Spatial and Temporal Variation of Watertype-Specific No-Effect Concentrations and Risks of Cu, Ni, and Zn

Anja J. Verschoor,[†,‡,*] Jos P. M. Vink,[§] Geert. R. de Snoo,[†] and Martina G. Vijver[†]

[†]Institute of Environmental Sciences-CML, Leiden University, P.O. Box 9518, 2300 RA Leiden, The Netherlands
[‡]National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[§]Deltares, P.O. Box 85467, 3508 AL Utrecht, The Netherlands

Ⓢ Supporting Information

ABSTRACT: Geographical and temporal variations in metal speciation were calculated and water-type specific sensitivities were derived for a range of aquatic species, using surveillance water chemistry data that cover almost all surface water types in The Netherlands. Biotic ligand models for Cu, Zn, and Ni were used to normalize chronic no-effect concentrations (NOEC) determined in test media toward site-specific NOEC for 372 sites sampled repeatedly over 2007−2010. Site-specific species sensitivity distributions were constructed accounting for chemical speciation. Sensitivity of species as well as predicted risks shifted among species over space and time, due to changes in metal concentrations, speciation, and biotic ligand binding. Sensitivity of individual species (NOEC) and of the ecosystem (HC5) for Cu, Ni, and Zn showed a spatial variation up to 2 orders of magnitude. Seasonality of risks was shown, with an average ratio between lowest and highest risk of 1.3, 2.0, and 3.6 for Cu, Ni, and Zn, respectively. Maximum risks of Cu, Ni, and Zn to ecosystems were predicted in February and minimum risks in September. A risk assessment using space-time specific HC5 of Cu and Zn resulted in a reduction of sites at risk, whereas for Ni the number of sites at risks increased.

■ INTRODUCTION

It is widely recognized that surface waters differ in observed toxicity of metals due to differences in water characteristics and consequently in speciation of metals.[1] Total dissolved metal concentrations only give a crude impression of potential effects of metals on aquatic organisms. A more realistic assessment of toxicological impacts of metals may be accomplished when site-specific characteristics that affect chemical speciation are taken into account. Free aqueous metal ion concentrations, rather than the total dissolved concentration, appear to largely determine toxicological or biological effects in aquatic organisms exposed to water or sediment containing heavy metals.[2,3] The explanation of toxic effects can be further improved by accounting for metal binding to biological ligands, for instance to fish gill.[4,5] These findings supported the development of biotic ligand models (BLM), starting in the 1990s, which describe the binding of metals to biotic ligands.[6] Complexation of metals to abiotic ligands (e.g., organic acids, OH^-, Cl^-, SO_4^{2-}, and HCO_3^-) and competition between metals and other cations like H^+, Ca^{2+}, Mg^{2+}, and Na^+ for binding sites on biotic ligands directly affects toxicity. Similar to speciation models that describe binding of metals to abiotic ligands in solution, biotic ligand models describe the binding of metals and competing macro-ions to biotic ligands with stability constants.[7] After fish-gill BLM, equations have been developed for gill-less organisms such as invertebrates and algal species, based on the idea that biotic ligand binding is related to initial effects.[8−10]

The EU water framework directive (WFD) commits European Union member states to achieve a good qualitative and quantitative status of all water bodies.[11] The WFD prescribes steps to reach common goals rather than adopting the generic limit value approach. Although the first tier assessment consists of comparison of total dissolved water concentrations with generic environmental quality standards, it is recognized that this is only a first step in risk assessment. The risk assessment may be refined, and the concept of bioavailability of toxic compounds is adopted for the second tier risk assessment. There are several options for implementation of bioavailability. One is to replace generic laboratory-based quality standards with HC5 that are protective for most cases under normal field conditions (HC5 is defined as the dissolved metal concentration that protects 95% of species in an ecosystem). Another option is to allow site-specific risk assessment to prioritize sites for risk management measures. To make these options operational, more evidence-based knowledge

Table 1. Overview of Numbers (n) and Percentages of Samples and Sites at Risk in 2007, Based on Single Metal Risk, As Well As the Total Added Risk of Cu + Ni + Zn

	Individual Samples					Sites				
	total	generic risk		sample specific-risk		total	generic risk		site specific-risk	
	n	n	%	n	%	n	n	%	n	%
Cu	1554	1187	76	2	0	237	201	85	0	0
Ni*	1616	135	8	349	22	264	21	8	45	17
Zn	1457	590[a]	41[a]	319	22	222	135[b]	61[b]	44	20
Cu + Ni + Zn	1430			674	47	213			97	46

[a] Concentrations compared with Maximum admissble Zn concentration. [b] Concentrations compared with Annual average Zn quality standard.

about geographical and seasonal variation of metal bioavailability and species sensitivity is required.

Until now, geographical and temporal variation of bioavailability and species sensitivity only has been studied on a small scale on isolated samples, on single species or based on annual average concentrations of single metals.[12−16] This study aims to quantify spatial and temporal variation of ecological risks of Cu, Ni, and Zn, accounting for chemical speciation in a wide range of surface water characteristics. Spatial variation is studied on a national scale, whereas temporal variation is studied in one river basin. We show that it is possible to identify vulnerable conditions and time periods, based on changes in water chemistry, and the level of which aquatic species or taxonomic groups are at risk.

■ METHODS

Outline of Calculations. To calculate water-type specific effect concentrations, we used a step-by-step approach that combines experimental laboratory-based effect concentrations with water type−specific characteristics.[13] Toxicity database and chemical surveillance data of fresh waters were both subjected to speciation modeling to compute free ion activities of ions and complexes. Free ion activities were used as input for BLM to normalize test-NOEC (from the toxicity data) to site-specific NOEC (for sites in the water-monitoring database). Species sensitivity distributions (SSD) were constructed based on site-specific NOEC.[17] The SSD is a cumulative probability distribution, that shows the relation between toxicant concentrations and the potential affected fraction of the ecosystem. Risks for species and taxa at particular sites can be derived from site-specific SSD. Finally, HC5 expressed as free ion activity, were 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 straight-forward comparison between HC5 and field measurements or generic quality standards was enabled.

