Consideration of the bioavailability of metal/metalloid species in freshwaters: experiences regarding the implementation of biotic ligand model-based approaches in risk assessment frameworks

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Abstract

After the scientific development of Biotic Ligand Models (BLMs) in recent decades these models are now considered suitable for implementation in regulatory risk assessment of metals in freshwater bodies. The approach has been developed over several years and has been described in many peer-reviewed publications. The original complex BLMs have been applied in prospective risk assessment reports for metals and metal compounds and are also recommended as suitable concepts for the evaluation of monitoring data in the context of the European Water Framework Directive. Currently, several user-friendly BLM-based bioavailability software tools are available for assessing the aquatic toxicity of a limited number of metals (mainly copper, nickel, and zinc). These tools need only a basic set of water parameters as input (e.g., pH, hardness, dissolved organic matter and dissolved metal concentration). Such tools seem appropriate to foster the implementation in routine water quality assessments. This work aims to review the existing bioavailability-based regulatory approaches and the application of available BLM-based bioavailability tools for this purpose. Advantages and possible drawbacks of these tools (e.g., feasibility, boundaries of validity) are discussed, and recommendations for further implementation are given.

1. Introduction

<u>Development of concepts for the consideration of metal bioavailability</u>

Metals and metal compounds may be a threat to living organisms and processes in the environment. They are a specific group of substances in the context of chemical risk assessment and monitoring because they are present in surface waters naturally due to geochemical processes. Some metals are even essential elements for organisms, and previous research has demonstrated that the availability and toxicity of metals towards organisms can strongly depend on local aquatic conditions. 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.

This recognition has led to the development of both analytical and modeling techniques for the determination of the concentrations of metal species that cause toxicity such as voltammetry and resin equilibration as analytical approaches (Tessier and Turner 1995) or the free ion activity model (FIAM; Morel 1983; Campbell 1995). The basic assumption of the FIAM is that the toxicity of a metal varies with its chemical speciation. In general, toxicity closely correlates with the free (i.e., the non-complexed) metal ion (Vink 2009). However, in some cases other species (or oxidation states) of the metal may contribute to toxicity.

The effect of water physico-chemistry on metal toxicity is related to metal bioavailability. A metal is considered bioavailable when it is free for uptake by an organism and can react with its metabolic machinery, which may result in a toxic effect (Campbell et al. 1988, Newman and Jagoe 1994). The main idea behind the bioavailability concept is that the toxic effect of a metal does not only depend on the total (or 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 FIAM (Morel 1983; Campbell 1995) recognized that coexisting protons and cations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+) affect metal accumulation and toxicity by competing for toxic action sites independent of metal species (Zitko and Carson 1976; Plette et al. 1999; De Schamphelaere et al. 2005; Kalis et al. 2006). 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 two distinctly different approaches have been developed to predict metal bioavailability in aquatic environments. One is the equilibrium modeling (e.g., the Biotic Ligand Model, BLM; Di Toro et al. 2001, Campbell and Fortin 2013). Alternatively, metal bioavailability is estimated based on biodynamic modeling (e.g., the DYNBAM model; Luoma and Rainbow 2005, Rainbow 2007).

The BLM aims to predict how dissolved metals interact with, and eventually affect, aquatic organisms. The epithelial surface of the organism, including the so-called biotic ligand (BL), is assumed to be in equilibrium with the ambient water, and the biological response (metal uptake/toxicity) is predicted on the basis of the concentration of the metal-biotic ligand complex (M-BL). The main idea behind the BLM concept is that the toxic effect of a metal does not only depend on the activity of that metal in the surrounding environment and the complex interaction between physico-chemical and biological factors, but also on the activity of ions competing for the same biotic ligand. Thus the same metal activity does not result in the same degree of toxic effect under all environmental conditions.

The BLM approach was originally developed to predict acute (short-term) toxicity to fish (Pagenkopf 1983; Di Toro et al. 2001, Paquin et al. 2002) based on a combination of existing chemical, toxicological, biological and physiological data. More recently, and specifically in Europe, the focus has shifted toward predicting chronic toxicity (i.e. not only effects on survival but also on growth and reproduction) to aquatic organisms belonging to different trophic levels such as algae, invertebrates and fish. This seems necessary if the BLMs are to be used in the framework of chemicals risk assessment in Europe (REACH, EC 2006) or for the setting of Environmental Quality Standards (EQSs) for metals for the EU Water Framework Directive (WFD; EC 2000, EU 2013).

An important assumption of the BLM approach is that metal toxicity can be predicted on the basis of waterborne exposures and that metals in food can be ignored. However, chronic BLMs implicitly include the dietary exposure route, too. Based on this implication and on studies demonstrating that the contribution of dietary metal to the overall toxicity value is rather small, an EU expert panel (SCHER 2010) concluded that the relative importance of uptake pathways other than the free ionic form of metals can be considered to be of minor importance.

In contrast, the biodynamic approach considers uptake of metals from both the ambient water and the ingested solids, and also takes into account metal loss (excretion; elimination of fecal matter; Luoma and Rainbow 2005). However, due to its complexity the biodynamic approach seems not applicable for routine assessments of specific sites.

Consideration of metal bioavailability in regulatory frameworks

Despite the large body of evidence on the effect of water chemistry on metal toxicity generated over the past 30 years, only few regulatory systems have taken this knowledge into account. This is due in part to a lack of quantitative tools. However, the BLM concept has gained interest from the academic, industrial and regulatory communities as this (conceptual) model is able to predict metal toxicity by integrating the most important effects of water chemistry. BLMs reflect the latest scientific knowledge and integrate decades of work regarding metal speciation, accumulation, toxicity, and physiology (Paquin et al 2002, Erickson 2013). In addition, efforts are made to develop user-friendly BLM-based bioavailability tools that are fit for use by non-expert water managers and environmental risk assessors.

Several regulatory authorities worldwide have developed or currently are developing methods to derive quality targets like EQSs for surface waters and to evaluate the potential environmental risks of metals and other substances. However, EQS derivation and the risk assessment of metals in the water compartment are still predominantly based on total or dissolved metal concentrations (Bergmann and Dorward-King 1997, Janssen et al. 2000, Sigg 2013), although it has been extensively demonstrated that neither total nor dissolved concentrations of metals are good predictors of their potential effects on ecosystems. Indeed, the physico-chemistry of water, described by characteristics such as dissolved organic carbon (DOC), pH and hardness can modify the toxicity of metals by several orders of magnitude. In this context BLM are now used for risk assessment and management (e.g., Zinc EU RAR 2010, Nickel EU RAR 2008, Copper RAR 2008).

Currently BLMs are only available for a limited number of metals (e.g., Cu, Ni, Zn or Ag), and are not widely accepted by regulatory agencies. In 2007 the United States Environmental Protection Agency (US EPA) recommended that aquatic life criteria incorporate the use of a BLM, but only for Cu and only in freshwater (US EPA 2007a). While the US EPA approved the use of the Cu BLM because it represents the "best available science", state and federal agencies may choose not to use the BLM. In Europe, some countries (e.g., UK, France) tested the use of BLM-based bioavailability tools for deriving region-specific PNECs (Predicted No Effect Concentrations), e.g. considering regional water conditions (David et al. 2011). Moreover a recently launched guidance document (EC 2011) encourages the derivation of BLM based EQSs for priority metals and metal compounds to allow compliance checking for specific local situations in the context of the EU WFD (EC 2000). Consequently, a recently released revision of the WFD EQS directive states for two metals (Pb, Ni) EQS based on bioavailable concentrations (2013/39/EU, EU 2013; to be implemented till end of 2015). To this end, efforts are made to adapt the originally complex BLMs to allow the use by risk managers by providing easy-to-use BLM-based bioavailability tools based on the full BLM (e.g., Bio-met 2011, PNEC.pro 2013).

