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Relationship between pesticide accumulation in transplanted zebra mussel (*Dreissena polymorpha*) and community structure of aquatic macroinvertebrates☆

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ABSTRACT

This study examined to what degree bioaccumulated pesticides in transplanted zebra mussels can give an insight to pesticide bioavailability in the environment. In addition, it was investigated if pesticide body residues could be related to ecological responses (changes in macroinvertebrate community composition). For this at 17 locations, 14 pesticide concentrations and nine dissolved metals were measured in translocated zebra mussels and the results were related to the structure of the macroinvertebrate community. Critical body burdens in zebra mussel, above which the ecological status was always low, could be estimated for chlorpyrifos, terbuthylazine and dimethoate being respectively 8.0, 2.08 and 2.0 ng/g dry weight.

With multivariate analysis, changes in the community structure of the macroinvertebrates were related to accumulated pesticides and dissolved metals. From this analysis, it was clear that the composition of the macroinvertebrate communities was not only affected by pesticides but also by metal pollution. Two different regions could be clearly separated, one dominated by metal pollution, and one where pesticide pollution was more important.

The results of this study demonstrated that zebra mussel body burdens can be used to measure pesticide bioavailability and that pesticide body burdens might give insight in the ecological impacts of pesticide contamination. Given the interrelated impacts of pesticides and heavy metals, it is important to further validate all threshold values before they can be used by regulators.

1. Introduction

The extensive application of pesticides for agricultural and non-agricultural uses results in elevated concentrations of pesticides and their residues in surface and groundwater resources. Pesticide residues have been reported as common organic contaminants worldwide (Aktar et al., 2009; Ali et al., 2014; Szekacs et al., 2015). Not all pesticides are easily degradable and due to their lipophilic properties, they can bioaccumulate in organisms, biomagnify in food chains, and consequently influence the health of organisms including humans (Andreu and Pico, 2012; Connell, 1988; Emanuela et al., 2017). Elevated pesticide levels may have adverse effects on macroinvertebrate communities including the loss of certain sensitive taxa (Munze et al., 2017; Schafer et al., 2012). As such, biological indices, based on the occurrence of macroinvertebrate species might be a good indicator of the ecological impact of pesticides. However, other stressors are present as well and there is no direct causal relationship between the measured pesticide concentrations and the macroinvertebrate community. Accumulated pesticides, on the other hand, can provide a better insight on bioavailability and ecological risks of pesticides.

Recent studies have shown that accumulated micropollutants

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can be used to predict ecological effects on macroinvertebrate community (Bervoets et al., 2016; Luoma et al., 2010; Rainbow et al., 2012) and allow to set safe body burden thresholds. The use of invertebrate body burdens as an indicator for metal toxicity was evaluated in several studies. In some studies, a significant relationship has been determined between metal accumulation in caddisfly (*Hydropsyche* sp.) and ecological responses such as a decrease in invertebrate taxa richness (Luoma et al., 2010; Rainbow et al., 2012). Also, metal concentrations in caged zebra mussels were found to be predictive for the ecological quality of a river along a metal pollution gradient (De Jonge et al., 2012).

Active biomonitoring using transplanted organisms has been used for more than two decades to assess bioavailability and effects of micropollutants in aquatic ecosystems (Bervoets et al., 2004; De Jonge et al., 2012, Mersch et al., 1996). Active biomonitoring can provide an integrative measure of bioavailable pesticides in the aquatic environment and help to detect pollutants that are usually present in the surface water at concentrations below detection limits of routine analytical methods (Bervoets et al., 2005a; Szekacs et al., 2015). In this regard, zebra mussel *Dreissena polymorpha* has been suggested as a reliable freshwater biomonitoring organism (Bervoets et al., 2005b; Bourgeault et al., 2010; De Jonge et al., 2012, Kraak et al., 1994).

Understanding the relationship between pesticide accumulation in zebra mussels and their effects on aquatic communities such as macroinvertebrates can provide better insight into ecological risks from pesticide contamination and is crucial for accurately monitoring and predicting the impacts of these contaminants in aquatic environments (Rainbow et al., 2012). Although the use of invertebrate body burdens was tested in different studies as an indicator of the ecological effects of metal toxicity, this approach has not been tested yet for effects of organic micropollutants including pesticides.

The objective of this study was to investigate to what extent a selection of accumulated pesticides in transplanted zebra mussels reflects bioavailable pesticide concentrations in the environment, and how the tissue residues can be related to alterations in macroinvertebrate community structure. Additionally, it was investigated whether we could estimate accumulated pesticide concentrations (thresholds) above which ecological quality is always low. Since in many cases pesticide pollution will not be the only stressor, the contribution of other stressors, i.e. accumulated metals on the invertebrate communities has been investigated as well.

