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Contaminant curiosity and pollutant puzzles: Conceptual insights in ecotoxicity and practical implementation of higher-tiered risk assessment.

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Since the soil quality Tool for Risk Identification, Assessment and Display approach introduced the "three lines of evidence" accounting for chemical, toxicological and ecological stressors to explain adverse effects in biota, the assessment of contaminant risks in the environment has significantly evolved. The concept of chemical speciation, related to water characteristics, boosted the understanding of the role of free-ion activities in the overall accumulation of pollutants in biota. New modeling concepts (e.g. biotic ligand models) and measuring techniques were developed. This in turn triggered widespread research addressing the quantitative role of sediment in the overall water quality, focusing on redox interfaces. For contaminant mixtures in river catchments, complex relations between (bio)availability of compounds, including nutrients, help to explain aquatic toxicity. Variation in ecological patterns and processes across environmental or spatiotemporal gradients occur, which may identify ecological factors that influence contaminant fate and effects. Empirical evidence by meta-analysis and theoretical underpinning by modelling showed relationships between population growth rates and carrying capacities, across chemicals and across species. The potentially affected fraction (PAF) of species may be related to the mean species abundance, an often-used indicator in global change studies. Knowledge gaps remain on how pollutants travel through ecological communities and which species and species-relationships are affected. Outdoor experimental systems that examine the natural environment under controlled conditions may be useful at the higher biological level to investigate the impact of stressors on a variety of species, including mutual interactions.

Keywords: bioaccumulation, bioavailability, environmental chemistry, sediment quality, speciation, TRIAD

Evolution of risk assessment: principles and gaps

Struggling to understand the complicated reactions of biota to environmental pollution, Cees van de Guchte (†2021) was one of the early pioneers starting in the late 1980s to perform water and sediment tests to study the effects of pollutants on aquatic organisms. A bioassay was generally performed in a controlled environment with a single species, observing a biological reaction to various doses of a contaminant (Guchte vd and Maas, 1987;

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Guchte vd et al., 1989). Most puzzling at that time was the observation that biota react very differently when exposed to surface waters of different geographic origin. An early, unpublished result from the early 1990s is shown in Figure 1; Trying to determine the 50% effect concentration (EC50-mortality) of copper on *Daphnia magna* in surface waters, and anticipating possible seasonal effects induced by e.g. temperature and food availability, the 10-fold variation over the various locations exceeded the variation over the four seasons by far. This location-specific dominance, presumed to be caused by the (chemical) composition of the surface water, motivated further research in search of causal relationships between water and/or sediment characteristics and the biological response.

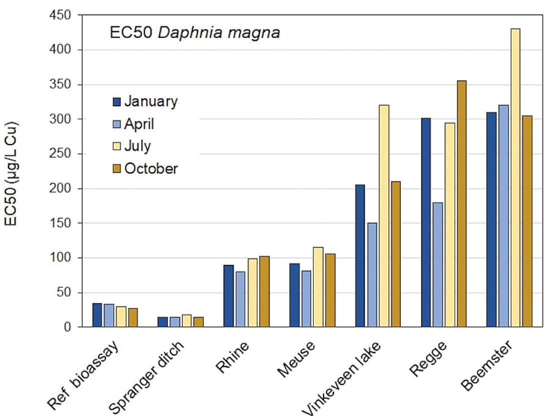


Fig. 1. Variance in toxicity of Daphnia magna with copper spike in surface waters from 7 different locations (after Guchte vd, unpublished). The reference bioassay is the standard test with protocolized water composition.

The early research on interactions between pollutants and organisms mainly focused on the target organism (e.g. life stages and toxic endpoints). Experimental protocols dictated that a compound of interest was added to a standard growth medium, followed by short term (acute) or long term (chronic) tests. However, it gradually became clearer that the medium itself may affect the chemical speciation of the compound, and therefore the actual exposure of organisms. Lewis et al. ,1972) were one of the first to report on the behavior of a compound over phases, thereby affecting biological uptake.

