# Chapter 4

## Spatial and temporal variation of water type-specific no-effect concentrations and risks of Cu, Ni and Zn


## 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 extrapolate chronic no-effect concentrations (NOEC) determined in test media towards 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 two 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 risk increased.




Published in: *Environmental Science and Technology*, 2011, Vol. 45, 6049-6056.

## 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 (Tessier et al., 1995). 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 (Sanders et al., 1983; Vink, 2009). The explanation of toxic effects can be further improved by accounting for metal binding to biological ligands, for instance fish gill (Pagenkopf, 1983; Zitko et al., 1976). These findings supported the development of Biotic Ligand Models (BLMs), starting in the 1990s, which describe the binding of metals to biotic ligands (Di Toro et al., 2001). 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 $H^+$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$) 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 (Playle et al., 1993). After fish-gill BLMs, 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 (De Schamphelaere et al., 2002b; Heijerick et al., 2002a; Keithly et al., 2004).



The EU Water Framework Directive (WFD) commits European Union member states to achieve a good qualitative and quantitative status of all water bodies (EC, 2000). 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 compliance with EQS 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 by 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 in order to prioritize sites for risk management measures. To make these options operational, more evidence-based knowledge 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. (De Laender et al., 2005; Heijerick et al., 2005a; van Genderen et al., 2008; Van Sprang et al., 2009; Vijver et al., 2008) 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 as presented by Vijver et al. (2008). A toxicity database and a database containing 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 to BLM to extrapolate 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 (Posthuma et al., 2002). 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 straightforward comparison between HC5 and measurements and 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 (Denmark, 2008; ECI, 2008; The Netherlands, 2008). When necessary, original studies from the EU-RARs were used to add information about chemical composition of test media, e.g. 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 of 27 aquatic species. The Zn database contained 19 species (132 test results) (Van Sprang et al., 2009), the Ni database contained 24 species (128 test results). The species in the databases were tested for various toxic end points e.g., reproduction, mortality and growth.

Fish, invertebrates, algae, insects, mollusks and amphibians were present in the database. An overview of species and taxa in the toxicity database is given in Tables S4.1, S4.2 and S4.3**.**



**Collection of surface water data.** To determine geographical variation, water chemistry data and soluble metal concentrations were collected from surveillance monitoring programs of different water managers who share information of chemical, physical and biological parameters. 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. BLM calculations were performed on monthly mean concentrations. The selected data contained 2573 monthly subsets of 11 BLM parameters for 372 sites, available for HC5-computations. 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 4.2.

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 January 2007 until June 2010 were obtained directly from the water-board authority. The selected data, contained 2760 monthly records (samples) of 237 parameters, varying from nutrients, total and dissolved metals, pesticides, chlorophyll-a, oxygen-content, pH to temperature and water depth 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 binding of metals to DOC was computed with WHAM6 (Tipping, 1998). Stability constants of the relevant metal complexes were modified to comply with values described in the NIST-database (NIST, 2004). DOC was considered to contain 50% active fulvic acids only, and complies with current EU-RAR procedures (Guthrie et al., 2005).

**Biotic ligand modeling.** Biotic ligand models as reported in the EU-RARs for chronic toxicity of Cu, Ni and Zn were used (Denmark, 2008; ECI, 2008; The Netherlands, 2008). BLMs translate NOEC from toxicity tests to water-specific NOEC by the following general formula:

$$\text{NOEC} = \text{Intrinsic Sensitivity} \times \text{Environmental Modulator (EM)}$$

**(Eq 4.1)**

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

$$\text{Intrinsic Sensitivity} = \text{NOEC}_{\text{test}} / \text{EM}_{\text{test}}$$ **(Eq. 4.2)**

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

$$\text{NOEC}_{\text{sample}} = \text{Intrinsic Sensitivity} \times \text{EM}_{\text{sample}}$$ **(Eq. 4.3)**

The environmental modulator is defined as:

$$\text{EM} = \frac{1 + \text{K}_{\text{H-BL}} \times [\text{H}^{+}] + \text{K}_{\text{Na-BL}} \times [\text{Na}^{+}] + \text{K}_{\text{Ca-BL}} \times [\text{Ca}^{2+}] + \text{K}_{\text{Mg-BL}} \times [\text{Mg}^{2+}]}{\text{K}_{\text{Me-BL}}}$$

**(Eq. 4.4)**

For copper, also CuOH and $CuCO_3$ are considered as toxic components; in that case the denominator in Eq. 4.4 is extended to:

$$\text{K}_{\text{Cu-BL}} + (\text{K}_{\text{CuOH-BL}} \times \text{K}_{\text{CuOH}} \times [\text{OH}^{-}]) + (\text{K}_{\text{CuCO3-BL}} \times \text{K}_{\text{CuCO3}} \times [\text{CO}_3^{2-\,-}])$$