2. Material and methods

2.1. Study area and sampling sites

Adult individuals of zebra mussels (*Dreissena polymorpha*) were collected in September 2013 from a shallow lake, Blaarmeersen (Gent, Belgium) and transported to the lab. From former analysis (2011; unpublished data) it was demonstrated that these mussels were uncontaminated with regard to metals, polychlorinated biphenyls (PCBs), organic chloride pesticides (OCPs) and polyaromatic hydrocarbons (PAHs). All mussels were kept in dechlorinated, aerated and filtered tap water in plastic tanks (1000 L) for at least 1 month at 15 °C and fed with a mixture of algae (*Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*).

After this acclimatization period, zebra mussels were transplanted to cages deployed at 17 sites in Flanders (Belgium) in October 2013 (Fig. 1, Table 1). Nine sites were located in the southeast (1–9) and 6 sites in the northeast (10–15) of Flanders. In addition, two sites in the west of Flanders were selected, one in the upper part of the Scheldt (16; southwest) and one in the canal Ghent Terneuzen (17; northwest). Sites 1 to 9 were selected because of a broad range in pesticide pollution (Flemish Environment Agency data), whereas lower pesticide concentrations were expected at sites 10 to 15 although elevated metal concentrations might be present and even follow a gradient of high zinc and cadmium levels at site 10 which gradually decrease until site 15 (Bervoets et al., 2005a). Sites 16 and 17 were selected because of general industrial pollution.

Before the start of the exposure 15 mussels of comparable length (15–20 mm) were collected and soft tissue was pooled for analysis of background metals and pesticides.

At every site, 24 individual mussels of comparable length (15–20 mm) were randomly placed in 2 polyethylene cages (11 × 11 × 22 cm with a mesh size of 2 × 4 mm), which allowed free circulation of water (Bervoets et al., 2004). To standardize the exposure and to prevent the accumulation of sediment all cages were free floating at a maximal depth of 30 cm below the water surface. After an exposure period of six weeks, all mussels were collected and processed (see below).

2.2. Macroinvertebrate sampling

Benthic macroinvertebrates were sampled at each site using a standard invertebrate hand net (500 mm-mesh, 200–300 mm frame and 500 mm bag depth). The samples were collected in October 2013 covering a river length of 20 m for 5 min (Depauw and Vanhooren, 1983). In the lab, samples were rinsed with tap water through a sieve of 0.5 mm to remove sediment and the organic fraction. The invertebrates were transferred to a white tray with gridlines and sorted, counted and transferred into small glass vials with denatured ethanol (70%) for fixation. The macroinvertebrates were identified up to family or genus level according to De Pauw and Vannevel (1991). The Multimetric Macroinvertebrate Index Flanders (MMIF, Gabriels et al. (2010)) was used to assess the biological water quality. This index combines 5 different characteristics of the macroinvertebrate community, i.e.: the total number of taxa (taxa richness), the number of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera), the number of other sensitive taxa, not belonging to the EPT, the mean tolerance score and the Shannon-Wiener diversity index. For sites 16 and 17 existing MMIF data originating from the Flemish Environment Agency were used (www.vmm.be/geoview).

2.3. Pesticide analysis

After exposure, the zebra mussels were collected and in the lab placed in 10 L buckets at 15 °C in filtered (0.45 μm) site water for 18 h to allow depuration. After removal of the byssus threads, the soft tissue of the mussels was dissected and rinsed with MQ-water. All mussels were pooled per site and homogenized by a rotor-stator homogenizer (Tissue Ruptor, Qiagen). The homogenized tissue was transferred to polypropylene vials (50 mL) and kept at −80 °C until freeze dried. For the pesticide analysis, the procedure of Wille et al. (2011) was adapted: samples were freeze-dried for 48 h and the dry tissue was extracted by a combination of pressurized liquid extraction (PLE) and solid phase extraction (SPE). All solvents were prepared according to Wille et al. (2011). The PLE was performed on a Dionex ASE® 350 accelerated Extractor with Solvent Controller (Dionex Crop., Sunnyvale, CA, USA). A cellulose filter (27 mm, Dionex Crop) was located on the bottom of a 22 mL stainless extraction cell. Subsequently, 0.25 g of a sample (freeze-dried sample), 2 g of aluminum oxide and 4.5 g of diatomaceous earth were placed in each Extraction cell. Each extraction cell was spiked with the internal standards at a concentration of 100 ng/g (100 ppb). The ASE-extract obtained was reduced to 0.5 mL by