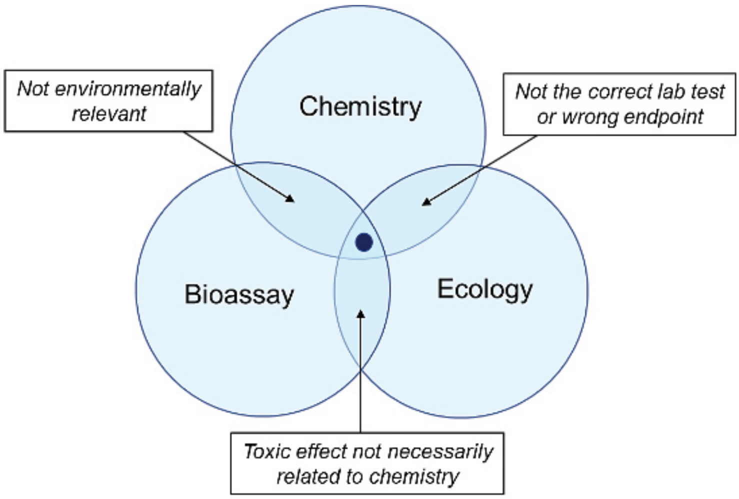
TRIAD approach

In the 1980's, Chapman (1986) indicated that "the combination of three lines of evidence can lead to a comprehensive understanding of the possible effects to the aquatic community". This principle became known as the TRIAD (Tool for Risk Identification, Assessment and Display approach). The "three lines" consist of: 1) chemistry; 2) toxicology; and 3) ecology. (see Fig. 2). TRIAD guidelines, proposed by Chapman et al. (1997) stated that a balanced TRIAD requires efforts in all three lines, and that parameters should be quantitative rather than integer expressions or text. TRIAD does not provide a cause-and-effect relationship, linking concentrations of individual chemicals to adverse biological effects, but it does provide an assessment to explain the (quantitative) effect of environmental characteristics (Grootelaar et al., 1994; Guchte vd, 1992; Guchte vd et al., 1987; et al., 1988; et al., 1989).

In a long-term study (SKB, 2009), TRIAD proved to generate more useful insights in the adverse effects than could be established by the generic, "first-tier" assessment: the compliance check of a compound's concentration with a generic environmental quality standard. When the three different approaches – being chemical monitoring, biological surveillance and laboratory-based experiments – point to the same direction, site-specific risk assessment is easy. However, the TRIAD not always generates the necessary results to predict ecological effects in all cases (SKB, 2009). Hence, each of TRIAD's lines of assessment required more detailed knowledge so realistic site-specific assessments can be made in a robust way, so-called "higher tiered" assessment. These inconsistent outcomes in chemical, toxicological and ecological monitoring can be theoretically attributed to the causes summarised in Figure 2. Yet, the way forward is to a more detailed analysis of discrepancies. Legal implementation of TRIAD, and consequences for e.g. remediation, did not emerge until some decades (NEN-5737, 2010; Mesman et al., 2011; ISO-19204, 2017) and TRIAD became part of the Dutch Soil Protection Act (Agentschap NL, 2012).

Developments in chemical speciation

Interestingly, innovative insights in environmental chemistry developed almost parallel; in the late 70's, chemical speciation



	Chemistry	Bioassay	Ecology	Possible conclusion
1	+	+	+	Evidence for pollution-induced degradation
2	-	-	-	Evidence of no pollution-induced degradation
3	+	-	-	Contaminants are not bioavailable
4	-	+	-	Unmeasured chemicals or conditions cause degradation
5	-	-	+	Alterations not due to chemicals
6	+	+	-	Chemicals are stressing the system
7	-	+	+	Unmeasured chemicals cause degradation
8	+	-	+	Chemicals are not bioavailable or alteration not due to chemicals

Fig. 2. Principle of TRIAD; combining three lines of evidence to assess overall risk of pollutants.

became a key playing ground for chemists with an interest in toxicology. Studies of Anderson et al. ,1978 Allen et al. ,1980) and others, resulted in the concept of the free ion activity model (FIAM). Biological response was attributed to the most (bio)available chemical species, the freely dissolved ion. FIA is determined not only via the total dissolved concentration, but also by (organic) ligands in solution (Morel, 1983; Sanders et al., 1983). The concept of FIAM triggered a huge amount of both theoretical and practical research in the forthcoming years, focusing on the need to measure, or indirectly quantify, the concentration of free ions and/or labile phases in solution. Promising techniques developed over the years, based on an array of principles such as electrostatic induced transport (ISE, ASV), membrane separation (DMT, PLM), exchange with competing ligands (CLE), or techniques that use molecular diffusion over permeable gels or films (DGT, DET). Table 1 summarizes some of the most studied and applied techniques (Vink et al., 2005).

Although FIAM is indeed a large step forward in understanding the biological reaction to contaminants, uncertainties remain, specifically for chronic exposure. The TRIAD approach is still conceptually valid; the implementation of sophisticated methods and models that became available in last decades have been incorporated in the higher tiered assessments with the TRIAD approach. Much scientific effort is put into the quantitative role of the composition of surface water, and its effect on the extent of toxicity of heavy metals, allowing for higher tiered, site-specific risk assessment (section 2). Also, the interaction of surface water and its overlying sediment receives much attention, addressing the complex interaction of both chemical exchange over water-sediment interfaces and the relationships of multiple (polluting) compounds (section 3). Next, the differences in field-versus-lab observations (section 4) need to be further explained, allowing

Table 1. Operational metal speciation techniques.

