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Environmental Quality Standards for trace metals in the aquatic environment

Science Report – SC030194

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Steve Killeen

Head of Science

Executive summary

The scientific basis for the regulation of metals that are potentially toxic to aquatic life is widely debated, especially with respect to bioavailability. Environmental Quality Standards (EQSs) based on total metal concentrations and laboratory toxicity data for conditions unrepresentative of field situations may both underprotect and overprotect. The setting of EQSs could be informed by knowledge about dose–response relationships in the field. The work described in this report was performed to attempt to establish dose-response relationships for macroinvertebrates and diatoms in streamwaters contaminated to different extents with trace metals (nickel, copper, zinc, cadmium, lead). Different possible variables were used to express metal concentrations and bioavailability. The results obtained were then used to evaluate different approaches to EQS setting.

We carried out field studies of the ecology and chemistry of upland streams, influenced by past mining activity. We conducted field sampling and performed analyses at carefully chosen field sites, and analysed the data to attempt to establish quantitative relationships between the chemical and ecological variables. The chosen sites were perceived to have several advantages, notably similarity in their physical characteristics, appreciable ranges of water and metal chemistries, and the absence of other pollutants. Therefore they made it possible to explore the effects of differences in streamwater chemistry on ecological response, with few confounding factors.

The ecological part of the fieldwork was conducted in spring 2006, and comprised sampling and enumeration of benthic invertebrates and diatoms. Chemical characterisation of the streamwaters was achieved by repeated sampling during the 6 weeks before the ecological fieldwork. As well as carrying out conventional chemical analyses, we also used analytical speciation techniques (Diffusive Gradients in Thin films, DGT, and the Donnan Membrane Technique, DMT), and applied the WHAM chemical speciation model to calculate free metal ion activities and other variables. We collected separate samples of key invertebrate taxa and analysed them for metal body burdens. We also collected samples of stream bryophytes and analysed them for metals. In another supporting study, we collected water samples from the study sites and used them in laboratory toxicity experiments with an algal and a daphnid species. We carried out a literature review of metal mixture effects, to aid interpretation of field data from those sites (the majority) where more than one metal may exert a toxic effect. We also reviewed methods for setting EQSs.

The chemical compositions of the study streams covered appreciable ranges of acidity, alkalinity, total ionic concentration and dissolved organic carbon (DOC) concentration. The nutrient concentrations were sufficiently low for there to be negligible effects on either macroinvertebrates or diatoms. Suspended particulate matter concentrations were in the range 0–50 mg l⁻¹. As expected because of the historical mining activities, most of the streamwaters were contaminated with trace metals, at levels exceeding EQS values. The study sites included several acid streamwaters, some of which were contaminated with trace metals, but all of which had high levels of aluminium. The results of DGT and DMT measurements, and of chemical speciation calculations, indicated that zinc and cadmium are present largely in inorganic forms in the streamwaters, whereas there was appreciable organic complexation of aluminium, copper, lead, and to a lesser extent nickel. The metal contents of both bryophytes and macroinvertebrates varied considerably with streamwater chemistry. The metal contents of both types of organism were approximately predictable using chemical

speciation concepts, which is direct evidence that biota respond to the chemistry of the medium.

Analysis of the relationships between ecological and chemical variables, and of the results of the laboratory toxicity measurements, led to the following conclusions:

1. Toxic metals were demonstrated to reduce species numbers of both macroinvertebrates and diatoms in the field.
2. Laboratory tests confirmed that eight of the streamwaters in which field effects were observed contained metals at toxic concentrations.
3. The macroinvertebrate community provides an effective and sensitive tool for detecting metal toxicity. In the study sites, toxicity could be attributed definitely to aluminium and zinc, and there was some evidence of toxic effects due to H^+ and copper.
4. For diatoms, which were less sensitive than macroinvertebrates, the most likely toxicant was zinc, but cadmium may also have been active.
5. A function combining free metal ion concentrations and pH provided the best solution-based measure of metal toxicity towards macroinvertebrates, because it accounted best for aluminium effects.
6. Toxicity towards diatoms was expressed best, in terms of solution concentrations, by either total dissolved metal concentration or the metal concentration measured by DGT.
7. The field data permitted the formulation and partial parameterisation of the Toxicity Binding Model (TBM), which is based on Biotic Ligand Model (BLM) principles, but applicable to mixtures of metals. The model permits clear toxicity thresholds to be identified, and quantifies the contributions of different metals to the overall toxicity.
8. Because of the dominant effect of zinc, among the trace metals, the available data did not permit conclusions to be drawn about the effect of chemical speciation on copper or lead toxicity. However, the finding that aluminium effects are best expressed in terms of the free ion, and the promising results from the TBM, imply that speciation-based measures provide the best way to describe the toxic effects of metals in the field.

We considered the implications of the results of this work in the assessment and setting of EQSs for metals in freshwaters. The following points can be made.

1. The results of the present study add significant weight to the use of bioavailability and chemical speciation in deriving EQSs.
2. A full assessment of the effects of toxic metals in contemporary waters requires knowledge about past 'pristine' conditions. In the contaminated streamwaters of the present study, dissolved concentrations of trace metals were considerably higher (by 10 to 1000 times) than those estimated for similar uncontaminated sites under pristine conditions. As well as solution concentrations, calculated loadings of biotic ligands with toxic metals can be compared, and when this is done with the TBM it appears that at present-day uncontaminated sites the loadings can be substantial, not much lower than threshold values.
3. If bioavailability and chemical speciation are to be used in EQS setting, the biotic ligand approach is currently the best way forward. Although its application relies on calculations, the chemical speciation results can be tested with *in situ* analytical devices (DGT and DMT), lending robustness to the method.
4. There may be regulatory advantages to confining EQS setting to individual metals. However, the results of the present study suggest a significant role of

'natural' aluminium in field toxicity, and this implies that the issue of mixtures should be addressed. This can be done using biotic ligand concepts.

By showing that metal toxicity operates in the field, with dose–response relationships, and in line with bioavailability and chemical speciation concepts, the study provides support for metals regulation through EQSs. It further shows that targeted fieldwork can provide the information necessary for EQS evaluation and modification.

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1 Introduction

The scientific basis for the regulation of metals that are potentially toxic to aquatic life is widely debated, especially with respect to bioavailability. Environmental Quality Standards (EQSs) based on total metal concentrations, and set from laboratory toxicity data obtained under conditions unrepresentative of field situations, may both underprotect and overprotect.

The setting of EQSs could be informed by knowledge about dose–response relationships in the field. The work described in this report was performed to attempt to establish dose–response relationships for macroinvertebrates and diatoms in streamwaters contaminated to different extents with trace metals (Ni, Cu, Zn, Cd and Pb). Different possible variables to express metal concentrations and bioavailability were compared. The results obtained were then used to evaluate different approaches to EQS setting.

1.1 Current aquatic Environmental Quality Standards

Currently used EQSs for freshwaters are based on acute and chronic laboratory toxicity data. In some countries, a single concentration, usually of dissolved metal, is used. In other countries, including England and Wales, the beneficial (protective) effect of water hardness is taken into account, so that harder waters have higher EQSs.