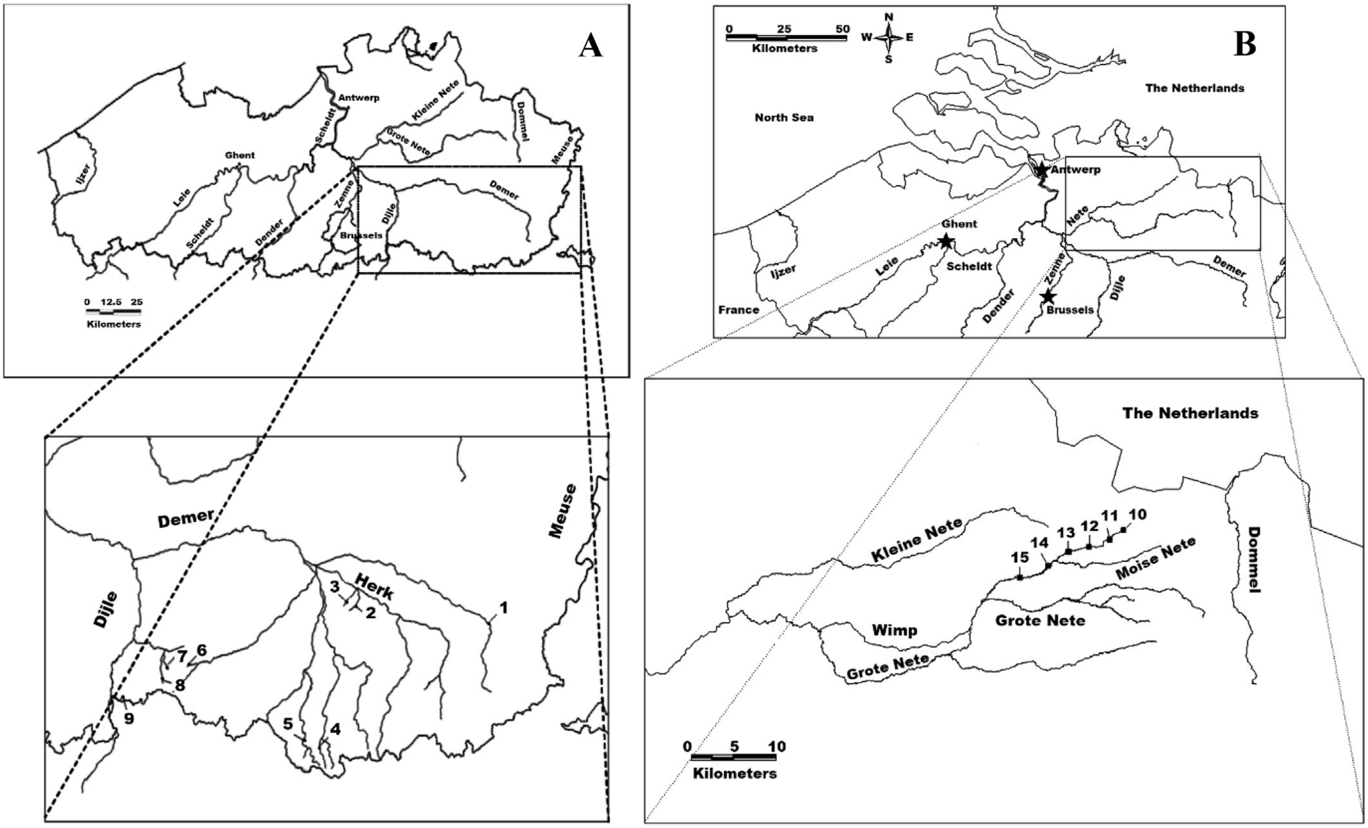

Fig. 1. Map of (A) sampling sites (1–9), (B) sampling sites (10–15). Sites 1 to 9 are pesticide polluted sites and site 10 to 15 are metal polluted sites.

Table 1
Location, General water quality characteristics and Multimetric Macroinvertebrate Index Flanders (MMIF) of the sampling sites.

Site	Name	VMMnr[a]	T (°C)	PH	O_2 (mg/L)	O_2 (%)	Conductivity (μS/cm)	MMIF
1	Demer	401000	10	7.43	9.21	86.7	506	0.7
2	Terbermenbeek	449800	8	7.06	5.09	44.8	411	0.2
3	Hoevenbeek	449750	8	7.26	5.3	46.3	545	0.15
4	Molenbeek	436920	9.7	6.82	6.94	63.6	882	0.15
5	Zevenbronnenbeek	445250	8.2	7.63	8.52	75.6	892	0.5
6	Kleine Vondelbeek	426910	9.3	7.54	9.32	80.7	733	0.5
7	Bovenheidebeek	483320	7.4	7.72	9.66	79.7	692	0.6
8	Bierbeek	483300	9	7.57	8.95	77	673	0.5
9	Paddenpoel	487200	8.8	7.55	9.74	83.1	450	0.7
10	Scheppelijke Nete	333750	NA	7.54	9.49	78	444	0.7
11	Scheppelijke Nete	333600	5.4	7.3	10.9	90	461	0.5
12	Scheppelijke Nete	333500	5.7	7.37	10.7	88	416	0.15
13	Scheppelijke Nete	NA	5.6	7.46	11.3	92	408	0.3
14	Molse Nete1	333100	5.7	7.39	10.2	84	378	0.5
15	Molse Nete2	330200	6.1	7.35	9.49	60	394	0.4
16	Scheldt	179000	NA	NA	NA	NA	NA	0.25
17	Terneuzen	NA	NA	NA	NA	NA	NA	0.25
Rf1	Blaarmeersen	NA	NA	NA	NA	NA	NA	NA

