediment-spiking, which ensures a homogenous distribution of lindane in the sediment. In contrast, Goedkoop and Peterson (2003) spiked the overlying water likely resulting in an adsorption of this pesticide to the very surficial sediment layer. The latter procedure may have favoured the remobilisation of lindane by *Chironomus* despite its bio-irrigating mode of bioturbation and hence less intensive mixing of the surficial sediment layer.

The importance of the bioturbating mode of action (intensity) for the remobilisation of sediment-bound contaminants is also supported by the present study. The bio-diffusing amphipod *M. affinis*, which is actively mixing the sediment in the upper few cm (Lopez and Elmgren, 1989; Van de Bund et al., 1994), increased the lindane water concentration with increasing density, but levels out at the highest density tested (i.e., 8 individuals per replicate, Fig. 1C). Therefore, the present study suggests that bio-irrigating organisms like deposit-feeding chironomids and oligochaetes tend to reduce – in a density dependent manner – the remobilization of lindane in homogenously contaminated sediments, while the opposite was observed for bio-diffusing species like amphipods (Fig. 1). The potential for contaminant remobilisation from sediments, however, also depends on the depth at which these substances are buried and on the aging of these contaminants in the sediment. Apparently, bio-irrigating species that build deep burrows in the sediment are capable of remobilizing substances from deep sediment layers, while bio-diffusors that only feed in the oxidized surface layer of the sediment obviously cannot remobilize contaminants from deep sediment layers (Josefsson et al., 2010, 2011).

Table 2
Output of two-factorial ANOVA assessing differences in the lindane sediment concentration depending on the three invertebrate species (*C. riparius*, L. *variegatus* and *M.a affinis*) at four densities (i.e., 0, 2, 4 and 8 individuals per replicate).

Factors	df	SS	MS	F	P
Species	2	11,687,271	5,843,635	2.629	0.086
Density	3	19,681,971	6,560,657	2.952	0.046
Species × Density	6	16,749,510	2,791,585	1.256	0.302
Residuals	36	80,015,229	2,222,645		

3.2. The role of pesticide properties on remobilization

Our two-factorial repeated measures ANOVA for treatments with *M. affinis* showed a significant effect of both pesticide, the density of the test organism, and their.

interaction term on the remobilization of the pesticides (Table 3). These parameters did, however, not statistically significantly influence the remaining sediment concentrations at the termination of the experiment (Table 4). For lindane – the less hydrophobic compound a non-significant tendency to an increased water phase concentrations co-occurred with an increasing invertebrate densities (Fig. 1C). However, the opposite, i.e. declining water concentrations across the *M. affinis* density gradient, was found for chlorpyrifos – the more hydrophobic compound (repeated measure ANOVA: $p=0.017$; Fig. 3). A similar pattern was found for copper, which also strongly sorbs to sediments, relative to the more mobile zinc (Remaili et al., 2016). At the same time, we observed declining, although not significant, sediment concentrations of chlorpyrifos with increasing densities of *M. affinis* (ANOVA: $p=0.184$; Fig. 2). While chlorpyrifos concentrations in both the sediment and the water phase decreased with increasing amphipod density, the amount of chlorpyrifos associated with suspended particles was only marginal (far below 1%). These findings suggest that chlorpyrifos is lost from our test systems by an increased volatilization. This would, however, first imply an increased remobilization from the sediment into the water phase and subsequent volatilization to the air phase. An increased flux from the sediment to the overlying water, however, was not observed in any of our treatments (Fig. 3). Therefore, considering also our relatively low experimental temperature of 11 ± 1 °C, this pathway is likely of minor importance. Alternatively, and more plausible, is the density-dependent accumulation of this hydrophobic pesticide in the amphipods, which has a log octanol/water partitioning coefficient (log K_{ow}) of 4.7. Unfortunately, this could not be quantified in our study. This conjecture is further supported by the absence of a similar pattern in the remaining sediment concentration for lindane (log $K_{ow}=3.5$).

A direct comparison of both experiments seems, however, difficult as the relative recovery, i.e. the total amount of pesticide at the termination of the experiment divided by that added at the start of the experiment, of lindane and chlorpyrifos from the whole test-system was approximately 20% and 100%, respectively, in absence of *M. affinis* (data not shown). These data suggest only a marginal loss of both pesticides as a consequence of adsorption to the microcosm walls. Moreover, a similar pattern of recovery was observed in presence of *M. affinis* although at a lower effect size, which may be explained by the accumulation of significant

Table 3
Output of three-factorial ANOVA corrected for pseudoreplication due to repeated measures (=the factor time was considered as random factor) assessing differences in the remobilisation of two pesticides (lindane and chlorpyrifos) from the sediment by *M. affinis* at four densities (i.e., 0, 2, 4 and 8 individuals per replicate) over time.