Collection of Toxicity Data. Databases containing toxicity data were composed from EU risk assessment reports (EU-RAR) of Cu, Ni, and Zn, containing chronic NOEC (expressed as dissolved concentrations) for a large number of species of various taxa.[18−20] When necessary, original studies from the EU-RAR were used to add information about chemical composition of test media, for example concentrations of DOC, H^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, OH^-, Cl^-, SO_4^{2-}, and HCO_3^-. The database of Cu contained 136 chronic toxicity test results (NOEC and EC10 values, further denoted as NOEC) of 27 aquatic species. The Zn database contained 19 species (132 test results), the Ni database contained 24 species (128 test results).[16] The species in the

databases were tested for various toxic end points, for example, reproduction, mortality, and growth. Fish, invertebrates, algae, insects, molluscs, and amphibians were present in the database. An overview of species and taxa in the toxicity database is given in Table S1 of the Supporting Information.

Collection of Surface Water Data. To determine geographical variation, water chemistry data and dissolved metal concentrations were collected from surveillance monitoring programs of different water managers who share information of chemical, physical, and biological parameters.[21] Data of January until December 2007 were selected and covered almost all water types that are described in the WFD. Data below the limit of quantification were divided by 2.[22] BLM calculations were performed on monthly mean concentrations. The selected data contained 2573 monthly subsets of 11 BLM parameters for 372 sites. Not all of the 2573 records contained dissolved metal concentrations, so for the risk assessment a lower number of samples and sites was available, exact numbers are given in Table 1. The resulting normalized NOEC were further aggregated to annual means to show spatial variability.

To determine temporal variation, the river Meuse tributary Dommel was selected and detailed surveillance monitoring data over the period between January 2007 and June 2010 were obtained directly from the waterboard authority. The selected data, contained 2760 monthly complete records (samples) of BLM parameters and dissolved metal concentrations over 76 sites. BLM parameter records were evenly distributed over the seasons.

Speciation Modeling. Free ion activities were computed for the metal and macro-ions in the test-media of the toxicity databases, as well as for the surface water data. Chemical speciation and competitive binding of metals to DOC was computed with WHAM6.[23] Stability constants of the relevant metal complexes were modified to comply with values described in the NIST-database.[24] The DOC was considered to contain 50% active fulvic acids only and complies with current EU-RAR procedures.[25]

Biotic Ligand Modeling. Biotic ligand models as reported in the EU-RARs for chronic toxicity of Cu, Ni, and Zn were used.[18−20] BLM translate NOEC from toxicity tests to watertype-specific NOEC by the following general formula:

$$\text{NOEC} = \textbf{I}\text{ntrinsic } \textbf{S}\text{ensitivity} \times \textbf{E}\text{nvironmental } \textbf{M}\text{odulator (EM)} \quad (1)$$

The species intrinsic sensitivity is derived from the toxicity test, using the measured NOEC and the chemical speciation in the test media:

$$\text{Intrinsic Sensitivity} = \text{NOEC}_{test}/\text{EM}_{test} \quad (2)$$

The watertype-specific NOEC is computed by correction of the intrinsic sensitivity with the chemical speciation in the water sample:

$$NOEC_{sample} = Intrinsic\ Sensitivity \times EM_{sample} \quad (3)$$

The environmental modulator is defined as:

$$EM = \left(1 + K_{H-BL} \times [H^+] + K_{Na-BL} \times [Na^+]\right.$$
$$\left.\frac{+ K_{Ca-BL} \times [Ca^{2+}] \times + K_{Mg-BL} \times [Mg^{2+}])}{K_{Me-BL}}\right) \quad (4)$$

For copper, also CuOH en CuCO$_3$ are considered as toxic components, in that case the denominator in eq 4 is extended to:

$$K_{Cu-BL} + (K_{CuOH-BL} \times K_{CuOH} \times [OH^-])$$
$$+ (K_{CuCO_3-BL} \times K_{CuCO_3} \times [CO_3^{2-}])$$

BLM were developed for *Pseudokirchneriella subcapitata*, *Daphnia magna*, and *Oncorhynchus mykiss*, as representatives of three taxonomic groups: algae, crustacean, and fish.[26−32] The model concepts are identical for each substance and taxon; differences are caused by the biotic ligand binding constants. Read-across of BLM over species means that species within the same taxonomic group use the same set of biotic ligand binding constants. Read-across is used to predict end point information for one species by using measured data from the same end point from another species, which is considered to be comparable. For species classified as other taxa (Table S2 of the Supporting Information), the trophic level is used as shared property for read-across. In this way, NOEC for plants are normalized with algae BLM, frogs, and toads were treated as fish and rotifers, insects, bivalves, molluscs, and hydrozoa are treated as crustacea. Biotic ligand binding constants for Cu, Ni, and Zn for different taxa are given in Table S2 of the Supporting Information.

Environmental Quality Criteria. The generic environmental quality standards used in this study were: Cu: 1.5 μg/l and Ni: 20 μg/l.[33,34] For Zn two values set by European policy were used: an annual average concentration of 7.8 μg/l and a maximum admissible concentration of 15.6 μg/l for individual samples.[20]

Statistics. For construction of SSD-curves, mean (monthly) site-specific NOEC were computed per toxicity end point for each species. For each occasion, lowest NOEC per species were included to compile the SSD. HC5 were computed as the fifth percentile of modeled SSD-curves assuming a log-normal frequency distribution of NOEC of all taxa.[22] BLM computations and descriptive statistics were performed with software package R.[35]

Risk characterization ratios (RCR), were calculated as ratios of metal concentrations over HC5. Multimetal RCRs were calculated assuming concentration addition, as follows:[36]

$$\sum RCR = \frac{[Cu]}{HC5_{Cu}} + \frac{[Ni]}{HC5_{Ni}} + \frac{[Zn]}{HC5_{Zn}} \quad (5)$$

An analysis of seasonality was performed using a generalized linear mixed model which computes the *systematic* effect of months while taking random error due to different years or sites into account.[37] ANOVA was applied to test significance of the monthly model compared to a model assuming only random effects. Criteria for significance were $\chi^2 < 0.05$ and $\Delta AIC > 2$.