Method		Chemical separation	Measured species	Response time	Remark	Ref
ASV	Adsorptive stripping voltammetry	Electrochemical	Free metal + labile species	Minutes	Complex detection	Kaldova & Koopanica, 1989
CLE	Competing ligand exchange	Exchange reaction with ligands	Free metal + very labile species	Seconds	Critical modification and handling	Apte & Batley, 1995
DET	Diffusive equilibrium in thin film	Porous gel	Free metal + penetrable complexes	Days	Ionic strength limitations	Davison e.a., 1991
DGT	Diffusive gradient in thin film	Porous gel	Free metal + labile penetrable complexes	Days	Critical gel composition	Zhang & Davison, 1995
DMT	Donnan membrane technique	Charged pore membrane	Free metal + cationic penetrable complexes	Days	Critical pore size	Lampert, 1982
GIME	Gel impregnated microelectrode	Porous gel + electrochemical	Free metal + labile penetrable complexes	Minutes	Surface/lability interaction	Tercier & Buffle, 1996
ISE	Ion selective electrode	Liquid partition without countercharge transport	Free metal	Seconds	Interference from other metals and organic matter	Berner, 1963
PLM	Permeation liquid membrane	Liquid partition with countercharge transport	Free metal + labile complexes	Minutes	Diffusion-controlling step	Buffle e.a, 2000

for a reliable assessment of multi-contaminant fate in whole ecosystems.

Water composition and toxicity

As demonstrated in its simplest way by Fig. 1, ecotoxicity in water varies with the chemical characteristics specific to each water body. One of the earliest recognitions of this came from Zitko and Carson (1975) who coupled potential hazards of inorganic pollutants to water hardness. To date, many countries have implemented regulatory jurisdictions for environmental quality values (e.g. cadmium) that vary according to the water hardness. However, water hardness is just one of the several important parameters that explain the observed differences in metal ecotoxicity. Dissolved organic carbon (DOC) has been shown to be a key parameter to predict the behavior and toxicity of metals in aquatic systems (e.g. Tipping and Hurley, 1992; Kinniburgh et al., 1996; Christensen et al., 1996; Milne et al., 2003; Guthrie et al., 2005; Environment Agency, 2012). There is extensive peer-reviewed literature demonstrating that metal toxicity in waters is poorly correlated with total metal concentrations (e.g. Erickson et al., 1996; Paquin et al., 2002; Peijnenburg et al., 2002; Vink, 2002; Erickson, 2013) and it has been recognized that only a portion of the total amount of metal in the environment can actually be taken up by organisms and subsequently induce adverse effects. The main idea behind this "bio-availability concept" is that the toxic effect of a metal does not only depend on the total (dissolved) concentration of that metal in the surrounding environment but also on the complex interactions between physico-chemical and biological factors.

The development of the free ion activity model (FIAM) for metals recognized that coexisting protons and cations (e.g. Ca^{2+}, Mg^{2+}, K^+, Na^+) affect accumulation and toxicity by competing for toxic action sites (Pagenkopf, 1983; Playle and Dixon, 1993; Di Toro et al., 2001; De Schamphelaere et al. 2005). For example, cations were assumed to reduce toxicity in fish by competing with toxic metal ions for binding sites on gills or other biological surfaces (Erickson, 2013). On this basis, the Biotic Ligand Model (BLM) was developed. BLMs are metal and organism specific, requiring the incorporation of empirically determined metal-binding constants and intrinsic metal sensitivity of different biological species. These concepts (SCHER, 2010; Rüdel et al., 2015) and the practical application (Paquin et al., 2002; Vijver et al., 2008; EU, 2019) are well reviewed and documented. To calculate water-type specific effect concentrations, a step-by-step approach is used to combine experimental laboratory-based effect concentrations (from bioassays) with water type specific characteristics. Data from toxicity tests are subjected to speciation modeling to compute free ion activities of the metal of interest. These are used as input for the BLM to normalize the test-NOEC to a site-specific NOEC. Depending on the amount of toxicity data, and the represented amount of biota from various taxa, Species Sensitivity Distributions (SSD) are constructed. The SSD is a cumulative probability distribution showing the relation between toxicant concentrations and the potential affected fraction of the ecosystem (Posthuma et al., 2002). Finally, HC5 (hazardous concentration at which 5% of the species in the SSD exhibit an effect) expressed as free ion activity, are derived from site-specific SSD and subsequently transformed to site-specific total dissolved HC5, using the same speciation model. By expressing HC5 as total dissolved metal concentration, a straightforward comparison between HC5 and field measurements or generic quality standards can be done (Verschoor et al., 2011; 2012; 2017).

Figure 3 shows an example for zinc at various sites, geographically distributed over The Netherlands (data from Verschoor et al., 2011). Monitoring data included 11 water characteristics that were used as input for chemical speciation calculations using WHAM software (Tipping, 1994) and full-BLM modeling (Heijerick et al., 2002; 2005; DeSchamphelaere and Janssen, 2004, 2010; De Schamphelaere et al., 2005). To visualize the variation over various water types, we constructed SSD-curves for 19 different aquatic organisms, varying from algae to fish (validated toxicity data from Zn-Risk Assessment Report, EU, 2008). For comparison, the SSD on which the generic environmental quality standard (EQS) was derived, is also incorporated. The EQS is the cut-off value for which 95% of all species are supposed to be protected, resulting in a hazard concentration HC5.

