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Identification of threshold body burdens of metals for the protection of the aquatic ecological status using two benthic invertebrates

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ABSTRACT

In this study accumulated concentrations of As, Cd, Cr, Cu, Ni, Pb and Zn in two benthic invertebrate taxa, *Chironomus* sp. and Tubificidae are related to ecological responses expressed as changes in macro invertebrate communities. In addition critical body burdens were estimated above which ecological quality was always lower than a certain threshold. Data from existing studies on bioaccumulation of the metals in both taxa were combined with different biological water quality indices. For all metal-endpoint combinations threshold values could be calculated above which ecological quality was always low. Safe threshold body burdens could be estimated for both species for all metals although the data set was more extended for Chrionomus sp. with estimated threshold values being 65, 3.2, 10, 57, 6.5, 73 and 490 μg/g dw for As, Cd, Cr, Cu, Ni, Pb and Zn.

This study demonstrated that metal accumulation in resistant species such as chironomids and tubificid worms have the potential to be used as predictors of ecological effects in aquatic ecosystems. However, the estimated threshold values have to be validated and supported by more lines of evidence before they can be used by regulators.

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1. Introduction

Monitoring of micro pollutants in aquatic ecosystems is mainly based on measurements in the environment, i.e. in the water column, suspended matter and/or sediment (e.g. Kördel et al., 2013; Loos et al., 2008; Roots and Roose, 2013). However, these measurements only reflect the momentary pollution status and do not take into account possible differences in bioavailability, affected by a-biotic factors such as pH, water hardness and temperature and by biotic factors such as feeding habits (Luoma, 1983; Tessier et al., 1984; Bervoets et al., 1997; Clearwater et al., 2002). As a consequence, current water or sediment quality criteria for micro pollutants are not necessarily adequate and well related to effects on aquatic communities observed in the real world (Bervoets et al., 2005; De Jonge et al., 2012; Van Ael et al., 2014). Direct measurement of pollutants in biota could tackle these problems. For this, indicator species are needed which are tolerant of pollution and can accumulate high levels of micro contaminants in their tissues. Moreover, these tissue levels should be related to changes in ecological relevant end-points such as the structure of aquatic communities. Recent studies have demonstrated that accumulated micro-pollutant levels in some aquatic invertebrates (e.g. Luoma et al., 2010; Rainbow et al., 2012; De Jonge et al., 2012, 2013) can be potentially used to predict ecological effects of micro contamination on aquatic communities and to estimate safe pollutant levels. As stated by Rainbow et al. (2012) risk assessments should put a greater emphasis on 'lateral risk assessment' by integrating biological and ecological principles from field observations. Several countries worldwide use biotic indices based on the macro invertebrate composition to evaluate the general status of the ecological quality of surface waters (e.g. Metcalfe, 1989; Abbasi and Abbasi, 2011; Nichols and Dyer, 2013). Within the aquatic macro invertebrates especially Ephemeroptera, Plecoptera and certain families of Trichoptera, the so-called EPT-taxa proved to be sensitive to micro pollutants and more specifically metals (Beasley and Kneale, 2003; De Jonge et al., 2008; Rainbow et al., 2012). In order to comply with the guidelines of the European Water Framework Directive (WFD) many countries moved from biotic indices, which are based only on the presence and sensitivity of macro invertebrate taxa, towards a multimetric index that provides a more complete description description of the community (Böhmer et al., 2004; Rosenberg et al., 2004; Solimini et al., 2008; Teixeira

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et al., 2009; Mondy et al., 2012). In Flanders (Belgium) until about 4 years ago the Belgian Biotic Index (BBI; De Pauw and Vanhooren, 1983) was used to evaluate the biological water quality based on macro invertebrates. The BBI combines the diversity of a sample based on certain taxonomic groups with the tolerance to pollution of individual taxa into one score, ranging from 0 (very bad) to 10 (very good). Since 2010 the Multimetric Macroinvertebrate Index Flanders (MMIF) developed by Gabriels et al. (2010) is used by the Flemish Environment Agency (FEA). The MMIF combines 5 metrics including taxa richness, number of EPT-taxa, number of sensitive taxa (exclusive EPT-taxa); Shannon–Weiner diversity and mean tolerance score. The resulting MMIF-score will change from 0 to 1, with score 0 representing a very low score and score 1 a very high ecological quality.

Rainbow et al. (2012), who related metal accumulation in hydropsychid (Trichoptera) caddisfly larvae to mayfly abundance to estimate threshold values for metals, recommend in their paper that this approach should be tested in other metal-contaminated freshwater rivers in the world. In Flanders however, even under uncontaminated conditions hydropsychid larvae are not very abundant (from absent to <1% of taxa; www.vmm.be/geoview). In addition, the number of mayfly species is rather limited under undisturbed conditions (Lock and Goethals, 2011). This is probably due to the fact that almost all water courses have mainly soft sediments as substratum. Therefore we had to consider other suitable species or taxa. Good candidates are larvae of the non-biting midge (Chironomidae) and oligochaeta worms of the family of the tubificidae. Both taxa are sediment dwelling organisms and good accumulators of metals and tolerate low levels of oxygen, making them suitable as biomonitors in a broad range of water qualities (e.g. Bervoets et al., 1997; Chapman, 2001; Di Veroli et al., 2014). In addition, a good relationship was found between cadmium body burdens in Chironomidae and ecological endpoints on a restricted data set by De Jonge et al. (2012). Instead of mayfly abundance the MMIF-index and the number of EPT-taxa seem good endpoints to assess the ecological quality of freshwaters with a soft substratum (Beasley and Kneale, 2003; De Jonge et al., 2008; Rainbow et al., 2012).