The current work aims to review available bioavailability-based regulatory approaches and the application of proposed BLM-based bioavailability tools for this purpose. Advantages and possible drawbacks of these tools (e.g., feasibility, boundaries of validity) are discussed and recommendations for an informed use are given. This paper is the first outcome of the project "Consideration of bioavailability of metals/metal compounds in the aquatic environment" supported by the IUPAC "Division of Chemistry and the Environment" (#2011-060-1-600).

2. Bioavailability approaches to be covered

Based on the increasing knowledge of factors which influence metal bioavailability, different approaches were developed (Table 1). Here we focus on mature concepts which are already used (or are expected to be used in the near future) in regulatory contexts. Full or complex BLMs provide sound assessment of metal bioavailability but require a high number of measured water quality parameters as input. Moreover, most BLM programs are proprietary software and often require expert knowledge.

A major obstruction for a widespread, practical use and implementation of BLMs is the conceptual complexicity, requiring advanced chemical speciation calculations (e.g., Tipping 1994) and normalization procedures with toxicity data. Moreover, "full" BLMs require a large number of measured input parameters, some of which are not readily available in standard monitoring programmes. For this reason, user-friendly bioavailability tools were developed in recent years. Often, but not always, these tools are based on simplifications of the available "full" original BLMs. This is achieved by applying fixed values for the least relevant BLM parameters or exclusion of these parameters (Vijver et al. 2008). Other approaches apply empirical equations (transfer functions) to correlate water parameters and metal toxicity. Such bioavailability tools allow assessments by requiring only a few additional parameters in addition to the dissolved metal concentration (< 0.45 µm-filtered fraction) which serves as an

approximate of the bioavailable metal fraction (Table 1). Simplified bioavailability approaches are applied in cases where metal bioavailability is dominated by one parameter (e.g., hardness in case of cadmium).

BLMs are metal and organism specific, requiring the incorporation of empirically determined metal binding constants and intrinsic metal sensitivity of different biological species. The full BLMs that have been developed for, e.g., Cu, Ni, and Zn have been accepted as the basis for the aquatic risk assessment of these metals in the EU (Zinc EU RAR 2010, Nickel EU RAR 2008, Copper RAR 2008). The basis of these risk assessments are validated applications of the BLMs to calculate site-specific species sensitivity distributions (SSDs). In a subsequent step, either site-specific SSDs are integrated towards larger geographic scales (regional, national) or a pre-set percentile of the required input parameters for the BLMs (e.g., the x-th percentile of the regional distribution of pH, DOC, etc.) is used to derive SSDs at a higher level of geographical integration.

Table 1: Different approaches for consideration of metal bioavailability in aquatic systems.

Approach	Basis	Monitoring requirements	Example
Full or complex BLM (partly including chemical speciation models)	Consideration of metal speciation and interactions between metal ions, organic matter content, particles, and abiotic and biological ligands	Dissolved target metal concentration, pH, concentrations of major cations and anions, DOC, suspended particles, temperature, others	Cu-BLM (Copper RAR 2008)
User-friendly BLM-based bioavailability tools	Simplification of the complex BLM, based on calculations with complex BLM or based on transfer functions; validated against the complex BLM	Minimum: dissolved target metal concentration, DOC concentration, pH, hardness (e.g., as Ca concentration) and concentration; optional: Mg, Na concentrations	Bio-met (2011), M-BAT (WFD-UKTAG 2012) PNEC.pro (2013)
Simplified bioavailability approaches	E.g., organic matter/DOC related assessments or hardness banding	Dissolved target metal concentration, hardness or DOC concentration depending on approach	Hardness banding of Cd EQS for EU WFD (directives 2008/105/EC, EC 2008, and 2013/39/EU, EU 2013)

3. Overview of BLM literature

General BLM literature

In this section recent BLMs' developments and their applications to study metal toxicity are reviewed. Over the past decade, a number of BLMs, for both acute and chronic exposures, have been improved and applied to study metal toxicity of different trophic levels (see comprehensive list of publications in Table S1, Electronic Supplementary Material). Several reviews covered the genesis of the BLM approach for addressing impacts of exposure water chemistry and discussed challenges (e.g. Niyogi and Wood 2004; Slaveykova and Wilkinson 2005).

Cu remains the most studied metal followed by Zn and Cd. BLMs have also been adapted to predict metal toxicity of Ag, Ag nanoparticles, Hg, Pb, Ni, Mn, Co, Tl, U as well as multi-metal exposures (see Table S1 in the Electronic supplementary material). By applying BLMs it was demonstrated that Al^{3+} competed with Cu^{2+} (Chappaz and Curtis 2013) and Ni^{2+} (Worms and Wilkinson 2007) for the same binding sites, but very few studies applied BLMs directly to investigate the toxicity of trivalent cations. Recently, using

a freshwater alga (*Chlamydomonas reinhardtii*) Cremazy et al. (2013) found that a BLM could be applied to accurately predict the uptake of Sc only at pH values below 6.5.

The studies confirmed that water quality parameters such as DOC (or Dissolved Organic Matter, DOM), pH, alkalinity, water hardness, sulfide and thiosuphide ions significantly affect metal toxicity. Physicochemistry, exposure time (Hatano and Shoji 2010) and bioaccumulation kinetics of metals (Veltman et al. 2010) have also been incorporated into the models in order to improve their accuracies.

The limnic organisms that have been studied were predominantly *Chlamydomoas sp.* (representing microalgae), lemna (aquatic plants), *Oncorhynchus mykiss* (fish) and *Daphnia sp.* (invertebrates). A small number of studies were also carried out on molluscs. Cross-species application of BLM has been demonstrated (e.g., Casares et al. 2012; Schlekat et al. 2010). The use of gill cell lines to study silver toxicity in rainbow trout (Zhou et al. 2005) and copper effects in blue crab (Paganini and Bianchini 2009) promise a cost-effective in vitro alternative to using live animals.

BLMs have been applied to assess freshwater quality in Japan (e.g., Hayashi 2013), South and Central America (e.g., Natale and Leis 2008; Villavicencio et al. 2011; Casares et al. 2012), North America (e.g., Khan et al. 2012), certain European countries (e.g., Croatia, Vukosav et al. 2014) and the European Union (e.g., van Sprang et al. 2009). The application of BLM as an evaluative tool has clearly been demonstrated and it continues to serve as a useful interface amongst scientific disciplines and regulatory authorities as outlined by Paquin et al. (2002).

In the estuarine and marine environment, BLMs have been applied to assess Ag, Cu, and Zn toxicity (Table S1 in the Electronic supplementary material) where salinity and the availability of food appeared to significantly affect the metal toxicity (e.g., Leaes Pinho and Bianchini 2010). In addition to gills, in marine organisms also sites involved in osmotic and ionoregulation in other tissues should be considered as biotic ligands (Bianchini et al. 2005; Blanchard and Grosell 2005; de Polo and Scrimshaw 2012). Due to such differences, BLMs for marine species will not be covered in this paper.

Special aspects of metalloids for application of BLM/bioavailability-based tools

Metalloids are elements found in groups 13 - 17 in the periodic table and may act as metals and non-metals depending on their physical and chemical conditions. B, Si, Ge, As, Sb, Te, Po and At are all considered metalloids. Most of these metalloids are toxic in high concentrations (with the exemption of Si). However, Ge, Te, Po and At are typically present in very low levels in the environment. The deficiency of B is by far more critical that its toxicity to most organisms (Nielsen 1991; Sposito 2008). Therefore the metalloids which are important to consider as relevant in terms of environmental health are As and Sb (Sun 2011).