[a] VMM (Vlaamse Milieumaatschappij) is the Flemish Environment Agency which monitors (both chemically and biologically) Flemish surface waters and sediment on a regular basis.

evaporation under nitrogen at 40 °C and subsequently diluted with 10 mL of ultra-pure water. SPE was performed using Isolute EnV + cartridges (6 mL, 200 mg, Biotage, Uppsala, Sweden). The cartridges were conditioned with 10 mL of methanol and 10 mL of ultra-pure water with methanol (5%). Further, the cartridges were loaded with the sample and then washed with 5 mL of ultra-pure water. Elution was carried out by 5 mL of methanol and acetonitrile. Next, the eluate was evaporated under nitrogen at 40 °C to 1 droplet and reconstituted in 50 μL methanol and 150 μL of 2 mM aqueous ammonium carbonate.

Analysis for the following pesticides was carried out: dichlorvos, dimethoate, diazinon, pirimicarb, linuron, metolachlor, chloridazon, chlorpyrifos, simazine, isoproturon, terbuthylazine, diuron, atrazine, and kepone. Atrazine-D_5 and isoproturon-D_6 were used as an internal standard. Separation of pesticides was conducted by Ultra-high performance liquid chromatography (U-HPLC). The apparatus consisted of an Accela™ High-Speed LC and an Accela™ Autosampler (Thermo Scientific, San Jose, CA, USA). Pesticides detection was performed using a TSQ Vantage Triple-Stage Quadrupole Mass Spectrophotometer (Thermo Electron) equipped with a heated electrospray ionization probe (HESI-II). Separation, detection, identification and quantification of target analytes followed the same methods as described in Wille et al. (2011). Based on the concentration range found in Van Praet et al. (2014) we expected the accumulated concentrations to be lower than 100 ng/g. Therefore we slightly modified the method of Wille et al. (2011) by spiking the samples (freeze-dried mussels) with a standard mixture to obtain final concentrations in the range of 0–100 ng/g instead of 0.1–250 ng/g.

Quality assurance was performed in the same way as explained in Wille et al. (2011). However, the concentration ranges at which samples were spiked with standard solution ranged from 1 to 100 ppb instead of 0.1–100 ppb.

2.4. Metal analysis

Since metal pollution was known to be present at some sites, the mussel tissue was analyzed for the presence of nine metals. From each pooled sample (see above) a sub-sample of about 0.5 g was transferred into pre-weighed acid-washed polypropylene vials and dried for at least 24 h at 60 °C. After re-weighing, the samples were digested using 500 μL ultra-pure nitric acid (HNO_3, 69%) followed by microwave-heating (Bervoets et al., 2005a). Metal Ag, Al, Cd, Cr, Cu, Fe, Ni, Pb and Zn) were measured using a quadrupole inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7700x ICP-MS, Santa Clara, USA). Quality control included analysis of procedural blanks and certified reference material metals (mussel tissue; BCR278R) from the community bureau of reference (European Union, Brussels, Belgium) (Bervoets et al., 2005b). Recoveries were all within 15% of the certified values (recoveries of 90–112%).

2.5. Statistics

Relationships between the individual accumulated pesticides and the calculated indices were visualized using scatterplots. Quantile regression was used to investigate the possible relationship between invertebrate body burdens and calculated indices. Quantile regression analysis allows to calculate the 90th quantile of ecological responses (calculated indices) as a function of an environmental stressor (zebra mussel body burden) and therefore to determine the maximum ecological response. Using the maximal response can reduce the influence of non-modeled factors that might affect invertebrate taxa richness. The detailed information regarding this model is described in De Jonge et al. (2013).

To find the concentration threshold, above which a good ecological status was never reached, concentrations were selected where the MMIF score was higher than 0.6. The concentration thresholds were set as the 95th percentile of the internal pesticides concentrations.

Among the detected pesticides, chlorpyrifos, dichlorvos, terbuthylazine and dimethoate were used to investigate the relationship between pesticides and biological water indices since they have been found in most of the sampling sites. Pesticide concentrations below the limit of quantification (LOQ) were replaced by LOQ/2 (Bervoets et al., 2004; Custer et al., 2000).