Significant differences between monthly values and annual average HC5 and RCR were tested by a *t* test with Bonferroni correction.

Temporal factors (F) were derived that describe deviation of monthly parameters relative to the annual average:

$$F = \frac{X_{month}}{X_{year}} \quad (6)$$
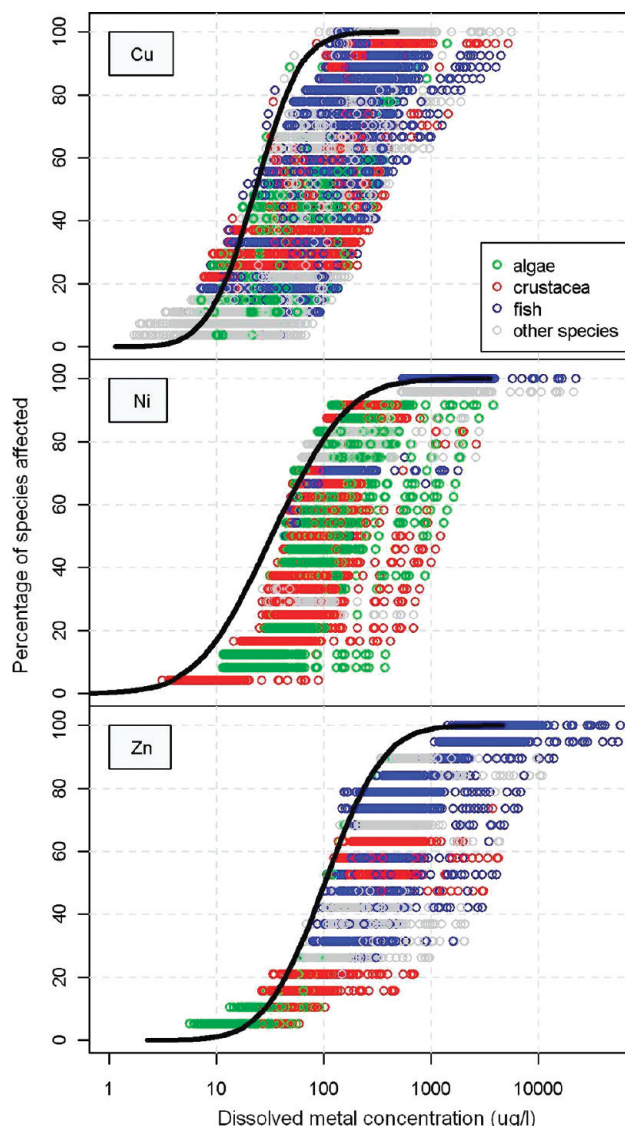
Figure 1. Annual average SSD for chronic Cu, Ni, and Zn toxicity at 372 sites in The Netherlands in 2007 computed with BLM. The line represents the modeled SSD based on measured NOEC from the toxicity database. Colors indicate different taxonomic groups.

in which X_{year} = annual average parameter value, X_{month} = parameter value in month t, and F = temporal factor.

RESULTS

Spatial Variation. In Figure 1, the spatial variation of Cu, Ni, and Zn SSD in 2007 is shown, with the percentage of affected species as a function of total dissolved metal concentrations. The colored points represent 372 site-specific SSD, obtained by application of the BLM to measured laboratory NOEC. Major taxonomic groups in the SSD are indicated with colors to get an impression of data richness and relative sensitivity of taxa within ecosystems.

In general, it appeared that normalized NOEC are higher in more than 95% of the cases compared to measured NOEC in the toxicity database, implying that natural waters contain more protective components than toxicity test media. Site-specific
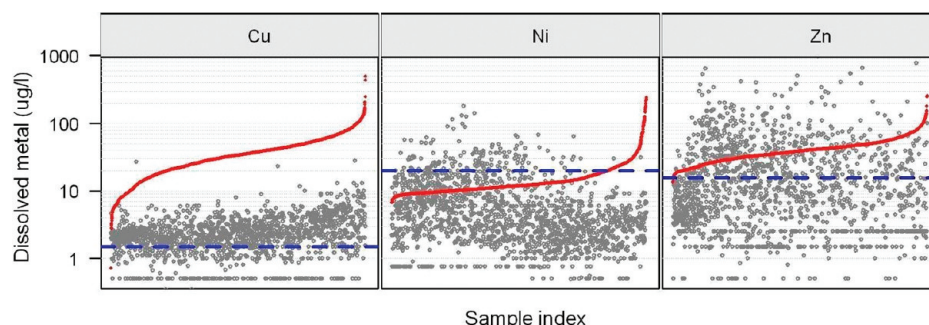
Figure 2. Comparison of total dissolved Cu, Ni, and Zn concentrations (Gray dots) in 2573 individual samples of 273 sites in 2007 with generic environmental quality standards (blue dashed lines) and sample-specific HC5 (red dots). Sample-specific HC5 were derived from normalized NOEC, computed with chronic BLM for algae, crustacea and fish. Individual samples were ranked with increasing HC5 before plotting.

HC5 ranged from 5.9 to 121 μg Cu/l, 7.9–197 μg Ni/l and 17.5–368 μg Zn/l.

The distribution of taxa within SSD is not constant; application of BLM and thus accounting for chemical speciation and bioavailability leads to changes in relative sensitivity of species. Their position within SSD varies among sites and within sites among sampling times. This is a consequence of taxa-specific BLM causing different relations between NOEC and site-specific water chemistry parameters. Taxa showed large variation of where they occurred in SSD, implying that taxa are more sensitive at some sites than at others. At the intersect with 5% affected species (below HC5) only representatives of one or two taxa are found. In general the most sensitive species in site-specific SSD resemble the most sensitive species in lab-based SSD (Table S1 of the Supporting Information).