A shortcoming of the current standards is that they are derived from experiments with metal salts in systems where much of the dissolved metal would have been in inorganic forms, and therefore more bioavailable. Translation of the toxicity results to the field situation should ideally take into account the more complex nature of natural waters, where metals may be significantly complexed by natural organic matter. At present in the UK, this is only possible qualitatively (Smith, 1993). Another shortcoming is that pH is not taken into account, although there is much evidence (e.g. Mance, 1987; Heijerick *et al.*, 2002) that this can be an important factor in toxicity. The factoring-in of hardness acknowledges that water chemistry influences toxicity. However, results summarised by Crommentuijn *et al.* (1997) suggest that chronic toxicity is much less affected by hardness than is acute toxicity, and so its use may be questioned. More recent studies show that hardness may affect Zn chronic toxicity to invertebrates and fish at least to a similar extent as for acute toxicity (Heijerick *et al.*, 2005). Hardness did not affect the chronic toxicity of Cu to *Daphnia*, while it did substantially affect acute Cu toxicity (De Schampelaere and Janssen, 2004b).

The application of metal EQSs that are too simple (i.e. overlook speciation and bioavailability issues) may lead to costly 'overprotection' of waters in some cases, for example where natural organic matter significantly decreases bioavailability. EQS failures for both Cu and Zn are relatively common in the UK, although there is little evidence to suggest that significant exceedences of the EQSs result in adverse effects in the ecosystem. In other instances, the toxicity of a metal might be underestimated by a pH-independent standard, with consequent damaging 'underprotection'.

To attempt to improve EQSs, information about the metal speciation of natural waters, and about the ways in which the metals interact with organisms, can be considered. Such information is increasingly becoming available, and the time is ripe to explore the development of new EQS methodologies.

1.2 Chemical speciation

The term chemical speciation means the distribution of a chemical component, in this case a metal, among different chemical forms. A simple schematic example is shown in Figure 1.1.

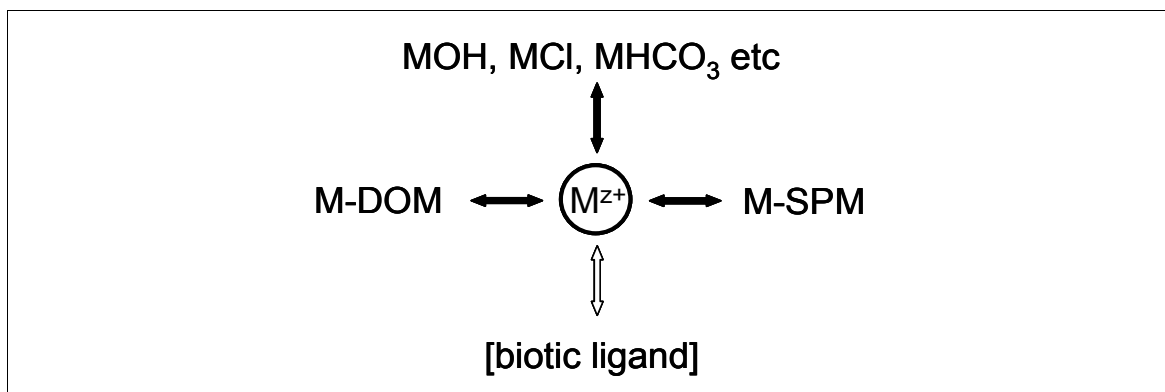


Figure 1.1 Schematic diagram of metal speciation, including binding to a 'biotic ligand'. Note that in a natural water, all the reactions are subject to competition by other metal cations and H^+ . DOM = dissolved organic matter, SPM = suspended particulate matter.

Recent years have seen significant advances in both practical and theoretical aspects of metal chemical speciation in natural waters. The state of knowledge is now such that reasonable confidence can be placed in our ability to describe metal chemistry, although there remain unanswered questions and a need for continuing testing of the available methods, especially in the field.

A key chemical variable in all speciation and bioavailability work is the concentration of the free metal ion, for example Cu^{2+} in the case of copper, or Zn^{2+} for zinc. All chemical speciation models use free ions as the 'master species' in computing the distribution of metals among the various possible chemical forms. The free ion is also seen as the most useful indicator of bioavailability, although other chemical variables must also be taken into account (see below). From the analytical viewpoint, the direct determination of free ion concentrations would be the ultimate goal, but other speciation methods can also provide data for testing models, and may also provide practical measures of bioavailability.

