Safeguarding Freshwater Organisms From Chemicals

From the Application of Evolutionary Concepts in Ecotoxicology to Large-Scale Risk Assessment of Chemicals

by
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Summary

The increasing anthropogenic demand for chemicals has created large environmental problems with repercussions for the health of the environment, especially aquatic ecosystems. As a result, the awareness of the public and decision makers on the risks from chemical pollution has increased over the past half-century, prompting a large number of studies in the field of ecological toxicology (ecotoxicology). However, the majority of ecotoxicological studies are laboratory based, and the few studies extrapolating toxicological effects in the field are limited to local and regional levels. Chemical risk assessment on large spatial scales remain largely unexplored, and therefore, the potential large-scale effects of chemicals may be overlooked. To answer ecotoxicological questions, multidisciplinary approaches that transcend classical chemical and toxicological concepts are required. For instance, the current models for toxicity prediction - which are mainly based on the prediction of toxicity for a single compound and species - can be expanded to simultaneously predict the toxicity for different species and compounds. This can be done by integrating chemical concepts such as the physicochemical properties of the compounds with evolutionary concepts such as the similarity of species. This thesis introduces new, multidisciplinary tools for chemical risk assessments, and presents for the first time a chemical risk assessment on the continental scale. After a brief introduction of the main concepts and objectives of the studies, this thesis starts by presenting a new method for assessing the physiological sensitivity of macroinvertebrate species to heavy metals (Chapter 2). To compare the sensitivity of species to different heavy metals, toxicity data were standardized to account for the different laboratory conditions. These rankings were not significantly different for different heavy metals, allowing the aggregation of physiological sensitivity into a single ranking. Furthermore, the toxicological data for macroinvertebrates were used as input data to develop and validate prediction models for heavy metal toxicity, which are currently lacking for a wide array of species (Chapter 3). Apart from the toxicity data, the phylogenetic information of species (evolutionary relationships among species) and the physicochemical parameters for heavy metals were used. The constructed models had a good explanatory power for the acute sensitivity of species to heavy metals with the majority of the explained variance attributed to phylogeny. Therefore, the integration of evolutionary concepts (relatedness and similarity of species) with the chemical parameters used in ecotoxicology improved prediction models for species lacking experimental toxicity data. The ultimate goal of the prediction models developed in this thesis is to provide accurate predictions of toxicity for a wide range of species and chemicals, which is a crucial prerequisite for conducting chemical risk assessment. The latter was conducted for the first time on the continental scale (Chapter 4), by making use of a dataset of approximately 4,000 sites distributed throughout 27 European countries and 91 respective river basins. Organic chemicals were likely to exert acute risks for one in seven sites analyzed, while chronic risk was prominent for almost half of the sites. The calculated risks are potentially underestimated by the limited number of chemicals that are routinely analyzed

in monitoring programmes, and a series of other uncertainties related with the limit of quantification, the presence of mixtures, or the potential for sublethal effects not covered by direct toxicity. Furthermore, chemical risk was related to agricultural and urban areas in the upstream catchments. The analysis of ecological data indicated chemical impacts on the ecological status of the river systems; however, it is difficult to discriminate the effects of chemical pollution from other stressors that river systems are exposed to. In Chapter 5, the hypothesis of multiple stressors in German streams was tested and the relative importance of organic toxicants was investigated against abiotic (habitat degradation and nutrients enrichment) and biotic (invasive species) stressors. The results indicated that almost all sites were influenced by more than one stressor. Stream size and ecoregions influenced the distribution of risks, e.g., the risks for habitat degradation, organic chemicals and invasive species increased with the stream size; whereas organic chemicals and nutrients were more likely to influence lowland streams. In order to successfully mitigate the effects of pollutants in river systems, co-occurrence of stressors has to be considered. Overall, to successfully apply integrated water management strategies, a framework involving multiple environmental stressors on large spatial scales is necessary. Furthermore, to properly address the current research needs in ecotoxicology, a multidisciplinary approach is necessary which integrates fields such as toxicology, ecology, chemistry and evolutionary biology.

Contents

Chapter 1

Introduction and Objectives

1.1 Chemical pollution in freshwater ecosystems

Human activities over the last several decades have changed ecosystems rapidly and extensively, with deleterious and irreversible consequences for the environment and human well-being (MEA, 2005). A recent study found that the global boundaries for biodiversity loss and the nitrogen cycle have already been exceeded, whereas the boundaries for climate change and land use change will soon follow, if the current trends prevail (Rockström et al., 2009a,b). More than 60% of world ecosystem services are degraded or used in an unsustainable way, especially with regard to aquatic ecosystems (MEA, 2005; Vörösmarty et al., 2005). It is estimated that 65% of the biodiversity in global river systems is moderately to highly threatened, with water pollution amongst the main contributors to the threat (Vörösmarty et al., 2010). Ultimately, the effects from pollutants will compromise the capacity of ecosystems to provide goods and services for society now and in the future (Cardinale et al., 2012).

With more than half of accessible freshwater systems used by humanity (Vitousek et al., 1997) mainly for industrial, domestic and agricultural processes (Jackson et al., 2001), the occurrence of chemical pollutants – comprising heavy metals and a wide range of man-made organic compounds – in freshwater is inevitable (Schwarzenbach et al., 2010). Until the 1960s, the use of chemical compounds (e.g., pesticides) was perceived only as beneficial, with few repercussions for the environment. This perception changed with the seminal book *Silent Spring* (Carson, 1962), which raised awareness on the impacts of chemicals in the environment and spurred a series of long-standing public policies to safeguard ecosystems and human health (for a summary of the policies see Suter, 2008). This awareness was followed by decades of pollution research, monitoring and management, including the banning of several persistent pollutants and the development of improved wastewater treatment technologies. Despite these efforts, chemicals are still widespread and ubiquitously found in freshwater systems. Currently, more than 100,000 registered synthetic chemicals are in use as consumer products and have ultimately entered and impacted our environment (Schwarzenbach et al., 2006). Furthermore, compounds such as heavy metals or metalloids are frequently found in freshwater systems due to human-induced mobilization of naturally occurring compounds (Schwarzenbach et al., 2006). Some of these compounds can persist in the water system for a long time, because of slow or lack of degradation (e.g., heavy metals, persistent organic pollutants or polychlorinated biphenyls; Schwarzenbach et al., 2006; Fenner et al., 2013). Some compounds also form metabolites, which can pose a comparable or even larger risk to aquatic biota than the parent compounds (Boxall et al., 2004; Fenner et al., 2013), whereas others (e.g., Hg) have the potential to biomagnify up the food web, causing mortality, reproductive failure or other adverse effects on freshwater species or human health (Cristol et al., 2008). Overall, there are indications that the exposure to chemical compounds has profoundly affected

ecosystems (Schwarzenbach et al., 2006; Köhler & Triebskorn, 2013; Beketov et al., 2013) with direct or indirect repercussions for human health (Sharpe & Irvine, 2004; Guillette & Iguchi, 2012).

1.2 Chemical Risk Assessment

Faced with the enormous task of assessing numerous chemicals, while protecting different species, ecological toxicology (hereafter ecotoxicology) provides the basis for assessing the potential adverse effects of chemicals on ecosystems; and therefore, sets the foundations for managing chemicals and informing chemical risk assessment (Calow & Forbes, 2003).

Ecotoxicology developed in the 1980s as an extension of human toxicology. The fundamental theoretical basis of (eco)toxicology is to establish dose-response relationships under laboratory conditions, which estimate the number of individuals that responds to different doses of a chemical. This is typically expressed as a fixed percentile, e.g., LC50, which is the concentration where 50% of the individuals of test populations would suffer lethal effects (Calow & Forbes, 2003). The toxicological information creates the basis for assessing the likelihood of adverse effects in aquatic ecosystems (e.g., chemical risk assessment). Risk assessment - a term borrowed from the insurance industry - estimates (i) the magnitude of the potential adverse effects of chemicals and (ii) the probability that these effects will occur given the current trends of exposure (Suter, 2008). Typically, the risk assessment process follows a tiered approach, with the first tier designed to be coarse and require minimum data, and the last tier designed to be detailed and require a large number of input data (Brock & Wijngaarden, 2012; details in Box 1 and Figure 1.1).

Box 1: Tiered Risk Assessment

The first tier of the risk assessment procedure is based on acute laboratory toxicity tests, performed on a limited number of standard test species (e.g., the fish *Pimephales promelas*, the arthropod *Daphnia magna*, and the algae *Pseudokirchneriella subcapitata*). A threshold concentration (also known as predicted-no-effect-concentration: PNEC) is calculated based on (i) the toxicity data of the most sensitive organism and (ii) a safety factor (also known as an application, extrapolation, or uncertainty factor). The safety factor for acute toxicity data generally equals 1,000 and accounts for the uncertainties related with differences between (i) laboratory and field conditions, (ii) acute and chronic conditions and (iii) sensitivities of the various species groups. In Tier-2 toxicity data for more than the standard test species are used to assess threshold concentrations. For instance, species sensitivity distributions (SSD) allow for the calculation of a concentration which is assumed to protect x% of species of interest (usually 95%). In higher tiers, micro-/mesocosm tests or field studies, and food-web and/or population models are used. Test systems or field studies account for the ecological complexity and realism, indirect effects (Fleeger, Carman & Nisbet, 2003), and recovery from chemicals (Forbes, Calow & Sibly, 2008). On the other hand, population models (e.g., individual-based models; Martin et al., 2012) provide a mechanistic understanding of the ecological processes and an improved understanding of the extrapolation of effects from the individual to the population level.

Increasing the level of complexity of data would increase ecological realism; however,

data required for high tier risk assessment are generally scarce. Low tier risk assessment (tier-1 and tier-2) are more feasible and conducted more often on river basins scales when a wide range of chemicals are present in the water system (e.g., Schäfer et al., 2011; von der Ohe et al., 2011; Schäfer et al., 2013). Although, ecotoxicology has developed beyond single-species laboratory tests, the frequent use of low tier risk assessment is owed to the fact that acute toxicity data are the most abundant ecotoxicological data currently available (Calow & Forbes, 2003; Brock & Wijngaarden, 2012; Scholz et al., 2013). Toxicity information is practically required in all tiers of risk assessment: (i) tier 1 and tier 2 depend entirely on toxicity data, (ii) tier 3 uses LC50 values for the estimation of site toxicity in field studies (e.g., Schäfer et al., 2012), and (iii) tier 4 uses toxicity values of different species for model parametrization (Forbes, Calow & Sibly, 2008).

Despite the abundance of acute toxicity data, it is almost impossible (or at least impractical) to exhaustively conduct experimental toxicity tests for all species-chemical combinations. Furthermore, extensive animal testing has raised ethical concerns, where only in 2008 in the European Union, more than one million fish were used as experimental animals, from which 10% were used for toxicological purposes (Scholz et al., 2013). Thus, prediction models are necessary to fill the gap in missing toxicity information for untested species and chemicals.

Box 2: Methods to predict toxicity

Toxicity of organic compounds can be predicted by two methods: first, the Quantitative Structure-Activity Relationships (QSARs) typically predict baseline toxicity (also know as narcosis), or the minimal toxicity of a chemical. The ability of organic chemicals to elicit toxic effects, such as narcosis, is influenced by the partitioning tendency between a certain target site in the organism and water (Gobas et al., 1988; Escher & Hermens, 2002). Since partitioning of organic chemicals is predominantly occurring in the organic or lipid phase of the organisms (e.g., membranes), the octanol-water partition coefficient (K_{ow}) of an organic compound is considered as an appropriate parameter to develop QSARs (Gobas et al., 1988). Second, read-across methods predict the toxicity of organic chemicals based on the toxicity of structurally similar compounds, such as atom-centered fragments (ACFs). ACFs are parts of the structure of the chemical, i.e., they contain a central atom surrounded by one or several shells of atoms with the same topological distance from the central one (Kühne, Ebert & Schüürmann, 2009; Schüürmann, Ebert & Kühne, 2011; Baskin & Varnek, 2013). Structural similarity is quantified by the ratio between the number of identical ACFs of two compounds and the total number of ACFs of both compounds (Kühne, Ebert & Schüürmann, 2009; Schüürmann, Ebert & Kühne, 2011).

Toxicity of heavy metals has been predicted with Quantitative Ion Character-Activity Relationships (QICARs). Typically, metals are more active in their ionic form, therefore metal toxicity is correlated to the characteristics of ion bindings to biomolecules (Newman, McCloskey & Tatara, 1998). The best characteristics were those which reflected the binding stability with ligands of the groups possessing O-, N-, and S- donor atoms (e.g., softness parameter or covalent index; Newman, McCloskey & Tatara, 1998; Wu et al., 2013). Similar to QSARs, a relationship is established between the toxicity of a test dataset and the respective physicochemical parameters.

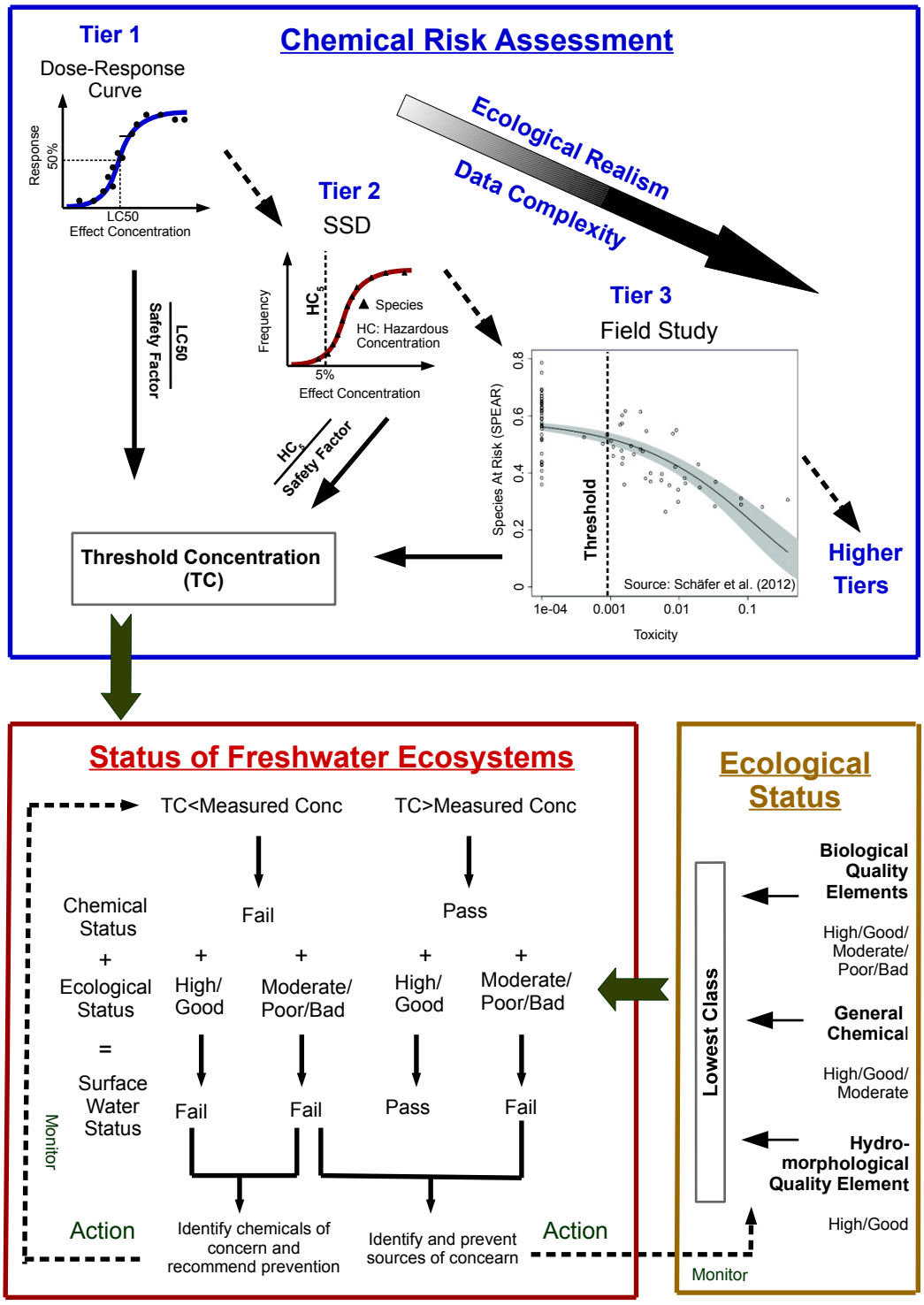

Figure 1.1: Illustration of the main steps to assess the status of freshwater ecosystems. Details on methods can be found in Box 1 for chemical risk assessment and in Box 4 for the ecological status and the overall status of freshwater ecosystems.

1.3 Predicting the sensitivity of species using evolutionary concepts

Predicted effect concentrations (i.e., LC50) for untested organic compounds are typically derived from Quantitative Structure-Activity Relationships (QSARs) or read-across models. The former predicts the toxicity using empirical relationships between chemical properties of the compounds and their toxicity, whereas the latter uses the structural similarities between compounds to make predictions (for details on the methodologies see Box 2).

The integration of experimental information of chemically similar compounds makes read-across models more robust than QSARs (Schüürmann, Ebert & Kühne, 2011). Similar to QSARs for organic compounds, several metal characteristics have explained the toxicity of heavy metals in the so-called Quantitative Ion Character-Activity Relationships (QICARs; see Box 2).

Predicting effect concentrations for untested species is an unexplored field in ecotoxicology (Guénard et al., 2011). Similar to predicting the toxicity of untested compounds - where the structural similarity between compounds is considered - to predict the sensitivity to untested species, the similarity between species can be used. Conceptually, similarity is assumed to increase with the relatedness of species. The similarities between closely related species are attributed to inheritance, i.e., the fact that species descend from common ancestors in a hierarchical fashion, or a tree like process (Delsuc, Brinkmann & Philippe, 2005; Stone, 2011; see Box 3 for details).

Box 3: Phylogenetic similarities of species

To identify the similarities between species, it is necessary to identify characters that descend from common ancestors (also known as homologous characters), which are shared between different organisms (Delsuc, Brinkmann & Philippe, 2005). These evolutionary relationships can be captured in the so-called phylogenetic trees, which were originally constructed based on taxonomy – observations of the phenotypes of species – whereas nowadays, they are mainly constructed using the molecular information of species (e.g., by using mitochondrial DNA and RNA sequences). To summarize, the use of molecular data helped to (i) identify homologous characters among species and (ii) reconstruct phylogenetic trees based on comparison of the homologous characters in species (Delsuc, Brinkmann & Philippe, 2005). Several statistical methods are used for tree construction including distance methods, maximum parsimony or likelihood methods (for details see Felsenstein, 1988; Pagel, 1999; Delsuc, Brinkmann & Philippe, 2005).

This concept is the basis of the theory of natural selection introduced by Charles Darwin 150 years ago in his seminal book on the origin of species (Darwin, 1859). The tendency of closely related species to be similar with regard to a particular quantitative variable is measured by the strength of the phylogenetic signal. This is valid also for the sensitivity of species, where the strength of the phylogenetic signal is related to the change in the sensitivity to chemicals, e.g., if the change in sensitivity for a clade is small, the phylogenetic signal is strong (Carew, Miller & Hoffmann, 2011). Recently, the phylogenetic relationship of species has been used to predict the toxicity of organic chemicals to untested species by making use of the phylogeny of tested species (Guénard et al., 2011). To enhance the predictive power of models based solely on phylogeny, the so-called bilinear models were developed (Guénard et al., 2014). These models integrate

phylogeny with other chemical parameters (e.g., K_{ow}; see Box 2 for details) that explain the toxicity of organic chemicals.

Models that accommodate the phylogenetic relationships of species are lacking for inorganic pollutants such as heavy metals. Similar to organic compounds, we can investigate the influence of phylogenetic information in explaining the sensitivity to heavy metals and integrate parameters used in QICARs to construct prediction models for heavy metals. Sensitivity rankings for heavy metals can be developed by data mining (Wogram & Liess, 2001; von der Ohe & Liess, 2004; Baird & Van den Brink, 2007; Rubach, Baird & Van den Brink, 2010) of toxicological datasets such as the Ecotoxicological Database System (ECOTOX; USEPA, 2007). These rankings would serve as a basis for the development of bilinear models.

Overall, bilinear models for heavy metals and those previously developed for organic compounds would help to predict the toxicity of untested species, and therefore, influence all tiers of risk assessment procedures. Furthermore, by integrating toxicological and chemical information with phylogenetic concepts, bilinear models would bridge the gap between ecotoxicology and evolutionary biology.

1.4 Large-scale assessment of freshwater ecosystems

The majority of studies dealing with the quality of freshwater have traditionally targeted pressures such as hydromorphological degradation and/or nutrients, whereas organic chemicals are typically under-represented (see discussion in Chapter 5). However, river basin management plans might fail if widespread stressors such as organic chemicals are ignored. For instance, the Water Framework Directive (WFD) represents an integrated water management approach adopted in Europe with the aim of achieving a good ecological and chemical status for surface waters in a 15 year timeframe (CEC, 2011; see Box 4 for details on the methods and Figure 1.1). If organic chemicals are of ecological concern the status of freshwater ecosystems would be compromised, and therefore, mitigation measures would be required to manage the risk from chemicals (Figure 1.1).

Box 4: Status of freshwater ecosystems

Good chemical status is assessed by comparing environmental concentrations with the environmental quality standards (EQS) for 41 chemicals which are classified as priority pollutants (CEC, 2012). By analogy to the low tier risk assessment, EQS are derived by the ratio between the toxicity of the chemicals and a safety factor. Based on the type of toxicological data used, safety factors equal (i) 1,000 when acute toxicity data are used (similar with PNEC), (ii) 100 when chronic toxicity data are available for at least one organism group (e.g., as the No-Observed-Effect-Concentration: NOEC), (iii) 10 when chronic toxicity data are available for the main organism groups, and (iv) reviewed on a case by case basis for field data (Forbes, Calow & Sibly, 2008; CEC, 2011). To account for long-term and short-term effects from chemicals, two types of EQS are derived: (i) using the annual average concentration (AA-EQS) for protection against chronic effects and (ii) using the annual maximum acceptable concentration (MAC-EQS) for protection against short term acute effects (for details see CEC, 2011).

Good ecological status is assessed in terms of the quality of (i) the biological communities, such as the characteristics (e.g., abundance and community

composition) of the Biological Quality Elements (BQE; i.e., fish, invertebrates, diatoms, plants and phytoplankton), (ii) the hydrological characteristics, such as the hydrological regime and morphological conditions, and (iii) general chemical characteristics, such as salinity, acidification, nutrients and oxygen conditions. The characteristic - otherwise known as the "metric" - is defined as the measurable part of a system that changes its value along a gradient of human influence (Karr & Chu, 1999; Hering et al., 2006). The metric results in scores reflecting the intensity of the stressor and are numerically expressed as a value between zero and one. Based on these scores, water quality is then divided into five classes that correspond to the levels of impairment: high, good, moderate, poor and bad. Assessment methods across Europe are inter-calibrated to make the assessment of different national systems comparable (e.g., see Bennett et al. (2011) for macroinvertebrates).

To this end, no comprehensive large-scale analysis exists for chemical compounds, which is one of the reasons for a missing planetary boundary for chemicals (Rockström et al., 2009a). However, Rockström et al. (2009a) stress that there are indications that chemical pollution has affected ecosystem health on the global scale. The lack of large-scale analysis for chemicals can be attributed to the (i) unavailability of monitoring data on large spatial scales (e.g., continental), and (ii) scarcity of toxicity data for a wide range of chemicals. The development of centralized monitoring datasets (e.g., the European Waterbase Dataset (EEA, 2012)) and enhanced toxicity prediction tools discussed above can close these gaps.

1.5 Co-occurrence of stressors

Effects observed on ecosystems are rarely the result of a single stressor. A stressor is defined as an abiotic (e.g., organic chemicals or nutrients) or a biotic (e.g., introduction of alien species) variable for which adverse effects on individuals or populations are statistically significant (see Vinebrooke et al. (2004) and the references therein). Typically, multiple stressors interact and their combined effects have been documented from local to regional scales (Comte et al., 2010; de Deckere et al., 2012; de Zwart et al., 2006; Schinegger et al., 2012; Vörösmarty et al., 2010). A global analysis found that stressors co-occur in the majority of cases (Vörösmarty et al., 2010), whereas a pan-European study found that >50% of the river sites are subject to alteration of water quality, hydrology, and morphology (Schinegger et al., 2012). Furthermore, biological, chemical and habitat data revealed that most of the sites in the Ohio (USA) stream network were impaired as a result of multiple stressors (de Zwart et al., 2006). Similar results were found on regional scales, where the co-occurrence of organic and inorganic pollutants has impaired the quality of freshwater (Comte et al., 2010; de Deckere et al., 2012). However, to date, the relative importance of organic chemicals in scenarios with multiple stressors has rarely been assessed. So far, studies related with multiple stressors have mainly focused on habitat degradation and nutrients, whereas chemical pollution has typically been assessed as organic load (see discussion in Chapter 5). Considering the likelihood of ecological effects from chemicals (see Chapter 4) and the assumption that organic compounds co-occur with other stressors (especially nutrients), there is an urgent need for studies which assess the contribution of chemicals in relation to other stressors.

1.6 Objectives and structure of the thesis

The two main objectives of this thesis are (i) to introduce new, multidisciplinary methods for chemical risk assessment and (ii) to estimate the likelihood of chemical effects on the continental scale (see Figure 1.2 for an illustration of the main research work). The thesis starts with a method for developing the physiological sensitivity of macroinvertebrate species to heavy metals (Chapter 2). Specific goals were to:

- develop sensitivity rankings for different bivalent heavy metals using an existing, up-to-date ecotoxicological dataset
- standardize toxicity data to (i) account for different laboratory conditions and (ii) derive comparable sensitivity rankings for different heavy metals
- test for differences between individual heavy metal rankings

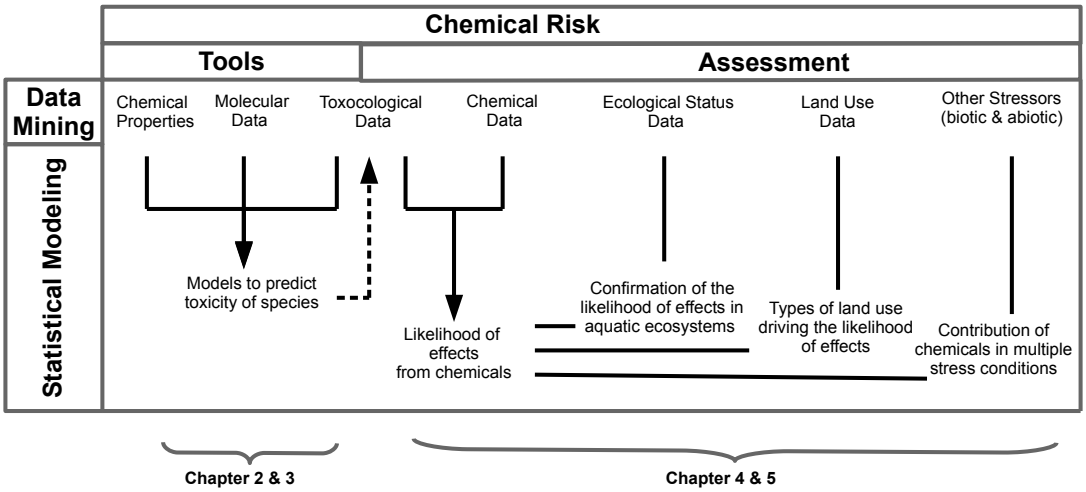

Figure 1.2: Conceptual model of the chemical risk procedure followed in this thesis.

The toxicological information is further used in Chapter 3 to develop and validate bilinear models which integrate the phylogenetic information of species and the physicochemical properties of compounds to make predictions for heavy metal toxicity. Specific goals were to:

- investigate whether the sensitivity of heavy metals is phylogenetically structured
- investigate the accuracy of the predictions made by bilinear models for untested species and heavy metals

The ultimate goal of the heavy metals bilinear models and the models previously developed for organic compounds is to provide accurate predictions of toxicological information, which is crucial for conducting chemical risk assessment. Chemical risk assessment of organic compounds was conducted for the first time on the European scale and represents the most complete and holistic analysis to date (Chapter 4). The following main goals were addressed:

- quantification of the chemical risk for the European river basins

- identification of the ecotoxicologically relevant compounds and their influence on the chemical risk in Europe
- influence of land use on chemical risk
- influence of chemical risk on the ecological status of rivers

Finally, an analysis investigating the risk of organic chemicals in the frame of multiple stressors was conducted (Chapter 5). The specific goals were to:

- analyze the individual occurrence of organic toxicants, habitat degradation, nutrients and invasive species in German streams
- analyze the joint co-occurrence of organic toxicants with the above mentioned stressors
- investigate the relationships between stressors and stream sizes or ecoregions

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Chapter 2

Physiological Sensitivity of Freshwater Macroinvertebrates to Heavy Metals

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"We are rightly appalled by the genetic effects of radiation; how then, can we be indifferent to the same effect in chemicals we disseminate widely in our environment?"
Rachel Carson

[1]ET&C is one of the most important scientific journals in the field of environmental toxicology. It has an impact factor of **2.62** as reported by the 2012 Journal Citation Reports (Thomson Reuters, 2013)

2.1 Abstract

Macroinvertebrate species traits, such as the physiological sensitivity have successfully been introduced in trait-based bioassessment approaches and are important predictors of species sensitivity in the field. The authors ranked macroinvertebrate species according to their physiological sensitivity to heavy metals using toxicity data from acute laboratory assays. Rankings for each of the heavy metals, Cd, Cu, Cr, Ni, Pb, Zn, and Hg, were standardized based on all available species data. Rankings for different heavy metals on the species level showed no significant difference between compounds and were reasonably well correlated pairwise ($0.50<r<0.73$). Thus, an aggregated heavy metal ranking was developed, which assigns a single physiological sensitivity value (S_{metal}) to macroinvertebrate taxa. Considering the high variation, especially for higher taxonomic levels, i.e., in the order level, it is recommended to use S values of the genus or species level for meaningful analyses. In terms of taxonomic ranking, crustaceans were overall the most sensitive taxonomic group, whereas insects were generally the most tolerant taxonomic group. Species in the order of Cladocera were three orders of magnitude more sensitive than insects of the order of Trichoptera. By contrast, mollusks covered a wide range of sensitivities, with bivalves being on average one order of magnitude more sensitive than gastropods. The authors concluded that physiological sensitivity represents a promising trait for trait-based risk assessment that together with other demographic and recolonization traits may help to identify the effects of heavy metal pollution in aquatic ecosystems.

2.2 Introduction

Heavy metals are natural elements found in the earth's crust. Their principal sources in ecosystems are either natural (geological weathering of rocks) or anthropogenic (industrial effluents, mining activities, domestic wastewater, or atmospheric deposition from industrialized areas; Förstner & Wittmann, 1983). In terms of anthropogenic inputs, heavy metals are of major concern in lotic and lentic freshwater ecosystems (Förstner & Wittmann, 1983). The respective effects of heavy metal pollution on the biota depend on the concentration and speciation of the metals present, on environmental factors determining bioavailability, and especially on the sensitivity of the target organisms.

To classify metals based on their biological, toxicological, and environmental relevance, insight into the toxic mode of action of metal ions and their chemistry is necessary. For this purpose, Nieboer & Richardson (1980) classified metals as class A metals (e.g., Mg, Ca, Al, Ba, Be), which are oxygen-seeking metals, class B metals (e.g., Hg or Ag), which are sulfur- or nitrogen-seeking metals, and borderline metals (e.g., Cd, Cu, Ni, Pb, Zn, or Cr), which bind to both sulfur/nitrogen and oxygen groups. Sulfur- and nitrogen-seeking metals are considered to be more toxic than oxygen seeking metals (Nieboer & Richardson, 1980; Rainbow, 2002). Furthermore, some metals (Hg, As, or Pb) can form water stable organometallic cations, well known for toxic effects as they accumulate within cells (Nieboer & Richardson, 1980).

Generally, heavy metals are accumulated by aquatic organisms, either from the surrounding aquatic medium or from food, regardless of whether these metals are essential to metabolism (Rainbow, 2002). Toxic effects are influenced by intrinsic processes, such as accumulation, transport, detoxification or excretion (Rainbow, 2002). Toxicity is also influenced by environmental factors. For instance, heavy metal toxicity is inversely related to water hardness, as a result of competition of metal cations with Ca^{2+} and Mg^{2+} cations in the water phase (Di Toro et al., 2001). The influence of aqueous ligands (e.g.,

pH, water hardness) on metal toxicity is generally acknowledged, for example, from the biotic ligand model (BLM) that is used to predict toxic effects of metal ions under different environmental conditions (Di Toro et al., 2001). Furthermore, toxicity can be altered by other exposure factors such as temperature and exposure time (Wang, 1987). Therefore, sensitivity to heavy metals varies greatly between organisms.

Freshwater aquatic macroinvertebrates have been widely used as biological indicators to assess water quality in ecological risk assessments (Rosenberg & Resh, 1993). An approach recently receiving more attention is trait-based ecological risk assessment, which relies on ecological and/or physiological traits to determine the vulnerability of individuals, populations, or communities to different stressors (Rubach et al., 2011; Rubach, Baird & Van den Brink, 2010; Baird & Van den Brink, 2007; Liess & Von Der Ohe, 2005; Schäfer et al., 2011). According to a framework proposed by Rubach et al. (2011), traits can be classified according to their vulnerability factor into the following: (i) external exposure (e.g., habitat choice, migration); (ii) intrinsic sensitivity (e.g., target site distribution, elimination rate); (iii) demography (e.g., generation time, voltinism); and (iv) recolonization (e.g., drift, trophic level). The intrinsic sensitivity of Rubach et al. (2011) is equivalent in the present study, to the physiological sensitivity measured in single species toxicity tests. Hence, data from these tests can be used to rank macroinvertebrate species from the most to the least sensitive with regard to the specific stressor of concern (Rubach, Baird & Van den Brink, 2010; Baird & Van den Brink, 2007; Wogram & Liess, 2001; von der Ohe & Liess, 2004).

There is a paucity of trait-based risk assessment approaches for heavy metals in freshwater ecosystems. One crucial prerequisite for the development of trait-based approaches for heavy metals is the availability of physiological sensitivity rankings. Such rankings represent the backbone of the species at risk (SPEAR) trait-based index, which has been successfully used to assess the effects of organic toxicants in various lotic systems across Europe (von der Ohe et al., 2007; Schäfer et al., 2007) and Australia (Schäfer et al., 2011). This index has successfully been adapted to assess the impact of organic compounds and heavy metals on nematodes in freshwater soft sediments (Höss et al., 2011). Physiological sensitivity represents one of the best descriptors of the effects in the field from organic toxicants, although other traits such as demographic traits and/or recolonization traits significantly improved predictability of the SPEAR index (Liess & Von Der Ohe, 2005; Schäfer et al., 2007; Schäfer et al., 2011).

Previous physiological sensitivity studies provided no information on the metals or metal compounds used in the analysis and were limited in their taxonomic range (Wogram & Liess, 2001; von der Ohe & Liess, 2004). Therefore, the aim of the present study was to derive a revised heavy metal ranking for a representative set of heavy metals (Cd, Cu, Cr, Ni, Pb, Zn, and Hg) using acute laboratory assays of aquatic invertebrate. The approach from Wogram & Liess (2001) and von der Ohe & Liess (2004) was revised with regard to a wider taxonomic range, an unbiased standardization method (not using *Daphnia magna* as reference species), and the consideration of intraspecies variation from diverse laboratory test conditions (i.e., hardness, temperature, and exposure time). Furthermore, we tested the hypothesis of difference between heavy metal rankings.

2.3 Methods

2.3.1 Database Compilation

Test data were retrieved from the Ecotoxicology Database System (ECOTOX; USEPA, 2007) on January 10, 2011. The database was selected because it is the largest freely available toxicity database to our knowledge. Furthermore, using the ECOTOX dataset facilitated the process of comparing the results to previous studies (Wogram & Liess, 2001; von der Ohe & Liess, 2004). In total, 16,827 toxicity test records were obtained for 18 metal ions and their salts. This dataset comprised data on Ag, Al, As, Ba, Be, Cd, Co, Cr (VI), Cr(III), Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, V, and Zn. Initially, duplicate entries and entries with missing taxonomic information or missing concentration values were omitted. Where no mean values were reported, the average of the maximum and minimum values was taken. Units were standardized for lethal concentrations and for all other physicochemical parameters. Similarly, inconsistencies in the taxonomic classification (mainly order level) between the ECOTOX database and previous studies on the physiological sensitivity of species (Wogram & Liess, 2001; von der Ohe & Liess, 2004) were harmonized to ensure taxonomic consistency. For example, entries retrieved from ECOTOX belonging to the order of Diplostraca were replaced with Cladocera. Furthermore, data were restricted to freshwater macroinvertebrates. In total 34.4% of the original data were omitted due to these restrictions.

Toxic effect concentrations, expressed as median lethal concentrations (LC50) and median effect concentrations (EC50), corresponding to the endpoints of mortality and immobility, respectively, were used equivalently, and hereafter are referred to as LC50. Acute tests were used instead of chronic tests because the latter were rather scarce with regard to the taxonomic range. A combined use of acute and chronic data is not suitable if physiological sensitivity is determined, since chronic toxicity is also influenced by other traits (i.e., demographic). The dataset was restricted to acute toxicity data following standardized procedures that recommend fixed exposure times of 24-, 48-, 72- and 96-h tests for acute effects (USEPA, 2002). In total, 20.5% of the original data were excluded as a consequence of endpoint and exposure time restrictions. The removed data contained 17 different endpoints and 74 different exposure durations.

Furthermore, entries of toxicity tests performed in water hardness above 200 mg/L $CaCO_3$ (Meyer, 1999) and in temperatures below 5°C or above 35°C were removed to limit the potential bias resulting from extreme hardness and temperature conditions, which are unlikely to occur in the field. In total, another 4% of the data were omitted due to hardness restriction and 14 entries due to temperature restriction.

Availability of species data for different metals was inconsistent, therefore making the comparison between heavy metals difficult. To address this problem, we requested that each species had toxicity data available for at least three metals. From the remaining dataset, only metals with at least 35 species were considered to allow for a statistical comparison between heavy metal sensitivities, which requires a minimum number of observations. Therefore, the metals Ag (19 species; 15 genera), Co (19 species; 17 genera), Fe (13 species; 12 genera), As (12 species; 10 genera), Mn (10 Species; 10 genera), Se (9 species; 6 genera), Al (8 species; 15 genera), Ba (7 species; 7 genera), Be (3 species; 3 genera), Sb (3 species; 3 genera), V (3 species; 3 genera), and Cr(III) (2 species; 2 genera) were removed from the analysis. In total, 11.3% of the original data were omitted due to these restrictions.

The analysis was finally conducted for Cd, Cr(VI)(hereafter referred to as Cr), Cu, Hg, Pb, Ni, and Zn. In the present study, the term heavy metal is used as a description

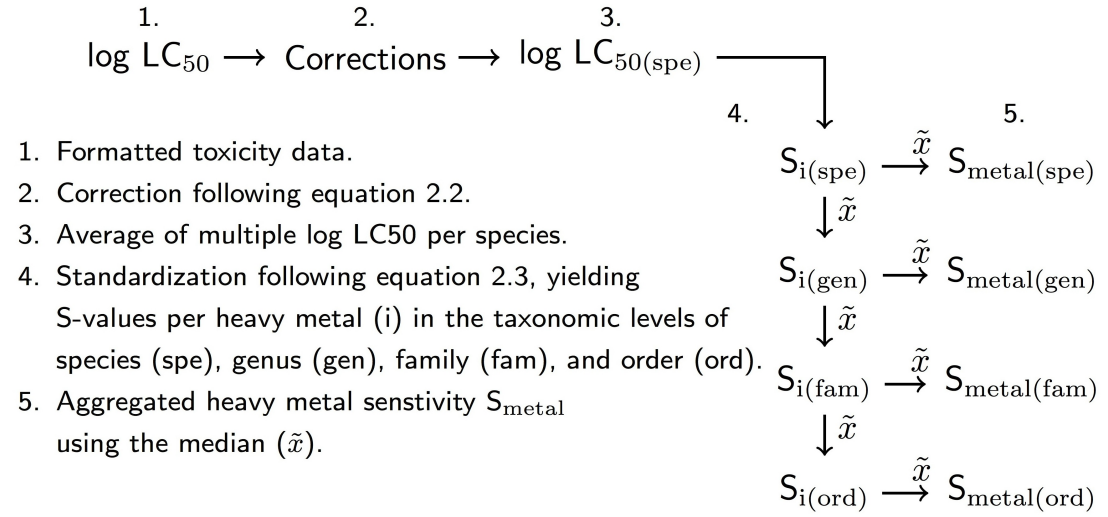

Figure 2.1: Steps to derive heavy metal-based rankings for freshwater macroinvertebrates.

of metals with ions of class B (Hg^{2+}) and borderline (Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}) according to Nieboer & Richardson (1980).

The toxic concentrations were generally reported on the basis of the ionic form of the compound. In those cases when LC50s have been reported for the total compound (5.5%), they were transformed to ionic toxic concentrations ($LC50_{HM}$) using:

$$LC50_{\mathrm{HM}} = (\frac{molwt_{\mathrm{HM}}}{molwt_{\mathrm{c}}})LC50_{\mathrm{c}} \tag{2.1}$$

where $mol\ wt_{HM}$ is the heavy metal's molecular weight, $mol\ wt_c$ is the compound's molecular weight, and $LC50_c$ is the reported lethal concentration of the compound.

The toxic concentrations were log transformed to center the data and allow an easier interpretation of the outliers. In some cases, differences of up to four orders of magnitude were noticed for the same species-metal combination and the same endpoint (e.g., log LC50 for Cd for *D. magna*). Aberrant entries can result from human errors (e.g., during the conduction of tests, reporting of results, or data insertion in the database), temporal variation related to changing test designs over the past decades, or various laboratories performing the tests, which differ in analytical methods or quality control procedures. If the values differed by more than two standard deviations from the mean, they were considered as outliers. This rule was applied only to species with a large number of entries and source references (e.g., log LC50 for Cd for *D. magna* with 457 entries from 75 different references). For the remaining species, if values differed by more than a factor of 30 from the closest one in a group of at least three other references, the aberrant value was discarded as described in previous studies (von der Ohe & Liess, 2004; Guénard et al., 2011). In case of further doubt, the references were either verified using the original publications, or if this was not possible, priority was given to the more recent data sources. In total, 5.5% of the original data were omitted due to outlier removal.

Summing up all the aforementioned restrictions, 75.7% of the original data were omitted. Subsequently, 4,103 entries fulfilled the above-mentioned requirements for the seven selected heavy metals, with a diverse taxonomic range of 114 species, based on 412 different sources over the last 50 years. Copper had the most entries (37%), followed by Cd (24%), Zn and Cr (12% each), Hg (7%), and Ni and Pb (4% each). Cadmium covered most of species (104), followed by Cu (97), Zn (88), Hg (67), Cr (57), Pb (42), and Ni

(35). Number of entries (n) for each species, for each heavy metal, is shown in Appendix A (Table A.5).

2.3.2 Normalization of the toxicity data

Due to the expected influence of exposure time, hardness, and temperature on heavy metal toxicity (Wang, 1987), the original log LC50 (Figure 2.1, Step 1) values were normalized to standard laboratory conditions to reduce bias when pooling data generated under different test conditions (Figure 2.1, Step 2). Toxicity tests from the final dataset were mainly performed under 48-h of exposure time (80%), 20°C of temperature (75%), and 50 mg/L $CaCO^3$ of hardness (45%). When hardness and temperature conditions were not reported (exposure time was always reported), standard laboratory conditions were assumed.

Normalization for exposure time followed Haber's rule, stating that the product of exposure time and exposure concentration results in a constant toxic effect (Haber, 1924). Hence, an exposure time of 96-h would result in a fourfold lower LC50 value compared to the 24-h test. With regard to temperature, as a rule of thumb (van't Hoff's or Q10 rule), an increase of 10°C in laboratory conditions results in up to three-fold increase in the metabolism rate (Cairns, Heath & Parker, 1975), and hence toxicity. We used the maximum value of three-fold change in this analysis. Hence, temperature accounted for almost one order of magnitude difference in LC50 values for species tested under 5°C or above 35°C. Finally, hardness normalization was done following an equation proposed by de Zwart et al. (2006), based on hardness criteria available from the Ohio Environmental Protection Agency (USEPA, 1996). The equation from de Zwart et al. (2006), assumed that for a water hardness of 200 mg/L, a fivefold decrease in metal ion toxicity could be observed compared to hardness below 25 mg/L. Hence, laboratory conditions could account for more than two orders of magnitude in the toxicity of heavy metals. It is worth noting that normalizing only for hardness was a simplification under the assumption of interrelation of hardness with other physicochemical parameters, e.g., pH and alkalinity (Meyer, 1999; USEPA, 2001; Schmidt et al., 2010). These considerations resulted in the following overall normalization equation:

$$logLC50_{\text{norm}} = (logLC50_{\text{org}}) \times (\frac{t}{0.50}) \times (\frac{0.58}{12.52 \times H^{-0.79}}) \times (\frac{0.33}{3e^{-(0.11\text{xT})}}) \qquad (2.2)$$

where $LC50_{\text{norm}}$ is the normalized LC50, $LC50_{\text{org}}$ is the original LC50, and t, H, and T represent constants for time, hardness, and temperature, respectively. Details on the normalization procedure mentioned above and on the steps to derive equation 2.2 can be found in the Appendix A, Supplementary Methods and Figure A.1.

The BLM was not used in the present study due to the large number of input parameters required (up to 12). Most of these parameters were not available in the database, or were rather limited, such as dissolved organic carbon that is considered as one of the most sensitive parameter for application of the Cu-BLM (Di Toro et al., 2001). Furthermore, BLMs have been developed only for Cu, Cd, Ag, and Zn, thus only for three out of seven of our heavy metals of interest in the present study. Hardness-normalized procedures, due to a paucity of physico-chemical parameters were also found in other studies (Brix, DeForest & Adams, 2011; Buchwalter et al., 2007).

To quantify the reduction in data variability achieved by normalization, the standard deviation from the mean log LC50 per heavy metal and species was calculated for corrected and non-corrected data. In detail, the standard deviation was first calculated for each heavy metal and species and subsequently averaged over all species to quantify the

variation in the genus level. Similarly, the variation in the family and order level was obtained by averaging across the standard deviations of the next lowest taxonomic level. Sequential averaging was done to avoid bias from species rich genera (e.g., gammarids). A schematic presentation of this procedure is given in Appendix A, Figure A.2.

2.3.3 Calculation of physiological sensitivity to heavy metals

Initially, the log transformed normalized concentrations for multiple entries of the same species and heavy metal were averaged (Figure 2.1, Step 3; values in Appendix A, Table A.5). These values were then standardized (Figure 2.1, Step 4) according to:

$$S_{\mathrm{i(spe)}} = \frac{logLC50_{\mathrm{i(spe)}} - \hat{\mu}_{\mathrm{i}}}{\hat{\sigma}_{\mathrm{i}}} \tag{2.3}$$

where LC50 is the species *spe* toxicity value, $\hat{\mu}$ is the mean, and $\hat{\sigma}$ is the standard deviation for each heavy metal *i*.

Using equation 2.3, we calculated standardized toxicity values ($S_{\mathrm{i(spe)}}$), for each heavy metal *i*, for the lowest taxonomic group represented by the species *spe*. Standardization was done so that each heavy metal dataset had a mean of zero and a standard deviation of one, in accordance with the method from Rubach, Baird & Van den Brink (2010). Therefore, the $S_{\mathrm{i(spe)}}$ values for each heavy metal had a similar range.

The *S* values will represent hereafter the physiological sensitivity values in the respective taxonomic levels for a given heavy metal. If ranked, negative *S* values indicate more sensitive taxa, and positive *S* values less sensitive (or more tolerant) taxa.

The *S* values for taxonomic levels other than species, namely $S_{\mathrm{i(gen)}}$, $S_{\mathrm{i(fam)}}$, and $S_{\mathrm{i(ord)}}$ were calculated using the median sensitivity of the subjacent taxonomic units for each heavy metal *i* (Figure 2.1, Step 4). The dispersion of data around the median for $S_{\mathrm{i(ord)}}$, $S_{\mathrm{i(fam)}}$, and $S_{\mathrm{i(gen)}}$ was measured as median absolute deviation (MAD), and is given in Appendix A, Table A.2, Table A.3 and Table A.4, respectively. Median and MAD were used as robust alternatives to arithmetic mean and standard deviation for the *S* values under the condition of a small heavy metal sample size (maximum of seven). The last step (Figure 2.1, Step 5) included calculating the median heavy metal sensitivity (S_{metal}), after concluding that heavy metals species sensitivity could be aggregated (discussed below). The overall S_{metal} values were calculated as the median heavy metals sensitivity in each taxonomic level, namely $S_{\mathrm{metal(spe)}}$, $S_{\mathrm{metal(gen)}}$, $S_{\mathrm{metal(fam)}}$, and $S_{\mathrm{metal(ord)}}$.

Furthermore, the $S_{\mathrm{metal(ord)}}$ was compared to the previous rankings by Wogram & Liess (2001) for both heavy metal and organic compound sensitivities values, expressed as S_{m} and S_{o}, respectively. Taxa were compared in the order level, because it was the highest level reported in the previous study (Wogram & Liess, 2001). The Pearson correlation coefficient (r) was used to investigate the relationships between S_{o} and $S_{\mathrm{metal(ord)}}$ and S_{m} and $S_{\mathrm{metal(ord)}}$.

2.3.4 Statistical Analysis

All data transformations and standardizations, as well as statistical procedures and generations of graphics, were performed in the free open source software R, version 2.11 (R Core Team, 2011).

Considering that an aggregated S_{metal} value for all heavy metals would be of greater practical value for further applications and easier to use than separate *S* values for each heavy metal, we tested whether there are significant differences in the $S_{\mathrm{i(spe)}}$ values.

A linear mixed-effect (LME) model was employed to check for significant differences between $S_{i(spe)}$ values, under the condition that heavy metal rankings were represented by an unequal number of species, and therefore had an unbalanced design. For this purpose, the lmer function in the R package lme4 (Bates & Maechler, 2010) was used to fit a linear mixed effect model. Moreover, the function pvals.fnc in the R package languageR (Baayen, 2010) was used to calculate p values and Bayesian highest posterior density (HPD) intervals for the parameters of the model fitted with lmer. Because we were interested in potential differences between heavy metals, the latter were considered as fixed factors and species as random factors. Model diagnostics included checking for normality and heterogeneity using graphical tools (Appendix A, Figure A.3) as recommended in Zuur et al. (2009). The shapes of histograms were used to check for normality of fixed factors (Figure 2.2), which suggested normal distribution of the S values for each heavy metal.

Unbalanced datasets suffer from the deficiency of properly calculating degrees of freedom, and therefore, the p values generated from a normal Student t-distribution tend to be incorrect (for details Baayen, Davidson & Bates (2008)). To tackle this problem, we simulated a Markov Chain Monte Carlo (MCMC) sample from the Bayesian posterior distribution (Baayen, Davidson & Bates, 2008), using the function mcmcsamp in the R package lme4. Furthermore, we calculated the Bayesian HPD 95% confidence intervals (CIs) of the MCMC sample for fixed effects using the function HPDinterval in the R package coda (Plummer et al., 2010). This function calculated the mean values of fixed factors with bootstrapped 95% CIs, which we set at 50,000 iterations. This is considered to be the most efficient technique to evaluate LME parameters (Baayen, Davidson & Bates, 2008). However, for ease of interpretation, corresponding p values were provided for posterior distribution calculated with the function pvals.fnc in the package languageR (Baayen, 2010).

Whereas linear mixed models are used to investigate the differences in heavy metal rankings, the Pearson correlation coefficient r was employed to examine the strength of relationships between the fixed effects represented by the $S_{i(spe)}$. Furthermore, the sensitivity of a single heavy metal was compared to the median sensitivity of the remaining heavy metals in the species level. For example, $S_{Cd(spe)}$ was correlated to the median sensitivity calculated from $S_{Cu(spe)}$, $S_{Cr(spe)}$, $S_{Ni(spe)}$, $S_{Pb(spe)}$, $S_{Zn(spe)}$and $S_{Hg(spe)}$. This was repeated for all heavy metals. In this way, it was possible to determine the predictive power of the median sensitivity value for the prediction of the corresponding S value of a nonincluded heavy metal.