Most sensitive species in the Cu-SSD are molluscs (*Juga plicifera*, 96% of the samples), and occasionally algae (*Chlamidomonas reinhardtii*, 2% of the samples) or fish (*Onchorhynchus kisutch*, 2% of the samples). For Ni, crustacea (*Ceriodaphnia dubia*, 100% of the samples) are the most sensitive species. For Zn, algae and crustacea are the most sensitive species in respectively 74% and 26% of the samples.

Additional to evaluation of annual average concentrations, the WFD requires evaluation of peak concentrations of sites. Figure 2 shows the distribution of computed HC5 of individual samples in 2007 and corresponding measured metal concentrations. Figure 2 shows that the variation in HC5, based on individual samples is higher than HC5 based on annual average site-characteristics. Sample-specific HC5 ranged from 0.7 to 490 μg Cu/l, 6.7–244 μg Ni/l, and 13–1300 μg Zn/l. HC5 are variable due to differences in free ion activities and water chemistry between the samples.

For Cu and Zn, sample-specific HC5 are in most cases higher (less stringent) than the generic environmental quality standards. For Ni, it is shown that HC5 is generally lower than the WFD-value of 20 μg/l. Sample-specific HC5 of Zn are higher than the maximum admissible concentration of Zn.

A full overview of HC5-exceeding samples of generic, site-specific, and sample-specific quality standards is given in Table 1, and corresponding maps are shown in Figure S2 of the Supporting Information. Predicted risks of Cu and Zn to ecosystems decreased significantly by including bioavailability in risk assessment. This holds for individual samples as well as for annual average risks at sites. No site-specific Cu-HC5 were exceeded, whereas the exceedance of site-specific Zn-HC5 was reduced with approximately $^2/_3$. However, HC5

of Ni are often lower than the generic EU quality standard, leading to an increase of sites indicated to be at risk. Apparently, the EU generic quality standard of 20 μg Ni/l is under-protective at a significant number of sites in The Netherlands.

The total risk of Cu, Ni, and Zn was calculated by adding risk characterization ratios. Table 1 and the maps in Figure S2 of the Supporting Information show that 45 and 44 sites exceed site-specific HC5 of Ni and Zn, whereas 97 sites exceed HC5 when risks of Cu, Ni, and Zn are added. This implies that single metal risks are uncorrelated and/or that there are many sites where the single metal risk is below 1 whereas total risk is above 1. It appeared that 28 new sites become at risk, when Ni and Zn toxicity is combined. There are 39 sites with either Ni or Zn risks and an overlap of 25 sites with both Ni and Zn risk. There are 5 sites becoming at risk when also copper is taken into account as a third toxicant, whereas there are no sites with a risk due to Cu only. The HC5-exceeding sites are clustered in the southern part of The Netherlands (the Dommel catchment area), due to regional high metal concentrations and low site-specific HC5 (Figure S1 of the Supporting Information).

Temporal Variation. Temporal variation of BLM parameters in 76 sites of the Dommel-database over a period of 42 months is shown in Figure 3. BLM-input parameters are shown (1st and second row) and calculated risk assessment related output, such as metal concentrations, HC5, and risks of Cu, Ni, and Zn are presented (3rd row).

In Figure 3, Cu-concentrations are relatively stable whereas calculated Cu-HC5 show fluctuation. Unlike Cu, measured Ni- and Zn-concentrations are highly variable whereas their HC5 are relatively stable. Variation coefficients for mean Cu-, Ni-, and Zn-concentrations over time are respectively 35, 28, and 64%. Variation coefficients for mean Cu-, Ni-, and Zn-HC5 are respectively 18, 2, and 8%.

Cu-concentrations never exceeded the HC5 in the period 2007–2010, whereas Ni and Zn-concentrations showed a seasonal pattern of HC5-exceedances, implying seasonal effects on the aqueous ecosystem. The predicted Ni- and Zn risks occurred respectively 44 and 40% of the time and usually took place during winter and spring.

