Variables determining the response of invertebrate species to toxicants, A case study on the River Meuse.
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Chapter IV

SPECIES SPECIFIC RESPONSES TO METALS IN ORGANICALLY ENRICHED RIVER WATER, WITH EMPHASIS ON EFFECTS OF HUMIC ACIDS

Abstract
Invertebrate communities in polluted rivers are often exposed to a wide variety of compounds. Due to complex interactions, “pollution tolerant” species are not necessarily the most tolerant species to toxicants tested under standard laboratory conditions. It was hypothesized that the distribution of species in polluted rivers is not only dependent on the tolerance of species to toxicants, but also on species specific capacities to modify or compensate for negative effects of toxicants. To test this hypothesis, species specific responses to metals in organically enriched river water were studied under controlled conditions. The zebra mussel *Dreissena polymorpha* and the midge *Chironomus riparius* were exposed to metal polluted water from the River Dommel. Additionally, the (interactive) effects of metals and humic acids (HA) on both species were evaluated. In spite of a lower tolerance of *C. riparius* to metals in laboratory studies, the midge was the most tolerant of the two test species to metal polluted site water. The results indicated that the sensitivities of the two test species determined in laboratory tests were inversely related to their sensitivities to polluted river water. In accordance with these results, midge larvae were protected from Cu toxicity by HA, while metal toxicity was not reduced (Cu) or even amplified (Cd) by HA for the zebra mussel. Thus, the presence of (naturally occurring) HA in site water may partly account for discrepancies between responses of species to bioassays and toxicity tests. It is suggested that these differences in responses to metals in site water are strongly influenced by species specific preferences for organic compounds (like HA). It is concluded that the response to organic compounds present in site water largely determines whether a species is classified as “pollution tolerant” or “pollution sensitive”. 
CHAPTER IV

Introduction

Rivers that suffer from anthropogenic disturbances often contain a wide variety of compounds that can have divergent effects on aquatic communities. Due to complex interactions, species observed to be tolerant to pollution in the field are not necessarily the most tolerant species to various chemical compounds tested under standard laboratory conditions (Nalepa and Landrum, 1988; Hickey and Vickers, 1994). For example, the “pollution tolerant” midge Chironomus riparius occurs in heavily metal contaminated rivers (Postma et al., 1995), while the zebra mussel Dreissena polymorpha has not been found at these sites. Laboratory toxicity tests, however, showed that zebra mussels are generally more tolerant to metals than C. riparius (Kraak et al., 1994 vs. Postma et al., 1995). For C. riparius, the inconsistency between it’s performance in toxicity tests and it’s tolerance to polluted water has been demonstrated to be partly caused by a compensation of toxic effects by nutritional effects of particulate organic matter (Stuijfzand et al., subm.). The distribution of this “pollution tolerant” species is therefore partly explained by trophic preferences.

Besides beneficial trophic effects of organic matter, organisms may benefit from a reduced bioavailability of toxicants. Several studies have indicated that sorbed or complexed toxicants are less toxic than the soluble compound (Allen et al., 1980), due to reduced bioavailability (e.g. Nugegoda and Rainbow, 1988; Landrum et al., 1996). However, this is not always the case; dissolved organic matter can either decrease or increase toxicity of metals and organic xenobiotics (Klein et al., 1995; Kukkonen, 1995). Humic acids (HA) are an important representative within the group of dissolved organic compounds. In the presence of HA, changes in toxicity of the essential metal copper (Cu) and the non-essential metal cadmium (Cd) were observed to be opposite for Daphnia magna (Oikari et al., 1992), demonstrating that the alteration of toxicity due to HA is metal specific. Hanssten et al. (1996), however, observed that for another species (Anodonta anatina) the effects of HA on both Cu and Cd were similar. It is unknown whether these differences in responses between species were caused by different test conditions or are due to species specific capacities to cope with metals in the presence of HA. In the present study it is hypothesized that aside from taxa sensitivity to toxicants, the distribution of species in polluted rivers depends on species specific capacities to modify or compensate for negative effects of toxicants. The response to organic compounds present in site water, like humic acids, may be essential in ranking pollution tolerant and pollution sensitive invertebrates. To test this hypothesis, species specific responses to metals in organically enriched river water were studied for the midge C. riparius and the zebra mussel D. polymorpha. In addition, the (interactive) effects of HA and metals on both species were tested, using growth and filtration rates as sublethal parameters, respectively.
Materials and methods

