Evaluation of the use of bioavailability corrections for zinc under low pH and low calcium conditions

by
Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

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Executive summary

The Environment Agency and other UK environmental regulators wish to use the zinc biotic ligand model (ZnBLM) in a compliance-based framework for the assessment of freshwaters under the Water Framework Directive. The ZnBLM has been validated for use between pH 6 and 9 and Ca concentrations between 5 and 150 mg l^{-1}. However, under conditions of very low water hardness (below 24 mg l^{-1} $CaCO_3$, or below 7 mg l^{-1} Ca) a "soft water" predicted no effect concentration (PNEC) has been recommended (European Union, 2008). This soft water PNEC was established because the generic PNEC for Zn may not be sufficiently protective under very soft water conditions. The soft water PNEC derived in the EU risk assessment is 3.1 µg l^{-1} dissolved Zn (European Union,2008).

The ZnBLM (Version 4b) predicts that Zn bioavailability will be greatest to invertebrates under conditions of low pH and low Ca concentrations, although high bioavailability conditions are also expected to occur at circumneutral pH for fish and at high pH for algae. Zinc toxicity is predicted to be greatest for invertebrates at the lower limit of the BLM boundaries for pH and Ca, and greatest to algae at pH values above 6.4. An acute ZnBLM for *Daphnia pulex* which can be applied to soft water conditions has recently been developed (Clifford *et al.* 2009) demonstrating that the BLM principles can be applied to very low Ca concentrations (down to 2.6 mg l^{-1} Ca), although no comparable tests have been reported using chronic studies.

Calculations of Zn speciation under soft water conditions show that Zn binding to DOC could be important, especially where DOC concentrations are relatively high and Ca concentrations are low. At pH values much lower than 5.5, Zn binding to DOC will generally be relatively low, but between pH 5.5 and 6.0 a reduction in Zn bioavailability due to DOC binding could be important. Performing a correction of Zn exposure concentrations relative to DOC concentrations may take account of the chemical availability of Zn, although confirmation that this results in decreased bioavailability over chronic exposures from ecotoxicity tests may not be available.

A field study into the effects of metals on invertebrate and diatom communities in mining impacted streams was able to identify effects on invertebrates, although it was necessary to take account of the presence of other potential stressors such as aluminium and acidity in addition to Zn. Diatom communities appeared to show a response to Zn, and a comparison of the toxicity coefficients employed in modelling suggests that diatom communities may be more sensitive than invertebrate communities to Zn under the range of conditions included in the study. A relatively large proportion of soft waters were included in this study, suggesting that the findings may be particularly applicable to consideration of the soft water PNEC. Any acclimation of organisms to elevated exposure of metals at these mining sites over many generations may, however, complicate application to more typical exposure situations.

An analysis of matched chemical and ecological field monitoring data for effects of Zn in waters that predominantly would not be covered by the soft water PNEC suggests that benthic macroinvertebrate communities may not be particularly sensitive to the effects of Zn. There are no clear indications of invertebrate communities being more sensitive to Zn toxicity under soft water conditions, although data under these conditions are very limited.

There is a possible difference between the ZnBLM predictions, which suggest that invertebrates are likely to be the most sensitive trophic level under soft water conditions, and the available field data which suggest that invertebrates may not be particularly sensitive to the effects of Zn, and that algae may be more sensitive. It is difficult to draw firm conclusions about the relative sensitivity of the different trophic levels due to differences between the studies and types of data available.

Analysis of the available data does not show any clear requirement for a difference in approaches between waters with Ca concentrations of less than 7 mg l^{-1} and waters with Ca concentrations of greater than 7 mg l^{-1}. It appears that similar principles, in terms of the competition between Zn and major cations such as Ca and Mg, and bioavailability reduction through Zn binding to DOC can still be applied across the complete range of conditions. The current ZnBLM will require considerable modification before it can make such bioavailability corrections.

For waters which lie within the validated pH range of the ZnBLM, but with low Ca concentrations, it is recommended that the Ca concentrations are assumed to be equal to the lower limit for the BLM, as the error associated with making this assumption is likely to be less than 25 percent. This means that the generic PNEC, which used an assessment factor of two in its derivation, should still be adequately protective of this difference.

A bioavailability correction approach based on prediction of the chemically available Zn species using chemical speciation models such as WHAM 6 is proposed as a practical means of correcting dissolved Zn concentrations for their likely bioavailability to aquatic organisms. Look-up tables of bioavailability correction factors are provided in Tables 4.1 to 4.4.

Contents

Tables and Figures

1 Introduction

The Environment Agency and other UK regulators wish to use the Zn biotic ligand model (ZnBLM) in a compliance-based framework for the assessment of freshwaters under the Water Framework Directive. The ZnBLM has been validated for use between pH 6 and 9 and calcium (Ca) concentrations between 5 and 150 mg l^{-1}. However, under conditions of very low water hardness (below 24 mg l^{-1} $CaCO_3$ or below 7 mg l^{-1} Ca) a “soft water” predicted no effect concentration (PNEC) has been recommended (European Union, 2008). This soft water PNEC was established because the generic PNEC for Zn may not be sufficiently protective under very soft water conditions. Application of this soft water PNEC was in relation to findings that at Ca concentrations below 7 mg l^{-1} bioavailability is maximised, and is not affected by the pH or dissolved organic carbon (DOC) concentration of the water. The resulting soft water PNEC is 3.1 µg l^{-1} dissolved Zn.

Background concentrations of dissolved Zn have been derived, on a hydrometric area basis, for approximately 50 hydrometric areas within the UK (UKTAG 2012). Background concentrations were not derived for hydrometric areas with insufficient data (less than 100 samples reported) or where more than 30 percent of the reported data were less than the reporting limit of 5 µg l^{-1}. Background concentrations were derived as the 5th and 10th percentiles of the available monitoring data, and in many cases this was less than the reporting limit of 5 µg l^{-1}. This value is higher than the soft water PNEC.   The background concentration was derived from such data as half of the reporting limit value, resulting in a typical background concentration of 2.5 ug l^{-1} dissolved Zn, which is close to the PNEC for soft waters (although the PNEC would be applied as a $PNEC_{add}$, and so would be added to the derived background concentration).

The soft water PNEC is derived by applying a water effects ratio (WER) to the generic PNEC. Testing was undertaken on three species (*Pseudokirchneriella subcapitata*, *Daphnia longispina*, and *Salmo trutta*) in two very soft waters (hardness around 6 and 8 mg l^{-1} $CaCO_3$), and also in the same waters after adjustment to 100 mg l^{-1} $CaCO_3$. WER values were similar when based on the no observed effect concentration (NOEC) or lowest observed effect concentration (LOEC) values from the tests, and an overall arithmetic mean value of 2.5 was selected for calculation of the soft water PNEC from the generic PNEC. The lowest NOEC from the tests undertaken in soft waters was 42 µg l^{-1} Zn, for *Daphnia longispina*, and applying an assessment factor of 10 to this NOEC would have resulted in a soft water PNEC of 4.2 µg l^{-1} Zn. *Daphnia longispina* is not included in the ecotoxicity database which was used to derive the generic PNEC, although tests on the related crustacean species *Daphnia magna* and *Ceriodaphnia dubia* are. The lowest NOEC for crustaceans in the database used for the generic PNEC was 37 µg l^{-1} Zn. Although the results of the soft waters testing indicated greater sensitivity of the tested species under conditions of very low hardness compared to tests in hard waters, the resulting NOEC values were not appreciably different from those included in the database used to derive the generic PNEC.

Many field sites in the UK meet the criteria for using the soft water PNEC, although some Environment Agency regions do not appear to have any waterbodies where the criterion is met. Table 1.1 indicates the proportion of sites where the soft water criterion is likely to be met in some of the Environment Agency regions in England and Wales (Environment Agency 2009b).

Table 1.1 Percentage of sites potentially meeting the soft water criterion in some areas of the UK

Region	Percentage soft waters
Midlands	>30
North West	>20
Scotland	>33
South West	>8
Wales	>35

The aims of this project are to assess the validity and relevance of the Zn soft water PNEC through the use of field data from several independent sources. These data are used to determine whether a PNEC for soft water conditions is required and, if so, to define the characteristics of those waters that require additional protection. The lower pH boundary limit of the ZnBLM is also assessed using these field-based data.

The approach to assessing the validity of the soft water PNEC is based on an assessment of field data from a variety of sources, including previously published Environment Agency reports and datasets which combine information on chemical exposure and ecological effects (see Section 2). A study of the effects of metals from historic mining sites in North West England (Environment Agency 2008) included 15 (out of 35) sites where Ca concentrations were below 7 mg l^{-1}, and involved ecological monitoring of benthic macroinvertebrates and benthic diatoms. Many of the sites included in the study were impacted by mixtures of metals, although Zn was the most common metal contaminant across all sites included in the study.

Low Ca concentrations are commonly found in waters of low pH, and it is therefore appropriate to consider the relevance of the soft water PNEC *alongside* the lower limit of the validated range of pH for ZnBLM. The relationship between Ca concentrations and pH for several upland streams is shown in Figure 1.1, which suggest a relatively close relationship between these parameters for sites from a limited geographical area.

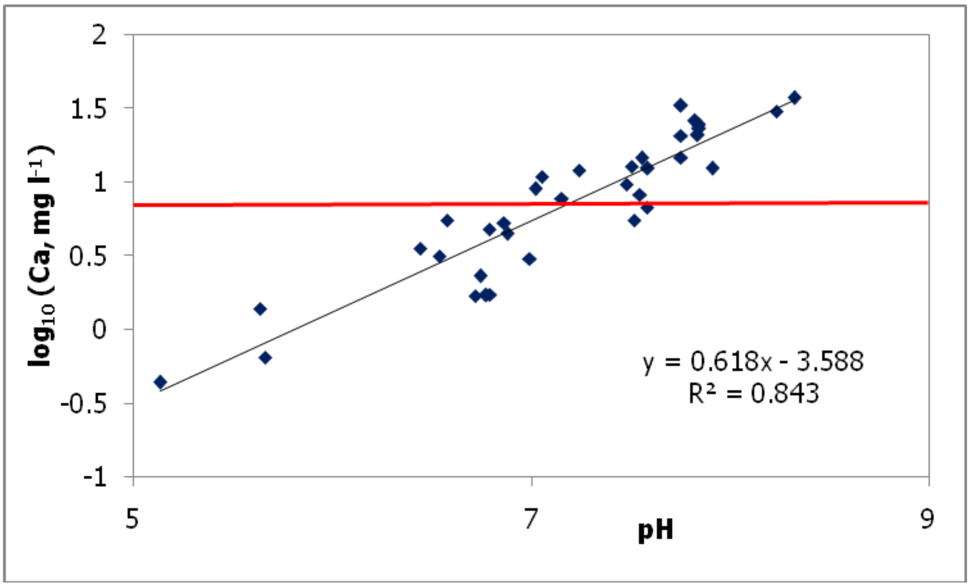

Figure 1.1 Relationship between pH and log_{10}(Ca concentration) at 35 upland sites in North West England; red line indicates cut-off for soft water PNEC

Identification of the soft waters scenario in the Zn risk assessment (European Union, 2008) is based on the Ca concentrations in waters in which ecotoxicity tests have been undertaken for Zn. It is not based on any distinction between waters which would typically be considered to be hard or soft. The Drinking Water Inspectorate identifies soft waters as those with $CaCO_3$ concentrations below 100 mg l^{-1} (roughly equivalent to 30 mg l^{-1} Ca) and hard waters as those with higher $CaCO_3$ concentrations. When Ca concentrations are compared against pH no distinction can be made between hard or soft waters, and a continuum appears to exist between relatively soft acid waters and harder, more alkaline, waters (Figure 1.2). Conditions considered as soft water within the Zn risk assessment represent a relatively small proportion of waters which would generally be considered to be soft waters. One aspect of this study is to identify whether these waters, with Ca concentrations below 7 mg l^{-1}, need to be treated with a conceptually different approach from other waters with Ca concentrations below 30 mg l^{-1} or higher.