The aim of this study is therefore to investigate the possible relationships between accumulated metals in *Chironomus* sp. and/or Tubificidae and ecological endpoints expressed as the BBI, the MMIF, the total number of taxa, the number of EPT-taxa and the relative abundance of Ephemeroptera. Furthermore, we also investigated the possibility to define thresholds of accumulated metals which can be considered as critical tissue concentrations to protect the aquatic ecosystem.

2. Materials and methods

2.1. Metal concentrations in chironomids and tubificidae

Data on metal body burdens in *Chironomus* sp. (Chironomidaen Diptera) and Tubificidae (Oligochaeta) were derived from several sampling campaigns between 1990 and 2010. All sites were situated in Flanders with most of them in the Campine region in the catchments of the Grote and Kleine Nete (Scheldt basin) or the Meuse River basin. Data on the metal body burdens were partially published elsewhere (Bervoets et al., 1994, 1997, 2004; De Jonge et al., 2009, 2010, 2012 and partially unpublished).

In general the sampling procedure was similar at all sites. Invertebrate samples were taken with a pond net (500 mm mesh, 200–300 mm frame and 500 mm bag depth) fitted to a 1.5 m handle. From all sites invertebrates that were selected for metal analysis were identified as *Chironomus* sp. gr. thummi and/or Tubificidae if present in sufficient numbers. Concerning *Chironomus* sp. only fourth instar larvae were collected. Between 5 and 20 individuals were pooled per taxon per site. The collected invertebrates were depurated by placing them for 24 h in artificial OECD (Organization of Economic Cooperation and Development) water (2 mM $CaCl_2.2H_2O$, 500 mM $MgSO_4.7H_2O$, 771 mM $NaHCO_3$ and 77.1 mM KCl). The samples were dried for at least 24 h at 60 °C in polypropylene 14 ml tubes. Subsequently, the biological material was digested with concentrated ultrapure nitric acid (69% HNO_3) in a microwave (Blust et al., 1988) and stored until analysis for maximally one month. Metals were analysed with inductively coupled plasma mass spectrometer (ICP-MS) from different types and brands. For the already published data see for details on the type of instruments and quality control in the papers mentioned above. For the unpublished data, metals were analysed using a high resolution ICP-MS (HR-ICP-MS; Thermo Scientific, Finnigan Element 2, Waltham, MA, USA) or a Q-ICP-MS (Varian Ultra Mass 700, Australia). Analytical quality was assured by measuring blanks and certified reference material (mussel tissue CRM 278) from the Community Bureau of Reference (European Union, Brussels). Recoveries were always within 10% of the certified values. Depending on the study different metals were analysed. However, at all selected sites Cd, Cu, Pb and Zn were analysed and at most sites also As, Cr and Ni.

2.2. Biological quality data

In total 88 sites were sampled but although most sites were sampled only once, some sites were repeatedly sampled between 1990 and 2009 (2–7 times), resulting in a total of 156 samples from which either *Chironomus*, Tubificidae or both were analysed on metals.

From about 97 samples all macro-invertebrats were collected from the net samples and identified. As a result we could calculate the Belgian Biotic Index (BBI) according to De Pauw and Vanhooren (1983), and the MMIF according to Gabriels et al. (2010). For an additional 59 samples we were able to retrieve the ecological data from the monitoring network of the FEA (www.vmm.be/geoview). Only assessments of biotic data collected by the FEA that were within 30 days before or after our sampling were included in the dataset unless drastic changes in the water courses took place after our collection, e.g. due to removal of discharges or dredging of the river. In the latter case these data were excluded from the analysis. In addition to the BBI and the MMIF it was possible to assess the total number of taxa, the number of EPT taxa and the relative abundance of Ephemeroptera (mayfly larvae) at most sites. Combining the data on the tissue residues of metals with own biological assessment or by coupling to the VMM database resulted in a maximum (depending on the metal considered) of 137 samples for *Chironomus* and 62 samples for Tubificidae for which body burdens could be related to ecological endpoints. From all samples information on BBI and total number of taxa was available, whereas from 122, 132 and 127 samples information on respectively MMIF, EPT taxa and Ephemeroptera abundance was obtained.

2.3. Physical and chemical analysis

From each site, water chemistry variables that largely affect macro invertebrate communities were available including conductivity, pH, oxygen content, ammonium and orthophosphate. In addition, data on dissolved metal concentrations were available from about half of the sites. These variables, were either measured by ourselves according to the methods described in Bervoets et al. (1994, 1997, 2004) and De Jonge et al. (2009, 2010, 2012) or were measured by the FEA and retrieved from their website (www.vmm.be/geoview).

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