2.4 Results

2.4.1 Normalization of the toxicity data

Normalization of the data to standard laboratory conditions (exposure time of 48-h, temperature of 20°C, and hardness of 50 mg/L $CaCO_3$) reduced the variability deriving from various exposure time, temperature, and hardness conditions. Variability was reduced by 24% for Cd, 22% for Pb, 19% for Cr and Ni, 17% for Cu, 13% for Hg, and 11% for Zn. Aggregated standard variation of normalized and non-normalized concentration values can be found in Appendix A, Table A.1.

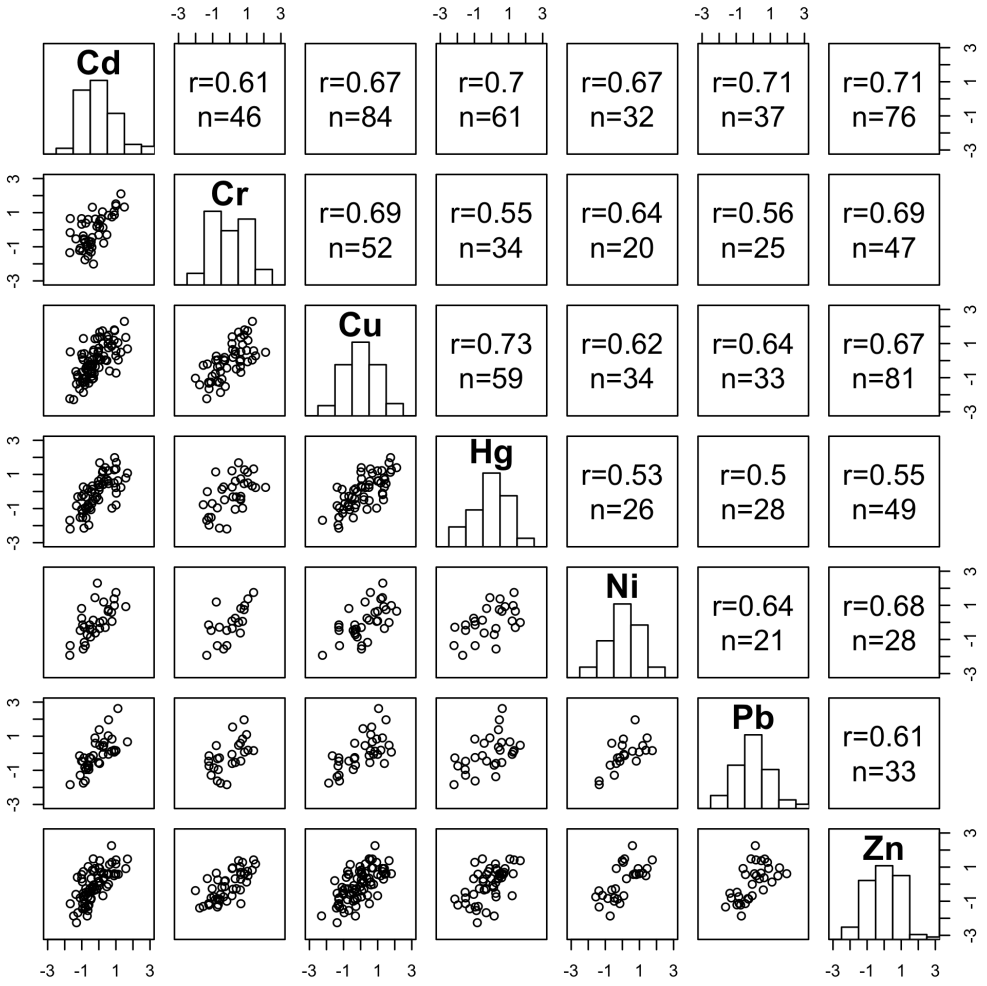

Figure 2.2: Pairwise mean comparison of physiological sensitivity values for each taxa of the seven heavy metals expressed as S_{Cd}, S_{Cr}, S_{Cu}, S_{Hg}, S_{Ni}, S_{Pb}, and S_{Zn}. Histograms show the distribution of the standardized sensitivity values for each heavy metal. The upper panel prints the Pearson correlation coefficient r and the number of pairwise comparisons n. The lower panel prints the plotted values for each pair of heavy metals.

2.4.2 Relationship of physiological sensitivity for different heavy metals

The Bayesian HPD 95% CIs of the MCMC sample for fixed factors ($S_{i(spe)}$) suggested a lack of statistical significant differences between all separate heavy metal rankings (Table 2.1). It is important to note that 95% CIs that include zero are equivalent to a p value greater than 0.05, resulting in statistically insignificant results. Relationships between each pair of heavy metal's S values ranged from moderate correlation (r=0.50, n=28) between Hg and Pb to good correlations (r=0.73, n=59) between Cu and Hg (Figure 2.2). The $S_{Hg(spe)}$ exhibited the weakest inter relationship with other heavy metals, e.g., with $S_{Ni(spe)}$ (r=0.53, n=26), $S_{Cr(spe)}$ (r=0.55, n=34) and $S_{Zn(spe)}$ (r=0.55, n=49). By contrast, correlations between $S_{Cd(spe)}$ and other heavy metals were among the highest, e.g., with $S_{Zn(spe)}$ (r=0.70, n=76) and $S_{Pb(spe)}$(r=0.71, n=37).

Furthermore, sensitivity of a single heavy metal was compared to the median sensitivity of the remaining heavy metals in the species level. Values of $S_{Zn(spe)}$(r=0.75, n=87),

Table 2.1: Summary of results for the fixed factors of the mixed effect model. Parameters are presented by: model estimate calculated (Estimate), mean estimate across MCMC samples (MCMC mean), lower (-) and upper (+) 95% highest posterior density (HPD) confidence intervals (CIs), and p-values based on the posterior distribution (pMCMC).

Fixed effects	**Estimate**	**MCMC mean**	**HPD 95% CIs**		**p(MCMC)**
			-	+	
$S_{Cd(spe)}$	0.041	0.023	-0.142	0.195	0.793
$S_{Cr(spe)}$	0.154	0.077	-0.181	0.325	0.552
$S_{Cu(spe)}$	0.113	0.036	-0.176	0.247	0.739
$S_{Hg(spe)}$	-0.002	-0.023	-0.266	0.208	0.849
$S_{Ni(spe)}$	0.061	0.004	-0.297	0.302	0.980
$S_{Pb(spe)}$	0.069	0.009	-0.274	0.293	0.954
$S_{Zn(spe)}$	0.163	0.067	-0.144	0.292	0.548

$S_{Cu(spe)}$(r=0.75, n=97), $S_{Ni(spe)}$(r=0.74, n=35), $S_{Pb(spe)}$ (r=0.72, n=41), and $S_{Cd(spe)}$ (r=0.71, n=95) correlated slightly better than $S_{Hg(spe)}$ (r=0.70, n=65), and $S_{Cr(spe)}$ (r=0.68, n=55) with the respective medians of the remaining heavy metals.

2.4.3 Physiological sensitivity to heavy metals (S_{metal})

Because no significant differences in $S_{i(spe)}$were found, an overall heavy metal sensitivity value (S_{metal}) was calculated for each taxon (Table 2.2). It is worth noting that the restriction of three metals per species, required for the statistical analysis, excluded insect species of the orders of Zygoptera, Trichoptera, Heteroptera, Anisoptera, and Megaloptera from the statistical analysis to avoid bias from underrepresented taxa. Nevertheless, the S_{metal} values were reinserted in Table 2.2 considering the importance of these orders for biomonitoring. At the taxonomic level of the species, S_{metal} values showed cladocerans like *Moina irrasa* (-1.76), *Ceriodaphnia dubia* (-1.22), *Ceriodaphnia reticulate* (-1.18), *Daphnia pulex* (-1.07), and *D. magna* (-1.09) to be among the most sensitive taxa. These species belonged to the most sensitive families of Moinidae (-1.16), Daphniidae (-0.96), and Diaptomidae (-0.85), respectively. The family Moinidae was more sensitive to Hg (-2.15) as compared to the other heavy metals, whereas the other two families had more consistent S values for all heavy metals. These families belonged to the most sensitive crustacean order of Cladocera (-0.93). Other sensitive orders also belonged to the taxonomic group of crustaceans, such as Calanoida (-0.85), Anostraca (-0.74), and Amphipoda (-0.45). Some differences were noticed in the S values of these orders for specific heavy metals, where the order of Cladocera was more sensitive to Hg (-1.49), Calanoida to Zn (-1.67), and Anostraca to Cr (-1.29), while Amphipoda had similar values for all heavy metals. Isopods represented a rather tolerant taxa from the group of crustaceans (0.91), followed by intermediate sensitivities for the orders of Podocopida (0.40) and Decapoda (0.13).

Furthermore, S_{metal} values showed that some of the least sensitive species were *Hydropsyche angustipennis* (2.07) and *Ischnura elegans* (1.39), corresponding to the least sensitive insects families of Hydropsychidae (2.05), and Coenagrionidae (1.70) respectively. The latter two families represent the two most tolerant orders of Trichoptera (1.97) and Zygoptera (1.71). In addition, Plecoptera, represented by only one species (*Acroneuria lycorias*) was among the most tolerant taxa with a S_{metal} value of 1.39. All the aforementioned insect orders had comparable S values for most of the heavy metals. On the

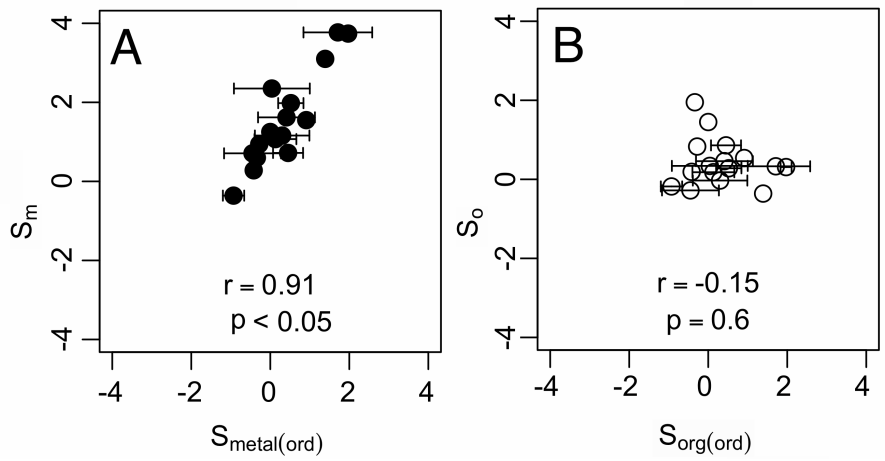

Figure 2.3: Relation between (A) metal species sensitivity (S_m) and (B) organic species sensitivity (S_o), from Wogram & Liess (2001) both in the order level, with $S_{metal(ord)}$ from this study. Error bars represent median absolute deviation (MAD) from the $S_{metal(ord)}$.

contrary, there were also other orders of insects, which were more sensitive, for example, Diptera (0.52), Ephemeroptera (0.30), and Heteroptera (0.04).

Regarding taxa with low to high S_{metal} values, mollusks spanned the whole range of sensitivities. However, species of the class Bivalvia, represented by the orders of Veneroida (-0.28) and Unionoida (-0.41), were more sensitive than mollusks of the class of Gastropoda, represented by the orders of Architaenioglossa (0.95), Neotaenioglossa (0.60), and Basommatophora (0.45). Similar to mollusks, annelids covered a wide range of sensitivities, starting from their most sensitive order of Lumbriculida (-0.28), the intermediate sensitive order of Hirudinea (0.001), and the least sensitive order of Aciculata (0.59).

Finally, there was a significant strong relationship ($r=0.91$, $p<0.05$, $n=16$) between the $S_{metal(ord)}$ and the previous S_m values, reported in Wogram & Liess (2001) (Figure 2.3A), both addressing heavy metal rankings for the order level. However, there was no significant correlation ($r=-0.15$, $p=0.60$, $n=16$) between the $S_{metal(ord)}$ and S_o values for organic compounds reported in Wogram & Liess (2001) (Figure 2.3B). The variation in $S_{metal(ord)}$, expressed by MAD error bars in Figure 2.3 was large. Variation was especially high for the orders of Heteroptera and Amphipoda, whereas it was low for Trichoptera (Appendix A, Table A.2). For instance, families of the order of Heteroptera showed high differences in $S_{metal(fam)}$, ranging from rather sensitive (-0.28 for Belostomatidae) to tolerant (0.87 for Corixidae). A similar observation was made for the order of Amphipoda, where differences in $S_{metal(fam)}$ spanned from -0.53 (Gammaridae) to 0.39 (Crangonyctidae). Number of taxa and MAD values in each level can be found in Appendix A, Table A.2-A.4.

Table 2.2: Physiological sensitivity (S) values for Cd, Cr, Cu, Hg, Ni, Pb, and Zn for each taxonomic level. S_{metal} is the median of the seven heavy metal sensitivity values for each taxonomic level. S-values in the higher taxonomic levels equal the S-values in the lower taxonomic levels, when only one lower taxon is present. Negative values indicate more sensitive taxa and positive values less sensitive taxa.

Class/Order	Family	Genus	Species	S_{Cd}	S_{Cr}	S_{Cu}	S_{Hg}	S_{Ni}	S_{Pb}	S_{Zn}	S_{metal}[a]
Bivalvia											
Unionoida	Unionidae			−0.45	−0.36	0.07	1.23	−1.01		−0.60	−0.41
		Actinonaias	*A. pectorosa*	−0.49		−0.24		−0.40		−0.23	−0.32
		Anodonta	*A. imbecillis*	−0.89	−0.36	0.06	0.27	−1.56		−0.69	−0.52
		Epioblasma	*E. capsaeformis*			0.08		−1.38		−0.35	−0.35
		Lamellidens	*L. marginalis*	0.99		1.76	1.99				1.76
		Lampsilis	*L. straminea*	−0.83		0.42		−1.17		−0.60	−0.72
		Parreysia	*P. favidens*	0.61		1.25	1.23				1.23
		Utterbackia	*U. imbecillis*	−0.33		−0.30		−0.41			−0.33
		Villosa	*V. vibex*	−0.45		−0.19		−0.85		−0.78	−0.62
Veneroida				−0.59	−1.08	0.03	0.70	−1.93		0.60	−0.28
	Corbiculidae	Villorita	*V. cyprinoides*	−1.69	−1.34	−2.21	−1.69	−1.93			−1.69
	Donacidae	Donax	*D. faba*	0.52		1.42	0.70			0.60	0.65
	Mactridae	Rangia	*R. cuneata*		−0.82	0.03	1.15				0.03
Clitellata											
Haplotaxida				0.14	1.00	0.08	0.49	−0.62	0.59	0.30	0.30
	Naididae	Nais		0.19	0.65	0.07	0.74			0.69	0.65
	Tubificidae			0.10	1.36	0.09	0.24	−0.62	0.59	−0.09	0.10
		Branchiura	*B. sowerbyi*	1.37	2.10	0.50	0.24				0.93
		Limnodrilus	*L. hoffmeisteri*	0.10		0.09	0.25			0.12	0.11
		Tubifex	*T. tubifex*	−0.17	0.62	−0.33	−0.43	−0.62	0.59	−0.30	−0.30
Hirudinea	Erpobdellidae	Erpobdella	*E. octoculata*	0.06		−0.38	0.07			−0.05	0.00
Lumbriculida	Lumbriculidae	Lumbriculus	*L. variegatus*	−0.99	0.65	−0.67	−0.28	0.81	−0.46	0.57	−0.28
Crustacea											
Amphipoda				−0.65	−0.79	−0.18	−0.53	0.72	−0.45	0.25	−0.45
	Crangonyctidae	Crangonyx	*C. pseudogracilis*	0.33	−0.79	0.27		1.20	0.45	0.65	0.39
	Gammaridae			−0.65	−0.90	−0.18	−0.53	0.24	−1.35	0.25	−0.53
		Echinogammarus	*E. tibaldii*	−0.41	−1.31	−0.18	−0.01			0.34	−0.18
		Gammarus		−0.88	−0.50	−0.18	−1.05	0.24	−1.35	0.16	−0.50
			G. sp.	−0.98	0.32	0.39	−1.05	0.24		0.34	0.28
			G. fasciatus		−0.70	0.05				0.27	0.05
			G. italicus	−0.35	−0.46	−1.07	0.13			−0.16	−0.35

Table 2.2 – continued from previous page

Class/Order	Family	Genus	Species	S_{Cd}	S_{Cr}	S_{Cu}	S_{Hg}	S_{Ni}	S_{Pb}	S_{Zn}	S_{metal}[a]
			G. lacustris	−0.94		0.20				0.16	0.16
			G. pseudolimnaeus	−0.88	−0.54	−1.85			−1.75		−1.31
			G. pulex	−0.72		−0.41	−1.54		−0.95	−0.43	−0.72
	Hyalellidae	Hyalella	*H. azteca*	−1.33	−0.53	−0.63				−0.55	−0.59
Anostraca				−0.52	−1.29	−0.58	−0.74			−1.04	−0.74
	Streptocephalidae	Streptocephalus		−0.44	−1.03	−0.90	−0.71			−0.84	−0.84
			S. proboscideus	−0.37	−1.03	−0.90	−0.71			−0.22	−0.71
			S. rubricaudatus	−0.44	−0.85	−0.75				−0.84	−0.79
			S. texanus	−0.78	−1.76	−1.39				−1.32	−1.36
	Thamnocephalidae	Thamnocephalus	*T. platyurus*	−0.60	−1.55	−0.26	−0.78			−1.23	−0.78
Calanoida	Diaptomidae			−1.01		−0.94	−0.30	−0.71	−0.76	−1.67	−0.85
		Diaptomus	*D. leptopus*	−1.29		−1.36	−0.84			−2.14	−1.33
		Eudiaptomus	*E. padanus*	−0.65		−0.29	0.16	−0.71	−0.70	−1.76	−0.68
		Heliodiaptomus	*H. viduus*	−0.80					−0.83	−1.06	−0.83
		Skistodiaptomus	*S. oregonensis*	−1.22		−0.94	−0.30			−1.58	−1.08
Cladocera				−0.93	−0.79	−1.22	−1.49	−0.60	−0.62	−1.29	−0.93
	Chydoridae	Chydorus	*C. sphaericus*	−0.93		−1.04	−1.27			−1.36	−1.16
	Daphniidae			−0.70	−0.97	−1.22	−1.49	−0.92	−0.96	−0.95	−0.96
		Ceriodaphnia		−0.56	−0.97	−1.22	−1.47	−1.37	−1.18	−1.15	−1.18
			C. dubia	−0.77	−0.73	−1.22	−0.96	−1.37	−1.62	−1.26	−1.22
			C. reticulata	−0.56	−1.21	−1.30	−1.97		−0.74	−1.15	−1.18
			C. rigaudi	−0.26		0.63				−0.01	−0.01
		Daphnia		−0.70	−0.72	−0.98	−1.52	−0.47	−0.96	−0.75	−0.75
			D. ambigua	−1.67	0.65	0.53				−1.08	−0.27
			D. carinata	−0.42	−0.72	−0.36			−0.32	−0.78	−0.42
			D. curvirostris	−0.56	−0.67				−0.96	−1.14	−0.82
			D. hyalina	−1.68	−0.17		−2.20	−1.35	−1.84		−1.68
			D. lumholzi	−0.35		−0.73				−0.23	−0.35
			D. magna	−1.07	−1.09	−1.25	−1.52	−0.47	−1.29	−0.75	−1.09
			D. obtusa	−0.61	−0.93				−0.85	−0.67	−0.76
			D. pulex	−0.93	−1.24	−1.25	−1.52	−0.15	−0.47	−1.07	−1.07
			D. rosea	−0.70		−0.98	−1.05			−0.55	−0.84
			D. similis			−1.36	0.25		−1.37	−0.48	−0.92
		Simocephalus	*S. vetulus*	−1.11	−1.21	−1.63			−0.14		−1.16
	Moinidae	Moina		−1.16	−0.60	−1.76	−2.15	−0.29	−0.28	−1.29	−1.16
			M. irrasa	−1.47		−2.27				−1.76	−1.76
			M. macrocopa	−0.85	−0.60	−1.26	−2.15	−0.29	−0.28	−0.81	−0.81

Table 2.2 – continued from previous page

Class/Order	Family	Genus	Species	S_{Cd}	S_{Cr}	S_{Cu}	S_{Hg}	S_{Ni}	S_{Pb}	S_{Zn}	S_{metal}[a]
Cyclopoida	Cyclopidae			−0.30	0.16	−0.42	−0.41	−0.48	−0.55	−0.93	−0.42
		Cyclops		0.02	0.49	0.95	0.06		−0.55	0.32	0.19
			C. abyssorum	0.02	0.33	0.55	0.60		−0.55		0.33
			C. sp.		0.64	1.35	−0.48			0.32	0.48
		Mesocyclops	*M. pehpeiensis*		−0.17	−0.42		−0.48		−0.93	−0.45
		Tropocyclops	*T. prasinus*	−0.61		−0.68	−0.89			−1.46	−0.78
Decapoda				−0.29	0.49	−0.10	0.05	0.13	1.31	0.62	0.13
	Astacidae	Austropotamobius	*A. pallipes*	−0.50		−0.32	−0.96	−0.15	−0.26		−0.32
	Atyidae			−0.71	1.32	0.05	0.33		1.44	0.50	0.41
		Caridina	*C. nilotica*			0.16			1.44	0.50	0.50
		Neocaridina	*N. denticulata*	−0.34	1.32	0.05	0.33			0.82	0.33
		Paratya		−1.06		−0.92				−0.03	−0.92
			P. australiensis	−1.24		−0.82				0.42	−0.82
			P. compressa	−0.89		−1.02				−0.48	−0.89
	Cambaridae			0.45		0.79	0.05	0.13	1.24	1.45	0.62
		Orconectes	*O. limosus*	−0.29		0.52	−0.53	0.13	−0.13	1.45	0.00
		Procambarus	*P. clarkii*	1.19		1.06	0.63		2.62		1.12
	Palaemonidae	Macrobrachium		−0.67	0.49	−0.25	−0.32			0.73	−0.25
			M.hendersodayanus	−0.88		1.30	−0.19			0.73	0.27
			M. kistnensis	−0.43		−0.62	−1.07				−0.62
			M. lamarrei	−0.74	0.39	−0.48	−0.32			−0.22	−0.32
			M. rude	−0.59	0.60	−0.02				1.02	0.29
	Parastacidae	Cherax	*C. destructor*	−0.04		1.31		2.30			1.31
	Parathelphusidae	Paratelphusa	*P. hydrodromus*	0.06			0.41		1.38		0.41
	Penaeidae	Penaeus	*P. chinensis*	−0.28	−2.01	−1.02				−0.33	−0.67
Harpacticoida	Ameiridae	Nitocra	*N. spinipes*	−0.07	0.19	0.20	−0.32			−0.26	−0.07
Isopoda	Asellidae	Asellus	*A. aquaticus*	−0.19		1.29	−0.09	1.45	0.90	0.92	0.91
Podocopida	Cyprididae			0.40		0.76	0.41		0.15	0.33	0.40
		Cypris		0.17		0.74	0.41		0.36	0.64	0.41
			C. sp.	0.30			0.41		0.36	0.34	0.35
			C. subglobosa	0.04		0.74				0.94	0.74
		Diacypris	*D. compacta*	0.77			−0.06	0.02	0.32		
Gastropoda											
Architaenioglossa	Viviparidae	Viviparus	*V. bengalensis*	0.95	1.07	1.19	0.50	1.38	0.18	0.51	0.95
Basommatophora				0.53	1.04	−0.06	0.31		1.10	0.37	0.45
	Lymnaeidae	Lymnaea		0.53	1.25	0.51	0.31		1.10	0.37	0.52

Table 2.2 – continued from previous page

Class/Order	Family	Genus	Species	S_{Cd}	S_{Cr}	S_{Cu}	S_{Hg}	S_{Ni}	S_{Pb}	S_{Zn}	S_{metal} [a]
			L. emarginata		0.99	0.61			1.10	0.15	0.80
			L. luteola	1.06	1.51	0.29	0.23			1.17	1.06
			L. stagnalis	0.00		0.51	0.39			0.37	0.38
	Physidae	Physa	*P. integra*		−0.26	−1.50				0.23	−0.26
	Planorbidae	Planorbella	*P. trivolvis*		1.04	−0.06				0.76	0.76
Neotaenioglossa				0.60	−0.20	0.59	0.61	0.64	1.53	0.31	0.60
	Hydrobiidae	Amnicola		0.60		1.00	0.61	0.64		0.58	0.61
	Pleuroceridae				−0.20	0.18			1.53	0.04	0.11
		Anculosa			−0.57	−0.30				−0.87	−0.57
		Elimia	*E. livescens*		0.17	0.66			1.53	0.95	0.80
Hydrozoa											
Hydroida	Hydridae	Hydra	*H. vulgaris*	−0.24		−0.75				0.76	−0.24
Insecta											
Anisoptera [b]	Corduliidae	Macromia		−0.35							−0.35
Diptera				0.27	0.52	1.40	1.00	−0.14	−0.07	1.00	0.52
	Ceratopogonidae	Culicoides	*C. furens*	0.09	0.15	1.40	0.26	−0.36	−0.60	0.92	0.15
	Chironomidae	Chironomus		0.56	0.52	1.19	1.00	0.07	0.47	1.09	0.56
			C. plumosus	0.10	0.52	1.67	1.68	−0.01	0.47	1.36	0.52
			C. riparius	1.66		1.37	0.79	0.92		0.91	0.92
			C. sp.	0.83	0.75	0.95	−0.98	0.06	0.84	1.26	0.83
			C. tentans	0.29	0.13	1.01	1.21	0.08	−0.06	−0.62	0.13
	Culicidae	Aedes	*A. aegypti*	0.27	1.05	1.75	1.13				1.09
Ephemeroptera				0.69		−0.59	0.30	−0.30		0.87	0.30
	Baetidae	Cloeon	*C. dipterum*	1.07		−0.71	−0.79			0.60	−0.06
	Ephemerellidae	Ephemerella	*E. subvaria*	0.42		0.05	1.39	−0.30			0.24
	Heptageniidae	Rhithrogena	*R. hageni*	0.69		−0.59				1.15	0.69
Heteroptera				1.85			0.20		−0.28	−0.12	0.04
	Belostomatidae	Lethocerus							−0.28		−0.28
	Corixidae			1.85			−0.10				0.87
		Corixa	*C. punctata* [c]	1.09			−0.10				0.50
		Sigara	*S. dorsalis* [c]	2.61							2.61
	Naucoridae	Aphelocheirus	*A. aestivalis* [c]	2.88							2.88
	Nepidae			−0.36			0.50			−0.12	−0.12
		Laccotrephes					0.50				0.50
		Ranatra	*R. elongata* [c]	−0.36						−0.12	−0.24
Megaloptera [b]	Sialidae	Sialis	*S. lutaria*	2.67							2.67

Table 2.2 – continued from previous page

Class/Order	Family	Genus	Species	S_{Cd}	S_{Cr}	S_{Cu}	S_{Hg}	S_{Ni}	S_{Pb}	S_{Zn}	S_{metal} [a]
Plecoptera	Perlidae	Acroneuria	*A. lycorias*			2.12	1.39	0.66			1.39
Trichoptera				1.97	2.05		1.39				1.97
	Hydropsychidae	Hydropsyche		2.07	2.05		1.39				2.05
			H. angustipennis [c]	2.07							2.07
			H. betteni [c]				1.39				1.39
			H. sp. [c]		2.05						2.05
	Rhyacophilidae	Rhyacophila	*R. dorsalis* [c]	1.87							1.87
Zygoptera				1.85	1.89		1.70		1.03	1.71	1.71
	Calopterygidae	Calopteryx	*C. splendens* [c]	2.46							2.46
	Coenagrionidae			1.25	1.89		1.70		1.03	1.71	1.70
		Argia								1.71	1.71
		Enallagma		1.62	1.89				1.03		1.62
			E. aspersum [c]		1.89						1.89
			E. cyathigerum [c]	2.63							2.63
			E. sp. [c]	0.61					1.03		0.82
		Ischnura		0.87			1.70				1.28
			I. elegans [c]	1.09			1.70				1.39
			I. heterosticta [c]	0.65							0.65
Nematoda											
Aphelenchida	Aphelenchidae	Aphelenchus	*A. avenae*	1.76		0.70	1.08		0.67	1.45	1.08
Rhabditida				0.97	1.13	0.85	1.30	0.99	0.12	1.40	0.99
	Cephalobidae	Cephalobus	*C. persegnis*	0.81		0.85		0.59	0.12	2.20	0.81
	Panagrolaimidae	Panagrellus	*P. silusiae*	1.07	1.40	0.59	1.32	1.75	0.15	1.40	1.32
	Rhabditidae	Caenorhabditis	*C. elegans*	0.97	0.87	1.81	1.27	0.99	0.07	0.62	0.97
Polychaeta											
Aciculata	Nereididae	Hediste	*H. diversicolor*	1.21		0.05				0.59	0.59
Aeolosomatida	Aeolosomatidae	Aeolosoma	*A. headleyi*	0.52	−0.29	0.02				−0.11	−0.05
Turbellaria											
Tricladida				0.33	0.27	0.85	−0.01	0.75	1.96	0.41	0.41
	Dugesiidae	Girardia	*G. tigrina*	0.04	−0.31	0.20	−0.49			0.19	0.04
	Planariidae	Dugesia	*D. tigrina*	0.62	0.84	1.50	0.46	0.75	1.96	0.62	0.75

[a] S_{metal} is the median of the seven metal sensitivity values for each taxonomic level. S values in the higher taxonomic levels equal the S value in the lower taxonomic levels, when only one lower taxon is present. Negative values indicate more sensitive taxa and positive values indicate less sensitive taxa.
[b] One entry for Cd
[c] Less than three heavy metals

2.5 Discussion

2.5.1 Similarities in the physiological sensitivity of heavy metals

The individual heavy metal rankings were not significantly different at the species level and correlated reasonably well with each other. Similarities in rankings can be explained by a similar mode of action for the analysed heavy metals. Heavy metals often occur in mixtures in the environment (Di Toro et al., 2001; Wang, 1987; Schmidt et al., 2010). Previous studies on heavy metal mixtures suggested that a similar mode of action applied for the heavy metals analysed (e.g., Cd, Cu, Zn; Wang, 1987; Schmidt et al., 2010). Furthermore, it was shown for the nematode *Caenorhabditis elegans* that toxicity for bivalent metals (Ca, Cd, Cu, Hg, Mg, Mn, Ni, Pb, and Zn) can effectively be predicted by using ions characteristics (e.g., the first hydrolysis constant; Tatara et al., 1997), thus these metals had the same mode of action. Other studies (Nieboer & Richardson, 1980; Vaal et al., 1997) found that metals such as Cu, Cr, Cd, Ni, Pb, and Zn were less toxic than Hg, therefore excluding the latter from a shared mode of action with the other five metals. Overall, similarities between the heavy metal rankings in the present study can be explained by a similar mode of action between heavy metals.

For future application, we provide an aggregation of the separate rankings in an overall heavy metal ranking (S_{metal}), making use of all available data. Considering that heavy metals in the field are found in mixtures, a unified ranking should be applied to detect respective mixture effects (see below for the potential application of the ranking in trait-based risk assessment). However, an individual ranking may be applied when a single heavy metal is of concern. Special attention should be given to metals other than those considered in the present study, where species deviated in their relative sensitivities from the S_{metal} values presented here.

2.5.2 Physiological sensitivity to heavy metals (S_{metal})

D. magna has been repeatedly used as a reference species to compare sensitivities to various chemicals (Wogram & Liess, 2001; von der Ohe & Liess, 2004). Other authors suggested Ceriodaphnia as an alternative standardization species, because it is more sensitive to heavy metals than Daphnia (Wong et al., 2009), which was also confirmed in the present study. Furthermore, Rubach, Baird & Van den Brink (2010) argued that the use of *D. magna* as a benchmark organism is not appropriate, considering that it is a clonal organism with varying sensitivities. Hence, the use of a single species for standardization could potentially lead to errors and was avoided in the present study. Instead, we standardized using all data available per heavy metal following the approach suggested by Rubach, Baird & Van den Brink (2010).

Generally, crustaceans with representatives of the order Cladocera have been ranked as the most sensitive group with regard to heavy metals, which was in line with other studies (Wogram & Liess, 2001; von der Ohe & Liess, 2004; Wong et al., 2009; Vaal et al., 1997). Similar to von der Ohe & Liess (2004), isopods were the least sensitive group of crustaceans. In the present study, isopods were represented by a single species (*Asellus aquaticus*), which was reported to have rapid elimination patterns especially for Zn and Pb (Rainbow, 1998), potentially explaining the lower sensitivity compared to other crustaceans.

Insects of the order of Trichoptera and Zygoptera were the most tolerant group of macroinvertebrates analyzed. By contrast, the insect orders of Ephemeroptera, Heteroptera, and Diptera had rather intermediate S values compared to all other inverte-

brate orders and were the most sensitive insect orders. Similar results were found with laboratory toxicity data from Brix, DeForest & Adams (2011), where Ephemeroptera and Diptera (mainly chironomids) were more sensitive than Trichoptera and Plecoptera. In addition, we found the orders of Heteroptera and Ephemeroptera to be more sensitive than gastropods or crustaceans of the order of Decapoda and Podocopida. It is worth noting that although the ECOTOX database (USEPA, 2007) has the largest toxicological information for aquatic species currently available, there are large differences in the representation of species (Baird & Van den Brink, 2007). Insects, for instance, were highly underrepresented in our database. Therefore, the S_{metal} values for Zygoptera, Trichoptera, and Heteroptera should be considered as tentative values. Caution should be taken when using S values especially for the orders of Megaloptera and Anisoptera that had only one datum for Cd. Further testing of these species for heavy metal toxicity appears necessary to consolidate the given values.

On the order level, our ranking for heavy metals was in agreement with the previous study by Wogram & Liess (2001), regardless of the different methodologies used. This possibly resulted from the overlap in the input toxicity data, both deriving from the ECOTOX database (USEPA, 2007). Regardless of the similarities with previous metal rankings, grouping at higher taxonomic levels reduced the predictive accuracy and would result in a considerable error if used for determination of the sensitivity at lower taxonomic levels, such as the species level (von der Ohe & Liess, 2004). In this context, Buchwalter & Luoma (2005) found that the metal uptake was similar among species of the same genus, but not within the same orders. For the latter study, the variability within orders was found to be as large as, or even larger than between orders. Rainbow (2002) suggested that any meaningful comparison of relative toxic concentrations in aquatic invertebrates should be intraspecific, and certainly not between families or higher systematic levels. In another metal ranking study, von der Ohe & Liess (2004) presented sensitivity values on lower taxonomic levels, but these values were only available for 18 taxa of macroinvertebrates. This could be attributed to their restriction to use at least five heavy metals per taxon (von der Ohe & Liess, 2004). In the light of these considerations, we suggest using the genus or species level for meaningful analyses.

Finally, the rankings of heavy metals and organic compound in the order level were not significantly correlated. Dissimilarities in sensitivities between these two groups of compounds were most pronounced for insects of the orders of Plecoptera, Tricoptera, and Zygoptera, which were sensitive to organic toxicants, but the most tolerant orders to heavy metals. On the contrary, bivalves and annelids of the order of Lumbriculida were more sensitive to heavy metals than to organic compounds. These differences can be explained by the specific modes of action of heavy metals and organic compounds (Vaal et al., 1997) and might have large impacts on bioassessment.

2.5.3 Application of physiological sensitivity to heavy metals in ecological risk assessment

Species traits are increasingly being used in ecological risk assessment procedures (Rubach et al., 2011; Baird & Van den Brink, 2007; Liess & Von Der Ohe, 2005; Schäfer et al., 2011). Rankings representing the physiological sensitivity of macroinvertebrate species have been introduced previously as one of the main traits explaining effects of stressors (e.g., organic toxicants and salinity) in the field (Liess & Von Der Ohe, 2005; Schäfer et al., 2011). Furthermore, Schäfer et al. (2011) suggested that the relevance of different traits would depend on the disturbance type. Heavy metals are considered as presses, which are disturbances that may arise sharply and reach a constant level that is maintained

throughout time (Lake, 2000). For this type of disturbance, physiologically sensitivity would be of primary importance.

The SPEAR index represents an example of how different traits including physiological sensitivity can be used to establish a mechanistic link between community traits and environmental stressors. Using physiological sensitivity, demographic and recolonization species traits, macroinvertebrates were classified as species at risk (SPEAR) or species not at risk (SPEnotAR; Liess & Von Der Ohe, 2005). The fraction of the abundance of SPEAR in communities was then related to the exposure levels in the environment, demonstrating a relatively high explanatory power for the respective stressors (e.g., organic toxicants or salinity; Liess & Von Der Ohe, 2005; Schäfer et al., 2011).

In the present study, we have revised the physiological sensitivity approach with the purpose of using it as one of the traits in trait-based risk assessment of heavy metals in freshwater ecosystems. However, it is worth noting that the relevance of the physiological sensitivity is based on *a priori* assumptions. If the physiological sensitivity ranking would be used in a trait-based risk assessment for heavy metals, the relevance of other demographic and recolonization species traits should be further examined. Traits other than physiological sensitivity can be particularly important for some groups of macroinvertebrates, which means that the sensitivity of species in the field may differ from the physiological sensitivity derived from laboratory data. For example, in acute and chronic laboratory toxicity studies some insect orders were insensitive to heavy metals, whereas field studies categorized them among the most sensitive (Brix, DeForest & Adams, 2011; Buchwalter et al., 2007). Species in the field are generally exposed to long-term concentration levels; therefore toxic effects would also depend on other species traits related with field exposure (e.g., life span, generation time or dietary exposure; Buchwalter et al., 2007). Influencing ultimately the population growth rate, these traits were considered important for the recovery rate of the community (Rubach et al., 2011; Liess & Von Der Ohe, 2005), and therefore potentially important to explain the sensitivity of species in the field.

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Chapter 3

Evolutionary Patterns and Physicochemical Properties Explain Macroinvertebrate Sensitivity to Heavy Metals

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"We can allow satellites, planets, suns, universe, nay whole systems of universe, to be governed by laws, but the smallest insect, we wish to be created at once by special act."
- Charles Darwin

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3.1 Abstract

Ecological risk assessment depends strongly on species sensitivity data. Typically, sensitivity data are based on laboratory toxicity bioassays, which for practical constraints cannot be exhaustively performed for all species and chemicals available. Bilinear models integrating phylogenetic information of species and physicochemical properties of compounds allow to predict species sensitivity to chemicals. Combining the molecular information (DNA sequences) of 33 invertebrate species with the physicochemical properties of six bivalent metals, we built bilinear models that explained 70-80% of the variability in species sensitivity to heavy metals. The major part of the explained variance was attributed to phylogeny (>40%). Predicted values were in strong agreement with experimental values (>50%), therefore, this approach can be used to infer toxicity values for untested invertebrate species based on similar species for which toxicity has been tested. Despite their good performance, development of bilinear models would likely benefit from an expanded phylogenetic and toxicological dataset. Our analysis is one of the few examples linking evolutionary biology with applied ecotoxicology, and its potential applications can be expanded to other stress factors or trait properties influencing aquatic organisms.

3.2 Introduction

Sensitivity data for aquatic species to organic and inorganic chemicals are a crucial prerequisite for assessment and management of ecosystems, especially in the face of current environmental changes (MEA, 2005). For example, species sensitivity data are frequently used for ecological risk assessment (CEC, 2011). They are often based on acute laboratory toxicity tests of chemicals (e.g., exposure of 24- to 72-h) that are used to estimate the lethal concentration for 50% of the test population (LC50). The paucity of toxicity data for a wide range of species has resulted in the use of standard test species as representatives for entire taxonomic groups (e.g., a single invertebrate species used to represent all Protostomia, while a single fish used to represent all Deuterostomia). On the other hand, community-based risk assessment is often conducted using the species sensitivity distribution (SSD), which require an expanded toxicological dataset (Schäfer et al., 2013). Given that laboratory toxicity tests for all species-chemical combinations cannot be conducted exhaustively due to practical, financial and ethical constrains, modeling of species sensitivities represents an appealing alternative.

Species are likely to show a phylogenetic signal as a result of shared ancestry, which means that closely related species tend to resemble each other more than distant ones. As a result, toxicity values for species with unknown toxicity (untested species) can be estimated based on phylogenetically similar species for which toxicity values were experimentally determined (tested species). This approach has rarely been used in ecotoxicological studies, and the few existing studies have only compared a few closely related species or compounds. For instance, phylogenetic signal was used to describe the uptake and elimination of Cd and Zn in aquatic insects (Buchwalter et al., 2008; Poteat et al., 2013), to explain the toxicity of the pesticide endosulfan for amphibians (Hammond et al., 2012), or to explain the differences in sensitivity to herbicide for 14 diatom species (Larras et al., 2014). None of these studies established prediction models between the phylogenetic signal and toxicity.

Recently, phylogenetic models have been introduced as a tool to make predictions based on shared ancestry (Guénard et al., 2011; Guénard, Legendre & Peres-Neto, 2013; Fagan et al., 2013). Typically, the similarities originating from evolutionary processes are estimated using phylogenetic trees, the branches of which represent the course of evo-

lution to descendants (Felsenstein, 1981; Pagel, 1999). While phylogenetic trees can be inferred from taxonomy, DNA sequences provide better estimates for their construction (Felsenstein, 1981; Pagel, 1999; Delsuc, Brinkmann & Philippe, 2005; Guénard et al., 2011). Once the phylogeny is estimated, it is used to calculate Phylogenetic Eigenvector Maps (PEM; Guénard, Legendre & Peres-Neto, 2013), which are subsequently used as predictors in modeling approaches such as multiple regression, bilinear modeling (Gabriel, 1998), or generalized linear modeling (Diniz-Filho, de Sant'Ana & Bini, 1998). Based on this method, phylogenetic information of species explained up to 80% of the variation in the toxicity data for four organic chemicals (Guénard et al., 2011). Moreover, to account for variation in toxicity for multiple chemicals, physicochemical characteristics of the compounds are often related to the observed toxicity (Newman, McCloskey & Tatara, 1998). A recent study bridged the gap between evolutionary and toxicological concepts by relating the phylogenetic information of species and physicochemical properties in a bilinear model (Guénard et al., 2014). Bilinear models (Gabriel, 1998) can combine two types of descriptors: (i) variation among rows, which in the present case represents the sensitivity of multiple species (as rows) to multiple compounds (as columns), and (ii) variation among columns, which represents the change in toxicity for different compounds. By complementing the phylogenetic information with the physicochemical properties, Guénard et al. (2014) were able to explain 70-85% of the variation in the pesticide sensitivity for aquatic species.

To our knowledge, neither the phylogenetic modeling approach alone nor complemented with other data in a bilinear model has been applied to predict species sensitivity to heavy metals. The availability of toxicological (USEPA, 2014) and genetic databases (Benson et al., 2010) facilitates the development of phylogenetic models for heavy metals. Furthermore, considering that heavy metal toxicity is often explained by physicochemical properties (e.g., the softness parameter or the covalent index (Newman, McCloskey & Tatara, 1998; Walker, Enache & Dearden, 2003; Wu et al., 2013)), including physicochemical properties of heavy metals in a bilinear model is a promising approach.

Here, we present a statistical method to explain the multivariate response of macroinvertebrate species to heavy metals using the aforementioned phylogenetic bilinear modeling framework developed for organic compounds. The bilinear models for heavy metals were validated for additional species and heavy metals. The relevance of these results are discussed in terms of potential application in ecological risk assessment.

3.3 Methods

3.3.1 Data Mining

The toxicological database comprised LC50 values for macroinvertebrate species as described in Malaj et al. (2012) (original data from Ecotoxicology Database System by USEPA (2007)). To remove the variation derived from the large number of laboratories performing the tests, toxicity values went through quality control check (see Malaj et al. (2012) for details). Briefly, entries for the same species-heavy metal combination were removed for (i) extreme test conditions (e.g., tests performed in $<5^{o}$C and $>35^{o}$C for temperature and >200mg/L $CaCO_3$ for hardness), or (ii) large fluctuations in LC50 values (e.g., >two standard deviation from the mean LC50 value). To avoid bias from pooling data related to different test conditions, each entry was standardized to standard laboratory conditions of 20^{o}C of temperature, 200 mg/L $CaCO_3$ of hardness, and exposure duration of 48-h (see Malaj et al. (2012) for details). After quality control, toxicity

values were available for Cd(II), Cu(II), Zn(II), Hg(II), Pb(II), Ni(II), and Cr(VI). The geometric mean of the LC50 values for each combination of species and heavy metal was taken. Finally, 41 macroinvertebrate species had toxicity information available for at least four heavy metals.

Genetic information for the 41 macroinvertebrate species were queried from the U.S. National Center for Biotechnology Information's, GenBank (Benson et al., 2010). When possible, the whole mitochondrial DNA sequences was used, as well as the sequences for nuclear 28S, 18S, and 5.8S ribosomal RNA (rRNA) transcripts and internal transcribed spacers (ITS 1 and ITS 2). If more than one sequence was available, the longest sequence was used. The minimum requirement for the genetic information was the availability of the cytochrome oxidase sub-unit 1 (COX1) which is recognized to (i) generate a substantial phylogenetic signal and (ii) have a slow change of amino acids sequences, which allows to borrow missing species DNA sequences from higher taxonomic groups (Hebert, Cywinska & Ball, 2003). Species without any genetic information were substituted with the taxonomically most similar species (which were mainly on the genus level; Appendix B, Table B.1) for which genetic information was available. To avoid a coarse phylogenetic tree, taxa with borrowed sequences on the family level were requested to have at least one sequence on the lower levels (species or genus). Five species (*Diaptomus leptopus*, *Daphnia rosea*, *Tropocyclops prasinus*, *Nitocra spinipes*, and Amnicola *ssp.*) were omitted due to this condition. Furthermore, six species which had information only in the genus level were renamed (2 species for each genus; Ceriodaphnia *ssp.*, Macrobrachium *ssp.*, and Schmidtea *ssp.*). Finally, 33 species had toxicity and genetic information available.

To build and validate phylogenetic models, a complete data matrix (no missing values) is required. To represent scenarios with the greatest possible number of species or the greatest possible number of heavy metals (given data availability), two models were build. The first model had 33 species and four heavy metals (Cd(II), Cu(II), Zn(II), Hg(II); hereafter called 4HM model), whereas the second model had 15 species and six heavy metals (Cd(II), Cu(II), Zn(II), Hg(II)), Pb(II), and Ni(II); hereafter called 6HM model). We restricted our analysis to divalent heavy metals, thus Cr(VI) was not considered. Finally, the experimental LC50 values were represented as two rectangular response matrices $\mathbf{Y_{exp}}$=[$LC50_{i,j}$], where i is the number of species and j is the number of heavy metals.

3.3.2 Construction of the phylogenetic tree

We estimated the phylogenetic tree based on genetic sequences collected for the 33 invertebrate species. Multiple-sequence alignment was performed for each gene using Muscle version 3.8.31 (Edgar, 2004). Individual genes were concatenated in a single super-alignment. Phylogenetic trees were based on the analysis of nucleotides with a maximum likelihood (ML) method (Felsenstein, 1981), which is considered appropriate for super-alignments derived from sequence-based methods (Delsuc, Brinkmann & Philippe, 2005). The calculation was performed with the program fdnaml within the software EMBOSS version 6.3.1. We ordered species in fdnaml in descending order of their number of obtained bases, to ensure that species with sparse genetic data were added to a tree that was already well-supported.

3.3.3 Phylogenetic bilinear models

To build the bilinear models, we used the phylogenetic bilinear modeling approach described in Guénard et al. (2014). This method involves a bilinear regression model, i.e., a

multivariate regression model that has two sets of descriptors; one set models the variation among rows (species) of the multivariate response matrix ($\mathbf{Y_{exp}}$), whereas the other set models the variation among its columns (heavy metals). The set modeling among-row variation consists of eigenvectors from a Phylogenetic Eigenvector Map (PEM); a method recently introduced by Guénard, Legendre & Peres-Neto (2013), and which we will refer to as matrix **U** (Figure 3.1). The eigenvectors of a PEM are obtained from a decomposition of the among-species covariances and represent a set of candidate patterns of phylogenetic variation of the response variables (i.e., toxicity to different heavy metals). In addition to represent phylogenetic patterns of trait variation, PEM also allows the user to calculate scores for species not involved in model building. These scores enable prediction of toxicity for untested (i.e., out of the model) species, using the information of the tested species. The set of descriptors modeling among-column variation, which we will hereafter refer to as **Z**, is composed of physicochemical variables related to the compounds. We explored eight physicochemical properties that have been successfully used to explain the toxicity of the metals (Appendix B, Supplementary Methods, and Table B.2). They comprised (i) Pearson softness parameter (σp), (ii) covalent bond stability ($\Delta\beta$), (iii) first hydrolysis constant ($|\log_{10}\mathrm{KOH}|$), (iv) metal hydroxide solubility product ($\log_{10}\mathrm{K_{SO}MOH}$), (v) atomic ionization potential (AN/ΔIP; AN is the atomic number and IP is the ionization potential), (vi) electrochemical potential ($\Delta\mathrm{E_o}$), (vii) ionic index ($\mathrm{Z^2/r}$; Z is the charge and r is Pauling ionic radius), and (viii) covalent index ($\mathrm{X_m{}^2r}$; $\mathrm{X_m}$is the electronegativity).

In a bilinear model, the response matrix **Y** is modeled as follows:

$$\langle Y\rangle_{\mathrm{exp}} = (Z \otimes U)\langle B\rangle + \langle E\rangle \tag{3.1}$$

where $\langle ...\rangle$ denotes the lexicographic concatenation (unfolding) of the columns of a matrix into a single column vector, $\otimes$ is the Kronecker product, **Z** is a matrix of size (jxl), **U** is the influence matrix of size (ixk), where j represents the heavy metals and l represents the physicochemical properties, i being the specie and k representing the eigenvectors, **B**=[$\mathrm{c_{j,l}}$] is a matrix of bilinear regression coefficients, and **E** = [$\varepsilon_{\mathrm{i,j}}$] is a matrix of error terms (Gabriel, 1998).

Regression methods can be used to estimate the matrix **B** based on the type of model which best fits to the data (e.g., least-squares, generalized linear models, or mixed-effect models). Here, we used the ordinary least-square regression on log-transformed LC50 values, which is the transformation typically used for toxicity data.

The Kronecker product of **Z** and **U** matrix resulted in a block matrix which has nxm rows, where n is the number of species and m is the number of heavy metals, and pxq columns, where p and q are the number of columns in **Z** and **U**, respectively (corresponding to the physicochemical properties and the eigenvectors, respectively). Because there are many (n-1) phylogenetic eigenvectors, we regularized the model to avoid over-fitting and obtain parsimonious models. Therefore, a subset of the columns of the block matrix was selected on the basis of the smallest Akaike Information Criteria (AIC; Hurvich & Tsai (1993)).

Predictions of toxicity for untested species were calculated in a new (target) influence matrix $\mathbf{U}_{\mathrm{target}}$ based on the position of these species in the phylogenetic tree. Using the bilinear coefficient matrix **B** (calculated in equation 3.1), and the target influence matrix $\mathbf{U}_{\mathrm{target}}$, we can predict LC50 values ($\mathbf{Y}_{\mathrm{pred}}$) for untested species as:

$$Y_{\mathrm{pred}} = U_{\mathrm{target}} B Z^{\mathrm{T}} \tag{3.2}$$

where $\mathrm{Z^T}$ is the transposed matrix of **Z**.