An especially important issue in metal speciation is the contribution of organically complexed metal to the total concentration. Due to the widespread occurrence of dissolved organic matter (DOM) in natural waters, some metals (e.g. copper, lead) are often present mostly in organically complexed forms, and this substantially reduces their bioavailability. This is one of the most significant advantages of taking a chemical speciation approach towards metal toxicity.

1.2.1 Analytical methods

Two techniques offering particular promise for the practical determination of metal speciation in natural waters are Diffusive Gradients in Thin films (DGT), introduced by Davison and Zhang (1994), and the Donnan Membrane Technique (DMT), originally developed for soils (Weng *et al.*, 2001) and adapted for waters by Kalis *et al.* (2006).

The DGT method has been used widely in research work (e.g. Odzak *et al.*, 2002; Gimpel *et al.*, 2003; Zhang, 2004), and also in monitoring and managing water quality

(Denney *et al.*, 1999; Martin *et al.*, 2003). The method involves a device containing a hydrogel disc exposed on one side to the water sample of interest, and on the other to an exchange resin that acts as a sink for labile forms of metals that have diffused through the gel. Knowing the time of deployment and the diffusion characteristics of the gel, measurement of the metal content of the exchange resin allows the concentration of metal in the external fluid to be calculated. DGT has the advantages of being a multi-element method, amplifying the actual water concentration, and providing a time-averaged concentration. More recently the technique has been extended to include the use of a restricted diffusion gel (Zhang and Davison, 2000), which excludes solutes of larger size, especially fulvic and humic acids, and this provides a more refined estimation of solution speciation. Davison and Zhang (1994) proposed that the metal flux measured by open-gel DGT could be used as a surrogate for bioavailability in soils and sediments where the rate of supply of metal, by release from the solids, is the key process. Later, Zhang and Davison (2000) suggested that the labile metal concentration measured by restricted-gel DGT might be a useful measure of aquatic bioavailability.

The DMT technique provides a well-defined chemical measure (i.e. the free ion concentration), and therefore differs from DGT, which estimates only 'labile' metal. In DMT, a cation exchange membrane is employed and a Donnan membrane equilibrium is established between the sample solution and an acceptor solution with a similar salt level to that of the sample. In the set-up designed by Fitch and Helmke (1989) the volume of the acceptor solution is very small (200 μl), which limits the possibilities for further analyses. Temminghoff *et al.* (2000) adjusted the technique towards a more efficient solution flow and a higher acceptor volume. Thus it could be used in combination with ICP-MS and/or ICP-AES, making it possible to measure many elements in a single sample. Moreover, in comparison with other methods, the disturbance of the sample equilibrium and interference from other components are minimal. Recently Kalis *et al.* (2006) have applied the method to relatively uncontaminated surface waters. They increased the sensitivity of the method by including metal-binding ligands in the acceptor compartment.

1.2.2 Chemical speciation models

The last decade has seen the development of comprehensive models of metal speciation in natural waters and soils. The most prominent are WHAM (Tipping, 1994, 1998) and NICA–Donnan (Kinniburgh *et al.*, 1999). The key advance in these models is their ability to account for cation binding by humic substances and thereby dissolved organic matter (DOM). The models are parameterised with laboratory data describing proton and metal binding by isolated humic matter. In the case of WHAM, which is based on conventional chemical reactions, the parameter values are consistent with the known chemical properties of the metal cations.

Application of these models to the field requires assumptions about the nature of dissolved organic matter, and field-testing is an ongoing process, aided by developments in analytical techniques for speciation measurement. Tipping (2002) showed that WHAM6 could account for a variety of published data. Bryan *et al.* (2002) reported that WHAM6 could successfully describe the results of experiments in which natural waters were titrated with copper and the concentration of Cu^{2+} measured. Zhang (2004) found that field DGT measurements of nickel, copper and zinc speciation agreed with WHAM6 predictions. However, some work has produced results that do not agree with model outputs. The speciation measurements of Meylan *et al.* (2004) on some Swiss waters suggested that Cu^{2+} concentrations were appreciably lower than WHAM6 and NICA predictions. Thus, although the models show great promise for the interpretation of natural water chemistry, and are indeed in use in many laboratories around the world, there is still a need for further model evaluation.