Calculated site-specific HC5-values show a large variation of several factors over the sites, resulting from different chemical composition and subsequent chemical speciation. Compared to the generic Zn-EQS, both over- and underestimation

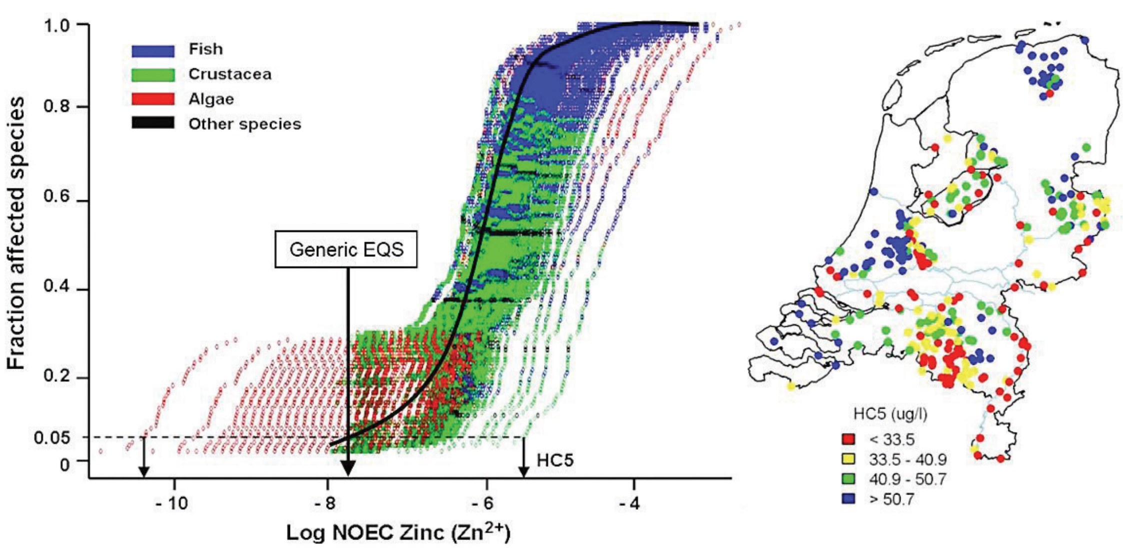


Fig. 3. Species sensitivity distributions for zinc in various water bodies across The Netherlands. Compared to the generic environmental quality standard (black solid curve) large variations occur as a result of water composition and chemical speciation.

of zinc toxicity occurs. In other words: some locations are more sensitive, while others are more robust against zinc toxicity. The capability of BLM to differentiate locations in terms of risks was reviewed by many authors (e.g. DeSchamphelaere et al., 2005; Van Sprang et al., 2009; McLauglin, 2015).

In recent years, the development of BLMs for various metals and target organisms (both flora and fauna) has increased dramatically. The full BLMs that have been developed for, e.g. Cu, Ni, Pb and Zn have been accepted as the basis for the aquatic risk assessment in the EU (Zinc EU RAR 2010, Nickel EU RAR 2008, Copper RAR 2008). The bases of these risk assessments are validated applications of the BLMs to calculate site-specific SSDs. In a subsequent step, either site-specific SSDs are integrated toward larger geographic scales (regional, national) or a preset percentile of the required input parameters for the BLMs (e.g. the x^{th} percentile of the regional distribution of pH, dissolved organic carbon (DOC etc.) is used to derive SSDs at a higher level of geographical integration (Rüdel et al., 2015; EU, 2019).

A major barrier to widespread, practical use and implementation of BLMs is the conceptual complexity of the approach, requiring advanced chemical speciation calculations and normalization procedures with toxicity data. Moreover, BLMs may require a large number of measured data, some of which are not readily available in standard monitoring programs. For this reason, simplified and user-friendly tools have been developed over the past years. These tools may replace complicated BLM procedures and increase the applicability for "routine", water type-specific risk assessment. A simplified model mimics the BLM upon which it is based, but with a slightly reduced level of predictive performance (Environment Agency, 2012; Verschoor et al., 2012). It uses a basic set of commonly determined water parameters as input (i.e. DOC, pH, hardness, and dissolved metal concentration). Currently, there are three operational user-friendly tools: M-Bat (EPA, 2007); Bio-met (Peters et al., 2011); and PNEC-pro (Vink et al., 2016). These tools are implemented in regulatory protocols of European member states for water quality assessment or mandatory WFD-reporting (Rüdel et al., 2015; EU, 2019).