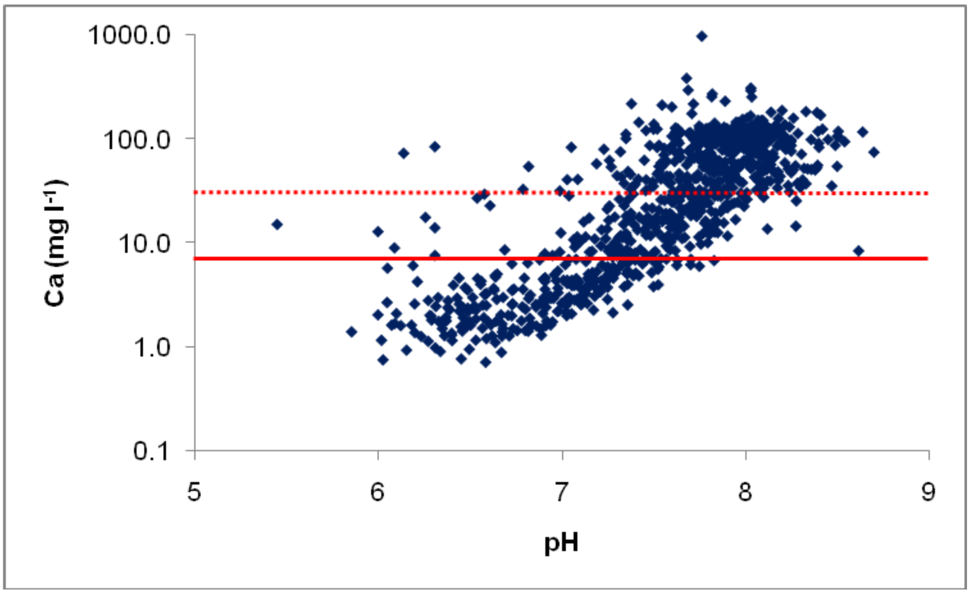

Figure 1.2 Annual average pH and calcium concentrations at 916 sites in Great Britain. Horizontal lines indicate 30 mg l^{-1} (dotted line) Ca and 7 mg l^{-1} Ca (solid line)

Ecological quality can be summarised as the ecological quality index (EQI, or observed/expected (O/E)), which expresses observed benthic macroinvertebrate assemblages relative to the expected assemblages in an unimpacted state. Three EQI metrics can be derived from monitoring of communities and predictions of communities using RIVPACS. These are the Biological Monitoring Working Party (BMWP) score, the number of scoring taxa (N-Taxa), and the average score per taxon (ASPT). Comparison of EQI against water quality parameters such as pH, Ca, and hardness concentrations may enable the relevance of the soft waters scenario to be assessed. Figure 1.3 shows this type of information for sampling sites which have dissolved Zn concentrations greater than, or equal to, the generic PNEC for Zn (7.8 µg l^{-1}). This simple assessment, although only including a small number of soft water sites, does not suggest that observable impacts on benthic macroinvertebrate assemblages should necessarily be expected when Ca concentrations are low and Zn concentrations are above the soft waters PNEC identified in the Zn risk assessment.

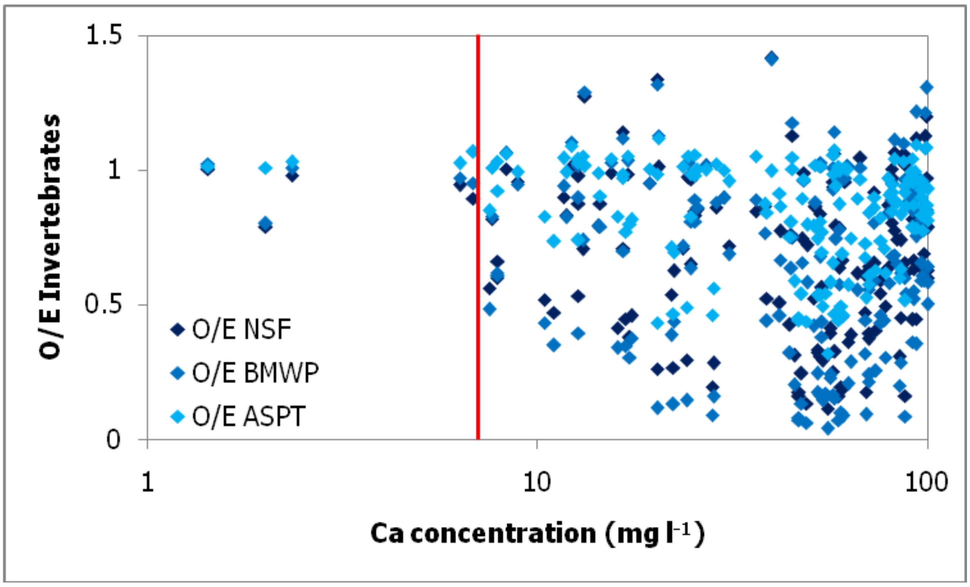

Figure 1.3 Relationship between ecological quality (benthic macroinvertebrates) and Ca concentrations at sites with Zn concentrations of 7.8 µg l^{-1} and above; the red line indicates the cut-off for the soft water PNEC.

Data of this type are assessed in more detail in Section 2.2, to assess whether there are any indications of increased sensitivity to Zn under soft water conditions in benthic macroinvertebrate communities.

A weight of evidence is developed below to assess the validity of the soft water PNEC and the need to undertake compliance assessments of waters with pH below 6 or Ca below 7 mg l^{-1} in a distinctly different manner (by not making bioavailability corrections). Most of the ecological information available for such an assessment is for benthic macroinvertebrates, but other possible sources of information are also considered, where available, such as data for benthic diatoms.

Conductivity and alkalinity, both of which co-vary with Ca concentrations, were found to be important variables in defining the assemblages of diatoms in Spain (Negro and de Hoyos 2005). That study did not, however, include any sites with very low alkalinity which would be likely to meet the soft water criteria for the Zn PNEC. The minimum alkalinity measured in the study was 12 mg l^{-1}, which is equivalent to 7 mg l^{-1} Ca based on the observed co-variation of these parameters in European surface waters.

Dissolved organic carbon (DOC) is known to have a considerable influence on Zn bioavailability to aquatic organisms, and the potential to apply corrections for Zn bioavailability due to DOC binding are considered in Section 4.

2 Review of available field data

2.1 Environment Agency study of mining impacted streams in Northern England

This study investigated the effects of trace metals on benthic macroinvertebrates and benthic diatoms in 36 upland streams in North West England (Environment Agency 2008). The majority of sites included in the study were impacted by historic metal mining activities, although a number of control sites were also included. The trace metal contamination at the study sites was dominated by Zn, although elevated concentrations of Cd, Cu, and Pb were also found in several cases. This makes the results of this study particularly relevant when considering the effects of Zn on ecological assemblages. A detailed analysis of the speciation of trace metals at study sites indicated that Zn was predominantly present as dissolved inorganic species. The maximum metal concentrations measured were 9 $\mu g\ l^{-1}$ Cu, 19 $\mu g\ l^{-1}$ Cd, 76 $\mu g\ l^{-1}$ Ni, 160 $\mu g\ l^{-1}$ Pb, and 11,000 $\mu g\ l^{-1}$ Zn.

This study included a relatively large proportion of sites with very soft water conditions, with 15 out of 35 sites having Ca concentrations below 7 mg l^{-1}. Only four of the sites studied had a pH below six. This makes the results of this study particularly applicable when considering the effects of Zn in soft waters, although the presence of other potential pressures at the study sites makes interpretation difficult.

Free zinc ion activities, as calculated using speciation models (WHAM6 and ECOSAT), were found to be similar to dissolved zinc concentrations in virtually all cases. This indicates that much of the zinc present was likely to have been in a bioavailable form. Dissolved zinc concentrations at the study sites ranged from a mean concentration of 1.1$\mu g\ l^{-1}$ to greater than 10000ug l^{-1}.

Assemblages of benthic macroinvertebrates were assessed in terms of the ratio of their observed to expected occurrence (O/E). In order to improve the sensitivity of this metric to metals, O/E for EPT taxa (Ephemeroptera, Plecoptera, and Tricoptera), O/E for the 67 percent most sensitive taxa, and O/E for the 50 percent most sensitive taxa (not shown) were compared against O/E for total scoring taxa (see Figure 2.1)

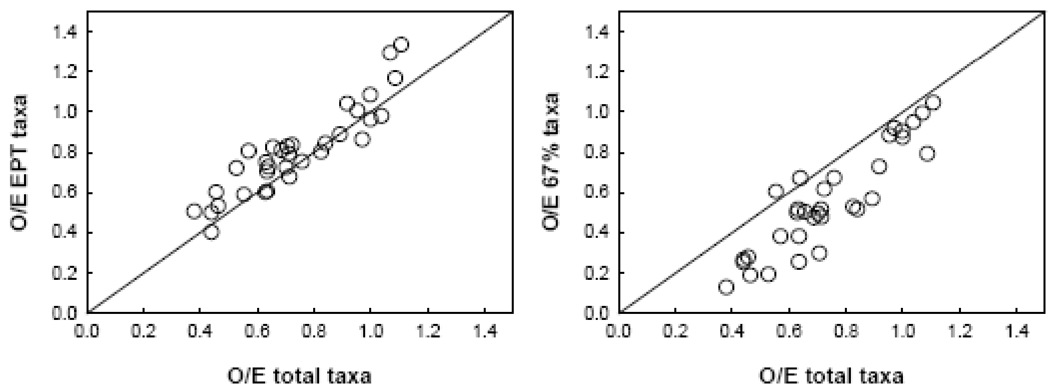

Figure 2.1 Relationship between two metrics of invertebrate communities (O/E EPT taxa, and O/E 67 percent most sensitive taxa) and O/E for total scoring taxa (from Environment Agency 2008)

EPT taxa have often been reported as being relatively sensitive to the effects of trace metals (see Kiffney and Clements 1996, Beltman *et al.* 1998, Hickey and Clements 1999), although some studies have reported relatively poor sensitivity for EPT richness metrics (Carlisle and Clements 1999). This study found EPT taxa to be slightly less sensitive than the overall community response, whereas the 67 per cent most sensitive taxa were found to be slightly more sensitive than the overall community response. Analyses were conducted on the basis of the 67 per cent most sensitive taxa.