Natural waters may also contain appreciable concentrations of mineral colloids, able to bind trace metals (see, e.g., Stumm and Morgan, 1996). These include aluminosilicate clay minerals and the oxides of Al, Si, Mn and Fe. Modelling of trace metal–mineral colloid interactions, taking account the effects of pH and competition by major solute cations, can be performed with SCAMP (Lofts and Tipping, 1998), which comprises models for these individual phases, parameterised with laboratory data.

1.3 Bioavailability and toxicity

If the chemical speciation of a metal is known, the information can be used to explain or predict toxic effects, in terms of bioavailability. The most recent work in this area recognises that neither total metal nor the free metal ion alone provide adequate guides to the toxic effects of metals. The best predictions are obtained by taking account of (a) the concentration of the free metal ion, and (b) the concentrations of other ions that may compete with, or otherwise interfere with, the interaction of the metal with the organism. The use of the free ion concentration deals with the fact that complexation reduces bioavailability by competing with the organism for the metal, while the 'chemical medium' characterisation takes account of the competition by other metals for the organism.

The most prominent interpretive system in this context for aquatic systems is the Biotic Ligand Model (BLM; Paquin *et al.*, 2000), while simpler critical limit functions (CLFs), in which only pH dependence is taken into account have been developed for soils by Lofts *et al.* (2004).

1.3.1 The Biotic Ligand Model (BLM)

The BLM has gained prominence in recent years, especially in the USA, where the Environmental Protection Agency (EPA) has fostered its development for the evaluation of speciation effects on toxicity (Paquin *et al.*, 2000; Di Toro *et al.*, 2001). The central notion is that organisms possess a key binding site – the biotic ligand – that interacts with the toxic metal and with other cationic components of the water. The degree of loading of the biotic ligand with toxic metal determines the toxic effect. The loading depends upon the binding of competing cations, and the interactions of the toxic metal with other 'conventional' ligands in the solution, notably dissolved organic matter.

In the initial work with fish, parameters for cation binding to the biotic ligand were derived from experimental measurements on excised fish gills (e.g. Playle *et al.*, 1992), but more recent applications have derived the parameters directly from toxicity data, both acute (De Schamphelaere and Janssen, 2002) and chronic (De Schamphelaere and Janssen, 2004). BLMs have been developed, or are in the process of development, for several metals including copper (e.g. Santore *et al.*, 2001; De Schamphelaere and Janssen, 2002), cadmium (e.g. Playle *et al.*, 1993; Wu *et al.*, 2001), zinc (e.g. Heijerick *et al.*, 2002; De Schamphelaere *et al.*, 2005), nickel (Keithly *et al.*, 2004; Deleebeeck *et al.*, 2007) and lead (MacDonald *et al.*, 2002). Within the BLM, solution speciation is computed using WHAM (see Section 1.2.2).

Development of BLMs has so far been based almost entirely on data from laboratory studies. These take solution complexation into account, and also competition by protons and base cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+}), by defining and determining equilibrium constants for the reactions of these species, as well as the metal of interest, with the biotic ligand. To date there have been no measurements and associated parameterisations of mixture effects, although Playle (2004) considered mixtures from

a theoretical perspective (see also Appendix 1). The BLM has not yet been used to interpret field data, although there has been recent work on microcosms.