**(Eq. 4.5)**

BLMs were developed for *Pseudokirchneriella subcapitata*, *Daphnia magna*, and *Onchorhynchus mykiss*, as representatives of three taxonomic groups: algae, crustacean, and fish (De Schamphelaere et al., 2002b; De Schamphelaere et al., 2003; De Schamphelaere et al., 2004b; De Schamphelaere et al., 2004c; De

Schamphelaere et al., 2005; Deleebeeck et al.; Heijerick et al., 2005b). The model concepts are identical for each substance and taxon; differences are caused by the biotic ligand binding constants. Read across of BLMs over species means that species within the same taxonomic group use the same set of biotic ligand 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 (see Table S4.1 of the Supporting information), the trophic level is used as a shared property for read-across. In this way, NOEC for plants are normalized with algae BLMs, frogs and toads were treated as fish and rotifers, insects, bivalves, mollusks and hydrozoa are treated as crustaceans. Biotic ligand binding constants for Cu, Ni and Zn for different taxa are given in Table 4.1**.**

**Environmental quality criteria.** The generic environmental quality standard used in this study were: Cu: 1.5 ug/L and Ni: 20 µg/L. (EC, 2008; RIVM). 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 (EC, 2010).



**Table 4.1 Overview biotic ligand binding constants. Me = metal**

| $logK_{BL}$ | Algae | | | Crustacea | | | Fish | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cu | Ni (1) | Zn | Cu (2) | Ni (1) | Zn (3) | Cu (4) | Ni (1) | Zn (5) |
| Me | | 4.0 | | 8.02 | 4.0 | 5.3 | 8.02 | 4.0 | 5.5 |
| MeOH | | - | | 8.02 | n.r. | n.r. | 7.32 | n.r. | n.r. |
| $MeCO_3$ | | | | 7.44 | n.r. | n.r. | 7.01 | n.r. | n.r. |
| H | n.a. | 5.9 | n.a. | 6.67 | 5.9 | 5.8 | 5.4 | 6.8 | 6.3 |
| Ca | | 2.1 | | n.r. | 3.1 | 3.2 | 3.47 | 3.7 | 3.6 |
| Mg | | 3.3 | | n.r. | 3.3 | 2.7 | 3.58 | 4.0 | 3.1 |
| Na | | - | | 2.91 | n.r. | 1.9 | 3.19 | n.r. | n.r. |
| Regression | Cu (6) | Ni | Zn (7) | Cu | Ni | Zn | Cu | Ni | Zn |
| Slope | -1.140 | n.r. | -0.754 | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. |
| Intercept | -0.812 | | -1.294 | | | | | | |

n.a.= not available; n.r. = not relevant since a full BLM is available

1) (Deleebeeck et al.); 2) (De Schamphelaere et al., 2004c) ; 3) (Heijerick et al., 2005b) ; 4) (De Schamphelaere et al., 2002b) ; 5) (De Schamphelaere et al., 2004b) ; 6) (De Schamphelaere et al., 2003) ; 7) (De Schamphelaere et al., 2005)

**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 5$^{th}$ percentile of modeled SSD-curves assuming a log-normal frequency distribution of NOEC of all taxa (EC, 2011). HC5 were further aggregated to annual average values for Figure 4.3**.**

BLM computations and descriptive statistics were performed with software package R (http:/cran.r-project.org/).

Risk characterization ratios (RCR), were calculated as ratios of metal concentration over HC5. Multimetal RCRs were calculated assuming additivity of risks, as follows (Posthuma et al., 2008):

$$\sum RCR = \frac{[Cu]}{HC5_{Cu}} + \frac{[Ni]}{HC5_{Ni}} + \frac{[Zn]}{HC5_{Zn}} \qquad \textbf{(Eq.4.6)}$$

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 (Bates et al., 2010). ANOVA was applied to test significance of the monthly model compared to a model assuming only random effects. Criteria for significance were Pr $\chi^2$ <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 parameter relative to the annual average:



$$F = X_{month} / X_{year} \qquad \textbf{(Eq. 4.7)}$$

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

# RESULTS

**Spatial variation.** In Figure 4.1 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 2573 sample-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 HC5 ranged from 5.9-121 µg Cu/L, 7.9-197 µg Ni/l and 17.5-368 µg Zn/L.

