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Title: The power of biotic ligand models : site-specific impact of metals on aquatic communities
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Summary

Many surface waters in The Netherlands do not comply with environmental quality standards for metals. This doesn't mean that the aquatic ecosystem is actually affected. Toxic effects of metals on aquatic life (for example fish, crustaceans and algae) are often higher in test with "standard" water, than in natural surface water. This implies that environmental quality standards, which are derived from tests with standard water, may be stricter than necessary to protect aquatic life. As a result, unnecessary and ineffective measures aiming to reduce emissions may be taken.

Differences in toxicity are caused by the fact that the total metal concentration in water consists of different chemical species, for example free metal ions, metal complexes with carbonates, sulphate or hydroxides or metal bound to suspended matter or dissolved organic matter. The free metal ion is mainly responsible for the toxic effect. Natural surface waters contain substituents that reduce the free ion activity of metals. Moreover, effects to organisms only occur when actual metal exposure takes place (=bioavailability). Binding to biological membranes is a first step in exposure of the organism to the free metal ion. Fish gills are examples of biotic ligands, where metal binding can be measured. Subsequently, further uptake into the organism can take place, potentially followed by a toxic reaction. Binding of free metal ions can be reduced by macro-ions of similar size and charge. Sodium, calcium and magnesium are able to compete with metals for binding sites on the biotic ligand, thus inhibiting the uptake of free metals into organisms.

The composition of surface water affects the bioavailability of metals. This offers the opportunity to improve water quality standards and risk assessment of metals. The European Water Framework Directive allows that differences in bioavailability between water types are taken into account. The method to do so, is however not specified.

The past decade, significant progress has been made in the development of biotic ligand models (BLMs) that are able to account for bioavailability in toxicity tests. BLMs calculate at what level of metal binding to the biotic ligand effects are to be expected. Most developed and tested are BLMs of copper, nickel and zinc, which predict effect-concentrations for acute and chronic exposure of fish, crustaceans, algae and plants.

The regulatory implementation and routine application of BLMs is obstructed by several factors. BLMs are complex models, which require chemical speciation calculations and normalization of large numbers of toxicity data. Moreover, upto10 chemical characteristics of the surface water need to be measured in order to be able to calculate a water type-specific quality standard. Finally, some reluctance exists because BLMs are based on laboratory tests and the validity of the models for field predictions is largely unknown. The aims of this PhD research project were therefore 1) to verify and optimize the predictive power of BLMs for field exposures and 2) to facilitate the regulatory implementation of BLMs.

To verify and optimize the predictive power of BLMs for field exposures (1st aim) twelve small rivers in the catchment area of the Dommel, The Netherlands, were selected. The area contains several brooks and small rivers with relatively high concentrations of several metals, caused by industrial discharges and leaching of fertilizers and historical soil contaminations.

In these surfaces waters, we investigated the effect of metals on survival, growth and reproduction of the water flea *Daphnia magna* and the fresh water crustacean *Gammarus roeseli.* The organisms were cultured in the laboratory and the juveniles were transferred to cages in natural brooks and rivers in the field. The cages were covered by gauze and therefore permeable for water; organisms could not escape and were also protected against predators. Simultaneously, daphnids were tested in the lab, in water taken from the same brooks and rivers. The experiments lasted for 3 weeks, during which the juvenile water fleas matured and reproduced. Survival and reproduction was counted at the end of the field test, and in the laboratory test also at intermediate intervals of 4-5 days. At the end of the test, organisms were weighed and the uptake (accumulation) of metals by *D. magna and G. roeseli* was measured. Physico-chemical characteristics of the surface waters, required for BLM calculations, were measured; metal concentrations, acidity, dissolved organic matter, sodium, calcium, magnesium, chloride, sulphate and carbonate. Also, temperature, oxygen-concentrations and salinity (expressed as electric conductivity) were measured, in order to check if conditions were suitable to maintain normal biological functions of the organisms.

In chapter 2, it was shown that the bioaccumulation of cadmium, cobalt and manganese in *Daphnia magna and Gammarus roeseli* in various natural surface waters could be explained by differences in calculated metal-binding to the biotic ligand (f_{BL}). Higher water- and body-concentrations of cadmium, cobalt and manganese had a negative impact on growth (body weight). Organisms are able to regulate body concentrations of essential elements. This is confirmed by the field experiments, in which concentrations of calcium, sodium, potassium, copper, selenium and zinc in the body stayed constant, while large differences in metal-concentrations in the water existed.

In chapter 3, large differences in population growth of *Daphnia magna* between surface waters and between laboratory and field tests with the same waters is described. In the field test, this could be explained by bioaccumulation of cadmium and zinc and by a higher metal binding to the biotic ligand. Effects were found at concentrations that were 30 times lower than predicted by the BLM.