Although the development of BLMs is a good step forward in underpinning (site-specific) risk assessment, following TRIAD's concept, challenges remain. Next to the effect of environmental variations on population dynamics (Hendriks et al., 2005; Heugens et al., 2006 surface waters mostly carry more than just single pollutants for which BLMs are available, and combined effects (Enserink et al., 1991; Posthuma et al., 2008) and synergistic toxicity (Chu and Chow, 2002) of pollutants are likely to occur. Brix et al. , 2022) proposed that the continued development of mixture biotic ligand models (m-BLMs) may be the most effective way to achieve the goal of a more holistic approach to regulating metals in aquatic ecosystems. Given the need to further develop and validate m-BLMs, a weight-of-evidence approach is suggested that includes m-BLMs, macroinvertebrate community bioassessment, and measurement of metals in key macroinvertebrate species. This approach provides a near-term solution and simultaneously generates data needed for the refinement and validation of m-BLMs.

Sediment: sink or contributor to toxicity?

The role of sediment in the overall contribution of aquatic toxicity has long been studied. In the early days, some bioassay protocols were available for monitoring sediment and (pore-)water quality (e.g. Hendriks and Guchte vd, 1997). Later, additional tests became available, especially for water, allowing for a comprehensive assessment using so-called Species Sensitivity Distributions (SSDs) (Posthuma et al., 2002). Nowadays, SSDs are available for over 12,000 substances allowing for more comprehensive assessments (Posthuma et al., 2019). Combining all data on sediment pollution in major waterways in the Netherlands and Flanders collected for decades, the potentially affected fraction (PAF) of species was demonstrated to range from 15% to 30% for different catchments (Den Besten et al., 1995, Wang et al., 2020). The largest contribution came from metal and PAH pollution. Of the effects in toxicity assays, 60-100%$^{+}$ and 20%-90% could be attributed to the identified chemicals for *Daphnia magna* and *Hyaella azteca*, respectively. Moreover, the fraction explained for *Hyaella azteca* and

Heterocypris incongruens increased from 20% to 90% after accounting for chromium, tin, ammonia and phosphate availability (Nolte et al. 2020, 2021). Yet, contributions of other substances not covered in monitoring programs could have contributed, as was demonstrated for organophosphate pesticides in Spanish sediments (De Castro-Català et al., 2016). Remarkably, macrofauna abundance sampled in the same Dutch and Flemish sediments was not correlated to chemical concentrations and toxicological effects (Wang et al., 2020).

The role of sediment in the assessment of ecological risks of contaminants in the aquatic environment is historically linked to generic quality standards of total contents in sediment. However, sediment quality standards are mostly derived from aquatic tests, converting LC5-values (lethal concentration for 5% of test species) via equilibrium partitioning to solid phase. The correlation between total sediment concentrations and bioconcentrated metals of exposed biota is generally very poor (Ciutat et al., 2005; Simpson and Batley, 2007 with a possible exception for arsenic for which methylation was generally promoted by eutrophication via microbial metabolism (Farag et al., 2007; Düster et al., 2008 most probably resulting in an underestimation of arsenic remobilization from polluted sediments.

Over the last two decades, the development of new techniques enabled experimenters to study chemical speciation over aerobe-anaerobe gradients over the water-sediment interface. Redox potential, nutrients and contaminants could be measured simultaneously in the water column and sediment porewater at different depths (Düster et al., 2008; Saaltink et al., 2018 while observing biological response to exposed organisms. Studies integrating water, sediment and biota measurements during an exposure period show that bioaccumulated contaminants in aquatic organisms, including soil dwellers, use the oxygenated, overlying water as a contaminant source (Vink, 2002; 2009). The fact that the overlying water plays such a significant role in metal uptake is actually not very surprising: sediment dwellers are in close contact with sediment pore water, but species that live in burrows are actually in closer contact with overlying water because: i) the burrow inhibits direct contact between the organism and pore water, and ii) organisms exchange the water in their burrows with overlying water, either actively by physical motion or passively through diffusion (Ciutat et al., 2005; Simpson and Batley, 2006). Saaltink et al. , 2018) for example showed that Tubificidae oxygenated the upper 15 mm of the sediment, resulting in a sixfold increase in NO_x concentrations in porewater and the water column, and simultaneously decreasing phosphorus concentrations as a result of pyrite oxidation and the formation of iron(hydr)oxide formation as a sorption phase.