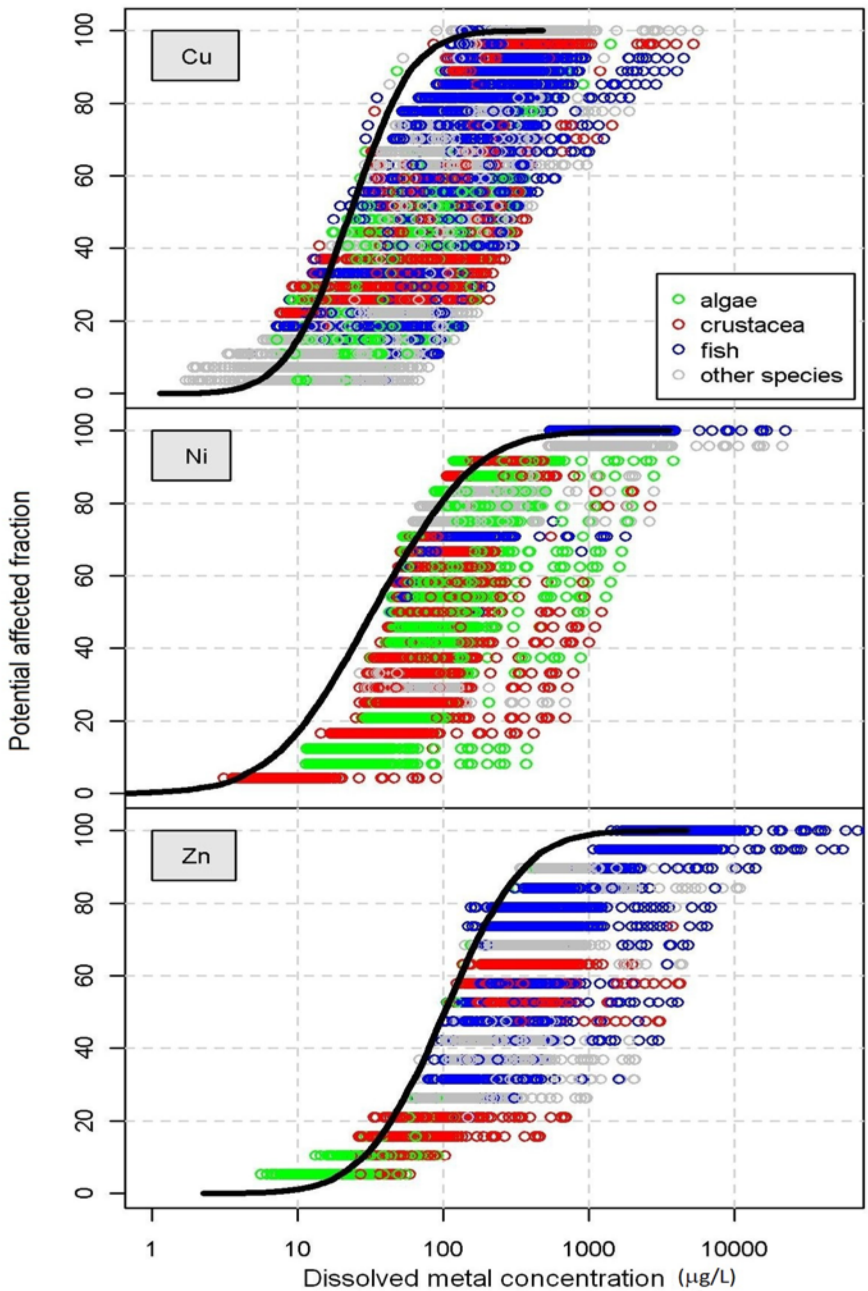




**Figure 4.1 Annual average SSD for chronic Cu, Ni and Zn toxicity at 372 sites in the Netherlands in 2007 computed with BLM. The line represents the modeled SSD based**

**on measured NOEC from the toxicity database. Colors indicate different taxonomic groups.**

The distribution of taxa within SSD is not constant; application of BLMs 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 BLMs 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 (see Tables S4.1, S4.2 and S4.3 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 (*Oncorhynchus kisutch*, 2% of the samples). For Ni, crustacean (*Ceriodaphnia dubia*, 100% of the samples) is the most sensitive taxon. For Zn, algae and crustacean are the most sensitive species in respectively 74% and 26% of the samples.