The survival, growth and reproduction of the water flea were better in the laboratory than in the field test, because additional food was provided and an optimum temperature was maintained. Differences in population growth between water samples were explained by differences in cobalt and nickel bioaccumulation and by differences in calculated metal binding to the biotic ligand. Effects occurred at 20 times lower concentrations than predicted by the BLMs.

Deviations in the effect prediction of BLMs (like the factor 20 and 30 mentioned above) may be caused by different experimental set-ups, different cultures or species and different water types. A way to improve the BLM-prediction for field predictions is the adjustment of the intrinsic sensitivity of the organism (=calibration). Literature values of calibration factor upto a factor 100 lower were reported. The calibration factors in our study are within the range of reported values, 0.05 (20 x lower) for cobalt and nickel and 0.033 (30 x lower) for cadmium and zinc.

To facilitate the regulatory implementation (2nd aim), we investigated if the BLMs of copper, nickel and zinc could be simplified and if the required monitoring effort could be reduced, while an acceptable confidence of calculated quality standards is maintained. Aquatic quality standards are generally derived by testing the sensitivity of a variety of species. The sensitivity of each species is expressed as a no-observed effect concentration (NOEC). Several toxicological endpoints are considered, for example growth, reproduction, survival or mobility. Ideally, the quality standard, aimed to protect the whole ecosystem, is set to a concentration that affects not more than 5% of the species (=HC5). To derive a water type-specific quality standard, the BLM must be applied to each individual NOEC of the species sensitivity distribution. This requires that the original test conditions, i.e. metal concentrations and specific water chemistry must be known. For copper, nickel and zinc, all test data (approximately 130 tests for each metal) were subjected to BLM calculations. For each test, the level of metal binding to the biotic ligand that coincides with no observed effects was computed (=$f_{BL,NOEC}$). This is the metric for the intrinsic sensitivity of an organism for a metal.

In the next step, the BLM computes at which dissolved metal concentration in a concerning water sample, the metal binding to the biotic ligand equals $f_{BL,NOEC}$. In chapter 4, water type-specific quality standards were derived for copper, nickel and zinc in this way for 372 Dutch surface waters, with chemical monitoring data of 2009. Metal binding to the biotic ligand strongly depends on the physical-chemical composition of the surface water. The calculated environmental quality standard, calculated with BLMs, may differ a factor 100 between water types, while providing the same protection level. When the bioavailability was taken in to account, the number of sites where concentrations exceeded the calculated quality standard dropped from 85 to 0% for copper, from 61 to 20% for zinc. For nickel an increase of sites at risk was estimated; from 8% when concentrations were compared to the generic European quality standard of 20 µg/L, to 17% when a BLM-derived water type-specific quality standard was used.

In chapter 4, variations in water type-specific quality standards for copper, nickel and zinc in time were determined for 76 sites in the catchment of the Dommel river. For which monthly data of the physical-chemical water characteristics were available over a period of 3½ years (2007-2010). Metal concentrations as well as quality standards were subject to seasonal fluctuations. Highest risks were found in February and minimum risks were found in September. The month May was a good proxy for the annual average risks of metal exposures.

In chapter 5, copper, nickel and zinc BLMs of fish, crustaceans and algae (that are 9 BLMs) were simplified to one equation per metal, that demanded only a limited set of monitoring data. The quality standards (HC5-values) that were computed in chapter 4 for 372 sites in The Netherlands, were related to dissolved organic matter, acidity, sodium, calcium, magnesium, chloride, sulphate and carbonate concentrations in the same samples. As a first step, one linear equation with all these parameters was derived. The probability that the BLM computes a value that was outside the confidence interval of the linear equation was very small (<0.05). Less important parameters were eliminated one by one, by a stepwise multiple regression, while the probability of the equation was checked after each step. An equation with fewer parameters results in a larger uncertainty of the predicted HC5. Our results show that the dissolved organic matter content is the most important estimator for the HC5, with a probability of 72-75%. A second parameter enhances the probability of the equation to 87-94%. When also the acidity and calcium, magnesium or sodium are included in the equation, it is possible to predict 88-97% of the HC5 variation.

The sensitivity of organisms in natural surface waters is higher than predicted by BLMs (Chapter 3). The application of a lab-to-field calibration factor may correct for this difference. The calibration factors in this thesis are applicable to surface waters that are contaminated with a mixture of metals, that represents emission from zinc industries. One of the major challenges for environmental research is to develop risk assessment methodology that acknowledges the complex interactions between chemistry and biology and that is practical and useful in a regulatory context. The simplified equations and guidance for efficient monitoring offer opportunities for regulatory implementation of bioavailability and routine application, and give information on the uncertainties involved. The simplified equations are the foundation of the recently developed software package PNEC-Pro, which provides regulators and risk managers with a simple tool to calculate aquatic quality standards, and that requires only 1, 2, or 3 monitoring parameters (www.pnec-pro.com). To estimate actual risks caused by exposure to several stressors, additional investigations remain necessary. BLMs or PNEC-Pro are able to prioritize sites where additional research is most urgent, and risk mitigation measures will be most effective.