A statistical analysis of the data was performed that computed the effect of the factor months while taking random error due to different years or sites into account. The monthly model performed significantly better than a model with only random effects, for all BLM-parameters, metal concentrations, HC5 and RCR, except for the Cu-RCR. For the Cu-RCR the probability of
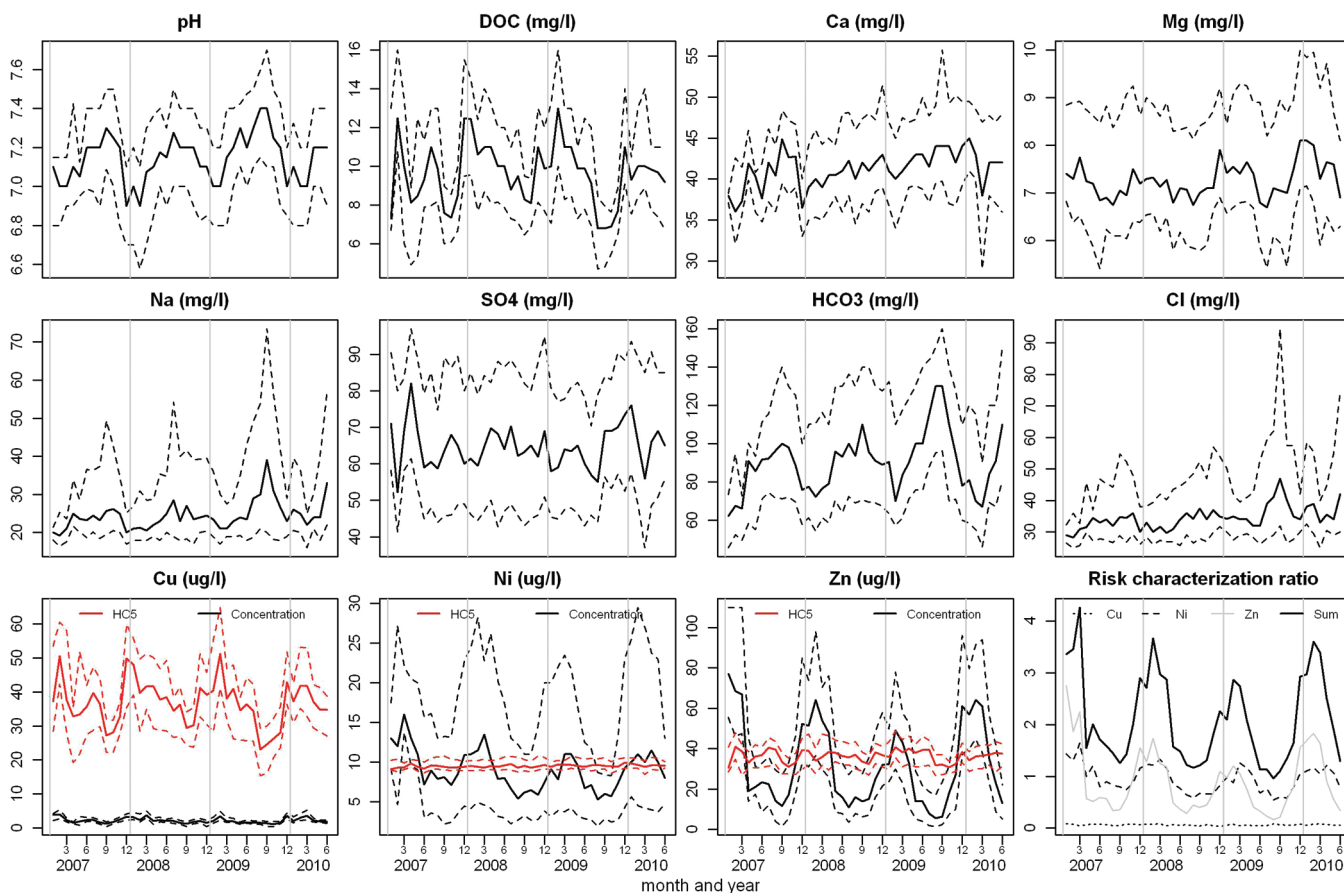
Figure 3. Temporal variation in BLM parameters, HC5 values, and risks in 76 sites of the Dommel database from January 2007 until June 2010. Bold lines represent mean values, dashed lines represent 25th and 75th percentiles. January 1st is indicated by gray vertical lines. HC5 are derived from normalized NOEC. Risk characterization ration = dissolved metal concentration/sample-specific HC5.

Table 2. Temporal Factor F for Seasonal Effects of HC5 and Risk Characterization Ratios (RCR)[a]

	HC5 (µg/l)			RCR (-)			SumRCR (-)
	Cu	Ni	Zn	Cu	Ni	Zn	
annual mean	**37.5**[(0.39)]	**10.0**[(0.06)]	**38.0**[(0.002)]	**0.075**[(0.03)]	**1.35**[(0.03)]	**1.36**[(0.06)]	**2.79**[(0.07)]
Month			Temporal factor of monthly value relative to annual mean (F)				
January	1.13*[(0.04)]	0.98*[(0.03)]	1.00 [(0.03)]	1.05 [(0.09)]	1.21 [(0.12)]	1.37*[(0.13)]	1.29*[(0.09)]
February	1.25*[(0.03)]	0.97*[(0.02)]	1.03 [(0.02)]	1.11*[(0.09)]	1.30*[(0.03)]	1.85*[(0.08)]	1.57*[(0.05)]
March	1.14*[(0.03)]	0.97*[(0.03)]	1.03 [(0.02)]	1.18*[(0.09)]	1.33*[(0.03)]	1.43*[(0.11)]	1.38*[(0.06)]
April	0.84 [(0.05)]	1.03 [(0.02)]	1.07 [(0.02)]	1.19 [(0.09)]	1.29 [(0.03)]	1.24 [(0.13)]	1.26 [(0.07)]
May	0.94 [(0.04)]	1.03 [(0.02)]	1.04 [(0.02)]	0.97 [(0.11)]	1.06 [(0.04)]	0.85 [(0.19)]	0.96 [(0.09)]
June	0.96 [(0.04)]	1.06 [(0.02)]	1.06 [(0.02)]	0.94 [(0.11)]	0.85 [(0.05)]	0.71 [(0.22)]	0.78 [(0.11)]
July	0.93 [(0.04)]	1.01 [(0.03)]	1.03 [(0.02)]	0.96 [(0.12)]	0.77*[(0.05)]	0.44*[(0.38)]	0.61*[(0.14)]
August	0.94 [(0.04)]	1.00 [(0.03)]	1.02 [(0.02)]	0.96 [(0.12)]	0.72*[(0.06)]	0.55*[(0.30)]	0.64*[(0.14)]
September	0.70*[(0.06)]	1.01 [(0.03)]	0.89*[(0.03)]	0.91 [(0.12)]	0.69*[(0.06)]	0.45*[(0.37)]	0.58*[(0.15)]
October	0.73*[(0.06)]	0.99 [(0.03)]	0.86*[(0.03)]	0.94 [(0.12)]	0.76*[(0.05)]	0.61*[(0.27)]	0.69 [(0.13)]
November	0.93 [(0.04)]	1.01 [(0.03)]	0.95 [(0.02)]	0.93 [(0.12)]	0.92 [(0.04)]	0.89 [(0.18)]	0.91 [(0.10)]
December	1.17*[(0.04)]	0.99 [(0.03)]	1.01 [(0.02)]	0.90*[(0.12)]	1.16 [(0.04)]	1.36*[(0.12)]	1.25*[(0.07)]
χ^2 of model	<0.001	<0.001	<0.001	0.051	<0.001	<0.001	<0.001

[a] F is the ratio of the monthly HC5 or RCR over the annual average HC5 or RCR. Asterisks indicate a significant difference between monthly and annual average values. Between brackets the standard error.

the χ^2-test was 0.051, which is an indication of weak or lacking monthly effects. For Ni-RCR, Zn-RCR, and SumRCR the monthly effect was confirmed by the χ^2-test.