Treatments
Two types of experiments were performed:

I Bioassays
Mussels and midges were exposed to site water from the River Dommel in the laboratory. The River Dommel is organically enriched due to the input of municipal waste water. At a small inlet (Eindergatloop) and further downstream, the Dommel becomes critically polluted with metals due to the presence of a zinc factory. The contamination of the Dommel is described into more detail by Postma (1995). Water samples were collected on August 20, 1996, from the following locations along the Dommel: Eindergatloop Upstream (EGU; 51°14'00" N, 5°25'30" E), Eindergatloop (EG; 51°14'00" N, 5°25'17" E), Neerpelt (NP; 51°14'20" N, 5°25'23" E), Turfheide (TH; 51°15'40" N, 5°25'20" E). A map of the river Dommel, including the sample sites, is presented in Fig. 4.1. Immediately after transfer to the lab, the river water was filtered (1.2 μm; glass fiber filter). The water was kept in vessels in a dark room, at 4°C, until further use (2 days maximum).

After filtration, samples were taken for metal and (in)organic carbon analysis. Metal concentrations were measured using furnace (Cu, Cd) and flame (Zn, Fe) AAS (Perkin Elmer 5100PC and 1100B, respectively), and (in)organic carbon concentrations were measured using a TIC/TOC analyzer (Model 700 OI Analytical) (Table 4.1). Dutch Standard Water (DSW) was used for controls. DSW is a standardized synthetic analogue of common Dutch surface waters (Maas et al., 1993).
Table 4.1: Concentrations of four metals, total organic carbon (TOC) and total inorganic carbon (TIC) in filtered site water. EGU: Eindergatloop Upstream; EG: Eindergatloop, NP: Neerpelt, TH: Turfheide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cd (µg/L)</th>
<th>Zn (µg/L)</th>
<th>Cu (µg/L)</th>
<th>Fe (µg/L)</th>
<th>TOC (mg/L)</th>
<th>TIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGU</td>
<td>&lt;0.1</td>
<td>31</td>
<td>5.2</td>
<td>302</td>
<td>5.7</td>
<td>13</td>
</tr>
<tr>
<td>EG</td>
<td>11.5</td>
<td>463</td>
<td>5.6</td>
<td>102</td>
<td>5.8</td>
<td>33</td>
</tr>
<tr>
<td>NP</td>
<td>3.3</td>
<td>228</td>
<td>5.8</td>
<td>248</td>
<td>5.6</td>
<td>20</td>
</tr>
<tr>
<td>TH</td>
<td>0.2</td>
<td>84</td>
<td>1.7</td>
<td>158</td>
<td>5.0</td>
<td>21</td>
</tr>
</tbody>
</table>

II Toxicity tests

Mussels and midges were exposed in the laboratory to a metal (Cu or Cd) and/or to humic acids (HA), dissolved in standard water (DSW). The test species were subjected to the following treatments: DSW (control), HA, Cu, Cu+HA, Cd, Cd+HA. The concentration of HA (Aldrich) was 2.5 mg/l (nominal). Concentrations of metals (CuCl₂ and CdCl₂) were based on reported or derived EC₅₀ values from similar tests with *D. polymorpha* and *C. riparius* (Kraak et al., 1994; Postma et al., 1995 resp.), or on pilot experiments (midge: Cu). Water samples were taken for furnace and flame AAS metal analysis as described below. Nominal and actual metal concentrations and EC₅₀ values are listed in Table 4.2.