Originally, the application of the BLMs has been aimed at improving the prediction of safe environmental concentrations of metals as function of up to ten specific water parameters (Slaveykova and Wilkinson 2005). However, whilst some metalloids induce toxicological effects in water-based organisms similar to metals, the application of the BLM concept to predict safe environmental concentrations of important metalloids seems less appropriate. This is because some parameters that are important for trace metal cations have not been shown to be influential in the partitioning of trace metalloids (Lombi and Holm 2010).

There are two major differences between metals and metalloids. Firstly, metalloids are mainly present in the environment as negatively charged oxyanions; as such these anions behave differently from metal cations under some principles of the BLM such as pH and cation competition. This behaviour applies to any toxic oxyanion present in the environment, e.g., selenate, selenite, chromate and dichromate (Kinraide 2003). In contrast to the BLM assumptions, redox potential and the reactive sorbents (clay

minerals, Al, Fe and Mn oxides) in solids (sediment, suspended particulate matter, and soil) are key factors in controlling the partitioning, speciation and bioavailability of the metalloid elements B, Si, As and Sb (Lombi and Holm 2010; Sun 2011; Lui et al. 2011). Cell membranes are in general negatively charged; the presence of cations in the media can change the charge in the membrane and thus cause an increase in anion surface attraction (Kinraide 2006; Wang et al. 2011). Therefore the inclusion of the surface membrane potentials in availability models could improve our understanding of metalloids toxicity in the future and should represent a robust approach in the prediction of metalloid toxicity (Zhou and Wang 2011; Wang et al. 2008). The effect of the presence of competing cations on the bioavailability of metalloid oxyanions is not known; however other oxyanions such as phosphates, in the particular case of arsenate, have shown to impact on bioavailability and therefore on toxicity (Levy et al. 2005). The second difference to metals is that some metalloids are present as uncharged molecules (e. g. $As(OH)_3$, $Sb(OH)_3$ and $B(OH)_3$). These toxic molecules are very similar in structure to glycerol and other uncharged molecules and consequently, aquaporins and aquaglyceroporins facilitate their transport across cell membranes (Jahn and Bienert 2010).

<u>Metal nano-particles and application of BLM/bioavailability tools</u>

In this section the application of BLM for the effect assessment of nanoparticles (NPs; size < 100 nm for all dimensions) is discussed. Although a large number of publications are dealing with effects of NPs, only a few consider BLMs. In general, bioavailability models are assumed to be be appropriate tools for estimating NPs bioavailability and toxicity. However, there is also the common view that the application of bioavailability models to NPs will require further experimental and modelling development (Fabrega et al. 2011; Reidy et al. 2013).

Metal NPs, including copper oxide, iron oxide, zinc oxide, titanium dioxide, silver and gold NPs, are used across a wide variety of industries and within thousands of consumer products (Mytych and Wnuk 2013; Arora et al. 2012). Characterization of NPs is required not only for the manufacturing and application of NPs, but also to understand their hazards and risks. There is a lack of more fundamental understanding related to the mechanism of NPs toxicity. Most NPs may be bioaccessible since it is known that particles < 100 nm can enter cells (Mytych and Wnuk 2013). However, it is not yet clear what physico-chemical properties have the greatest influence on the free ion activities of metal NPs and thus their bioavailability. Further questions are whether the assumptions that aqueous phase chemistry is at equilibrium and that uptake and flux across the membrane is rate limiting are valid (Fabrega et al. 2011; Xiu et al. 2011). BLM calculations may be helpful for studying those influences.

In a recent study by Kennedy et al. (2012) the effects of 20 and 100 nm silver NPs to zooplankton in the presence and absence of DOC were investigated. The authors report a decrease of acute toxicity of Ag NPs with larger size and higher DOC concentration. The decrease in toxicity after DOC additions was greater for the 20 nm particle (2.5 - 6.7 fold). Interestingly the correlation of the effects with the NPs surface area was better than that for the NPs mass in tests without DOC as compared to experiments with DOC. Under all conditions tested the dissolved Ag in the test suspensions was the best predictor of the acute toxicity. Calculations based on this dissolved Ag fraction in the Ag NPs suspensions with a BLM which considers sulfides, chlorides, DOC, Ca^{2+}, Mg^{2+} and Na^+ were consistent with the observed effects. However, this outcome contradicts data by Choi et al. (2009) who reported that a BLM could not help explaining Ag NPs toxicity to nitrifying bacteria on base of dissolved Ag ions. The difference may be explained by the different organisms used or the different test solutions applied. Further BLM supported investigations are required to explain effects of Ag (and other) NPs in aqueous solutions.

4. Relevant metal bioavailability/BLM approaches in regulations

<u>General remark</u>

In the concept of the EU WFD (EC 2000) an EQS is derived as an overall quality standard (QS), thus considering the protection goals water phase organisms, sediment organisms, prevention of secondary poisoning of predators by prey, and drinking water. Then, the lowest QS of these protection goals is chosen as the respective EQS. The BLM-based QS is only related to the $QS_{freshwater, eco}$ (= PNEC), the QS to protect the aquatic community exposed via exposure in the water phase. The overall EQS which considers all protection goals may be lower than the $QS_{freshwater, eco}$. The maximum value of the calculated QS (e.g. derived from a user-friendly BLM-based bioavailability tool) is determined by the lowest QS derived for the other protection goals (e.g., secondary poisoning). Thus it has to be decided on a case by case basis if other compartments or other protection goals are more relevant (i.e. more sensitive) for EQS setting (Hommen and Rüdel 2012). In the following the term QS_{local} will be used for outputs from the BLM / BLM based bioavailability tools.

<u>Europe</u>

In the European Union, there are two major fields of BLM applications in regulations. First, BLMs are used in the risk assessment of chemicals. For a number of metals, Risk Assessment Reports (RAR) were prepared under the former Existing Substances Regulation (EEC 793/93), e.g., for Zn (Zinc EU RAR 2010), Ni (Nickel EU RAR 2008) and Cu (as voluntary RAR by industry stakeholders, Copper RAR 2008). One major conclusion in the RARs was that only by consideration of site-specific bioavailability, metal ions concentrations and effects of metals on aquatic organisms became clearly correlated. While the data base for the BLM development is described in detail in the RARs, the BLM software with the complete models are proprietary and not freely available. Under the new EU chemicals management regulation REACH (Regulation EC 1907/2006; EC 2006), BLM may also be used for risk assessment. The BLM approach is covered in a guidance document on the environmental risk assessment for metals and metal compounds (ECHA 2008). This guide asks for expressing the effect data preferentially on a "bioavailable" basis in cases where ambient dissolved metal concentrations are reported and chronic BLMs have been validated.

The second field where BLMs are applied in European regulations is the surface water monitoring according to the WFD (EC 2000). The subsequent directives of the WFD (2008/105/EC, EC 2008, now to be replaced by directive 2013/39/EU, EU 2013), state that in case of metals the EQS refers to the dissolved concentration (0.45 μm membrane-filtered). When assessing the monitoring results against the EQS, Member States may take into account the natural background concentrations for metals as well as water quality parameters like hardness, pH or others that affect metal bioavailability. In case of cadmium, the measurement of hardness is mandatory for directives 2008/105/EC and 2013/39/EU. For two metals (Pb and Ni) EQSs that are based on bioavailable concentrations were derived (2013/39/EU, EU 2013; to be implemented till end of 2015).The current knowledge on metal bioavailability is described in a technical guidance document for the derivation of new EQS (EC 2011). To support implementation of bioavailability-based assessments, easy-to-use BLM based bioavailability tools were developed (currently for for Cu, Ni and Zn; e.g., Bio-met 2011, PNEC.pro 2013).

BLM were derived for a range of water characteristics. Tests reveal that most water types found in Europe are covered (Table 2, derived from Vijver et al. 2008). For example, water types I - III cover about 90% of freshwaters in The Netherlands, and types IV - VI cover the remaining 10%. The latter typically represent more extreme freshwater sites. However, some specific surface waters, e.g. those having extreme water hardness or extreme acidity, are outside the verified boundaries of the currently applied BLMs and the bioavailability tools based on them.