In 2005, the "standard sediment test" of the TRIAD guidelines 1993 was compared head-to-head to an exposure test using the technique described above (Vink et al., 2005). In the standard sediment test, a sediment field sample is

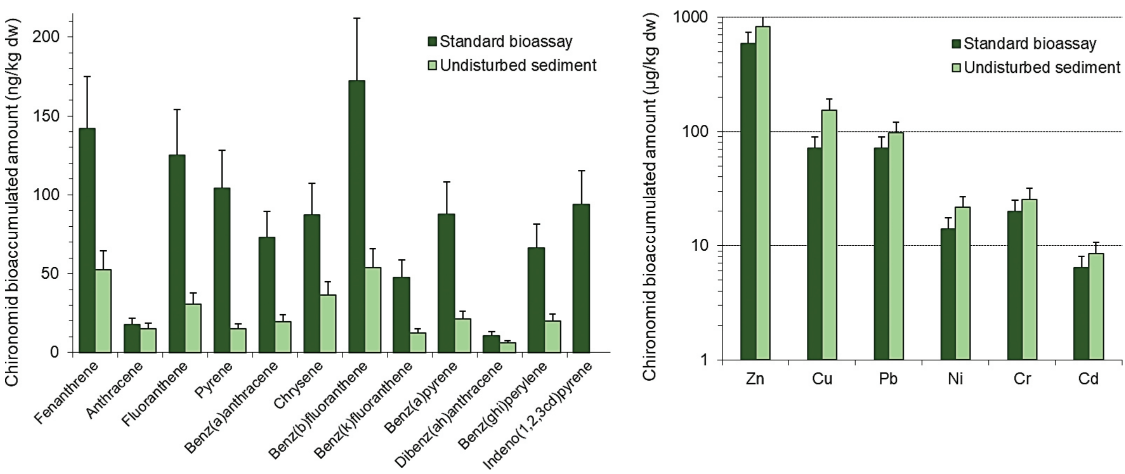


Fig. 4. Bioaccumulated contaminants in chironomids exposed in a TRIAD standard sediment assay and in an undisturbed water-sediment system.

mechanically homogenized, mixed 1:4 v/v with artificial composed water (DSW), shaken for 24 hours and the suspension is decanted into dishes to settle after which test species (*Chironomus riparius)* were introduced for 28 days. From the same site, an undisturbed sediment/water core was sampled using a SOFIE® device (Vink, 2002 leaving physical conditions untouched. Figure 4 shows the biological response, in terms of accumulated organic PAHs (polycyclic aromatic hydrocarbons) and inorganic (metals) compounds, from both tests. In the standard sediment test, chironomids take up PAHs in significant larger amounts than in the undisturbed sediment test. It was suggested that pre-treatment of sediment has liberated PAH from the sediment matrix by unlocking and exposing organic surface areas. For metals, oxidation in the standard test leads to the production of reactive (hydr)oxides of Fe and Mn that act as strong sorbents for free dissolved metals, thereby - at least temporarily - immobilizing part of the reactive metal concentrations.

Studies that provide an integral, quantitative overview of contaminant distribution and fate over environmental compartments, including sediment, water, and biota, are very scarce. In order to mass-balance the compounds of interest over the various (a)biotic compartments, data collection must occur practically simultaneously to prevent non-equilibrium situations to occur. Examples of such studies from USA (Farag et al., 2007) and Europe (Schipper et al., 2009; Vink et al., 2010) provide a range of (in)organic contaminants, nutrients, from multiple locations over water-sediment interfaces. Farag et al. (2007) concluded that transfer of metals associated with Fe colloids to biological components of biofilm is an important pathway, where metals associated with abiotic components are first available to biotic components. Schipper et al. (2009) and Vink et al. (2010) examined whether substances were released from aquatic sediments to the water column by means of diffusive transport, using a range of techniques including passive sampling (IVPS), Empore disk, SOFIE, and modelling. Results showed that substances may be released from sediment by diffusion (in particular phosphate, As, Zn, Ni, Pb, and a number of PAH and PCB compounds). The redox-sensitivity of substances, particularly at the transition between oxic and anoxic conditions, determines mobilization to a large extent. In general, compounds that act as electron donor (e.g. NH_4^+, Fe^{2+}, HPO_4^{2-}, $HAsO_4^-$, HCO_3^-) tend to emit from sediment to surface waters, while electron acceptors (e.g. NO_3^-, SO_4^{2-}, Mg^{2+}, Cl^-) tend to diffuse from surface water into the sediment.

Ecological variability

The response of communities to contaminant exposure depends on “the context”: Clements et al. , 2012) introduced the concept of context dependency, referring to variation in ecological patterns and processes across environmental or spatiotemporal gradients. These natural variations among communities may identify ecological factors that influence contaminant fate and effects. Similar to the way in which abiotic factors are associated with contaminant bioavailability, observations about context dependency could be used to test the underlying ecological mechanisms responsible for differences among communities. It also means that when lab-derived experiments are executed, a translation towards field situations needs to be done with caution. As empirical research is severely limited by financial, practical and ethical constraints, modelling is indispensable. In addition to the modelling of chemical speciation (see section 2 ecotoxicological extrapolation of effects in lab to field communities is needed.