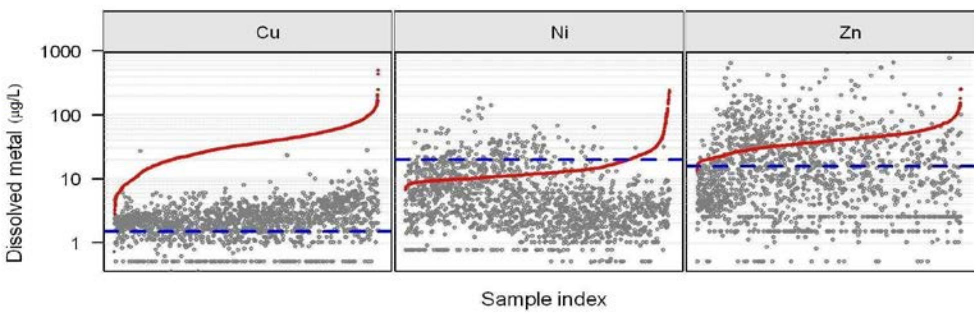


**Figure 4.2 Comparison of total dissolved Cu, Ni and Zn concentrations (gray dots) in 2573 individual samples of 273 sites in 2007 with generic environmental quality standards (dashed line) and sample-specific HC5 (red dots) were derived from normalized NOEC computed with chronic BLMs for algae, crustaceans and fish. Individual samples were ranked with increasing HC5.**

**Sample-specific HC5.** Additional to evaluation of individual sample quality, the WFD requires evaluation of annual average concentration of sites. Figure 4.2 shows the distribution of computed site-specific HC5 of individual samples in 2007 and corresponding measured metal concentrations. The figure shows that the variation in HC5, based on individual samples, is higher than the variation in HC5 based on annual average site characteristics. Sample-specific HC5 ranged from 0.7-490 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 samples.

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

**Table 4.2 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 | 319 | 22 | 222 | 135[b] | 61[b] | 44 | 20 |
| Cu+Ni+Zn | 1430 | - | | 674 | 47 | 213 | - | | 97 | 46 |

[a] Concentrations compared with MAC-Zn; [b] Concentrations compared with AA-Zn



A full overview of HC5-exceeding samples of generic, site-specific and sample-specific quality standards is given in Table 4.2**.** 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 risk limit 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 4.2 and the maps in Figure 4.3 show that 45 and 44 sites exceed site-specific HC5 of Ni and/or 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 the 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 Cu is taken into account as a third toxicant, whereas there are no sites with a risk due to copper only. The HC5-exceedings are clustered in the southern part of The Netherlands (the Dommel catchment area) due to regional high metal concentrations and low site-specific HC5 (see Figure S4.1 of the Supporting Information).

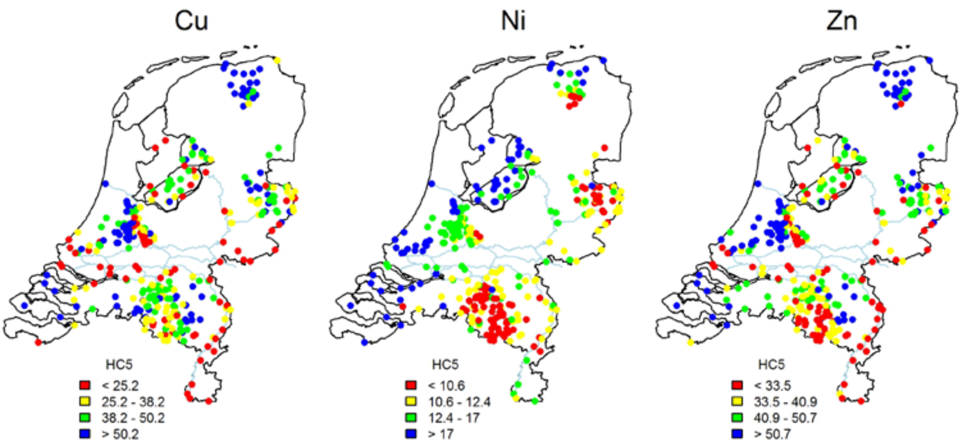


**Figure 4.3 Spatial variation of site-specific annual average HC5 (µg/L).**



**Temporal variation.** Temporal variation of BLM parameters in 76 sites of the Dommel-database over a period of 42 months is shown in Figure 4.4**.** BLM-input parameters are shown (1st and 2nd 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 4.4**,** 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 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 lasted respectively 44 and 40% of the time and usually took place during winter and spring.