In Table 2, temporal factors are presented for HC5 and risks of Cu, Ni, and Zn. Temporal factors of 1 imply that the monthly HC5 or RCR equals annual average. Factors >1 indicate HC5 or RCR higher than annual average, whereas factors <1 indicate parameters lower than annual average.

Asterisks show which months were significantly different from the annual average value. In general May represented the annual average risks for Cu, Ni, and Zn well. The highest total risks were found from December until March and the lowest risks were found from July until October. Zn- and Ni-risk show the same pattern as total metal risk. Temporal variation of Cu-risks is the least pronounced, but also less relevant than variations in Ni- and Zn-risks, because Cu-HC5 are not exceeded at any site at any time.

Seasonal patterns of risks were not reflected by variation in HC5. Cu showed highest HC5 in February, when also risks peaked. Ni-HC5 showed slightly, though significantly, lower HC5 from January until March, whereas Zn-HC5 showed minima in September and October. Theoretically at constant concentrations, lower risks were expected when HC5 rises. Apparently, seasonal variation in Ni and Zn concentrations dictated the seasonal effect of the HC5, as was also shown in the Ni and Zn-graphs of Figure 3.

Finally, Table 2 indicates that monitoring in February, May, and September would result in a good reflection of the annual range of risks.

■ DISCUSSION

Derivation of HC5 from SSDs is an accepted way of dealing with variation between species in risk assessments.[38] The possibility to account for spatial variation in risk limits for ecosystems caused by differences in bioavailability is provided by environmental policy higher tier risk assessments.[34] Temporal variation is only reflected in risk limits for Zn, where the EU-WFD set the annual average risk limit at half of the maximum admissible concentration in individual samples. The idea behind site-specific risk assessment is to establish site-specific ecosystem sensitives, expressed as HC5. Application of BLM is a way to refine the estimation of species exposure to metals. Effects of geographical or seasonal changes in water chemistry on physiology, toxicodynamics or behavior as well as seasonal differences in intrinsic sensitivity of different life-stages are not explicitly accounted for in BLM. These phenomena, which define ecosystem composition and stability in the field, are beyond the scope of current risk assessment methodology, which focuses on *potential* risk of chemicals.

In Figure 1, most of the sites have higher Cu and Zn NOEC than the generic environmental quality standards, implying that they are more robust than test media. In a tiered approach, it is a prerequisite that lower-tiered criteria are more strict than higher-tier criteria to minimize the chance of overlooking risks. The BLM fits in the strategy of a tiered approach of risk assessment, with toxicity test data that are relatively low compared to site-specific data.

SSD give an overview of variation of sensitivity between species and between taxa. The ranking of sensitives of species and taxa in the SSD changes when test-NOEC are normalized to site-specific NOEC, because binding of metals to biotic ligands is not equally affected by waterchemistry for each taxon, as a results of different stability constants (Table S2 of the Supporting Information). Still, it is only one or two species or taxa that are consistently the most sensitive ones over all watertypes. Moreover, most sensitive organisms in toxicity tests are also most sensitive after site-specific extrapolation.

In the Cu-SSD, two molluscs are below HC5. The species-collection in the SSD is meant to be a representation of an ecosystem. Therefore, molluscs *Juga plicifera* and *Campeloma decisum* are representatives for many species of that taxa. The application of a bioavailability model across species assumes similar mode of actions (e.g., similar stability constants between the cations and the biotic ligands). Justification for use of the Daphnia-BLM is found for the rotifer *Brachionus calyciflorus* (Cu, Ni, and Zn), the mollusc *Lymneae stagnalis* (Ni and Zn), and the insect *Chironomus tentans* (Ni).[39−41]

Crustacea were indicated as the most sensitive taxon in the Ni-SSD. However, new data exist indicating that the mollusc *Lymneae stagnalis* is more sensitive than crustacea.[41] This would lead to lower Ni-HC5 values than currently predicted. The Ni case clearly shows the need for a wide variety of species in toxicity databases, covering relevant species and taxa.

For Zn, it appears that the normal distribution does not fit well to the lower part of SSD for all of the samples. The normal function predicts higher values than the available NOEC data suggests. We assume that sensitivities are normally distributed and assume that the deviation of the current data collection is an artifact of the limited number of tests. For Zn, algae or crustacea determined the HC5, depending on the water chemistry. In 74% of the sites, algae were the most sensitive taxon and in 26% of the sites crustacea were the most sensitive taxon. The average HC5 were not significantly different; algae Zn-HC5 was 42.9 ± 35.0 μg/l and crustacea Zn-HC5 was 41.5 ± 18.4 μg/l. The relative sensitivity between algae and crustacea depended on water characteristics. Algae were the most sensitive taxon in watertypes with relative lower DOC and higher pH. The Zn-concentrations were significantly lower in watertypes where algae were the most sensitive group compared to watertypes where crustacea were the most sensitive taxon (9.7 versus 40.5 μg/l, respectively), indicating that crustacea are more at risk than algae. Lower Zn-concentrations in watertypes where algae were the most sensitive taxon coincided with relative higher pHs.

The impact of spatial variation of water compositions translates directly into the broad range of all SSD and is a combined effect of all individual BLM parameters. The results of spatial variation can help to select monitoring sites and monitoring parameters. The choice of monitoring sites will depend on the monitoring goal, but is usually more intense in areas where generic quality criteria are exceeded. For site-selection, the origin of spatial variation in BLM-parameters should also be considered. Major BLM parameters such as pH and Ca are primarily determined by the geology of the area. DOC concentrations are likely to be controlled by land use. Trace metals like Cu, Ni, and Zn have natural origins, though their concentrations can be heavily elevated by industrial or historic point sources as well as diffuse sources related to population density and agriculture.