Table 2: Chemical characteristics of different water types applied for BLMassessments (average and standard deviation; values given between brackets are number of locations / number of observations; Vijver et al. 2008).

Water type description	Water type	DOC [mg/L	pH [-]	Ca [mg/L]	Mg [mg/L]	Na [mg/L]	Cl [mg/L]	Alkalinity [mg/L]
Large rivers	I	3.1±0.9 (12 / 926)	7.7±0.2 (12 / 4024)	70.0±4.8 (11 / 26)	9.1±1.9 (11 / 26)	25.5 (2 / 2)	55.5 (2 / 2)	120 (2 / 8)
Canals, large lakes & small lakes	II	8.4±4.4 (16 / 425)	8.1±0.4 (15 / 443)	56.6±15.8 (8 / 13)	19.5±5.5 (6 / 9)	65.8 (7 / 7)	120 (7 / 7)	128 (6 / 9)
Streams and brooks	III	18.2±4.3 (3 / 12)	7.4±0.1 (5 / 401)	75.8±39 (5 / 58)	8.5±0.4 (3 / 56)	58.4 (3 / 8)	102 (3 / 8)	151 (1 / 4)
Ditches	IV	27.5±12.2 (4 / 7)	6.9±0.8 (4 / 7)	86.7±28 (4 / 7)	31.9±5.6 (1 / 4)	60.5 (4 / 4)	115 (4 / 4)	265 (1 / 4)
Sandy spring	V	2.2±1.0 (1 / 4)	6.7±0.1 (1 / 4)	20.3±0.4 (1 / 4)	6.7±0.4 (1 / 4)	17.0 (1 / 4)	31 (1 / 4)	8.6 (1 / 4)
Small acid ponds	VI	17.3±4.4 (7 / 22)	5.1±0.8 (7 / 22)	1.5±0.9 (7 / 22)	0.8±0.3 (7 / 22)	3.7 (1 / 3)	7.0 (1 / 3)	0.27 (1 / 4)

DOC - Dissolved Organic Carbon; alkalinity - $CaCO_3$ concentration.

USA

The United States Environmental Protection Agency (US EPA) is one of several federal agencies tasked with protecting water quality under the Clean Water Act and was specifically tasked with implementing pollution control programs. The BLM is currently accepted by the US EPA as an acceptable model for use by risk assessors to develop aquatic life criteria for copper in freshwater (US EPA 2007a). The US EPA's Scientific Advisory Board initially reviewed the BLM (US EPA 2000) and proposed it is suited to be used for copper and silver in both freshwater and saltwater bodies. However, it was later ruled that the model was not acceptable for use in saltwater bodies (US EPA 2003).

In the United States, the 1972 Clean Water Act section 304(a)(1) requires the US EPA to develop and publish criteria for water quality that reflect the latest scientific knowledge to provide guidance to states and tribal nations. The criteria concentrations, 1) are not legally enforceable unless adopted by states/tribes or specifically superseded by other federal regulations, 2) are the basis for regulatory control of pollutant discharges to the waters of the US, and 3) states may consider the economic and technical implications of the established criteria. If recommendations from the US EPA are not adopted, the states/tribes are required to adopt other water quality criteria that are scientifically valid. Because the US EPA is obligated by the Clean Water Act to establish criteria based upon the latest scientific knowledge the use of the BLM for copper was accepted via recommendations made in the 1985 Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (US EPA 1985a). The 1985 guidelines provided methodologies recommended for establishing toxic values of pollutants on diverse set of aquatic taxa largely from laboratory experiments. The empirical relationship between water hardness and copper was considered determinatant for copper toxicity in the EPA's Water Quality Criteria (US EPA 1985b). The use of the BLM more accurately reflects toxic values of copper on fish in freshwater bodies in part because it considers physical and chemical factors affecting toxicity. The implementation of the BLM by the US EPA was appealing because it is assumed to account

more appropriately for periods of enhanced copper bioavailability. Several guidance documents on the implementation of the BLM in the USA are available (cited in US EPA 2007b).

UK

Following the implementation of the WFD many European countries began to feature BLMs in their regulations for monitoring water quality and risk assessment. In the UK, the Environment Agency (2009) published a "step-change" scientific report proposing the introduction of BLMs into compliance assessment procedures. To overcome the major challenges in implementing BLMs in routine regulatory monitoring (e.g., model complexity, data requirement, site specificity), the Environment Agency developed simplified versions of the original complex BLMs that require fewer data inputs (i.e., only dissolved metal concentrations, pH, DOC and Ca concentrations). These were implemented in the Metals Bioavailability Assessment Tool (M-BAT). The M-BAT is incorporated into a tiered-approach monitoring concept that comprises three stages: classification, investigation and management (Fig. 1). The report concludes that accounting for bioavailability offers considerably larger ecological relevance than, e.g., the currently applied hardness-based corrections for copper. A revision of the calculation procedure and finalisation of process and terminology was published by WFD-UKTAG (2012).
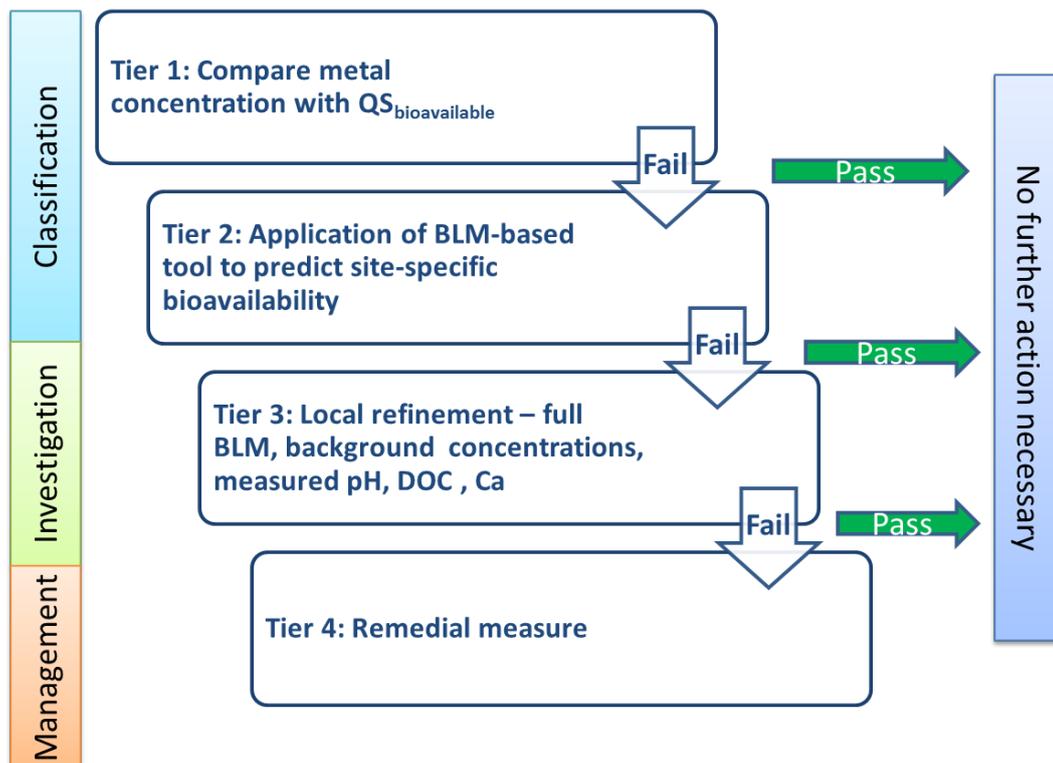
Fig.1: UK Environment Agency QS compliance assessment scheme under the Water Framework Directive (adapted from WFD-UKTAG 2012).