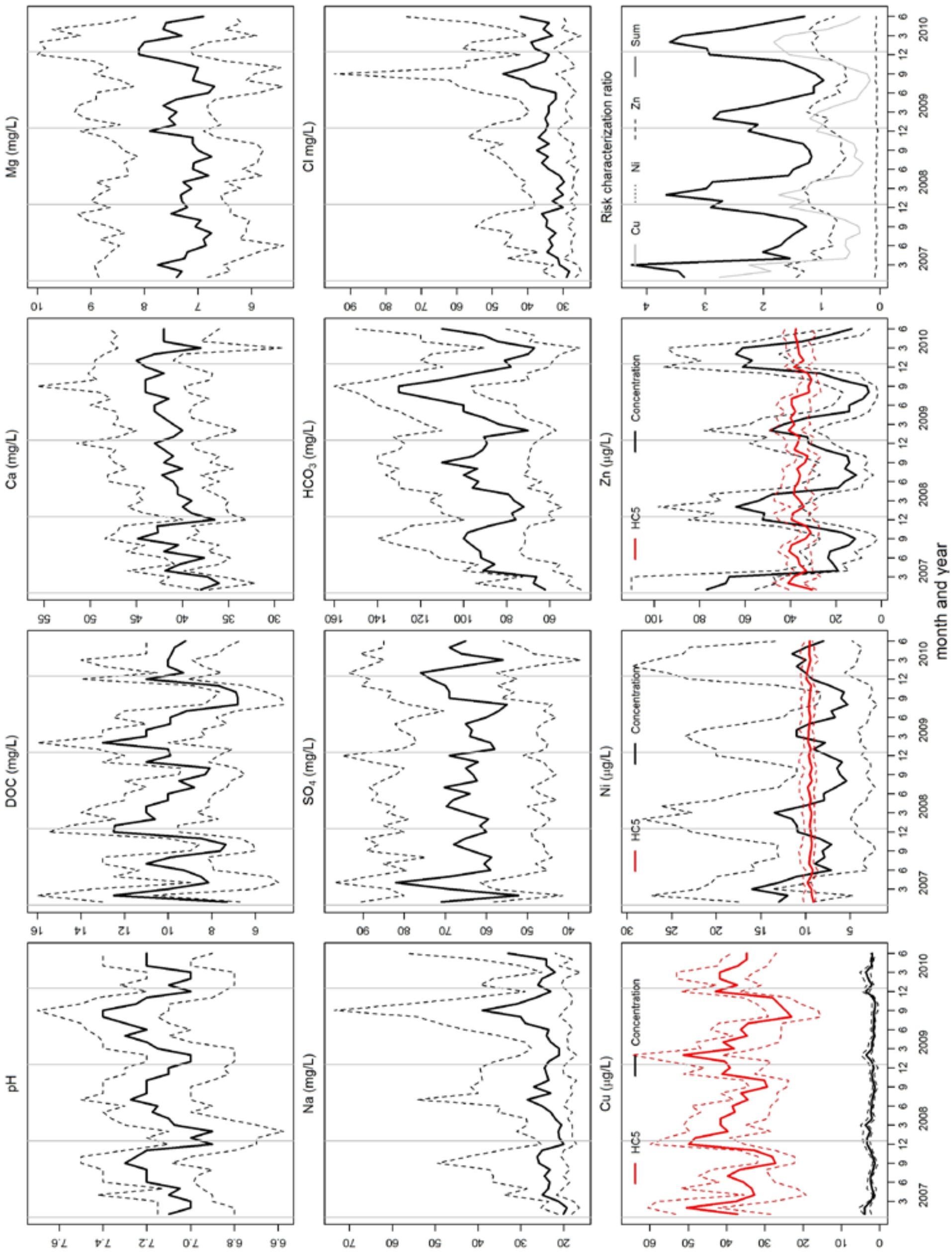


**Figure 4.4 Temporal variation in BLM parameters, HC5 values and risks in 76 sites of the Dommel database from January 2007 until June 2010. Bold lines represent mean values, dashed lines represent 25th and 75th percentiles.**

A statistical analysis of the data was performed which 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 the $\chi^2$-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 $\chi^2$-test.

In Table 4.3 temporal factors are presented for HC5 and risks of Cu, Ni and Zn. Temporal factors of 1 imply that the parameter equals annual average. Factors >1 indicate parameters higher than annual average, whereas factors <1 indicate parameters lower than annual average.