Table 4.2: Nominal and average actual concentrations of Cd and Cu. EC₅₀ values are given as well: *: Kraak et al., 1994; **: Postma et al., 1995, or ***: pilot experiment.

<table>
<thead>
<tr>
<th>species</th>
<th>metal</th>
<th>nominal conc. (µg/L)</th>
<th>actual conc. (µg/L)</th>
<th>EC₅₀ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. polymorpha</em></td>
<td>Cd</td>
<td>500</td>
<td>410</td>
<td>388 *</td>
</tr>
<tr>
<td><em>D. polymorpha</em></td>
<td>Cu</td>
<td>50</td>
<td>23</td>
<td>41 *</td>
</tr>
<tr>
<td><em>C. riparius</em></td>
<td>Cd</td>
<td>60</td>
<td>43</td>
<td>59 **</td>
</tr>
<tr>
<td><em>C. riparius</em></td>
<td>Cu</td>
<td>50</td>
<td>35</td>
<td>50 ***</td>
</tr>
</tbody>
</table>

*Dreissena polymorpha*

Effects of toxicants on filtration rates of *D. polymorpha* were determined according to Kraak et al. (1994). Zebra mussels (*D. polymorpha*) were collected from Lake Markermeer (The Netherlands), a relatively clean site (Kraak et al., 1991). In the laboratory, the mussels were sorted by length (1.5-2.5 cm) and placed in glass aquaria (6 l), each aquarium containing 25 mussels and 3l of DSW. The average length of the mussels in each experiment was equal for all treatments. Each treatment was carried out in triplicate. The
water in the aquaria was aerated and kept at 15°C. The aquaria were covered with glass plates to prevent evaporation. A 16 : 8 hr light : dark regime was applied.

The animals were allowed to acclimatize to the experimental set-up for one day. At the start of the experiment, each aquarium was supplied with 3 L treatment water. The water was renewed after 24 and 48 h. During the toxicity tests, water samples for metal analysis were taken at the start of the experiment, and before and after renewal of the water. After 48 h, directly after renewal of the treatment water, 60 mL of a continuous culture of the green alga Scenedesmus acuminatus was added to the water to measure filtration rates. The density of the algal cells in the aquaria was approximately 20,000 cells/mL. The algal concentration decreased due to the filtration activity of the mussels. When the mussels started filtrating, 5 min after addition of the algae, three water samples were taken (5 mL). This was repeated 10 and 20 minutes after the first sampling. The concentration of algae in the water samples was measured using a Coulter Counter. The filtration rate was calculated from the declining number of algae, using Coughlan’s (1969) formula:

\[ m = \frac{M}{nt} \ln \frac{C_0}{C_t} \]

in which

- \( m \) = volume of water filtered by D. polymorpha (mL/mussel/h)
- \( M \) = volume of water in the aquaria (3 L)
- \( n \) = number of mussels in each aquarium (25)
- \( t \) = duration of filtration measurement (h)
- \( C_0 \) = concentration of algae at the start of the filtration measurement
- \( C_t \) = concentration of algae after \( t \) hours

Control mussels always filtrated more than 50 mL/mussel/h, and the average filtration rates of controls was 77.4 mL/mussel/h. No mortality occurred in the control group.

Chironomus riparius
The methods for determining effects of toxicants on C. riparius were derived from Postma et al. (1995). An experimental treatment consisted of a glass jar (180 mL), supplied with 100 mL treatment water. Water samples for metal analysis were taken at the start and the end of the experiment. Treatments consisted of 3 (bioassay, Cd/HA toxicity test) or 2 (Cu/HA toxicity test) replicates.