Within this dataset, zinc was found to be relatively important when describing the response of benthic macroinvertebrates to metal concentrations. The sampling sites included in the study were also assessed using the chronic ZnBLM for *Daphnia magna*. It was only possible to apply the BLM to a limited number of the sites, because the water quality at all but 12 of the sites would not have been tolerable for *D. magna* in the absence of metal stressors. Many of the sites included in the study had a water hardness of less than 25 mg l^{-1} $CaCO_3$ or a pH below six, thus excluding application of the chronic ZnBLM.

An alternative model, the toxicity binding model (TBM), which takes account of the effects of mixtures of contaminants, was developed from the field data as part of the reported study (Environment Agency 2008) and this did not require a distinction to be made between waters of different Ca or hardness concentrations. The TBM includes the effects of Al^{3+} and H^+ along with the toxic metals Zn^{2+}, and Cu^{2+}. The application of the TBM to these data suggests that a speciation-based approach can be taken when assessing the toxicity of metals under the conditions studied. However, zinc was found to have a relatively high chemical availability at all of the study sites, although free metal ion concentrations were lower than total dissolved metal concentrations in some cases, particularly at lower total dissolved Zn concentrations (see Figure 2.2).

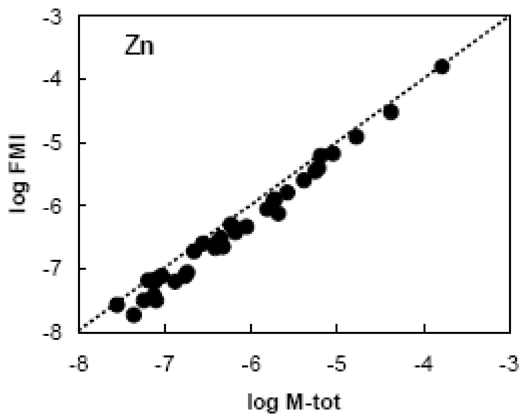

Figure 2.2 Comparison of free Zn^{2+} ion (FMI) concentrations and total dissolved Zn (tot) concentrations in mining impacted streams of Northern England (from Environment Agency 2008). The dotted line indicates a 1:1 relationship between total dissolved Zn and Zn^{2+} concentrations.

The study also considered the effects of metals on benthic diatom communities, but did not reveal any clear effect of metals on these communities. It was not possible to normalise the diatom communities to a reference condition, as was the case for benthic macroinvertebrates, and this may have limited the potential to identify any effects due to metals. Recently, efforts have been made to predict benthic diatom communities in unstressed conditions (Kelly *et al.* 2008) although so far it has only been possible to explain around 35 percent of the community variation at reference sites.

The TBM was also applied to the diatom data and a relationship was observed between the number of diatom species and the toxicity parameter, although only a limited amount of the overall variation in the number of diatom species could be explained by the effects of metals. Given that it was not possible to account for the effects of habitat factors, such as altitude, this is not unexpected. Toxicity coefficients for Zn that were applied in the model when fitting it to the invertebrate and diatom data were two and 11, respectively. This suggests that diatoms may be more sensitive than invertebrates to the effects of Zn at the sites considered in this study. This observation is broadly consistent with ecotoxicity data for Zn (European Union, 2008) which suggests that the most sensitive tested algal species are approximately twice as sensitive to the effects of zinc as the most sensitive invertebrates.

The concentrations of Zn at the sites at which diatoms were considered to be impacted by Zn ranged from 130 $\mu g\ l^{-1}$ to 2.8 mg l^{-1} (mean 765 $\mu g\ l^{-1}$, median 416 $\mu g\ l^{-1}$). The lowest Ca concentration at these sites was 4.8 mg l^{-1}. Two of the sites at which diatom diversity was not considered to be impacted by Zn had dissolved Zn concentrations of 14 and 18 $\mu g\ l^{-1}$, DOC concentrations of less than one mg l^{-1} and Ca concentrations of up to 3 mg l^{-1}. This suggests that diatoms at these sites are not as sensitive to Zn as is implied by the soft water PNEC. It is, however, possible that prolonged exposure to metals at these sites has resulted in greater tolerance in the communities relative to less stressed sites without historic metal impacts.

There are indications from this study that the approach taken in the TBM can be applied across the range of Ca concentrations covered by the study. The TBM considers the concentrations of a range of cations at sites on a hypothetical ligand, and relates these to the quality of ecological assemblages. This approach is conceptually similar to the BLM approach, suggesting that similar approaches can be applied beyond the current range of the ZnBLM. The ability of the TBM to predict effects on benthic macroinvertebrates, as a function of pH, Al, Zn, and Cu, does indicate that BLM-type principles can be applied to a wider range of conditions than are covered by the current ZnBLM. It does not, however, suggest that the current ZnBLM could be applied to bioavailability corrections under the conditions.

It does not appear to be necessary to separate sites with Ca concentrations of less than 7 mg l^{-1} from sites with Ca concentrations of greater than 7 mg l^{-1} when considering the effects of Zn on ecological systems. This does not, however, necessarily imply that the chronic ZnBLM will be adequately protective of Zn toxicity under conditions of very low Ca concentrations.

2.2 Environment Agency ecological monitoring data

A comprehensive dataset from Environment Agency monitoring of benthic macroinvertebrates, associated water quality parameters (including general water quality, sewage-related parameters, and trace metals), and perceived stresses was provided by the Environment Agency. This dataset includes a limited number of sites with very low Ca concentrations (less than 7 mg l^{-1} Ca).

In the UK the ecological quality indices (EQI) for macroinvertebrates used by environmental regulatory agencies is based on the BMWP scoring system, and may be expressed in several ways (Clarke *et al.* 2003). There are 85 BMWP scoring families and predictions of the presence or absence of particular taxa allows several metrics to be calculated. The number of scoring taxa (N-Taxa) and the total BMWP score can be calculated from River InVertebrate Prediction and Classification System (RIVPACS) software and compared with field observations. A further metric, the average score per taxon (ASPT), may also be calculated from these metrics as the total BMWP score divided by N-Taxa.

The BMWP scoring system is principally concerned with the sensitivity of organisms to organic pollution from sewage. The commonly used metrics cannot, therefore, be assumed to be sensitive to any particular chemical toxicants and they may have low sensitivity if low-scoring species are more sensitive to a particular toxicant than high-scoring species. Conversely, if high-scoring species are sensitive to the chemical toxicant of interest these metrics may be very sensitive to chemical pollution. It follows that metrics such as ASPT should not be used unless there is evidence to suggest that high-scoring taxa are more sensitive than lower scoring taxa. This is because ASPT may *increase* in response to pollution when low-scoring taxa are more sensitive than high-scoring taxa to the chemical toxicant.

Observations of the presence or absence of benthic macroinvertebrates can be rather sensitive to the sampling effort, which can result in variability in the resulting EQI scores. Of the three EQI metrics ASPT is the least sensitive to variations in sampling effort, whereas BMWP and N-Taxa are more sensitive to this source of variation. The cut-off values for very good quality are 1.00 and 0.85 for EQI ASPT and EQI N-Taxa respectively, and the cut-off values for good quality are 0.90 and 0.70 for EQI ASPT and EQI N-Taxa respectively.

The dataset was initially assessed to determine whether it was possible to identify any decline in the quality of benthic macroinvertebrate assemblages as a result of exposure to Zn. Samples with matched data for dissolved Zn, pH, Ca and dissolved Fe were identified. Dissolved iron was used as an indicator of likely DOC concentrations in the absence of any other information on actual DOC concentrations, following observations of a relatively strong correlation between these two parameters in several UK datasets (see Figure 2.3).

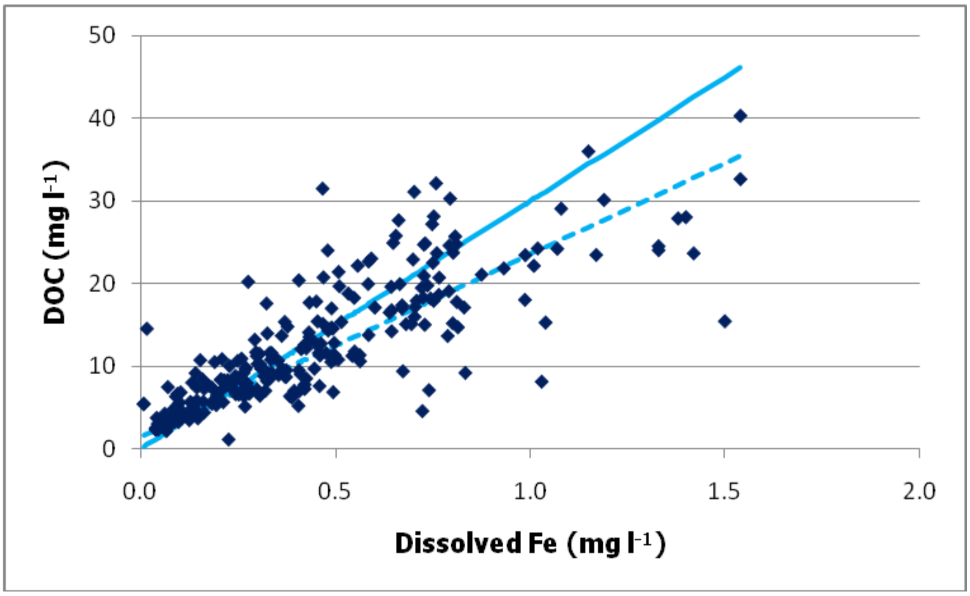

Figure 2.3 DOC and dissolved iron monitoring data from South West and North Scotland. Blue lines indicate the relationship obtained from a separate dataset assuming a non-zero intercept (dashed line) or a zero intercept (solid line).

Ecological quality indices for benthic macroinvertebrate communities were compared with predicted risk characterisation ratios for Zn calculated using the ZnBLM Version 4 (see Figures 2.5 to 2.7). Predictions of ecological quality under unimpacted conditions were calculated using RIVPACS software. Three EQI metrics, BMWP, N-Taxa, and

ASPT, were assessed against predicted risk characterisation ratios for Zn. Sites at which the soft water PNEC would need to be applied were excluded from this analysis, which was based on risk characterisation ratios (RCR), because relatively large risks are commonly predicted for soft waters due to the very low $PNEC_{add}$ value of 3.2 $\mu g\ l^{-1}$.