1.3.2 Free metal ion concentrations and species sensitivity

Parameterisation of the BLM for a single metal with a single organism requires a considerable amount of data. In order to make use of less detailed data, but applying to many more organisms, and for five toxic metals (nickel, copper, zinc, cadmium, lead), Lofts *et al.* (2004) re-evaluated soil chronic ecotoxicity data, taking into account solution chemical speciation, and also the differing sensitivities of different organisms. The organisms in question were those whose exposure to metals is via the soil solution, and therefore the approach is also applicable in principle to aquatic organisms. It was found that the toxicity data could be described by a model of the following form:

$$\log [M^{2+}]_{\text{crit}} = a \text{ pH} + b \quad (1.1)$$

where $[M^{2+}]_{\text{crit}}$ is the critical free ion metal concentration, and a and b are constants. The advantage of the regression approach is that species sensitivity is readily taken into account, since it is attributed to the scatter of points around the regression line. Thus a 'critical limit function' can be obtained with the same form as equation 1.1, but with b replaced by a lower value c , that ensures protection of a desired proportion (we used 95%) of the organisms considered. An example is shown in Figure 1.2.

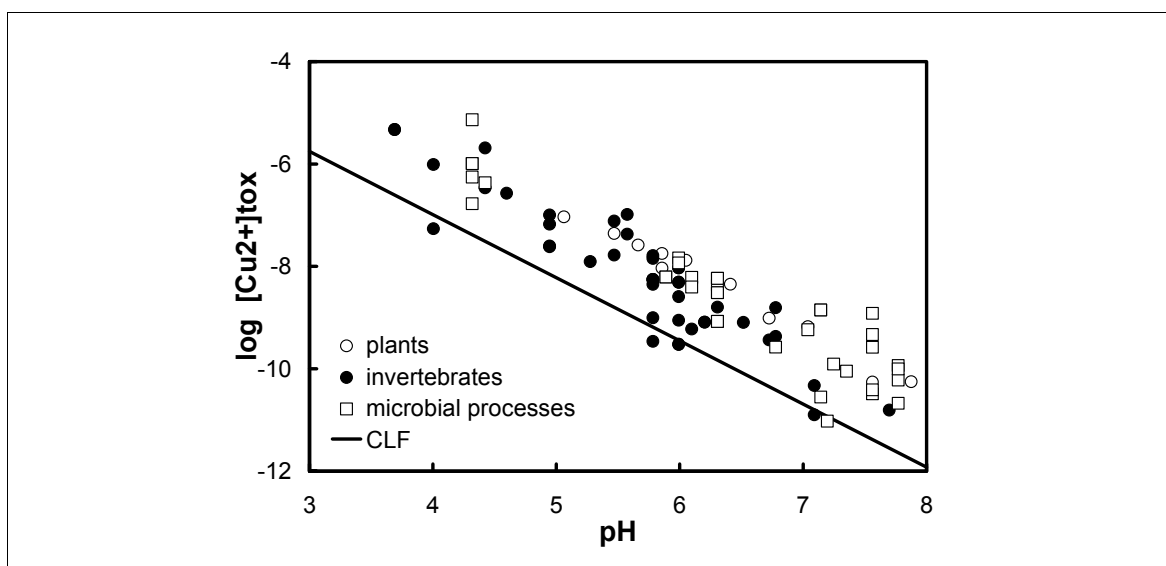


Figure 1.2 Soil toxicity data for Cu. Each point represents a chronic toxicity endpoint expressed as the free metal ion concentration. The line is the derived critical limit function (CLF), protective of 95% of the species.

This free metal ion approach is related to biotic ligand modelling based on observed toxicity (Section 1.3.1), the main point of which is to describe efficiently the variability of the toxic endpoint with solution chemistry. An advantage of the CLF is the ease of incorporation of species sensitivity.

Although it may appear a considerable extrapolation to apply critical limits derived for soils to waters, there is no theoretical reason why the sensitivities of soil and water organisms to metals should not be similar, assuming that uptake of the free ion from

the aqueous phase is the significant mechanism leading to toxicity. Furthermore, there is convincing overlap between $[M^{2+}]_{\text{tox}}$ values for soils and waters for both zinc and cadmium. There is also some overlap for copper and lead, although the numbers of chronic toxicity data available for aquatic organisms are too few to permit a conclusive comparison.