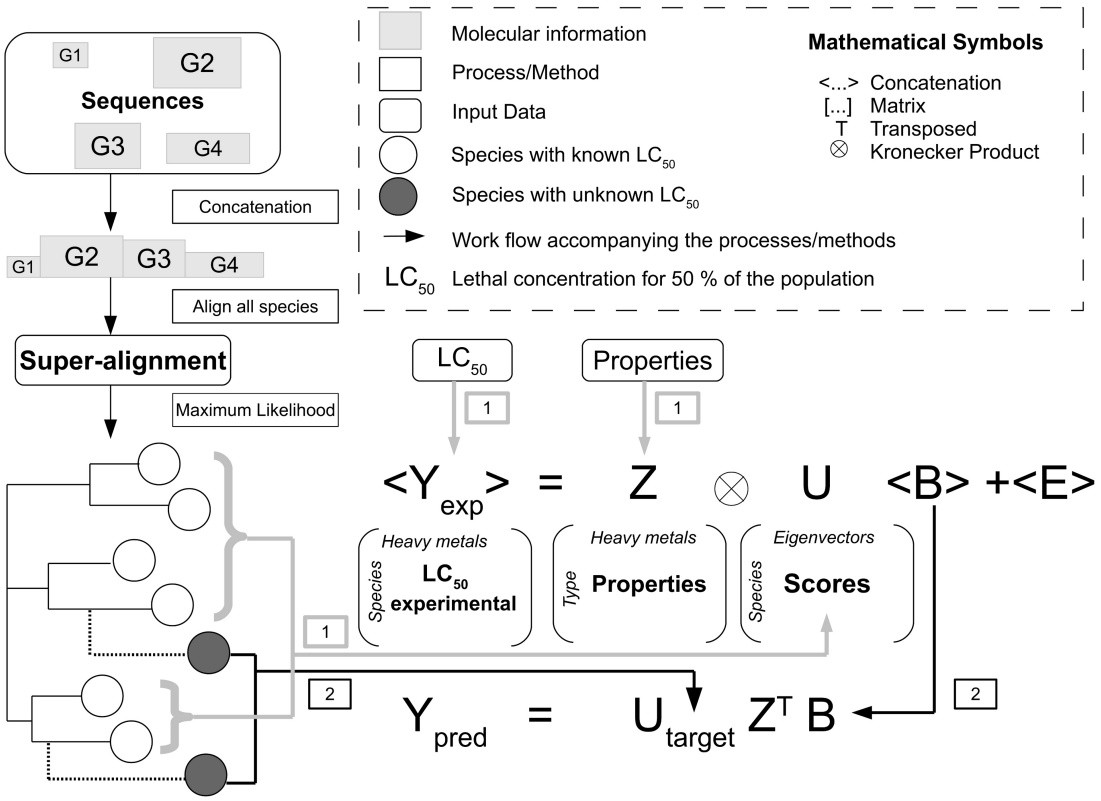

Figure 3.1: Illustration of the calculations and predictions of a bilinear model. The workflow starts with the genetic sequence collection, concatenation of the genes (represented by G1-G4) and construction of the super-alignment, which contains the molecular information for all species. Using a maximum likelihood method, the phylogenetic tree is constructed from the super-alignment. The work flow is then separated in two steps. Step 1 represents the calculation of a bilinear matrix of correlation coefficients (**B**) based on the phylogenetic information (**U**) of species with known LC50 values (open circles in the tree), experimental LC50 values ($\mathbf{Y}_{\mathrm{exp}}$), and physicochemical properties (**Z**). Step 2 uses the bilinear matrix of correlation coefficients (**B**) to predict LC50 values ($\mathbf{Y}_{\mathrm{pred}}$) for species with unknown LC50 values (gray circles in the tree) for which the phylogenetic information is stored in a new matrix $\mathbf{U}_{\mathrm{target}}$.

The calculation of $\mathbf{Y}_{\mathrm{pred}}$ was performed employing one physicochemical property for the 4HM and 6HM data sets and employing two physicochemical properties for the 6HM dataset. Two properties models for the 4HM data set were not calculated because of the small number of heavy metals present. In total, 36 models were run for the 6HM case and eight models for the 4HM case (Appendix B, Table B.4). The proportion of the variation was estimated with the adjusted coefficient of multiple determination ($r_{adj}{}^2$) for each model. The physicochemical properties that resulted in the highest $r_{adj}{}^2$ for both 4HM and 6HM models were selected (Appendix B, Table B.4) and subsequently used in validations.

The predictive power of the models was also checked by using the non-standardized LC50s as input values. By comparing the non-standardized with the standardized model predictions, we would check if the reduced variance in the LC50 values due to standardization has an effect on model predictions. This would allow to extend the models beyond the standardized dataset in Malaj et al. (2012), if more data would become available.

Resampling techniques were employed to evaluate the ability of the models to make predictions for new species (Type 1A and 1B), and for new metals (Type 2). Type 1A

prediction was a typical leave-one-out cross-validation procedure that included: (i) removing a species i, (ii) building the aforementioned bilinear model for n-1 remaining species (equation 3.1), (iii) predicting the LC50 value for species i (equation 3.2) and (iv) quantifying the difference between the predicted and experimental values for species i. This procedure was repeated for all species i in the dataset and for both the 4HM model and the 6HM model.

The number of species was different between the 4HM (33 species) and 6HM models (15 species), because the earlier used the maximum number of species, while the latter used the maximum number of heavy metals. Type 1B and Type 2 predictions employed this difference in the number of species to validate the models. Type 1B prediction included: (i) removing species t from the 4HM model with 33 species, which had toxicity values for Ni and Pb (Appendix B, Table B.3 for the species used), (ii) building the aforementioned bilinear model for 33-t remaining species (equation 3.1), (iii) predicting the LC50 values for the t species (equation 3.2) and (iv) quantifying the difference between the predicted and experimental values for the t species.

To evaluate the models with respect to the prediction of new heavy metals, Type 2 prediction was employed, where we (i) extracted the phylogenetic scores for s species which had toxicity values for Ni and/or Pb (Appendix B, Table B.3 for the species used), (ii) extracted the physicochemical properties for Ni and Pb, (iii) used the species scores and the physicochemical properties in the 4HM model (Cd, Cu, Hg, and Zn) to predict species values s for new metals (Ni and Pb; equation 3.2) and (iv) quantified the difference between predicted and experimental species values. The species used in each prediction are displayed in Appendix B, Table B.3.

Predicted values were compared with experimental values globally and individually. Globally, the experimental and predicted values were compared with the prediction coefficient (q^2; for mathematical details see Schüürmann et al. (2008)) which is a common approach to evaluate the robustness and the prediction ability of the models. The q^2 ranges from $-\infty$ (worst model) to 1 (perfect model), and models with q^2>0.5 are considered as having a high predictive power (Tropsha, Gramatica & Gombar, 2003). Therefore, q^2 behaves similar to the ${r_{adj}}^2$, while also informing on the accuracy of the predictions.

Individually, the experimental and predicted values were compared using a deviation factor ($d_{i,j}$), which quantified how much the predictions were above ($d_{i,j}$>0) or below ($d_{i,j}$<0) the experimental values for each species i and each heavy metal j (Guénard et al., 2011).

$$Y_{\mathrm{i,j}} = \begin{cases} 10^{Y_{\mathrm{pred\ i,j}} - Y_{\mathrm{exp\ i,j}}} - 1 & : Y_{\mathrm{pred\ i,j}} \geq Y_{\mathrm{exp\ i,j}} \\ 1 - 10^{Y_{\mathrm{exp\ i,j}} - Y_{\mathrm{pred\ i,j}}} & : Y_{\mathrm{pred\ i,j}} < Y_{\mathrm{exp\ i,j}} \end{cases} \tag{3.3}$$

This factor is the number of times that the predicted toxicity for a species is overestimated (positive values) or underestimated (negative values). For example, when $d_{i,j}$=0 the predicted LC50 value equals the experimental LC50 value, whereas $d_{i,j}$=-2 indicates that the predicted LC50 value is two-fold lower then the experimental LC50 value. Similarly, when $d_{i,j}$=2 the predicted LC50 value is two-fold higher than the experimental LC50 value.

All calculations and plotting were performed in the free open source software R, version 3.0.3 (R Core Team, 2014) using the R package ape for the phylogenetic tree construction (Paradis, Claude & Strimmer, 2004) and the package MPSEM for phylogenetic bilinear models (Guénard, Legendre & Peres-Neto, 2013).

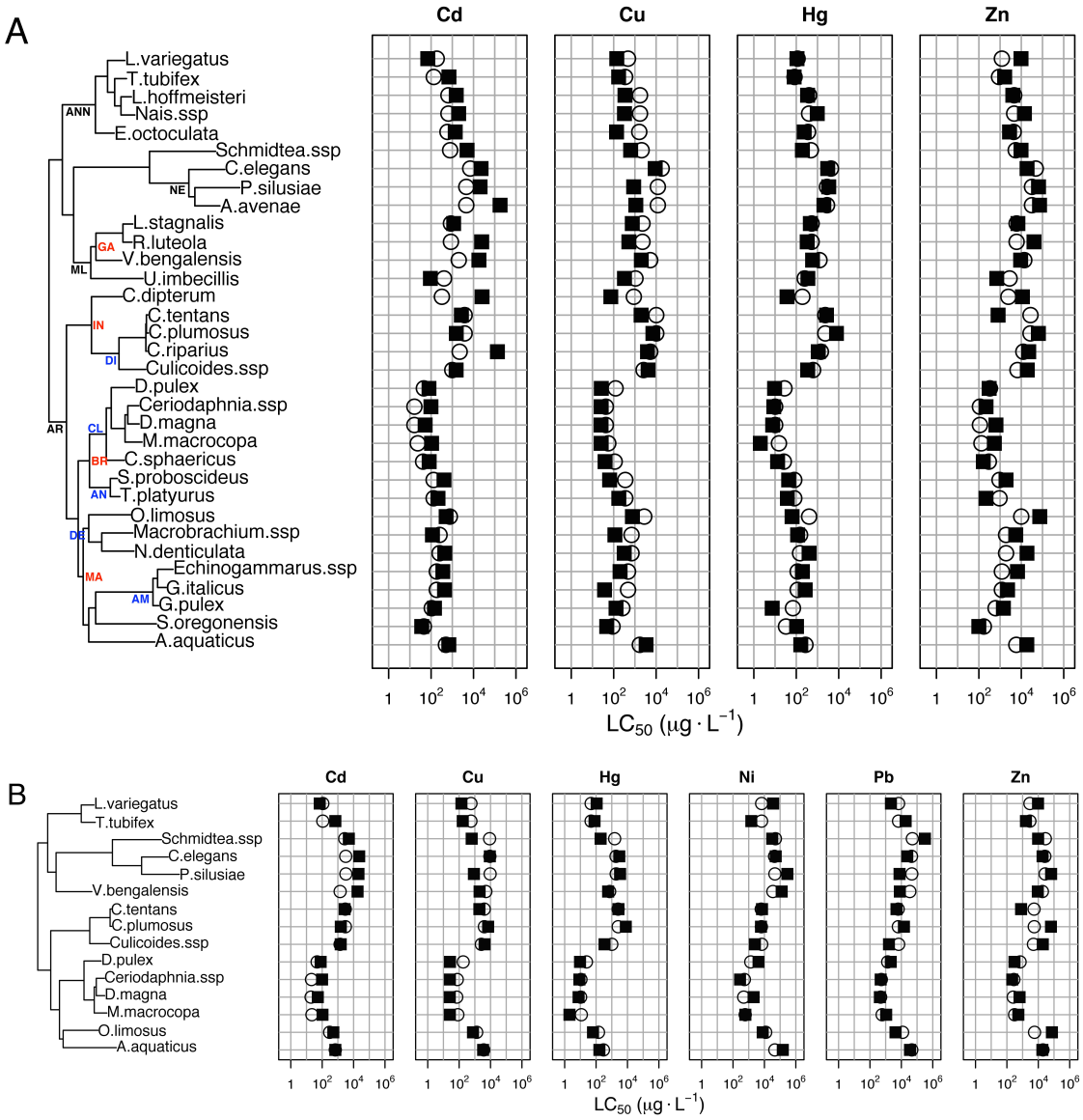

Figure 3.2: The experimental (closed squares) and predicted (open circles) median lethal concentration (LC50 in μg/L) for the four heavy metal model (4HM) with 33 species (A) and for the six heavy metal model (6HM) with 15 species (B). For both bilinear models the softness parameter (σp) of the heavy metals was used as a second descriptor. The adjusted coefficient of determination (r_{adj}^2) was 0.63 for the 4HM (A) and 0.77 for the 6HM (B). The complete phylogenetic tree is shown in part A where the main divisions were: (i) *phylum* (black): AR=Arthropoda, NE=Nematoda, ANN=Annelida, ML=Mollusca; (ii) *class* (red): BR=Branchiopoda, MA=Malacostraca, IN=Insecta, GA=Gastropoda; and (iii) *order* (blue): AM=Amphipoda, DE=Decapoda, AN=Anostraca, CL=Cladocera, DI=Diptera. For details on the taxonomic divisions see Appendix B, Table B.3.

3.4 Results

3.4.1 Sensitivity to heavy metals

Species in the orders of Cladocera and Anostraca were consistently the most sensitive for all heavy metals, whereas nematodes (e.g., *Panagrellus silusiae*) and insect species (e.g.,

Chironomus plumosus) were among the least sensitive (Figure 3.2 and Table B.3 in Appendix B). Some species had high variability within their taxonomic units (e.g., species from the genus Chironomus differed by two-orders of magnitude for Cd), whereas in other cases sensitivity patterns differed based on the heavy metals tested (e.g., nematode *Aphelenchus avenae* differed by two-orders of magnitude between Cd and Cu). The complete dataset of experimental $\log_{10}$LC50 values is presented in Appendix B, Table B.3.

3.4.2 Phylogenetic Tree

We found at least one DNA sequence on the species level for 26 species, whereas for the remaining species the genetic information was borrowed from other species of the same genus (five species), or from the same genus and family (two species; Appendix B, Table B.1). The most common genes were the cytochrome oxidase sub-unit 1 (COX1; all species; 7 complete sequences), the nuclear small ribosomal sub-unit (18S; all species; 13 complete sequences), and the nuclear large ribosomal sub-unit (28S; 29 species; 2 complete sequences). For 12 species the complete mitochondrial sequences was available.

The tree obtained from the molecular information placed almost all species within their taxonomic divisions (see Figure 3.2A for the complete tree and Table B.3 in Appendix B for details on taxonomy). Classes for which phylogeny was resolved included: (i) Branchiopoda (represented by the orders Cladocera and Anostraca), (ii) Insecta (represented by Diptera), or (iii) Malacostraca (represented by the orders Amphipoda and Decapoda). Inconsistencies in phylogenies were found for those species which were the sole representatives of taxonomic groups such as (i) *Skistodiaptomus oregonensis* for the order Copepoda, (ii) *Asellus aquaticus* for the order Isopoda, or (iii) Schmidtea *ssp.* for the phylum Platyhelminthes.

3.4.3 Bilinear Models

The best bilinear models for the 4HM resulted from using ${X_m}^2$r and σp with a minimal difference (4%) between their prediction coefficients (${r_{adj}}^2$=0.67 for ${X_m}^2$r and ${r_{adj}}^2$=0.63 for σp; see Table B.4 in Appendix B for a complete list of models). Similarly, the differences (2-4%) between the 6HM models employing only σp (${r_{adj}}^2$=0.77), and 6HM models combining σp with the $|\log_{10}$KOH$|$(${r_{adj}}^2$=0.79), ΔE_o(${r_{adj}}^2$=0.78) or $\log_{10}K_{SO}$MOH(${r_{adj}}^2$=0.81) were minimal (Appendix B, Table B.4). Furthermore, the ${r_{adj}}^2$ values for the 6HM models with two properties were likely to be influenced by the correlation between the properties (Pearson $|r|>0.4$; Appendix B, Table B.4). Under these considerations, σp was preferred for both 4HM and 6HM models, and the one parameter model was preferred over the two parameter model for 6HM. The selected models are presented in Figure 3.2, and their bilinear parameters are given in the Appendix B, Table B.5. The major part of the explained variance was attributed to the phylogeny for both models (4HM model: ${r_{adj}}^2$=0.45, and 6HM model: ${r_{adj}}^2$=0.41).

The prediction coefficient (q^2) following leave-one-out cross-validation (Type 1A prediction) equaled 0.53 and 0.71 for the 4HM model and 6HM model, respectively (Figure 3.3A and B). The predicted values were within a deviation factor of 10 from the experimental values (in absolute values $|d|<10$; equation 3.3) for 86% and 92% of the cases for 4HM model and 6HM model, respectively. The deviation $|d|<10$ ranged from 82% (Zn) to 94% (Hg) for 4HM model, and from 80% (Zn) to 100% (Hg and Pb) for 6HM model. The most severe cases of underestimation of toxicity were for *Chironomus riparius* and Cd (d=-75.8; 4HM model), *Orconectes limosus* and Zn (d=-50.2; 4HM model), *Viviparus bengalensis* and Cd (d=-31.5; 6HM model), as well as *A.aquaticus* and Ni (d=-25.2; 6HM

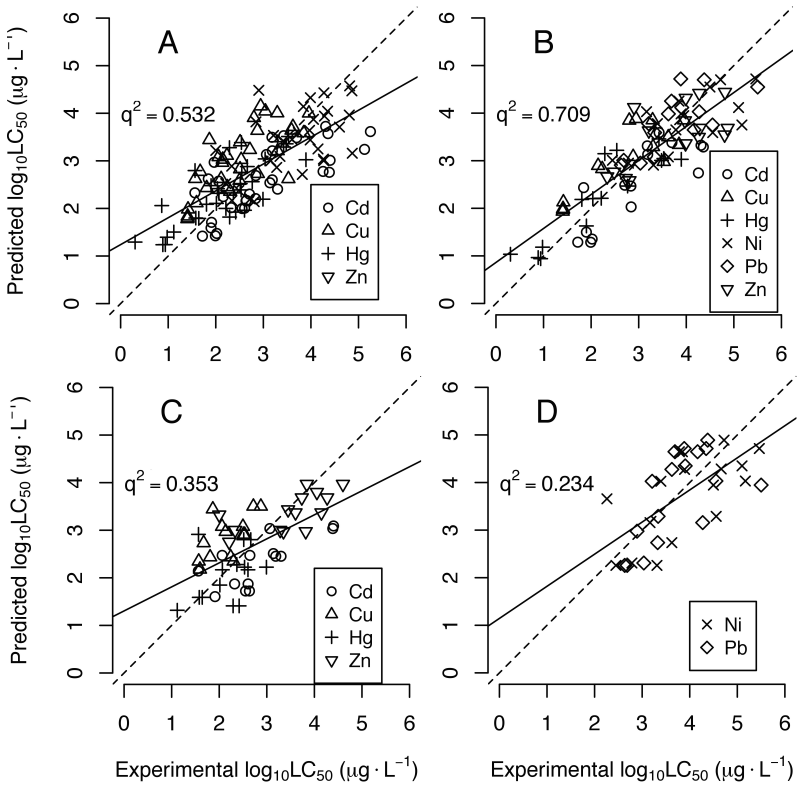

Figure 3.3: Relationships between the experimental and predicted median lethal concentration (log_{10}LC50 in μg/L^{-1}) of the heavy metals for type 1A prediction (new species; A: 4HM model; B: 6HM model), type 1B prediction (new species: C) and type 2 prediction (new metals: D). The dashed line is the 1:1 relationship which represents the perfect prediction by the model, and the solid line is the regression line. The prediction coefficient (q^2) demonstrates the accuracy of the predicted values. For all types of predictions, bilinear models employing the softness parameter (σp) as second descriptor were used.

model). The most severe cases of overestimation of toxicity were *Chironomus dipterum* and Cu (d=36.6; 4HM model), and *Chironomus tentans* and Zn (d=15.4; 6HM model; Appendix B, Figure B.1). The prediction coefficient following Type 1B prediction was lower (q^2=0.35; Figure 3.3C), with 50 out of 56 cases (89%) which had $|d|$<10, and with *C. dipterum* (d=36.9 for Cu) and *Radix luteola* (d=-21.4 for Cd) at the extremes. Finally, the prediction coefficient for Type 2 prediction equaled 0.23 and the predicted values scattered relatively homogeneously around the 1:1 line (Figure 3.3D). We found 28 out of 34 cases (82%) had $|d|$<10, mainly overestimating the LC50 values (6 out 7 cases had $|d|$>10), with Schmidtea *ssp.* (d=-34.9 for Pb) and *Utterbackia imbecillis* (d=-24.0 for Ni) as the most extreme disagreements.

3.5 Discussion

We demonstrated that the variation in heavy metal sensitivity of macroinvertebrate species can be explained and predicted using phylogenetic information of taxa and physicochemical properties of heavy metals. Similarly, using phylogenetic information (Guénard et al., 2011) or combining phylogenetic information and physicochemical properties (Guénard et al., 2014) to explain variation in LC50 has produced satisfactory results for organic chem-

icals. We obtained similar findings for heavy metal toxicity: phylogeny explained a large proportion of the variation in sensitivity and we were able, using a sufficient number of tested species sharing common evolutionary history, to successfully predict species sensitivity to heavy metals. Note that these models are constructed under the assumption that the tested species were unbiased representatives of their taxonomic groups. However, toxicological and genetic databases are typically biased towards common test species used for regulatory, practical or economical purposes. Other factors that influence the predictive power of bilinear models are discussed below.

3.5.1 Influence of inter-species variation

Large inter-laboratory differences in experimental toxicity values would obscure phylogenetic signals. Although, the variability was reduced by standardizing the LC50 values to reference conditions (Malaj et al., 2012), the differences in prediction power between the models using standardized and non-standardized LC50 values were minimal (between 2-3% higher for the models with standardized LC50). We recommend the use of standardized toxicities as it is likely to reduce variability. It is unclear whether high variability in experimental toxicity data in the same taxonomic group (e.g., chironomus species) originates from inter-laboratory differences or originates from a lack of phylogenetic signal. The observed inter-laboratory differences in LC50 values are likely a result of (i) different physicochemical conditions (not always reported) under which toxicity test are performed (e.g., pH, alkalinity, etc alter toxicity to metals; see Di Toro et al. (2001)) or (ii) the lifestages of species employed in the experiments (e.g., tolerance to heavy metals increases with the size of the instars of aquatic insects (Clements, Cadmus & Brinkman, 2013)). Alternatively, evolutionary events may have lead to distinct sensitivities (Guénard et al., 2011), or other biological parameters e.g., related to bioaccumulation and detoxification (Buchwalter et al., 2008) might better explain inter-species variation to heavy metals. If factors determining inter-species variation would be available, they can be used as further model parameters to explain species sensitivity (Guénard et al., 2014).

3.5.2 Influence of physicochemical properties

Physicochemical properties used in the present study were good predictors of the toxicity of heavy metals (Newman, McCloskey & Tatara, 1998; Walker, Enache & Dearden, 2003; Wu et al., 2013) for a good share of the explained variance (15% for the 4HM model and 29% for the 6HM model). Furthermore, the predictive power of bilinear models for the toxicity of new metals depended directly on the representativeness of the physicochemical properties. In fact, the capacity of metals to form stable complexes with biologically active molecules through association with the O-, N- or S- ligands is often responsible for metal toxicity (Walker, Enache & Dearden, 2003). The best physicochemical parameter for both models was σp, a parameter that is consistently found as the best descriptor of heavy metal toxicity for aquatic organisms (e.g., see Wu et al. (2013); Walker, Enache & Dearden (2003) and the references therein). Based on σp, metals are divided into Class A (e.g., Mg^{2+}, Al^{3+}) or O-seeking metals (affinity for hard ligands), Class B (e.g., Hg^{2+}) or S- and N- seeking metals (affinity for soft ligands), and borderline metals (e.g., Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}), or S-, N-, O- seeking metals. Therefore, our investigated metals were all borderline, with the exception of Hg^{2+}. The higher explanatory power (4%) of the $X_m{}^2r$ in comparison with σp for the 4HM model can be explained with the higher influence of Hg in the 4HM model than in the 6HM model. Hg is a class B metal (high affinity for S-and N-ligands, low σp values), and therefore, it is probably closely related to

$X_m{}^2r$, which has a higher descriptive power for this class of metals (Nieboer & Richardson, 1980)(see Appendix B, Supplementary Methods for details). Physicochemical properties have been shown to improve the explanation of toxicity when metals were grouped based on their valence, or on their ligand affinity (Newman, McCloskey & Tatara, 1998). The inclusion of the metal valence into bilinear models may lead to further improvements in the presented models, though models involving metals with other valences need further investigation.

3.5.3 Influence of molecular information

Bilinear models can be influenced by uncertainties related with the estimated phylogenetic tree. Firstly, the super-alignment method uses the concatenation of individual genes usually of unequal length, which results in a sparse overlap of sequences and empty cells in the super-alignment. However, the sparse genetic data would not influence our tree estimation because phylogenetic accuracy has been related to sufficient characters (e.g., amino acids), rather than to missing data (up to 90% without compromising the accuracy (Wiens, 2003; Crandall & Buhay, 2004)). This is because many DNA sequences are invariant, especially for lower taxonomic classes, and therefore do not contribute to phylogenetic reconstructions. By contrast, data on the same (variant) gene regions was available for all species (COX1 profiles and 18S rRNA sequences), which are recognized to posses high phylogenetic signals (Hebert, Cywinska & Ball, 2003). Secondly, unrelated species can be erroneously clustered together. For instance, the mollusc *V. bengalis* is connected with the nematode clade (Figure 3.2B), or *A.aquaticus* (order Isopoda) that is connected to *S. oregonensis* (order Copepoda) and together are attached to the order Ampipoda (Figure 3.2A). These connections were due to lack of data on other species in their respective taxonomic groups, which also explains in most cases the high deviation (as $|d|$) of predicted from experimental values. These questionable connections would probably be broken, if more species data would become available for the respective orders. In this context, Driskell et al. (2004) suggested that unrooted trees with fewer than four taxa would contain unreliable information on species relations. Although phylogenetic regression is considered robust to tree misclassification (Stone, 2011), the precision of predictions for untested species would improve with new data on close relatives, rather than data on species elsewhere in the tree (Fagan et al., 2013). Finally, we did not consider the influence of phylogenetic uncertainty in the model prediction and we assumed that the phylogenetic tree does not contain large errors. In cases when the phylogeny is difficult to estimate, more robust methods could potentially be used (e.g., Bayesian methods; Kolaczkowski & Thornton, 2004).

3.5.4 Implications for ecological risk assessment

Our approach has direct applications in ecotoxicology. Firstly, it provides managers with a tool for predicting heavy metal toxicity for untested species, which in turn will enhance the applicability of community-based risk assessment methods such as SSDs. As a result, this would improve the reliability of protection levels for aquatic communities. Moreover, this approach could be used to predict bioaccumulation parameters of heavy metals (Buchwalter et al., 2008; Poteat et al., 2013), which might reconcile the differences in effect concentrations between bioassays and field observations for aquatic invertebrates. By being dominant, diverse and abundant, the composition of invertebrate communities is considered as the backbone of the biotic indices used to evaluate the ecological status of freshwater ecosystems (Clements, Cadmus & Brinkman, 2013). Secondly, details about

the phylogenetic structure of among-species sensitivity variation would inform managers on the optimal taxonomic resolution for biomonitoring (Carew, Miller & Hoffmann, 2011; Poteat et al., 2013). For instance, if the phylogenetic signal is strong (low residual variance) for the species sensitivity on the genus level, then any species constituting this genus would adequately represent the group and species-level identification could be dropped. On the contrary, if the phylogenetic signal is weak (high residual variance) at that same level, species-level identification would be required to reliably determine the sensitivity. Finally, our study provides an example of how concepts of evolutionary biology can be applied in ecotoxicology. However, this method is largely applicable to predict a wider range of endpoints, including other toxicological and stress factors or other species traits. Phylogenetic methods are a promising tool to explore the among species patterns and to optimize our ability to extrapolate across species making use of existing data.

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Chapter 4

Organic Chemicals Jeopardize the Health of Freshwater Ecosystems on the Continental Scale

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"Risk assessment is the product of a shotgun wedding between science and the law"
- William Ruckelshaus

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4.1 Abstract

Organic chemicals can contribute to local and regional losses of freshwater biodiversity and ecosystem services. However, their overall relevance regarding larger spatial scales remains unknown. Here, we present, to our knowledge the first risk assessment of organic chemicals on the continental scale comprising 4,000 European monitoring sites. Organic chemicals were likely to exert acute lethal and chronic long-term effects on sensitive fish, invertebrate, or algae species in 14% and 42% of the sites, respectively. Of the 223 chemicals monitored, pesticides, tributyltin, polycyclic aromatic hydrocarbons, and brominated flame retardants were the major contributors to the chemical risk. Their presence was related to agricultural and urban areas in the upstream catchment. The risk of potential acute lethal and chronic long-term effects increased with the number of ecotoxicologically relevant chemicals analysed at each site. As most monitoring programs considered in this study only included a subset of these chemicals, our assessment likely underestimates the actual risk. Increasing chemical risk was associated with deterioration in the quality status of fish and invertebrates communities. Our results clearly indicate that chemical pollution is a large-scale environmental problem and requires far-reaching, holistic mitigation measures to preserve and restore ecosystem health.

4.2 Introduction

The majority of streams and rivers are ecologically impaired or threatened with high losses in biodiversity, which compromise the future provisioning of vital ecosystem services (Vörösmarty et al., 2010; Cardinale et al., 2012). Understanding the causes of these impairments is crucial to inform freshwater management and for directing restoration efforts (Stendera et al., 2012). Despite their ubiquitous global use, organic chemicals have only been shown to affect aquatic communities locally or regionally, whereas the overall extent of their impact is largely unknown (Rockström et al., 2009; Schwarzenbach et al., 2006). Previous studies on the risk assessment of organic chemicals have been limited to a few sites (Belden et al., 2007), regions (Schäfer et al., 2011), or compounds (de Zwart et al., 2006) rendering the extrapolation to larger spatial scales questionable. To date, large-scale analyses have been hindered by the lack of large-scale monitoring databases for organic chemicals and by the scarcity of empirical toxicity data (Strempel et al., 2012). Gaps in missing experimental toxicity data can be filled by modeled or predicted toxicity data from read-across methods (Schüürmann, Ebert & Kühne, 2011) or quantitative structure-activity relationships approaches (Altenburger, Walter & Grote, 2004), which serve as surrogates for experimental data. Once toxicity data are compiled, the availability of chemical datasets such as Waterbase (EEA, 2012), which accommodates information on the chemical concentrations for more than 8,200 European sites, allows chemical risk assessment to be conducted on large spatial scales.

Chemical risk assessment is typically conducted by comparing measured or predicted environmental concentrations with the respective risk thresholds, which are usually derived from ecotoxicological tests in the laboratory, at or above which effects on aquatic organisms cannot be excluded (Calow & Forbes, 2003). In particular, when data on acute toxicity of species are the only data available, safety factors (e.g., 100-1,000 (CEC, 2011)) are applied to the lowest median lethal concentration (LC50) from three representative taxonomic groups (usually a crustacean, a fish, and an algae). These safety factors are supposed to protect the non-target species from the likely effects of chemicals. More sophisticated approaches such as species sensitivity distributions (SSDs), which allow for

derivation of thresholds that are assumed to protect a distinct percentage of species (usually 95%), are generally not applicable for data sets with a high number of chemicals, due to a paucity of toxicity data (Schäfer et al., 2013). However, establishing effect thresholds that are protective for the entire ecosystem is an on-going challenge, due to difficulties with addressing the inherent differences between laboratory test systems and field situations and due to the required balance of ecosystem protection and economic development (Calow & Forbes, 2003). The plausibility of the chemical risk assessment can, however, be determined by comparison with ecological endpoints from real ecosystems, if ecological data are available.

In this study, we present, to our knowledge, the first comprehensive chemical risk assessment on the continental scale encompassing three major organism groups in freshwater ecosystems (fish, invertebrates, and algae, represented by *Pimephales promelas*, *Daphnia magna*, and *Pseudokirchneriella subcapitata*, respectively). We combined measured concentrations of 223 chemicals for 4,001 sites distributed over 91 European river basins with their respective toxicity information to determine the spatial distribution of chemical risk on the continental scale. For this purpose, the CR per river basin was calculated using two risk thresholds, the acute risk threshold (ART), and the chronic risk threshold (CRT) for each organism group (Appendix C, Supplementary Methods for rationale). Compounds whose concentrations exceeded the ART at any site were considered as the most relevant compounds for risk assessment and classified as acute-risk chemicals (ARCs). We checked if the CR increased with the number of ARCs analysed and which compounds contributed most. Furthermore, we identified to what extent different land use types drove the chemical risk. Finally, we compared the chemical risk to the ecological status of fish, invertebrate and diatom communities at selected sites.

4.3 Methods

4.3.1 Data mining

Chemical concentrations were retrieved from the Waterbase (version 12) dataset of the European Environmental Agency (EEA, 2012). The database quality control comprised (i) removal of duplicates entries, entries with missing concentrations and entries with missing coordinates or with coordinates outside of Europe; (ii) treatment for concentrations reported as below the limit of quantification (LOQ); (iii) treatment of sites spatially autocorrelated; and (iv) restriction of the dataset to the most recent data available (2006-2010) for organic chemicals (Appendix C, Figure C.1 and Supplementary Methods). The chemical concentrations (in μg/L) for each monitoring site were reported as mean (C_{mean}), and maximum (C_{max}) annual values, typically used to characterise chronic and acute exposure, respectively. For sites with few measurements per year (e.g., n$\leq$12) , the C_{mean} can be potentially influenced by the (C_{max}) and/or non-detects (reported as a fraction of LOQ). To account for this bias, we adjusted the reported (C_{mean}) as three times lower for n$\leq$12 ($C_{c\text{-}mean}$) based on the C_{max}/C_{mean} relationship for the sites with n>12, for which C_{mean} values were considered as representative of chronic exposure (Appendix C, Supplementary Methods).

Short-term toxicity values (i.e., LC50) were collected for each chemical and each of the three test species: (i) the fish *P. promelas* (96 h); (ii) the invertebrate *D. magna* (48 h); and (iii) the green algae *P. subcapitata* (formerly known as *Selenastrum capricornutum*; 48-96 h). In a sequential order, LC50 values were compiled by using experimental, predicted or baseline (from the octanol-water partitioning coefficient) toxicity data. Toxicity values

were excluded when (i) they exceeded 10-fold the water solubility, and (ii) the application domain for baseline toxicity was violated (Appendix C, Table C.1 for sources of toxicity data, and Appendix C, Supplementary Methods for details). Finally, 223 compounds were considered in this analysis.

4.3.2 Threshold selection

To quantify the potential effects of chemicals on ecosystem health, for each site within a river basin, we compared (i) C_{max} to the ART, defined as 1/10 of the LC50 values for each of the three standard test organisms; and (ii) C_{mean} (or $C_{c\text{-}mean}$) to the CRT, defined as 1/1,000, 1/100, and 1/50 of the LC50 values for invertebrate, fish, and algae, respectively. Concentrations exceeding these thresholds may cause acute and chronic ecological effects, respectively (Appendix C, Supplementary Methods).

4.3.3 Chemical risk calculation

First, CR index for each organism group was calculated on the river basin scale as:

$$CR_{\mathrm{j,o,b}} = \frac{N_{\mathrm{j,o,b}}}{N_{\mathrm{total,b}}} \tag{4.1}$$

where N represents the number of sites for which one of the chemical concentrations exceeded the respective risk threshold j for each organism group o within a river basin b, and N_{total} represents the total number of sites within that river basin. The risk thresholds j are either the CRT or the ART. Fewer than six monitoring sites were considered as unrepresentative for a river basin, which were subsequently omitted from the analysis (basins in grey in Figure 4.1 and Figure C.4 in Appendix C). Furthermore, the chemical risk index for each threshold j was calculated as the aggregation over all organism groups o per basin b:

$$CR_{\mathrm{j,b}} = \frac{N_{\mathrm{j,b}}}{N_{\mathrm{total,b}}} \tag{4.2}$$

Overall chemical risk on the continental scale for each organism groups o was calculated as:

$$CR_{\mathrm{j,o}} = \frac{N_{\mathrm{j,o}}}{N_{\mathrm{total,o}}} \tag{4.3}$$

Overall chemical risk on the continental scale was calculated as:

$$CR_{\mathrm{j}} = \frac{N_{\mathrm{j}}}{N_{\mathrm{total}}} \tag{4.4}$$

We created maps on the distribution of the chemical risk in Europe by dividing the $CR_{j,b}$ and $CR_{j,o,b}$ indices into five classes: (i) 0-10% as very low; (ii) 10-25% as low; (iii) 25-50% as moderate; (iv) 50-75% as high; and (v) 75-100% as very high (Figure 4.1 and Figure C.4 in Appendix C for $CR_{j,b}$ and $CR_{j,o,b}$, respectively). We based the definition of the likelihood of observing acute and chronic effects on a literature review summarized in Appendix C, Table C.3.

Finally, the Kendall tau correlation coefficient (τ) was used to check the relationship between the chemical risk in river basins ($CR_{j,b}$) and the number of sampling sites.

4.3.4 Acute-Risk Chemicals

Chemicals for which the C_{max} exceeded the ART at any site for any organism group were classified as ARCs. We hypothesised that the chemical risk at a monitoring site would be positively correlated with the number of ARCs analysed. Therefore, we (i) calculated the chemical risk for groups of sites at which a given number of ARCs were analysed; (ii) fitted a cubic smoothing spline to visualise the trend between the calculated chemical risk and ARCs; and (iii) used the nonparametric rank-based Mann–Kendall test (McLeod, 2011) to assess the significance of the trend ($P<0.05$; for details on the calculations, Appendix C, Supplementary Methods).

4.3.5 Temporal Variation

Large differences in the monitoring frequencies among sites raised the question whether temporal variability biased the chemical risk. Therefore, the chemical risk was calculated for each year to check for potential differences among the years 2006-2010. The chemical risk was calculated as:

$$CR_{\mathrm{a,j,b}} = \frac{N_{\mathrm{a,j,b}}}{N_{\mathrm{total,a,b}}} \tag{4.5}$$

where a represents the year, ranging from 2006 to 2010. Only sites that had data for more than one year and basins that had more than six sites were included in the analysis. To test for differences in the chemical risk between years, one-way analysis of variance (ANOVA) with Welch correction was used ($P<0.05$).

4.3.6 Land Use Practices

To identify the potential origin of pollution, we retrieved the land use information from the same dataset (EEA, 2012), which was reported as the percentage of the upstream catchment land use of each monitoring site (available for 14% of the sites; Appendix C, Table C.5). We investigated the difference in chemical risk between two types of land use categories (i) natural vegetation (NV); and (ii) anthropogenically influenced areas (AI; Appendix C, Table C.6 for sub-categories), as we assumed that organic chemicals would originate primarily from agricultural or urban areas. The land use was further restricted to (i) sites with more than 80% natural vegetation (n=117) and (ii) sites with more than 50% anthropogenically influenced areas (n=189). Chemical risk for the sites from the two land use categories was calculated as:

$$CR_{\mathrm{u,j,b}} = \frac{N_{\mathrm{u,j,b}}}{N_{\mathrm{total,u,b}}} \tag{4.6}$$

where u is natural vegetation (NV) or anthropogenically influenced areas (AI). Basins with less than six sites for each land use category were omitted. Chemical risk between the two land use categories was compared with the Student t-test ($P<0.05$).

4.3.7 Ecological status

We compared the chemical risk to the ecological status using sites from the French National Monitoring Program, because this program measured the highest number of ARCs and had the highest match of chemical and ecological data (Appendix C, Table C.7). The ecological data for 2007-2010 were extracted from the French National Network (Réseau de Contrôle de Surveillance (RCS)) performed by 22 regional environmental agencies (for details see Mondy et al. (2012)). The ecological status classification (high, good, moderate,

poor and bad) was based on biotic indices, namely (i) the multimetric Indice Poisson Rivière (IPR+ (Marzin et al., 2014)) for fish, (ii) the MultiMetric Invertebrate Index (I_2M_2 (Mondy et al., 2012)) for invertebrates, and (iii) the Indice Biologique Diatomées (IBD (Coste et al., 2009)) for diatoms. To avoid pseudoreplication for sites with multiple years of matching chemical and ecological data, the year with the highest chemical risk was selected and matched with the lower ecological status of the corresponding or the following year. The rationale was that the sampling dates were unknown and the ecological data for the same year might have predated the chemical data that drove the chemical risk classification. Furthermore, we selected the ecological data corresponding to small (>90% of the sites between 5 and 15 m width) and lowland (<200 m altitude) streams, to minimize confounding effects from other stressors (e.g., the number of stressors increases with stream size; see Schinegger et al. (2012)) or from different ecoregions (e.g., Alpine streams). Based on the CR thresholds and the chemical concentration, sites with ecological status were divided into three classes: (i) sites with chemical concentrations exceeding ART, which were the sites acutely affected by chemicals; (ii) sites with chemical concentrations exceeding CRT, but not ART, which were the sites chronically affected by chemicals; and (iii) sites with chemical concentrations lower than CRT, which were the sites with no or negligible risk from chemicals. Finally, the frequency of sites with high or good ecological status was calculated per class.

All data analyses, statistical computations and graphics were generated with the open source software R (R Core Team, 2013). The R code and data are made available to enable reproducibility of our analysis (http://www.uni-koblenz-landau.de/campus-landau/faculty7/environmental-sciences/landscape-ecology/publications/Malaj/).

4.4 Results and Discussion

4.4.1 Chemical Risk

On the continental scale, 14% of the monitoring sites were likely to be acutely affected by organic chemicals (Figure 4.1A) and 42% were likely to be chronically affected by organic chemicals for at least one organism group (Figure 4.1B). For each organism group, at 3%, 6%, and 9% of sites, the maximum chemical concentrations exceeded the ART for fish, invertebrates, and algae, respectively, and at 6%, 38%, and 13% of sites, the mean chemical concentrations exceeded the CRT (Appendix C, Figure C.4). Note that the differences in CRT exceedances between organism groups may partly be attributed to the different sources of effect thresholds, which were field based for invertebrates, and extrapolated from laboratory-based acute toxicity data for fish and algae (Appendix C, Supplementary Methods). In general, these results suggest that organic chemical pollution is an important large-scale pressure.

On the regional level, river basins in the north of Europe had higher chemical risks than those situated in the south. For the northwestern river basins, the acute and chronic CR reached high (50-75%) to very high (>75%) levels, respectively. This is in agreement with other studies that predicted loads of chemicals in European rivers (e.g., perfluorinated compounds (Pistocchi & Loos, 2009) and insecticides (Kattwinkel et al., 2011)). In Southern Europe, the low chronic and acute CR (<25%) were presumably due to the low number of ecotoxicologically relevant chemicals measured and the result of unreliable limits of quantifications for part of the data (e.g., Spain; Appendix C, Supplementary Methods). On the contrary, the high acute and chronic risks in the French river basins probably resulted from good monitoring practices, such as a dense monitoring network

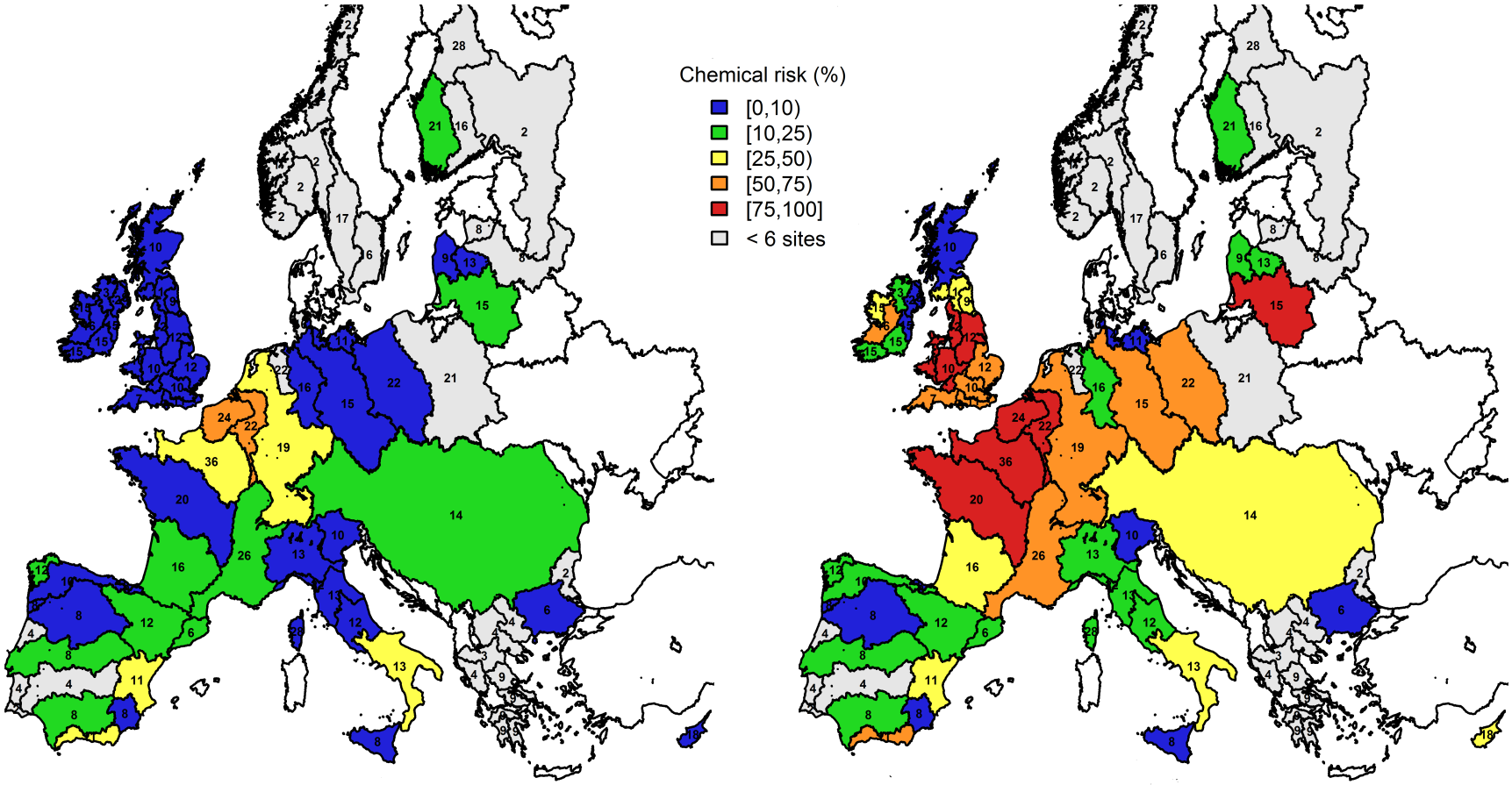

Figure 4.1: Chemical risk (by percentage range) in European river basins. The map displays the fraction of sites where the maximum chemical concentration exceeds the acute risk threshold (A) and the mean chemical concentration exceeds the chronic risk threshold (B) for any organism group. The colour code shows the level of chemical risk, from low chemical risk (blue) to high chemical risk (red). River basins with up to six sites are displayed in grey (Appendix C, Table C.5), whereas river basins without data are displayed in white. The numbers denote the median of the acute-risk chemicals analysed at the monitoring sites of each river basin. Direct comparisons between river systems is potentially biased by the ecotoxicologically relevant compounds analysed and the limit of quantification of the compounds (Appendix C, Figure C.2 and Table C.2).

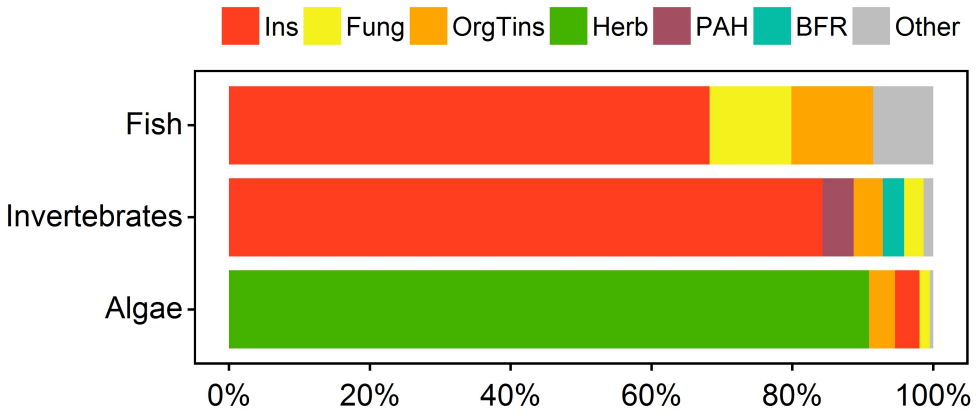

Figure 4.2: Proportion of sites acutely affected by different chemical groups. The chemical groups analysed were Insecticides (Ins), Fungicides (Fung), Organotin compounds (OrgTins), Herbicides (Herb), Polycyclic Aromatic Hydrocarbons (PAH), Brominated Flame Retardants (BFR), and other compounds (chemical groups with five or fewer sites acutely affected which comprised Polychlorinated biphenyls, Halogenated alkanes, and Phenols). The groups of organisms considered were fish (represented by *P. promelas*), invertebrates (represented by *D. magna*), and algae (represented by *P. subcapitata*). Acutely affected sites were all sites with maximum concentrations exceeding 1/10 of the LC50.

and the inclusion of most ecotoxicologically relevant chemicals (i.e., the ARCs). Hence, comparisons between river basins are potentially biased by spatial and temporal sampling density and the number of ARCs analysed. One example is the generally poor spatial sampling density for river basins in the Scandinavian and Baltic countries (fewer than six sites), hampering a reliable CR estimation. Furthermore, only 5% of the sites were regularly monitored every year, whereas 53% of the sites were sampled only once in the 5-year interval investigated. Nevertheless, we found only a weak relationship between the number of sampling sites and CR (ART: Kendall τ=0.42 CRT: Kendall τ=0.33), and no significant difference between the CR from different years (one-way ANOVA, ART: $F_{4,41}$=1.37, P=0.26, CRT: $F_{4,44}$=0.47, P=0.75). Overall, standardized monitoring programs with regard to spatial and temporal sampling density, as well as the inclusion of ARCs in monitoring schemes (see below for discussion on ARCs) would enhance the comparability of individual basins on large scales. Note that deficiencies in monitoring programs can only result in underestimation of risk, never in overestimation.

4.4.2 Contributors to chemical risk

Pesticides were responsible for 81%, 87% and 96% of the observed exceedances of the ART related to fish, invertebrates and algae, respectively (Figure 4.2). Despite extensive regulation and technological advances in terms of specificity and degradability, pesticides continue to threaten non-target species, especially those groups exhibiting physiological similarity to pest species (Stark, Banks & Vargas, 2004). Herbicides accounted for most of the exceedances in algae, whereas insecticides accounted for most of the exceedances for invertebrates and fish (Figure 4.2, and Table C.4 in Appendix C). Whereas pesticides are designed to acutely affect invertebrates and algae, fish typically suffer from compounds affecting development, fitness, or reproduction (e.g., by endocrine disruptors), which are not covered here, but might increase the risk to fish communities (Jobling et al., 1998).

Additional ARCs were (i) organotin compounds, mainly the banned biocide tributyltin,

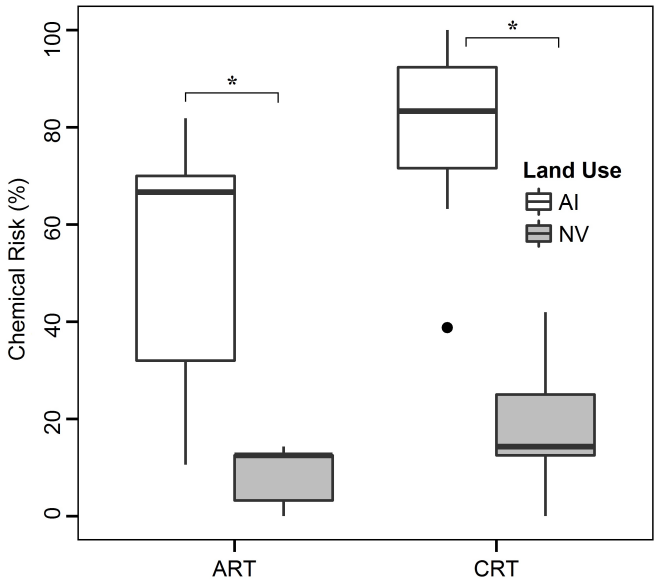

Figure 4.3: Box-and-whisker plots of the chemical risk for different land use categories. The two categories used comprised anthropogenically influenced areas (AI) and natural vegetation (NV) for the acute risk threshold (ART) and chronic risk threshold (CRT). The categories analyzed were significantly different for both thresholds ($P<0.05$).

which is an antifouling agent that primarily leaches from the hulls of ships; (ii) brominated diphenyl ethers, which are widely used as flame retardants in consumer products; and (iii) polycyclic aromatic hydrocarbons, which are released by petroleum products or by combustion of organic matter. These chemical groups have raised concerns of persistence and biomagnification in the environment (Schwarzenbach et al., 2006). For the majority of the ARCs, experimental toxicity data (82% for *P. promelas*, 89% for *D. magna*, and 71% for *P. subcapitata*; Appendix C, Table C.4) were available, reducing the uncertainty related to predicted toxicity values. Here, we frame the chemical risks primarily for the environment, but maintaining environmental integrity is directly and indirectly relevant to human health and welfare (Sharpe & Irvine, 2004). Protection of freshwater from pollution safeguards ecosystem services such as water quality, which is pivotal for clean drinking water at an acceptable cost, and recreational values (Vörösmarty et al., 2010).