Matched data for the Zn RCR and ecological metrics, expressed as EQI values, were analysed by quantile regression to assess whether estimates of Zn RCR values are protective of the benthic invertebrate community. The quantile regression approach considers a limiting function and is particularly useful where it is not possible to remove all of the potentially confounding factors; it has been applied to the concept of limiting factors as constraints on organisms (Scharf *et al.* 1998, Cade *et al.* 1999, Cade and Noon 2003). The approach taken in this study was similar to that applied in previous assessments of field data (Pacheco *et al.* 2005, Linton *et al.* 2007, and Crane *et al.* 2007). An advantage of statistical techniques such as quantile regression is that they do not require the datasets to be screened for potentially interfering pressures.

Quantile regression was applied to the datasets using a log linear model (log(response) = *a* + *b*.(exposure)) using R (Version 2.9.0; http://cran.r-project.org). The quantile regression was applied to the 90th, 95th and 99th quantiles of the datasets, although in some cases the model could not be fitted to all of the quantiles assessed. Higher quantiles may be more susceptible to outliers in the data, although both 90th quantiles (Linton *et al.* 2007) and 99th quantiles (Crane *et al.* 2007) have both been successfully applied previously.

The principle of the quantile regression approach to derive a limiting function is shown in Figure 2.4. Data with unimpacted responses at low exposures effectively serve as controls, and impacted data at low exposures are effectively not considered in the analysis. A decline in the maximum response with increasing exposure is assumed to be due to the contaminant of interest.

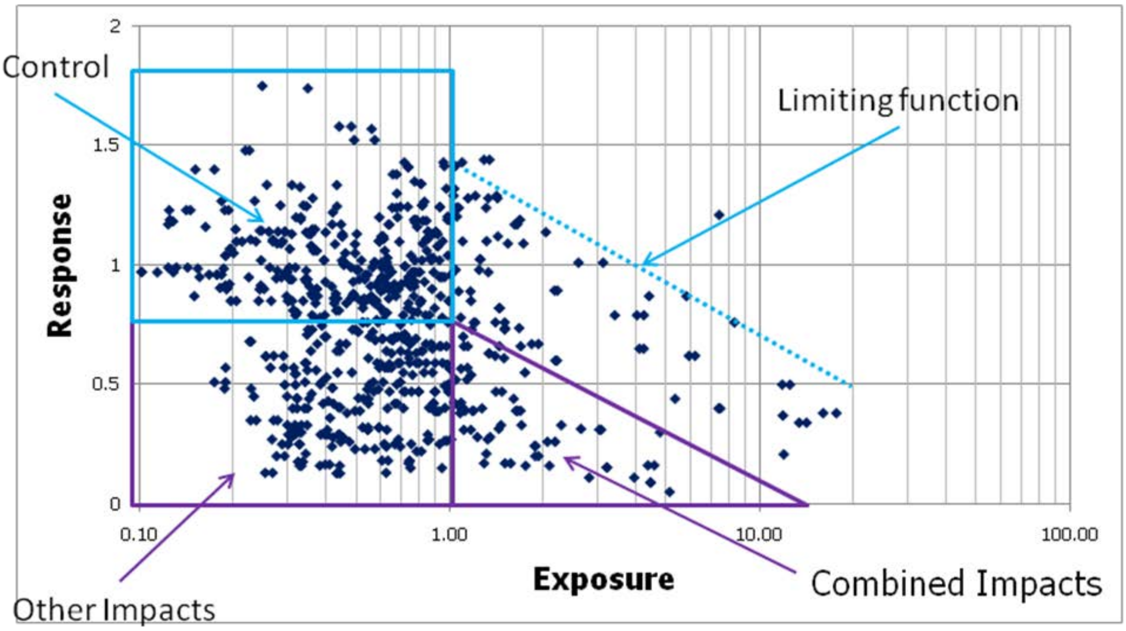

Figure 2.4 Interpretation of combined chemical and ecological field data

Assessments using EQI ASPT as the response metric did not show a major decline with increasing Zn risk characterisation ratios, suggesting that this metric is insensitive to the effects of Zn (Figure 2.5). High EQI scores of greater than one were observed at five out of nine sites with risk characterisation ratios of between 19 and 103 (equivalent to “bioavailable Zn” concentrations of between 148 and 803 $\mu g\ l^{-1}$).

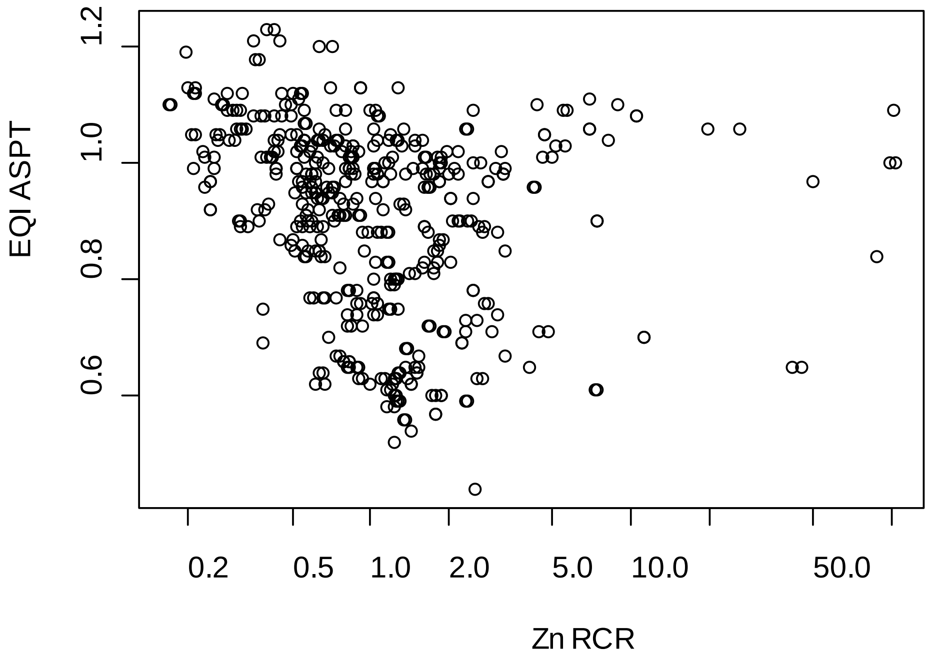

Figure 2.5 Ecological quality (EQI ASPT) as a function of predicted risk characterisation ratios for Zn (Zn RCR)

Assessments using EQI BMWP and EQI N-Taxa both showed a notable decline with increasing Zn risk characterisation ratios ($p<0.001$, and $p=0.001$ respectively). These data were assessed using quantile regression analysis, a technique suitable for assessing datasets where a number of potentially limiting factors cannot be taken into account. In this case the limiting factors are toxic pressures due to other contaminants.

The results of the quantile regression analysis for EQI BMWP and EQI N-Taxa are shown in Figures 2.6 and 2.7, respectively, along with the original data. Whilst a decline in ecological quality with increasing risk characterisation ratios for Zn is apparent, it is relatively slight and does not appear to occur until relatively high risk characterisation ratios are reached. The Zn risk characterisation ratios at which the 99th quantile declines by 10 percent were determined (referred to as the $EC10_{99\%ile}$). The Zn risk characterisation ratios relating to the $EC10_{99\%ile}$ were 14.1 (95% confidence interval 8.8 to 35.7) on the basis of EQI BMWP, and 16.6 (95% confidence interval 10.6 to 38.3) on the basis of EQI N-Taxa. These values relate to “bioavailable Zn” concentrations of 110 and 130 $\mu g\ l^{-1}$ respectively.

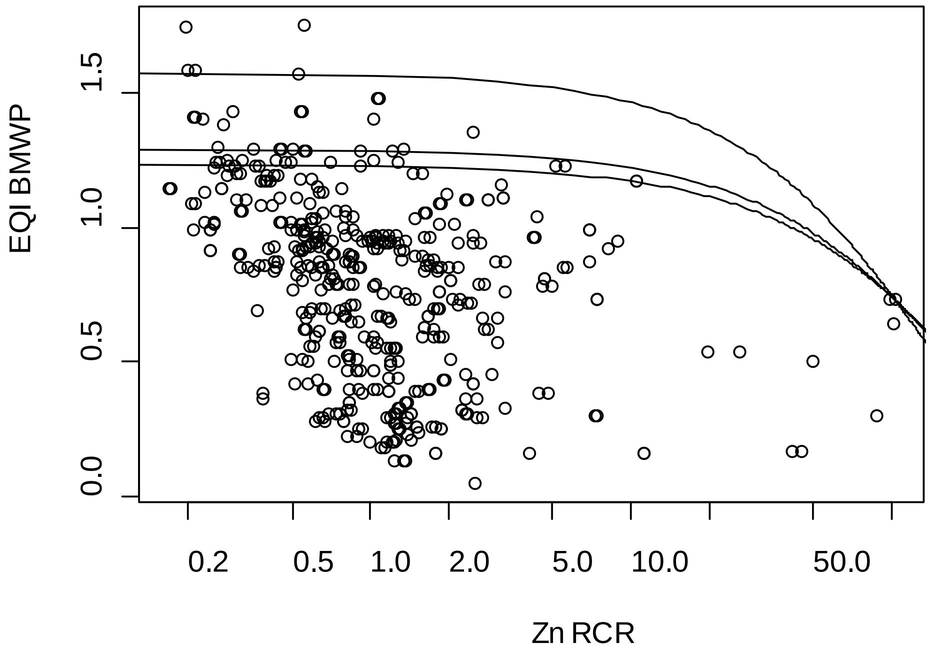

Figure 2.6 Ecological quality (EQI BMWP) as a function of predicted risk characterisation ratios for Zn (Zn RCR). Fitted lines show the quantile regression analysis for the 90th, 95th, and 99th quantiles of the dataset.

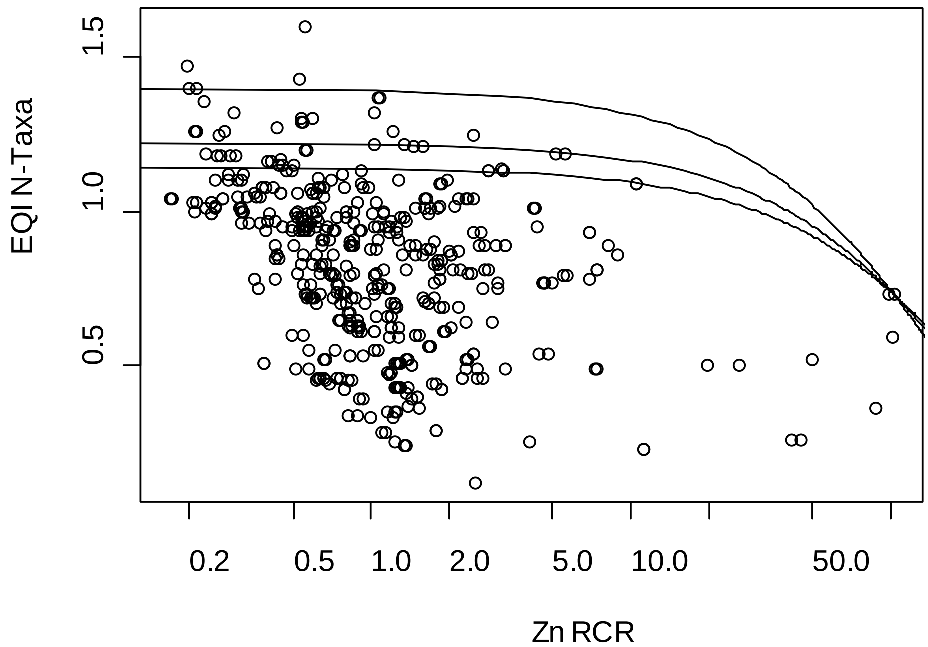

Figure 2.7 Ecological quality (EQI N-Taxa) as a function of predicted risk characterisation ratios for Zn (Zn RCR). Fitted lines show the quantile regression analysis for the 90th, 95th, and 99th quantiles of the dataset.