Seasonal risk patterns are dominated by seasonal concentration patterns of metals, shown by correlation coefficients of 0.69 for Cu~RCR-Cu, 0.99 for Ni~ RCR-Ni and 0.97 for Zn~RCR-Zn. Metal concentrations peak during wintermonths, probably caused by higher input of runoff water in combination with a low biological mineral uptake. With plant growth starting in spring,

uptake of minerals will increase, and trace metal concentrations will drop.[42,43] Again, it is emphasized that variations of risks predicted in our study relate to changes in water chemistry, which affects exposure of species. A relevant question is whether predicted risk-periods coincide with the sensitive life-stages of species.

The results of temporal variations (Figure 3, Table 2) have implications for a cost-effective setup of metal risk monitoring. It is a challenge to minimize the number of samples and parameters without loss of information about risks. This study shows that monitoring in February, May, and September would result in a good reflection of the annual range of risks.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. NOECs for Cu, Ni, and Zn for species and taxa in the toxicity database, Log KBL for BLMs, number of sites at risk, geographical variation of HC5, and Geographical variation of risks. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: anja.verschoor@rivm.nl; phone:+31 30 2742655; fax:+31 30 2744413.

■ ACKNOWLEDGMENT

■ REFERENCES

(1) *Metal Speciation and Bioavailability in Aquatic Systems*; Tessier, A., Turner, D. R., Eds.; John Wiley & Sons: New York, NY, 1995.

(2) Sanders, B. M.; Jenkins, K. D.; Sunda, W. G.; Costlow, J. D. Free cupric ion activity in seawater: Effects on metallothionein and growth in crab larvae. *Science* **1983**, *222*, 53–55.

(3) Vink, J. P. M. The origin of speciation: Trace metal kinetics over natural water/sediment interfaces and the consequences for bioaccumulation. *Environ. Pollut.* **2009**, *157* (2), 519–527.

(4) Zitko, V.; Carson, W. G. A mechanism of the effects of water hardness of the lethality of heavy metals to fish. *Chemosphere* **1976**, *5*, 299–303.

(5) Pagenkopf, G. K. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, pH and water hardness. *Environ. Sci. Technol.* **1983**, *17*, 342–347.

(6) Di Toro, D. M.; Allen, H. E.; Bergman, H. L.; Meyer, J. S.; Paquin, P. R.; Santore, R. C. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ. Toxicol. Chem.* **2001**, *20* (10), 2383–2396.

(7) Playle, R. C.; Dixon, D. G. Copper and Cadmium binding to fish gills: Estimates of Metal-Gill Stability constants and modelling of metal accumulation. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 2678–2687.

(8) Heijerick, D. G.; De Schamphelaere, K. A. C.; Janssen, C. R. Biotic ligand model development predicting Zn toxicity to the alga *Pseudokirchneriella subcapitata*: Possibilities and limitations. *Comp. Biochem. Physiol., C* **2002**, *133*, 207–218.

(9) De Schamphelaere, K. A. C.; Janssen, C. R. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*; the effects of

calcium, magnesium, sodium, potassium, and pH. *Environ. Sci. Technol.* **2002**, *36* (1), 48–54.

(10) Keithly, J.; Brooker, J. A.; DeForest, D. K.; Wu, B. K.; Brix, K. V. Acute and chronic toxicity of nickel to a cladoceran (*Ceriodaphnia dubia*) and an amphipod (*Hyalella azteca*). *Environ. Toxicol. Chem.* **2004**, *23*, 691–696.

(11) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. 2000; http://circa.europa.eu.

(12) Heijerick, D. G.; Bossuyt, B. T. A.; De Schamphelaere, K. A. C.; Indeherberg, M.; Mingazzini, M.; Janssen, C. R. Effect of varying physicochemistry of European surface waters on the copper toxicity to green alga *Pseudokirchneriella subcapitata*. *Ecotoxicol.* **2005**, *14*, 661–670.

(13) Vijver, M. G.; de Koning, A.; Peijnenburg, W. J. G. M. Uncertainty of water type-specific hazardous copper concentrations derived with biotic ligand models. *Environ. Toxicol. Chem.* **2008**, *27* (11), 2311–2319.

(14) De Laender, F.; De Schamphelaere, K. A. C.; Verdonck, F. A. M.; Heijerick, D. G.; Van Sprang, P. A.; Vanrolleghem, P. A.; Janssen, C. R. Simulation of spatial and temporal variability of chronic copper toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata* in Swedish and British surface waters. *Human and Ecological Risk Assessment: An International Journal* **2005**, *11* (6), 1177–1191.

(15) Van Genderen, E.; Adams, W.; Cardwell, R.; van Sprang, P.; Arnold, R. S. R. An evaluation of the bioavailability and aquatic toxicity attributed to ambient copper concentrations in surface waters from several parts of the world. *Integrated Environmental Assessment and Management* **2008**, *4* (4), 414–424.

(16) Van Sprang, P. A.; Verdonck, F. A. M.; Van Assche, F.; Regoli, L.; De Schamphelaere, K. A. C. Environmental risk assessment of zinc in European freshwaters: A critical appraisal. *Sci. Total Environ.* **2009**, *407* (20), 5373–5391.

(17) *Species Sensitivity Distribution in Ecotoxicology*; Posthuma, L., Suter II, G. G., Traas, T. P., Eds.; Lewis Publishers: USA, 2002.

(18) *European Union Risk Assessment Reports, Nickel and nickel compounds, Final version*; European Communities: 2008; http://tcsweb3.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/nickelreport311.pdf.