At the start of the experiment, first instar larvae from at least three newly hatched egg ropes were distributed randomly with glass Pasteur pipettes over the different treatments. The midge larvae originated from a laboratory culture (Stuijfzand et al., 1998). Each treatment
contained 15 (bioassay) or 20 (toxicity tests) first instar larvae. Food stock suspensions were made by adding Trouvit (5 g) and Tetraphyll (0.25 g) to 100 mL water of the corresponding treatment. Larvae were given food *ad libitum* (0.6 mL suspension). At the start of the experiment, the lengths of 15 first instars were measured using a binocular microscope. After 96 h, lengths were measured of the surviving larvae. Growth of the individual larvae was calculated by subtracting the final length of each larva from the average initial length. The average initial length per experiment varied from 1.09 (± 0.01) to 1.22 mm (± 0.02). Average growth of control larvae was between 0.90 (± 0.04) and 2.13 (± 0.06) mm, and mortality in the controls was always less than 3%. The experiments were carried out in a climate room under identical conditions as the laboratory culture of the larvae.

The results were tested using one-way ANOVA, when appropriate. When a significant difference was found, a Student Newman Keuls test was applied to indicate which groups differed from controls. If data were not homogenous or normally distributed, a non parametrical test (Kruskal Wallis) was performed.

**Results**

![Graph](image)

*Fig. 4.2:* Growth and filtration rate (with standard error) of *C. riparius* and *D. polymorpha* after exposure to Dommel water. See Fig. 4.1 for site codes. Growth and filtration rates presented as percentage of corresponding controls. Filtration rates in EG and NP water differed from controls (*p*<0.05). Midge growth differed significantly between treatments (Kruskal Wallis, *p*<0.05).
No mortality of *D. polymorpha* and *C. riparius* occurred in either the bioassays or the toxicity tests. Filtration rates of zebra mussels were, however, strongly inhibited (*p*<0.05) after exposure to water from the heavily polluted site Eindergatloop (EG), and this effect was equally severe (*p*<0.05) in water from the less polluted downstream site Neerpelt (NP) (Table 1, Fig. 4.2). Although not significant, filtration rates of mussels were slightly reduced (24%) by water from the upstream site (EGU) which contained only an elevated background concentration of Zn (Table 4.1). In contrast, only the most polluted water (EG) caused a minor decrease (21%) in midge growth, and the larvae were not inhibited by water from NP or the other sites compared to controls.

The observed responses of both species to the site water were compared to their tolerance to the individual metals present in the river water (Table 4.3). Therefore, the expected inhibition of filtration rates and growth due to Zn and Cd was determined by calculating a dose-response relationship for both metals according to Haanstra *et al.* (1985), using toxicity data from Kraak *et al.* (1994) on mussels and Postma *et al.* (1995) on midges. These published toxicity tests using additions of Zn and Cd were carried out under similar conditions.

**Table 4.3:** Actual inhibition (mean %) of test species in site water, and expected inhibition (%) due to individual metals (Zn, Cd) measured in site water. Expected values were calculated from toxicity data reported in Kraak *et al.*, 1994 (*) and Postma *et al.*, 1995 (**). A: *D. polymorpha*, B: *C. riparius*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Actual Inhibition (%)</th>
<th>Expected Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>81</td>
<td>4</td>
</tr>
<tr>
<td>NP</td>
<td>85</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TH</td>
<td>31</td>
<td>&lt;1</td>
</tr>
<tr>
<td>EGU</td>
<td>24</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Actual Inhibition (%)</th>
<th>Expected Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>21</td>
<td>48</td>
</tr>
<tr>
<td>NP</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>TH</td>
<td>-2</td>
<td>16</td>
</tr>
<tr>
<td>EGU</td>
<td>-9</td>
<td>7</td>
</tr>
<tr>
<td>EG</td>
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<td>NP</td>
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<td>33</td>
</tr>
<tr>
<td>TH</td>
<td>-2</td>
<td>16</td>
</tr>
</tbody>
</table>

59
procedures as used in the present study. Based on the measured Zn and Cd concentrations in the river water, the expected inhibition of filtration rates of mussels was insignificant (4% at most) (Table 4.3A). Midge growth, on the other hand, was calculated to be strongly inhibited by Zn alone (48% in EG water) (Table 4.3B). These expectations are in sharp contrast with the actual reduction of filtration rates (up to 85%), while growth of *C. riparius* was not or only barely (21% at most) affected in site water (Table 4.3).