4.4.3 Chemical risk and land use

Chemical risks strongly depended on the land-use in the upstream catchments of the monitoring sites. We found a significant difference in the chemical risk between sites with intensive agriculture and/or urban practices (>50% land use) and those with natural vegetation (>80% land use) (all, $P<0.05$, t-test, CRT: $t=5.61$, $df=10$, ART: $t=4.13$, $df=7$; Figure 4.3). Adverse effects on the biota of small agricultural streams are well documented (Schäfer et al., 2012), but our study suggests that these effects can occur catchment-wide, presumably originating from the interconnectedness of freshwater ecosystems. Hence, management tools such as land sparing, i.e., high-intensity agriculture in defined areas to spare land for conservation in other parts, appear to be less plausible for freshwater biodiversity conservation than land sharing through extensive agriculture (Phalan et al.,

2011). Control of diffuse sources of pollution from agriculture remains a challenging task but can, for example, be achieved by implementing riparian buffer strips (especially edge of field), grassed paths, or vegetated treatment systems (Stehle et al., 2011; Reichenberger et al., 2007). Risk from other chemicals of concern relates mainly to point-source-pollution (e.g., input of waste water from households or industry), implying the requirement of optimised treatment technologies (e.g., ozonation (Hollender et al., 2009)) and better source control approaches.

4.4.4 Underestimation of chemical risk

Notwithstanding the high-quality data used for this analysis, the retrospective risk assessment presented here most likely underestimated the real risk of chemicals and can be considered as the best case scenario for the following reasons: First, the significantly increasing trend of the CR with the number of ARCs that were analysed (Figure 4.4) suggested that the acute and chronic risks would be higher if more ARCs were analysed. River basins with more than 15 ARCs analysed exhibited generally higher chemical risks (Figure 4.1). For a more realistic risk assessment, monitoring programs should be designed to measure at least all ARCs, unless there is strong evidence that a specific ARC is ecotoxicologically irrelevant in a basin. However, emerging chemicals other than those frequently monitored are likely to be present in ecotoxicologically relevant concentrations in water samples (e.g., Slobodnik et al. (2012)) and should be progressively identified and included in monitoring programs.

Second, potential threshold exceedances would go unnoticed due to high LOQs. For 18% of the analysed chemicals, in the majority of cases (>50%), the reported LOQ values were above the CRT (Appendix C, Table C.2). The LOQs provide the smallest concentrations that can be reliably quantified by the analytical method used and should be substantially lower than the risk threshold (Lepom et al., 2009). Thus, analytical measurements with higher sensitivity are required.

Third, other considerations, not addressed here, could exacerbate the chemical risk (i) chemicals usually occur in mixtures, which have been shown to exhibit stronger combined adverse effects than single compounds, especially for chemicals with similar modes of action (Altenburger, Walter & Grote, 2004); (ii) transformation products may be more ecotoxicologically potent than their parent compounds (Fenner et al., 2013); and (iii) current monitoring relies on point grab water samples at monthly or quarterly intervals, which are very likely to underestimate the real maximum concentrations (Stehle, Knäbel & Schulz, 2013). Moreover, very hydrophobic chemicals were omitted from the analysis due to uncertainty with regard to the effect concentrations derived from experiments exceeding the water solubility. Nevertheless, these compounds may bioaccumulate, as well as have other ecological effects such as endocrine disruption, which have been shown to impact ecosystems on large spacial scales (Jobling et al., 1998) (for details, Appendix C, Supplementary Discussion).

4.4.5 Ecological status and the relationship with chemical risk

The ecological status decreased strongly with increasing chemical risk for fish and invertebrates, whereas no clear trend was observed for diatoms (Figure 4.5). Similarly, a recent study found losses of invertebrate biodiversity above the CRT (Beketov et al., 2013). However, these results should be interpreted with caution, because European streams are subject to multiple stress (e.g., >90% of lowland streams (Schinegger et al., 2012)) and the indices employed in our study indicate general ecological degradation of a site and are

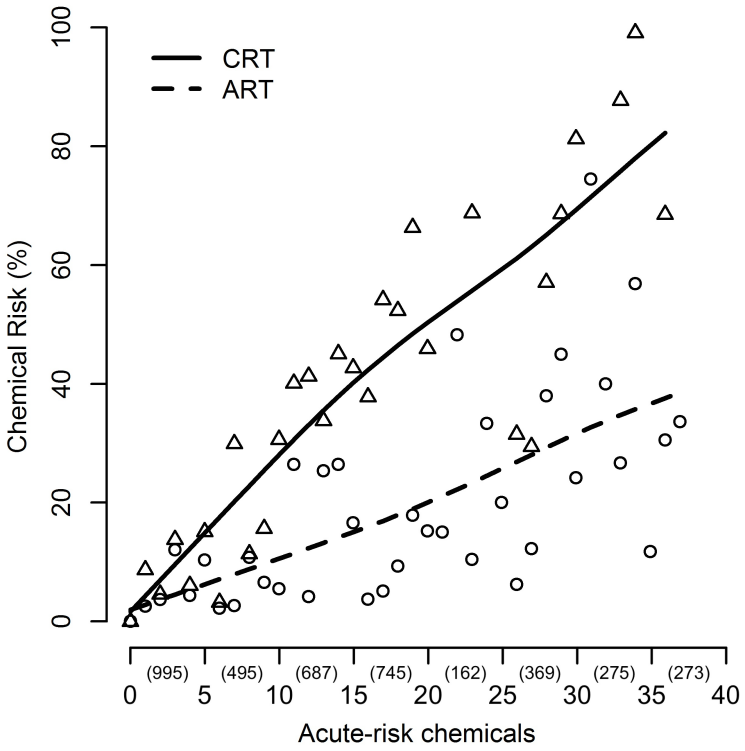

Figure 4.4: Mean chemical risk of the river basins to exceed the risk thresholds as a function of the number of acute-risk chemicals (ARCs) analyzed. ARCs are chemicals for which the maximum concentration exceeds 1/10 of the lethal effect concentration at any site. Dots correspond to the acute risk threshold (ART), and triangles are for the chronic risk threshold (CRT). The total number of sites for each ARC interval are given in parentheses on the x-axis. For the relationship between the number of acute-risk chemicals analyzed and the chemical risk, a cubic smoothing spline (all, df=3) was fitted to the data to visualise the significant increasing trend (all, $P<0.05$, n=30; ART, dashed line: Kendall τ=0.53, CRT, solid line: Kendall τ=0.74).

not toxicant-specific. The invertebrate I_2M_2 (Mondy et al., 2012) and fish IPR+ (Marzin et al., 2014) indices are multimetric, hence they are designed to respond to a large range of stressors (e.g., nutrients, hydromorphological alterations and land use (Mondy et al., 2012; Mondy & Usseglio-Polatera, 2013)) including toxic chemicals. Therefore, they may be more suitable to detect chemical risk than the IBD diatom index that was tailored to detect the effects of eutrophication (Coste et al., 2009). With respect to diatoms, confounding factors such as the light regime, turbulence or current velocity may also mask chemical effects (Marcel, Bouchez & Rimet, 2013). With respect to fish, the low number of sites impacted by chemicals (Appendix C, Table C.7) could hamper the relationship with ecological status. Furthermore, the difficulty in linking chemical stress to ecological status for fish in a given location likely originates from their high mobility. Therefore, fish indices are primarily regarded as indicators of habitat degradation and flow regulations, rather than as indicators of water pollution (Hering et al., 2006). By contrast, invertebrates are considered as good site-specific bioindicators, due to their low mobility. Note that the standard test species used for estimating chemical risk may not represent the chemical sensitivity of entire communities (e.g., Stark, Banks & Vargas (2004)), which may add to inconsistencies between the chemical risk and the ecological status of a site. Finally, toxicant-specific indices (e.g., the invertebrates' Species At Risk of pesticides;

$SPEAR_{pesticides}$index (Liess & Von Der Ohe, 2005)) would be more appropriate for detecting chemical effects. However, its application requires access to raw biological data (e.g., species abundance), whereas governmental agencies only provide ecological status information for the Biological Quality Elements (phytoplankton, aquatic macrophytes, benthic macroinvertebrates and fish) based on general indices. Providing access to raw data would foster our understanding of the links between anthropogenic stressors and populations or communities.

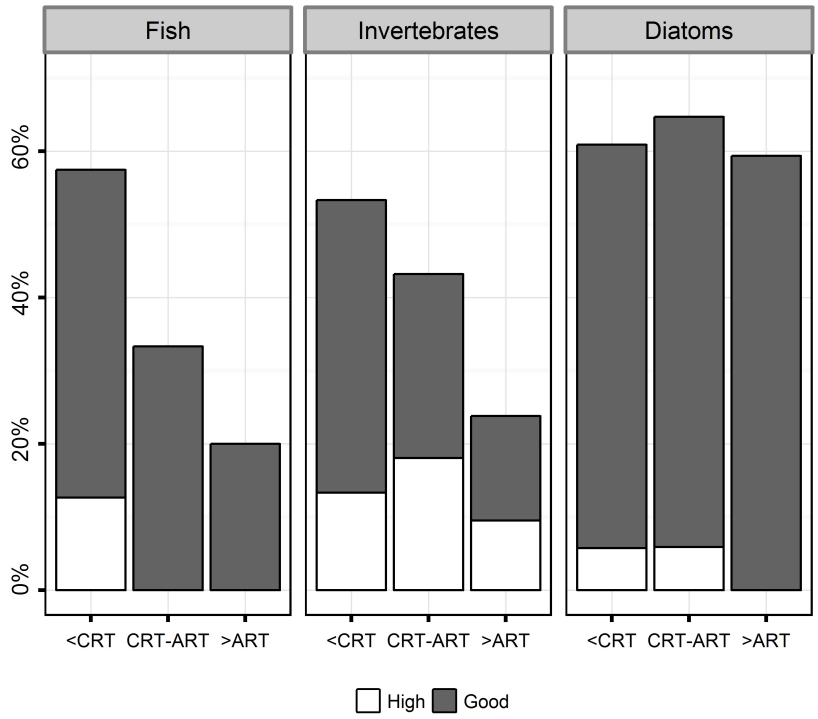

Figure 4.5: Proportion of sites in high and good ecological status for fish, invertebrates and diatoms. Sites were classified as acutely affected by chemicals (>ART), chronically affected by chemicals (CRT-ART) and not affected by chemicals (<CRT; Methods for details; Appendix C, Table C.7 for the number of sites).

Chemical and ecological data were matched on the basis of sampling years, because precise sampling dates were unavailable. Hence, the temporal lags between the chemical and the ecological samplings sites may have allowed for recovery, if effects occurred, which is especially relevant for diatoms that have reproduction times of few hours to days. A harmonisation of biological and chemical monitoring schemes would reduce the temporal and spatial bias in estimating ecological effects from chemicals.

Overall, we suggest that the decrease in ecological status for fish and invertebrates with increased chemical risk is an indication of water quality deterioration in aquatic ecosystems in response to chemicals.

4.4.6 Conclusions and prospective

Our study suggests that chemical pollution is a continental-scale problem and as such requires large-scale integrated solutions, which are not always provided by end-of-pipe

technologies. New frontiers in pollution prevention, such as designing chemicals according to the principles of green chemistry and substitution of hazardous chemicals preferably by nonchemical solutions, closed cycles of chemicals, specific treatment of unavoidable effluents at the source, innovative take-back systems from consumers, as well as new approaches in communication and education, should be promoted (Schwarzenbach et al., 2006; Kümmerer, 2007). Furthermore, considering that approximately 100,000 organic chemicals are currently in daily use and may enter freshwater ecosystems via different routes (Schwarzenbach et al., 2006), the success of mitigation measures obviously cannot be based on chemical monitoring of a limited set of target chemicals only, but requires a smart combination of stressor-specific indices, effect-based monitoring tools and chemical screening (Brack et al., 2009). Holistic basin-scale assessments (e.g., European Water Framework Directive (CEC, 2000)) and chemical regulations (e.g., Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH)(CEC, 2006)) are good starting points for addressing large spatial-scale pollution problems. However, more effort is necessary to integrate and advance these regulations towards the reduction of toxic pollution. Our study suggests that a paradigm change in chemical regulation and management is required to achieve a holistic approach, which assesses the toxic pressure as a whole rather than from individual chemicals, and complements specific case studies by large scale analyses.

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Chapter 5

How Much do Organic Toxicants Contribute to Multiple Stress in Freshwater Ecosystems?

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"The whole is more than the sum of its parts."
- Aristotle

[1]Freshwater Biology is ranked as the 3rd journal in Marine & Freshwater Biology research field. It has an impact factor of **3.93** as reported by the 2012 Journal Citation Reports (Thomson Reuters, 2013)

5.1 Abstract

Stream ecosystems are threatened by multiple stressors including habitat degradation, pollution and invasive species. Freshwater ecologists have largely disregarded the additional contribution of toxicants to multiple stress in streams, whereas ecotoxicologists have primarily examined ecological effects of toxicants in artificial systems. Hence, there is a paucity of studies on the relative importance of organic toxicants for the ecological status of streams and their co-occurrence with other stressors. We used monitoring data to analyse the individual and joint occurrence of four stressors, namely habitat degradation, invasive species, excessive nutrients and organic toxicants. All stressors were examined for potential ecological effects in German streams based on the exceedances of low and high risk thresholds. At approximately 80% of the sites nutrients and habitat degradation exceeded ecological thresholds, whereas in 40% of the sites the thresholds for invasive species and organic toxicants were exceeded. Almost all sites (96%) were subject to more than one stressor. Toxicity was weakly positively correlated with nutrients and habitat degradation ($0.27<$Spearman's $\rho<0.32$, all, $p<0.001$). The risks of ecological effects from toxicants, habitat degradation and invasive species increased with stream Strahler order. Nutrients and habitat degradation were more likely to affect lowland streams, whereas organic toxicants thresholds were more frequently exceeded in highland streams. Our assessment demonstrates that habitat degradation and nutrients are dominant stressors, although we likely underestimated the risk from organic toxicants and invasive species. Most sites are at risk from multiple stressors and mitigation focusing on individual stressors is unlikely to improve the ecological status. The risk of ecological effects from organic toxicants is prevalent and they may interact with other stressors in complex ways. Hence, integrating freshwater ecology and ecotoxicology is pivotal to tackle the challenge of multiple stressors.

5.2 Introduction

A multitude of stressors that are associated with anthropogenic land use or economic activities contribute to the ecological deterioration of freshwater ecosystems including habitat degradation, biological invasions and pollution with a wide range of substances (MEA, 2005; Dudgeon et al., 2006; Vörösmarty et al., 2010; Carpenter, Stanley & Vander Zanden, 2011). More than 50% of European river water bodies fail achieving ecological quality targets; in Germany and neighbouring countries almost all water bodies are degraded (EEA, 2012a). This situation has resulted in political frameworks that aim at improving the quality of freshwater ecosystems such as the European Water Framework Directive (WFD; CEC, 2000) and the Blueprint to Safeguard Europe's Water Resources (CEC, 2012). For example, the WFD requires all member states to achieve or preserve at least good ecological status (or good ecological potential) of their surface waters by 2015 or at the latest by 2027 in the case of reasoned exceptions (CEC, 2000). Targeted restoration requires knowledge on the relevance of stressors and their interactions. While the monitoring focus of the WFD is on "biological quality elements" (i.e., organism groups such as fish, benthic invertebrates, macrophytes and diatoms), which reflect river deterioration in general, the presence and strengths of stressors are regarded as supporting elements for monitoring.

Several studies assessed the relevance of different stressors for freshwater ecosystems. A global study evaluated the relevance of 23 stressors comprising (i) land use, (ii) water pollution (e.g. excessive nutrients and pesticides), (iii) water resource development (e.g. habitat degradation and water abstraction) and (iv) biotic factors (e.g., invasive species

and fishing) for freshwater biodiversity (Vörösmarty et al., 2010). Stressors associated with the groups of water pollution and water resource development were identified as major ecological threats (Vörösmarty et al., 2010). A study on 9,330 European sampling sites assessed the relative prevalence of water pollution and habitat degradation (Schinegger et al., 2012). Water pollution occurred most frequently in the sampling sites (59%) compared to habitat degradation in terms of hydrological pressures (41%), hydromorphological degradation (39%) and connectivity disruption (35%) (Schinegger et al., 2012). Finally, a study on 2,302 German and Austrian streams estimated the ecological effects from four stressors, which comprised hydromorphological degradation, water pollution, riparian and catchment land use (Dahm et al., 2013). Water pollution and catchment land use were identified as dominant drivers of potential ecological effects in invertebrate, fish and diatom communities. Overall, the three studies targeting the global, European and Central European scales all identified water pollution and habitat degradation as key drivers of ecological impairment.

Freshwater ecological studies have regarded water pollution mainly in the form of excessive nutrients, acidification or organic pollution (i.e., excessive organic matter), whereas organic toxicants, such as pesticides were rarely considered. In five major freshwater ecological journals, publications related to stressors focused dominantly on eutrophication, followed by climate change, invasive species and habitat degradation (Appendix D, Table D.1). Despite their widespread occurrence and potential ecological effects (Schwarzenbach et al., 2006; Beketov et al., 2013), the coverage of organic toxicants in these journals was negligible. Most studies on ecological effects of organic toxicants have been published in ecotoxicological journals. However, this "division of labor" between freshwater ecologists and ecotoxicologists has resulted in a blind spot: a paucity of studies on the field effects of toxicants. Beketov & Liess (2012) emphasised the legacy of laboratory orientation of ecotoxicology, with only 0.6% of studies on the effects of pesticides conducted under field conditions. Accordingly, Gessner (2014) highlighted the potential for fruitful exchange of concepts and methods between freshwater ecologists and ecotoxicologists. For instance, freshwater ecologists may benefit from ecotoxicological approaches related to ecoepidemiology (De Zwart et al., 2014) or to community change such as the pollution induced community tolerance concept (Blanck, Eriksson & Gamfeldt, 2014). Conversely, ecological insights on intraspecific and interspecific relationships that can moderate toxicant effects may strengthen the "eco" in ecotoxicology (Gessner, 2014). Importantly, toxicants may co-occur with other stressors in the field leading to potential interactive effects (Culp & Glozier, 2014; Alexander et al., 2014); models predicting riverine assemblage composition may therefore have a higher explanatory power when considering toxic substances along with other stressors. However, previous studies on the ecological relevance of toxicants have been restricted to a few sites (Belden et al., 2007; Schäfer et al., 2011) or compounds (De Zwart et al., 2006) and did not examine their association with other stressors (Malaj et al., 2014). Thus, there is a paucity of studies on the relative importance of toxicants for the multiple stress in freshwater ecosystems.

Here, we analyse the individual and joint occurrence of four stressors, namely habitat degradation, invasive species, excessive nutrients and organic toxicants above effect thresholds in German streams. We hypothesised that toxicants affect a similar percentage of water bodies as compared with the other stressors. Moreover, we hypothesised that toxicants (in particular pesticides) frequently co-occur with excessive nutrients and habitat degradation because of their joint association with agricultural land use.

Table 5.1: Data sources, measured parameters, temporal coverage and sample size n (for sampling points) for the stressors. See text for further details.

Stressor	Data source/provider	Measured parameters	Temporal coverage	n
Toxicity	EEA (2012[b])	Concentrations (μg/L) of up to 103 organic chemicals	2006-2010	173
Nutrients	German federal states[a]	Nitrate and phosphate concentrations (mg/L)	2006-2010[b]	4214
Invasive Species	German federal states[a]	Abundances of taxa	2006-2010	7381
Habitat degradation	LAWA (2000)	Hydromorphological index	2001	31616[c]
Stream typology and ecoregion	Pottgiesser et al. (2004)	Stream type and ecoregion	2004	-
Stream order	Guth (2011)[d]	Strahler order	2008	-

[a] Except for city states (Berlin, Hamburg, Bremen) and the smallest federal state Saarland
[b] For three states data from adjacent years was included to obtain a similar coverage
[c] Number of stream segments after snapping to stream network layer
[d] see also http://www.usna.edu/Users/oceano/pguth/srtm/hydrosheds_geomorph.htm

5.3 Methods

5.3.1 General rationale

We compiled data on habitat degradation, invasive species, nutrients and organic toxicants for German streams and rivers for the years 2006 to 2010 (Table 5.1). Based on these data, we evaluated the exceedance of stressor-related thresholds for low risk (LR) or high risk (HR) of ecological effects. Non-exceedances of stressor-related thresholds were classified as negligible risk (NR). In addition, the number of stressors above the thresholds per sampling site was examined. Finally, we checked the correlation between stressors, and their relationships with broadly defined ecoregions and stream orders.

5.3.2 Stream network including stream typology and ecoregion

A German stream network including information on stream typology and ecoregion sensu Illies (1978) was provided by Pottgiesser et al. (2004)(Table 5.1). The stream typology relies on characteristics such as ecoregion, altitude, catchment size and geology (Sandin & Verdonschot, 2006; Lorenz, Feld & Hering, 2004). For Germany, 24 stream types and subtypes have been established based on these characteristics and have largely been validated using macroinvertebrate data (Lorenz, Feld & Hering, 2004; Haase et al., 2004b). Approximately 2/3 of the stream types in our data set were small and mid-sized highland rivers, sand-dominated rivers or streams in riverine floodplains (Appendix D, Table D.2, cf. Figure D.1).

5.3.3 Stream order

We used the stream order layer associated with Guth (2011) (Table 5.1). This layer is based on the HydroSHEDS global hydrography data (Lehner, Verdis & Jarvis, 2008) and gives the Strahler order (Strahler, 1957) for basins with an area of at least 100 km^2. Almost 90% of the sampling sites were located at 1.-3. order streams, 9% at 4.-5. order streams and 2% at 6.-7. order streams.

5.3.4 Habitat degradation and risk thresholds

For assessing habitat degradation, we used hydromorphological index data for German streams provided by the Länderarbeitsgemeinschaft Wasser (LAWA, 2000; Gellert et al., 2014)(Table 5.1). The hydromorphological index results from a top-down approach based on geographic information system (GIS) data. It classifies stream segments (100 m - 1000 m) into seven categories ranging from 1 (pristine) to 7 (excessively impaired)(Appendix D, Figure D.1). We aggregated: categories 1, 2 (slightly modified) and 3 (moderately modified) into the NR class, 4 (clearly modified) and 5 (notably impaired) into the LR class and categories 6 (strongly impaired) and 7 into the HR class. Stream segments with missing hydromorphological index values were omitted from the analysis.

5.3.5 Invasive species and risk thresholds

To evaluate the threat imposed by invasive species, we acquired georeferenced governmental monitoring data on macroinvertebrates from the agencies of twelve German federal states for the years 2006 to 2010 (Table 5.1). The macroinverebrate monitoring was based on the multi-habitat sampling procedure (Haase et al., 2004a) and covered 7,381 sampling sites (Appendix D, Figure D.2). The taxa list for these sites was corrected for misspelled taxon names and duplicates. From this list, taxa absent in the German taxa list (Mauch et al., 2003; Mauch et al., 2011) or in the freshwaterecology.info database with respect to German ecoregions (Schmidt-Kloiber & Hering, 2012) were extracted. Subsequently, we used databases and literature to identify neozoans among these taxa (Appendix D, Table D.3). The risk of ecological effects from invasive species was evaluated using the invasion success model of Colautti & MacIsaac (2004). Briefly, they discriminate neozoans that are constrained by environmental, community or dispersal filters from widespread and dominant (as opposed to rare) neozoans. The latter are assigned invasion category 5 (Colautti & MacIsaac, 2004). We classified a neozoan as "widespread" if their geographical distribution exceeded 100 km in latitude and longitude (maximum distance of Germany: approximately 875 and 640 km, respectively), and as "dominant" if the species occurred in more than 1% (i.e., >73) of the sampling sites (Appendix D, Table D.3). Sites with category 5 neozoans (i.e., widespread and dominant) were considered as HR of ecological effects from invasive species, whereas sites with non-category 5 neozoans were assigned LR. Sites without neozoans were assigned as NR. Note that the reported results are insensitive to changes in the classification (i.e., 100 km and 1%), because several widespread and dominant neozoans occurred in most invaded sites (Appendix D, Table D.3). For instance, the most widespread and dominant neozoan *Potamopyrgus antipodarum* occurred in 64% of invaded sites.

5.3.6 Nutrients and risk thresholds

Data on nitrate and phosphate concentrations were obtained from the same twelve German federal states that are described above (Table 5.1) and comprised 4,214 sites (Appendix D, Figure D.3). The risk thresholds were based on environmental quality targets of the LAWA (1998; 2007). For nitrate, following LAWA (1998) the annual 90th quantile (Q90) for a sampling site was employed for classification: >2.5 and >10 mg/L NO3-N were assigned as LR and HR threshold corresponding to the exceedance of the class "moderate load" and "enhanced load" in the original classification, respectively. For phosphate, LAWA (2007) established stream type specific annual average values indicating the threshold between a good and moderate ecological status in terms of the WFD. These values were used as LR threshold. Accordingly, 0.07 mg/L o-PO4-P represented the LR threshold for all stream

types, except for stream types 2, 3, 11, 12 and 19 (0.1 mg/L) as well as for stream type 22 (0.2 mg/L) (see Appendix D, Table D.2 for details on stream types). Given that LAWA (2007) provided no threshold values for discriminating higher pollution levels, we set the HR threshold to 0.4 mg/L o-PO4-P for the annual Q90 of the concentrations following LAWA (1998). This value corresponded to the exceedance of the class "enhanced load" in the original classification. For both nitrate and phosphate, if less than five samples were available per year, we used the maximum value instead of the 90th quantile. For final site classification with respect to the ecological risks of nutrients, we used the maximum risk class of both nitrate and phosphate per sampling site, if data for multiple years were available. Sites with nitrate and phosphate below the LR threshold were assigned as NR.

5.3.7 Organic toxicants and risk thresholds

Georeferenced data on the concentrations of organic toxicants were available for 173 german sampling sites for the years 2006 to 2010 as part of the European Environment Agency (EEA) Waterbase database (EEA, 2012b; Table 5.1). Formatting of the dataset followed the methodology described in Malaj et al (2014). Between 1 and 103 organic toxicants were measured and between 1 and 86 chemicals were detected in the sampling sites (see Appendix D, Table D.4 for the compounds and Appendix C, Table C.1 for the toxicity values). The toxic unit (TU) indicator (Sprague, 1970; von der Ohe & de Zwart, 2013) was used to estimate the potential toxicity from chemical concentrations for the organism groups of invertebrates, fish and algae. Briefly, the TU for a chemical i was calculated as:

$$TU_{\mathrm{i}} = \frac{c_{\mathrm{i}}}{LC50_{\mathrm{i,j}}} \tag{5.1}$$

where c is the concentration of i and $\mathrm{LC50_{i,j}}$ is the median lethal concentration of i for a standard test species j. We employed three standard test organisms *Pimephales promelas*, *Daphnia magna*, and *Pseudokirchneriella subcapitata* as proxies for potential risks for fish, invertebrates, and algae, respectively (see Appendix D, Table D.4 for the compounds and Appendix C, Table C.1 for the toxicity values). This represents a standard procedure in the ecological risk assessment of chemicals (cf. Schäfer et al., 2011). Moreover, the calculation was done using the maximum and average annual concentration of a chemical in a site. Maximum concentrations were considered as indicative of short-term episodic exposures that may cause acute toxic effects, whereas average concentrations were considered indicative of long-term exposure that may result in chronic toxic effects. The thresholds for the maximum and average concentrations were taken from Malaj et al. (2014). The HR was equivalent to their acute risk threshold (ART) of 0.1 TU for the maximum annual concentration for invertebrates, fish and algae. For LR, we used the chronic risk threshold (CRT) for the average annual concentration. Malaj et al. (2014) adopted different CRTs for the organism groups based on a literature review. Thus, the LR was set to a TU of 0.001, 0.01 and 0.02 for invertebrates, fish and algae, respectively (see discussion in Malaj et al. (2014)). For final site classification regarding the ecological risks of organic toxicants, we used the maximum risk class per site.

5.3.8 Combination of data sets

All layers were converted to the coordinate reference system EPSG:31467 (DHDN/ 3-degree Gauss-Krüger zone 3). For joint analyses of stressors such as correlations, sampling points from different stressors within a 3 km distance were regarded as matching cases. Previous studies showed a reasonably high autocorrelation for biota and abiotic factors for

site pairs within a 6 km distance (Lloyd, Mac Nally & Lake, 2005; Peterson et al., 2006). Information on ecoregion and stream order were added to sampling points by snapping the points to the nearest stream segment of the stream network layer. All geographical information system operations were done in Quantum GIS (QGIS Development Team, 2014) and R (R Core Team, 2014).

Table 5.2: Pairwise Spearman correlation coefficient ρ (above diagonal), P-values and sample size n (both below diagonal) for the stressors. See text for stressor information.

	TU[a]	**Q90 NO3-N (mg/L)[b]**	**Q90o-PO4-P (mg/L)[b]**	**Invasion category**	**HM index[c]**
TU	-	0.27	0.32	-0.05	0.31
Q90 NO3-N (mg/L)	P=0.003, n=111	-	0.42	-0.07	0.1
Q90 o-PO4-P (mg/L)	P=<0.001, n=111	P<0.001, n=4214	-	0.05	0.14
Invasion category	P=0.62, n=116	P<0.001, n=3375	P=0.008, n=3375	-	0.12
HM index[c]	P<0.001, n=159	P<0.001, n=1623	P<0.001, n=1623	P<0.001, n=1322	-

[a] The toxic unit (TU) based on the maximum annual concentration was used in calculation, but the results would be similar for the TU based on the average annual concentration
[b] Q90=90th quantile
[c] HM=Hydromorphological

5.3.9 Data analysis

To assess the number of stressors that affect a sampling site, the three-class evaluation (NR, LR and HR) was dichotomised. Stressors occurring in the LR and HR class were considered as affecting a site, whereas stressors in the NR class were regarded as non-affecting a site. Subsequently, the percentage frequency distribution of the number of stressors per sampling site was calculated. The pairwise association between the stressors was calculated using Spearman's correlation coefficient ρ (see Table 5.2 for input data). The relationship of each stressors risk classes with ecoregions and stream orders was analysed using Multiple Correspondence Analysis (MCA) with adjusted inertias (Greenacre, 2007), which improve the measurement of goodness of fit. MCA is an eigenvalue-based ordination method for categorical data, and its interpretation follows that of principal component analysis. Individual categories of stream types or ecoregions with very low sample size, defined as <0% of the category with maximum sample size, were aggregated with adjacent categories (cf. Figure 5.3) or omitted from analysis to improve the visual accuracy in terms of quality scores of the ordination. For the same reason, we present the results for ecoregions rather than stream types (details and quality scores are given in the caption of Figure 5.3). All data analyses and graphics were done in R (R Core Team, 2014).

5.4 Results

5.4.1 Frequency of stressors in the sampling sites and joint occurrences

The majority (78-79%) of sampling sites was affected by nutrients and habitat degradation above the LR threshold (Figure 5.1). By contrast, for invasive species and organic toxicants approximately 60% of sites were at negligible risk of ecological effects. For invasive species, sites above the NR category were mainly classified as HR and consequently exceeded the

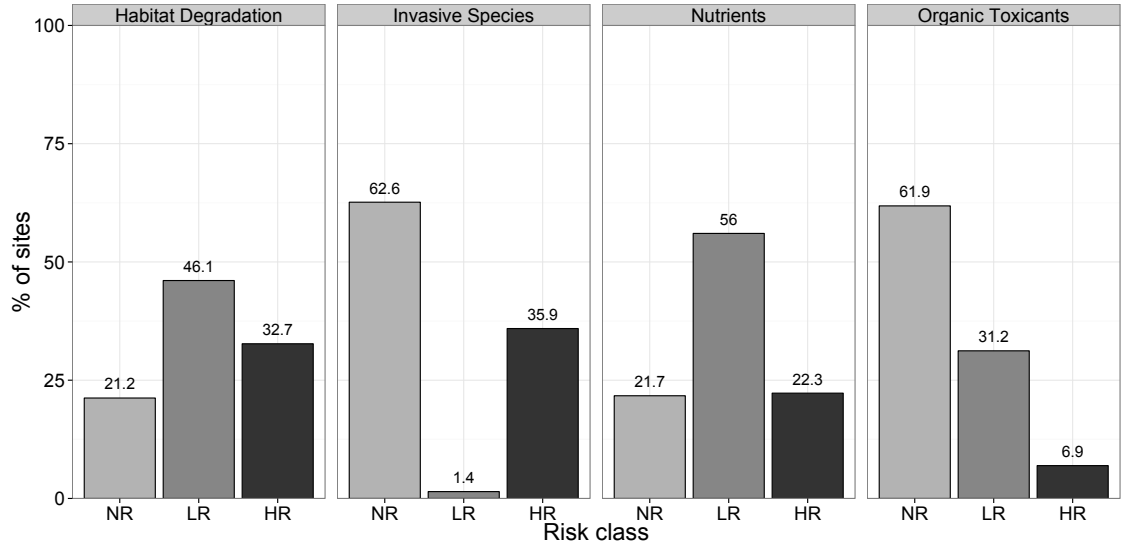

Figure 5.1: Threshold exceedances of the different stressors. Details on stressors and their thresholds are given in Table 5.1 and text.

HR threshold most frequently of all stressors (Figure 5.1). The herbicide diuron, the combustion product benzo[a]pyrene, the insecticide chloropyrifos and the antifouling agent tributyltin were primarily accountable for exceedances of toxicant thresholds (Appendix D, Table D.4). Only 1% of the sampling sites were not at risk of ecological effects from any of the four stressors (Figure 5.2). Most sites were at risk from three stressors and in 26% of the sites, all four stressors co-occurred above their LR threshold.

5.4.2 Association among stressors and with stream type and order

Phosphate and nitrate exhibited the highest pairwise correlation (ρ=0.42, p<0.001). Toxicity in terms of TU was significantly correlated with nutrients and the hydromorphological index, albeit the relationship was rather weak (0.27 <ρ<0.32, all, p<0.001). The remaining pairwise associations between the stressors were very weak (all, ρ<0.14, Table 5.2). The risk of ecological effects from habitat degradation and nutrients increased from independent stream types over highland streams to lowland streams (Figure 5.3A, C). The risk from invasive species followed a reverse order (Figure 5.3B). For toxicants, highland streams were at higher risk than lowland streams (Figure 5.3D). An increase in Strahler order was associated with increasing risks of ecological effects from habitat degradation, invasive species and toxicants (Figure 5.3A,B, D). For nutrients, no clear relationship with Strahler order was found (Figure 5.3C).

5.5 Discussion

5.5.1 Stressor occurrence and caveats

Habitat degradation and excessive nutrients were the dominant stressors with approximately 80% of sites at risk of ecological effects. Organic toxicants and invasive species occurred only in approximately 40% of sites above risk thresholds, though for invasive species this was primarily above HR. Hence, our hypothesis of a similar fraction of sites affected by organic toxicants was not supported with respect to habitat degradation and

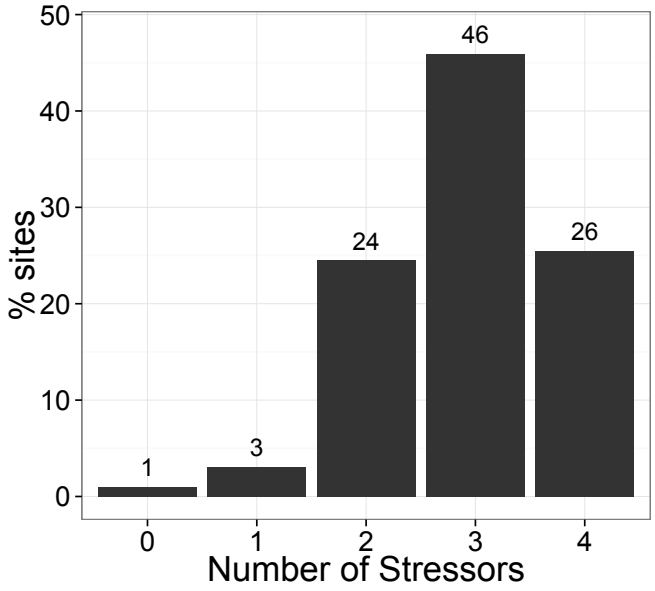

Figure 5.2: Percentage frequency distribution of the number of stressors per sampling site. Stressors exceeding the LR threshold were considered as occurring in a site. Details on stressors and their thresholds are given in Table 5.1 and text.

nutrients. The governmental monitoring data was biased towards impacted sites, which may result in a potential overestimation of all stressors (Dahm et al., 2013). However, other studies found a similar fraction of affected sites and identified habitat degradation and excessive nutrients as dominant stressors. On the European scale, a study focusing on fish-related stressors identified between 45 and 72% of small and large streams at risk of nutrients and habitat degradation in terms of hydromorphological degradation (Degerman et al., 2007). Toxicity, which was mainly measured as acidification, affected between 9 and 26% of the large and small streams, respectively. Another study using European monitoring data determined 60 to 100% of sites in German ecoregions (see Appendix D, Table D.2) as affected by habitat degradation and nutrients (Schinegger et al., 2012). An analysis employing a similar data set to ours, identified nutrients and other water quality variables as the most important predictors for several biotic metrics of fish, invertebrates and diatoms (Dahm et al., 2013). However, the above-mentioned analyses disregarded organic toxicants. A global analysis combining empirical and modeled data identified water pollution as a dominant stressor for freshwater biodiversity, on par with the stressor water resource development, which contained several drivers of habitat degradation (Vörösmarty et al., 2010). Among the nine drivers of water pollution, the nutrients phosphorus and nitrogens were ranked third and fourth after sedimentation and organic loading, whereas pesticides ranked sixth concerning contribution to stressor occurrence. Finally, the loss of fish species in 695 river sites in Ohio was mainly attributed to habitat quality and water quality in terms of pH, oxygen, hardness, suspended solids, heavy metals and ammonium, whereas modelled toxicity from heavy metals, ammonium and a few household chemicals were irrelevant in most sites (De Zwart et al., 2006). However, half of the variation in fish species loss remained unexplained and the authors conceded that organic toxicants, as well as nutrients, which were both excluded from analysis, might explain parts of this variation (De Zwart et al., 2006). The comparatively high proportion of sites affected by toxicants in our study may be attributed to a more comprehensive chemical data set and toxicity assessment (cf. Malaj et al. (2014)).

The risk of threshold exceedances increased with stream size in terms of Strahler order, except for nutrients where the pattern was ambiguous (Figure 5.3). Similarly, two studies on the European scale reported an increase in the fraction of affected sites with stream size for several pressures related to water quality and habitat degradation (Degerman et al., 2007; Schinegger et al., 2012). The increase of invasion risk with Strahler order matches with a study on 981 German sampling sites that found an increased invasion risks for larger, navigable waterways (Früh, Stoll & Haase, 2012). Interestingly, streams in the lowland ecoregion were at highest risk of effects from habitat degradation and excessive nutrients, whereas threshold exceedances related to toxicity were highest in the highlands ecoregion (Figure 5.3 and Table D.2 in Appendix D). The latter may be attributed to higher slopes, which are an important driver of surface runoff (Schriever & Liess, 2007). For nutrients, a modelling study identified groundwater and tile drainage as the main input paths in German streams (Hirt et al., 2012; Venohr et al., 2011). Given that the groundwater-surface water exchange is higher in regions with lower slopes, this may explain the higher threshold exceedances for nutrients in the lowland ecoregion.

We suggest that the stressors organic toxicants and invasive species were most likely underestimated in their threshold exceedances (Table 5.3). Regarding organic toxicants, only a small fraction of potentially ecotoxicologically relevant chemicals is included in monitoring programs (Schwarzenbach et al., 2006; Malaj et al., 2014). For instance, most chemicals responsible for exceedances of thresholds related to invertebrates were measured in <55% of sites (Appendix D, Table D.4). Moreover, monitoring programs rely mostly on monthly or quarterly grab sampling (cf. Appendix D, Table D.4) that has been shown to strongly underestimate the exposure and consequently threshold exceedances, especially in small streams (Stehle, Knabel & Schulz, 2013). However, this holds as well for the sampling of nutrients. Regarding invasive species, non-identification of neozoans is very likely in routine biomonitoring. Furthermore, our assessment of neozoans was restricted to invertebrates, whereas neozoans from the groups of fish or plants may also result in ecological changes (Hussner et al., 2010; Kornis et al., 2013).

Previous studies demonstrated that our thresholds are ecologically relevant. Dahm et al. (2013) reported change points of biotic metrics for diatoms in the range of 0.01 to 0.1 mg/L total PO4-P and of 2 to 5 mg/L NO3-N. These values conform with our LR and HR thresholds of 0.07 to 0.2 and 0.4 mg/L for o-PO4-P and 2.5 and 10 mg/L NO3-N, respectively. Similarly, the LR and HR thresholds for organic toxicants are in agreement with chronic and acute toxic effects determined in meta-analyses of field and artificial pond studies (Brock, Lahr & Van den Brink, 2000; Van Wijngaarden, Brock & Van Den Brink, 2005; Schäfer et al., 2012), though uncertainties remain concerning the LR threshold (Brock, 2013; see discussion in Malaj et al. (2014)). Regarding habitat degradation, the LR threshold agreed with a study of Lorenz et al. (2004). Finally, several of the most widespread and dominant neozoans such as *Potamopyrgus antipodarum*, *Dikerogammarus villosus* and *Dreissena polymorpha* (Appendix D, Table D.3) have been reported to alter ecosystem structure and functioning (Gergs, Grey & Rothhaupt, 2011; Alonso & Castro-Díez, 2012; MacNeil et al., 2013; Riel et al., 2006). Thus, exceedance of the invasive species HR threshold corresponds to the risk of ecological effects.

5.5.2 Relevance of stressors and management of multiple stress

The ecological relevance of threshold exceedances depends strongly on several other factors such as landscape context (Kail & Hering, 2009), environmental conditions including co-occurring stressors (Liess & Beketov, 2011; Culp & Glozier, 2014; Alexander et al., 2014) and disturbance history (Landis, Matthews & Matthews, 1996; Harding et al., 1998). Con-

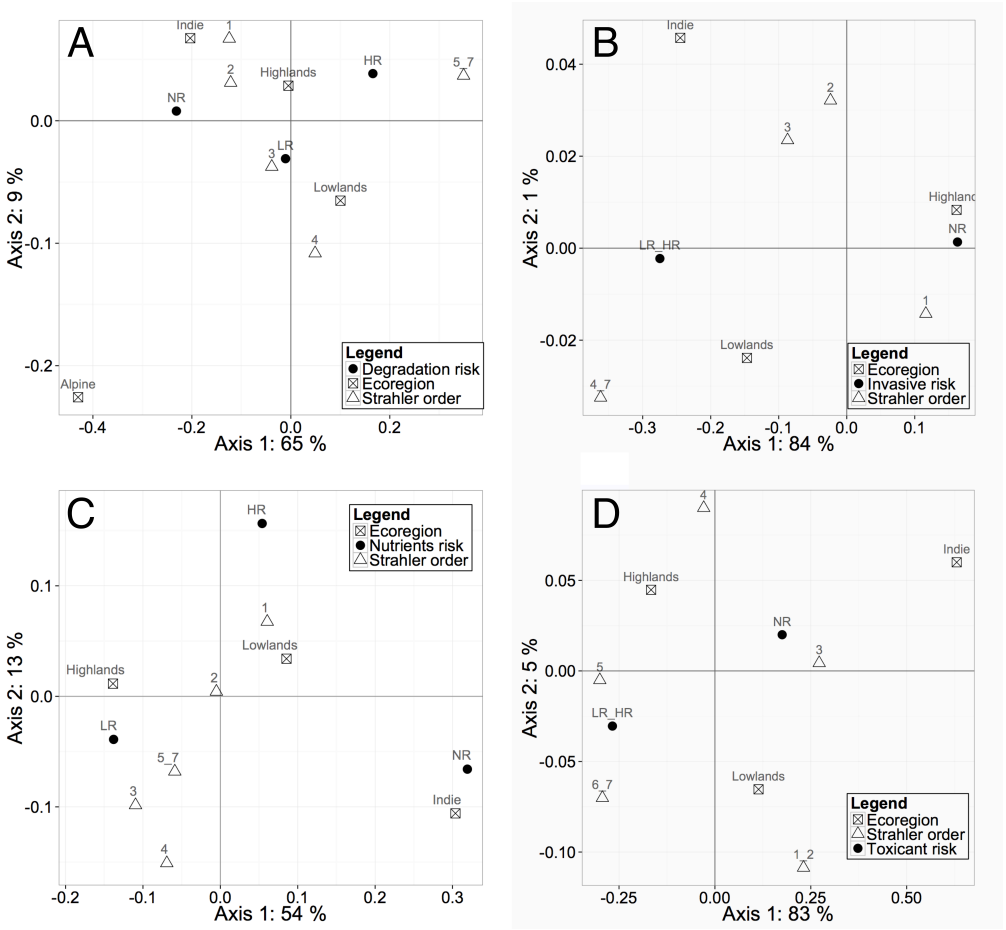

Figure 5.3: Multiple correspondence analysis of risk classes, ecoregion and stream order for (A) habitat degradation, (B) invasive species, (C) nutrients and (D) organic toxicants. Axis labels give the adjusted inertia (Greenacre, 2007). Underscores between plot labels indicate aggregation of categories. The quality scores, which indicate the visualisation accuracy, for the two-dimensional ordination (see Glynn (2014) for details) exceeded: (A) 53.7% except for Strahler order 3 with 39%, (B) 80.9% except for Strahler order 2 with 58.6%, (C) 46.9% except for Strahler order 2 and 5 to 7 with 2.2 and 14%, respectively and (D) 76.7% except for Strahler order 4 with 43.6%. For categories with a quality score less than 50%, the ordination point may not accurately reflect the relation to other features and the category may exhibit strong variation (Glynn, 2014).

sequently, risk threshold exceedances should be interpreted as potential, and not actual, ecological effects. More importantly, the ecological relevance of threshold exceedances depends on the pressure type and reversibility of potential effects (Table 5.3).

Invasive species and habitat degradation represent ramp or press disturbances, i.e., the related disturbance increases or remains constant through time. Thus, affected streams usually show no recovery as long as the stressor persists (Niemi et al., 1990). The magnitude of ecological effects from habitat degradation depends on the degree of habitat change, whereas the effects from invasive species depend on species identity and may greatly differ even in the HR class. While the assemblages of large rivers (e.g., the Rhine) are almost entirely composed of invasive species, smaller streams might be a habitat for some neozoans but still the fauna will be mainly composed of native species. Thus, effects from invasive species can be minor or even positive for biodiversity (Davis et al., 2011). The effects of toxicants and nutrients depend on their concentration and they occur as pulse

or press disturbance (Table 5.3). In case of pulses, affected sites usually recover within a few years, even if parts of the community were eradicated (Whiles & Wallace, 1995; Niemi et al., 1990; Zwick, 1992). However, the reversibility of effects from nutrients and pesticides depends also on the landscape context e.g., whether recolonisation pools exist (Schäfer et al., 2012). Similarly, recolonisation pools have been identified as prerequisite for the success of habitat restoration projects (Sundermann, Stoll & Haase, 2011; Haase et al., 2012).

Table 5.3: Characteristics of the stressors.

Stressor	Data uncertainty	Pressure type[a]	Reversibility	Driver of effects
Toxicity	high	Pulse or Press	high	Chemical concentration
Nutrients	low	Pulse or Press	high	Chemical concentration
Invasive Species	medium	Ramp or Press	low	Species identity
Habitat degradation	low	Press	low to medium	Degree of habitat change

[a] sensu Lake (2000)

From the perspective of freshwater management, mitigation of nutrient or pesticide inputs from diffuse sources (e.g., agricultural surface runoff) may be more cost-effective than addressing point sources (e.g., waste water treatment plants) or habitat restoration. Mitigation options include riparian buffer strips or vegetated treatment systems for diffuse sources and advanced waste water treatment for point sources (Reichenberger et al., 2007; Bechmann et al., 2008), which at the same time may also enhance habitat quality, sediment input and water temperature. However, 96% of sites in our study were subject to multiple stressors (Figure 5.2). This result agrees with a study on the two stressors water pollution and habitat degradation in European streams that found co-occurrence in 50 to 90% of sites (Schinegger et al., 2012). In accordance with our hypothesis, toxicity exhibited a statistically significant, albeit rather weak, correlation with nutrients and hydromorphological degradation. The correlation may be partly due to the large agricultural area in Germany (*ca.* 40%), which is a source of agrochemicals including fertiliser and pesticides. In the study of Vörösmarty et al. (2010), the pairwise association between pesticide toxicity and nutrient input was one of the strongest among the 23 stressors considered. In 1,724 French sampling sites, risk classes for phosphate and nitrate as well as for nitrogen and habitat degradation exhibited the strongest correlations among 16 stressors (Mondy & Usseglio-Polatera, 2013). By contrast, the correlation of the risk classes for organic toxicants such as pesticides and polycyclic aromatic hydrocarbons with the risk classes for nutrients or habitat degradation was low (Mondy & Usseglio-Polatera, 2013). This may be attributed to the use of concentrations instead of toxicity (e.g., TU) for risk classification, as well as of risk categories instead of raw data in correlation analysis. Overall, our results provide further evidence that multiple stressor occurrence is the norm rather than the exception. The management implication is that mitigation of all stressors that affect a site may be required to improve its ecological status. For example, the multiple stressor situation may have contributed to the failure of restoration projects that were only focused on improving habitat quality (Haase et al., 2012; Wahl, Neils & Hooper, 2013). Moreover, interactions between stressors may exacerbate the effects of individual stressors and result in unanticipated ecological effects (Shears & Ross, 2010; Townsend, Uhlmann & Matthaei, 2008). Organic toxicants have been shown to interact with habitat degradation (Rasmussen et al., 2012), nutrients (Alexander et al., 2014) and a wide range of other environmental factors (Laskowski et al., 2010). Hence, this multiple stressor

context calls for an inclusion of toxicants in freshwater ecological studies and a stronger field-orientation in ecotoxicological studies. To tackle the challenge of multiple stressors, a stronger cooperation between freshwater ecologists and ecotoxicologists is needed and other contributions to this Special Issue provide further insights on this topic (e.g., De Zwart et al., 2014; Blanck, Eriksson & Gamfeldt, 2014; Gessner, 2014).

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Chapter 6

General Discussion

6.1 Tools in ecotoxicology

Toxicity data were employed to develop sensitivity rankings for heavy metals and were subsequently used as input data to build and to validate prediction models for invertebrate species. Note that development (Chapter 2) and modeling (Chapter 3) of species sensitivities were done for heavy metals only. Organic compounds have already well established (i) sensitivity rankings (von der Ohe et al., 2005), which are currently used in trait-based risk assessment (e.g., SPEAR index; Liess & Von Der Ohe, 2005; Schäfer et al., 2011a) and (ii) phylogenetic and bilinear models for the prediction of the sensitivity of species to organic compounds (Guénard et al., 2011; Guénard et al., 2014). Therefore, similar analyses - which were missing to date for heavy metals - are provided in Chapter 2 and Chapter 3.

6.1.1 Sensitivity to heavy metals: Applications and limitations

Toxicity data for various chemical groups (e.g., heavy metals or organic compounds) are useful for ranking the sensitivity of species. In Chapter 2, we describe the sensitivity of species with regard to seven heavy metals. In contrast to other rankings provided so far (Wogram & Liess, 2001; von der Ohe et al., 2005), we present an un-biased standardization method which removes the dependency from the highly criticized (e.g., Rubach, Baird & Van den Brink, 2010) standardization method with *D.magna* (cf. Wogram & Liess, 2001; von der Ohe et al., 2005). In contrast to other studies that used toxicity data from different laboratory conditions (Wogram & Liess, 2001; von der Ohe et al., 2005; Rubach, Baird & Van den Brink, 2010), in this study, toxicity values were normalized to avoid bias in the comparison of species. Ideally, the biotic ligand models (BLM) that predict the bioavailable fraction in water samples should be used (Santore et al., 2001; Di Toro et al., 2001; Paquin et al., 2002; de Schamphelaere & Janssen, 2004). However, BLM models were not applicable here because they: (i) require a multitude of input parameters (pH, hardness, alkalinity, dissolved organic carbon, etc) which were not available, (ii) are generated only for standard test species, and (iii) are developed only for a few metals (Cd, Cu, and Ni).