A site is classified by comparing the generic $QS_{bioavailable}$ (derived by assuming complete bioavailabiliy of the metal ion) with the dissolved metal concentration in the assessment at Tier 1. However, this step does not take into account of the parameters modifying the bioavailability locally. "Failure" sites then proceed to Tier 2 where M-BAT is implemented to derive the site-specific bioavailable metal concentration (QS_{local}). Sites that fail the first two tiers will proceed to the investigation stage (Tier 3). At tier 3, observed metal concentrations are compared with a bioavailable QS_{local} derived from a "full" BLM. Here measured supporting parameters which modify the bioavailability and possibly background

concentrations are taken into account. At Tier 4, the failure of sites to achieve the QS has been demonstrated and remedial measures are required to manage the pollution.

Currently, the M-BAT is applied to measure potential risks of Cu, Ni, Zn and Mn in the aquatic eco-systems. However, a number of challenges remain, including addressing operational boundary conditions of low pH and low Ca concentration, allowing for background metal levels, the necessary input of DOC data (currently not routinely gathered), and possible integration into issues of emission permits that are currently expressed as total concentrations.

Israel

In Israel, the water resources, lakes and aquifers, are calcareous and are to be characterized as relatively hard water. These waters are not considered as potentially at risk when based on hardness EQS. Soft waters which are likely most sensitive to metal exposures, are found in seasonal streams. However, water in the streams, often have high DOC levels and therefore metals, if present, may not be of risk. In most cases, the presence of metals in surface water derives from anthropogenic sources releasing metals into wastewater, soil, atmosphere and to the groundwater through infiltration processes. The degree of metal toxicity and bioavailability is related to water chemistry composition, including DOC, pH, and hardness among other, but also to factors such as the redox state, chelation, complexation, digestion, prey and grazing by zooplankton, protozoan and algae grazing. BLM or BLM-based bioavailability tools are currently not applied in Israel.

5. Available BLM software and user-friendly BLM based bioavailability tools

The overview of availabe BLM software and BLM-based bioavailabiliy tools is split in two parts, giving examples for BLMs as well as for simplified user-friendly BLM-based tools to predict metal bioavailability (including those applying a pragmatic but nevertheless most user-friendly approach of using algorithms/transfer functions).

BLMs

BLM EU-RAR

In the risk assessment of several metals in Europe optimized and validated BLMs were applied which are documented in the EU RARs for copper, nickel and zinc (see section 4, Europe). A detailed discussion is given by Vijver et al. 2008 (comparing full and limited normalization scenarios, case study Cu). The BLMs applied in the RARs have been made operational by several stakeholders, resulting in different models. Short descriptions of the available tools are given below.

EPA tool Cu

In 2007 the US EPA recommended BLM-based aquatic life ambient freshwater quality criteria (US EPA 2007a). The criteria are expressed as dissolved copper concentrations since these approximate the toxic fraction better than the total metal concentrations. For compliance testing a designated tool is available which requires chemical water body characteristics to calculate local copper criteria (Biotic Ligand Model, Version 2.2.3, Hydroqual 2007). The US EPA copper criteria calculations are a special mode ("Cu WQC calculation") in the Hydroqual BLM which also covers other metals. The BLM tool provides the computational basis for predicting the amount of dissolved metal that will be toxic to organisms. The input parameters required are: temperature, pH, alkalinity and concentrations of DOC, Ca, Mg, Na, K, SO_4^{2-}, and Cl^-. The BLM tool allows also the input of sulfide concentrations but these are currently not affecting the BLM calculation. How sulfide may affect metal levels is still under investigation and will possibly be covered in revisions of the BLM tool (HydroQual 2007). The required water parameters are

of different importance and the user has to decide how to proceed if certain data are not available. The user guide gives advice how important the input parameters are; generally, the Cu BLM is most sensitive to pH and DOC (HydroQual 2007). For DOC, also the fraction of humic acids has to be defined (a default value of 10% humic acid is recommended for natural waters). For parameters known to be absent in the water, a nominal, negligible concentration should be applied (not zero). Because some of the parameters are known to vary seasonally, the US EPA suggests at least one sampling event per season (US EPA 2007c). Finally, the measured dissolved copper concentrations are used as input in the BLM tool. However, they are not applied in the calculations but used for comparisons with the calculated quality criteria for the given water sample. The BLM tool calculates the US EPA copper criteria recommendations for acute and chronic effects and they are expressed as criteria maximum concentrations (CMC) and criteria continuous concentrations (CCC).

<u>Simplified, user friendly BLM-based tools</u>

The currently available chronic BLM software tools for undertaking bioavailability calculations are data-demanding (about 10 physico-chemical input parameters are required), and often insufficiently user-friendly and time-consuming. To address these barriers, user-friendly tools were developed. Currently, there are three readily available user-friendly tools which are more or less implemented in regulatory protocols of European member states. These are:

- Bio-met (current version 2.3) – a "lookup table" based tool in both MS Excel spreadsheet and online formats developed by ARCHE (Belgium) / WCA-environment (UK) (www.bio-met.net);

- M-BAT – an algorithm-based tool in MS Excel developed by WCA-environment (UK);

- PNEC.Pro (current version 5) – an algorithm-based tool in MS Excel developed by Deltares (The Netherlands) (www.pnec-pro.com).

Bio-met tool

The Bio-met tool is a collaborative initiative led by the European Copper Institute (ECI), International Zinc Association (IZA) and the Nickel Producers Environmental Research Association (NiPERA). The tools and resources on bio-met.net have been developed collaboratively by ARCHE and WCA environment. The Bio-met tool reports results obtained from the originally developed chronic BLMs, but has the advantages of only requiring few input parameters and being able to process large numbers of samples quickly. The basic approach behind the Bio-met bioavailability tool is a large database of more than 20,000 different combinations of key input parameters, i.e. pH, DOC, and Ca concentrations, and corresponding HC5 (Hazardous Concentration for 5% of the species assuming a lognormal SSD) calculations for Cu, Zn and Ni, using the original chronic BLMs for each metal. Other less critical parameters such as, e.g., Mg, Na, and alkalinity have been reasonably accurately calculated from Ca or pH (Table 3).

Table 3: Relationships implemented in the Bio-met tool (2011) to estimate the concentrations of Mg, Na, and alkalinity in surface water.

Relationship	Reference
Mg (mg/L) = 10^(0.6113 * log10(Ca) - 0.2754)	Peters et al., 2011
Na (mg/L) = 10^(0.7068 * log10(Ca) + 0.1029)	Peters et al., 2011
Alkalinity (mg CaCO$_3$/L) = 10^(1.0439 * log10(Ca) + 0.1881) (for Zn, Ni)	Peters et al., 2011
Alkalinity (mg CaCO$_3$/L) = 10^(1.0665 * pH - 6.5978) (for Cu)	Calculated from De Schamphelaere and Janssen 2004

For the purposes of simulations, small steps were taken for pH (0.125 pH units from pH = 6 to pH = 8.5) and DOC concentrations (0.15 log (DOC) units from DOC = 0.1 mg/L to DOC = 100 mg/L). The selected values for Ca were: 1, 14, 40, 80, 200 mg/L, while for Na the following values were selected, 14, 20, 40, 80 mg/L. The negligible to low impact parameters were set at following reasonable worst-case values: temperature = 5°C, K = 25 mg/L, SO_4^{2-} = 100 mg/L and Cl^- = 160 mg/L. The database then serves as a lookup table. The physico-chemistry of new sites are compared to existing simulations in this lookup table and the minimum HC5 of the two best matching lookup table entries is selected for QS_{local} derivation (after application of an additional assessment factor which varies between the metals).