These data suggest that the benthic macroinvertebrate indices considered above are relatively insensitive to adverse effects from Zn, although a decline in the maximum achievable quality (relative to predicted reference conditions) decreases at very high predicted risk characterisation ratios for Zn.

There is uncertainty in the concentrations of DOC used in the calculation of Zn bioavailability. Bioavailable Zn concentrations were calculated for 112 samples from England and Wales using DOC information which was measured or estimated from dissolved iron concentrations. A comparison of the results is shown in Figure 2.8 which suggests that bioavailable Zn concentrations predicted using estimates of DOC concentrations, which are based on dissolved iron, are generally within a factor of two of the true result.

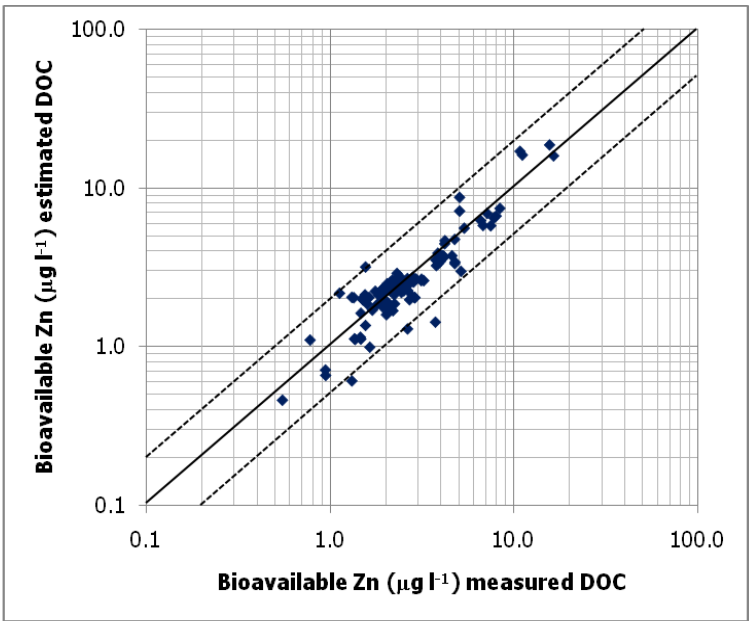

Figure 2.8 Comparison of predicted bioavailable Zn concentrations, calculated using measured or estimated DOC concentrations for 112 data points from England and Wales

EQI metrics can also be compared against Ca concentrations; Figures 2.9 and 2.10 show EQI values at 841 monitoring sites with Ca concentrations ranging from 1.2 to 264 mg l^{-1}, and Zn concentrations ranging from 2.5 $\mu g\ l^{-1}$ to 1.5 mg l^{-1}.

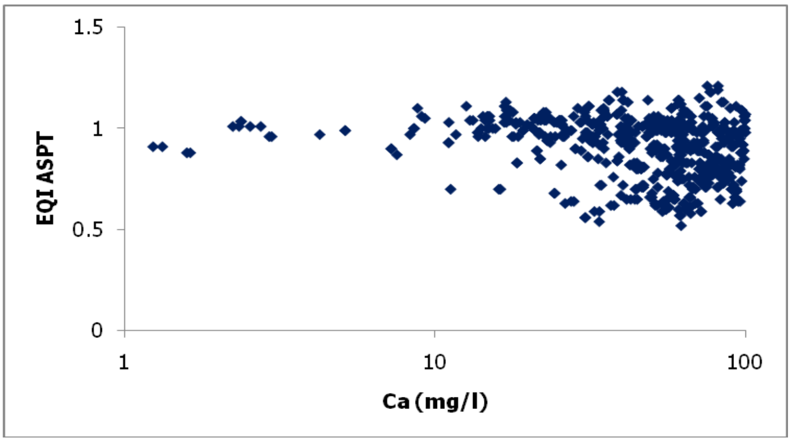

Figure 2.9 Ecological quality (EQI ASPT) as a function of Ca concentrations

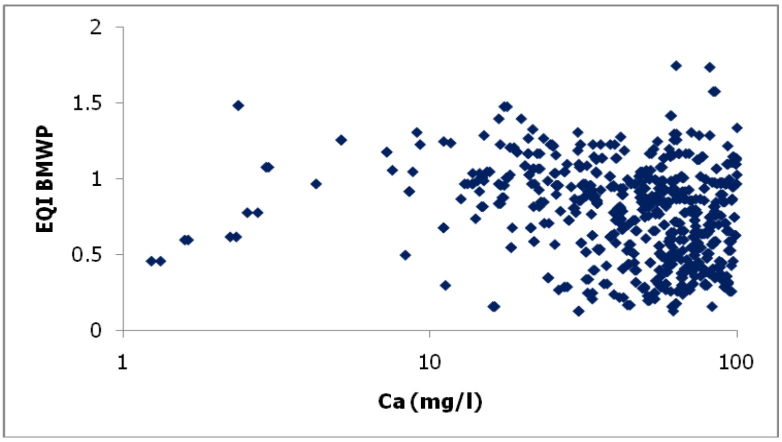

Figure 2.10 Ecological quality (EQI BMWP) as a function of Ca concentrations

There are relatively few samples available for very low Ca concentrations where the soft water PNEC for Zn may need to be applied. Fifteen samples have Ca concentrations of less than 7 mg l^{-1}, and 24 samples have Ca concentrations of less than 10 mg l^{-1}. There is, however, a slight tendency for EQI values to be less than the threshold for good quality (0.7 for EQI N-Taxa) at very low Ca concentrations (Figure 2.11), and consideration of possible causes for this apparent deterioration is warranted.

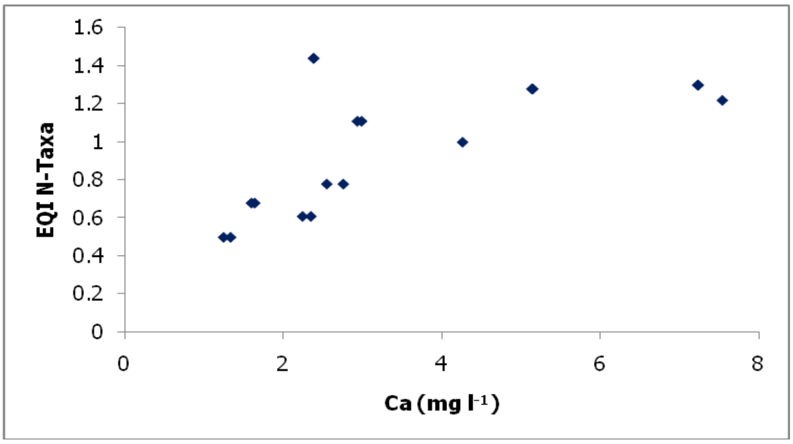

Figure 2.11 Ecological quality (EQI N-Taxa) as a function of Ca concentrations for samples with very low Ca concentrations

Whilst there is an apparent decline in EQI values at very low Ca concentrations of less than 7.5 mg l^{-1} (Figure 2.10) there is no apparent relationship between dissolved Zn concentrations and EQI values in the same data (Figure 2.12). These data may therefore be reflecting pressures other than Zn. One site shows an EQI N-Taxa value of 1.4 at a Ca concentration of less than 3 mg l^{-1}.

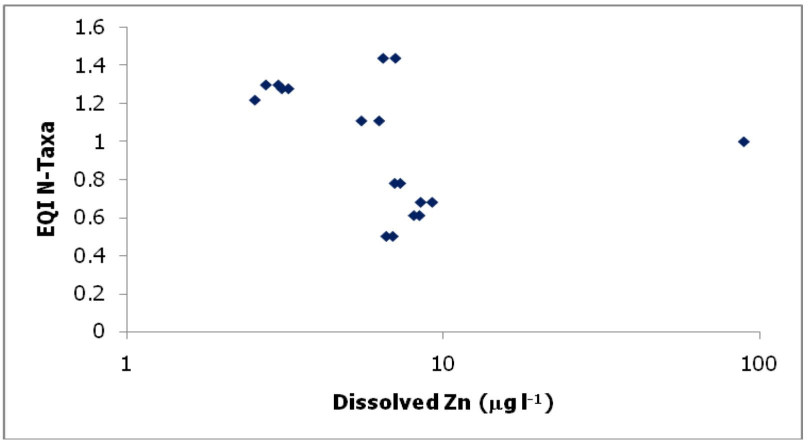

Figure 2.12 Ecological quality (EQI N-Taxa) as a function of dissolved Zn concentrations for samples with very low Ca concentrations

Inspection of the sites with very low Ca concentrations which show impacts on the benthic invertebrate communities reveals that there are only two sampling sites, both of which have been sampled on four occasions. Additional information on perceived stressors at the sites is also recorded in the database. Both sites are perceived to be stressed by acid conditions. One of them is also expected to be affected by flow-related pressures and the other by natural effects. Thus, benthic macroinvertebrates are not likely to be impacted in an observable way by exceedances of the PNEC for soft waters, up to approximately 10 $\mu g\ l^{-1}$ dissolved Zn. EQI N-Taxa values of as high as 1.4 are observed at Zn concentrations close to this level; whilst some sites show an impact at similar Zn concentrations, these impacts may not be due to Zn toxicity.

To assess the potential effect of Ca on Zn toxicity, an assessment was made of the slopes of 90th quantile regression functions. These estimate the maximum EQI BMWP as a function of the dissolved Zn concentration for datasets with progressively reducing maximum Ca concentrations. The results in Table 2.1 appear to indicate that the slope steepens as data for higher Ca concentrations are progressively removed from the dataset. A steeper slope of the regression function may be interpreted as representing an increased sensitivity to Zn, as the maximum EQI BMWP score reduces more rapidly with increasing dissolved Zn concentrations.