![Fig. 4.3: Growth and filtration rate (with standard error) of *C. riparius* and *D. polymorpha* after exposure to humic acids (HA) and/or Cu. Growth and filtration rates presented as percentage of corresponding controls. A significant difference in filtration rates was found between treatments (Kruskal Wallis, p<0.05), and for *C. riparius*, the Cu treatment differed significantly from controls (p<0.05).](image)

To gain more insight into the divergent responses of *D. polymorpha* and *C. riparius* to metal polluted, organically enriched site water, standard tests were conducted in which a metal and/or humic acids were added to reference water. As mentioned above, two metals were selected that were reported to have opposing effects on biota in the presence of HA: Cu and Cd. Exposure to the EC50's of Cu for both species (Table 4.2) caused, as expected, a decrease in filtration rates (Kruskal Wallis, p<0.05) and growth (p<0.05) (Fig. 4.3). However, midge larvae experienced no adverse effect of Cu in the presence of HA. In contrast, in the presence of Cu and HA, filtration rates dropped to the same level (38%) as those of zebra mussels exposed to Cu alone (36%).

Filtration rates of mussels seemed to be slightly inhibited (19%) in the presence of HA (Figs. 4.3 and 4.4), although this was not significant. The presence of Cd alone did also not
result in a significant reduction of filtration rates, even though the mussels were subjected to
the EC50 of Cd in synthetic medium (Fig. 4.4). However, Cd plus HA reduced the filtration
rates (66%) significantly (p<0.05). As in the previous experiment with Cu, C. riparius
responded differently to the combination of Cd and HA than D. polymorpha. As expected,
growth was reduced by Cd, but Cd toxicity remained unchanged in the presence of HA
(Kruskall Wallis, p<0.05) (Fig. 4.4).

Fig. 4.4: Growth and filtration rate (with standard error) of C. riparius and D. polymorpha
after exposure to humic acids (HA) and/or Cd. Growth and filtration rates presented as
percentage of corresponding controls. For D. polymorpha, the HA+Cd treatment differed
significantly from controls (p<0.05), and a significant difference in growth was found
between treatments (Kruskal Wallis, p<0.05) for C. riparius.

Discussion

The response of two test species, C. riparius and D. polymorpha, to water from the River
Dommel could not be predicted from sensitivities to metals in synthetic water. Different
performances of species in site water compared to standard water have been reported
before (Giesy et al., 1977; Diamond et al., 1997), and have often been attributed to
variation in speciation of the test compound. A “Water Effect Ratio” is often applied to
account for these differences between laboratory water and site water (Diamond et al.,
1997). Indeed, the results in the present study showed that naturally occurring compounds
like humic acids (HA) altered metal toxicity for D. polymorpha and C. riparius. However, if
speciation alone would play a role, it would be expected that the responses of different
species would alter in a similar way. This was not the case in the present study; metal toxicity was not reduced or increased for the zebra mussel, while larvae of *C. riparius* were not affected or even benefited from HA when metals were present. Likewise, midge larvae were less inhibited by polluted river water than expected from toxicity tests, while the inhibition of filtration rates was eminently stronger than expected. The metal sensitivities of the two test species determined in laboratory toxicity tests seemed to be even inversely related to their sensitivities to metal polluted river water. Therefore, the observed differences between effects of metals in site water and those in standard water cannot be ascribed to effects of speciation alone.