In order to relate changes in macroinvertebrate community composition to accumulated pesticides and metals, direct ordination techniques (canonical correspondence analysis; CCA) were used. Before the analysis, all data were log (X+1) transformed and the gradient length within the taxa data was analyzed. Detrended correspondence analysis (DCA) revealed that the total gradient length of species distribution was >2 times standard deviation. Therefore, the unimodal (Gaussian distribution) response model was selected. For CCA, interspecies distance and Hill's scaling were applied. Down-weighting of rare species was used to give less weight to taxa which occurred only infrequently. All ordination models were calculated using CANOCO version 4.5.

3. Results

3.1. Background information

The environmental variables (temperature (°C), pH, oxygen content (mg/L) and conductivity (μS/cm) of the surface water at the sampling sites are presented in Table 1. The lowest pH was 6.82 observed at site 4. The oxygen concentration was the lowest with 5.3 mg/L at site 3 and the highest with 11.3 mg/L at site 13. The maximum conductivity was 892 μs/cm, observed at site 5. For most sites, the water oxygen level met the water quality standard ($\geq$6 mg/L; $\leq$ 12 mg/L) with the exception of sites 2 and 3. Conductivity was well below the quality standard of 1000 μS/cm at all sites (Flemish Government, 2000).

3.2. Pesticide and metal accumulation in zebra mussels

The results of the concentrations of pesticides and metals in mussels exposed at the different sites are presented in table SI. 1 and SI. 3 respectively. The concentration in mussels from Blaarmeersen after one month of acclimatization (called ‘Start’ in the tables) are reported as well. Among the fourteen pesticides analyzed for in the present study, simazine and kepone were below the detection limit in mussel tissue at all sites, while dimethoate, dichlorvos, pirimicarb, terbuthylazine, chlorpyrifos were detectable in mussel tissue at most of the sampling sites (Fig. 2). The concentrations of diuron, linuron, metolachlor, isoproturon, atrazine and diazinon were below the LOQ at most of the sites. The LOQ of the analyzed pesticides was 1 ng/g except for dimethoate and simazine which had a LOQ of 10 and 5 ng/g respectively. It should be noted that high background concentrations of pirimicarb (5.48 ng/g dry weight) in mussels were measured (in mussels originating from Blaarmeersen used for exposure at the other sites; called “start” in SI. 1).

In mussels from the sites 2 and 3 nearly all measured pesticides were higher than LOQ while at some sites (9,10, 11, 13, 14, 15) almost all pesticides were below the LOQ. The highest concentration of pesticides found was for chlorpyrifos (2203 ng/g dry weight and 103 ng/g dry weight at site 2 and 3 respectively) and for dichlorvos with concentrations up to 128 ng/g dry weight. Accumulated metals are summarized in SI. 3. Elevated concentration of Cd, Pb and Zn were measured in mussels from 2, 11, 12, 13, 14 and 15.

3.3. Macroinvertebrate community composition

In total, 34 macroinvertebrate taxa have been identified. The highest abundance was observed for Gammaridae and Chironomidae representing >95% of all invertebrates at site 9 and 12 respectively (SI. 2). The highest taxonomic richness for Trichoptera and Ephemeroptera was observed at sites 1, 7 and 14, while no EPT families were present at sites 2–4 and 12. Plecoptera was not found at pesticide nor metal polluted sites. The presence of the EPT taxa resulted in a high score for 1, 7, 10 and 14. The highest total number of taxa was observed at site 1 and the lowest at sites 2 and 3. According to the MMIF index 1, 9 and 10 were classified as having a good biological quality (MMIF $\geq$ 0.7), the sites 2, 3, 4, 12, 13 and 17 had a bad biological (MMIF $\leq$ 0.3) water quality and the remaining sites were considered as having a moderate biological water quality (Table 1).

At the agricultural sites (1–9), predominant taxa were

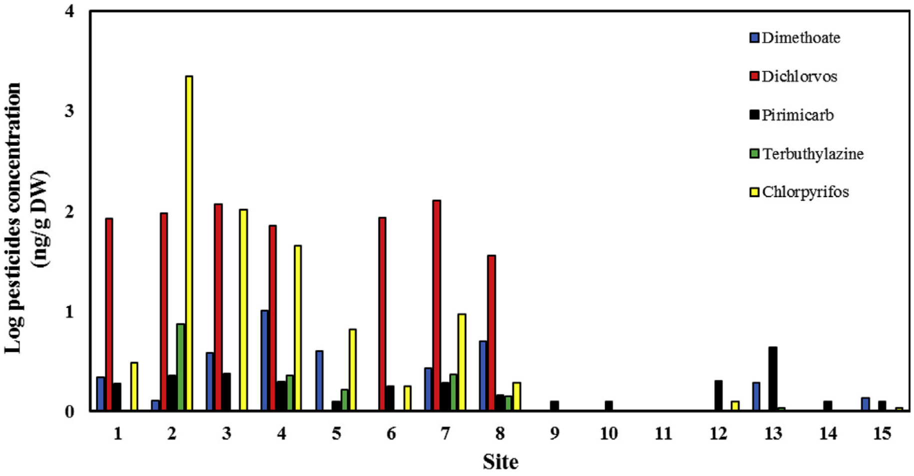

Fig. 2. Detected pesticide concentrations in zebra mussels at pesticide polluted sites (1–9) and metal polluted sites (10–15).