The individual heavy metal rankings were not significantly different; therefore an aggregated sensitivity ranking is provided for potential application in trait-based risk assessments. By using the physiological sensitivity and other biological traits (e.g., generation time and size) of species, a trait based index (SPEAR) has been used to link the exposure of organic toxicants to effects in macroinvertebrate communities in streams of Europe, Siberia and Australia (Liess & Von Der Ohe, 2005; Beketov & Liess, 2008; von der Ohe et al., 2009; Beketov et al., 2009; Schäfer et al., 2011a). A similar approach is missing for heavy metals. Adapting the SPEAR approach for heavy metals may be more challenging than for organic compounds, because of the large discrepancies in the sensitivities between

laboratory and field conditions, which are frequently reported for metals (Luoma & Rainbow, 2005; Buchwalter et al., 2007; Brix, DeForest & Adams, 2011; Clements, Cadmus & Brinkman, 2013; Poteat & Buchwalter, 2013). For instance, aquatic insects respond to dissolved metals in the laboratory at concentrations orders of magnitude higher than the concentrations found in highly metal-contaminated sites, where insect communities were depleted (Poteat & Buchwalter, 2013; Clements, Cadmus & Brinkman, 2013). There are two important factors related to these discrepancies: first, short-term exposure in laboratory conditions limits metal accumulation (Buchwalter et al., 2007; Brix, DeForest & Adams, 2011; Clements, Cadmus & Brinkman, 2013). For instance, it was estimated that the median time to steady state concentration from dissolved Cd exposure was 405 days for Ephemeroptera, 70 days for Plecoptera and 50 days for Trichoptera (Poteat & Buchwalter, 2013). Therefore, these exposure durations exceed by far the duration of the acute toxicity tests (e.g., 24- to 72-h). Second, diet is the predominant route of exposure, which has a stronger influence on metal toxicity than the dissolved exposure via the water phase (Xie & Buchwalter, 2011; Brix, DeForest & Adams, 2011; Poteat & Buchwalter, 2013). For instance, Xie & Buchwalter (2011) argued that dietary Cd exposure is potentially more toxic due to its influence on the antioxidant enzymes, which was not observed for the dissolved Cd exposure route. Considering that physiological sensitivity derived from laboratory conditions is the backbone of the SPEAR approach, reconciling the differences between the sensitivity of metals in the laboratory and in the field will be crucial for developing SPEAR approaches for metals.

6.1.2 Evolutionary patterns explain sensitivity to heavy metals

The toxicity data derived from Chapter 2 were used as input data to build and validate bilinear models for heavy metals. Using species for which toxicity values were available, we were able to successfully predict the toxicity of untested species. Models improved substantially when both the phylogenetic information of the species and physicochemical properties of the heavy metals were included. Several studies have attempted to integrate the phylogenetic information when answering ecotoxicological questions (Buchwalter et al., 2008; Carew, Miller & Hoffmann, 2011; Hammond et al., 2012; Larras et al., 2014; Poteat et al., 2013; Poteat & Buchwalter, 2014), however, phylogenetic modeling remains a largely unexplored field in ecotoxicology (Guénard et al., 2011). Chapter 3 provides an example of how evolutionary concepts can be applied in ecotoxicology. To increase the level of prediction, other relevant variables which explain the sensitivity of species can be included in the model, such as (i) species traits (e.g., morphological or life history traits; Baird & Van den Brink, 2007; Rubach, Baird & Van den Brink, 2010) and/or (ii) different modes of action (e.g., Guénard et al., 2014). When more molecular and toxicological data become available, these methods could expand to other (i) organism groups (e.g., fish, amphibians, or diatoms), (ii) chemicals (e.g., include all the metals), (iii) endpoints, e.g., predict chronic toxicity, and (iv) improve precision of the predictions as the phylogenetic tree would have less misclassified species. Considering the fast development of the genetic datasets, such as Genbank, which stores nucleotide sequences for >100,000 distinct species (Benson et al., 2010), and of ecotoxicological datasets, such as ECOTOX (USEPA, 2007), phylogenetic and/or bilinear models represent a promising tool for future research and application in ecotoxicology.

6.2 Likelihood of effects from organic chemicals

In Chapter 4, a risk assessment of organic compounds is presented on a large spatial scale encompassing 4,000 sites in 91 European river basins. The results were striking: acute effects were likely for at least one in seven sites, whereas chronic effects were likely for almost half of the sites. This analysis demonstrates that despite the regulatory efforts and developments in ecotoxicology, chemical pollution remains a large-scale stressor of freshwater ecosystems and pesticides are the main contributors to this risk. This analysis represents the first large-scale risk assessment, and provides a framework for holistic assessments of chemicals on the continental scale.

6.2.1 Effect thresholds

A crucial point of the chemical risk assessment presented in Chapter 4 is the establishment of threshold levels. As mentioned above, the threshold concentration is the acceptable level at which the concentration of a compound is assumed to have no or only slightly adverse effects on the communities (Brock et al., 2006). However, community studies reporting effect thresholds of chemicals are limited (Brock, Lahr & Brink, 2000; Van Wijngaarden, Brock & Van den Brink, 2005; Schäfer et al., 2012). The literature research conducted for this analysis (see Appendix C, Table C.3) revealed that chemicals were likely to exert adverse effects on fish (41% of the cases), invertebrate (71% of the cases) and algal (51% of the cases) communities at 1/10 of the LC50 value. For chronic threshold levels (i.e., 1/1,000 of the LC50 value), there is only one meta-analysis for invertebrates, for which there was a likelihood of effects for 71% of the affected cases (Schäfer et al., 2012). This result was confirmed by a recent study, which found a reduction in the species richness at 1/1,000 of the LC50 values (Beketov et al., 2013). However, similar meta-analyses are missing for algal and fish communities, therefore, empirical approaches (e.g., acute-to-chronic ratios; Ahlers et al., 2006) were applied instead, which established relatively high effect thresholds. Therefore, it is likely that the chronic effects for these communities are underestimated. The future establishment of cause-effect relationships in field/artificial test communities could elucidate more ecologically meaningful threshold levels.

6.2.2 How much complexity is feasible?

In risk assessment, increasing the complexity of the data would increase the ecological realism. However, such analysis is not always possible especially for large-scale assessments with >200 compounds available. Therefore, approaches such as SSD, which are species and compound-rich, would be difficult to perform. For instance, a recent study found that 70% of the compounds used (72 out of 103 compounds) had toxicity data for less than six species available (Schäfer et al., 2013a). In fact, the majority of the toxicological data used for SSDs are biased towards standard test species (Calow & Forbes, 2003). Additionally, acute toxicity data were not always available, even for the most common chemicals found in the water samples (e.g., pesticides) and for the standard test species, hence, necessitating prediction models to fill the gaps in toxicity data (Chapter 4). Due to the limited availability of toxicity data, it is challenging to perform large-scale risk assessments for higher tiers. The most feasible alternative, with the current knowledge, is to employ a set of representative species and apply effect thresholds which are validated for higher tiers (e.g., field analysis or artificial test systems), as was done in Chapter 4.

6.2.3 Minimum Risk from Chemicals

To arrive at a minimum risk, this analysis under- rather than overestimates the risk. Uncertainties, discussed in Chapter 4, were related to the quality of monitoring data (the number and identity of compounds analyzed), availability of toxicological data, the ecotoxicological relevance of the compounds analyzed, or the timing of sampling. Future studies should focus on integrating and/or quantifying these uncertainties for a more accurate risk estimation.

We suggest that the only potential overestimation of the risk to organic compounds might be related to the bioavailability of compounds. Typically, the concentration reported by monitoring agencies refers to the unfiltered water samples, which is driven by regulatory frameworks that recommend measuring total concentrations in water samples (CEC, 2000). An unavailable fraction of the chemical contractions can be measured when analyzing total concentrations, due to the adsorption or binding of the compounds to the particulate matter of the water phase. To avoid this discrepancy, total concentrations can be normalized, assuming that the water phase and the carbon in the solid phase of the particles are in equilibrium and that the concentrations are related to a partition coefficient (e.g., K_{oc}; Di Toro et al., 1991; Schäfer et al., 2011b). In Chapter 4, it was not possible to account for the bioavailability due to the lack of information on the organic carbon in the water samples. However, when considering all the underestimations of the chemical risk, it is unlikely that the bioavailability would decrease the real, overall risk from organic chemicals.

Metal compounds were not considered in the chemical risk assessment analysis, due to high uncertainties related to their chemical and toxicological concentrations. Total metal concentrations are unrelated to the toxicological effects because of the processes that modify the availability of the metals rendering them unavailable for uptake (CEC, 2011). Moreover, only a small fraction of the metal concentrations is found in the water phase, as they are mainly bound to particles or precipitate in the sediment (Malaj et al., 2012). As mentioned above, correcting with the BLM model was not feasible due to data limitations. Although, the metal bioavailability issue is largely acknowledged in the scientific literature (Di Toro et al., 2001; Paquin et al., 2002; de Schamphelaere & Janssen, 2004), and in the regulatory context (CEC, 2000), no harmonized method exists for the quantification of bioavailable concentrations (Schmidt et al., 2010). Furthermore, because this analysis relies primarily on acute toxicity data, the large discrepancies between laboratory and field data for metals would have likely misled the risk estimation from metals. Overall, (i) developments of BLM models which would reliably account for the bioavailable fraction, (ii) the availability of chemical parameters alongside with dissolved metal concentrations, and ideally (iii) the integration of ecological and physiological mechanisms for long-term metal exposures would allow a proper estimation of the likelihood of effects with respect to metals.

6.2.4 Ecological status of freshwater

In Chapter 4, it was hypothesized that chemical stress has reduced the ecological status of rivers. Despite the recent efforts to store the ecological status data in a centralized dataset (e.g., Waterbase-Biology; EEA (2012)), this information is generally lacking on the continental scale. Therefore, this hypothesis was tested only on a limited number of representative sites (5% of the total number of sites; see Chapter 4 for details). We noticed a decrease in the ecological status for invertebrates and fish for sites with high chemical risks, but this trend was not prevalent for diatoms. Both the fish and the invertebrate

indices were developed as the result of multimetric approaches (I_2M_2 for invertebrates (Mondy et al., 2012) and IPR+ for fish (Marzin et al., 2014)), which are likely to respond to chemical stress better than the single stressor indices (e.g., Indice Biologique Global Normalise (IBDN) method in France (Mondy et al., 2012)). In general, macroinvertebrates would respond to a series of stressors including organic pollution, whereas primary producers are mainly used for assessing nutrient enrichment, and fish are used as indicators of habitat degradation (Hering et al., 2006). Therefore, macoinvertebrates might be more suitable to represent chemical stress, as was the case in our analysis. However, we only had access to the ecological status data which might not necessarily be informative for the chemical risk. Ideally, access to the "raw" biological data (taxonomic composition and abundance of the BQEs communities) would help to elucidate the effects of chemicals in the field, e.g., by using stressor-specific indices such as SPEAR. However, "raw" biological data are scarce and logistically and legally difficult to collect. For instance, compilation of macroinvertebrate monitoring data for different German federal states took about one year, with one federal state refusing to deliver data (Schäfer et al., 2013b). Availability of this type of data would allow the estimation of effects in the field rather than only predicting the risk of effects as presented in this analysis.

6.2.5 Multiple stressors in freshwater

Apart from the quantification of the chemical risk on large spatial scales, to successfully implement integrated river basin strategies, it is necessary to investigate the effects of chemical pollution under multiple stressor conditions. A regional analysis on the relative importance of organic chemicals is presented in Chapter 5, which demonstrates that almost all sites (96%) were influenced by more than one stressor. This analysis was regional (only Germany) due to the lack of data on multiple stressors on the continental scale. The hypothesis that chemical pollution was among the dominant stressors was not confirmed, as we found that pressures such as habitat degradation and nutrients were more important for the likelihood of ecological effects in the environment. As discussed in Chapter 4, chemical risk was likely underestimated, mainly because only a small fraction of ecotoxicologically relevant compounds were included in the assessment. Furthermore, the combined effects of stressors cannot always be estimated as the sum of the single-stressors, because the interactions of stressors do not always produce additive effects (Vinebrooke et al., 2004). For instance stressors can be synergistic or antagonistic, when their combined effects are respectively larger or smaller than the sum or the product of their individual effects (Folt et al., 1999). An example are nutrients and toxic chemicals that typically co-occur in the environment and the effects from their interactions cannot be predicted based on single stressors (Skei et al., 2000; Roessink, Koelmans & Brock, 2008). Studies have demonstrated that an increase in nutrient levels in the presence of toxicants would: (i) increase the biomass, therefore, it will dilute toxicant levels, which will lower the internal exposure, (ii) increase organic matter, therefore, it will increase the partitioning of chemicals to dissolved organic carbon, which will reduce the chemical exposure, and (iii) increase the flow of dead organic matter to the sediment, therefore, it will increase the sedimentation of toxicants (Skei et al., 2000; Roessink, Koelmans & Brock, 2008). On the other hand, a decrease in nutrient levels (less phytoplankton) can lead to more sensitive populations, e.g., Daphnia populations were 2-3 times more sensitive to insecticides under low food conditions (Pieters et al., 2005; Skei et al., 2000; Roessink, Koelmans & Brock, 2008). Overall, there is an urgent need to develop an understanding of the interactive effects of stressors on ecosystems, which will in turn, allow the estimation of the effects from multiple stressors.

6.3 Future challenges

As highlighted by Vörösmarty et al. (2010), to test the degree to which water resources are impacted by stressors, it is necessary to develop a spatial picture of the exposure to different stressors. Large-scale spatial analyses are crucial not only to identify chemical hot-spots, but also to direct the protection and rehabilitation efforts for aquatic ecosystems as a whole. These analyses would greatly benefit from long-term monitoring studies; however, this information has only recently become publicly available (e.g., Waterbase (EEA, 2012)). Apart from quantifying chemical risk, temporal trends would allow to make future predictions of the chemical loads (cf. Schriever & Liess, 2007). Unfortunately, monitoring data in Europe are highly biased towards an *a-priori* selection of chemicals. Special attention should be given to chemicals (and their metabolites) different from priority pollutants or commonly measured compounds (e.g., pesticides). For instance, emerging pollutants, such as pharmaceuticals are potentially harmful to ecosystems, because they were designed to have biological effects at low concentrations (Arnold et al., 2013). Furthermore, developments in metal bioavailability would allow to reliably estimate the risk from metals. Perhaps one of the most urgent needs is the availability of "raw" biological data, which would enable the calculation of stressor-specific metrics. This in turn will allow the establishment of cause-effect relationships in field conditions which will help to quantify the ecological effects from chemicals. In order to successfully apply integrated water management strategies under the WFD, a framework involving multiple environmental stressors is deemed necessary. To address the multiple stressor situation from the ecotoxicological perspective, the combined knowledge from a multitude of fields, such as chemistry, ecotoxicology, and ecology, and a multiscale approach, from laboratory to the field, is required (Guasch et al., 2012). In fact, including interdisciplinary concepts is a general requirement for ecotoxicology. The coupling of genetic information with chemical and toxicological data is an example of how a field such as evolutionary biology can inform ecotoxicology. As our analytical and molecular techniques advance, we will presumably become more proficient in integrating methods from different fields with the aim of assessing the health of aquatic ecosystems.

To reduce the potential effect of chemical pollution on ecosystems, precautionary policies (i.e., acting on an early indication of the harm) are necessary. Prevention mechanisms are very effective as they give producers incentives to develop products according to the principles of green chemistry or life cycle analysis, by either producing safer products or by ensuring a less wasteful chemical process, respectively. As a result the damage to the environment would decrease, while optimized technologies might even result in reduced costs (Fahrenkamp-Uppenbrink, 2002; Domènech et al., 2002; Schwarzman & Wilson, 2009). It is worth noting that despite their impacts on the environment, the development of chemicals have profoundly improved, prolonged and changed human life. For instance, only by producing greater yield per unit of land, modern agriculture has (i) averted shortfalls in food supply, (ii) improved nutrition (therefore the ability of people to reach their mental and physical potential), and (iii) spared land by reducing conversion to agriculture land (Tilman et al., 2002). However, with the world's population expected to increase to nine billion and the global chemical production projected to double over the coming decades (Wilson & Schwarzman, 2009), the reconciliation between providing for a rapidly growing population and protecting the environment for future generations is one of the major challenges of our century.

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Appendix A

Supplementary information for: Physiological Sensitivity of Freshwater Macroinvertebrates to Heavy Metals

A.1 Supplementary Methods

A.1.1 Normalization of the toxicity data

Normalization ratios corresponding to the value of 1 stand for normalization at the lowest level of 24 hours for exposure time, 25 mg/L $CaCO_3$ for hardness, and 10°C for temperature. This means that for all the toxic concentrations at the lowest normalization point no change in the above mentioned parameters is expected. Our aim was to normalize at standard test condition, where most of the data in the database was found. Thus, normalization was made at exposure time of 48 hours (Figure A.1B) corresponding to Rt(48) (0.50), at hardness of 50 mg/L $CaCO_3$ (Figure A.1A) corresponding to RH(50) (0.58), and at temperature of 20°C (Figure A.1C) corresponding to RT(20) (0.33). The normalization constants for exposure time ($\mathrm{Norm_t}$), hardness ($\mathrm{Norm_H}$) and temperature ($\mathrm{Norm_T}$) are presented in equation A.1, A.2 and A.3 respectively:

$$Norm_{\mathrm{t}} = \frac{t^{\text{-}1}}{Rt(48)} \tag{A.1}$$

where t is exposure time and Rt(48) is the ratio for time at 48 hours.

$$Norm_{\mathrm{H}} = \frac{12.52 \times H^{\text{-}0.79}}{RH(50)} \tag{A.2}$$

where H is hardness and RH(50) is the ratio for hardness at 50 mg/L $CaCO_3$.

$$Norm_{\mathrm{T}} = \frac{3e^{\text{-}(0.11\mathrm{xT})}}{RT(20)} \tag{A.3}$$

where T is temperature and RT(20) is the ratio for temperature at 20°C.

Steps for normalization of log LC50 values ($\mathrm{logLC50_{norm}}$) deriving the final equation A.4 by substituting equation A.1, A.2, and A.3:

$$\log LC50_{\mathrm{norm}} = \frac{LC50_{\mathrm{org}}}{Norm_{\mathrm{t}} \times Norm_{\mathrm{H}} \times Norm_{\mathrm{T}}} \tag{A.4}$$

$$= (logLC50_{\mathrm{org}}) \times (\frac{t}{0.50}) \times (\frac{0.58}{12.52 \times H^{\text{-}0.79}}) \times (\frac{0.33}{3e^{\text{-}(0.11\mathrm{xT})}}) \tag{A.5}$$

where $\mathrm{LC50_{org}}$ are the original LC50 values.

A.2 Supplementary Figures

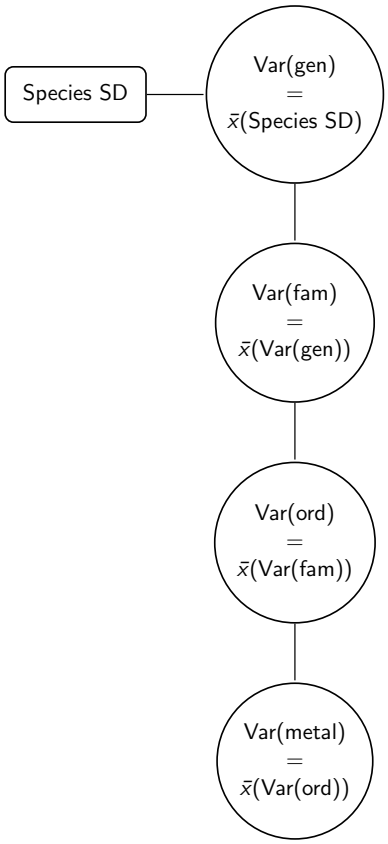

Figure A.2: Steps for calculating heavy metal variance.

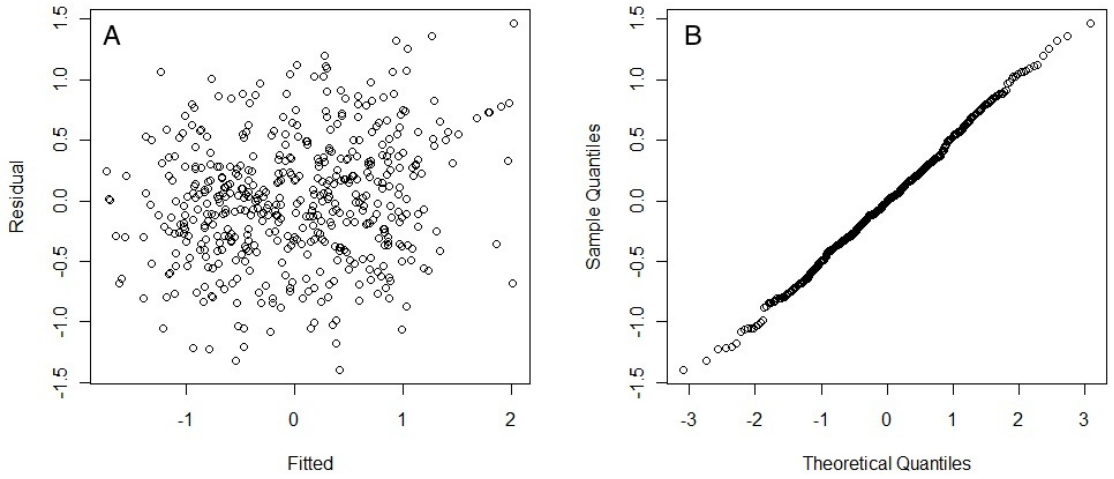

Figure A.3: Diagnostic plots for the linear mixed model. Residuals based on REML fit are plotted against fitted values for the explanatory variables (heavy metals) to check for homogeneity (A) and qqplots are used to check for normal distribution of residuals (B).

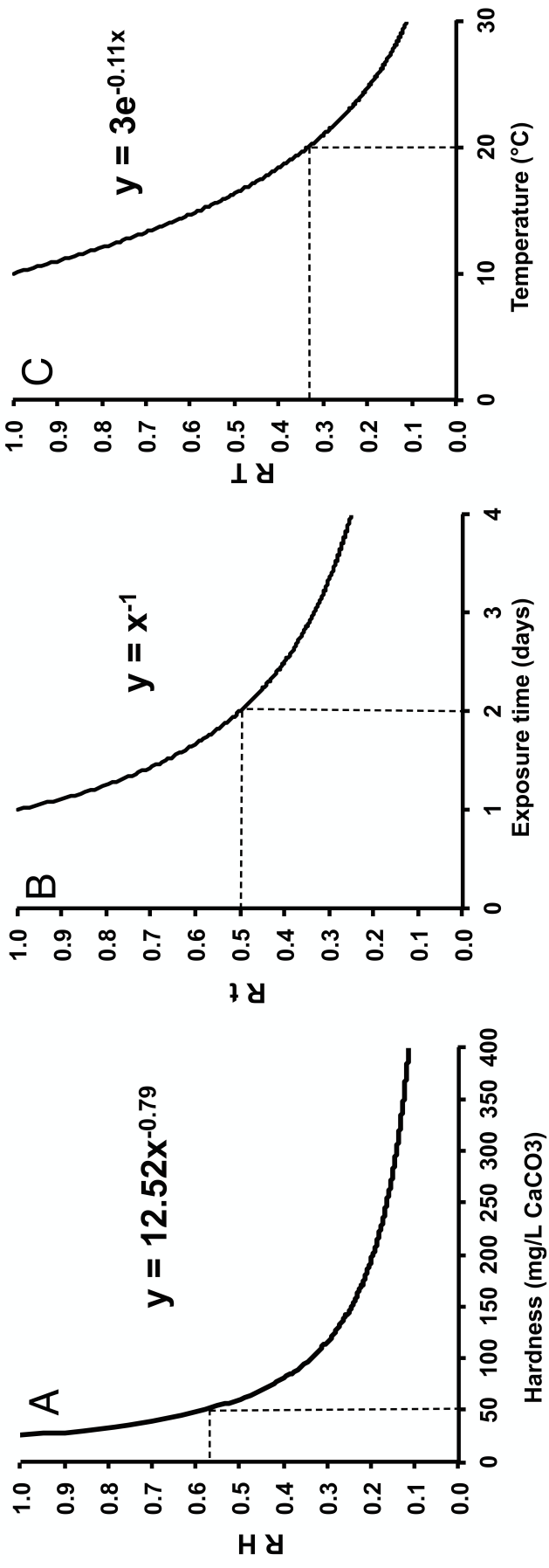

Figure A.1: Normalization of log LC50. For hardness the methodology in de Zwart et al. (2006) using hardness criteria from USEPA (1996) was applied. For exposure time the normalization followed Haber's rule (Haber, 1924). For temperature the Q10 rule was followed (Cairns & Parker, 1975)

A.3 Supplementary Tables

Table A.1: Variance of the original (Var(HM)org) and normalized (Var(HM)norm) log LC50 values.

	Var(HM)org	**Var(HM)norm**
Cd	0.36	0.27
Cr	0.44	0.36
Cu	0.43	0.36
Hg	0.31	0.27
Ni	0.30	0.24
Pb	0.27	0.22
Zn	0.31	0.28

Table A.2: Median absolute deviation (MAD) of S values and number of families (n) in the order level. MAD presents the median of the absolute difference between datapoints and family median.

Order	**Cd**		**Cr**		**Cu**		**Hg**		**Ni**		**Pb**		**Zn**	
	MAD	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**
Aciculata		1				1								1
Aeolosomatida		1		1		1								1
Amphipoda	0.98	3	0.17	3	0.66	3		1	0.78	2	1.33	2	0.6	3
Anisoptera		1												
Anostraca	0.12	2	0.39	2	0.48	2	0.55	2					0.29	2
Aphelenchida		1				1		1				1		1
Architaenioglossa		1		1		1		1		1		1		1
Basommatophora		1	0.38	3	0.84	3		1				1	0.21	3
Calanoida		1				1		1		1		1		1
Cladocera	0.33	3	0.27	2	0.27	3	0.16	3	0.47	2	0.47	2	0.13	3
Cyclopoida		1		1		1		1		1		1		1
Decapoda	0.49	7	1.22	3	0.83	6	0.53	5	0.41	3	0.14	4	0.76	4
Diptera	0.25	3	0.56	3	0.32	3	0.19	3	0.32	2	0.79	2	0.13	2
Ephemeroptera	0.47	4		1	0.57	4	1.62	2		1			0.82	3
Haplotaxida	0.66	2	0.53	2	0.26	2	0.37	2		1		1	0.58	2
Harpacticoida		1		1		1		1						1
Heteroptera	1.48	3					0.45	2				1		1
Hirudinea		1				1		1						1
Hydroida		1				1								1
Isopoda		1				1		1		1		1		1
Lumbriculida		1		1		1		1		1		1		1
Megaloptera		1												
Neoloricata		1				1								1
Neotaenioglossa		1		1	0.61	2		1		1		1	0.4	2
Plecoptera						1		1		1				
Podocopida		1				1		1				1		1
Rhabditida	0.14	3	0.4	2	0.39	3	0.38	2	0.59	3	0.52	3	1.15	3
Trichoptera	0.14	2		1				1						
Tricladida	0.43	2	0.85	2	0.96	2	0.72	2		1		1	0.32	2
Unionoida		1		1		1		1		1				1
Veneroida	1.59	2	0.39	2	2.66	3	0.67	3		1				1
Zygoptera	0.87	2		1				1				1		1

Table A.3: Median absolute deviation (MAD) of S values and number of genera (n) in the family level. MAD presents the median of the absolute difference between datapoints and family median.

Family	Cd		Cr		Cu		Hg		Ni		Pb		Zn	
	MAD	n	MAD	n	MAD	n	MAD	n	MAD	n	MAD	n	MAD	n
Aeolosomatidae		1		1		1								1
Ameiridae		1		1		1		1						1
Aphelenchidae		1				1		1				1		1
Asellidae		1				1		1		1		1		1
Astacidae		1				1		1		1		1		
Atyidae	0.52	2		1	0.15	3		1				1	0.48	3
Baetidae		1				1		1						1
Belostomatidae												1		
Calopterygidae		1												
Cambaridae	1.62	2			0.45	2	0.86	2		1	2.43	2		1
Cephalobidae		1				1				1		1		1
Ceratopogonidae		1		1		1		1		1		1		1
Chironomidae		1		1		1		1		1		1		1
Chydoridae		1				1		1						1
Coenagrionidae	0.54	2		1				1				1		1
Corbiculidae		1		1		1		1		1				
Corduliidae		1												
Corixidae	1.88	2						1						
Crangonyctidae		1		1		1				1		1		1
Culicidae		1		1		1		1						
Cyclopidae	0.46	2	0.49	2	0.38	3	0.70	2		1		1	0.78	3
Cyprididae	0.32	2			0.26	2		1			0.36	2	0.46	2
Daphniidae	0.14	3	0.36	3	0.35	3	0.13	2	0.66	2	0.41	3	0.33	2
Diaptomidae	0.36	4			0.62	3	0.69	3		1	1.00	2	0.42	4
Donacidae		1				1		1						1
Dugesiidae		1		1		1		1						1
Ephemerellidae		1				1		1		1				
Erpobdellidae		1				1		1						1
Gammaridae	0.34	2	0.62	2	0.24	2	0.77	2		1		1	0.13	2
Heptageniidae		1				1								1
Hyalellidae		1		1		1								1
Hydridae		1				1								1
Hydrobiidae		1				1		1		1				1
Hydropsychidae		1		1				1						
Ischnochitonidae		1				1								1
Leptophlebiidae		1		1		1								1
Lumbriculidae		1		1		1		1		1		1		1
Lymnaeidae		1		1		1		1				1		1
Mactridae				1		1		1						
Moinidae		1		1		1		1		1		1		1
Naididae		1		1		1		1						1
Naucoridae		1												
Nepidae		1						1						1
Nereididae		1				1								1
Palaemonidae		1		1		1		1						1
Panagrolaimidae		1		1		1		1		1		1		1
Parastacidae		1				1				1				
Parathelphusidae		1						1				1		
Penaeidae		1		1		1								1
Perlidae						1		1		1				
Physidae				1		1								1
Planariidae		1		1		1		1		1		1		1
Planorbidae				1		1								1
Pleuroceridae			0.55	2	0.72	2						1	1.35	2

Rhabditidae		1		1		1		1		1		1
Rhyacophilidae		1										
Sialidae		1										
Streptocephalidae		1		1		1		1				1
Thamnocephalidae		1		1		1		1				1
Tubificidae	0.38	3	1.96	2	0.62	3	0.18	3		1		1	0.38	2
Unionidae	0.54	7		1	0.49	8	1.13	3	0.68	6			0.27	5
Viviparidae		1		1		1		1		1		1		1

Table A.4: Median absolute deviation (MAD) of S values and number of species (n) in the genus level for each heavy metal. MAD presents the median of the absolute difference between datapoints and genus median.

Genus	**Cd**		**Cr**		**Cu**		**Hg**		**Ni**		**Pb**		**Zn**	
	MAD	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**	**MAD**	**n**
Acroneuria						1		1		1				
Actinonaias		1				1				1				1
Aedes		1		1		1		1						
Aeolosoma		1		1		1								1
Amnicola		1				1		1		1				1
Anculosa				1		1								1
Anodonta		1		1		1		1		1				1
Aphelenchus		1				1		1				1		1
Aphelocheirus		1												
Argia														1
Asellus		1				1		1		1		1		1
Austropotamobius		1				1		1		1		1		
Branchiura		1		1		1		1						
Caenorhabditis		1		1		1		1		1		1		1
Calopteryx		1												
Caridina						1						1		1
Cephalobus		1				1				1		1		1
Ceriodaphnia	0.36	3	0.36	2	0.12	3	0.76	2		1	0.65	2	0.16	3
Cherax		1				1				1				
Chironomus	0.53	4	0.34	3	0.38	4	0.66	4	0.65	4	0.55	3	0.33	4
Chydorus		1				1		1						1
Cloeon		1				1		1						1
Corixa		1						1						
Crangonyx		1		1		1				1		1		1
Culicoides		1		1		1		1		1		1		1
Cyclops		1	0.23	2	0.59	2	0.83	2				1		1
Cypris	0.19	2				1		1				1	0.45	2
Daphnia	0.41	1	0.55	7	0.46	7	0.85	6	0.47	3	0.67	8	0.43	1
Deleatidium		1		1		1								1
Diacypris		1				1						1		1
Diaptomus		1				1		1						1
Donax		1				1		1						1
Dugesia		1		1		1		1		1		1		1
Echinogammarus		1		1		1		1						1
Elimia				1		1						1		1
Enallagma	1.45	2		1								1		
Ephemerella		1				1		1		1				
Epioblasma						1				1				1
Erpobdella		1				1		1						1
Eudiaptomus		1				1		1		1		1		1
Gammarus	0.15	5	0.19	4	0.72	6	0.73	3		1	0.59	2	0.26	5
Girardia		1		1		1		1						1
Hediste		1				1								1

Heliodiaptomus		1							1		1
Hyalella		1		1		1					1
Hydra		1				1					1
Hydropsyche		1		1				1			
Ischnochiton		1				1					1
Ischnura	0.32	2						1			
Laccotrephes								1			
Lamellidens		1				1		1			
Lampsilis		1				1			1		1
Lethocerus										1	
Limnodrilus		1				1		1			1
Lumbriculus		1		1		1		1	1	1	1
Lymnaea	0.77	2	0.39	2	0.15	3	0.12	2		1	0.32	3
Macrobrachium	0.25	4	0.16	2	0.45	4	0.23	3			0.43	3
Macromia		1										
Mesocyclops				1		1			1			1
Moina	0.45	2		1	0.75	2		1	1	1	0.76	2
Nais		1		1		1		1				1
Neocaridina		1		1		1		1				1
Nitocra		1		1		1		1				1
Orconectes		1				1		1	1	1		1
Panagrellus		1		1		1		1	1	1		1
Paratelphusa		1						1		1		
Paratya	0.25	2			0.15	2					0.66	2
Parreysia		1				1		1				
Penaeus		1		1		1						1
Physa				1		1						1
Planorbella				1		1						1
Procambarus		1				1		1		1		
Ranatra		1										1
Rangia				1		1		1				
Rhithrogena		1				1						1
Rhyacophila		1										
Sialis		1										
Sigara		1										
Simocephalus		1		1		1				1		
Skistodiaptomus		1				1		1				1
Streptocephalus	0.93	3	0.27	3	0.23	3		1			0.76	3
Thamnocephalus		1		1		1		1				1
Tropocyclops		1				1		1				1
Tubifex		1		1		1		1	1	1		1
Utterbackia		1				1			1			
Villorita		1		1		1		1	1			
Villosa		1				1			1			1
Viviparus		1		1		1		1	1	1		1

Table A.5: Standard deviation (SD) and number of entries (n) for the toxicity concentrations (log LC50) values in the species level for each heavy metal.

Taxon	Cd			Cr			Cu			Hg			Ni			Pb			Zn		
	LC50	SD	n	LC50	SD	n	LC50	SD	n	LC50	SD	n	LC50	SD	n	LC50	SD	n	LC50	SD	n
Acroneuria lycorias							4.22		1	3.6		1	4.41		1						
Actinonaias pectorosa	2.46	0.13	2				2.26	0.23	3				3.38		1				3.28		1
Aedes aegypti	3.4	0.34	4	4.35	0.22	3	3.92	0.08	3	3.35	0.63	5									
Aeolosoma headleyi	3.72	0.55	3	2.81	0.22	9	2.47	0.27	10										3.39	0.34	10
Amnicola sp.	3.82	0.37	2				3.3	0.59	4	2.87	0.67	4	4.4	0.12	2				4.04	0.37	2
Anculosa sp.				2.49	0.2	10	2.21	0.15	10										2.68	0.2	10
Anodonta imbecillis	1.97	0.52	4	2.73	0.62	4	2.51	0.26	4	2.55	0.1	4	2.26	0.18	4				2.85	0.2	4
Aphelenchus avenae	5.26		1				3.04	0.02	3	3.31	0.4	6				4.35	0.02	3	4.87	0.01	3
Aphelocheirus aestivalis	6.65		1																		
Argia sp.																			5.11		1
Asellus aquaticus	2.84	0.44	24				3.53		1	2.22	0.12	3	5.18	0.19	2	4.56	0.02	2	4.36	0.53	6
Austropotamobius pallipes	2.45		1				2.19		1	1.41		1	3.63		1	3.52		1			
Branchiura sowerbyi	4.77	0.98	11	5.56	0.1	3	2.88	0.45	4	2.53	1.14	2									
Caenorhabditis elegans	4.28	0.91	21	4.14	1.37	4	3.96	0.88	11	3.48	0.32	7	4.73	1.4	5	3.81	1.03	9	4.08	1	5
Calopteryx splendens	6.13	0.07	3																		
Caridina nilotica							2.59		1							5.04	0.01	2	3.97		1
Cephalobus persegnis	4.07	0.08	3				3.17	0.02	3				4.34	0.07	3	3.86	0.06	3	5.58	0.11	3
Ceriodaphnia dubia	2.11	0.53	13	2.31	0.41	7	1.45	0.57	245	1.41	0.26	6	2.45	0.54	7	2.29	0.58	15	2.31	0.38	34
Ceriodaphnia reticulata	2.38	0.53	8	1.75	0.11	4	1.38	0.74	8	0.47		1				3.09	0.3	4	2.41	0.81	2
Ceriodaphnia rigaudi	2.75		1				2.98		1										3.49		1
Cherax destructor	3.02	0.04	4				3.55	0.47	11				6	0.1	4						
Chironomus plumosus	3.19	0.37	11	3.74	0.25	7	3.85	0.96	41	3.87	0.69	29	3.76	0.44	29	4.17	0.77	19	4.78	0.23	9
Chironomus riparius	5.13	0.61	18				3.6	0.52	20	3.04	0.37	20	4.67	0.18	6				4.36		1
Chironomus sp.	4.11	1.17	4	4.01	0.43	39	3.25	2.2	3	1.4	0.09	2	3.83	0.37	2	4.5		1	4.69	0.94	4
Chironomus tentans	3.43	0.52	15	3.29	0.89	3	3.3	0.84	49	3.43	0.35	6	3.85	0.18	2	3.69	0.1	4	2.91	0.13	2
Chydorus sphaericus	1.91	0.36	6				1.6	0.51	16	1.12	0.57	3							2.21	1.09	3
Cloeon dipterum	4.41	0.34	17				1.87	0.76	2	1.57		1							4.06	0.32	4
Corixa punctata	4.43		1							2.21		1									
Crangonyx pseudogracilis	3.49	0.71	2	2.24	0.29	4	2.69	0.05	2				4.93	0.2	2	4.15	0.07	2	4.12	0.35	2
Culicoides furens	3.19	0.28	8	3.31	0.23	7	3.63	1.06	12	2.55	0.49	18	3.43	0.14	5	3.21	0.29	6	4.36	0.17	7
Cyclops abyssorum	3.1		1	3.52		1	2.92		1	2.86		1				3.26		1			
Cyclops sp.				3.88	0.25	3	3.58	0.31	17	1.85	0.37	17							3.8	0.39	2
Cypris sp.	3.45	0	2							2.69	0.08	2				4.07	0.17	2	3.82	0.15	2

Cypris subglobosa	3.13	0.55	4					3.08	0.84	4														4.39	0.16	3
Daphnia ambigua	1		1	3.9	0.76	2		2.9	1.32	2														2.48		1
Daphnia carinata	2.56	0.33	8	2.32		1		2.16	0.24	2								3.47	0.36	3				2.76	0.59	4
Daphnia curvirostris	2.38		1	2.37		1												2.89		1				2.42		1
Daphnia hyalina	0.99		1	2.95	0.86	2					0.26		1	2.46		1		2.1		1						
Daphnia lumholzi	2.64	0.37	3					1.86	0.42	6														3.28	0.3	3
Daphnia magna	1.74	0.59	334	1.89	0.85	167		1.42	1.03	557	0.89	0.65	35	3.31	0.55	31		2.6	0.8	39				2.79	0.54	125
Daphnia obtusa	2.31		1	2.08	0.42	2												2.99		1				2.86		1
Daphnia pulex	1.92	0.39	106	1.72	0.57	45		1.42	0.51	62	0.89	0.64	7	3.62	0.64	3		3.33	0.42	6				2.49	0.2	19
Daphnia rosea	2.2	0.4	2					1.65	0.06	2	1.33	0.16	2											2.98		1
Daphnia similis								1.33	0.21	4	2.54	0.04	2					2.52	0.01	2				3.04	0.13	2
Delecatidium sp.	5.03	0.2	3	4.67	0.66	3		4.37	0.17	3														6.69	0.15	3
Diacypris compacta	3.84		1					3.1		1								3.7		1				3.52		1
Diaptomus leptopus	1.47	0.01	2					1.33	0.03	2	1.53	0.15	2											1.47	0.03	2
Donax faba	3.72	0.09	4					3.64	0.07	4	2.96	0.01	4											4.06	0.07	4
Dugesia tigrina	3.85	0.22	4	4.11	0.41	3		3.71	0.23	5	2.73		1	4.5	0.03	3		5.51		1				4.09	0.23	9
Echinogammarus tibaldii	2.56		1	1.64		1		2.31		1	2.29		1											3.82		1
Elimia livescens				3.34	0.23	2		3.01	0.14	3								5.13	0.11	2				4.4	0.4	2
Enallagma aspersum				5.31		1																				
Enallagma cyathigerum	6.34	0.22	3															4.67	0	3						
Enallagma sp.	3.83	0.09	3																							
Ephemerella subvaria	3.6	0	2					2.5	0	2	3.6	0	4	3.48	0	2										
Epioblasma capsaeformis								2.53		1				2.44		1								3.17		1
Erpobdella octoculata	3.14	0.2	4					2.14	0.08	2	2.37		1											3.45	0.45	2
Eudiaptomus padanus	2.26		1					2.22		1	2.45		1	3.08		1		3.12		1				1.83		1
Gammarus fasciatus				2.34		1		2.5		1														3.75	0.34	5
Gammarus italicus	2.64	0.23	2	2.62		1		1.57	0.27	2	2.42		1											3.35		1
Gammarus lacustris	1.9		1					2.63		1														3.65		1
Gammarus pseudolimnaeus	1.98	0.23	5	2.53	0.38	4		0.92		1								2.18	0.23	3						
Gammarus pulex	2.18	0.65	58					2.11	0.32	15	0.87		1					2.9		1				3.09	0.28	32
Gammarus sp.	1.85	0.21	2	3.51	0.21	2		2.78	0.21	5	1.33	0.25	2	4.01	0.37	2								3.82	0.36	2
Girardia tigrina	3.12		1	2.79		1		2.63	0.28	37	1.85		1											3.68		1
Hediste diversicolor	4.57		1					2.5	0.19	3														4.06	0.33	2
Heliodiaptomus viduus	2.09	0.06	2															3.01	0.72	2				2.5	0.05	2
Hyalella azteca	1.42	0.71	16	2.53		1		1.94	0.64	31														2.98	0.13	7
Hydra vulgaris	2.78	0.21	9					1.84	0.21	9														4.22	0.31	9
Hydropsyche angustipennis	5.64		1																							
Hydropsyche betteni											3.6		1													

Hydropsyche sp.				5.5		1													
Ischnura elegans	4.43		1							3.88		1							
Ischnura heterosticta	3.88		1																
Laccotrephes sp.										2.77		1							
Lamellidens marginalis	4.3		1				3.93	0.13	3	4.15	0.21	2							
Lampsilis straminea	2.04		1				2.81	0.46	11				2.64		1				2.93		1
Lethocerus sp.																3.5	0.11	2			
Limnodrilus hoffmeisteri	3.19	0.56	9				2.54	0.24	4	2.54	0.51	5							3.61		1
Lumbriculus variegatus	1.84	0.13	3	3.89	0.39	3	1.9	0.53	4	2.04	0.18	2	4.56	0.11	2	3.34	0.01	2	4.04	0.28	7
Lymnaea emarginata				4.28	0.09	2	2.97	0.16	2							4.74	0.33	2	3.64	0.2	2
Lymnaea luteola	4.39	0.11	4	4.88	0.07	4	2.7	0.07	4	2.52	0.09	4							4.61	0.12	12
Lymnaea stagnalis	3.07	0.22	7				2.88		1	2.67	0.18	4							3.85	0.15	3
Macrobrachium hendersodayanus	1.98	0.21	2				3.54		1	2.13		1							4.19		1
Macrobrachium kistnensis	2.54		1				1.94		1	1.31		1									
Macrobrachium lamarrei	2.15	0.14	4	3.59	0.07	4	2.06	0.23	8	2	0.15	4							3.29	0.13	4
Macrobrachium rude	2.34	0.06	4	3.83	0.14	4	2.44	0.32	5										4.46	0.19	4
Macromia sp.	2.66		1																		
Mesocyclops pehpeiensis				2.95		1	2.11		1				3.31		1				2.62		1
Moina irrasa	1.25	0.39	21				0.57	0.26	21										1.83	0.23	17
Moina macrocopa	2.02	0.2	14	2.45	0.55	2	1.41	0.51	6	0.31	0.12	2	3.5	0.84	4	3.5	0.81	3	2.73	0.71	9
Nais sp.	3.31	0.12	2	3.89	0.35	2	2.52	0.57	2	3	0.23	2							4.15	0.38	2
Neocaridina denticulata	2.65	0.44	3	4.66	0.46	3	2.5	0.57	3	2.61	0.37	3							4.27	0.68	3
Nitocra spinipes	2.98	0.07	2	3.36	0.13	8	2.63		1	2.01		1							3.26	0.04	2
Orconectes limosus	2.71		1				2.89		1	1.81		1	3.9		1	3.63		1	4.87		1
Panagrellus silusiae	4.4	0.27	6	4.75	0.46	6	2.95	0.4	6	3.53	0.1	6	5.47	0.56	6	3.89	0.2	6	4.82	0.25	6
Paratelphusa hydrodromus	3.15	0.56	5							2.68	0.06	4				4.99		1			
Paratya australiensis	1.53	0.34	2				1.78	0.24	17										3.89	0.5	13
Paratya compressa	1.97	0.08	2				1.61	0.06	2										3.05	0.06	2

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Appendix B

Supplementary information for: Evolutionary Patterns and Physicochemical Properties Explain Macroinvertebrate Sensitivity to Heavy Metals

B.1 Supplementary Methods

B.1.1 Physicochemical properties

We examined eight physicochemical properties extracted from McCloskey, Newman & Clark (1996) (Table B2) that are listed and explained below:

1. Pearson softness parameter (σp) is calculated by dividing the difference between the coordinate bond energies of the metal fluoride and iodide, by the coordinate bond energy of the metal fluoride (Jones & Vaughn, 1978). Based on this parameter, metals are divided into Class A (e.g., Mg^{2+}, Al^{3+}) or O-seeking metals (affinity for hard ligands), Class B (e.g., Hg^{2+}) or S- and N- seeking metals (affinity for soft ligands), and borderline metals (e.g., Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}), or S-, N-,O- seeking metals.

2. Covalent bond stability ($\Delta\beta$) is the difference between the log stability constant of the metal fluoride and chloride (Turner, Whitfield & Dickson, 1981) and it represents the tendency to form covalent bonds with soft ligands (Newman & McCloskey, 1996).

3. The absolute value of the $\log_{10}$ of the first hydrolysis constant ($|\log_{10}$ KOH$|$) is a measure of the ability of the metal ion to form a metal hydroxide ($Mn^{+} + H_2O = MOH^{n-1}+H^{+}$)(Tatara et al., 1998). It represents the metal affinity to intermediate ligands such as those with O donor atoms (Newman & McCloskey, 1996).

4. The solubility product for the metal hydroxide ($\log_{10}$ K_{so}MOH) is a parameter which has also related the affinity of metals to hard ligands (McCloskey, Newman & Clark, 1996).

5. The quotient AN/ΔIP is a combination of the atomic number (AN) that reflects the size of an ion, and the ionization potential (IP) (Kaiser, 1980). The heavier metals have the capacity to form irreversible and stable complexes with biological molecules, therefore, these heavy metals are considered as toxic (Walker, Enache & Dearden, 2003). The IP is the amount of energy required to remove completely the most loosely bound electron (Walker, Enache & Dearden, 2003). The differential change in IP (ΔIP) is the difference in ionization potential between the actual oxidation number of the ion and the next lower one (Kaiser, 1980). When using AN/ΔIP, higher explanation power is expected when developing models separately for metals with more than one oxidation state (e.g., Hg, Pb, or Cr) or with one oxidation state (e.g., Zn or Cd) (Kaiser, 1980).

6. The electrochemical potential (ΔE_o) reflects the change between the actual ion

and its first stable reduced state (Kaiser, 1980; Walker, Enache & Dearden, 2003). It is generally used as a second parameter together with the quotient AN/ΔIP (Kaiser, 1980).

7. Ionic index (Z^2/r represents the property of isolated metal ions (charge; Z) and the ionic radius (r) (Nieboer & Richardson, 1980). In general, Z^2/r would explain well the toxicity of class A (hard ions) metals (Nieboer & Richardson, 1980).

8. The covalent index ($X_m{}^2r$) represents the property of complexes (electronegativity; X_m), and the ionic radius (r) (Nieboer & Richardson, 1980). The $X_m{}^2r$ would explain well the toxicity of class B (soft ions) metals (Nieboer & Richardson, 1980).

B.2 Supplementary Figures

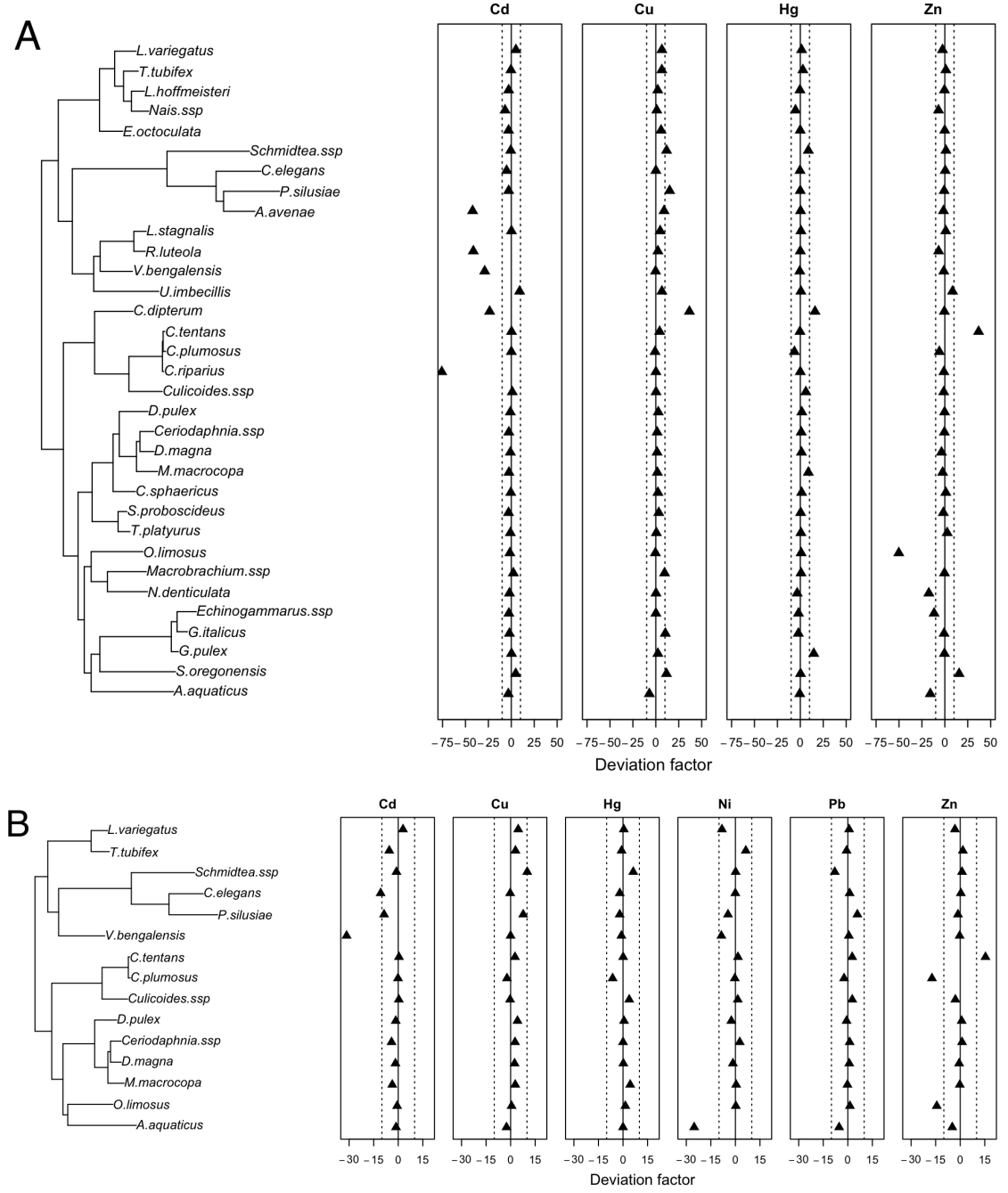

Figure B.1: Deviation factors following leave-one-out cross validation for the four heavy metal model with 33 species (A) and for the six heavy metal model with 15 species (B). Deviation factors are expressed as the number of times that the predicted tolerance values are overpredicted (positive values) or underpredicted (negative values). For both bilinear models the softness parameter of the heavy metals was used as a second descriptor. The straight line indicates equality between predicted and observed values, the dashed lines indicate 10-fold over- or under-prediction, respectively.