Table 2.1 Analysis of the slopes of 90th quantile regressions of EQI BMWP as a function of dissolved Zn concentrations ($\mu g\ l^{-1}$)

Dataset	Number of samples	90th Quantile slope	Standard error
All data	1,928	-0.00038	0.00009
Ca < 100 mg l^{-1}	1,457	-0.00028	0.00008
Ca < 50 mg l^{-1}	679	-0.00051	0.00004
Ca < 25 mg l^{-1}	310	-0.00075	0.00034
Ca < 12.5 mg l^{-1}	111	-0.00110	0.00100
Ca < 7 mg l^{-1}	42	-0.00128	0.00275

This analysis is limited by the fact that far fewer data are available for low Ca levels than for higher Ca concentrations, and whilst the slopes of the quantile regression functions appear to increase (become more negative), the slopes of the two smallest datasets (with Ca concentrations below 12.5 mg l^{-1} and 7 mg l^{-1}) were not found to be statistically significant. Furthermore, the standard errors of the slopes, which were estimated by bootstrapping, increase considerably as the size of the dataset is reduced. It is not possible, therefore, on the basis of this analysis to identify a real difference in the slopes of the different quantile regression functions between EQI BMWP and dissolved Zn over different ranges of Ca concentrations.

The dataset for Ca concentrations below 7 mg l^{-1} (n = 42) was compared to a similar dataset of 42 observations for concentrations between 7 and 11.2 mg l^{-1}. These two datasets represent situations which would be covered by the soft water PNEC in the first instance, and data which represent the lower limit of applicability of the ZnBLM with respect to Ca concentrations. A lower quantile (66th quantile) was assessed in this case due to the limited data available. This analysis initially appears to suggest that the slope of the regression at lower Ca concentrations is less steep than the slope at higher Ca concentrations. Both slopes were significant (at the 90 per cent confidence level for low Ca concentrations and at the 99.9 per cent confidence level for the higher Ca concentrations). A more detailed comparison of the slopes, however, reveals that they are not significantly different from each other. The slopes and standard errors (estimated by bootstrapping) are shown in Table 2.2 for the two datasets.

Table 2.2 Analysis of the slopes of 66th quantile regressions of EQI BMWP as a function of dissolved Zn concentrations (μg l^{-1})

Dataset	66th Quantile slope	Standard error
Ca < 7 mg l^{-1}	-0.149	0.088
Ca 7 to11 mg l^{-1}	-0.236	0.050

These analyses suggest that there is no evidence to support a distinction in the approaches taken towards the assessment of Zn toxicity above and below a Ca concentration of 7 mg l^{-1}. These assessments are limited in that they are based solely on benthic macroinvertebrate communities, which appear to be relatively insensitive to the effects of Zn. The assessments are also limited by the relatively small number of ecological monitoring sites with low Ca concentrations (below 7 mg l^{-1}) and reported Zn exposure (roughly two per cent of samples assessed here had Ca concentrations of less than 7 mg l^{-1}). A further limitation of these assessments is that changes to Zn bioavailability due to DOC binding is not taken into account. Changes to the slope of the quantile regression could result from differences in bioavailability where no correction for Zn bioavailability has been made. Estimates of Zn binding to DOC under soft water conditions suggest that relatively high DOC concentrations would be required to reduce Zn bioavailability substantially (see Section 4.2).

These assessments have considered only the community composition, in terms of BMWP score and the number of BMWP scoring taxa present. They have not considered the sensitivity of any of the individual taxa present, although some taxa may be more sensitive than others to the effects of Zn. The results do, however, suggest that benthic macroinvertebrate communities are relatively tolerant of Zn exposure, although information under very low Ca conditions is limited.

Observations that algae and other primary producers may be more sensitive than invertebrates to the effects of Zn suggest that assessments based on the quality of benthic invertebrate assemblages may be limited in their ability to identify potential impacts of Zn. The results of the Environment Agency study of mining-impacted streams in Northern England suggests that diatoms (see Section 2.1) may be more sensitive than benthic macroinvertebrates to the effects of Zn, and a more detailed consideration of the response of benthic diatoms to Zn exposure may provide valuable additional evidence of the effects of Zn on aquatic ecosystems. Primary producers appear to be relatively sensitive to the effects of Zn, although it is not clear whether they are especially sensitive under very soft water conditions. The development of a

ZnBLM for algae (Heijerick *et al.* 2002) suggests they are likely to be especially sensitive under high pH conditions, which are not typical of very soft waters.

3 Review of laboratory studies

3.1 Soft waters testing under the Existing Substances Regulations

A series of ecotoxicity tests was conducted to assess whether the generic PNEC derived within the Existing Substances Regulations (ESR) risk assessment of Zn would be adequately protective of soft water ecosystems (with hardness concentrations of less than 25 mg l^{-1} $CaCO_3$, or Ca concentrations of less than 7 mg l^{-1}). The study employed chronic ecotoxicity tests on an alga (*Pseudokirchneriella subcapitata*), a daphnid (*Daphnia longispina*) and a fish (*Salmo trutta*) in two natural soft waters, and also in the same waters with their hardness adjusted to 100 mg l^{-1} $CaCO_3$ (European Union, 2008). The two natural waters tested had hardness concentrations of 8.0 and 6.1 mg l^{-1} $CaCO_3$ for the algal and daphnid tests and 8.6 and 6.7 mg l^{-1} $CaCO_3$ for the fish tests. The pH of these waters was 6.7 and 6.4, respectively, and was unaffected by the modification of the waters to increase the hardness.

All three tested species showed relatively similar sensitivity to Zn in the tests in natural waters, with NOEC values between 42 $\mu g\ l^{-1}$ (daphnid) and 86 $\mu g\ l^{-1}$ (algae). There was much greater variability in the sensitivity of these species in the hardness-adjusted waters, with NOEC values varying between 57 $\mu g\ l^{-1}$ and 250 $\mu g\ l^{-1}$. The lowest NOEC from the hardness adjusted waters (57 $\mu g\ l^{-1}$ for fish) was similar to the result for the test on the same species in the unadjusted natural water (61 $\mu g\ l^{-1}$).

Whilst the tests did suggest sensitivity differences between moderately hard and very soft waters for the tested species, the differences were not consistent for the two waters. Much larger differences in sensitivity were observed for fish and daphnids in the lower pH water than in the higher pH water, with both species generally being much less sensitive in the harder waters than in the unadjusted waters. For algae, the higher pH water showed the greatest relative difference between the harder waters and the unadjusted waters, although this was less than a factor of two.

The results from the soft water testing programme did not produce any NOEC values which were lower than those for the same or similar species used in the derivation of the generic PNEC. The lowest NOEC from the testing programme is actually 13.5 times higher than the proposed $PNEC_{add}$ for soft waters of 3.1 $\mu g\ l^{-1}$.

Inspection of the toxicity data for *Pseudokirchneriella subcapitata* which were used to derive the generic PNEC suggests that algae may indeed be relatively sensitive to the effects of Zn, but that the sensitivity of this species tends to increase with increasing pH. This suggests that it is unlikely that algal sensitivity to Zn will be greatly increased by low water hardness conditions. This is in contrast to the observation from field studies (Environment Agency 2008) that diatoms may be more sensitive than invertebrates to the effects of Zn in low hardness upland streams which have been impacted by historic mining activities (see also Section 2.1).

3.2 Acute BLM for Zn toxicity to *Daphnia pulex* in soft waters

A recent study reported the development of an acute BLM to explain the effects of Zn on *Daphnia pulex* in soft waters (Clifford *et al.* 2009). Calcium concentrations between 2.6 and 97 mg l^{-1} were employed in univariate testing and a consistent effect of Ca on Zn toxicity was observed over this range. It was, however, noted that previous acute BLM for *Daphnia magna* developed for harder waters tended to underestimate the acute toxicity of Zn to *D. pulex* under low hardness conditions. This may indicate that the chronic BLM developed for *D. magna* may not necessarily be protective of similar species under low hardness conditions, although this is speculative.

Only the lowest of the tested Ca concentrations from this study was below the validation range of the chronic ZnBLM, and a linear relationship between Ca^{2+} activity and the EC50, expressed in terms of Zn^{2+} activity, was observed. The slope of the relationship between EC50(Zn^{2+}) and Ca^{2+} was 17 μmol per mmol, with an intercept of 1.01 μM Zn^{2+}. The intercept of the relationship can be interpreted as the EC50 at a Ca concentration of zero. A value of 1.01 μM Zn is equivalent to 66 μg l^{-1}, although this is not directly comparable to the Zn^{2+} activity except under conditions where Zn is almost entirely present as the free Zn ion (Zn^{2+}).

Zn toxicity, when expressed on the basis of the free Zn^{2+} ion concentrations, was not appreciably affected by DOC concentrations, suggesting that DOC acts to reduce the free Zn^{2+} ion concentrations, with a consequent effect on bioavailability of Zn. The experiments including DOC were, however, conducted at a Ca concentration of 6.8 mg l^{-1}, and a pH of 7.85.

This study shows that the BLM principles can be applied to soft water situations for acute Zn toxicity to *D pulex*, although it is not clear whether a similar situation would apply to chronic toxicity. There may be slight differences in the mechanisms of toxicity between acute and chronic exposures. Acute BLMs for Zn toxicity to *Daphnia* do not need to consider competition from protons in calculating metal concentrations on the biotic ligand (Clifford 2009, De Schamphelare *et al.* 2005), whereas the chronic Zn BLM for *Daphnia* takes account of proton competition (De Schamphelare *et al.* 2005).

4 Predictions of Zn behaviour in soft acid waters

4.1 ZnBLM calculations

The ZnBLM (Version 4b) was used to calculate "bioavailable Zn" concentrations for relatively soft, acid waters with pH values between 6 and 7, Ca concentrations between 5 and 10 mg l^{-1}, and DOC concentrations between 1 and 3 mg l^{-1}. Zinc bioavailability is predicted to be greatest for invertebrates and fish under these conditions, with invertebrates tending to have higher BioF (bioavailability factor) values at pH values below 6.5 and fish having higher BioF values at pH values above 6.5. The bioavailability correction applied by the ZnBLM does not, however, predict the relative sensitivity of the different taxa, but simply indicates the trophic level for which the bioavailability correction is least important. $BioF_{max}$ values (the BioF value for the species for which bioavailability has the least effect under the conditions) were predicted to be greater than one for all of the conditions considered here. This indicates that bioavailability under these conditions may be slightly greater than that under the conditions of the generic PNEC. Maximum bioavailability was predicted at neutral pH (pH 6.8 to 7.0) with a Ca concentration of 5 mg l^{-1} and a DOC content of 2.0 mg l^{-1} (Figure 4.1). This may indicate that under some conditions, bioavailability is greater than the high bioavailability conditions employed in the tests used to derive the generic PNEC. The use of an assessment factor of two in deriving the PNEC from the HC5 is, however, likely to ensure protection of these sensitive conditions.