**Table 4.3 Temporal factor $F_t$ for seasonal effects.**

| | HC5 (µg/l) | | | RCR | | | SumRCR |
|---|---|---|---|---|---|---|---|
| | Cu | Ni | Zn | Cu | Ni | Zn | |
| Annual mean | 37.5 | 10.0 | 38.0 | 0.075 | 1.35 | 1.36 | 2.79 |
| Temporal factor relative to annual mean | | | | | | | |
| January | 1.13* | 0.98* | 1.00 | 1.05 | 1.21 | 1.37* | 1.29* |
| February | 1.25* | 0.97* | 1.03 | 1.11* | 1.30* | 1.85* | 1.57* |
| March | 1.14* | 0.97* | 1.03 | 1.18* | 1.33* | 1.43* | 1.38* |
| April | 0.84 | 1.03 | 1.07 | 1.19 | 1.29 | 1.24 | 1.26 |
| May | 0.94 | 1.03 | 1.04 | 0.97 | 1.06 | 0.85 | 0.96 |
| June | 0.96 | 1.06 | 1.06 | 0.94 | 0.85 | 0.71 | 0.78 |
| July | 0.93 | 1.01 | 1.03 | 0.96 | 0.77* | 0.44* | 0.61* |
| August | 0.94 | 1.00 | 1.02 | 0.96 | 0.72* | 0.55* | 0.64* |
| September | 0.70* | 1.01 | 0.89* | 0.91 | 0.69* | 0.45* | 0.58* |
| October | 0.73* | 0.99 | 0.86* | 0.94 | 0.76* | 0.61* | 0.69* |
| November | 0.93 | 1.01 | 0.95 | 0.93 | 0.92 | 0.89 | 0.91 |
| December | 1.17* | 0.99 | 1.01 | 0.90* | 1.16 | 1.36* | 1.25* |
| $\chi^2$ of model | <0.001 | <0.001 | <0.001 | 0.051 | <0.001 | <0.001 | <0.001 |

AnnAvg=Estimated$_t$ / $F_t$. Asterisks indicate a significant difference between monthly and annual average values. Shaded rows indicate months that represent minimum, mean and maximum risks.

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, since 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 4.4.

Finally, Table 4.3 shows that monitoring in February, May and September would result in a good reflection of the annual range of risks.

## DISCUSSION

Derivation of HC5 from species sensitivity distributions is an accepted way of dealing with variation between species in risk assessments (Van Leeuwen et al., 1995). 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. 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 sensitivities, expressed as HC5. Application of BLMs 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 were not explicitly account for in BLMs. These phenomena, which define ecosystem composition and stability in the field, are beyond the scope of current risk assessment methodology, which focusses on *potential* risk of chemicals.



In Figure 4.1, most of the natural surface water 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 NOEC that are relatively low compared to site-specific NOEC.

SSD give an overview of variation of sensitivity between species and between taxa. The ranking of sensitivities of species and taxa in the SSD changes when test-NOEC are normalized to site-specific NOEC, because the binding of metals to biotic ligands is not equally affected by water chemistry for each taxon, as a result 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 water types. Moreover, most sensitive organisms in toxicity tests are also most sensitive after site-specific normalization.

In the Cu-SSD, two molluscs are below HC5. The species-collection in the SSD is meant to be a representation of an ecosytem. Therefore, molluscs *Juga plicifera* and *Campeloma decisum* are representative for many species of that taxa. The application of a bioavailability model across species assumes similar modes 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) (De Schamphelaere et al., 2006; De Schamphelaere et al., 2010; Schlekat et al., 2010).

Crustacea were indicated as the most sensitive taxon in the Ni-SSD. However, new data exist indicating the the mollusc *Lymneae stagnalis* is more sensitive than crustaceans (Schlekat et al., 2010). 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 the SSD for all of the samples. The normal function predicts higher values than the available NOEC suggest. 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 crustaceans 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 crustaceans were the most sensitive taxon. The average HC5 were not significantly different; algae Zn-HC5 was 42.9±35.0 µg/L and crustacean Zn-HC5 was 41.5±18.4 µg/L. The relative sensitivity between algae and crustaceans depended on water characteristics. Algae were the most sensitive taxon in water types with relative lower DOC (mean DOC= 10.8 mg C/l versus 13.0 mg C/L for crustaceans) and higher pH (mean pH= 7.7 versus 6.8 for crustaceans). The Zn-concentrations were significantly lower in water types where algae were the most sensitive group compared to water types where crustaceans were the most sensitive taxon (9.7 versus 40.5 µg/L respectively), indicating that crustaceans are more at risk than algae. Lower Zn-concentrations in water types 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 BLM 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 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 pattern were 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 winter months, probably caused by higher input of run-off 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 (Dushenkov et al., 1995; Römkens et al., 2002). 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 4.4**,** Table 4.3) 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.