B.3 Supplementary Tables

Table B.1: GenBank accession numbers for the 33 analysed species. The number of sequences in the three taxonomic levels is given for each species. The taxonomic level for each accession numbers is denoted in parenthesis (G: Genus, or F: Family, otherwise Species).

Species Name	No. sequences			Accession Number
	Species	Genus	Family	
Aphelenchus avenae	5	0	36	AF119048,JQ348399,JQ348400,NC_017899(F)
Asellus aquaticus	34	0	0	AF255701,GU130252
Caenorhabditis elegans	41	0	0	EU196001,JN636100,NC_001328,X03680
Ceriodaphnia ssp.	0	5	0	AF144208,AY822006,DQ470585,DQ470627,JN233851
Chironomus plumosus	8	35	0	AF192161,AJ296789,JF412099,JN016814,NC_016167(G)
Chironomus riparius	6	37	0	AY820919,DQ657920,GU053603,HM137935,NC_016167(G)
Chironomus tentans	7	36	0	AF109710,AF110158,NC_016167(G),X99212
Chydorus sphaericus	3	1	0	AF532891,AM490277,DQ310682(G),JN233896
Cloeon dipterum	7	1	0	AF461220,AF461249,AJ969725,AJ971773,HM185104(G),JN615361
Culicoides ssp.	0	41	0	AB462265,NC_009809
Daphnia magna	6	0	0	AF346515,AM490278,DQ132627,GQ343286,GU680597,JN903684
Daphnia pulex	39	0	0	AF014011,NC_000844
Echinogammarus ssp.	0	3	2	AF228046(F),AY529072(G),EF582918(G),FJ581623(G)
Erpobdella octoculata	4	3	0	AB679655(G),AF003274,AF099949,AF099954,AY364865
Gammarus italicus	3	0	0	JF965719,JF965906,JF966137
Gammarus pulex	4	0	0	AF202982,EF582877,JF965769,JF965940
Limnodrilus hoffmeisteri	5	0	0	AF325978,AF360992,AF534865,DQ459923,HM460076
Lumbriculus variegatus	6	0	0	AF209457,AY885578,DQ459885,GQ355466,GU453381
Lymnaea stagnalis	6	2	0	AF485661,EF489367,FR797836,FR797897,HM560968(G),JF960167(G), JN614412,Z73984
Macrobrachium ssp.	0	42	0	GQ369796,NC_015073
Moina macrocopa	2	3	0	AF339091,AF532882(G),DQ310693(G),EU702246, GQ503606(G)
Nais ssp.	0	5	0	DQ459894,DQ459940,GQ355427,GU902020,GU902104
Neocaridina denticulata	6	0	34	AB300191,AB524959,AB598478,DQ079770(F),DQ681269, FJ426068(F), FN995600,NC_008413(F)
Orconectes limosus	1	7	0	AF198565(G),AF198605(G),AF235965(G),AY071790(G), AY701199,JN800523(G), JQ397615(G),JQ397630(G)
Panagrellus silusiae	1	2	4	AF083007(G),AY878390(F),AY878406(F),EU195986(G), FJ591045,HM627507(F)
Radix luteola	1	6	0	AF485648,AY465067(G),EU413984(G),FR797817(G), FR797903(G),JF922879(G), JN614402(G)
Schmidtea ssp.	0	7	0	AF013152,AF047854,DQ665992,JF837054,JF837060
Skistodiaptomus oregonensis	7	1	0	EU582551,EU582582,EU582622,EU582656,EU598352(G)
Streptocephalus proboscideus	3	0	0	AJ238075,AY519813,AY519829
Thamnocephalus platyurus	4	0	0	AF209046,AF209057,AF209066,AJ238073
Tubifex tubifex	5	0	0	AF534866,DQ284762,GQ355398,GQ355465,HQ603822
Utterbackia imbecillis	39	0	2	AF091331(F),EU580117(F),L78858,NC_015479,U82333
Viviparus bengalensis	0	7	2	AF120516(G),AF120574(G),AF120634(G),AF131952(F), AY449491(G), DQ916415(F),U78677(G)

Table B.2: Metal physicochemical properties used in the bilinear models.

Properties	**Symbol**	**Cd**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
Pearson softness parameter	σp	0.08	0.10	0.07	0.13	0.13	0.12
Covalent bond stability	$\Delta\beta$	−0.89	1.12	−5.80	0.50	0.48	0.66
First hydrolysis constant	$\lvert\log_{10}$ KOH$\rvert$	10.08	8.00	3.40	9.86	7.71	8.96
Metal hydroxide solubility product	$\log_{10}$ K_{so}MOH	14.00	19.80	25.50	16.00	18.70	16.50
Ionization potential	AN/ΔIP	6.07	2.31	9.62	2.66	10.78	3.50
Electrochemical potential	ΔE_o	0.40	0.16	0.91	0.23	0.13	0.76
Ionic index	Z^2/r	4.21	5.48	3.92	5.79	3.39	5.40
Covalent index	$X_m{}^2r$	2.71	2.64	4.08	2.52	6.41	2.02

Table B.3: Toxicity of six bivalent metals expressed as $\log_{10}$ of the medial lethal concentration (LC50 in g/L) for the 33 species analysed. The standard deviation and the number of samples (in parenthesis) are displayed for species which had more than a single toxicity value. Species which were used for the model building 4HM (Cd, Cu, Hg and Zn), and 6HM (all metals), as well as species which were used for the predictions are displayed. Type 1A prediction and type 1B prediction is used to validate addition of new species in the model, wheras type 2 prediction is employed to validate the addition of new metals in the model.

Superphylum	**Phylum**	**Subphylum**	**Class**	**Subclass**	**Order**	**Family**	**Species**
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Phyllopoda	Cladocera	Moinidae	*Moina macrocopa*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Phyllopoda	Cladocera	Daphniidae	*Ceriodaphnia ssp.*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Phyllopoda	Cladocera	Chydoridae	*Chydorus sphaericus*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Phyllopoda	Cladocera	Daphniidae	*Daphnia magna*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Phyllopoda	Cladocera	Daphniidae	*Daphnia pulex*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Sarsostraca	Anostraca	Streptocephalidae	*Thamnocephalus platyurus*
Ecdysozoa	Arthropoda	Crustacea	Branchiopoda	Sarsostraca	Anostraca	Streptocephalidae	*Streptocephalus proboscideus*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Isopoda	Asellidae	*Asellus aquaticus*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Amphipoda	Gammaridae	*Echinogammarus ssp.*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Amphipoda	Gammaridae	*Gammarus italicus*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Amphipoda	Gammaridae	*Gammarus pulex*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Decapoda	Palaemonidae	*Macrobrachium ssp.*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Decapoda	Atyidae	*Neocaridina denticulata*
Ecdysozoa	Arthropoda	Crustacea	Malacostraca	Eumalacostraca	Decapoda	Cambaridae	*Orconectes limosus*
Ecdysozoa	Arthropoda	Crustacea	Maxillopoda	Copepoda	Calanoida	Diaptomidae	*Skistodiaptomus oregonensis*
Ecdysozoa	Arthropoda	Mandibulata	Insecta	Pterygota	Diptera	Chironomidae	*Chironomus plumosus*
Ecdysozoa	Arthropoda	Mandibulata	Insecta	Pterygota	Diptera	Chironomidae	*Chironomus riparius*
Ecdysozoa	Arthropoda	Mandibulata	Insecta	Pterygota	Diptera	Chironomidae	*Chironomus tentans*
Ecdysozoa	Arthropoda	Mandibulata	Insecta	Pterygota	Diptera	Ceratopogonidae	*Culicoides ssp.*
Ecdysozoa	Arthropoda	Mandibulata	Insecta	Pterygota	Ephemeroptera	Baetidae	*Cloeon dipterum*
Ecdysozoa	Nematoda	–	Secernentea	–	Aphelenchida	Aphelenchidae	*Aphelenchus avenae*
Ecdysozoa	Nematoda	–	Secernentea	–	Rhabditida	Rhabditidae	*Caenorhabditis elegans*
Ecdysozoa	Nematoda	–	Secernentea	–	Rhabditida	Panagrolaimidae	*Panagrellus silusiae*
Lophotrochozoa	Annelida	–	Clitellata	Hirudinea	Erpobdelliformes	Erpobdellidae	*Erpobdella octoculata*
Lophotrochozoa	Annelida	–	Clitellata	Oligochaeta	Haplotaxida	Tubificidae	*Limnodrilus hoffmeisteri*
Lophotrochozoa	Annelida	–	Clitellata	Oligochaeta	Haplotaxida	Naididae	*Nais ssp.*
Lophotrochozoa	Annelida	–	Clitellata	Oligochaeta	Haplotaxida	Tubificidae	*Tubifex tubifex*
Lophotrochozoa	Annelida	–	Clitellata	Oligochaeta	Lumbriculida	Lumbriculidae	*Lumbriculus variegatus*
Lophotrochozoa	Mollusca	–	Gastropoda	Basommatophora	Hygrophila	Lymnaeidae	*Lymnaea stagnalis*
Lophotrochozoa	Mollusca	–	Gastropoda	Orthogastropoda	Pulmonata	Lymnaeidae	*Radix luteola*
Lophotrochozoa	Mollusca	–	Gastropoda	Orthogastropoda	Architaenioglossa	Viviparidae	*Viviparus bengalensis*
Lophotrochozoa	Mollusca	–	Bivalvia	Palaeoheterodonta	Unionoida	Unionidae	*Utterbackia imbecillis*
Platyzoa	Platyhelminthes	–	Turbellaria	–	Tricladida	Dugesiidae	*Schmidtea ssp.*

Table B.3: – continued from previous page

Species	Cd	Cu	Hg	Zn	Pb	Ni	Model	Prediction
Moina macrocopa	2.021±0.204(14)	1.41±0.514(6)	0.304±0.125(2)	2.726±0.709(9)	3.035±0.092(2)	2.785±0.267(2)	4HM, 6HM	1A,1B
Ceriodaphnia ssp.	1.964±0.241(16)	1.442±0.576(245)	1.275±0.427(7)	2.275±0.378(34)	2.457±0.619(19)	2.448±0.54(7)	4HM, 6HM	1A,1B
Chydorus sphaericus	1.911±0.358(6)	1.595±0.505(16)	1.118±0.568(3)	2.209±1.088(3)			4HM	1A,2
Daphnia magna	1.72±0.576(274)	1.403±1.022(516)	0.887±0.651(35)	2.806±0.527(115)	2.634±0.798(37)	3.311±0.552(31)	4HM, 6HM	1A,1B
Daphnia pulex	1.9±0.373(87)	1.417±0.517(61)	0.978±0.652(6)	2.485±0.203(19)	3.326±0.419(6)	3.624±0.636(3)	4HM, 6HM	1A,1B
Thamnocephalus platyurus	2.323	2.241±0.836(3)	1.58	2.335±0.302(4)	–	–	4HM	1A,2
Streptocephalus proboscideus	1.561±0.271(2)	1.672±0.01(2)	2.013±0.31(2)	2±0.288(2)	–	–	4HM	1A,2
Asellus aquaticus	2.836±0.442(24)	3.527	2.213±0.114(3)	4.262±0.523(5)	4.551±0.02(2)	5.169±0.185(2)	4HM, 6HM	1A,1B
Echinogammarus ssp.	2.553	2.303	2.292	3.818	–	–	4HM	1A,2
Gammarus italicus	2.635±0.232(2)	1.569±0.274(2)	2.419	3.35	–	–	4HM	1A,2
Gammarus pulex	2.157±0.647(56)	2.113±0.316(15)	0.864	3.156±0.298(22)	2.892	–	4HM	1A,1B[a]
Macrobrachium ssp.	2.091±0.168(6)	2.059±0.225(8)	2.028±0.147(5)	3.464±0.422(5)	–	–	4HM	1A,2
Neocaridina denticulata	2.644±0.445(3)	2.499±0.565(3)	2.604±0.365(3)	4.268±0.68(3)	–	–	4HM	1A,2
Orconectes limosus	2.708	2.884	1.805	4.869	3.624	3.891	4HM, 6HM	1A,1B
Skistodiaptomus oregonensis	2.607±0.032(2)	1.809±0.343(24)	1.643±0.149(3)	3.281±0.088(2)	–	–	4HM	1A,2
Chironomus plumosus	3.189±0.368(11)	3.847±0.959(41)	3.895±0.684(28)	4.806±0.227(8)	4.165±0.773(19)	3.782±0.434(28)	4HM, 6HM	1A,1B
Chironomus riparius	5.127±0.609(18)	3.592±0.517(20)	3.037±0.372(20)	4.35	–	4.661±0.183(6)	4HM	1A,1B[b]
Chironomus tentans	3.429±0.516(15)	3.292±0.843(49)	3.426±0.349(6)	2.904±0.127(2)	3.689±0.099(4)	3.846±0.177(2)	4HM, 6HM	1A,1B
Culicoides ssp.	3.181±0.276(8)	3.622±1.058(12)	2.539±0.517(16)	4.284±0.175(4)	3.21±0.294(6)	3.393±0.143(4)	4HM, 6HM	1A,1B
Cloeon dipterum	4.403±0.339(9)	1.866±0.757(2)	1.564	4.053±0.396(2)	–	–	4HM	1A,2
Aphelenchus avenae	5.251	3.043±0.025(2)	3.301±0.396(6)	4.861±0.012(3)	4.345±0.015(3)	–	4HM	1A,1B[a]
Caenorhabditis elegans	4.358±0.912(19)	3.958±0.883(11)	3.481±0.319(7)	4.273±1.037(4)	4.377±0.475(5)	4.721±1.402(5)	4HM, 6HM	1A,1B
Panagrellus silusiae	4.31±0.177(5)	2.943±0.402(6)	3.528±0.102(6)	4.814±0.246(6)	3.884±0.2(6)	5.462±0.556(6)	4HM, 6HM	1A,1B
Erpobdella octoculata	3.138±0.196(4)	2.135±0.088(2)	2.371	3.448±0.447(2)	–	–	4HM	1A,2
Limnodrilus hoffmeisteri	3.187±0.56(9)	2.534±0.234(4)	2.532±0.506(5)	3.603	–	–	4HM	1A,2
Nais ssp.	3.299±0.12(2)	2.511±0.57(2)	2.992±0.229(2)	4.148±0.382(2)	–	–	4HM	1A,2
Tubifex tubifex	2.841±0.928(53)	2.233±0.299(30)	1.901±0.428(12)	3.214±0.27(8)	4.272±0.196(3)	3.165±0.405(7)	4HM, 6HM	1A,1B
Lumbriculus variegatus	1.843±0.122(3)	2.147±0.236(3)	2.036±0.184(2)	3.985±0.321(5)	3.336±0.018(2)	4.556±0.118(2)	4HM, 6HM	1A,1B
Lymnaea stagnalis	3.064±0.22(7)	2.875	2.663±0.184(4)	3.841±0.147(3)	–	–	4HM	1A,2
Radix luteola	4.387±0.105(4)	2.718±0.068(3)	2.515±0.09(4)	4.6±0.115(12)	–	–	4HM	1A,2
Viviparus bengalensis	4.255±0.232(5)	3.302±0.257(12)	2.767±0.278(2)	3.971±0.068(4)	3.905	5.099±0.068(4)	4HM, 6HM	1A,1B
Utterbackia imbecillis	1.966±0.518(4)	2.511±0.257(4)	2.547±0.1(4)	2.844±0.201(4)	–	2.26±0.188(4)	4HM, 6HM	1A,1B[b]
Schmidtea ssp.	3.698±0.376(5)	2.801±0.457(36)	2.288±0.623(2)	3.989±0.296(7)	5.501	4.497±0.035(2)	4HM, 6HM	1A,1B

a: only Pb; **b**: only Ni

Table B.4: Overview of the adjusted coefficient of multiple determination (r_{adj}^2) for the bilinear models with four heavy metals (4HM) and six heavy metals (6HM). The 4HM and 6HM models were run with one physicochemical property, whereas for the 6HM model the procedure was repeated with two physicochemical properties. The values in bold represent the highest r_{adj}^2. Pearson correlation (r) shows the relationship between each pair of parameters for the six heavy metals analysed.

	$\mathbf{r_{adj}^2}$		
Physicochemical property/ies	**4 HM**	**6HM**	**Pearson r**
σp	0.633	0.770	–
$\Delta\beta$	0.556	0.600	–
$\lvert\log_{10}$ KOH$\rvert$	0.620	0.605	–
$\log_{10}$ – K_{so}MOH	0.630	0.589	–
AN/ΔIP	0.522	0.425	–
ΔE_o	0.453	0.470	–
Z^2/r	0.512	0.441	–
$X_m{}^2r$	0.672	0.412	–
$\lvert\log_{10}$ KOH$\rvert$ + ΔE_o	–	0.589	-0.555
$\lvert\log_{10}$ KOH$\rvert$ + $\Delta\beta$	–	0.624	0.823
$\lvert\log_{10}$ KOH$\rvert$ + $\log_{10}$ K_{so}MOH	–	0.604	-0.977
$\lvert\log_{10}$ KOH$\rvert$ + $X_m{}^2r$	–	0.644	-0.421
$\lvert\log_{10}$ KOH$\rvert$ + Z^2/r	–	0.606	0.469
$\lvert\log_{10}$ KOH$\rvert$ + AN/ΔIP	–	0.617	-0.593
$\lvert\log_{10}$ KOH$\rvert$ + σp	–	0.798	0.513
ΔE_o + $\Delta\beta$	–	0.595	-0.716
ΔE_o + $\log_{10}$ K_{so}MOH	–	0.589	0.440
ΔE_o + $X_m{}^2r$	–	0.470	-0.235
ΔE_o + Z^2/r	–	0.492	-0.101
ΔE_o + AN/ΔIP	–	0.470	0.190
ΔE_o + σp	–	0.786	-0.522
$\Delta\beta$ + $\log_{10}$ K_{so}MOH	–	0.624	-0.722
$\Delta\beta$ + $X_m{}^2r$	–	0.600	-0.193
$\Delta\beta$ + Z^2/r	–	0.607	0.483
$\Delta\beta$ + AN/ΔIP	–	0.610	-0.564
$\Delta\beta$ + σp	–	0.770	0.752
$\log_{10}$ K_{so}MOH + $X_m{}^2r$	–	0.617	0.379
$\log_{10}$ K_{so}MOH + Z^2/r	–	0.596	-0.334
$\log_{10}$ K_{so}MOH + AN/ΔIP	–	0.589	0.468
$\log_{10}$ K_{so}MOH + σp	–	0.815	-0.434
$X_m{}^2r$ + Z^2/r	–	0.521	-0.830
$X_m{}^2r$ + AN/ΔIP	–	0.504	0.874
$X_m{}^2r$ + σp	–	0.771	0.141
Z^2/r + AN/ΔIP	–	0.441	-0.966
Z^2/r + σp	–	0.774	0.319
AN/ΔIP + σp	–	0.770	-0.236

Table B.5: Bilinear model parameters for the four heavy metal model with 33 species (A) and for the six heavy metal model with 15 species (B). Selected phylogenetic eigenvectors are represented by u and the main effects are represented as 1_{prop} and 1_{phylo} as the main effect of the physiochemical properties (represented by the softness parameter (σp)) and of the phylogeny, respectively. The F-test statistic and the P-values are given for the interaction terms in the given rows or columns of the models.

A	$\mathbf{1_{phylo}}$	σ**p**	**F**	**P**
1_{phylo}	0.876	21.094	–	–
u1	2.609	–	–	–
u2	1.326	–	–	–
u3	1.862	–	–	–
u4	-1.345	–	–	–
u5	0.844	–	2.76	0.1
u6	–	5.7	–	–
u8	-0.741	–	6.769	0.011
u14	–	-8.927	–	–
u16	-0.633	–	–	–
u19	0.706	–	2.615	0.109
u24	–	5.548	–	–
F	4.048	–	19.833	–
P	0.009	–	–	<0.001

B	$\mathbf{1_{phylo}}$	σ**p**	**F**	**P**
1_{phylo}	0.56	25.31	–	–
u1	2.151	–	130.273	<0.001
u2	3.686	-23.637	136.287	<0.001
u3	3.344	-24.177	28.852	<0.001
u4	–	11.124	–	–
u6	0.477	–	2.365	0.128
u10	–	-3.185	–	–
F	74.444	–	34.154	–
P	<0.001	–	–	<0.001

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Appendix C

Supplementary information for: Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale

C.1 Supplementary Methods

C.1.1 Chemical data

Monitoring data for chemical concentrations in the European River Basins were retrieved from the European Environmental Agency (EEA) in July 2012 as part of the Waterbase (version 12) dataset, table *HazSubs* (EEA, 2012a). In total, this dataset comprises >8,200 monitoring sites, covering 34 European countries. Quality control of the reported concentrations was performed following the decision flow chart in Figure C.1, which also provides the number of sites omitted for each step. The dataset was restricted to organic compounds that were identified by the Chemical Abstract Service (CAS) registry number. CAS numbers were checked for consistency and harmonised if necessary. Entries without CAS numbers, which was typically the case when organic compounds are reported as the sum of individual compounds (e.g., PCB congeners), were also removed from the dataset (Figure C.1).

The chemical concentrations were reported in μg/L as mean, minimum and maximum annual values for each monitoring site. The quality control document of the EEA provided guidance on the entries that were problematic, such as entries with missing concentrations or duplicate entries (EEA, 2012b) that were consequently removed from the analysis (Figure C.1).

For entries with limit of quantification (LOQ) or limit of detection (LOD) not reported (or reported as zero) the original files submitted by the national authorities to the EEA were checked and, when available, the LOQ was added to the dataset. Otherwise, the entries were omitted. Mean and maximum concentrations above the LOQ (or LOD) were considered as reliable measurements and were included in the analysis. These values were referred to as quantified concentrations. Mean and maximum concentrations below the LOQ (or LOD) were considered as unreliable measurements and were set to zero. These cases were referred to as non-detects. We note that this procedure is likely to underestimate the real exposure.

Analytical measurements, and consequently LOQ/LOD values, are highly dependent on the monitoring programs of the countries and on the laboratories involved. Given that a non-quantified or non-detected chemical is generally reported as equal to or a fraction of the LOQ/LOD value (e.g., 1/2, 1/4)(CEC, 2000), the ratios between the minimum reported concentrations and the LOQ/LOD values usually result in integer values (e.g., 1,

2, 4), which was the case for all countries, except Spain. In the case of Spain, no integer ratios were obtained, thus indicating that the reported LOQ values were incorrect. The error in reported LOQ values was further underpinned by the fact that the LOQ values did not follow the general rule of rounded values (e.g., 0.05μg/L) but had rather odd entries (e.g., 0.86μg/L). Moreover, due to several sub-basins in the Spanish dataset, more than one LOQ value was reported for each compound. Therefore, the frequency distribution of all maximum concentrations was calculated for each compound to identify LOQ values empirically. In the case of >3 identical maximum values, these repeatedly occurring values were considered to be LOQ values and, in turn, flagged as non-detects. Due to these considerations, the majority (94%) of entries for Spain were flagged as non-detects, which was higher than the average of the other countries (81%). This approach allowed to consider the remaining entries as real maximum concentrations. From this remaining Spanish dataset, 10 entries were removed for the chemical cyanide due to concentration values that were three order of magnitude higher than the mean value found in the other sub-basins.

Geographic coordinates for each site were retrieved from the EEA, Waterbase database, table *Station* (EEA, 2012c), which was connected with the *HazSubs* dataset via the unique site identifier *WatebaseID*. For 148 sites (all in Hungary), the WatebaseID was decapitalised to allow matching. Sites were linked to the river basins of the European Catchments and Rivers Network System database (ECRINS) version 1.0 (EEA, 2012c), which defined the river basins as *"the area of land from which all surface run-off flows through a sequence of streams, rivers and, possibly, lakes into the sea at a single river mouth, estuary or delta"* (CEC, 2000). A few sites (41) were removed due to missing coordinates or coordinates outside Europe.

Sampling sites were considered as spatially autocorrelated when they were (i) less than 5 km apart from each other and (ii) analysed in the same year. Sites that exhibited autocorrelation with several other sites were removed, whereas for pairs of autocorrelated sites, we removed one of the sites randomly. This process led to the omission of 532 sites (Figure C.1). Finally, 4,004 sites were available, covering 91 river basins in 27 European countries.

C.1.2 Concentrations for acute and chronic exposure

The number of samples taken in any monitoring program strongly biases the estimation of chronic exposure. To ensure that the mean annual concentration (C_{mean}) was related to multiple detections of a chemical and was not based on a single quantified concentration (i.e., the maximum concentration), we computed a test mean concentration (C_t) assuming that all other values were non-detects. As outlined above, in the regulatory context, non-detects were usually set as a fraction of the LOQ/LOD and reported as the minimum, which would consequently influence the reported C_{mean} (CEC, 2000). To check whether the reported C_{mean} was determined solely by a single maximum and otherwise minimum annual concentrations (C_{min} and C_{max}), we estimated C_t by:

$$C_t = \frac{(n-1)C_{min} + C_{max}}{n} \tag{C.1}$$

where n is the number of measurements for each entry.

A ratio C_{mean}/C_t >1 would indicate that C_{max} was not the only quantified concentration. However, given that the probability of measuring a compound likely depends on the n, we checked this relationship for sites with >12 measurements. For the cases where the n was >12 (342 sites) and >24 (25 sites), we found that 54% and 60% of the sites

with quantified concentrations had a ratio C_{mean}/Ct >1, respectively. Due to the high uncertainty when drawing conclusions from a small number of sites (e.g., only 5 sites had n >48), we considered n >12 as the best compromise between the number of measurements and the representativeness in terms of number of sites. The ratio C_{mean}/C_t for these sites indicated that at least two quantified values (the maximum annual concentration and another concentration between the maximum and minimum) were used for estimating the reported mean in approximately half of the samples. Thus, the mean can be considered representative for the exposure concentration of at least a few weeks.

However, only 20% of sites had n >12. To remove the dependency of the mean on the LOQ for sites with less frequent sampling (i.e., n≤12), we estimated a corrected mean concentration $C_{c\text{-}mean}$ using the C_{max}/C_{mean} relationship for the sites with n >12. The median for this relationship was estimated as approximately three. Thus, $C_{c\text{-}mean}$ for sites with n ≤12 was estimated as:

$$C_{\text{c-mean}} = \frac{C_{\max}}{3} \tag{C.2}$$

To assess the short-term (acute) exposure, the reported C_{max} was used. For 103 cases where this concentration was not reported, the C_{mean} was used instead. Although episodic peak exposures were likely to be missed (see above), we considered C_{max} as the best approximation of acute exposure in the investigated monitoring programs, especially when considering that several studies have reported a strong relationship between C_{max} and ecological effects (Schäfer et al., 2012; Schäfer et al., 2013; Schulz & Liess, 2000). C_{max} exhibits a higher analytical precision, as it often strongly exceeds the LOQ. The analytical methods are given in Table C.1, and although they were not reported for all compounds, analytical methods for chemical monitoring in Europe are considered to be well established and highly standardised (Lepom et al., 2009). Unless data for only one year were reported, the maximum concentration of each chemical over all years was used to represent the maximum exposure of a site.

C.1.3 Toxicity data

The standard test species used in this analysis (*Pimephales promelas*, *Daphnia magna*, and *Pseudokirchneriella subcapitata*) were selected because they are (i) standard test species for which a vast amount of experimental toxicity data are available; (ii) representatives of the three major organism groups of vertebrates, invertebrates, and plants; (iii) representatives of three major trophic levels of secondary consumers, primary consumers, and primary producers in freshwater ecosystems; and (iv) considered as relatively sensitive to chemical stressors (von der Ohe et al., 2011).

The endpoints used were either the LC50 (median lethal concentration) or the EC50 (median effect concentration). The LC50 is the concentration at which 50% of the test population suffers a lethal response (in the case of fish and invertebrate). The EC50 is the concentration at which 50% of the population suffers an equivalent effect, i.e., immobility for fish and invertebrate, or inhibition of cellular reproduction by 50% for primary producers. To improve readability, we refer to all endpoints as the LC50.

Experimental toxicity data were available for 80% of the compounds for *D. magna* and for 54% of the compounds for both *P. promelas* and *P. subcapitata* (see Table C.1 for sources). Predicted values based on a read-across methodology (Schüürmann, Ebert & Kühne, 2011; Kühne et al., 2013) were used for 15%, 46% and 31% of the compounds for *D. magna*, *P. promelas* and *P. subcapitata*, respectively. Read-across models extrapolate the acute toxicity of non-tested compounds based on the structural similarity of compounds

for which the acute toxicity is available. The similarity of two compounds is obtained by comparing the occurrence of identical atom-centred fragments (ACFs; see Schüürmann, Ebert & Kühne (2011) for details). An algorithm employing ACFs has been developed for the standard test species (Schüürmann, Ebert & Kühne, 2011; Kühne et al., 2013) and the method is fully automated in ChemProp (ChemProp, 2014). The latter was also used to extract physicochemical parameters of compounds (e.g, water solubility and the octanol-water partitioning coefficient: K_{ow}; Table C.1). The level of similarity between compounds would establish the reliability of the predicted values, which were classified from very high to low reliability (see Kühne et al. (2013) for details) and are presented in Table C.1. The prediction performance of the read-across models were assessed using a leave-one-out cross validation technique and explained 75-90% of the variance (Schüürmann, Ebert & Kühne, 2011; Kühne et al., 2013).

For the compounds where the read-across models were not applicable, baseline toxicity was estimated from the compounds' log K_{ow} using the species specific quantitative structure-activity relationship (QSAR; 5% for *D. magna*, and 15% for *P. subcapitata*; see Table C.1 for sources). Baseline toxicity assumes a narcotic mode of action (partioning into membranes), which is typically regarded as the minimal toxicity of an organic compound (Altenburger, Walter & Grote, 2004). Hence, this approach tends to underestimate the true toxicity. If the log K_{ow} was outside of the application domain of the baseline predictions ($1<\log K_{ow}<6$), then this compound was excluded from the analysis (Table C.1; 3 compounds for *P. promelas*, 4 compounds for *D. magna*, and 28 compounds for *P. subcapitata*).

Furthermore, compounds were removed when the respective experimental, predicted or baseline toxicity values were $\geq$10-fold higher (Table C.1; 18 compounds for *P. promelas*, 13 compounds for *D. magna*, 15 compounds for *P. subcapitata*). For three active enantiomers (metalaxyl-m, dichlorprop-p, and mecoprop-p) the measured or predicted values for the enantiomer mixtures were used. Finally, 223 compounds with acute toxicity values for at least one species were used in the analysis, covering 4,001 European sites.

C.1.4 Threshold selection for the assessment of chemical risk

Chemical concentrations above the acute risk threshold (ART - 1/10 of the LC50) are generally recognized to result in acute ecological effects (Schäfer et al., 2011; Schäfer et al., 2012; Van Wijngaarden, Brock & Van den Brink, 2005). Difficulties arise to estimate the threshold levels for ecological communities exposed to mixtures of chemicals for longer periods at lower concentrations. To our knowledge, there is only one meta-analysis that provides a potential threshold for such effects (Schäfer et al., 2012). These studies reported shifts in invertebrate communities towards more tolerant species when exposed to pesticides at 1/1,000 of the *D.magna* LC50 values. Furthermore, a recent study confirmed losses in biodiversity at 1/1,000 of the LC50 values (Beketov et al., 2013). Hence, this value was used as the chronic risk threshold (CRT) for invertebrates. In the absence of similar studies for fish and algae, we used acute-to-chronic ratios (ACR) from laboratory data to derive chronic effect thresholds for these organism groups. This method is based on the empirical calculation of the ratio between the acute toxicity data used here (e.g., LC50/EC50 values) and Chronic No Observed Effect Concentration (NOEC) values or Lowest Observed Effect Concentration (LOEC) for the same species and the same compound. Typically, the ACR is a factor of 10 for fish (Ahlers et al., 2006; Heger et al., 1995; Länge et al., 1998; Mayo-Bean et al., 2012), whereas for algae, the ACR is a factor of 4 to 5 (Mayo-Bean et al., 2012; Ahlers et al., 2006). Algae ACRs are limited due to insufficient acute data (Duboudin, Ciffroy & Magaud, 2004). In the absence of field studies, which

help in validating laboratory-driven thresholds, it is difficult to assess the magnitude of chronic effects for algae and fish, because (i) the test species used here are most likely not the most sensitive species for all substances consisting of various modes of action, (ii) laboratory chronic studies cover still only a short period compared to potential field exposures and the life cycle of many invertebrates and fish, and (iii) toxicity tests for single species cannot integrate ecological concepts, such as recovery or species interaction and the resulting indirect effects (Fleeger, Carman & Nisbet, 2003; Stark, Banks & Vargas, 2004; Relyea & Hoverman, 2006; von der Ohe et al., 2011; Köhler & Triebskorn, 2013).

The chronic risk for fish and algae estimated from the ACR-based threshold could be underestimated in our study, if field studies established lower effect thresholds for these organism groups. For instance, the use of the ACR-based threshold for invertebrates (1/100 of the LC50 values; ACR is approximately a factor of 10 (Ahlers et al., 2006)) would underestimate the chronic risk by approximately 68% compared to the field-based threshold (1/1,000 of the LC50 values).

C.1.5 Likelihood definition

We defined the likelihood for acute and chronic effects in field conditions based on the available literature on the ecological risk thresholds (Table C.3). Observation of acute effects was likely at sites exceeding 1/10 of the LC50 values, because on average in 55% of the cases, such concentrations led to adverse effects on organisms from insecticides and herbicides (Table C.3; Overall chemical). Similarly, it was likely to observe chronic effects on invertebrates at sites that exceed 1/1,000 of the LC50 values, as 71% of the cases reported shifts in invertebrate communities when exposed to pesticides (Table C.3). We note that only pesticide-related threshold studies were available, and this limited availability should be considered when interpreting our results, which relate to organic chemicals in general. However, pesticides were the major contributors to the chemical risk in our study, and in the absence of other studies, we deem our analysis to be based on the best available knowledge.

C.1.6 Suitability of reported limits of quantification for chemical risk assessment

No risk is generally assumed from a compound that is reported below the limit of quantification. However, if the LOQ (and, when not available, the LOD) is greater than any of the two risk thresholds (ART or CRT) for any of the three organism groups, the real concentration might still exceed the respective risk threshold(s). Therefore, for each chemical, we calculated the frequency of sites with non-detects, where the LOQ values exceeded one of the risk thresholds. This procedure was not followed for Spain because the LOQ values for Spain were considered unreliable.

C.1.7 Influence of acute-risk chemicals (ARCs) on chemical risk

The chemical risk was calculated for groups of sites for each river basin at which a given number of ARCs were analysed as:

$$CR_{\mathrm{r,j,b}} = \frac{N_{\mathrm{r,j,b}}}{N_{\mathrm{total,r,b}}} \tag{C.3}$$

where N represents the number of sites for which one of the chemical concentrations exceeded the respective risk threshold j within a river basin b, N_{total} represents the total

number of sites within that river basin, and r=0 to 36 and comprises all sites within a river basin with the respective number of analysed ARCs. A value of r=0 represents sites where no ARCs were analysed, while r=36 represents sites where all ARCs were analysed. The risk threshold j is either the CRT or the ART. All basins that had less than three sites for each r group were excluded from this analysis.

The overall chemical risk in each ARC group r for each threshold j was subsequently calculated as the mean of all basins:

$$CR_{\mathrm{r,j}} = \frac{1}{m}\sum_{i=1}^{m} N_{\mathrm{r,j,i}} \tag{C.4}$$

where i=1,...,m indicates the number of river basins. For each r group, at least three basins were required for the calculation of the mean ($\mathrm{CR_{r,j}}$).

C.2 Supplementary Discussion

C.2.1 Influence of hydrophobic compounds on chemical risk

Compounds with experimental or predicted effect concentrations that strongly exceeded the water solubility were omitted from the analysis (17, 13 and 15 compounds for fish, the invertebrate and algae, respectively, Table C.1). Exceedance of the water solubility usually results in suspensions in the form of undissolved test material, which compromises the quantification of exposure concentrations (Parkerton & Konkel, 2000). However, removal of these hydrophobic compounds from the dataset had only a negligible influence on the chemical risk, except for di-(2-ethylhexyl) phthalate (DEHP). This plasticizer is widely found at measurable concentrations in aquatic ecosystems because of its high production volume (included in more than 50% of the European plasticizers (Oehlmann, Oetken & Schulte-Oehlmann, 2008)). In addition, it has a high potential to persist in the environment and to bioaccumulate in biota (especially for invertebrates (ECB, 2008)) due to its high lipophilicity. Large discrepancies are reported for the experimental and predicted lethal concentrations of DEHP, which will be discussed below.

Short-term experimental toxicity values for DEHP exceeded the water solubility of 3μg/L (at 20^{o}C (ECB, 2008)) by up to five-orders of magnitude for fish, and by up to three-orders of magnitude for the invertebrate and algae (e.g., Adams & Heidolph (1985)). In several studies, a clear dose-response relationship could not be established for DEHP, suggesting that the reported lethal effects were caused through physical mechanisms, such as coating on fish (Parkerton & Konkel, 2000; ECB, 2008). The unrealistically high effect concentrations reported are typically nominal instead of measured concentrations, which leads to an underestimation of DEHP toxicity (ECB, 2008). Given the high uncertainty associated with the experimental effect concentrations for DEHP, we suggest that the risk assessment should be based on QSAR predictions instead.

Using baseline toxicity based on the log $\mathrm{K_{ow}}$ of DEHP (recommended value: 7.5 (ECB, 2008)), LC50 values resulted in 8.5μg/L, 2.2μg/L and 2.1μg/L, for fish, invertebrate and algae, respectively. Note that the quantitative structure-activity relationship (QSAR) models used for prediction are typically linear extrapolations, while it is argued that for hydrophobic compounds with a log $\mathrm{K_{ow}}$>5.5, this relationship is parabolic (see Parkerton & Konkel (2000) and the references therein). Accounting for the deviation from linearity would increase the predicted LC50, and consequently reduce the chemical risk from DEHP. Hence, there is great uncertainty regarding the prediction of effect concentrations and the risk predictions derived from that.

By including the highly uncertain QSAR based LC50 for DEHP in our assessment, the acute and chronic chemical risk would increase by 6% and 2%, respectively, and render this compound responsible for the majority of exceedances (53%) for the acute chemical risk. Thus, DEHP has the clear potential to increase the overall risk, although the quantification of risk with the current knowledge is highly uncertain. To avoid overestimation caused by highly uncertain predicted values, we omitted DEHP from the main analysis, which is also in line with the above mentioned criteria for baseline toxicity predictions' domain ($1<\log K_{ow}<6$). However, this does not rule out the potential of the DEHP or other hydrophobic compounds to cause ecological effects as a result of lethal or sublethal (e.g., endocrine disruption) effects (e.g., Planelló et al. (2011) and Carnevali et al. (2010)).

C.3 Supplementary Figures

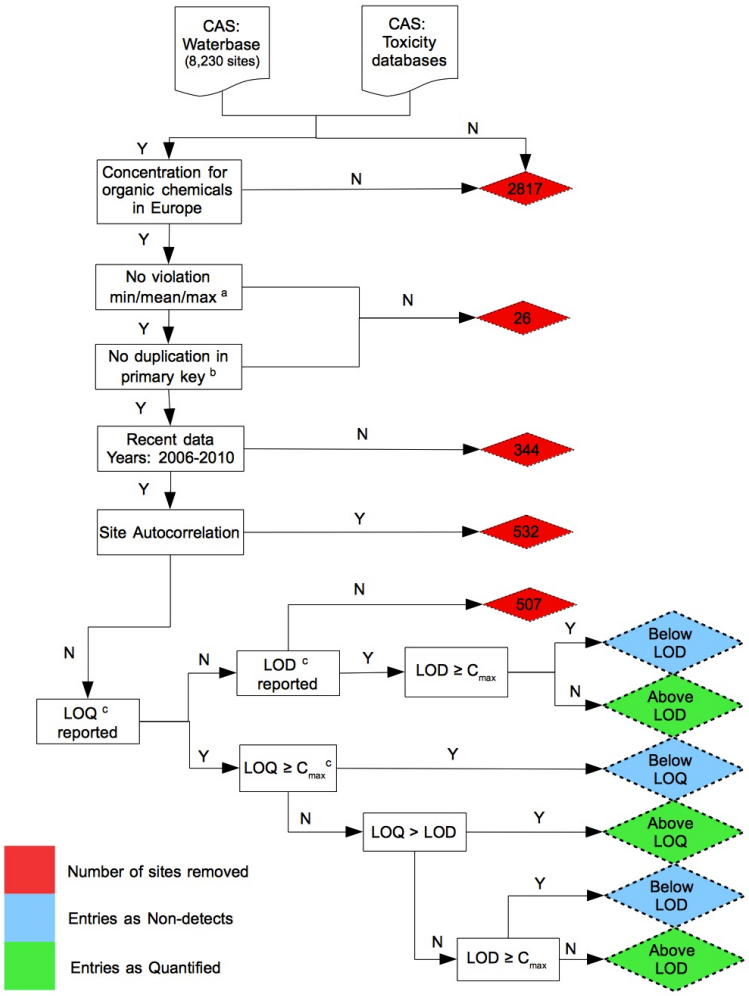

Figure C.1: Flow chart visualising the quality control of the chemical dataset.

[a] The reported concentrations which had the following violations: (i) the mean is missing, (ii) the mean is negative which is not allowed or possible, (iii) the mean is zero which is not allowed or possible, (iv) the minimum is higher than the mean, (v) the mean is higher than the maximum and (vi) the minimum is higher than the maximum (EEA, 2012b)
[b] Primary key is a field or combination of fields with values which have to be unique in the dataset.
[c] LOQ: limit of quantification, LOD: Limit of detection, C_{max} maximum concentration analyzed

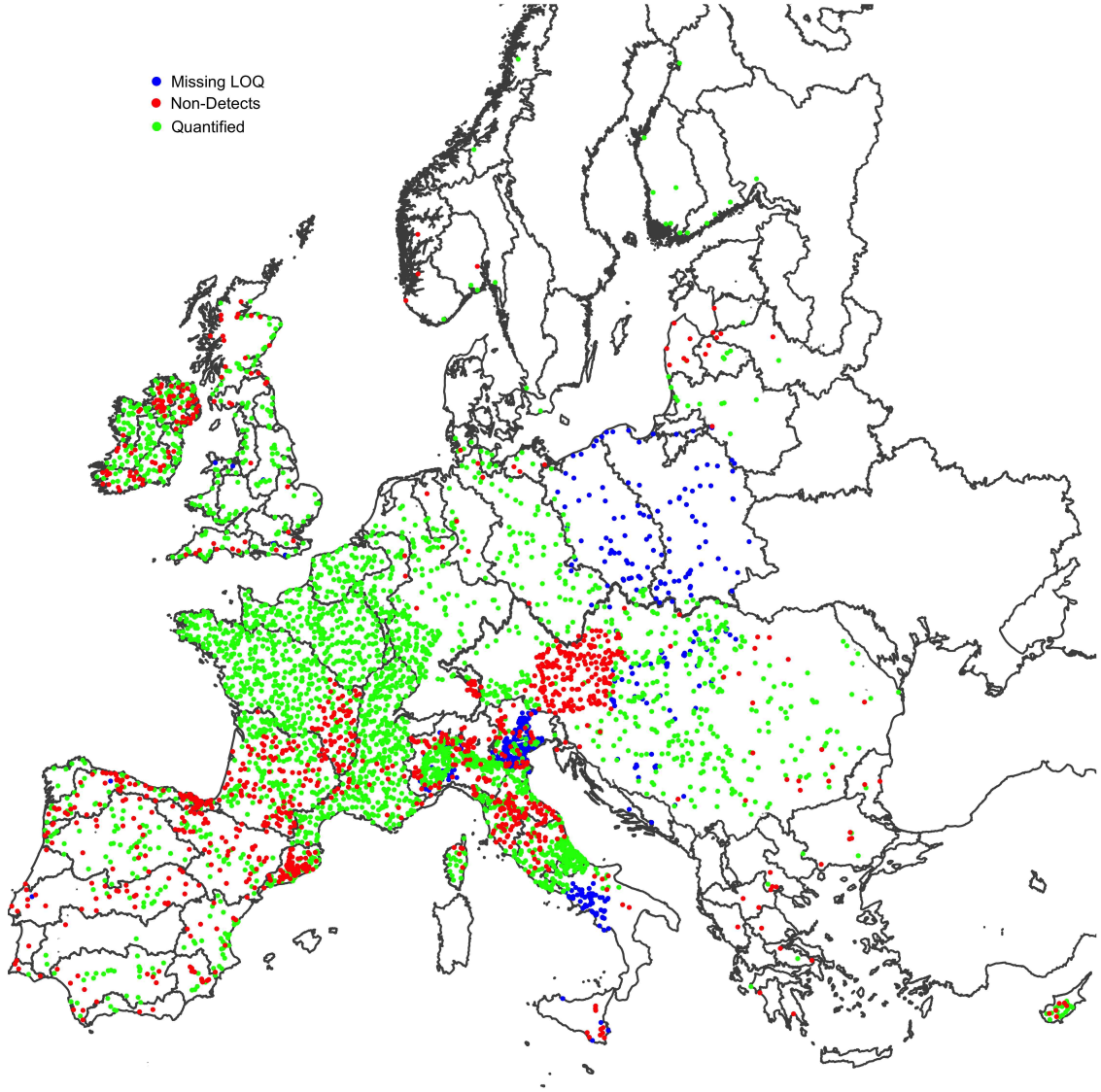

Figure C.2: Overview of monitoring sites in Europe. Sites that have at least one chemical with a maximum concentration above the limit of quantification/limit of detection are classified as "quantified" (n=2890). Sites with all measurements below the limit of quantification/limit of detection are classified as "non-detects" (n=1114), and "missing LOQ" represents the sites which have no information on the limit of quantification/limit of detection (n=506).

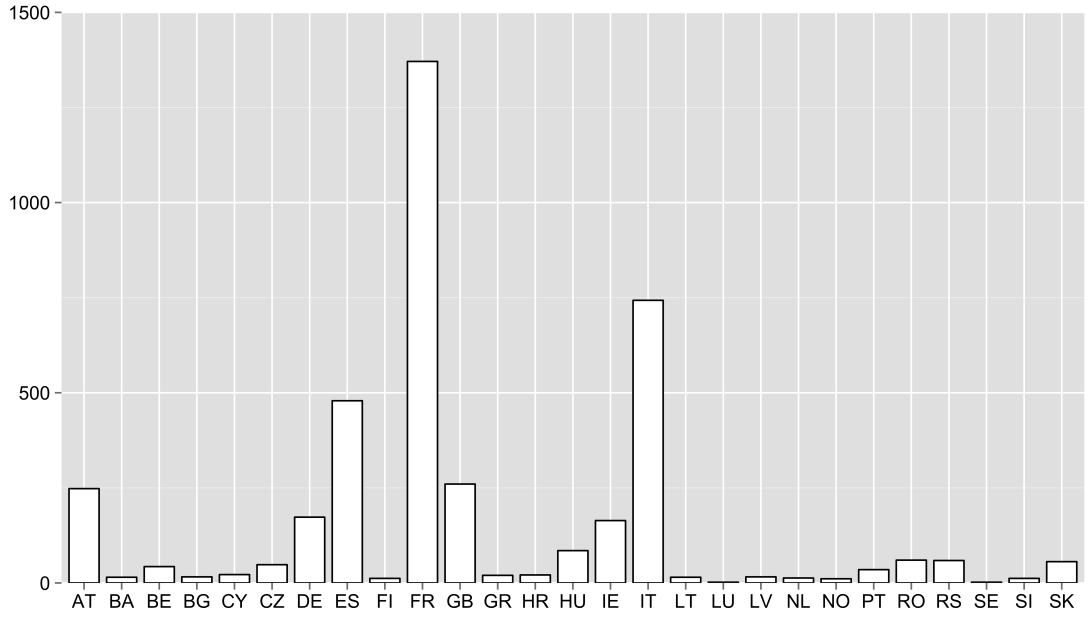

Figure C.3: Number of sites monitored in each country during the 2006-2010 period. The codes correspond to AT: Austria, BA: Bosnia and Herzegovina, BE: Belgium, BG: Bulgaria, CY: Cyprus, CZ: Czech Republic, DE: Germany, ES: Spain, FI: Finland, FR: France, GB: Great Britain, GR: Greece, HR: Croatia, HU: Hungary, IE: Ireland, IT: Italy, LT: Lithuania, LU: Luxembourg, LV: Latvia, NL: Netherlands, NO: Norway, PT: Portugal, RO: Romania, RS: Serbia, SE: Sweden, SI: Slovenia, SK: Slovakia.