In agreement with the results obtained with the midge, Diamond *et al.* (1997) reported that for the fathead minnow, metals (among which Cd) were less toxic in site water than in laboratory test water. *Ceriodaphnia dubia*, however, did not or barely benefit from changes in Cd toxicity in site water compared to synthetic water. In another study, Cd was observed to be much less toxic to the daphnid *Simocephalus serrulatus* in site water than in reference water, while the toxicity of Cd was similar in both waters for the fish *Gambusia affinis* (Giesy *et al.*, 1977). Since the different responses of organisms to complex waters containing metals do not seem to be related to taxonomic groups (Diamond *et al.*, 1997 vs. Giesy *et al.*, 1977), it appears that modification of metallic effects in site water depends on species specific capacities.

In the present study, the midge clearly benefited from compounds modifying metal toxicity, while zebra mussels were hampered by these compounds. The results imply that HA played a significant role in these different responses. The observed (species specific) effects of HA on the two test organisms suggest that (naturally occurring) HA in Dommel water may have amplified Cd toxicity and did not affect Cu toxicity for *D. polymorpha*, while for *C. riparius* Cd toxicity was not enhanced and Cu toxicity was even reduced. These species specific effects of HA may therefore partly explain the discrepancies found between responses of species in bioassays and toxicity tests.

It may be hypothesized that the underlying mechanism for species specific responses to complex effluents is due to differences in uptake and elimination mechanisms. However, a pilot experiment with *D. polymorpha* showed that the accumulation of Cd was similar with or without addition of HA, in spite of a stronger reduction in filtration rates when Cd plus HA are present (data not published). Other studies on invertebrate species also pointed out that bioaccumulation cannot be directly translated into toxicity (Winner and Gauss, 1986; Besser *et al.*, 1995; Rule and Alden, 1996). Hence, HA alter the toxic effects of metals, but this alteration does not (necessarily) seem to be related to increased or decreased metal accumulation.
Possibly, HA do not only interact with contaminants, but may also play a trophic role for invertebrate species. Dissolved organic matter (and possibly HA) can promote growth of macrofauna (Thomas, 1997). However, the present study did not clearly indicate that C. riparius could benefit from HA as a food source. Moreover, a decrease in filtration rates was observed when the mussel was exposed to DSW to which only HA were added. It has been reported that HA are potentially molluscicidal (Anya, 1992), however, toxic effects took place at much higher concentrations (1000 mg/L) than the concentration in the present study (2.5 mg/L).

Filtration rates were inhibited by more than 20% by water from the upstream site, as was the case with HA alone. Morton (1971) observed that filtration rates of D. polymorpha decreased or increased in water from which particles (algae and bacteria) were removed. These alterations in filtration rates were not attributable to toxicants, but to either algal or bacterial emanations. Apparently, mussels discriminate between waters containing different natural compounds. It is possible that, in the present study, filtration rates of the zebra mussel were not optimal in upstream water (or water containing HA) due to the unfavourable “taste” of the water. Therefore, the observed adverse effect on filtration rates in the other treatments (containing high metal levels) may not per se be caused by toxicity, but to a combination of metal toxicity and other “unfavourable” conditions. Midges, on the other hand, did favour the (food) conditions in the Dommel. Therefore, the effect of toxicants on invertebrates is strongly influenced by the food conditions in aquatic systems. Since various types of organisms (with diverse feeding habits) are used for monitoring purposes, the strong impact of trophic conditions on the effect of toxicants on macrofauna species calls for an integration of a “food quality factor” into monitoring procedures.

It is concluded that the response to organic compounds present in site water largely determines whether a species is classified as pollution tolerant or pollution sensitive. The distribution of species in polluted rivers depends on the tolerance of species to toxicants, but also on species specific capacities to modify or compensate for negative effects of toxicants.

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References


Morton, B (1971) Studies on the biology of *Dreissena polymorpha* Pall. V. Some aspects of filter-


Winner, RW and Gauss, JD (1986) Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. Aquat. Toxicol. 8, 149-161.