## Supplementary information

**Table S4.1 Cu NOEC lowest mean end point over taxa and species (µg total dissolved metal/L) and NOEC-range between brackets. (Source: (ECI, 2008))**

| | Species name and number of data | | Cu NOEC (µg/L) |
|---|---|---|---|
| algae | *Chlamydomonas reinhardtii* | 4 | 101 (22-178) |
| | *Chlorella vulgaris* | 7 | 183 (31-510) |
| | *Pseudokirchneriella subcapitata* | 12 | 43.1 (15.7-164) |
| crustacea | *Ceriodaphnia dubia* | 14 | 15.6 (4.0-122) |
| | *Daphnia magna* | 9 | 12.6 (12.6-181) |
| | *Daphnia pulex* | 9 | 18.2 (4-40) |
| | *Gammarus pulex* | 9 | 11.0 |
| | *Hyalella azteca* | 1 | 54.3 (30-82) |
| fish | *Catostomus commersoni* | 6 | 12.9 (12.9-12.9) |
| | *Esox lucius* | 2 | 34.9 (34.9-34.9) |
| | *Ictalurus punctatus* | 2 | 13.0 (13.0-13.0) |
| | *Noemacheilus barbatulus* | 2 | 120 |
| | *Oncorhynchus kisutch* | 1 | 21.0 (18.0-28.0) |
| | *Oncorhynchus mykiss* | 5 | 18.7 (2.2-45) |
| | *Perca fluviatilis* | 7 | 39.0 (39.0-188) |
| | *Pimephales notatus* | 2 | 57.9 (44.0-71.8) |
| | *Pimephales promela* | 3 | 16.1 (4.8-6.06) |
| | *Salvelinus fontinalis* | 12 | 14.0 (7.0-49.0) |
| other taxa | *Brachionus calyciflorus(rotifer)* | 4 | 47.5 (8.2-103) |
| | *Campeloma decisum (mollusk)* | 2 | 8.0 (8.0-8.0) |
| | *Chironomus riparius (insect)* | 1 | 16.9 |
| | *Clistoronia magnifica (insect)* | 2 | 10.7 (8.3-13.0) |
| | *Dreissenia polymorpha (bivalve)* | 2 | 18.5 (16.0-21.0) |
| | *Juga plicifera (mollusc)* | 1 | 6.0 |
| | *Lemna minor L.(plant)* | 1 | 30.0 |
| | *Paratanytarsus parthenogeneticus (insect)* | 2 | 40.0 |
| | *Villosa iris (bivalve)* | 1 | 19.1 |

**Table S4.2 Ni NOEC lowest mean end point over taxa and species (µg total dissolved metal/L) and NOEC-range between brackets. (Source: (Denmark, 2008))**

| | Species name and number of data | n | Ni NOEC (µg/L) |
|---|---|---|---|
| algae | *Ankistodesmus falcatus* | 2 | 33.8 (24.6-43.0) |
| | *Chlamydomos sp* | 2 | 17.9 (8.3-27.5) |
| | *Chlorella sp.* | 2 | 73.6(49.0-98.2) |
| | *Coelastrum microporum* | 4 | 36.9 (15.6-70.0) |
| | *Desmodesmus spinosus* | 4 | 28.3 (3.5-43.7) |
| | *Pediastrum duplex* | 2 | 31.5 (23.5-39.5) |
| | *Pseudokircheneriella subcapitata* | 12 | 107 (21.5-432) |
| | *Pseudokirchneriella sp.* | 2 | 8.7 (3.5-13.8) |
| | *Scenedesmus accumitus* | 2 | 7.7(3.1-12.3) |
| crustacea | *Alona affinis* | 2 | 25.0 (25-25) |
| | *Ceriodaphnia dubia* | 10 | 5.0 (2.8-15.3) |
| | *Ceriodaphnia pulchella* | 4 | 19.1 (9.9-28.2) |
| | *Ceriodaphnia quadrangula* | 8 | 9.1 (2.0-34.9) |
| | *Daphnia longispina* | 4 | 27.8 (26.6-118) |
| | *Daphnia magna* | 32 | 128 (50.5-389) |
| | *Hyalella azteca* | 1 | 29.0 |
| | *Peracantha truncata* | 4 | 14.2 (2.5-25.8) |
| | *Simocephalus vetulus* | 8 | 14.1(9.2-28.9) |
| fish | *Brachydanio rerio* | 1 | 40.0 |
| | *Oncorhynchus mykiss* | 5 | 750 (265-1770) |
| other taxa | *Bufo terrestris (toad)* | 5 | 640 (640-1360) |
| | *Gastrophryne carolensis(toad)* | 5 | 80.0 (70.0-450) |
| | *Hydra littoralis (hydrozoa)* | 1 | 60.0 |
| | *Xenopus laevis(frog)* | 6 | 88.2 (84.5-4790) |