Factors	df	SS	MS	F	P
Grouping factor					
Pesticide	1	184,511,099	184,511,099	393.288	< 0.0001
Density	3	8,927,145	2,975,715	6.343	0.003
Pesticide × Density	3	13,034,703	4,344,901	9.261	< 0.001
Residuals	20	9,383,006	469,150		
Time factor					
Time	4	38,437,651	9,609,413	328.351	< 0.0001
Pesticide × Time	4	45,455,197	11,363,799	388.298	< 0.0001
Density × Time	12	1,468,792	122,399	4.182	< 0.0001
Pesticide × Density × Time	12	1,987,045	165,587	5.658	< 0.0001
Residuals	80	2,341,254	29,266		

Table 4
Output of two-factorial ANOVA assessing differences in the sediment concentration of the two pesticides (lindane and chlorpyrifos) depending on the densities (i.e., 0, 2, 4 and 8 individuals per replicate) of *M. affinis*.

Factors	df	SS	MS	F	P
Pesticide	1	4,632,001	4,632,001	2.404	0.137
Density	3	6,700,419	2,233,473	1.159	0.350
Pesticide × Density	3	5,815,100	1,938,367	1.006	0.411
Residuals	20	38,533,214	1,926,661		

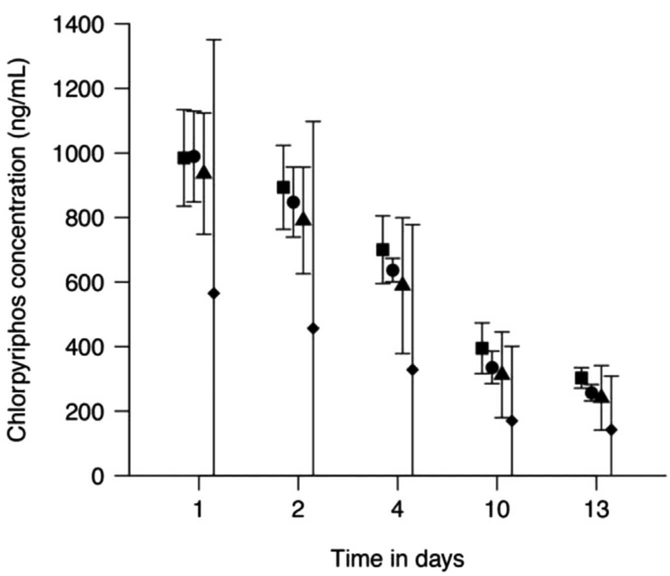

Fig. 3. Temporal changes in the chlorpyrifos water concentration for treatments with the amphipod *Monoporeia affinis*. Symbols represent treatment with 0 (squares), 2 (circles), 4 (triangles), and 8 (diamonds) individuals per replicate. Error bars represent the 95% confidence intervals of the mean (n=3).

amounts of chlorpyrifos in these amphipods (see above). In other words, more than 80% of the lindane introduced at the test initiation, was lost over the course of the study – potentially during the 24 h phase used to evaporate the solvent prior to the initiation of the experiment, despite the exclusion of test species. This suggests a massive volatilization of lindane during the study. Indeed, Goedkoop and Peterson (2003) reported with approximately 40% also a substantial loss of lindane in a comparable system with *C. riparius*, but a more than two-fold longer study duration. Especially due to the substantially longer study duration and the much lower effect size reported in their study, volatilization of lindane may only partly explain the effect uncovered in the present study. Conversely, the use of natural sediment in the present study may explain the substantial loss of lindane (Pesce and Wunderlin, 2004) as natural sediments exhibit a much more developed, diverse, and active microbial community than artificial sediments (Goedkoop et al., 2005). The latter can also explain the differences in the effect size between the present study and those by Goedkoop and Peterson (2003). Irrespective of the underlying processes, the very low recovery of lindane may have masked potential effects of species identity and density on the fate of this pesticide.

4. Conclusion

The present study underpins that both the bioturbation mode of the benthic invertebrates (i.e. bio-diffusor vs. bio-irrigation), their densities, and pesticide properties (i.e., hydrophobicity) affect the direction and magnitude of remobilisation of sediment-bound pesticides. Our results show that sediment-associated pesticides may be remobilised and that sediments are not by definition a sink for these compounds in agriculturally impacted water bodies. However, our results also show that the amount of pesticides remobilised by invertebrates with a bio-diffusing mode of bioturbation seems to be limited, thus questioning the relative importance of this exposure pathway under field conditions.

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