In the eighties, several groups (e.g. Borgmann and Whittle, 1983, Van Leeuwen et al., 1985) including Cees van de Guchte and his trainee (Hendriks, 1986) started to report ecologically relevant indicators in addition to slopes and medians of traditional concentration-response curves. Empirical evidence by a meta-analysis and theoretical underpinning by modelling later showed relationships between population growth rates and carrying capacities, across chemicals and across species (Hendriks et al., 2005). Expressing response to toxicants in quantities used in models by ecologists is a pre-requisite to predict field population dynamics, in particular in multi-stress conditions. Based on these models, toxic and non-toxic stressors were demonstrated to affect, e.g. copepods and eagles exposed to sediments in Dutch and Flemish deltas (Korsman et al., 2012; 2014).

Just as intraspecific concentration-response relationship parameters (EC_{50}, β) can be related to population dynamics of a species, one may mechanistically and statistically link interspecies sensitivity distributions quantities (HC_{50}, β) to community development. Initial attempts demonstrated that the potentially affected fraction (PAF) of species as described above can be related to the mean species abundance (MSA an often-used indicator in global change studies (Hoeks et al., 2020). Even more, impact of pesticides on MSA as calculated from PAF was confirmed by a decrease of macrofauna abundance in the field (Thunnissen et al., 2020; 2022). The next step will be to link to other ecological indicators that provide a snapshot of community health, such as size spectra (Dos Santos et al., 2017). Finally, we may relate changes in biodiversity indicated by PAF and MSA to ecosystem services (i.e. the benefits provided by the natural environment to humans; Wang et al., 2021a-c). Over decades, almost any environmental pressure has been subject to ecosystem services accounting, but chemical pollution has, so far, been largely ignored. Obviously, overlooking such an important stressor may have serious consequences in management of environmental problems as well as research funding.

Over the years, many toxicokinetic and -dynamic models were developed. While very useful for interpretation of a controlled experiment for a specific chemical and species, many have a limited potential for extrapolation to the field, to population and communities and to multi-stress conditions. By nature, models linking lab cohorts to field populations need to be simple, or possibly crude, as many chemicals and species need to be covered. While the models described above require more theoretical and empirical underpinning, they may aid toxicologists to team up with ecologists to predict future developments.

The various interactions among biota, and the possible (physiological) effects, are not accounted for in Species Sensitivity Distributions (SSD). Although PAF-MSA studies show that these biotic interactions may not be of decisive importance, there is evidence that many different interactions in biological communities occur in the field. Examples are shown in Table 2. Collecting ecological surveillance data under the TRIAD (Figure 2), this knowledge can be used to interpret toxicity data. It should be noted that this often relies on correlative interpretation, and therefore tiered experimental approaches were developed (see section 5).

Experimental mesocosms; bringing field realisms in testing

Ecotoxicological tests should provide the necessary data for making realistic predictions of the fate and effects of chemicals in natural ecosystems (Landner et al., 1989). Experiments executed at lower biological levels are often performed under standard laboratory conditions. The laboratory setting has obvious advantages: allowing for replication, relatively easy and simplified conditions that enable outcomes that are rather robust across different laboratories, the stressor of interest being more traceable under optimal stable conditions, and easy repetition of experiments. Consequently, at the lower biological level the responses of organisms to chemical stressors tend to be more tractable, or more causal, than those identified when studying effects at higher tiered levels. However, the fundamental problem is that testing under lab conditions is a simplification that cannot always provide a good prediction at environmental relevant conditions, which is not only due to water chemistry parameters (sections 2 and 3) and context (section 4) but also explained by biological parameters. The latter, as experimentally shown by Ieromina et al. (2014) specifically food

Table 2. Biotic interactions existing in communities.

Interaction	Effect	Ecological response
Predation, herbivory, parasitism	(+/-)	Positive effect on consumer or parasite and negative effect on the prey or host
Amensalism	(0/-)	Net negative effect on one
Competition	(-/-)	Net negative effect on both
Mutualism	(+/+)	Net positive effect on both
Commensalism/facilitation	(0/+)	Net positive effect on one

quality could explain differences. This was later confirmed for other species (Barmentlo et al., 2018). Nederstigt et al. (2022) showed that the impact of carbendazim was more pronounced than observed at similar test concentrations in smaller scale studies which have been performed to date (Daam et al., 2009; Van den Brink et al., 2000). This builds on previous observations of impacts of other stressors in the applied experimental setup, and indicates that the inclusion of natural (re) colonizing communities in ecotoxicological studies allows for a realistic and sensitive evaluation of macroinvertebrate community dynamics.

In recent years, biological interactions were studied intensively (e.g. Van Gestel et al., 2019).