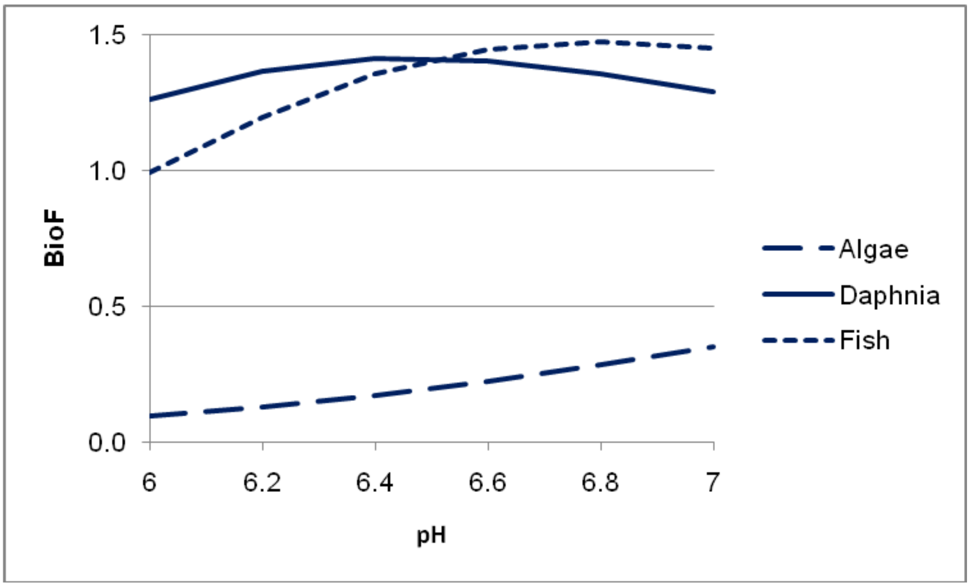

Figure 4.1 Predicted BioF values for algae, invertebrates and fish as a function of pH, at 5 mg l^{-1} Ca and 2 mg l^{-1} DOC

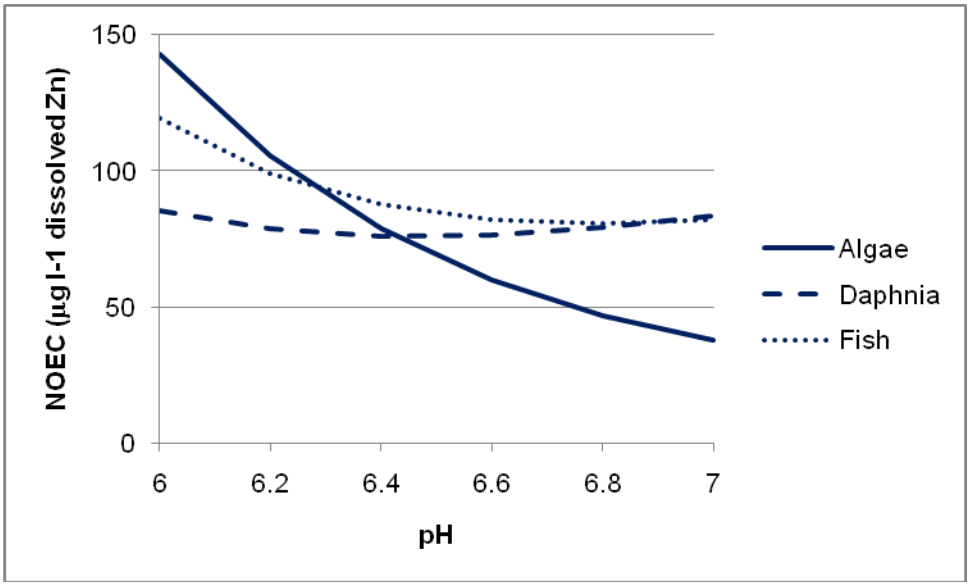

Figure 4.2 Predicted NOEC values for algae, invertebrates and fish as a function of pH, at 5 mg l^{-1} Ca and 2 mg l^{-1} DOC

NOEC values for algae, invertebrates and fish predicted by the ZnBLM show a rather different situation, because these take account of the relative sensitivity of the different taxonomic groups to Zn toxicity (Figure 4.2). Invertebrates are predicted to be the most sensitive trophic level at low pH, but algae are predicted to be much more sensitive than *Daphnia* or fish at pH values above 6.4 due to increasing bioavailability with increasing pH, and the higher inherent sensitivity of the tested species.

During development of the algal BLM for Zn (Heijerick *et al*. 2002) the test species (*Pseudokirchneriella subcapitata*) was found to be most tolerant of Zn under low pH conditions (pH 5.6 and 6.2). A protective effect was observed for both Ca and Mg, although tests on the effect of Ca and Mg were undertaken at a higher pH of 7.5. It was not possible to assume the presence of a single “biotic ligand” on the surfaces of algal cells due to the differences in response observed above and below pH 7.

The ZnBLM does not make bioavailability predictions for conditions which are outside its validation boundaries. BLM development is limited to some extent by the selection of species for which the models are developed, and their ability to tolerate a wide range of water quality conditions during chronic ecotoxicity tests. The composition of natural ecosystems may vary under different water quality conditions, for example between chalk streams with hard waters and soft, acid upland streams. It is not possible to cover the entire range of conditions with an individual species and there are therefore uncertainties about bioavailability outside these conditions.

4.2 WHAM calculations

Calculations in this section were undertaken using WHAM 6 (Version 6.0.13). The ZnBLM was developed from WHAM Model V, although this is unlikely to greatly influence the results. WHAM 6 is based on an increased set of Zn fulvic acid binding data, and has been reported to generate predictions consistent with measured data (Meylan *et al.* 2004). In order to take account of possible competition for binding to DOC between Zn and other metal ions which may be present in surface waters both iron and aluminium were assumed to be present, with their activities in solution controlled by the solubility of hydroxide precipitates. Log_{10} K_S values of 5.7 and 8.5 were used for iron and aluminium respectively (Webber *et al.* 2006, Environment Agency 2008). Zn binding to DOC was calculated over the pH range 4 to 7, at a Ca concentration of 3 mg l^{-1}, and at DOC concentrations of 0.5, 1, 2, and 4 mg l^{-1} (Figure 4.3). DOC has a rather limited effect on Zn availability below pH 5, due to increased competition from protons (H+). The effect is greater at higher pH values, and increases linearly with increasing DOC concentrations.

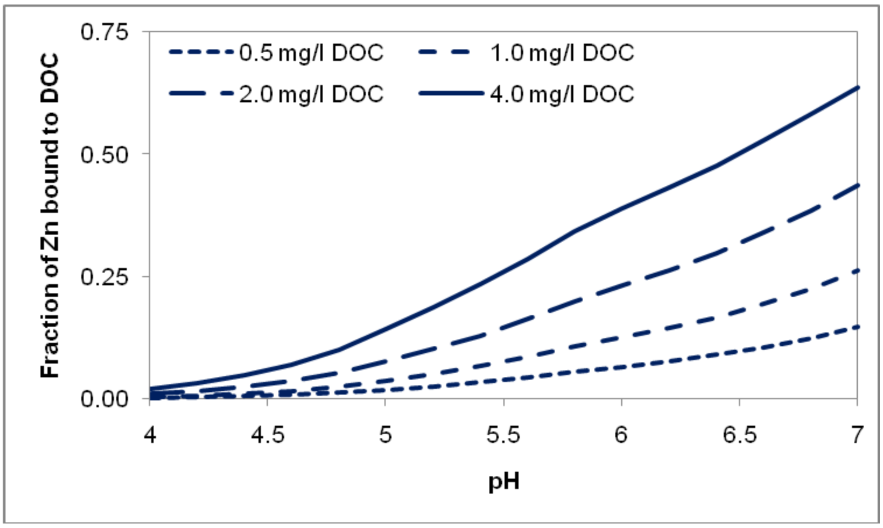

Figure 4.3 Fraction of total dissolved Zn predicted to be bound to DOC as a function of pH, at four different DOC concentrations between 0.5 and 4.0 mg l^{-1}

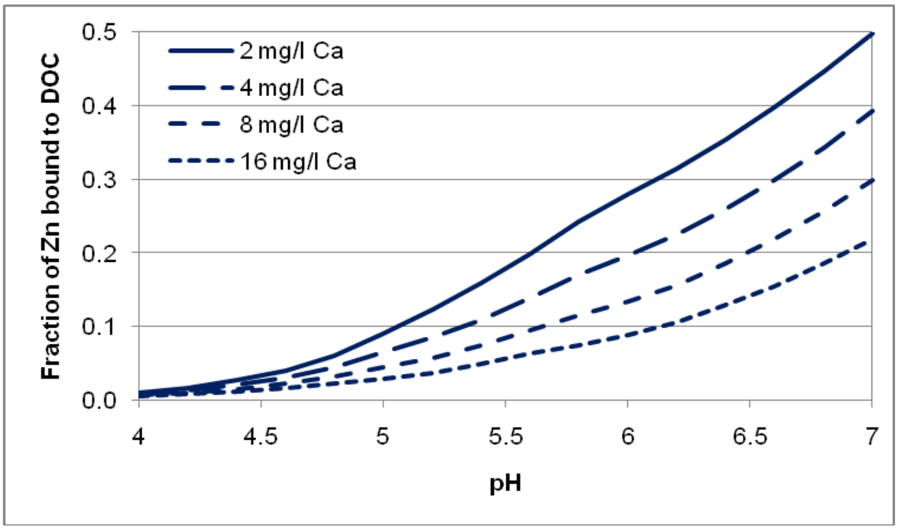

Figure 4.4 Fraction of total dissolved Zn predicted to be bound to DOC as a function of pH, at four different Ca concentrations between 2 and 16 mg l^{-1}

The Ca concentration can also have an effect on Zn binding to DOC, through reduced competition for binding sites, and also through an increase in electrostatic effects which result from the reduced solution ionic strength. The effect of this on Zn binding to DOC is shown in Figure 4.4 at a DOC concentration of 2 mg l^{-1} over a range of Ca concentrations from 2 mg l^{-1} to 16 mg l^{-1}. The result of this effect is that Zn binding to DOC is greater in waters with low Ca concentrations, under the same DOC and pH.

It may be possible to apply a simplified form of bioavailability correction to soft, acid waters by calculating the chemical availability of Zn under the site conditions, using a chemical speciation model such as WHAM 6. Applying such a correction would be based on the assumption that the chemical availability of Zn-DOC complexes is similar under lower and higher pH conditions, and during chronic exposures. Testing of this assumption may be limited by the need to test species which are compatible with the water quality conditions of low pH and very low Ca concentrations. It may also be necessary to assume that Zn has a greater intrinsic toxicity under lower Ca conditions than at higher concentrations, due to the diminished protective effect at lower concentrations (see Clifford *et al*. 2009).