Important to notice is that the BLMs were developed and validated for relevant ranges for each physico-chemical parameter (Table 4). When the user inputs a value for pH or Ca outside its validated range a prediction using the best-fitting combination of validated pH, DOC and Ca values is returned.

It should also be noted that the functions to calculate Mg and Na from Ca concentrations (Table 3) may not be "generic" or transferable to other European regions; for The Netherlands, for example, a systematic underestimation (up to an order of magnitude of 10) of both Mg and Na-concentrations occurred when compared to long-term monitoring data of these cations.

Table 4: Applicability boundaries of the different chronic BLMs (as implemented in the Bio-met tool, Bio-met 2011).

Metal	pH	Ca (mg/L)
Zn	6 - 8	5 - 160
Ni	6.5 - 8.7#	2.0 – 88#
Cu	6 - 8.5	3.1 - 93

for the current version 2.3; in the previous version (Bio-met 2011) the pH range was 6.5 - 8.2 and the Ca concentration range 3.8 - 88 mg/L.

To evaluate the performance of the Bio-met bioavailability tool against the original chronic BLMs for copper, nickel and zinc, individual monitoring data points from different surface waters from UK (> 900 sites) and The Netherlands (> 500 samples) were used to estimate HC5 using the "full" BLM and speciation models and using the Bio-met bioavailability tool. For all metals considered (i.e. Cu, Ni and Zn), the predictability of the full original BLMs by the user-friendly bioavailability tool is as or even more accurate (i.e. < factor of 2) and sufficiently conservative compared to the predictability of observed field toxicity by the full BLMs. Therefore, the user-friendly bioavailability tool is considered sufficiently accurate (David et al. 2011).

M-BAT

The Metal Bioavailability Assessment Tool M-BAT is developed for the UK-Environment Agency (see also section 4). It is not readily available on a website, nor has algorithms been published in scientific literature. The tool does not contain a database of predictions from BLMs, but is based on a mathematical relationship that attempts to simulate BLM calculations but uses a restricted set of input parameters (transfer functions). Algorithm based approaches are derived from training datasets of the results of bioavailability calculations from BLMs.

PNEC.pro

PNEC.pro is a user-friendly tool for professionals dealing with assessment of surface water quality developed by Deltares, NL (free download from www.pnec-pro.com). PNEC.pro processes local surface water monitoring data and automatically selects the most reliable calculation method for the available monitoring parameters. The algorithms (transfer functions) are published in scientific literature (Verschoor et al. 2012; Table 5). Minimum data requirement is DOC, and additional parameters, such as pH, Ca, Mg and Na can be used to improve the reliability of the predicted value of the PNEC (corresponds to QS_{local}). The tool calculates local, water-type specific no observed effect concentrations (NOECs) of copper, nickel and zinc, based on the original full BLMs. For nickel and zinc, free ion concentrations (operationally measured as dissolved concentrations after 0.45 µm membrane filtration) are processed in the BLMs. For copper however, the species CuOH and $CuCO_3$ are also considered in the Cu-BL binding (SSD calculated by applying the measured dissolved concentrations). BLMs, including chemical speciation calculations, are fully automated and validated. Local PNEC, RCR (risk characterization ratio = PEC/PNEC), and the probability that local concentrations exceed the local PNEC are provided. For the submitted datasets frequency distributions of monitoring parameters and PNECs for the three metals are shown, and a statistical summary of the sample set is produced.

The introduction and use of BLM amongst environmental regulators can be hampered by the complexity of the BLM procedure and the large number of required input parameters. However, Verschoor et al. (2012) concluded that a high level of accuracy of predicted HC5 can be maintained by applying simplified tools while the number of BLM parameters is reduced.

Table 5: Transfer functions for calculating local PNECs (as HC5 from SSDs; corresponds to QS_{local}) with decreasing number of monitoring parameters selected by stepwise statistical procedure (Verschoor et al. 2012). Concentrations of Ca, Mg, Na and DOC in mg/L.

HC5 (µg/L)	N	Transfer function (calculation of local PNECs)	adj. r^2
Ni	7	$DOC + pH + Ca + Mg + Na + SO_4 + HCO_3$	0.970
	3	-21.0 + 0.86 * DOC + 2.98 * pH + 0.43 * Mg	0.966
	2	0.25 + 0.81 * DOC + 0.58 * Mg	0.926
	1	5.06 + 0.90 * DOC	0.743
Cu	6	$DOC + pH + Ca + Mg + Cl + HCO_3$	0.895
	3	62.6 + 2.74 * DOC - 6.38 * pH – 0.23 * Ca	0.882
	2	18.8 + 2.80 * DOC - 0.30 * Ca	0.867
	1	1.05 + 2.75 * DOC	0.721
Zn	5	$DOC + pH + Na + SO_4 + HCO_3$	0.963
	3	-53.6 + 1.51 *· DOC - 7.79 * pH + 0.06 * Na	0.954
	2	-62.7 + 1.55 * DOC + 9.28 * pH	0.937
	1	7.30 + 1.48 * DOC	0.750

n - number of monitoring parameters; adj. r^2 - adjusted coefficient of determination (measure for the explained variance).

Table 5 shows that only few input parameters were required: DOC, and/or pH and Ca. Adding more water chemistry parameters gave more accurate predictions, but did not improve HC5 prediction significantly.

5 Case studies

Application of Bio-met tool to German waters

In a recent study the Bio-met tool (Bio-met 2011) was applied for the assessment of German surface water monitoring data (Hommen and Rüdel 2012). Based on example monitoring data sets from federal states in Germany the relevance of the validity range of the Bio-met tool for pH and Ca concentrations was analysed. For simplicity, only the ranges where the BLM for all three metals are valid were considered. From the more than10,000 analytical data sets available for one federal state (Northrhine-Westphalia), 1.5 % had pH values below 6.5 and 21.7 % pH values above 8. For Ca concentrations, 2.9 % of the samples were below 5 mg/L while 15.9 % were above 88 mg/L. In a second federal state, Baden-Wuerttemberg, the percentage of sites with values below the lower ranges was similar but more than 40 % showed pH values or Ca concentrations above the validity range (however, only one river was covered). For Sachsen-Anhalt, a third federal state, the data set was considerably smaller but the water bodies sampled often (41 %) had pH values above the validity range and all were above the validity range for the Ca concentrations. However, the Sachsen-Anhalt rivers were chosen for this evaluation because of their high salt concentrations and thus are not representative. Because of the high frequency of sites with high pH values (> pH 8) an optimization of the Bio-met tool for this condition was recommended. This was considered in a recent revision of the tool (Bio-met version 2.3; www.bio-met.net). The pH dependence of the QSs seems to be very variable between the metals and it cannot be assumed that higher pH values correspond to reduced bioavailability. For higher Ca concentrations the situation seems not critical because it can be assumed that the bioavailability will not increase at higher water hardness.