Barmentlo et al. (2018) showed that a detrivorous food chain as well as an algal-driven food chain responded differently to a trinary mixture of agrochemicals. This was illustrated by how the mayfly *Cloeon dipterum* reacted to the various combinations of pesticides. Compared to single substance exposures and binary mixtures, extreme low recovery of *C. dipterum* (3.6% of control recovery for both mixtures) was observed. However, after exposure to the trinary mixture, recovery of *C. dipterum* no longer deviated from the control, and was higher than expected. Unexpected effects of the mixtures were also obtained for both zooplankton species (*Daphnia magna* and Cyclops sp.) As expected, the abundance of both zooplankton species was positively affected by nutrient applications, but pesticide addition did not lower their recovery. These types of unanticipated results can only be identified when multiple species and multiple stressors are tested and cannot be detected in a lab-test with single species. Van den Brink et al. (2009) showed that lindane exposure caused a decrease in sensitive detritivorous macro-arthropods and herbivore arthropods. This allowed insensitive food competitors like worms, rotifers and snails to increase in abundance. Atrazine inhibited algal growth and hence also affected the herbivores. A direct result of the inhibition of photosynthesis by atrazine exposure were lower dissolved oxygen and pH levels and an increase in alkalinity, nitrogen and electrical conductivity.

Knowledge gaps remain on how pollutants travel through ecological communities and which species and species-relationships are affected. Hence, there is an urgent need to increase realistic, ecological-based toxicity tests by addressing chemical and particle mixtures effects, sub-lethal endpoints, chronic toxicity and multigeneration effects and species interactions, and incorporate this in predictive models. Outdoor experimental systems that examine the natural environment under controlled conditions (e.g. cosms) may be useful at the higher biological level to investigate the impact of a stressor on a variety of species, all having mutual interactions. This enables detecting both direct and indirect effects on the structure of species assemblages due to the chemicals. Therefore, mesocosm studies may bridge the laboratory derived results and the field observations. The crucial point in designing a model system may not be to maximize the realism, but rather to make sure that ecologically relevant information can be obtained. Reliability depends on the representativeness of biological processes or structures that are likely to be affected (OECD, 2004).

The merit to perform mesocosm studies is

Fig 5. Cosm approach; Left: indoor microcosm water-sediment interface. Right: outdoor mesocosms at the Living Lab facility of Universiteit Leiden.

that indirect effects can manifest as disruptions of species interactions, e.g. competition, predator-prey interactions and alike. A second reason is that abiotic interactions at the level of the ecosystem can be accounted for, allowing for measurement of effects of chemicals under more environmentally realistic exposure conditions. Examples are shown in Figure 5. Conditions that are likely to influence the behavior of chemicals are sorption to sediments, biofilms and plants, photolysis, changes in pH, and other natural fluctuations. By definition, mesocosms require an accurate equilibration of the system and well-designed realistic exposure scenarios.

In conclusion; more added value to TRIAD?

Only recently, scientists were able to make a quantitative assessment on the magnitude of synthesized contaminants exceeding the planetary boundaries (Persson et al., 2022). These studies show that awareness of the impact of contaminants on the overall functioning of our environment has been a challenging process. Without exception, European countries face levels of chemicals such as heavy metals and pesticides in our environment which are above environmental quality standards (Bouma, 1997; RIVM, 2011; CML, 2019; Santos et al., 2021) resulting in biological accumulation and biomagnification in higher trophic levels around the globe. Therefore, we need to further explore the mechanisms behind chemical-induced ecological effects, accounting for explicit field parameters to improve risk assessments in general and make them more realistic and scientifically sound.

Significant scientific progress was made over the last decades in understanding chemical speciation and biological responses of pollutants in water-sediment systems. The basic principles of TRIAD, accounting for the integration of chemical, toxicological and ecological data, are broadly accepted and practiced. Over the last decades however, many insights in each of these disciplines warrant improvement, not only in a monodisciplinary sense but particularly in integrating biotic and abiotic variables, and ecological context. For example, benthic organisms exert a significant effect on chemical availability, which must be considered when performing bioassays and chemical tests. Accumulated pollutants are regulated by temporal environmental variations and ecological (community) alterations. Biological kinetics (uptake) are for most metals faster than chemical kinetics (dissociation of less labile or complexed metals). Exposure can therefore only be estimated and modelled when time-related changes are considered.

The construction of generic models that transfer chemical information to a biological response has undoubtedly a high priority in future research. Dynamic metal speciation analysis is emerging as a powerful basis for development of predictions of bioavailability and reliable risk assessment strategies. Models need to be calibrated with robust, field-validated tests obtained by, e.g. (meso)cosms. Does this imply that all (upcoming) substances need to be tested under field conditions? And will this approach indeed allow us to characterize and quantify the risks posed by many thousands of substances? Preferably yes, but obviously this is costly and time consuming. Alternatively, and perhaps additionally, there is potential in the development of similarity modelling and ecological read-across extrapolation to derive better-founded estimates of environmental behavior, toxicity and fate of substances. Based on scenario analysis, more accurate evidence may be generated for which substances should be prioritized when it comes to in-depth testing. To expand quantification of cross-ecosystem impacts and the dynamic behavior of substances outside the domain of available data, it is essential that mechanistic modelling will further integrate the necessary disciplines. In this way, a weight-of-evidence approach (i.e. the basis of the TRIAD) is expanded with field realistic experimental data and processes based and mechanistic models and data-driven non-supervised models.

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