Such a bioavailability correction could be implemented through the use of chemical speciation calculations, or through a set of look-up tables based on such calculations. Tables 4.1 to 4.4 are examples of look-up tables which give the predicted inorganic fraction of dissolved Zn for different combinations of pH, DOC and Ca conditions. These bioavailability corrections could be applied to dissolved Zn measurements to correct for reduced bioavailability as a result of DOC complexation of Zn according to Equation 1. The bioavailable zinc concentration is then compared against the generic PNEC in order to perform the compliance assessment.

$[Zn]_{dissolved}$ x Bioavailability Coefficient = $[Zn]_{bioavailable}$

Table 4.1 Look-up table for Zn availability correction coefficients under low pH and low Ca conditions (1 mg l^{-1} Ca table)

pH	0.2 mg l^{-1} DOC	1 mg l^{-1} DOC	3 mg l^{-1} DOC	5 mg l^{-1} DOC	10 mg l^{-1} DOC
5	0.99	0.94	0.83	0.73	0.54
5.1	0.98	0.93	0.80	0.69	0.49
5.2	0.98	0.91	0.76	0.64	0.43
5.3	0.98	0.90	0.73	0.60	0.63
5.4	0.98	0.88	0.70	0.56	0.60
5.5	0.97	0.87	0.67	0.53	0.56
5.6	0.97	0.85	0.64	0.49	0.53
5.7	0.97	0.84	0.61	0.46	0.50
5.8	0.96	0.83	0.58	0.43	0.46
5.9	0.96	0.81	0.56	0.40	0.43
6	0.96	0.80	0.53	0.37	0.40

The bioavailability coefficient for Zn at pH 5.5 and in waters with 1 mg l^{-1} Ca and 3 mg l^{-1} DOC can be read from Table 4.1 as 0.67. If the measured dissolved Zn concentration is 10 mg l^{-1} then the bioavailable Zn concentration can be calculated (according to the equation above) and compared against the generic PNEC of 7.8 μg l^{-1}. This would result in a bioavailable Zn concentration of 6.7 μg l^{-1}, which is lower than the generic PNEC, indicating no concern for these conditions.

Table 4.2 Look-up table for Zn availability correction coefficients under low pH and low Ca conditions (3 mg l^{-1} Ca table)

pH	0.2 mg l^{-1} DOC	1 mg l^{-1} DOC	3 mg l^{-1} DOC	5 mg l^{-1} DOC	10 mg l^{-1} DOC
5	0.99	0.96	0.90	0.84	0.71
5.1	0.99	0.96	0.88	0.82	0.68
5.2	0.99	0.95	0.87	0.80	0.65
5.3	0.99	0.95	0.85	0.78	0.79
5.4	0.99	0.94	0.84	0.76	0.77
5.5	0.98	0.93	0.83	0.74	0.74
5.6	0.98	0.93	0.82	0.72	0.72
5.7	0.98	0.92	0.81	0.71	0.70
5.8	0.98	0.92	0.79	0.69	0.68
5.9	0.98	0.91	0.78	0.68	0.65
6	0.98	0.91	0.77	0.66	0.63

Table 4.3 Look-up table for Zn availability correction coefficients under low pH and low Ca conditions (5 mg l^{-1} Ca table)

pH	0.2 mg l^{-1} DOC	1 mg l^{-1} DOC	3 mg l^{-1} DOC	5 mg l^{-1} DOC	10 mg l^{-1} DOC
5	0.99	0.97	0.93	0.88	0.78
5.1	0.99	0.97	0.92	0.87	0.76
5.2	0.99	0.97	0.91	0.86	0.74
5.3	0.99	0.96	0.90	0.84	0.84
5.4	0.99	0.96	0.89	0.83	0.83
5.5	0.99	0.96	0.89	0.82	0.81
5.6	0.99	0.96	0.88	0.81	0.79
5.7	0.99	0.95	0.87	0.80	0.78
5.8	0.99	0.95	0.87	0.79	0.76
5.9	0.99	0.95	0.86	0.78	0.74
6	0.99	0.95	0.85	0.77	0.73

Table 4.4 Look-up table for Zn availability correction coefficients under low pH and low Ca conditions (7 mg l^{-1} Ca table)

pH	0.2 mg l^{-1} DOC	1 mg l^{-1} DOC	3 mg l^{-1} DOC	5 mg l^{-1} DOC	10 mg l^{-1} DOC
5	1.00	0.98	0.94	0.90	0.81
5.1	0.99	0.98	0.93	0.89	0.80
5.2	0.99	0.97	0.92	0.88	0.78
5.3	0.99	0.97	0.92	0.87	0.87
5.4	0.99	0.97	0.91	0.86	0.86
5.5	0.99	0.97	0.91	0.85	0.84
5.6	0.99	0.96	0.90	0.84	0.83
5.7	0.99	0.96	0.89	0.83	0.81
5.8	0.99	0.96	0.89	0.82	0.80
5.9	0.99	0.96	0.88	0.82	0.79
6	0.99	0.95	0.88	0.81	0.77

The development of ZnBLM for daphnia (Heijerick *et al.* 2005) and algae (Heijerick *et al.* 2002) investigated the protective effect of Ca on Zn toxicity and included an extrapolation of effects to a hypothetical zero concentration of Ca. The estimated Zn toxicity in the absence of Ca (although in the presence of several other major ions) was approximately 60 $\mu g\ l^{-1}$ for *Daphnia* and approximately 6 $\mu g\ l^{-1}$ for algae. In considering possible bioavailability corrections for Zn toxicity the use of a lower generic PNEC of 6 $\mu g\ l^{-1}$ may need to be considered, rather than the generic PNEC of 7.8 $\mu g\ l^{-1}$ derived in the ESR risk assessment (European Union, 2008).

The generic PNEC was, however, derived by applying an assessment factor of two to the HC5 from the species sensitivity distribution and this may, therefore, be adequately protective of Zn toxicity under high bioavailability conditions, such as in waters with very low Ca concentrations. The difference between the toxicity of Zn to algae at a Ca concentration of 7 mg l^{-1} and at infinite dilution is less than 10 percent (Heijerick *et al.* 2002). The difference in Zn toxicity between a Ca concentration of 7 mg l^{-1} and infinite dilution for *Daphnia magna* is 25 percent (Heijerick *et al.* 2005). This suggests that it may be appropriate to apply the ZnBLM to waters with low Ca concentrations and pH values above six, by assuming that their Ca concentrations are equal to the lower validated limit of the BLM for Ca, because the difference in toxicity is likely to be relatively small (up to 25 percent).

5 Conclusions

The Environment Agency and other UK environmental regulators wish to use the Zn biotic ligand model (ZnBLM) in a compliance-based framework for the Water Framework Directive. The ZnBLM has been validated for use between pH 6 and 9 and Ca concentrations between 5 and 150 mg l^{-1}. However, under conditions of very low water hardness (below 24 mg l^{-1} $CaCO_3$ or below 7 mg l^{-1} Ca) a "soft water" PNEC has been recommended (European Union, 2008). This soft water PNEC was established because the generic PNEC for Zn may not be sufficiently protective under very soft water conditions. Use of this soft water PNEC is based on the acceptance that at Ca concentrations below 7 mg l^{-1} bioavailability is maximised, and is not affected by the pH or DOC concentration of the water. The resulting soft water PNEC is 3.1 µg l^{-1} dissolved Zn.

Available information about the behaviour of Zn and its toxicity under soft water conditions has been reviewed, and the findings are summarised below.

The ZnBLM (Version 4b) predicts that Zn bioavailability will be greatest to invertebrates under conditions of low pH and low Ca concentrations, although high bioavailability conditions are also expected to occur at circumneutral pH for fish and at high pH for algae. Zinc toxicity is predicted to be greatest for invertebrates at the lower limit of the BLM boundaries for pH and Ca, and greatest to algae at pH values above 6.4. An acute ZnBLM for *Daphnia pulex* which can be applied to soft water conditions has recently been developed (Clifford *et al.* 2009) demonstrating that the BLM principles can be applied to very low Ca concentrations (down to 2.6 mg l^{-1} Ca), although no comparable tests have been reported using chronic studies.

Calculations of Zn speciation under soft water conditions show that Zn binding to DOC could be important, especially where DOC concentrations are relatively high and Ca concentrations are low. At pH values much lower than 5.5, Zn binding to DOC will generally be relatively low, due to competition from protons, but between pH 5.5 and 6.0 a reduction in Zn bioavailability due to DOC binding could be important. Performing a correction of Zn exposure concentrations relative to DOC concentrations may provide a means of taking account of the chemical availability of Zn, although confirmation that this results in decreased bioavailability over chronic exposures from ecotoxicity tests may not be available.

A field study into the effects of metals on invertebrate and diatom communities in mining-impacted streams was able to identify effects on invertebrates, although it was necessary to take account of the presence of other potential stressors such as aluminium and acidity, in addition to Zn. Diatom communities appeared to show a response to Zn, and a comparison of the toxicity coefficients employed in modelling suggests that diatom communities may be more sensitive than invertebrate communities to Zn under the range of conditions included in the study. A relatively large proportion of soft waters were included in this study, suggesting that the findings may be applicable to consideration of the soft water PNEC. Any acclimation of organisms to elevated exposure of metals at these mining sites over many generations may, however, complicate application to more typical exposure situations.

An analysis of matched chemical and ecological field monitoring data for effects of Zn in waters which predominantly would not be covered by the soft water PNEC suggests that benthic macroinvertebrate communities may not be particularly sensitive to the effects of zinc. There are no clear indications of invertebrate communities being more sensitive to Zn toxicity under soft water conditions, although data under these conditions are very limited. The assessments based on whole community metrics might not be able to identify any effects on particularly sensitive species

There is a possible difference between the ZnBLM predictions, which suggest that invertebrates are likely to be the most sensitive trophic level under soft water conditions, and the available field data which suggest that invertebrates may not be particularly sensitive to the effects of Zn, and that algae may be more sensitive. It is difficult to draw firm conclusions about the relative sensitivity of the different trophic levels due to differences between the studies and types of data available.

Analysis of the available data does not show any need for a difference in approaches between waters with Ca concentrations of less than 7 mg l^{-1} and waters with Ca concentrations of greater than 7 mg l^{-1}. It appears that similar principles, in terms of the competition between Zn and major cations such as Ca and Mg, and bioavailability reduction through Zn binding to DOC can still be applied across the complete range of conditions. The current ZnBLM will require considerable modification before it can be used to make such bioavailability corrections, given that the conditions considered here are outside the validation range of the BLM.

For waters which lie within the validated pH range of the ZnBLM, but with low Ca concentrations, it is recommended that the Ca concentrations are assumed to be equal to the lower limit for the BLM, as the error associated with making this assumption is likely to be less than 25 percent. This means that the generic PNEC, which used an assessment factor of two in its derivation, should still be adequately protective of this difference.

A bioavailability correction approach based on prediction of the chemically available Zn species using chemical speciation models such as WHAM 6 is proposed as a practical means of correcting dissolved Zn concentrations for their likely bioavailability to aquatic organisms. Look-up tables of bioavailability correction factors are provided in Tables 4.1 to 4.4.

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