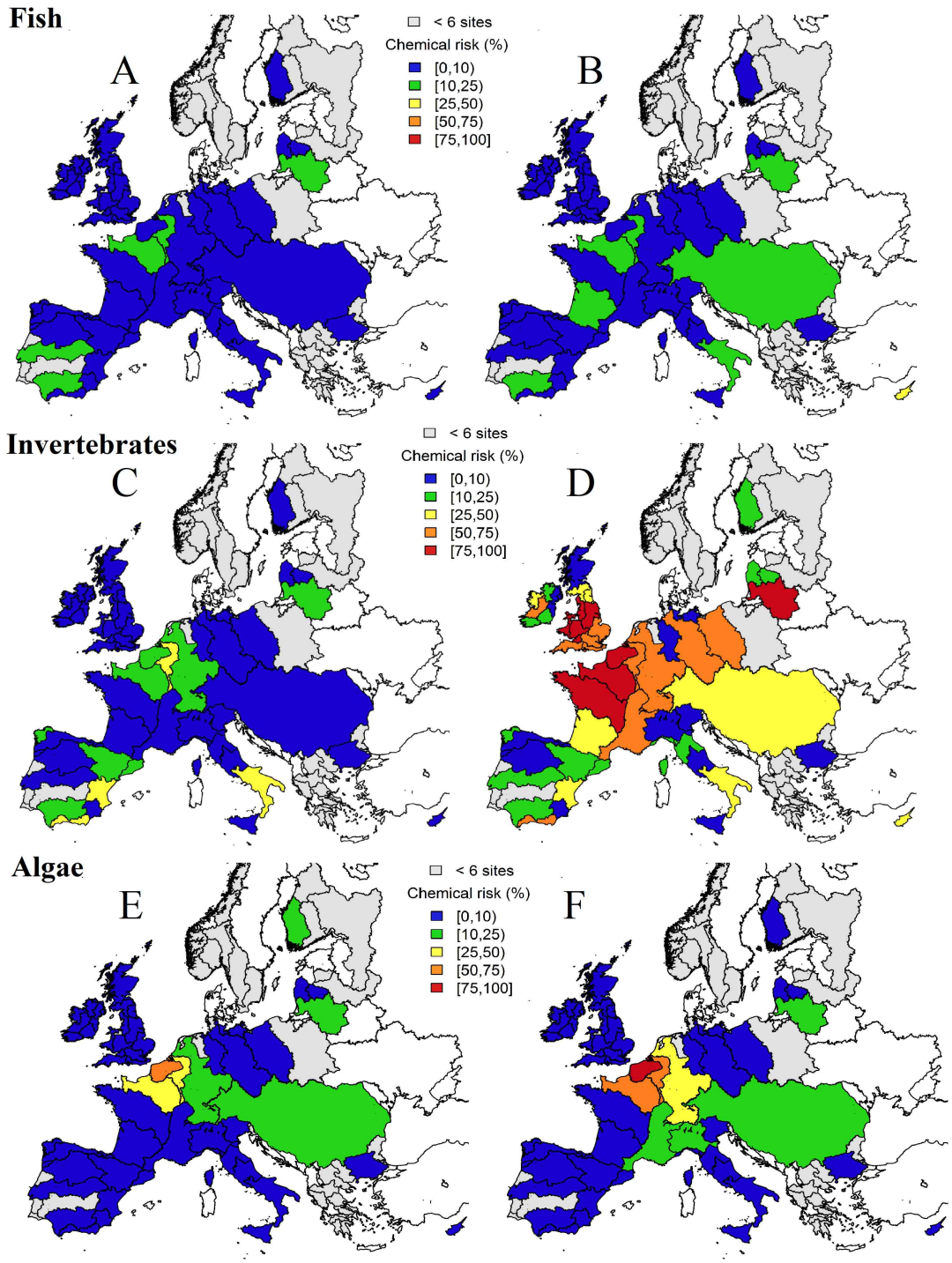

Figure C.4: Chemical risk (by percentage range) in the European river basins separately for the three organism groups. The map displays the fraction of sites which exceed the acute risk threshold (A, C, and E) and the chronic risk threshold (B, D and F) for fish (represented by *P. promelas*; A and B), invertebrates (represented by D. magna; C and D), and algae (represented by *P. subcapitata*; E and F). The colour range shows the level of chemical risk, from low chemical risk (blue) to high chemical risk (red). River basins with up to six sites are displayed in grey, while river basins without data are displayed in white. Direct comparisons between river systems is potentially biased by the ecotoxicologically relevant compounds analysed and the limit of quantification of the compounds.

C.4 Supplementary Tables

Table C.1: Overview of chemicals analyzed and their toxicity. Information on the chemical group, the main applications, the octanol-water partitioning coefficient (K_{ow}), the water solubility and the analytical methods (EN ISO) are given for each chemical, identified by the Chemical Abstract Service (CAS) number. Information on the toxicity comprises the median lethal concentration (LC50) in μg/L, sources of the toxicity values and the type of data for each chemical and each organism group represented by the fish *P. promelas* (PP), the invertebrate *D. magna* (DM), and the algae *P. subcapitata* (PS).

Chemical Group [a]	Main Application [b]	CAS	Chemical Name	log Kow	Water Solubility [μg/L]	LC50 [μg/L] PP	Source PP	Type of data PP [c]	LC50 [μg/L] DM	Source DM	Type of data DM [c]	LC50 [μg/L] PS	Source PS	Type of data PS [c]	Analytical Methods [d]
A	Ins	50293	4,4'- DDT	6.79	8.62	22.0	[1]	E	5.00	[3]	E	-	-	D	-
A	Ins	53190	2,4'-DDD	5.87	83.38	463	[2]	P/1	9.10	[2]	P/4	145	[6]	B	6468:1996
A	Ins	56382	Parathion-ethyl	3.73	3669	1519	[1]	E	2.50	[3]	E	1436	[2]	P/2	6468:1996
A	Ins	57125	Cyanide	-0.69	352690000	155	[1]	E	517	[1]	E	-	-	D	6468:1996
A	Ins	57749	Chlordane	6.26	33.14	96.0	[1]	E	98.4	[1]	E	-	-	WS	-
A	Ins	58899	g-Hexachlorocyclohexane	4.26	3194	82.0	[1]	E	1600	[3]	E	3385	[6]	B	-
A	Ins	60515	Dimethoate	0.72	7454800	34548	[2]	P/1	2000	[3]	E	37000	[1]	E	-
A	Ins	60571	Dieldrin	5.45	120	24.0	[1]	E	250	[3]	E	39.0	[2]	P/2	-
A	Ins	62737	Dichlorvos	0.60	10452000	6230	[1]	E	0.19	[3]	E	737	[1]	E	-
A	Ins	72208	Endrin	5.45	120	1.00	[1]	E	117	[1]	E	39.0	[2]	P/2	-
A	Ins	72435	Methoxychlor	5.67	264	24.0	[1]	E	50.0	[3]	E	1763	[2]	P/1	6468:1996
A	Ins	72548	4,4'- DDD	5.87	83.38	375	[2]	P/1	9.00	[1]	E	614	[2]	P/2	6468:1996
A	Ins	72559	4,4'- DDE	6.00	72.31	91.0	[2]	P/1	13.3	[2]	P/4	240	[2]	P/1	6468:1996
A	Ins	76448	Heptachlor	5.86	103	68.0	[1]	E	42.0	[3]	E	27.0	[3]	E	-
A	Ins	77474	Hexachlorocyclopentadiene	4.63	1241	74.0	[1]	E	46.0	[1]	E	190	[2]	P/4	17993:2003/6468:1996
A	Ins	87865	Pentachlorophenol	4.74	27202	245	[2]	P/4	450	[3]	E	347	[1]	E	-
A	Ins	115297	Endosulfan	3.50	75.69	1.00	[1]	E	440	[3]	E	427.8	[1]	E	-
A	Ins	115322	Dicofol	5.81	9.28	-	-	WS	-	-	WS	-	-	WS	-
A	Ins	121755	Malathion	2.29	6174200	14100	[3]	E	0.70	[3]	E	13000	[3]	E	6468:1996
A	Ins	122145	Phenitrothion	3.30	38355	4000	[3]	E	8.60	[3]	E	1300	[3]	E	-
A	Ins	143500	Kepon	4.91	4258	380	[1]	E	531	[1]	E	1539	[6]	B	-
A	Ins	297789	Isobenzan	4.51	84.38	-	-	WS	8.07	[2]	P/4	-	-	WS	10695:2000/6468:1996
A	Ins	298000	Methyl parathion	2.75	36445	7741	[3]	E	7.30	[3]	E	5400	[1]	E	-
A	Ins	309002	Aldrin	6.06	120	24.0	[1]	E	28.0	[3]	E	74.0	[2]	P/3	-

A	Ins	319846	a-Hexachlorocyclohexane	4.26	3194	82.0	[2]	P/4	370	[3]	E	3385	[6]	B	10695:2000
A	Ins	319857	b-Hexachlorocyclohexane	4.26	3194	82.0	[2]	P/4	1084	[2]	P/4	3385	[6]	B	-
A	Ins	319868	d-Hexachlorocyclohexane	4.26	3194	82.0	[2]	P/4	1084	[2]	P/4	3385	[6]	B	-
A	Ins	465736	Isodrin	6.06	120	167	[2]	P/1	69.3	[2]	P/4	74.0	[2]	P/3	-
A	Ins	470906	Chlorfenvinfos	4.15	1549	360	[2]	P/1	0.25	[3]	E	5226	[6]	B	6468:1996
A	Ins	608731	Hexachlorocyclohexane	4.26	3194	125	[1]	E	1084	[2]	P/4	3385	[6]	B	-
A	Ins	789026	2,4'-DDT	6.79	8.62	85.0	[2]	P/3	5.07	[2]	P/4	-	-	D	-
A	Ins	919868	Demeton-S-Methyl	1.01	4161300	295850	[2]	P/1	23.0	[3]	E	1888914	[6]	B	6468:1996
A	Ins	959988	Endosulfan I	3.50	75.69	1.00	[1]	E	440	[2]	P/4	43.0	[2]	P/2	-
A	Ins	1024573	Heptachloro Epoxide B	4.56	219	471	[2]	P/1	240	[3]	E	40.0	[2]	P/3	-
A	Ins	1113026	Omethoate	-0.79	176770000	-	-	D	21.0	[1]	E	-	-	D	-
A	Ins	1563662	Carbofuran	2.30	368070	844	[4]	E	9.40	[3]	E	6500	[3]	E	-
A	Ins	2310170	Phosalone	4.29	1048	231	[1]	E	0.74	[5]	E	880	[1]	E	-
A	Ins	2385855	Mirex	7.35	246	2.16	[2]	P/1	65.3	[2]	P/4	-	-	D	-
A	Ins	2921882	Chlorpyrifos	5.11	266	500	[3]	E	0.10	[3]	E	1586	[2]	P/3	-
A	Ins	3424826	2,4'-DDE	6.00	72.31	91.8	[2]	P/1	19.3	[2]	P/4	487	[2]	P/1	-
A	Ins	8001352	Toxaphene	6.79	10.37	12.0	[1]	E	14.1	[5]	E	-	-	WS	-
A	Ins	10265926	Methamidophos	-0.93	515720000	465820	[2]	P/1	270	[5]	E	-	-	D	10301:1997
A	Ins	13071799	Terbufos	4.49	2098	142	[1]	E	0.31	[5]	E	2111	[6]	B	-
A	Ins	13356086	Fenbutatin oxide	13.63	0.00	-	-	WS	-	-	WS	-	-	WS	10301:1997
A	Ins	16752775	Methomyl	0.61	20149000	2110	[4]	E	7.60	[5]	E	60000	[1]	E	-
A	Ins	17040196	Demeton-S-methylsulfon	-0.91	120920000	295850	[2]	P/1	259	[2]	P/4	-	-	D	-
A	Ins	18181709	Iodofenphos	5.39	63.43	139	[2]	P/1	1.60	[5]	E	-	-	WS	-
A	Ins	23103982	Pirimicarb	1.40	1748900	100000	[3]	E	17.0	[3]	E	140000	[3]	E	-
A	Ins	39765805	Trans-nonachlor	6.44	9.54	20.0	[2]	P/1	-	-	D	-	-	D	-
A	Ins	52645531	Permethrin	7.43	1.57	16.0	[4]	E	0.60	[5]	E	-	-	D	17353:2005
A	Ins	112410238	Tebufenozide	4.62	679	2116	[2]	P/1	3800	[5]	E	640	[1]	E	-
A	Ins	120928098	Fezaquin	5.76	136	179	[2]	P/1	4.10	[5]	E	208	[5]	E	-
A	Her	75990	Dalapon	1.68	74590000	290000	[1]	E	176220	[2]	B	303432	[6]	B	-
A	Her	88857	Dinoseb	3.67	19662	700	[4]	E	240	[7]	E	20496	[2]	P/3	-
A	Her	93721	Fenoprop	3.68	59374	23000	[3]	E	4379	[2]	B	218197	[2]	P/3	-
A	Her	93765	2,4,5-Trichlorophenoxyacetic Acid	3.26	193480	57711	[2]	P/3	5000	[3]	E	28042	[2]	P/3	-
A	Her	94746	MCPA	2.52	836260	134550	[2]	P/2	190000	[3]	E	15756	[1]	E	-
A	Her	94757	2,4-D	2.62	495230	63400	[5]	E	148281	[7]	E	30624	[1]	E	-
A	Her	94815	MCPB	3.50	117730	12500	[1]	E	55000	[3]	E	41000	[3]	E	6468:1996
A	Her	94826	2,4'-DB	3.60	127100	18000	[3]	E	25000	[3]	E	33636	[2]	P/3	-
A	Her	120365	Dichlorprop	3.03	690320	71895	[2]	P/3	100000	[5]	E	194000	[1]	E	-
A	Her	122349	Simazine	2.40	34471	34921	[2]	P/1	94000	[3]	E	275	[3]	E	-
A	Her	139402	Propazine	3.24	6371	5017	[2]	P/1	11000	[3]	E	29.0	[3]	E	-
A	Her	314409	Bromacil	1.68	680850	186000	[3]	E	121000	[1]	E	7.00	[3]	E	-
A	Her	330541	Diuron	2.67	63509	14200	[3]	E	5700	[3]	E	2.00	[3]	E	-
A	Her	330552	Linuron	2.91	117270	9481	[2]	P/1	477	[3]	E	16.0	[3]	E	-

A	Her	534521	Dinitro-o-Cresol	2.27	515150	1745	[4]	E	3200	[7]	E	50000	[2]	P/4	-
A	Her	834128	Ametryne	3.32	183640	10850	[1]	E	28000	[3]	E	4.00	[3]	E	6468:1996
A	Her	886500	Terbutryn	3.77	15673	3441	[2]	P/1	7100	[1]	E	3.00	[1]	E	-
A	Her	1007289	Desisopropylatrazine	1.36	1213500	89255	[2]	P/1	132340	[2]	P/3	77.0	[2]	P/3	6468:1996
A	Her	1014693	Desmetryn	2.82	114350	2859	[2]	P/1	26000	[1]	E	25.0	[5]	E	6468:1996
A	Her	1066519	Aminomethylphosphonic Acid	-2.47	141410000000	-	-	D	-	-	D	-	-	D	-
A	Her	1071836	Glyphosate	-4.77	6263500000	29867	[1]	E	40000	[5]	E	38964	[1]	E	6468:1996
A	Her	1194656	Dichlobenil	2.83	62544	6532	[3]	E	6200	[3]	E	11024	[2]	P/3	6468:1996
A	Her	1582098	Trifluralin	5.31	284	119	[3]	E	245	[3]	E	12.2	[3]	E	-
A	Her	1610180	Prometon	3.57	119970	6611	[2]	P/1	41167	[1]	E	98.0	[1]	E	-
A	Her	1689834	Ioxynil	3.94	128340	6800	[4]	E	3900	[5]	E	7276	[2]	P/1	-
A	Her	1689845	Bromoxynil	3.39	43879	12500	[3]	E	12500	[3]	E	4229	[1]	E	17993:2003
A	Her	1689992	Bromoxynil Octanoate	5.86	15.75	-	-	WS	46.0	[5]	E	-	-	WS	-
A	Her	1698608	Chloridazon	0.76	370690	545230	[2]	P/1	132000	[3]	E	3000	[3]	E	17993:2003
A	Her	1702176	Clopyralid	1.63	391450	63700	[2]	P/1	225000	[9]	E	30500	[5]	E	-
A	Her	1836755	Nitrofen	4.32	144	666	[2]	P/3	217	[3]	E	123	[2]	P/3	-
A	Her	1861401	Benfluralin	5.31	215	100.0	[1]	E	1143	[1]	E	-	-	WS	17993:2003
A	Her	1912249	Atrazine	2.82	14850	13525	[1]	E	54000	[3]	E	143	[3]	E	17993:2003
A	Her	1918009	Dicamba	2.14	497360	45796	[2]	P/1	110300	[2]	P/4	3700	[1]	E	-
A	Her	1918134	Chlorthiamid	2.96	8414	6444	[2]	P/1	6343	[2]	P/3	6538	[2]	P/1	-
A	Her	2008584	2,6-dichlorobenzamide	0.90	73738	469000	[3]	E	180000	[3]	E	5222	[2]	P/2	-
A	Her	2164081	Lenacil	3.09	158330	19357	[2]	P/1	8400	[3]	E	7.70	[3]	E	-
A	Her	3397624	Deisopropyldeethylatrazine	0.32	42275000	498310	[2]	P/1	-	-	D	115	[2]	P/2	6468:1996
A	Her	5915413	Terbutylazine	3.27	924	8782	[2]	P/1	13100	[1]	E	12.0	[3]	E	10301:1997
A	Her	6190654	Desethylatrazine	1.78	31628	86693	[2]	P/1	76529	[2]	P/4	2000	[1]	E	-
A	Her	7085190	Mecoprop	2.94	281400	35134	[2]	P/2	200000	[5]	E	10000	[1]	E	-
A	Her	7287196	Prometryn	3.73	23565	2513	[2]	P/1	12660	[3]	E	16.0	[3]	E	10301:1997
A	Her	13684565	Desmedipham	3.22	12861	9041	[5]	E	450	[5]	E	10	[5]	E	6468:1996
A	Her	15972608	Alachlor	3.37	170160	5000	[3]	E	10000	[3]	E	5.00	[3]	E	-
A	Her	16672870	2-Chloroethylphosphonic Acid	-0.25	131050000	130000	[1]	E	31700	[5]	E	1400	[1]	E	-
A	Her	21087649	Metribuzin	1.49	1970200	247100	[2]	P/1	49000	[3]	E	48.0	[3]	E	-
A	Her	21725462	Cyanazine	2.51	6754	18625	[1]	E	49000	[3]	E	22.0	[3]	E	-
A	Her	23950585	Propyzamide	3.57	19546	1658	[2]	P/1	5600	[1]	E	760	[1]	E	-
A	Her	25057890	Bentazone	1.67	1003000	185940	[2]	P/1	125000	[3]	E	4500	[3]	E	-
A	Her	26225796	Ethofumesate	2.89	119440	38663	[2]	P/1	14000	[3]	E	3900	[3]	E	-
A	Her	26259450	Secbumeton	3.64	96381	8527	[2]	P/1	3992	[2]	B	73.0	[2]	P/3	-
A	Her	30125634	Desethylterbutylazine	2.23	20915	41547	[2]	P/1	42000	[3]	E	11.0	[2]	P/3	-
A	Her	33693048	Terbumeton	3.60	83338	51500	[1]	E	40000	[5]	E	42.5	[1]	E	-
A	Her	34123596	Isoproturon	2.84	77471	17586	[2]	P/1	580	[3]	E	13.6	[1]	E	-
A	Her	34256821	Acetochlor	3.37	866070	6105	[2]	P/3	8600	[5]	E	1430	[1]	E	18857-1:2006
A	Her	40487421	Pendimethalin	4.82	1205	164	[2]	P/1	280	[3]	E	6.00	[3]	E	-
A	Her	41394052	Metamitron	1.44	2511300	133740	[2]	P/1	97000	[1]	E	34909	[2]	P/2	-

A	Her	51218452	Metolachlor	3.24	221820	8200	[1]	E	15595	[3]	E	38.0	[3]	E	-
A	Her	51235042	Hexazinone	2.15	334940	274000	[1]	E	85000	[3]	E	14.0	[3]	E	-
A	Her	55512339	Pyridate	5.73	52.80	97.0	[2]	P/1	-	-	WS	227	[6]	B	-
A	Her	64902723	Chlorsulfuron	2.26	3198	-	-	WS	-	-	WS	68.0	[5]	E	-
A	Her	67129082	Metazachlor	2.38	153130	81650	[2]	P/1	33000	[3]	E	16.0	[3]	E	18856:2005
A	Her	74223646	Metsulfuronmethyl	2.00	3828	-	-	WS	-	-	WS	395	[1]	E	-
A	Fun	50000	Formaldehyde	0.35	57018000	24100	[1]	E	19200	[1]	E	4249	[1]	E	-
A	Fun	82688	Pentachloronitrobenzene	5.03	270	55.0	[2]	P/1	770	[3]	E	493	[2]	P/2	-
A	Fun	96457	Ethylenethiourea	-0.49	3076200000	1957000	[2]	P/1	21600	[5]	E	93800	[5]	E	-
A	Fun	118741	Hexachlorobenzene	5.86	18.20	56.9	[2]	P/2	5.70	[3]	E	30.0	[1]	E	-
A	Fun	133062	Captan	2.74	55683	120	[1]	E	7100	[5]	E	1180	[5]	E	-
A	Fun	137268	Thiram	1.70	9613000	24.0	[1]	E	11.0	[5]	E	65.0	[5]	E	-
A	Fun	137304	Ziram	1.14	12291000	8.00	[1]	E	48.0	[5]	E	66.0	[5]	E	-
A	Fun	57837191	Metalaxyl	1.70	10208000	171160	[2]	P/1	28000	[3]	E	688	[2]	P/2	-
A	Fun	60207901	Propiconazole	4.13	2051	3246	[2]	P/1	10200	[3]	E	5333	[1]	E	-
A	Fun	67306030	Fenpropimorph	5.50	2397	530	[2]	P/1	2240	[5]	E	327	[5]	E	-
A	Fun	136426545	Fluquincozole	3.73	137	-	-	WS	-	-	WS	46.0	[5]	E	-
B	Lub,Pla	2051243	PCB 209	10.20	0.0014	-	-	WS	-	-	WS	-	-	WS	-
B	Lub,Pla	7012375	PCB 28	5.69	241	74.0	[2]	P/4	160	[3]	E	120	[2]	P/3	-
B	Lub,Pla	31508006	PCB 118	6.98	9.38	9.00	[2]	P/4	10.1	[2]	P/4	-	-	D	-
B	Lub,Pla	32598133	PCB 77	6.34	1.14	2.00	[1]	E	2.00	[7]	E	-	-	D	-
B	Lub,Pla	32598144	PCB 105	6.98	9.38	8.94	[2]	P/4	10.1	[2]	P/4	-	-	D	18857-1:2006
B	Lub,Pla	32774166	PCB 169	7.62	1.80	0.60	[2]	P/4	5.06	[2]	P/4	-	-	D	-
B	Lub,Pla	35065271	PCB 153	7.62	3.07	1.00	[1]	E	1.30	[3]	E	-	-	D	-
B	Lub,Pla	35065282	PCB 138	7.62	3.07	0.60	[2]	P/4	6.08	[2]	P/4	-	-	D	-
B	Lub,Pla	35065293	PCB 180	8.27	0.46	0.53	[2]	P/3	2.10	[2]	P/4	-	-	D	-
B	Lub,Pla	35693993	PCB 52	6.34	19.66	30.0	[1]	E	30.0	[1]	E	-	-	D	-
B	Lub,Pla	35694087	PCB 194	8.91	0.05	0.20	[1]	E	0.20	[1]	E	-	-	D	-
B	Lub,Pla	37680732	PCB 101	6.98	9.38	10.0	[1]	E	10.00	[1]	E	-	-	D	-
B	Lub,Pla	38380084	PCB 156	7.62	2.27	0.60	[2]	P/4	6.66	[2]	P/4	-	-	D	-
B	Lub,Pla	57465288	PCB 126	6.98	9.38	8.94	[2]	P/4	10.1	[2]	P/4	-	-	D	-
B	Lub,Pla	69782907	PCB 157	7.62	2.27	0.60	[2]	P/4	6.66	[2]	P/4	-	-	D	-
C	Byprod	50328	Benzo[a]pyrene	6.11	1.90	6.00	[8]	E	1.80	[1]	E	15.0	[3]	E	6468:1996
C	Byprod	53703	Dibenz[ah]anthracene	6.70	0.33	-	-	WS	-	-	WS	-	-	WS	6468:1996
C	Byprod	56553	Benz[a]anthracene	5.52	7.56	-	-	WS	71.6	[1]	E	-	-	WS	6468:1996
C	Byprod	83329	Acenaphthene	4.15	5239	1730	[3]	E	2350	[3]	E	520	[3]	E	-
C	Byprod	85018	Phenanthrene	4.35	665	1016	[2]	P/3	598	[3]	E	411	[3]	E	-
C	Byprod	86737	Fluorene	4.02	4081	2457	[2]	P/1	430	[3]	E	3400	[1]	E	-
C	Byprod	90120	1-Methylnaphthalene	3.72	39933	9000	[1]	E	1422	[1]	E	12000	[1]	E	-
C	Byprod	91203	Naphthalene	3.17	90836	6140	[3]	E	15000	[3]	E	25000	[3]	E	-
C	Byprod	91576	2-Methylnaphthalene	3.72	10479	8993	[2]	P/4	1670	[1]	E	3281	[2]	P/2	-
C	Byprod	120127	Anthracene	4.35	665	1015	[2]	P/3	428	[3]	E	225	[2]	P/3	-
C	Byprod	129000	Pyrene	4.93	27.52	200	[1]	E	195	[1]	E	-	-	WS	-
C	Byprod	135193	2-naphthalenol	2.69	204340	3500	[3]	E	3540	[3]	E	18800	[1]	E	-

C	Byprod	206440	Fluoranthene	4.93	16.83	-	-	WS	106	[3]	E	-	-	WS	-
C	Byprod	208968	Acephthylene	3.76	8394	3659	[2]	P/1	4204	[2]	P/3	509	[2]	P/3	-
C	Byprod	1634044	MTBE	1.43	59229000	672000	[4]	E	136000	[10]	E	73339	[2]	P/1	-
D	Sol	87616	1,2,3-Trichlorbenzene	3.93	27463	2987	[2]	P/4	1850	[3]	E	900	[3]	E	-
D	Sol	95501	1,2-Dichlorobenzene	3.28	118710	9470	[3]	E	2300	[3]	E	49800	[1]	E	-
D	Sol	108703	1,3,5-Trichlorobenzene	3.93	15096	2987	[2]	P/4	7295	[2]	P/4	1685	[2]	P/3	-
D	Sol	108907	Monochlorobenzene	2.64	199530	25185	[1]	E	19241	[3]	E	12500	[1]	E	-
D	Sol	120821	1,2,4-Trichlorobenzene	3.93	30356	2990	[3]	E	1700	[1]	E	16867	[1]	E	-
D	Sol	541731	1,3-Dichlorobenzene	3.28	118710	8030	[3]	E	9733	[3]	E	4509	[2]	P/3	-
D	Sol	608935	Pentachlorobenzene	5.22	821	306	[3]	E	1327	[1]	E	6600	[3]	E	-
E	Sol	56235	Carbon tetrachloride	2.44	388470	43000	[5]	E	29400	[3]	E	70443	[6]	B	6468:1996
E	Sol	67663	Trichloromethane	1.52	6622200	70700	[3]	E	228967	[3]	E	349909	[6]	B	-
E	Sol	71556	1,1,1-Trichloroethane	2.68	2276400	52900	[3]	E	11200	[1]	E	37645	[6]	B	6468:1996
E	Sol	75092	Dichloromethane	1.34	17630000	330000	[4]	E	220000	[3]	E	357960	[6]	B	-
E	Sol	75274	Bromodichloromethane	1.61	3215700	220912	[2]	P/1	234970	[2]	B	400476	[6]	B	6468:1996
E	Sol	75343	1,1-Dichloroethane	1.76	6154500	114673	[2]	P/1	92185	[2]	P/4	6460	[2]	P/1	6468:1996
E	Sol	75354	1,1-Dichloroethene	2.12	1846200	135060	[3]	E	51000	[3]	E	23923	[2]	P/1	-
E	Sol	75694	Trichlorofluoromethane	2.13	1120600	59442	[2]	P/1	63928	[2]	B	117591	[6]	B	6468:1996
E	Sol	75718	Dichlorodifluoromethane	1.82	503450	107990	[2]	P/1	110070	[2]	B	193462	[6]	B	-
E	Sol	79005	1,1,2-Trichloroethane	2.01	4998600	81600	[3]	E	107333	[3]	E	144600	[3]	E	-
E	Sol	79016	1,1,2-Trichloroethene	2.47	696870	44100	[3]	E	59000	[7]	E	56637	[6]	B	-
E	Sol	87683	Hexachlorobutadiene	4.72	1612	90.0	[4]	E	500	[3]	E	143	[2]	P/2	-
E	Sol	106934	1,2-Dibromoethane	2.01	3663400	5074	[2]	P/1	119000	[3]	E	204878	[6]	B	-
E	Sol	107062	1,2-Dichloroethane	1.83	9887600	136000	[3]	E	507000	[3]	E	16254	[2]	P/1	-
E	Sol	127184	Tetrachloroethylene	2.97	187090	13400	[3]	E	14000	[3]	E	5028	[2]	P/2	-
E	Sol	156592	Cis-1,2-Dichloroethylene	1.98	4980700	30498	[2]	P/1	62420	[2]	B	112317	[6]	B	-
E	Sol	156605	Trans-1,2-Dichloroethylene	1.98	4980700	30498	[2]	P/1	220000	[3]	E	112317	[6]	B	-
E	Sol	540590	1,2-Dichloroethene	1.98	4980700	30498	[2]	P/1	62420	[2]	B	112317	[6]	B	-
E	Sol	1070786	1,1,1,3-Tetrachloropropane	3.42	398470	6611	[2]	P/1	11184	[2]	P/4	11530	[6]	B	-
F	Sur	58902	2,3,4,6-Tetrachlorophenol	4.09	125820	1030	[4]	E	175	[3]	E	1300	[3]	E	10695:2000/6468:1996
F	Sur	59507	4-Chloro-3-Methylphenol	2.70	1055700	7380	[3]	E	2000	[3]	E	25157	[2]	P/3	10695:2000/6468:1996
F	Sur	80057	Bisphenol-a	3.64	367810	4650	[1]	E	10313	[1]	E	2900	[1]	E	-
F	Sur	87650	2,6-Dichlorophenol	2.80	8273000	7748	[2]	P/4	3400	[3]	E	29000	[3]	E	-
F	Sur	88062	2,4,6-Trichlorophenol	3.45	564320	2285	[2]	P/4	1710	[3]	E	3500	[3]	E	-
F	Sur	95487	2-Methylphenol	2.06	17303000	14000	[3]	E	14689	[3]	E	65000	[3]	E	12918:1999/10695:2000
F	Sur	95874	2,5-Dimethylphenol	2.61	1749300	18457	[2]	P/4	6750	[1]	E	7053	[2]	P/3	-
F	Sur	95954	2,4,5-Trichlorophenol	3.45	550940	902	[3]	E	900	[3]	E	1200	[3]	E	-
F	Sur	100027	4-Nitrophenol	1.91	7507500	45633	[1]	E	15200	[7]	E	4540	[1]	E	-
F	Sur	104405	4-Nonylphenol	5.99	215	140	[4]	E	135	[1]	E	515	[1]	E	-
F	Sur	106445	4-Methylphenol	2.06	15703000	16500	[3]	E	4550	[1]	E	10148	[2]	P/3	-
F	Sur	120832	2,4-Dichlorophenol	2.80	3602500	7750	[3]	E	2600	[3]	E	31520	[3]	E	-
F	Sur	140669	Para-tert-octylphenol	5.28	16110	48.0	[1]	E	90.0	[7]	E	1900	[2]	P/4	-
F	Sur	526750	2,3-Dimethylphenol	2.61	12016000	18457	[2]	P/4	22500	[1]	E	10215	[2]	P/3	-

F	Sur	732263	2,4,6-tri-tert-Butylphenol	6.39	268	61.0	[1]	E	110	[2]	P/4	-	-	D	-
F	Sur	1570645	4-Chloro-2-Methylphenol	2.70	1055700	5471	[2]	P/4	290	[3]	E	29706	[2]	P/3	-
F	Sur	1806264	4-Octylphenol	5.50	738	287	[2]	P/3	270	[2]	P/4	189	[2]	P/3	17993:2003
F	Sur	4901513	2,3,4,5-Tetrachlorophenol	4.09	122490	410	[3]	E	1760	[3]	E	877	[2]	P/3	10301:1997
F	Sur	84852153	4-Nonylphenol, branched	5.92	246	138	[1]	E	137	[2]	P/4	410	[1]	E	-
G	AOxi	71432	Benzene	1.99	1093300	24600	[3]	E	31277	[2]	E	35000	[3]	E	-
G	AOxi	95476	o-Xylene	3.09	189810	16400	[1]	E	17500	[3]	E	4450	[3]	E	-
G	AOxi	100414	Ethylbenzene	3.03	166690	12100	[3]	E	18870	[1]	E	5300	[3]	E	-
G	AOxi	100425	Styrene	2.89	292600	15928	[3]	E	4721	[2]	E	1218	[3]	E	-
G	AOxi	106423	p-Xylene	3.09	189880	8400	[1]	E	32400	[3]	E	3752	[3]	E	-
G	AOxi	108383	m-Xylene	3.09	189790	16000	[1]	E	17584	[1]	E	4368	[3]	E	-
G	AOxi	1330207	Xylene	3.09	189790	27710	[1]	E	17500	[3]	E	13099	[6]	B	6468:1996
G	AOxi	1825214	Pentachloroanisole	5.30	139	650	[1]	E	27.0	[7]	E	326	[2]	P/3	-
H	Sol	106434	4-Chlorotoluene	3.18	60804	3596	[2]	P/2	3570	[3]	E	7243	[2]	P/3	-
H	Sol	108883	Toluene	2.54	461360	36200	[3]	E	6000	[1]	E	10950	[3]	E	-
H	Sol	118967	2,4,6-Trinitrotoluene	1.99	215060	2190	[3]	E	9250	[3]	E	729	[3]	E	6468:1996
H	Sol	121142	2,4-Dinitrotoluol	2.18	301100	24300	[3]	E	35000	[3]	E	807	[1]	E	-
I	AFun	14488530	Dibutyltin	0.57	9566900	1776100	[2]	P/1	900	[3]	E	6544	[2]	P/3	10301:1997
I	AFun	56573854	Tributyltin	7.35	7.41	4.00	[1]	E	4.30	[3]	E	3.00	[1]	E	-
J	FlRet	85223	Pentabromoethylbenzene	7.48	0.29	-	-	WS	2.18	[2]	P/4	-	-	WS	-
J	FlRet	101553	4-Bromophenyl phenyl ether	4.94	2284	575	[1]	E	360	[7]	E	2339	[2]	P/2	-
J	FlRet	41318756	PBDE 28	5.88	38.91	88.7	[2]	P/1	248	[2]	P/4	180	[6]	B	-
J	FlRet	5436431	PBDE 47	6.77	0.31	-	-	WS	-	-	WS	-	-	D	-
J	FlRet	60348609	PBDE 99	7.66	0.01	-	-	WS	-	-	WS	-	-	D	-
J	FlRet	68631492	PBDE 153	8.55	3.07	-	-	WS	-	-	WS	-	-	D	-
J	FlRet	182346210	PBDE 85	7.66	0.01	-	-	WS	-	-	WS	-	-	D	-
K	Pla	84662	Di-ethyl phthalate	2.65	531890	21867	[1]	E	54235	[7]	E	48603	[1]	E	-
K	Pla	84742	Di-(n-butyl) phthalate	4.61	14851	1610	[1]	E	3700	[7]	E	400	[1]	E	-
K	Pla	85687	Butyl benzyl phthalate	4.84	2272	1533	[1]	E	2000	[7]	E	120	[1]	E	6468:1996
K	Pla	117817	Di-(2-ethylhexyl) phthalate	7.5	3	-	-	WS	-	-	WS	-	-	WS	-
K	Pla	131113	Dimethyl phthalate	1.66	1790100	121000	[4]	E	33000	[7]	E	70367	[1]	E	-
K	Pla	131168	Dipropyl phthalate	3.63	419510	5523	[2]	P/3	9274	[2]	P/4	15200	[2]	P/3	-
K	Pla	131180	Dipentyl phthalate	5.59	1162	153	[2]	P/3	391	[2]	P/4	5872	[2]	P/3	-
L	Mis	60004	EDTA	-3.86	4848600	59800	[1]	E	-	-	D	40842	[2]	P/1	-
L	Mis	75014	Vinylchloride	1.62	4837500	26624	[2]	P/1	87715	[2]	B	149716	[6]	B	-
L	Mis	107131	Acrylonitrile	0.21	69155000	16980	[1]	E	8800	[7]	E	2186680	[6]	B	-
L	Mis	139139	Nitrilotriacetic acid	-3.81	1839000000	-	-	D	-	-	D	30695	[2]	P/1	-
L	Mis	2599113	Hydroxysimazine	1.67	833040	132410	[2]	P/1	165760	[2]	P/3	36.0	[2]	P/3	-

[a] **A**: Pesticides and transformation products, **B**: Polychlorinated biphenyls, **C**: Polycyclic aromatic hydrocarbons and derivates, **D**: Halogenated benzenes and nitrobenzenes, **E**: Halogenated alkanes, **F**: Phenols and chlorophenols, **G**: Anilines, anisoles and alkylated benzenes, **H**: Toluenes and halogenated derivatives, **I**: Organotin compounds, **J**: Brominated flame retardants, **K**: Phthalates, **L**: Miscellaneous.
[b] **Her**: Herbicides, **Fun**: Fungicides, **Ins**: Insecticides, **Lub,Pla**: Lubricants, plasticizers, **Byprod**: Byproducts of petroleum processing or combustion, **Sol**: Solvent, **Sur**: Surfactant, **AOxi**: Anti-oxidants, **AFun**: Anti-fungal, **FlRet**: Flame retardants, **Pla**: Plasticizer, **Mis**: Miscellaneous

[c] **E**: Experimental toxicity data from literature, **B**: Baseline toxicity data estimated from the octanol/water partitioning coefficient, **P**: Predicted toxicity data from read across together with the level of reliability (1: low ; 2: moderate ; 3: high and 4: very high). Toxicity values were removed when they lay outside of the model domain ($1 < \log K_{ow} < 6$) for the baseline prediction **(D)**, or when they exceed water solubility by a factor of 10 **(WS)**.

[d] DIN EN ISO 6468:1996 - Water quality. Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes - Gas-chromatographic method after liquid-liquid extraction; DIN EN ISO 17993:2003 - Water quality. Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction; DIN EN ISO 10301:1997 - Water quality. Determination of highly volatile halogenated hydrocarbons – Gas chromatographic methods; EN ISO 10695:2000 - Water quality. Determination of selected organic nitrogen and phosphorus compounds – Gas chromatographic methods; EN ISO 18856:2005 - Water quality. Determination of selected phthalates using gas chromatography/mass spectrometry; EN ISO 17353:2005 - Water quality. Determination of selected organotin compounds. Gas chromatographic method; EN ISO 18857-1:2006 - Water quality. Determination of selected alkylphenols - Part 1: Method for non-filtered samples using liquid-liquid extraction and gas chromatography with mass selective detection; DIN EN 12918:1999 - Water quality. Determination of parathion, parathion-methyl and some other organophosphorus compounds in water by dichloromethane extraction and gas chromatographic analysis.

[e] [1] (USEPA, 2014a); [2] (ChemProp, 2014); [3] (Schäfer et al., 2011); [4] (USEPA, 2014b); [5] (PPDB, 2013); [6] (Arnoja et al., 2014); [7] (von der Ohe et al., 2005); [8] (Oris & Giesy Jr, 1987); [9] (Tomlin, 2006); [10] (IPCS, 2008)

Table C.2: Number of sites with quantified and non-detected concentrations for each chemical. The frequency of exceedance (%) and the number of sites are given for each chemical after comparing the limit of quantification (LOQ) for the non-detects with the chronic risk threshold (CRT) and the acute risk threshold (ART).

Chemical Name	**No. Cases**			
	Quantified	**Non-Detects**	**LOQ > CRT**	**LOQ > ART**
Fluoranthene	976	67	67(100)	12(18)
Benzo[a]pyrene	924	967	838(87)	65(7)
Diuron	859	1201	264(22)	32(3)
Naphthalene	826	919	17(2)	1(0)
Atrazine	820	2016	23(1)	14(1)
Isoproturon	727	1233	19(2)	0(0)
Metolachlor	480	736	23(3)	10(1)
Tributyltin	479	1005	581(58)	0(0)
Simazine	473	2284	14(1)	4(0)
g-Hexachlorocyclohexane	457	1904	33(2)	14(1)
MCPA	432	1024	0(0)	0(0)
Mecoprop	407	1082	0(0)	0(0)
Glyphosate	397	326	0(0)	0(0)
Phenanthrene	391	313	15(5)	0(0)
Pyrene	361	15	15(100)	0(0)
Alachlor	341	2261	18(1)	18(1)
Anthracene	327	1585	70(4)	4(0)
Terbutylazine	316	1008	12(1)	6(1)
Trichloromethane	299	1700	16(1)	0(0)
Bentazone	295	857	0(0)	0(0)
Benz[a]anthracene	289	7	7(100)	0(0)
Desethylatrazine	286	772	0(0)	0(0)
Metazachlor	285	693	0(0)	0(0)
Tetrachloroethylene	276	1753	21(1)	17(1)
2,4-D	265	892	0(0)	0(0)
Endrin	257	2158	436(20)	73(3)
Dichloromethane	254	1650	14(1)	0(0)
Linuron	233	1555	35(2)	0(0)
Pentachlorophenol	221	1704	186(11)	4(0)
4,4'- DDT	206	1005	1005(100)	61(6)
Carbofuran	204	780	753(97)	0(0)
Isodrin	201	1984	80(4)	4(0)
Chlorpyrifos	191	1935	1934(100)	1149(59)
Hexachlorobenzene	188	2139	1248(58)	81(4)
a-Hexachlorocyclohexane	183	1156	10(1)	10(1)
Toluene	183	1351	31(2)	17(1)
Para-tert-octylphenol	180	1066	370(35)	7(1)
1,3,5-Trichlorobenzene	177	874	0(0)	0(0)
Benzene	174	1975	21(1)	0(0)
b-Hexachlorocyclohexane	169	1146	10(1)	10(1)
Dieldrin	167	2256	73(3)	6(0)
Aldrin	164	2250	162(7)	4(0)
Trifluralin	153	2213	81(4)	12(1)
1,1,2-Trichloroethene	145	1755	17(1)	0(0)
1,2-Dichloroethane	142	2101	17(1)	0(0)
1,2,4-Trichlorobenzene	130	1028	9(1)	0(0)
4,4'- DDD	129	1441	911(63)	12(1)
Metalaxyl	127	831	10(1)	0(0)
2,4'-DDT	125	863	863(100)	12(1)
1,2,3-Trichlorbenzene	116	899	0(0)	0(0)
Fluorene	110	524	1(0)	0(0)
Propyzamide	103	849	0(0)	0(0)

Pirimicarb	100	527	388(74)	10(2)
4,4'- DDE	95	1491	116(8)	4(0)
Heptachlor	94	1075	49(5)	10(1)
Chlorfenvinfos	90	2049	2048(100)	588(29)
2-Methylnaphthalene	88	180	0(0)	0(0)
Endosulfan I	88	1859	440(24)	58(3)
Carbon tetrachloride	87	1957	21(1)	0(0)
Prometryn	86	770	0(0)	0(0)
Dimethoate	85	997	10(1)	0(0)
Acenaphthene	83	349	15(4)	0(0)
2,6-dichlorobenzamide	82	490	0(0)	0(0)
Hexachlorobutadiene	81	2048	128(6)	78(4)
Acetochlor	77	295	0(0)	0(0)
PCB 28	75	422	1(0)	1(0)
Ethofumesate	74	814	0(0)	0(0)
o-Xylene	72	151	0(0)	0(0)
Pentachlorobenzene	72	1719	4(0)	4(0)
Cyanide	71	73	73(100)	0(0)
Ethylbenzene	70	321	0(0)	0(0)
Chloridazon	67	466	0(0)	0(0)
1,1,1-Trichloroethane	63	1068	0(0)	0(0)
Parathion-ethyl	63	864	782(91)	14(2)
Pendimethalin	62	1044	8(1)	0(0)
Propazine	60	629	14(2)	8(1)
Di-(n-butyl) phthalate	58	48	20(42)	0(0)
Dibutyltin	58	500	0(0)	0(0)
Methoxychlor	58	385	0(0)	0(0)
Propiconazole	58	398	10(3)	0(0)
Dichlorvos	56	1020	1019(100)	560(55)
d-Hexachlorocyclohexane	54	641	0(0)	0(0)
Heptachloro Epoxide B	53	262	0(0)	0(0)
Desisopropylatrazine	51	902	0(0)	0(0)
PCB 118	49	8	8(100)	5(62)
2-naphthalenol	42	95	0(0)	0(0)
Terbutryn	42	160	23(14)	6(4)
1,1-Dichloroethene	38	561	0(0)	0(0)
Dicamba	37	707	0(0)	0(0)
Dichlorprop	37	414	0(0)	0(0)
4-Octylphenol	36	462	0(0)	0(0)
Bromodichloromethane	36	99	0(0)	0(0)
Ziram	36	46	46(100)	0(0)
1,2-Dichlorobenzene	35	674	1(0)	0(0)
Acephthylene	32	594	9(2)	0(0)
Dichlobenil	32	720	0(0)	0(0)
4-Nonylphenol	31	1065	143(13)	0(0)
Endosulfan	31	168	49(29)	0(0)
Pentabromoethylbenzene	31	18	18(100)	0(0)
Styrene	31	188	0(0)	0(0)
1,1-Dichloroethane	30	595	0(0)	0(0)
Malathion	30	1246	1225(98)	168(13)
Trichlorofluoromethane	30	51	0(0)	0(0)
1,1,2-Trichloroethane	29	241	0(0)	0(0)
1,3-Dichlorobenzene	29	240	6(2)	0(0)
Lenacil	29	597	0(0)	0(0)
4-Chlorotoluene	28	751	14(2)	0(0)
Bromacil	27	976	0(0)	0(0)
Metribuzin	27	812	0(0)	0(0)
Permethrin	27	600	600(100)	41(7)
Hexazinone	26	530	0(0)	0(0)
Monochlorobenzene	26	234	0(0)	0(0)
PCB 153	26	30	30(100)	1(3)

PCB 126	25	0	0(0)	0(0)
PCB 138	25	12	12(100)	1(8)
1,2-Dibromoethane	24	691	0(0)	0(0)
Methyl parathion	24	1014	869(86)	16(2)
Bisphenol-a	23	10	0(0)	0(0)
Desmedipham	23	24	1(4)	0(0)
Trans-1,2-Dichloroethylene	23	444	0(0)	0(0)
2-Chloroethylphosphonic Acid	21	82	0(0)	0(0)
2,3,4,5-Tetrachlorophenol	20	209	0(0)	0(0)
2,4,6-tri-tert-Butylphenol	20	5	5(100)	0(0)
Clopyralid	20	677	0(0)	0(0)
Dicofol	20	0	0(0)	0(0)
Phenitrothion	20	1239	1141(92)	10(1)
1,2-Dichloroethene	19	78	0(0)	0(0)
Dichlorodifluoromethane	18	36	0(0)	0(0)
2,4,5-Trichlorophenoxyacetic acid	17	769	0(0)	0(0)
Fenpropimorph	17	545	0(0)	0(0)
Methamidophos	17	0	0(0)	0(0)
PCB 101	17	3	3(100)	1(33)
Chlordane	16	0	0(0)	0(0)
Metamitron	16	691	0(0)	0(0)
PCB 52	16	1	1(100)	1(100)
Xylene	16	145	0(0)	0(0)
PCB 180	15	2	2(100)	1(50)
2,4-Dichlorophenol	14	49	17(35)	17(35)
Cis-1,2-Dichloroethylene	13	18	0(0)	0(0)
Terbumeton	13	618	0(0)	0(0)
Vinylchloride	13	60	7(12)	0(0)
Ametryne	12	406	12(3)	0(0)
1-Methylnaphthalene	11	93	0(0)	0(0)
2,4,6-Trichlorophenol	11	23	0(0)	0(0)
2,4'-DB	11	124	0(0)	0(0)
Desmetryn	11	485	0(0)	0(0)
4-Nonylphenol, branched	10	38	18(47)	11(29)
Benfluralin	10	0	0(0)	0(0)
Hexachlorocyclohexane	10	201	0(0)	0(0)
PCB 77	10	130	130(100)	0(0)
2,4'-DDD	9	85	54(64)	0(0)
Butyl benzyl phthalate	9	20	20(100)	20(100)
Demeton-S-Methyl	9	611	501(82)	0(0)
Fluquincozole	9	0	0(0)	0(0)
MCPB	9	38	0(0)	0(0)
Methomyl	9	448	383(85)	0(0)
MTBE	9	25	0(0)	0(0)
Prometon	9	398	0(0)	0(0)
2,4'-DDE	8	89	22(25)	0(0)
Bromoxynil	8	308	0(0)	0(0)
Demeton-S-methylsulfon	8	0	0(0)	0(0)
PCB 169	8	82	82(100)	0(0)
Phosalone	8	312	312(100)	19(6)
Chlorsulfuron	7	0	0(0)	0(0)
Formaldehyde	7	53	29(55)	0(0)
Ioxynil	7	254	0(0)	0(0)
Pentachloronitrobenzene	7	392	0(0)	0(0)
Cyanazine	6	460	0(0)	0(0)
Dipropyl phthalate	6	62	0(0)	0(0)
Fenoprop	6	8	0(0)	0(0)
Deisopropyldeethylatrazine	5	0	0(0)	0(0)
EDTA	5	0	0(0)	0(0)
2,4-Dinitrotoluol	4	110	19(17)	0(0)
4-Bromophenyl phenyl ether	4	40	19(48)	0(0)

Kepon	4	377	0(0)	0(0)
4-Chloro-3-Methylphenol	3	10	0(0)	0(0)
PCB 194	3	316	316(100)	0(0)
Pentachloroanisole	3	6	0(0)	0(0)
Tebufenozide	3	442	0(0)	0(0)
Trans-nonachlor	3	0	0(0)	0(0)
4-Nitrophenol	2	78	0(0)	0(0)
Desethylterbutylazine	2	39	0(0)	0(0)
Dimethyl phthalate	2	167	0(0)	0(0)
Hydroxysimazine	2	92	0(0)	0(0)
Iodofenphos	2	455	453(100)	0(0)
Metsulfuronmethyl	2	0	0(0)	0(0)
Nitrilotriacetic acid	2	0	0(0)	0(0)
Nitrofen	2	203	0(0)	0(0)
Pyridate	2	562	0(0)	0(0)
Terbufos	2	570	570(100)	179(31)
2-Methylphenol	1	0	0(0)	0(0)
2,3-Dimethylphenol	1	0	0(0)	0(0)
2,6-Dichlorophenol	1	15	0(0)	0(0)
Dinitro-o-Cresol	1	7	0(0)	0(0)
Dinoseb	1	8	0(0)	0(0)
Dipentyl phthalate	1	67	0(0)	0(0)
Hexachlorocyclopentadiene	1	19	19(100)	19(100)
m-Xylene	1	14	0(0)	0(0)
Omethoate	1	256	256(100)	0(0)
PBDE 28	1	72	0(0)	0(0)
PCB 105	1	0	0(0)	0(0)
PCB 157	1	0	0(0)	0(0)
1,1,1,3-Tetrachloropropane	0	153	0(0)	0(0)
2,3,4,6-Tetrachlorophenol	0	8	0(0)	0(0)
2,4,5-Trichlorophenol	0	14	4(29)	0(0)
2,4,6-Trinitrotoluene	0	13	0(0)	0(0)
2,5-Dimethylphenol	0	1	0(0)	0(0)
4-Chloro-2-Methylphenol	0	3	0(0)	0(0)
4-Methylphenol	0	218	0(0)	0(0)
Acrylonitrile	0	1	1(100)	1(100)
Captan	0	566	0(0)	0(0)
Chlorthiamid	0	279	0(0)	0(0)
Dalapon	0	3	0(0)	0(0)
Di-ethyl phthalate	0	20	0(0)	0(0)
Ethylenethiourea	0	1	0(0)	0(0)
Fezaquin	0	295	295(100)	0(0)
p-Xylene	0	25	0(0)	0(0)
Secbumeton	0	16	0(0)	0(0)
Thiram	0	53	53(100)	0(0)

[a] Sites from Spain were omitted, because the LOQ was not considered reliable.
[b] The percentage of sites which had LOQ>ART or LOQ>CRT was calculated as: (No. sites LOQ>ART OR No. sites LOQ>CRT / No. sites non-detects)x100

Table C.3: Community studies reporting effects from pesticides for the cases exceeding the levels of 1/10 and 1/1,000 of the LC50 values for the analyzed organism groups.