Application of a bioavailability based tool to UK waters

The UK Environment Agency considers the implementation of bioavailability based tools for surface water quality assessments (see section 4). In this context a user-friendly version of the original copper BLM (CuBLM) provided by the International Copper Association was developed as a rapid screening tool. The Cu-BAT (WFD-UKTAG 2012), the version for copper of the M-BAT (see above), was tested by comparing the estimated QS_{local} of the CuBLM for 916 surface waters from across the United Kingdom. According to this report, results of the tool tend to overestimate Cu toxicity under almost all circumstances. However, in waters with low Ca concentrations copper toxicity was underestimated by the Cu-BAT (WFD-UKTAG 2012). The consideration of local copper background concentrations changed the evaluation only at a small number of sites (< 3%). A further evaluation was performed for those sites of the data set which failed the currently applied hardness banded Cu QS in the UK (for example, 0 - 50 mg/L $CaCO_3$, 1 µg/L Cu; >50 - 100 mg/L $CaCO_3$, 6 µg/L Cu). In a tiered assessment approach the sites assessed were matched to annual average monitoring data for pH (based on measured values) and Ca and DOC concentrations. As a relatively conservative estimate the annual median value was used in case of DOC. For Ca, 50th percentile data were applied. If measured data were not available for either DOC or Ca, default concentrations were retrieved from an appropriate database for water bodies or even hydrometric areas. These input data were used to predict the bioavailability of Cu applying the bioavailability-based tool. From the about 500 sites 88 (17 %) also failed the assessment using the Cu-BAT. For most of these sites (86 %) also the original CuBLM resulted in an exceedance of the QS_{local}. The estimated QS_{local} values from the UK Cu-BAT were compared with the hardness based standards for the sites. From the comparison it was concluded that when bioavailability is taken into account the resulting QS_{local} can be much more variable than assumed by the hardness banded system. Sites which fall into the lowest of the hardness bands ($\leq$ 50 mg/L $CaCO_3$) had the largest variation in QS_{local} values. The evaluation revealed that the existing hardness banded standards had a poor predictive capacity to distinguish between sites of good status and sites with potential environmental risks associated with Cu.

David et al. (2011) report that the location of exceedances changed when using the bioavailability based tools as compared to the existing QS assessment. In the UK, many soft waters in Wales are considered as the most sensitive waters for metal exposures when based on hardness QS. However, since these waters often have relatively high DOC levels, the metals present limited risk when bioavailability is considered. On the other hand, calcareous streams of southern England, where water is relatively hard, are not considered as potentially at risk when using the hardness-based QS. Bioavailability consideration reveals that these waters which have very low DOC and high pH are very sensitive to metal additions.

Application of PNEC-pro for The Netherlands

The software PNEC.pro was developed after a four-year study on full-BLM modeling for water quality assessment in The Netherlands. A vast amount of water quality data (>209,000) were used covering more than 2800 sites, representing most of the water types that are covered by the EU WFD (EC 2000). A toxicity database was composed, containing chronic toxicity test results of 27 aquatic species for Cu, 19 species for Zn, and 22 species for Ni, tested for various toxic endpoints. Ecotoxicity data from the respective RARs (Copper EU RAR 2008, Nickel EU RAR 2008, Zinc EU RAR 2010) were adopted.

Water quality data were collected from monitoring programs covering a four-year period, thereby enabling both the geographic and the temporal (time dependent) variation analysis (Verschoor et al. 2011). Water compositions varied for pH: 5.7 - 8.7; DOC: 1.5 - 33 mg/L; Ca: 10.7 - 175 mg/L; Mg: 1.9 - 42.7 mg/L; Na: 7.1 - 153 mg/L. Limitations of other methods were analyzed and described (Vink and Verschoor 2010) and statistical methods were used to derive numerical functions based on major monitoring parameters (Verschoor et al. 2012). These functions were compared with full-BLM results and showed agreement of 88-97% depending on the metal of choice.

Maximum risks (based on HC5) of metals to ecosystems were predicted for February and minimum risks for September, while annual averages were best represented by the month of May. Compliance check with generic EQS showed a reduction of sites at risk for Cu and Zn using BLMs, whereas Ni showed a slight increase, identifying different sites as vulnerable. In the case of missing input data, PNEC.pro uses adapted sets of functions in order to calculate the local QS. The reliability of the calculation is shown in order to enable the user to evaluate the results.

Application of BLM-based bioavailability tools to Cantabrian Basin surface waters in Spain

Both the Bio-met tool and PNEC.pro were tested in the Cantabrian Basin (North of Spain), a geographically diverse landscape with a variety of water types. For the study monitoring data sets were chosen in which at least one of the Cu, Zn or Ni concentrations were above the current minimum EQS. The minimum hardness banded QS in Spain (Spanish QS corresponding to hardness class 1) is 5 µg/L for Cu and 30 µg/L for Zn (Spanish regulation RD 60 2011); 20µg/L for Ni (2008/105/EC, EC 2008). Most of these samples meet the hardness-banded QS in Spain because their hardness is higher than 10 mg/L CaCO$_3$ (hardness class 1). Thus, the total data sets were as follows:
- for Cu and Zn: 42 data sets including 23 sites (16 sites with 1 data set in 2010 or 2011, 2 sites with 2 data sets in 2010, 4 sites with 4 data sets in 2011 and 1 with 2 data sets in 2010 and 4 in 2011); and for Ni: 20 data sets including 17 sites (14 sites with 1 data set in 2010 and 3 sites with 2 data sets in 2010). Ca concentrations ranged from 4.1 to 108 mg/L Ca and pH values from 7.5 to 10.9.

The risk analysis applying both the Bio-met tool and PNEC.pro gave the following results:

- For copper 4 sites had Ca concentrations above the upper validated range in the Bio-met calculations. Only one of them was out of domain in the PNEC.pro calculations; 5 sites had a pH above the upper validated range in the Bio-met calculations. PNEC.pro gave 2 of these sites out of domain. No samples (no sites) showed a Cu concentration above the hardness-banded QS in Spain. However, one site (sampling in July with pH= 9.2, above the upper validated range in the Bio-met calculations) was at risk using the Bio-met tool. Another (different) site was at risk when using the PNEC.pro tool.

- For nickel 4 and 5 sites had Ca concentrations and pH values, respectively, above the upper validated range, in the Bio-met tool. None of the samples were out of domain in PNEC.pro. 4 sites with Ni concentration above the EU EQS (both individual measurements and annual average) were also at risk using both the Bio-met and PNEC.pro tools. All of them had Ca concentration above the upper validated range in the Bio-met calculations.

- For zinc 1 and 17 sites showed Ca concentration and pH, respectively, above the upper validated range, in the Bio-met tool. None of the sites were out of domain when the PNEC.pro tool was used. 2 sites showed Zn concentrations that exceeded the hardness-banded QS in Spain. These sites, and 10 others, were at risk according to both the Bio-met tool and PNEC.pro. PNEC.pro, but not the Bio-met tool, predicted 1 additional site to be at risk.

- In all cases (Cu, Ni and Zn), there were some sites (1, 2, 1, respectively) with annual averages for pH or Ca within the validated range in the Bio-met calculations but that had individual measures of these parameters during the year, out of the mentioned range (all of them were pH values above the upper validated range except for Zn calculations where 1 Ca concentration was below the validation range at 1 site in December).

In conclusion, for the Cantabrian basin in Spain, 25, 41 and 71% of the sites were out of the validated range of the Bio-met tool for pH, in Cu, Ni and Zn bioavailability calculations, respectively. This improved when annual average values were used particularly for Cu and Ni (22 and 29 %). There are very few sites (3 of 23 for Cu and none for Zn and Ni) out of domain with PNEC.pro. In general, there is a good agreement of predicted effects using the Spanish hardness-banded QS, and the two BLM-based bioavailability tools for the majority of studied sites. For Cu, slight differences for single sites occured between the two tools. For Ni, non-compliance results (sites at risk) were exactly the same using the Bio-met tool and PNEC.pro, and the hardness banded QS in Spain. The number of non-compliance sites for Zn was slightly higher using the PNEC.pro tool than the Bio-met tool.

6 Discussion

A variety of methods have been proposed to account for variations in the bioavailability of metals/metalloids and their compounds in risk assessment. Most of these approaches are based on the assumption of solution-mediated exposure and water-type specific impacts on metal bioavailability. The development of BLMs can be regarded as a milestone in the ecological risk assessment of metals as they integrate recent available interdisciplinary knowledge on metal bioavailability into a computerized framework.