Simuliidae, Gammaridae and Tubficidae while Chironomidae and Asellidae were present in higher abundance at the metal polluted sites (SI. 2).

3.4. Relationship between pesticide accumulation in zebra mussel and community structure

The relationship between accumulated pesticide concentrations and the biological water quality index (MMIF) was investigated for 4 pesticides (dichlorvos, dimethoate, terbuthylazine and chlorpyrifos), as many values for the other pesticides were below LOQ (Fig. 3). No significant relationships could be found (quantile regression analysis, $p > 0.05$). However, it was possible to derive accumulated pesticide threshold concentrations for three pesticides above which the ecological quality was always low ($MMIF < 0.6$), i.e. terbuthylazine, chlorpyrifos and dimethoate. Threshold values were calculated as the 95th percentile of the accumulated pesticide concentrations in zebra mussels collected at sites with good biological quality ($MMIF \geq 0.7$) (Table 2).

The CCA diagram that relates accumulated metals and pesticides to the macroinvertebrate composition (Fig. 4) reveals that the macroinvertebrate taxa distribution in sampling sites was influenced by accumulated pesticides and metals in zebra mussels, explaining respectively 26.4% and 15.2% of the variation. The sites which are dominated by pesticide pollution or metal pollution are separated in the CCA diagram. The sites 1, 3, 4–7 were more influenced by pesticides contamination and sites 2, 10–12 and 14–15 were more subjected to metal contamination.

In addition, Simuliidae and Planorbidae are more associated with sites where the concentration of dichlorvos, terbuthylazine and chloridazon were high, whereas sites with a high concentration of Pb and Cd were mainly dominated by Chironomidae and Asellidae (Fig. 4).

Table 2
The 95-percentile threshold concentrations (ng/g dry weight) in zebra mussel above which ecological status was always low ($MMIF < 0.6$).

Compound	Threshold
Chlorpyrifos	8
Terbuthylazine	2.08
Dimethoate	2

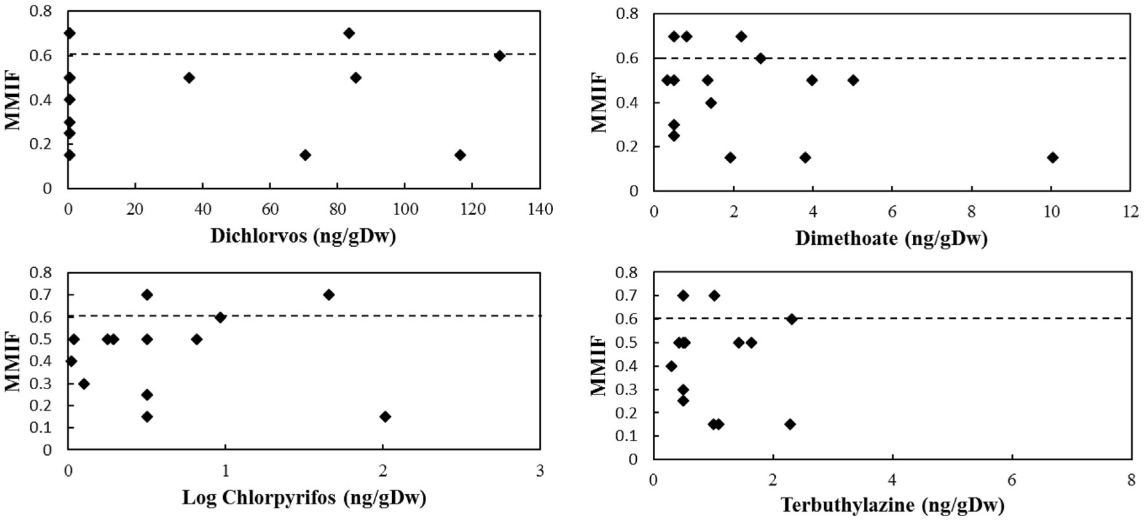

Fig. 3. The relationship between accumulated pesticides in zebra mussels and biological water quality indices (MMIF). The dashed line indicates the threshold value for the MMIF set at a score of 0.6. Due to the wide range of chlorpyrifos concentration the values were log transformed.