Threshold	Chemical group	MoA[a]	Organism groups	Total no. of cases	No.(%)of affected cases[c]	LoE[e]	Source[f]
1/10 -10 LC50	Insecticides	AChEI	Fish	22	9 (41)	About as likely as not	[1]
			Invertebrates	97	71 (73)	Likely	[1]
			Algae and macrophytes	25	5 (20)	Unlikely	[1]
		SCI	Fish	15	7 (47)	About as likely as not	[1]
			Invertebrates	99	75 (76)	Likely	[1]
			Algae and macrophytes	23	8 (35)	About as likely as not	[1]
	Herbicides	PSI	Fish	11	4 (36)	About as likely as not	[2]
			Invertebrates	32	16 (50)	More likely than not	[2]
			Algae and macrophytes	52	40 (77)	Likely	[2]
		GI	Fish	ns[b]	ns[b]	ns[b]	[2]
			Invertebrates	8	3 (38)	About as likely as not	[2]
			Algae and macrophytes	10	3 (30)	About as likely as not	[2]
1/10-1 LC50	Pesticides		Invertebrates	8	8 (100)	Very likely	[3]
			Fish	48[c]	20 (42)	About as likely as not	
>1/10 LC50	Overall chemical		Invertebrates	228[c]	162 (71)	Likely	
			Algae and macrophytes	110[c]	56 (51)	More likely than not	
1/1,000 LC50	Pesticides		Invertebrates	45	32 (71)	Likely	[3]

[a] Modes of actions groups: Acetylcholinesterase Inhibitor (AChEI) includes organophosphates and carbamates, which interrupt the transmission of nerve impulses by inhibition of the enzyme acetylcholinesterase. Sodium Channel Inhibitor (SCI) includes synthetic pyrethroids, which lead to paralysis by interfering with the sodium channels of the nervous system. Photosynthesis Inhibitors (PSI) includes triazines/triazinones and the urea compounds, which by interfering with the photosynthesis lead to disrupted plant growth. Growth Inhibitors (GI) includes all products that have no direct photosynthesis-inhibiting effects.

[b] ns - No study available

[c] Total number of cases is calculated as the sum of total number of cases for each chemical group and mode of action. For fish and algae: the sum of total no. cases for (i) Insecticides with AChEI, (ii) Insecticides with SCI, (iii) Herbicides with PSI, and (iv) Herbicides with GI. For invertebrates: the sum of total no. of cases for (i) Insecticides with AChEI, (ii) Insecticides with SCI, (iii) Herbicides with PSI, (iv) Herbicides with GI and (v) Pesticides for the level 1/10-1 of the LC50.

[d] Percentage of affected cases is calculated as ((Number of affected cases/Total number of cases)x100). Effects for the micro-/mesocosm reviews (Brock, Lahr & Brink, 2000; Van Wijngaarden, Brock & Van den Brink, 2005) ranged from slight, short-term (less than 8 weeks) effects, to pronounced, long-term (longer than 8 weeks) effects. All cases exhibiting at least slight acute effects for concentrations higher than the 1/10 of the LC50 were considered as affected cases. The exposure range was between 1/10 and 10 times the LC50 values. For the meta-analysis on the effects of pesticides on invertebrate communities (Schäfer et al., 2012) all sites exhibiting a statistically significant decline in the abundance of the sensitive invertebrate species when compared with both thresholds levels(1/10 - of the LC50 and 1/1,000-1/10 of the LC50) were considered as affected cases.

[e] Likelihood of effects classified based on the affected cases and divided into (i) unlikely (<33%), (ii) more likely than not (>50%), (iii) About as likely as not (33-66%), (iv) likely (>66%) and (v) very likely (>90%). Terminology is based on the guidelines for uncertainty treatment, which is used from the intergovernmental panel on climate change (Mastrandrea et al., 2010).

[f] [1] (Van Wijngaarden, Brock & Van den Brink, 2005); [2] (Brock, Lahr & Brink, 2000); [3] (Schäfer et al., 2012)

Table C.4: Number of sites for which the chemical concentration exceeds the CRT (chronic risk threshold) and the ART (acute risk threshold) for each organism group represented by the fish *P. promelas* (PP), the invertebrate *D. magna* (DM), and the algae *P. subcapitata* (PS).

Concentration exceeding ART/CRT (Type of data)[a]			
Chemical	**No. sites PP**	**No. sites DM**	**No. sites PS**
4,4'- DDE	8/9(P/1)	9/21(P/4)	8/8(P/1)
4,4'- DDT	7/8(E)	10/52(E)	-
2,4,6-tri-tert-Butylphenol	5/9(E)	3/17(P/4)	-
Hexachlorobenzene	4/4(P/2)	9/44(E)	5/4(E)
4,4'- DDD	4/4(P/1)	9/24(E)	3/1(P/2)
B-Hexachlorocyclohexane	3/3(P/4)	0/3(P/4)	0/0(B)
Endrin	26/71(E)	0/1(E)	0/0(P/2)
g-Hexachlorocyclohexane	20/51(E)	0/38(E)	0/0(B)
Atrazine	2/3(E)	0/7(E)	72/73(E)
Endosulfan	2/17(E)	0/0(E)	0/0(E)
Tributyltin	15/21(E)	15/363(E)	15/18(E)
Cyanide	13/22(E)	3/82(E)	-
Ziram	11/20(E)	1/24(E)	1/2(E)
Benzo[a]pyrene	1/9(E)	12/759(E)	1/1(E)
Hexachlorobutadiene	1/7(E)	0/7(E)	1/0(P/2)
PCB 194	1/2(E)	1/3(E)	-
Endosulfan I	1/17(E)	0/1(P/4)	1/1(P/2)
Aldrin	1/1(E)	1/5(E)	1/1(P/3)
Chlordane	1/1(E)	1/1(E)	-
Hexachlorocyclohexane	1/1(E)	0/1(P/4)	0/0(B)
Hexachlorocyclopentadiene	1/1(E)	1/1(E)	1/1(P/4)
Dichloromethane	1/0(E)	1/1(E)	0/0(B)
Pentachlorophenol	0/6(P/4)	0/7(E)	0/0(E)
PCB 180	0/2(P/3)	0/3(P/4)	-
2-naphthalenol	0/2(E)	0/6(E)	0/0(E)
4-Nonylphenol	0/1(E)	0/5(E)	0/0(E)
Chlorpyrifos	0/1(E)	146/235(E)	0/0(P/3)
Methoxychlor	0/1(E)	0/1(E)	0/0(P/1)
PCB 153	0/1(E)	0/4(E)	-
Permethrin	0/1(E)	13/27(E)	-
Pyrene	0/1(E)	0/15(E)	-
1,2,3-Trichlorbenzene	0/0(P/4)	0/1(E)	0/0(E)
2,4'-DDT	0/0(P/3)	0/21(P/4)	-
4-Octylphenol	0/0(P/3)	0/1(P/4)	0/0(P/3)
Anthracene	0/0(P/3)	0/6(E)	0/0(P/3)
Dipentyl phthalate	0/0(P/3)	0/1(P/4)	0/0(P/3)
Phenanthrene	0/0(P/3)	0/5(E)	0/0(E)
2,4'-DDD	0/0(P/1)	0/8(P/4)	0/0(B)
Chlorfenvinfos	0/0(P/1)	69/112(E)	0/0(B)
Demeton-S-Methyl	0/0(P/1)	0/9(E)	0/0(B)
Dibutyltin	0/0(P/1)	0/12(E)	0/0(P/3)
Iodofenphos	0/0(P/1)	0/1(E)	-
Isoproturon	0/0(P/1)	0/12(E)	25/51(E)
Lenacil	0/0(P/1)	0/0(E)	1/3(E)
Linuron	0/0(P/1)	0/1(E)	2/1(E)
Metazachlor	0/0(P/1)	0/0(E)	1/6(E)
Pendimethalin	0/0(P/1)	0/0(E)	1/1(E)
Terbutylazine	0/0(P/1)	0/0(E)	24/31(E)
4-Nonylphenol, branched	0/0(E)	0/1(P/4)	0/0(E)
Alachlor	0/0(E)	0/0(E)	10/14(E)
Bromacil	0/0(E)	0/0(E)	1/1(E)
Butyl benzyl phthalate	0/0(E)	0/1(E)	0/1(E)
Carbofuran	0/0(E)	11/114(E)	0/0(E)

Di-(n-butyl) phthalate	0/0(E)	0/1(E)	0/0(E)
Dichlorvos	0/0(E)	21/56(E)	0/0(E)
Diuron	0/0(E)	0/0(E)	230/345(E)
Heptachlor	0/0(E)	0/8(E)	0/0(E)
Malathion	0/0(E)	7/29(E)	0/0(E)
Methomyl	0/0(E)	1/3(E)	0/0(E)
Methyl parathion	0/0(E)	0/17(E)	0/0(E)
Metolachlor	0/0(E)	0/0(E)	2/7(E)
Para-tert-octylphenol	0/0(E)	0/23(E)	0/0(P/4)
Parathion-ethyl	0/0(E)	1/22(E)	0/0(P/2)
PCB 52	0/0(E)	0/1(E)	-
Pentachloroanisole	0/0(E)	0/1(E)	0/0(P/3)
Phenitrothion	0/0(E)	1/12(E)	0/0(E)
Phosalone	0/0(E)	0/8(E)	0/0(E)
Pirimicarb	0/0(E)	0/4(E)	0/0(E)
Terbufos	0/0(E)	2/2(E)	0/0(B)
Toluene	0/0(E)	0/1(E)	0/0(E)
Trichloromethane	0/0(E)	0/2(E)	0/0(B)
Benz[a]anthracene	-	0/9(E)	-
Fluoranthene	-	4/30(E)	-
Omethoate	-	0/1(E)	-
Pentabromoethylbenzene	-	11/31(P/4)	-

[a]Type of data: E: Experimental toxicity data from literature, B: Baseline toxicity data estimated from the octanol/water partitioning coefficient, P: Predicted toxicity data from read across together with the level of reliability (1: low ; 2: moderate ; 3: high and 4: very high). Toxicity values were removed when they lay outside of the model domain ($1<\log K_{ow}<6$) for the baseline prediction (D), or when they exceed water solubility by a factor of 10 (WS).

Table C.5: Number of sites monitored for each river basin (RB) and number of sites with land use (LU) for each river basin. The area for each river basin is given in km^2.

River basin	Area	No sites		River basin	Area	No sites	
		RB	LU			RB	LU
Danube	803554	569	343	Sicily	25684	10	-
Loire	156765	388	37	Northumbria	8499	9	-
Rhone	128355	360	103	UK South East	8288	9	-
Po Basin	73341	279	-	Schlei/Trave	6154	8	-
Adour-Garonne	116740	247	52	Venta	21915	8	-
Seine	94356	206	19	Cavado, Ave and Leca	3358	7	-
Middle Appenines	36192	193	-	Andalusia Atlantic Basins	68010	6	-
Rhine	186325	168	32	East Aegean	60875	6	-
Northern Appenines	38477	149	-	Galician Coast	13081	6	-
Internal Basins of Catalonia	16433	106	-	Kokemäenjoki	68628	6	-
Ebro	86012	84	1	Lielupe	17800	6	-
Minho and Lima	40829	75	-	Warnow/Peene	13631	6	-
Elbe	147527	73	1	Algarve Basins	3836	5	-
Eastern Alps	37179	70	-	Daugava	82793	5	-
Scheldt	36904	65	12	Ems	16315	5	-
Douro	97713	60	-	Central Macedonia	31811	4	-
Meuse	34238	58	9	Guadiana	66989	4	-
GB North Western	12375	53	-	Kymijoki-Gulf of Finland	50867	4	-
Scotland	67803	47	-	Vidaa-Krusaa	5718	4	-
Tagus and Western Basins	83120	42	-	Vistula	206058	4	-
Jucar	42959	41	-	Black Sea	20974	3	-
Shannon	18317	41	-	Eastern Sterea Ellada	12201	3	-
Andalusia	17958	35	-	North Adriatic	3852	3	-
Southern Appenines	67496	32	-	SE South West	29032	3	-
Neagh Bann	7920	30	-	NO West Bay	37529	3	-
Basque County internal basins	2267	29	-	Western Macedonia	19761	3	-
IE South Eastern	12859	29	-	Dee	2142	2	-
IE Western	12220	28	-	Gauja	14381	2	-
IE South Western	11363	27	-	Vouga, Mondego and Lis	11596	2	-
UK South West	17631	27	-	Eastern Peloponnese	8408	1	-
Corsica	8694	22	8	Epirus	15294	1	-
Cyprus	9248	22	-	Finnmark	48528	1	-
UK North Eastern	3067	17	-	Glomma	47429	1	-
Western Wales	12213	17	-	Nordland	38369	1	-
Humber	25415	16	-	Northern Peloponnese	7383	1	-
Weser	47291	16	-	Oulujoki-Iijoki	64731	1	-
IE Eastern	6275	14	-	Sado and Mira	10072	1	-
Segura	18905	13	-	Skagerrak and Kattegat	75986	1	-
Severn	21068	13	-	South Baltic	54753	1	-
Ucker	134390	13	-	Thessalia	13133	1	-
UK North West	11370	12	-	Troendelag	35297	1	-
Anglian	25093	11	-	Vuoksi	283356	1	-
Solway Tweed	15218	11	-	NO West	32815	1	-
Thames	15811	11	-	West Aegean	21584	1	-
Nemunas	100082	10	-	Western Peloponnese	7235	1	-
Serchio	1439	10	-				

Table C.6: Land use type and their respective categories.

Category	Type of land use
Natural vegetation	Scrub and/or herbaceous vegetation areas
	Open spaces with little or no vegetation
	Forested areas
Anthropogenically influenced areas	Arable land
	Permanent crops
	Heterogeneous agricultural areas
	Urban areas

Table C.7: Number of sites with ecological and chemical information. Thresholds comprise chronic risk threshold (CRT) and acute risk threshold (ART). Number of sites exceeding the risk thresholds, number of sites in high (H) and good (G) status which exceed the risk thresholds, as well as the total number of sites analysed are given for each organism group.

		No. of sites		
Species	**Threshold Classes**	**Exceeding**	**Exceeding (H+G)**	**Total**
Fish	>CRT	87	50	
	CRT-ART	3	1	
	<ART	5	1	**95**
Invertebrates	>CRT	15	8	
	CRT-ART	155	67	
	<ART	21	5	**191**
Diatoms	>CRT	87	53	
	CRT-ART	17	11	
	<ART	32	19	**136**

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Appendix D

Supplementary information for: How Much do Organic Toxicants Contribute to Multiple Stress in Freshwater Ecosystems?

D.1 Supplementary Figures

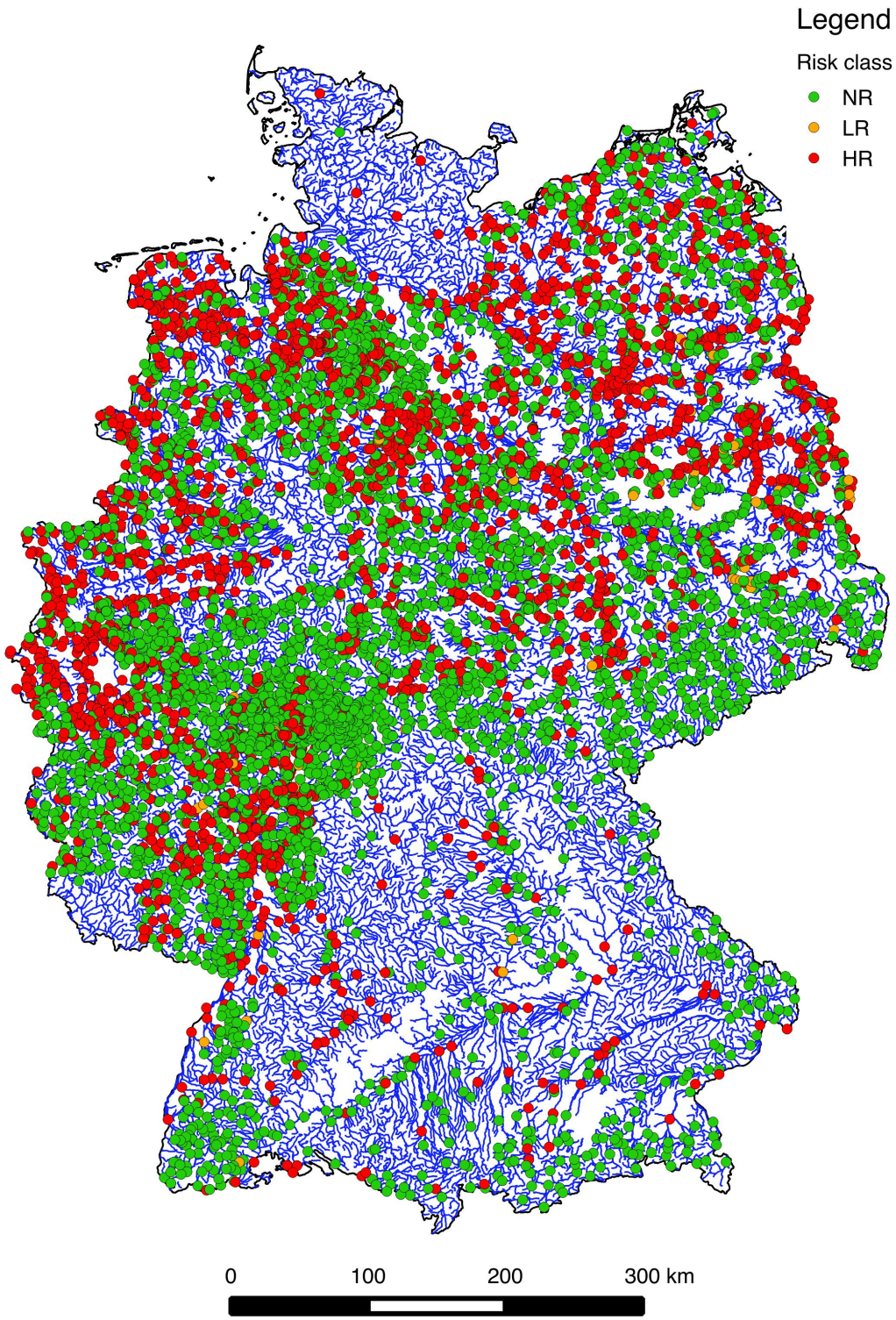

Figure D.1: Risk for effects from invasive species in german sampling sites over stream network. NR= Negligible risk, LR = Low risk, HR = High risk. See main document for information on risk thresholds and Table 5.1 for data source. Boundary for Germany obtained from GADM (2012).

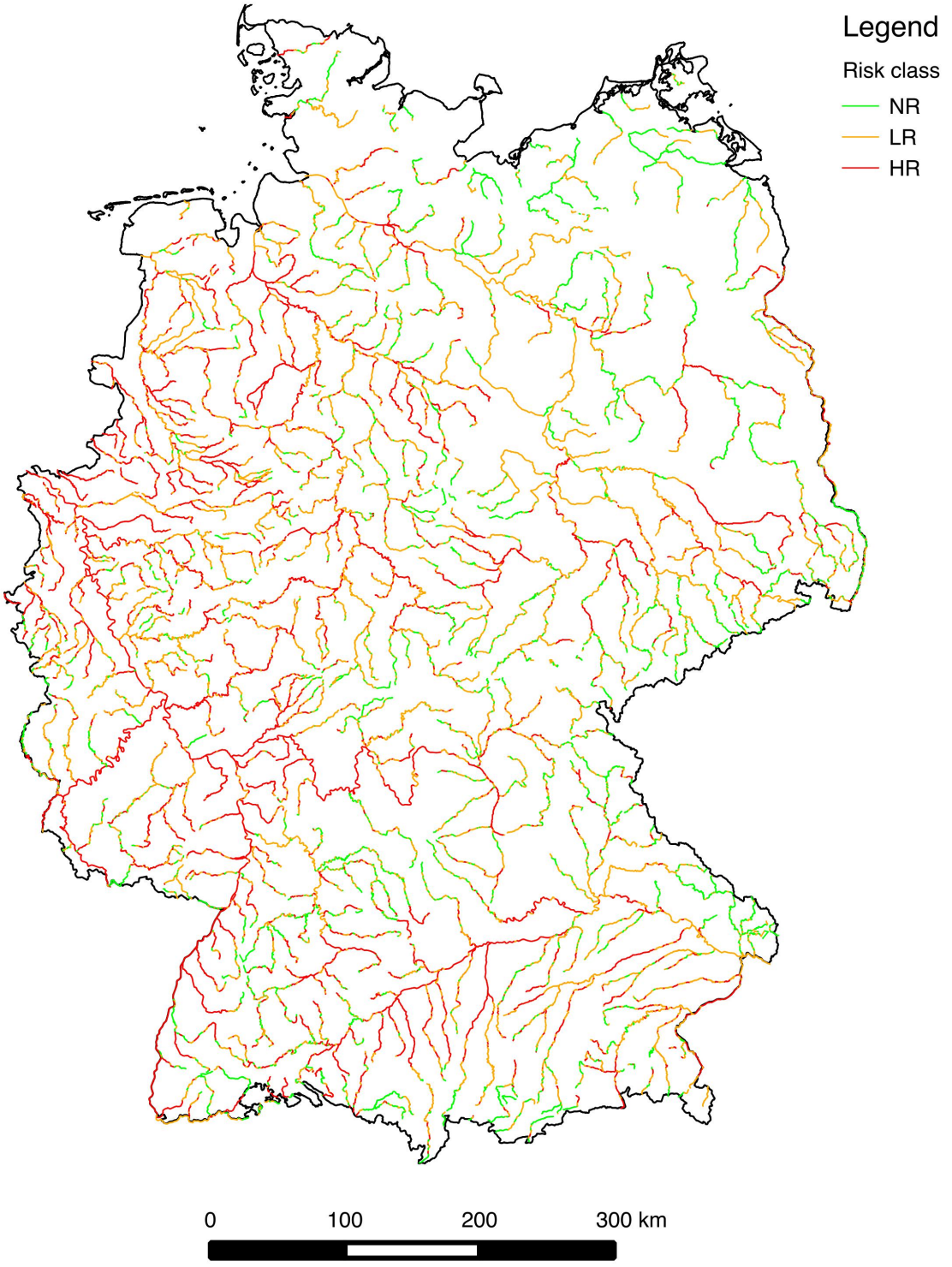

Figure D.2: Hydromorphological risk classes based on the german hydromorphological index. NR=Negligible risk, LR=Low risk, HR=High risk. See main document for information on risk thresholds and Table 5.1 for data source. Boundary for Germany obtained from GADM (2012).

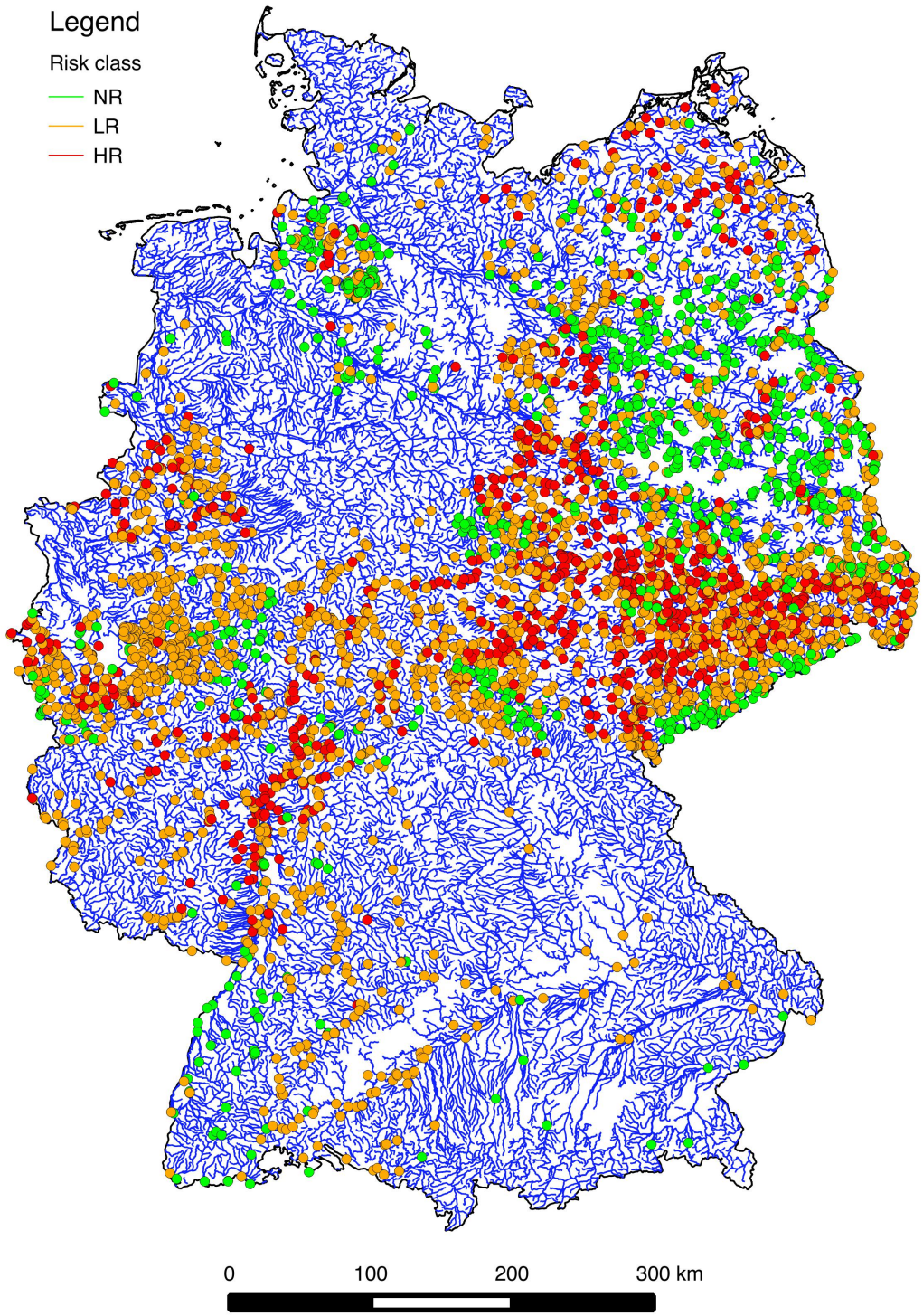

Figure D.3: Risk for effects from nutrients in German sampling sites over stream network. NR=Negligible risk, LR=Low risk, HR=High risk. See main document for information on risk thresholds and Table 5.1 for data source. Boundary for Germany obtained from GADM (2012).

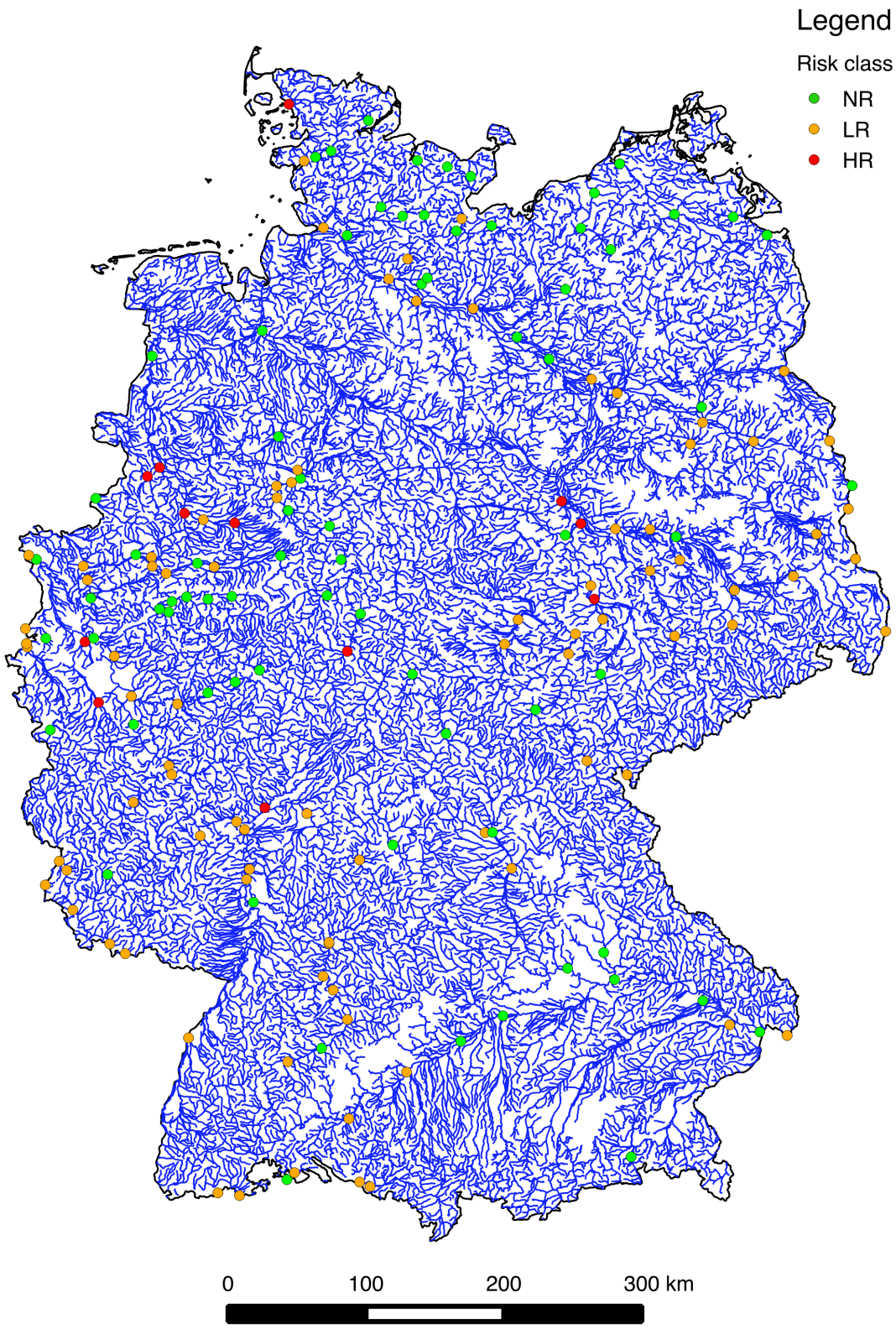

Figure D.4: Risk for effects from organic toxicants in German sampling sites over stream network. NR=Negligible risk, LR=Low risk, HR=High risk. See main document for information on risk thresholds and Table 5.1 for data source. Boundary for Germany obtained from GADM (2012).

D.2 Supplementary Tables

Table D.1: Number and % of papers related to different stressors in five freshwater ecology journals (Freshwater biology, Freshwater Science/ Journal of the North American Benthological Society, Aquatic Sciences, Canadian Journal of Fisheries and Aquatic Sciences, Hydrobiologia) published between 2009 and 2013 using the given search terms in Web of Science (Accessed January 17, 2014)

Stressor[a]	Search term	Number of publications	% of publications
EN	TOPIC: ((nutrient* OR nitrogen OR phosph* OR eutroph*) AND (stress* OR effect*) AND (stream* OR river*) AND PUBLICATION NAME: (Freshwater biology OR Freshwater Science OR Journal of the North American Benthological* OR Aquatic Science* OR Canadian Journal of Fisheries* OR Hydrobiologia)	340	52
CC	TOPIC: (("climate change" OR "climate warming") AND (stress* OR effect*) AND (stream* OR river*)) AND PUBLICATION NAME: (Freshwater biology OR Freshwater Science OR Journal of the North American Benthological* OR Aquatic Science* OR Canadian Journal of Fisheries* OR Hydrobiologia)	121	18
IS	TOPIC: ((invasi* OR neozoa* OR non-native) AND (stress* OR effect*) AND (stream* OR river*)) AND PUBLICATION NAME: (Freshwater biology OR Freshwater Science OR Journal of the North American Benthological* OR Aquatic Science* OR Canadian Journal of Fisheries* OR Hydrobiologia)	96	15
HD	TOPIC: (("habitat degradation*" OR "habitat loss*" OR hydromorpholog* OR geomorpholog* OR "stream restoration" OR "river restoration") AND (effect* OR stress*) AND (stream* OR river*)) AND PUBLICATION NAME: (Freshwater biology OR Freshwater Science OR Journal of the North American Benthological* OR Aquatic Science* OR Canadian Journal of Fisheries* OR Hydrobiologia)	87	13
OT	TOPIC: ((pesticide* OR herbicide* OR insecticide* OR fungicide* OR (organic AND toxic*)) AND (stress* OR effect*) AND (stream* OR river*)) AND PUBLICATION NAME: (Freshwater biology OR Freshwater Science OR Journal of the North American Benthological* OR Aquatic Science* OR Canadian Journal of Fisheries* OR Hydrobiologia)	14	2

[a] EN=Excessive nutrients; CC=Climate change; IS=Invasive species; HD=Habitat degradation; OT=Organic toxicants

Table D.2: Stream types with description and % sample size in monitoring data related to the invasive species data set.

Stream type code	**Description**[a]	**German ecoregion (with number)**[a]	%
1	Alpine streams	Alps (4)	0.6
2	Streams in the alpine foothills	Alps (4)	0.9
3	Streams in the Pleistocene sediments of the alpine foothills	Alps (4)	0.6
4	Large rivers in the Alpine foothills	Alps (4)	0.2
5	Small coarse substrate-dominated siliceous highland rivers	Western/Central highlands (8/9)	22.3
5.1	Small fine substrate-dominated siliceous highland rivers	Western/Central highlands (8/9)	6.7
6	Small fine substrate-dominated calcareous highland rivers	Western/Central highlands (8/9)	5.6
7	Small coarse substrate-dominated calcareous highland rivers	Western/Central highlands (8/9)	1.4
9	Mid-sized fine to coarse substrate-dominated siliceous highland rivers	Western/Central highlands (8/9)	7.1
9.1	Mid-sized fine to coarse substrate-dominated calcareous highland rivers	Western/Central highlands (8/9)	1.9
9.2	Large highland rivers	Western/Central highlands (8/9)	3
10	Very large gravel-dominated rivers	Western/Central highlands (8/9)	0.8
11	Small organic substrate-dominated rivers	Independent	2.7
12	Mid-sized and large organic substrate-dominated rivers	Independent	1.7
14	Small sand-dominated lowland rivers	Lowlands (14)	13.1
15	Mid-sized and large sand and loam-dominated lowland rivers	Lowlands (14)	5.8
16	Small gravel-dominated lowland rivers	Lowlands (14)	4.8
17	Mid-sized and large gravel-dominated lowland rivers	Lowlands (14)	1
18	Small loess and loam-dominated lowland rivers	Lowlands (14)	3.5
19	Small streams in riverine floodplains	Independent	8.1
20	Very large sand-dominated rivers	Lowlands (14)	0.6
21	Lake outflows	Independent	1.8
22	Marshland streams of the coastal plains	Lowlands (14)	1
23	Backwater and brackish water influenced Baltic Sea tributaries	Lowlands (14)	0.4

[a] taken from Wasserblick (2004)

[b] according to Illies (1978) and Lorenz et al. (2004)

Table D.3: Longitudinal (Long) and Latitudinal (Lat) distribution (dist.) and % occurrence in invaded (I) and all (A) sampling sites as well as distributional and dominance (Dom.) classification for neozoans.

Taxon	Source[a]	Long dist. (km)	Lat dist. (km)	Dist. class	% I	% A	Dom. class[b]
Atyaephyra desmarestii	1	485	477	high	0.5	0.2	nd
Balanus improvisus	1, 2	5	3	low	0.1	0	nd
Branchiura sowerbyi	1, 2	584	665	high	2	0.7	nd
Caspiobdella fadejewi	1, 2	577	497	high	1.5	0.6	nd
Chelicorophium curvispinum	2	535	593	high	2.4	0.9	nd
Chelicorophium robustum	3	356	443	high	1.1	0.4	nd
Chelicorophium sowinskyi	3	0	0	low	0	0	nd
Corbicula fluminalis	1, 2	448	506	high	0.4	0.2	nd
Corbicula fluminea	1, 2	593	563	high	4.4	1.7	dom
Cordylophora caspia	1, 2	495	155	high	0.6	0.2	nd
Crangonyx pseudogracilis	1, 2	96	551	low	0.5	0.2	nd
Dendrocoelum romanodanubiale	1, 2	152	361	high	0.3	0.1	nd
Dikerogammarus bispinosus	4	139	36	low	0.1	0.1	nd
Dikerogammarus haemobaphes	1, 2	544	557	high	4.2	1.6	dom
Dikerogammarus villosus	1, 2	576	720	high	8.3	3.1	dom
Dreissena polymorpha	1, 2	585	727	high	9.6	3.6	dom
Dreissena rostriformis bugensis	1	227	49	low	0.1	0	nd
Dugesia tigrina	1, 2	602	660	high	6.7	2.5	dom
Echinogammarus berilloni	1, 2	180	414	high	2.4	0.9	nd
Echinogammarus ischnus	1, 2	526	561	high	1	0.4	nd
Echinogammarus trichiatus	1, 2	506	436	high	0.5	0.2	nd
Gammarus tigrinus	1, 2	590	500	high	7.6	2.9	dom
Gyraulus chinensis	1	0	0	low	0	0	nd
Gyraulus parvus	1, 2	472	678	high	0.7	0.2	nd
Hemimysis anomala	1, 2	475	419	high	0.3	0.1	nd
Hypania invalida	1, 2	548	595	high	2.2	0.8	nd
Jaera istri	1, 2	570	619	high	2.6	1	nd
Limnomysis benedeni	1, 2	496	403	high	0.5	0.2	nd
Lithoglyphus naticoides	1, 2	427	596	high	0.5	0.2	nd
Menetus dilatatus	1, 2	211	198	high	0.8	0.3	nd
Musculium transversum	1	107	383	high	0.3	0.1	nd
Obesogammarus crassus	1, 2	156	107	high	0.6	0.2	nd
Orchestia cavimana	1, 2	175	406	high	0.1	0.1	nd
Orconectes limosus	1, 2	597	652	high	7.6	2.8	dom
Pacifastacus leniusculus	1, 2	364	550	high	1.6	0.6	nd
Pectinatella magnifica	1, 2	30	74	low	0.1	0	nd
Physella acuta	1, 2	577	621	high	8	3	dom
Physella heterostropha	1, 2	392	556	high	0.8	0.3	nd
Pontogammarus robustoides	1, 2	297	189	high	3.7	1.4	dom
Potamopyrgus antipodarum	1, 2	616	789	high	64.2	24	dom
Proasellus coxalis	1, 2	617	749	high	26.7	10	dom
Proasellus meridianus	1	309	284	high	1.1	0.4	nd
Rhithropanopeus harrisii	1, 2	20	17	low	0.2	0.1	nd

[a] Source of information that species is a neozoan: 1=Nehring (2014), 2=DAISIE (2014), 3=Borza et al. (2010), 4=Müller et al. (2002)

[b] dom=dominant; nd=not dominant

Table D.4: Chemicals with information on the main application, chemical family, % of sites monitored, % of sites with detections, and % threshold exceedances for *Pseudokirchneriella subcapitata* (PS), *Daphnia magna* (DM), *Pimephales promelas* (PP). Toxicity values for these species and chemicals can be found in Table C.1.

Chemical family[a]	Chemical	CAS number	Main application[b]	% of sites monitored	% of sites with detections	% exceedances PS[c]	% exceedances DM[c]	% exceedances PP[c]
A	2,4'-DDT	789026	Ins	23	4	0	0	0
A	4,4'- DDD	72548	Ins	37	5	0	1	0
A	4,4'- DDE	72559	Ins	37	6	0	0	0
A	4,4'- DDT	50293	Ins	40	4	0	1	0
A	A-Hexachlorocyclohexane	319846	Ins	47	9	0	0	0
A	Aldrin	309002	Ins	40	2	0	0	0
A	B-Hexachlorocyclohexane	319857	Ins	47	11	0	0	0
A	Chlordane	57749	Ins	24	3	0	0	0
A	Chlorfenvinfos	470906	Ins	50	2	0	2	0
A	Chlorpyrifos	2921882	Ins	47	5	0	5	0
A	D-Hexachlorocyclohexane	319868	Ins	43	6	0	0	0
A	Demeton-S-Methyl	919868	Ins	12	0	0	0	0
A	Demeton-S-methylsulfon	17040196	Ins	3	0	0	0	0
A	Dichlorvos	62737	Ins	65	1	0	1	0
A	Dieldrin	60571	Ins	40	3	0	0	0
A	Dimethoate	60515	Ins	75	4	0	0	0
A	Endosulfan I	959988	Ins	68	2	0	0	0
A	Endrin	72208	Ins	40	1	0	0	0
A	g-Hexachlorocyclohexane	58899	Ins	52	17	0	0	0
A	Heptachlor	76448	Ins	49	1	0	0	0
A	Heptachloro Epoxide B	1024573	Ins	22	3	0	0	0
A	Isodrin	465736	Ins	38	5	0	0	0
A	Malathion	121755	Ins	69	2	0	2	0
A	Methamidophos	10265926	Ins	24	0	0	0	0
A	Methyl parathion	298000	Ins	72	1	0	1	0
A	Omethoate	1113026	Ins	13	0	0	0	0
A	Parathion-ethyl	56382	Ins	76	1	0	1	0
A	Pentachlorophenol	87865	Ins	26	2	0	0	0
A	Phenitrothion	122145	Ins	64	1	0	0	0
A	Pirimicarb	23103982	Ins	25	9	0	0	0
A	2,4-D	94757	Her	84	13	0	0	0
A	2,4,5-Trichlorophenoxyacetic acid	93765	Her	71	1	0	0	0
A	Alachlor	15972608	Her	55	3	0	0	0
A	Ametryne	834128	Her	75	3	0	0	0
A	Atrazine	1912249	Her	81	32	0	0	0
A	Bentazone	25057890	Her	90	36	0	0	0
A	Bromacil	314409	Her	77	1	0	0	0
A	Bromoxynil	1689845	Her	45	0	0	0	0
A	Chloridazon	1698608	Her	65	9	0	0	0
A	Dichlorprop	120365	Her	53	9	0	0	0
A	Diuron	330541	Her	77	33	12	0	0
A	Hexazinone	51235042	Her	62	3	0	0	0
A	Isoproturon	34123596	Her	78	49	0	0	0

A	Linuron	330552	Her	77	2	0	0	0
A	MCPA	94746	Her	90	38	0	0	0
A	Mecoprop	7085190	Her	86	44	0	0	0
A	Metazachlor	67129082	Her	84	44	0	0	0
A	Metolachlor	51218452	Her	87	42	1	0	0
A	Metribuzin	21087649	Her	34	1	0	0	0
A	Prometryn	7287196	Her	77	8	0	0	0
A	Simazine	122349	Her	80	27	0	0	0
A	Terbutylazine	5915413	Her	84	55	2	0	0
A	Trifluralin	1582098	Her	60	2	0	0	0
A	Hexachlorobenzene	118741	Fun	46	9	0	0	0
A	Propiconazole	60207901	Fun	23	11	0	0	0
B	PCB 101	37680732	Lub,Pla	13	1	0	0	0
B	PCB 118	31508006	Lub,Pla	22	1	0	0	0
B	PCB 138	35065282	Lub,Pla	13	3	0	0	0
B	PCB 153	35065271	Lub,Pla	13	2	0	0	0
B	PCB 28	7012375	Lub,Pla	13	1	0	0	0
B	PCB 52	35693993	Lub,Pla	13	0	0	0	0
C	Anthracene	120127	Byprod	62	21	0	0	0
C	Benzo[a]pyrene	50328	Byprod	54	31	0	34	0
C	Fluoranthene	206440	Byprod	51	40	0	0	0
C	Naphthalene	91203	Byprod	61	38	0	0	0
C	Phenanthrene	85018	Byprod	35	21	0	0	0
D	1,2-Dichlorobenzene	95501	Sol	50	1	0	0	0
D	1,2,3-Trichlorbenzene	87616	Sol	57	1	0	0	0
D	1,2,4-Trichlorobenzene	120821	Sol	54	5	0	0	0
D	1,3-Dichlorobenzene	541731	Sol	38	0	0	0	0
D	1,3,5-Trichlorobenzene	108703	Sol	49	0	0	0	0
D	Monochlorobenzene	108907	Sol	35	0	0	0	0
D	Pentachlorobenzene	608935	Sol	22	0	0	0	0
E	1,1-Dichloroethane	75343	Sol	21	1	0	0	0
E	1,1-Dichloroethene	75354	Sol	37	2	0	0	0
E	1,1,1-Trichloroethane	71556	Sol	65	8	0	0	0
E	1,1,2-Trichloroethane	79005	Sol	31	1	0	0	0
E	1,1,2-Trichloroethene	79016	Sol	70	25	0	0	0
E	1,2-Dibromoethane	106934	Sol	38	0	0	0	0
E	1,2-Dichloroethane	107062	Sol	68	3	0	0	0
E	Carbon tetrachloride	56235	Sol	71	17	0	0	0
E	Dichloromethane	75092	Sol	69	9	0	0	0
E	Hexachlorobutadiene	87683	Sol	46	2	0	0	0
E	Tetrachloroethylene	127184	Sol	70	43	0	0	0
E	Trichloromethane	67663	Sol	72	35	0	0	0
E	1,2-Dichloroethene	540590	Mis	7	1	0	0	0
F	2,4-Dichlorophenol	120832	Sur	9	1	0	0	0
F	2,4,5-Trichlorophenol	95954	Sur	2	0	0	0	0
F	2,4,6-Trichlorophenol	88062	Sur	6	0	0	0	0
F	4-Chloro-3-Methylphenol	59507	Sur	3	1	0	0	0
F	4-Nonylphenol, branched	84852153	Sur	5	4	0	1	0
F	4-Octylphenol	1806264	Sur	10	1	0	0	0
F	Para-tert-octylphenol	140669	Sur	21	9	0	1	0
G	Benzene	71432	AOxi	56	24	0	0	0
G	Ethylbenzene	100414	AOxi	49	13	0	0	0
G	m-Xylene	108383	AOxi	4	0	0	0	0
G	o-Xylene	95476	AOxi	42	11	0	0	0
G	p-Xylene	106423	AOxi	4	0	0	0	0
H	4-Chlorotoluene	106434	Sol	24	1	0	0	0
H	Toluene	108883	Sol	60	25	0	0	0
I	Tributyltin	56573854	AFun	24	16	0	5	1
J	PBDE 28	41318756	FlRet	12	0	0	0	0
K	Vinylchloride	75014	Mis	20	0	0	0	0

[a] A: Pesticides and transformation products, B: Polychlorinated biphenyls, C: Polycyclic aromatic hydrocarbons and derivates, D: Halogenated benzenes and nitrobenzenes, E: Halogenated alkanes, F: Phenols and chlorophenols, G: Anilines, anisoles and alkylated benzenes, H: Toluenes and halogenated derivatives, I: Organotin compounds, J: Brominated flame retardants, K: Miscellaneous.
[b] Her: Herbicides, Fun: Fungicides, Ins: Insecticides, Lub,Pla: Lubricants and plasticizers, Byprod: Byproducts of petroleum processing or combustion, Sol: Solvent, Sur: Surfactant, AOxi: Anti-oxidants, AFun: Antifungal, FlRet: Flame retardants, Mis: Miscellaneous.
[c] Percentage of sites with exceedances of low or high risk thresholds

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Author's Contributions

Paper I

Title:	Physiological sensitivity of freshwater macroinvertebrates to heavy metals
Authors:	Egina Malaj, Matthias Grote, Ralf B. Schäfer, Werner Brack, & Peter C. von der Ohe
Status:	Published in 2012 in Environmental Toxicology and Chemistry, Vol. 31, pp 1754-1764
Contribution:	Malaj (>70%) Designed research, Analysed data, Discussed results, Wrote manuscript von der Ohe (10%) Designed research, Discussed results, Edited manuscript Schäfer (10%) Designed research, Discussed results, Edited manuscript Grote (5%) Discussed results, Edited manuscript Brack (2%) Discussed results, Edited manuscript

Paper II

Title:	Evolutionary Patterns and Physicochemical Properties Explain Macroinvertebrate Sensitivity to Heavy Metals
Authors:	Egina Malaj, Guillaume Guénard, Ralf B. Schäfer, & Peter C. von der Ohe
Status:	In Review in Environmental Science & Technology
Contribution:	Malaj (70%) Designed research, Analysed data, Discussed results, Wrote manuscript Guénard (20%) Analysed data, Designed research, Discussed results, Edited manuscript von der Ohe (5%) Designed research, Discussed results, Edited manuscript Schäfer (5%) Discussed results, Edited manuscript

Paper III

Title:	Organic Chemicals Jeopardize the Health of Freshwater Ecosystems on the Continental Scale
Authors:	Egina Malaj, Peter C. von der Ohe, Matthias Grote, Ralph Kühne, Cédric P. Mondy, Philippe Usseglio-Polatera, Werner Brack, & Ralf B. Schäfer
Status:	In Press, doi:10.1073/pnas.1321082111
Contribution:	Malaj (60%) Designed research, Analysed data, Discussed results, Wrote manuscript Schäfer (20%) Designed research, Discussed results, Wrote manuscript von der Ohe (10%) Designed research, Discussed results, Provided data, Edited manuscript Grote (5%) Discussed results, Provided data, Edited manuscript Kühne, Mondy, Usseglio-Polatera & Brack (5%) Provided data and/or Edited manuscript

Paper IV

Title:	How Much do Organic Toxicants Contribute to Multiple Stress in Freshwater Ecosystems?
Authors:	Ralf B. Schäfer, Bernhard Kühn, Egina Malaj, Anne König, René Gergs
Status:	In Review in Freshwater Biology
Contribution:	Schäfer (50%) Designed research, Analysed data, Discussed results, Wrote manuscript Kühn (20%) Analysed data, Discussed results, Edited manuscript Malaj (10%) Analysed data, Discussed results, Edited manuscript König (10%) Analysed data, Discussed results, Edited manuscript Gergs (10%) Analysed data, Discussed results, Edited manuscript

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Resume

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Research Interests

Ecological risk assessment and management of micropollutants

Effects of environmental stressors on benthic invertebrates in streams

Application of statistical and geostatistical models to understand ecological and chemical patterns in aquatic ecosystems

Research Activities

Current Oct 2010 | **PhD Candidate**
Helmholtz Center for Environmental Research-UFZ
Department of Directed-Effect Analysis
Leipzig, Germany

University of Koblenz-Landau
Institute for Environmental Sciences
Landau, Germany

Dissertation: Safeguarding freshwater organisms from chemicals: From the application of evolutionary concepts in ecotoxicology to large-scale risk assessment of chemicals

Apr-Jul 2010 | **Research Assisstant**
UNESCO-IHE Institute for Water Education
Delft, The Netherlands

Main Task: Laboratory work regarding heavy metal investigation in sediment including sequential extraction, analysis of heavy metals, and quality control analysis of the experiments.

Education

Mar 2013 – Oct 2010 | **Graduate School: HIGRADE**
Helmholtz Center for Environmental Research-UFZ

Core Subjects: Statistics (Introduction, Multivariate, & Geostatistics), Introduction to Environmental Chemistry and Ecotoxicology, Introduction to Hydrological Processes, and Water Resources Management

Apr 2010 – Oct 2008 | **MSc in Environmental Science**
UNESCO-IHE Institute for Water Education, Delft, The Netherlands
Specialization in Environmental Science & Technology

Core Subjects: Environmental Monitoring and Modeling, Environmental Engineering, Solid Waste Management, Cleaner Production and the Water Cycle

Dissertation: Distribution and fate of heavy metals in the Albanian part of Lake Ohrid

Jul 2008 – Oct 2004 | **BSc in Agro-Environment & Ecology**
Agricultural University of Tirana, Albania

Core Subjects: Chemistry (General, Organic, Analytical, Physical), Biostatistics, Microbiology, Earth science, Ecology

Dissertation: Qualitative assessment of coastal marine waters and sediments in Saranda, Albania (In Albanian)

Scholarships

Sep 2008 | MSc scholarship for outstanding results during the Bachelor studies. Granted by: Netherlands and Western Balkan Environmental Network (NEWEN)

May 2010 | Research Stay Grand from Waterpass Foundation, Amsterdam, The Netherlands

Publications

Journal Articles

Malaj E., Guénard G., Schäfer R. & Von Der Ohe P. (2015) Evolutionary patterns and physicochemical properties explain macroinvertebrate sensitivity to heavy metals. In Review in *Environmental Science & Technology.*

Schäfer R.B., Kuehn B., **Malaj E.**, Koening A. & Gergs R. (2015) How much do organic toxicants contribute to multiple stress in freshwater ecosystems? In Review in *Freshwater Biology.*

Malaj E., Von Der Ohe P.C., Grote M., Kühne R., Mondy C.P., Usseglio-Polatera P., Brack W. & Schäfer R.B. (2014) Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proceedings of the National Academy of Sciences*, USA, 111, (26), 9549-9554.

Malaj E., Grote M., Schäfer R.B., Brack W. & Von Der Ohe P.C. (2012) Physiological sensitivity of freshwater macroinvertebrates to heavy metals. *Environmental Toxicology and Chemistry*, 31, 1754-1764.

Malaj E., Rousseau D.P.L., Du Laing G. & Lens P.N.L. (2012) Near-shore distribution of heavy metals in the Albanian part of Lake Ohrid. *Environmental Monitoring and Assessment*, 184, 1823-1839.