**Table S4.3 Zn NOEC lowest mean end point over taxa and species (µg total dissolved metal/L) and NOEC-range between brackets. (Source:The Netherlands, 2008)**

| | Species name and number of data | n | Zn NOEC (µg/L) |
|---|---|---|---|
| algae | *Chlorella sp.* | 5 | 114 (5.9-350) |
| | *Pseudokircheneriella subcapitata* | 30 | 56.4 (4.0-358) |
| crustacea | *Ceriodaphnia dubia* | 8 | 40.6 (25-100) |
| | *Daphnia longispina* | 2 | 129 (91-209) |
| | *Daphnia magna* | 39 | 105 (25-491) |
| | *Hyalella azteca* | 1 | 42.0 |
| fish | *Cottus bairdi* | 2 | 99.50 (27-172) |
| | *Danio rerio* | 9 | 1282 (180-2900) |
| | *Jordanella floridae* | 2 | 50.5 (26-75) |
| | *Oncorhynchus mykiss* | 23 | 286 (32-974) |
| | *Phoxinus phoxinus* | 2 | 50.0 (50.0-50.0) |
| | *Pimephales promelas* | 1 | 78.0 |
| | *Salvelinus fontinalis* | 1 | 534 |
| | *Salmo trutta* | 2 | 154 (57-250) |
| other taxa | *Anuraeopsis fissa (rotifer)* | 1 | 50.0 |
| | *Brachionus rubens (rotifer)* | 1 | 50.0 |
| | *Dreissena polymorpha (mollusk)* | 1 | 382 |
| | *Ephoron virgo (insect)* | 1 | 718 |
| | *Potamopyrgus jenkinsi (mollusc)* | 1 | 72 |

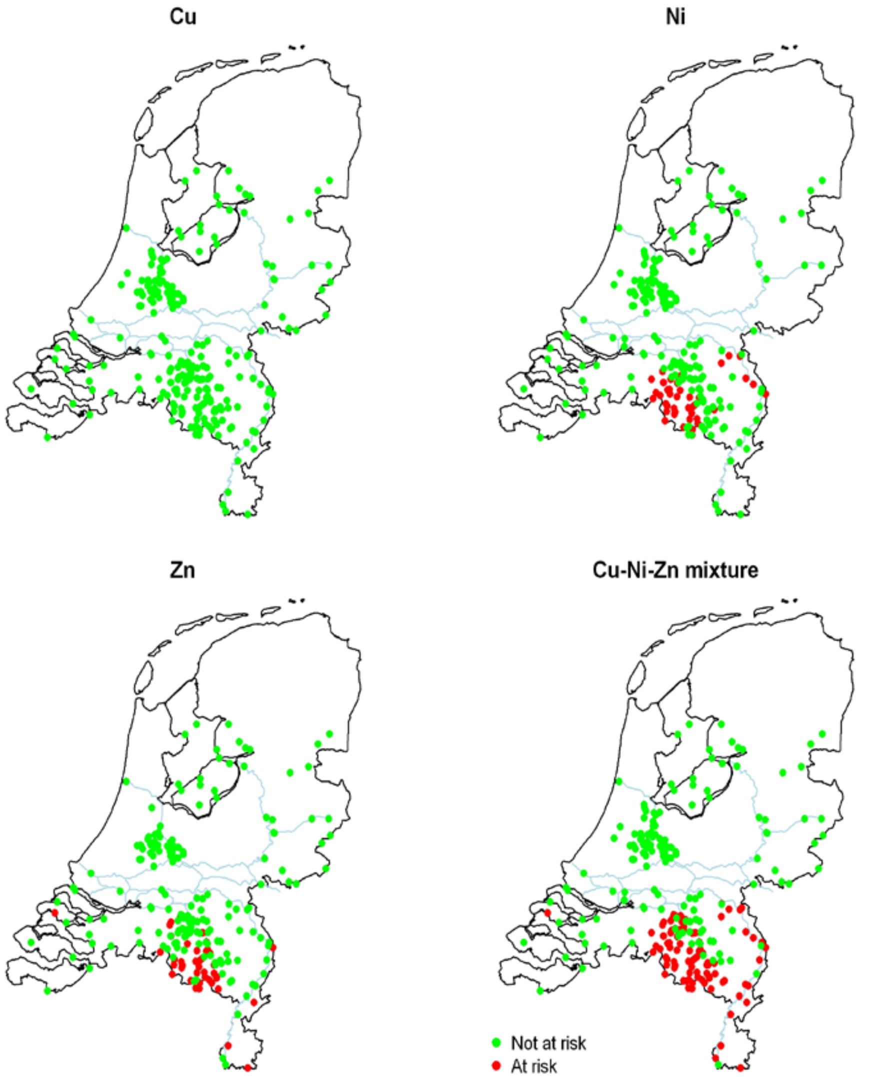


**Figure S4.1 Overview sites at risk for single metals and Cu, Ni and Zn mixture (SumRCR). A site is considered to be at risk when RCR>1.**