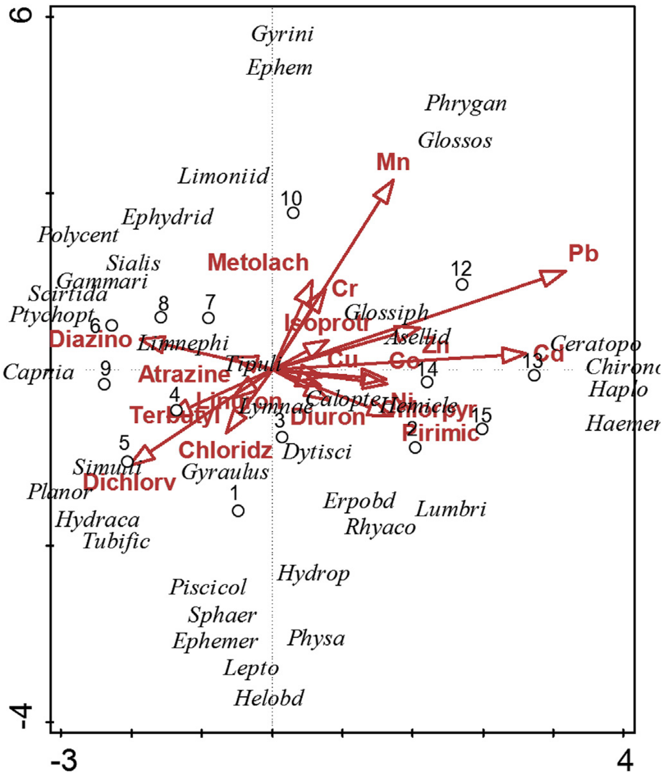

Fig. 4. CCA diagram of macroinvertebrate taxa composition. Direct ordination based on both accumulated pesticides and metals concentration. Axes represent the first two axes of the ordination analysis. Eigenvalues (%cumulative variance): axis 1:0.677 (26.4%), axis 2:0.392 (41.6%), axis 3:0.348 (55.2%), axis 4: 0.248 (64.8%).

At sites 2–4 where the concentration of chlorpyriphos was high and 12 with high Zn concentrations, no EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) occurred.

4. Discussion

4.1. Pesticide accumulation in zebra mussels

Literature on bioaccumulation of pesticides in freshwater biota, mainly invertebrates is limited. Wille et al. (2011) measured the same set of pesticides as this study in the marine mussel *(Mytilus edulis)* for an active biomonitoring program in the Belgian coastal zone. Only dichlorvos and chloridazon were found with concentrations four times lower (dichlorvos) or similar (chloridazon) compared with the present study. Van Praet et al. (2014) measured the same set of pesticides in the damselfly larvae *Ischnura elegans* (Zygoptera, Odonata) collected from sixteen ponds in Flanders. They reported four of the measured pesticides (chloridazon, dichlorvos, terbuthylazine and metolachlor) above the LOQ. In the present study, metolachlor in zebra mussel tissue was below LOQ at most sites, terbuthylazine and chloridazon concentrations were comparable to the concentrations in the damselfly larvae, while for dichlorvos, the concentration in the present study was twice as high. Neither Wille et al. (2011) nor Van Praet et al. (2014) detected simazine and kepone in invertebrates collected during their studies which is in agreement with the present study.

In a study by Miranda et al. (2008) three pesticides (atrazine, diuron, terbuthylazine) were detected in the muscle tissue of freshwater trahira fish *(Hoplias maladbaricus)* from Ponta Grossa Lake in south Brazil. Diuron and terbuthylazine were detected in the same range as in the present study while atrazine concentration was 2 times lower.

It should be noted that, although pirimicarb was found at all sampling sites in the present study, the measured accumulated concentration might not represent the site-specific bioavailability, since the concentration was already high (highest) at the start of the exposure in the mussels originating from Blaarmeersen (“start”). At most sites, however, the pirimicarb was lower in the mussel after 6 weeks of exposure compared to the start, so was probably eliminated from the mussel tissue. Almost all measured pesticides in this study (except dimethoate) had octanol-water partition coefficient greater than 1000 (SI. 4) and a soil half-life greater than 30 days. These properties result in accumulation in sediment and aquatic biota (Andreu and Pico, 2012; Van Praet et al., 2014). As most pesticides are difficult to quantify in surface water samples due to their hydrophobic characteristics (Wille et al., 2011), the results of this study suggest that using accumulated concentrations in zebra mussels is a good monitoring strategy.