In recent years BLMs for both acute and chronic exposures have been improved and applied to study metal toxicity of organisms from different trophic levels (Table S1, Electronic Supplementary Material). The review of full BLMs and subsequently developed simplified tools that are made available for use by informed and non-informed users clearly highlights that most efforts were put in developing BLMs for metals of primary (eco)toxicological concern, i.e. Cu, Ni, Zn. Metalloids are not covered due to physico-

chemically different interactions with biotic ligands compared to metals (since metalloids toxicity is caused by oxoanions, not by free ions). However, several publications have reported the use of BLM for arsenic toxicity without further discussing its suitability (see Table S1, Electronic Supplementary Material). Toxicity prediction is commonly restricted to the contribution of the free metal cation (only for Cu two other aqueous species are considered). An application of BLM seems also possible for nanoparticles (NP) if metal ions are set free (e.g., from AgNP).

BLM research confirmed that water quality parameters such as DOC, pH, alkalinity, water hardness and sulfide ions significantly affect metal toxicity. However, in most cases some parameters are dominating (e.g., for Cd water hardness or for Cu water hardness, DOC and pH). In research, BLMs are often applied to predict metal bioavailability for biological species for which experimental data are scarce. The basic underlying assumption is similarity of mechanisms of competition and similarity of metal binding to biotic ligands. Especially in case of sensitive organisms and/or sensitive endpoints it is essential that proper justification is provided for extrapolation of BLM outputs across biological species. Proper mechanistic underpinning of the BLMs as well as proper consideration of the relevant ecological traits are essential considerations in this respect.

In many studies BLMs were already successfully applied as evaluative tools. However, there is still need for further refinements. It is also important to consider some of the challenges to BLM development and application, as brought forward by, e.g., Slaveykova and Wilkinson (2005): for example, the critical assessment of the equilibrium assumption of the BLM, the relevance of the uptake of metals by feed, and the improved characterization of the relationship between bioaccumulation and toxicity. A recent editorial by Erickson (2013) discussed a better coverage of toxicodynamic processes that are potentially important to toxicity predictions, the improved modelling of metal complexation to natural organic matter and an optimized derivation of binding constants of the covered metals to the biotic ligands as challenges for further BLM developments.

Based on the scientific development of the BLMs, they have the potential to be implemented in other areas. The majority of the applications focus on using BLM predictions for regulatory purposes. The chief benefit for using bioavailability for the assessment as compared to the current approach (i.e. total metal concentrations) is that the BLMs offer a more realistic assessment of the potential effects of metals on the pelagic community.

For regulatory purposes a conservative approach is used in which appropriate assessment factors (AF) are applied to derive hazardous concentrations that constitute the integrated BLM output. AF are applied in the derivation of generic QS to account for the inherent uncertainties associated with the mere fact that it is not feasible to test all combinations of ecologically relevant endpoints and surface waters in which aquatic organisms reside. Issues to consider in the assessment of the proper AF of modelled HC-values include, for example, model uncertainty (including the issue of use of the model restricted to its boundaries), soundness of across-species extrapolation, and extent of model validation. For the use in a regulatory context, a transparent documentation of the BLM-based bioavailability tools is required. This includes a description of the derivation of the generic EQS and on the use of AF. The applied AF should be justified properly.

In general, a stepwise approach should be considered for site assessments under consideration of metal bioavailability and the approach suggested in the UK may serve as an example (Fig. 1, WFD-UKTAG 2012).

Currently, only a few countries adopted the BLM-based water quality assessments. The UK and the US have legally implemented tools at least for single metals (e.g., M-BAT in the UK), while in The Netherlands a tool is endorsed by the administration (i.e. PNEC.pro by the Dutch Ministry of Infrastructure and the Environment). Up to now only a few metals are covered by these regulatory approaches. In the EU as a whole, BLM tools will probably be implemented with the enactment of the new EQS directive which states bioavailable EQS for lead and nickel (EU 2013). For other countries (e.g., Egypt, South Africa, Israel, or Canada) no official use of BLMs or BLM-based bioavailability tools could be identified although research papers on BLMs from several other countries/geographical reasons are available (see section 4).

Experiences from countries which already apply BLM-based bioavailability tools reveal that special care needs to be taken in case of waters of which the physico-chemical conditions are near to or even outside of the boundaries of the tools. Especially pH and hardness are the most relevant parameters to be considered here, and alkaline as well as soft waters are of particular concern in this respect. An analysis of the influence of the measurement uncertainty on the results of the Bio-met tool (Bio-met 2011) revealed that especially pH value variations and DOC concentration variations (at low DOC levels) result in significant changes of QS_{local} (Hommen and Rüdel 2012). Thus, variability and measurement uncertainty of pH and DOC values can result in large uncertainty of the derived local quality standard.

For the practical implementation, the BLM-based bioavailability tools should allow the assessment of all relevant metals under the respective regulation (e.g., also those, which are regulated by hardness-banding like Cd in the EU WFD). In the case of the EU WFD, where different QS are covered, the tools should also consider the QS for all protection goals (in cases when the calculated QS_{local} exceeds these QS).

Further comparison of the performance of different tools should be initiated by competent authorities (as decribed above in the case study for the Cantabrian basin). Intercalibration studies with different BLM-based bioavailability tools could demonstrate the performance of different tools for the same datasets. Considering this, it should also be very clear that physico-chemical properties of water systems (and their underlying relations) may be region- and/or country-specific (see also Table 6 which reveals large differences in water characteristics, e.g. pH or DOC levels, for different European countries). Examples were given in section 5, such as the prediction of Mg and Na concentrations from Ca concentrations, and empirically derived transfer functions based on national situations. Hence, standardization may not necessarily be the best option for legal implementation per country. However, if outcomes of the assessments with different tools for the same scenarios are comparable this probably would foster the further implementation of the tools. Thus it may be also considered to harmonize the different bioavailability-based tools. For a broad implementation of BLM-based bioavailability tools into regulatory assessments, some more guidance from competent authorities (e.g., the European Commission) would be helpful. This would ensure that the implementation in different states follows the same principles. Aspects of guidance such as recommendations on how to proceed if water criteria are outside the validity range of the BLM tools; or how to deal with sites with high natural background levels of the assessed metals would be beneficial and could be be accompanied by webinars, workshops and (online) tutorials.

Table 6: Ranges of water chemistry data in European countries that are major BLM input parameters; concentrations in mg/L (modified after Verschoor et al. 2012).

Country	pH			DOC			Ca			Mg			Na		
Austria	6.9	-	8.5	0.5	-	9.3	4.7	-	223	0.95	-	45.1		unknown	
Belgium	5.8	-	8.5	1.1	-	19.7	8.6	-	165	2.0	-	26.8	12	-	199
Denmark	6.1	-	7.8	6.7	-	17.1	8.7	-	74.3	2.1	-	4.0	9.0	-	16.8
Finland	6.1	-	7.7	4.3	-	19.6	2.7	-	14.2	0.8	-	4.2	0.9	-	7.4
France	7.6	-	8.5	0.6	-	5.34	10.1	-	542	3.6	-	15.4	4.3	-	118
Germany	7.0	-	8.5	1.6	-	7.5	39	-	112	7.6	-	19.8	14	-	111
Ireland	5.6	-	8.0	6.1	-	16.9	2.8	-	157	0.9	-	10.8	3.7	-	134
Netherlands	5.7	-	8.7	1.5	-	33	10.7	-	175	1.94	-	42.7	7.15	-	153
Portugal	5.7	-	8.4	1.6	-	16.2	1.8	-	116	1.0	-	104	4.0	-	283
Spain	7.6	-	8.7	2.7	-	11.5	45	-	234	4.9	-	49.5	10.5	-	36.7
Sweden	5.5	-	8.4	0.45	-	20.2	0.3	-	89.7	0.1	-	12.8	0.3	-	59.9
UK	5.9	-	8.5	0.5	-	18	0.5	-	182	0.1	-	63	0.1	-	546

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