(19) *Voluntary European Union Risk Assessment Report. Copper, CopperII sulphate pentahydrate, Copper(I)oxide, Copper(II)oxide, Dicopper chloride trihydroxide*; European Copper Institute: 2008; http://echa.europa.eu/chem_data/transit_measures/vrar_en.asp.

(20) *European Union Risk Assessment Reports on Zinc and Zinc compounds, Final report, Part 1 Environment*; European Communities: 2008; http://tcsweb3.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/zincmetalreport072.pdf.

(21) iBever 3.7.200; http://www.helpdeskwater.nl/onderwerpen/applicaties-modellen/monitoring/ibever-3-7-200/

(22) *Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II*; European Communities: 2003; http://ecb.jrc.it/documents/TECHNICAL_GUIDANCE_DOCUMENT/.

(23) Tipping, E. Humic ion-binding model VI: An improved description of the interactions of protons and metal ions with humic substances. *Aquat. Geochem.* **1998**, *4*, 3–48.

(24) *NIST standard reference database 46 Version 8.0*; National Institute of Standards and Technology: Gaithersburg (MD) USA, 2004.

(25) Guthrie, J. W.; Hassan, N. M.; Salam, M. S. A.; Fasfous, I. I.; Murimboh, C. A.; Murimboh, J.; Charabarti, C. L.; Grégoire, D. C. Complexation of Ni, Cu, Zn, and Cd by DOC in some metal-impacted freshwater lakes: A comparison of approaches using electrochemical determination of free-metal-ion and labile complexes and a computer speciation model, WHAM V and VI. *Anal. Chim. Acta* **2005**, *528*, 205–218.

6055

dx.doi.org/10.1021/es2007963 |*Environ. Sci. Technol.* 2011, 45, 6049–6056

(26) Development and validation of biotic ligand models for predicting nickel toxicity to fish, daphnids and algae. Draft final report; Laboratory for Environmental Toxicology and Aquatic Ecology, Ghent University: Ghent, Belgium, 2005.

(27) De Schamphelaere, K. A. C.; Janssen, C. R. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna. Environ. Toxicol. Chem.* **2004**, *23* (6), 1365–1375.

(28) Heijerick, D. G.; De Schamphelaere, K. A. C.; Van Sprang, P. A.; Janssen, C. R. Development of a chronic zinc biotic ligand model for *Daphnia magna. Ecotoxicol. Env. Saf.* **2005**, *62* (1), 1–10.

(29) De Schamphelaere, K. A. C.; Janssen, C. R. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*; the effects of calcium, magnesium, sodium, potassium, and pH. *Environ. Sci. Technol.* **2002**, *36* (1), 48–54.

(30) De Schamphelaere, K. A. C.; Janssen, C. R. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. *Environ. Sci. Technol.* **2004**, *38* (23), 6201–6209.

(31) De Schamphelaere, K. A. C.; Vasconcelos, F. M.; Heijerick, D. G.; Tack, F. M.; Delbeke, K.; Allen, H. E.; Janssen, C. R. Development and field validation op a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata. Environ. Toxicol. Chem.* **2003**, *22*, 2454–2465.

(32) De Schamphelaere, K. A. C.; Lofts, S.; Janssen, C. R. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface water. *Environ. Toxicol. Chem.* **2005**, *24* (5), 1190–1197.

(33) National Institute for Public Health and the Environment Website, Risico's van stoffen; http://www.rivm.nl/rvs/normen/.

(34) Directive 2008/105/EC OF THE European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy; European Communities: 2008; http://eur-lex.europa.eu/.

(35) R Version 2.10.0, 1999−2010, R Foundation for Statistical Computing, http://cran.r-project.org/

(36) Posthuma, L., Richards, S. M., De Zwart, D., Dyer, S. D., Hickey, C. W., Altenburger, R., Mixture extrapolation approaches. In *Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals*; Solomon, K. R., Brock, T. C. M., De Zwart, D., Dyer, S., Posthuma, L., Richards, S. M., Sanderson, H., Sibley, P. K., van den Brink, P. J., Eds.; CRC, New York, 2008; pp 135.

(37) Bates, B. Maechler, M., lme4: Linear mixed-effects models using S4-classes; http://cran.r-project.org/web/packages/lme4/.

(38) Van Leeuwen, C. J., Hermens, J. L. M., Eds. *Risk assessment of chemicals: an introduction*; Kluwer Academic Publishers, Dordrecht, The Netherlands: 1995.

(39) De Schamphelaere, K. A. C.; Janssen, C. R. Cross-phylum extrapolation of the Daphnia magna chronic biotic ligand model for zinc to the snail *Lymnaea stagnalis* and the rotifer *Brachionus calyciflorus. Sci. Total Environ.* **2010**, *408* (22), 5414–5422.

(40) De Schamphelaere, K. A. C.; Heijerick, D. G.; Janssen, C. R. Cross-phylum comparison of a chronic biotic ligand model to predict chronic toxicity of copper to a freshwater rotifer, *Brachionus calyciflorus* (Pallas). *Ecotoxicol. Environ. Saf.* **2006**, *63*, 189–195.

(41) Schlekat, C. E.; Van Genderen, E.; De Schamphelaere, K. A. C.; Antunes, P. M. C.; Rogevich, E. C.; Stubblefield, W. A. Cross-species extrapolation of chronic nickel Biotic Ligand Models. *Sci. Total Environ.* **2010**, *408*, 6148–6157.

(42) Dushenkov, V.; Kumar, P. B. A. N.; Motto, H.; Raskin, I. Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Technol.* **1995**, *29* (55), 1239–1245.

(43) Römkens, P. F. A. M.; Plette, A. C. C.; Verstappen, G. G. C. Contribution of agriculture to the heavy metal loads in The Netherlands. In *Agricultural Effects on Ground and Surface Waters: Research at the Edge of Science and Society*; Steenvoorden, H. A. M., Claessen, F., Willems, J., Eds.; IAHS Press, 2002; pp 337−342.