4.2. Relationship between pesticide accumulation in zebra mussel and community structure

The ecological value of the investigated water bodies was always low at high accumulated concentrations of terbuthylazine, chlorpyrifos and dimethoate. Based on the results, pesticide threshold values of tissue burdens ranging from 2 to 8.0 ng/g dry weight have been calculated and are indicative of pesticide levels which is harmful to macroinvertebrates communities. The threshold concentrations indicate safe values above which a good ecological status was never reached. However, this is a conservative approach as the lower ecological quality is not directly related to the presence of pesticides as also other stressors are present.

Mayon et al. (2006) observed a lower IBI score (Index of Biotic Integrity or Fish Index) in a river polluted with pesticides, compared to a reference site. On the other hand, in a study of Eaton and Lydy (2000) no relationship was found between the IBI score and the present organochlorine pesticides in fish tissue on twenty sampling sites in the Arkansas river in Wichita, Kansas (USA). In the present study low MMIF values were observed at sites with low accumulated pesticide concentrations, indicating that other factors also negatively affect the macroinvertebrate community. Bervoets et al. (2016) and Van Ael et al. (2014) pointed out that the decrease in ecological quality may also be caused by others stressors such as other contaminants, physical characteristics of the water body or food and habitat availability. Also, the CCA analysis revealed that the macroinvertebrate community was affected not only by pesticide contamination but also by metals. The pesticide-polluted and the metal-polluted sites are clearly separated from each other which was expected since sites 1–9 were located in a fruit cultivation area with intensive pesticide application (information Flemish Environment Agency) while sites 10, 12, 13, 14 and 15 were situated in a metal contaminated river system (Bervoets et al., 2005a; Michiels et al., 2017). However, even within the pesticide-polluted sites, accumulated Cd, Mn, Ni and Cu concentrations at site 2 were elevated compared to uncontaminated Flemish lowland rivers and comparable to values in other metal contaminated sites in Flanders (Bervoets et al., 2005a).

Based on the quantile regression analysis, no significant negative relations were found between maximal (90th quantile) ecological response and accumulated pesticides in zebra mussel. This might be due to the fact that only 15 sites were considered.

The applicability of body tissue residues to biomonitor metal toxicity in aquatic ecosystems has been described before. De Jonge et al. (2013) found significant negative relationships between accumulated Cu, Pb and Zn concentrations in *Leuctra* sp., Simuliidae, *Rhithrogena* sp. and Perlodidae and the maximal ecological response. In addition, De Jonge et al. (2012) observed significant relationships between accumulated metal concentrations in *D. polymorpha* and Chironomidae and biological community response. Luoma et al. (2010) and Rainbow et al. (2012), showed that metal accumulation in larvae of caddisfly *Hydropsyche* sp. was highly correlated with ecological indicators such as mayfly richness and macroinvertebrates taxa, whereas Bervoets et al. (2016) could estimate safe body concentrations for a set of metals in Chironomidae larvae and Tubifidae worms. Awrahman et al. (2016) stated that the bioaccumulated concentrations of metals in larvae of *Hydropsyche* sp. can be used to predict reductions in local mayfly (particularly ephemerellid and heptageniid) abundance.

According to Weber et al. (2018), the most sensitive invertebrate species to organic pesticides belong to the EPT taxa. Thiere and Schulz (2004) and Colville et al. (2008) similarly found a high sensitivity of Ephemeroptera to pesticide pollution mainly to chlorpyrifos. Our results are in good agreement with their results.

As insects, Chironomidae may have been sensitive to insecticides such as chlorpyrifos. However, according to (Macchi et al., 2018), they were the most abundant taxa in a stream with high detected pesticides including chlorpyrifos. The result of the present study showed that Chironomidae are related rather to high metal concentrations than to high pesticide levels. This is in accordance with Bervoets et al. (2016) who found very high accumulated metals in Chironomidae.

In contrast to the study by Macchi et al. (2018) and Von Der Ohe and Liess (2004) who found the molluscs being the most tolerant taxa towards pesticides, we did not find molluscs at most of the studied sites, which of course could be due factors other than pesticide pollution. This is supported by the fact that at all sites the caged mussels survived during the 6 weeks exposure period.

5. Conclusion

From fourteen analyzed pesticides in zebra mussel tissue, dimethoate, dichlorvos, pirimicarb, terbuthylazine, chlorpyrifos were detected at most of the sampling sites. The results of pesticide body burdens suggest that using the zebra mussel is a promising monitoring strategy to measure bioavailable pesticides that are difficult to quantify in water.

The results of the present study also demonstrate that for four out of the five detectable (not for dichlorvos) pesticides the ecological status was always low (MMIF <0.6) when accumulated concentrations in zebra mussel were detected. Thus, we suggest that body residues of pesticides in zebra mussels can be used to predict the ecological effects of pesticides on the macroinvertebrate community. However, to exclude possible effects of co-occurring stress factors such as dissolved metals, more research and larger database mainly are needed. Additional, evidence is needed to assess the effects of the accumulated pesticides in the environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.05.140.

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