An important point to emerge from both this analysis and the principles of the BLM is that metal toxicity, expressed in terms of the free metal ion concentration, is lower at low pH. Therefore, although trace metal concentrations tend to be higher at low pH, due to greater geochemical mobility, it does not follow that their toxic effects are greater at low pH.

1.3.3 DGT and bioavailability

An example of the practical application of DGT in metal regulation is the work of Martin *et al.* (2003). These workers used the open-gel DGT method to determine labile copper in mine discharge waters, and concluded that the measurements provided a reliable proxy for copper bioavailability, in particular because they took account of the relatively large fraction of Cu bound by organic matter. If this is generally true, then replacement of conventional total or dissolved metal concentrations by DGT-labile concentrations could provide a straightforward means of improving EQSs, in many natural waters.

1.4 Benthic invertebrate and diatom assemblages

Supported by the Environment Agency, English Nature and government departments, the River Communities Group at the Centre for Ecology and Hydrology (CEH) have developed biological assessment in running waters based on their macroinvertebrate fauna. The biological classification of unpolluted running water sites in Great Britain has led to the development of the River Invertebrate Prediction and Classification System (RIVPACS; Clarke *et al.*, 2003). Using RIVPACS, the observed fauna can be compared with fauna expected at that type of site if it was unstressed and of high quality or 'Reference' state. This is often done through the use of the ratios of observed to expected values (O/E) of a range of biotic indices. This approach to biological assessment has subsequently been adopted by other countries (Australia, Poland, Sweden, Czech Republic, Spain and groups within the USA) and has influenced the European Union Water Framework Directive (Wright *et al.*, 2000).

The O/E ratio for taxon richness is a non-specific index of general deterioration in diversity, which may be particularly responsive to the effects of trace metals, as indicated by the work of Armitage (1980). Metal effects on stream invertebrates have been reported by Hirst *et al.* (2002) and by Clements *et al.* (1992, 2000).

Benthic diatoms are also used to assess nutrient status in running waters within the UK. The Trophic Diatom Index (TDI; Kelly and Whitton, 1995) reflects community response to prevailing nutrient concentrations. Similar indices have been developed elsewhere in Europe. Most of these evaluate responses along a nutrient/organic gradient but none have been developed specifically to examine responses to toxic pollutants. Work by Whitton and colleagues has shown effects of trace metals on stream algal assemblages (Harding and Whitton, 1976; Say and Whitton, 1981). As diatoms have grown in popularity for pollution monitoring, so some studies have focused on the effect of metals on diatom assemblages (e.g. McFarland *et al.*, 1997; Medley and Clements, 1998; Hirst *et al.*, 2002).

1.5 Objectives, plan and benefits of this research

The combination of chemical speciation, and bioavailability-based toxicity assessment improves our ability to interpret toxicity data in waters of different chemical composition, and on this basis the US EPA is moving ahead with implementation of the BLM in the setting of discharge consents. However, there is little evidence that any speciation-based description of toxicity can explain toxicity in the field, although there are microcosm, mesocosm and field enclosure studies which demonstrate that BLM-corrected HC5 values are protective (Van Sprang *et al.*, in preparation). The present project was undertaken to address this issue, and to consider the implications of the findings for the derivation of EQSs. The overall objective, as stated in the original tender document from the Environment Agency, was 'to investigate the relationships between metal speciation and ecological quality in the field and assess a number of potential methods for EQS derivation, including the ecotoxicological validity of the current system of EQSs for trace metals'.

We divided this into two separate objectives, as considered below. For easy reference, a schematic diagram of the project activities and how they relate to the objectives is shown in Figure 1.3.

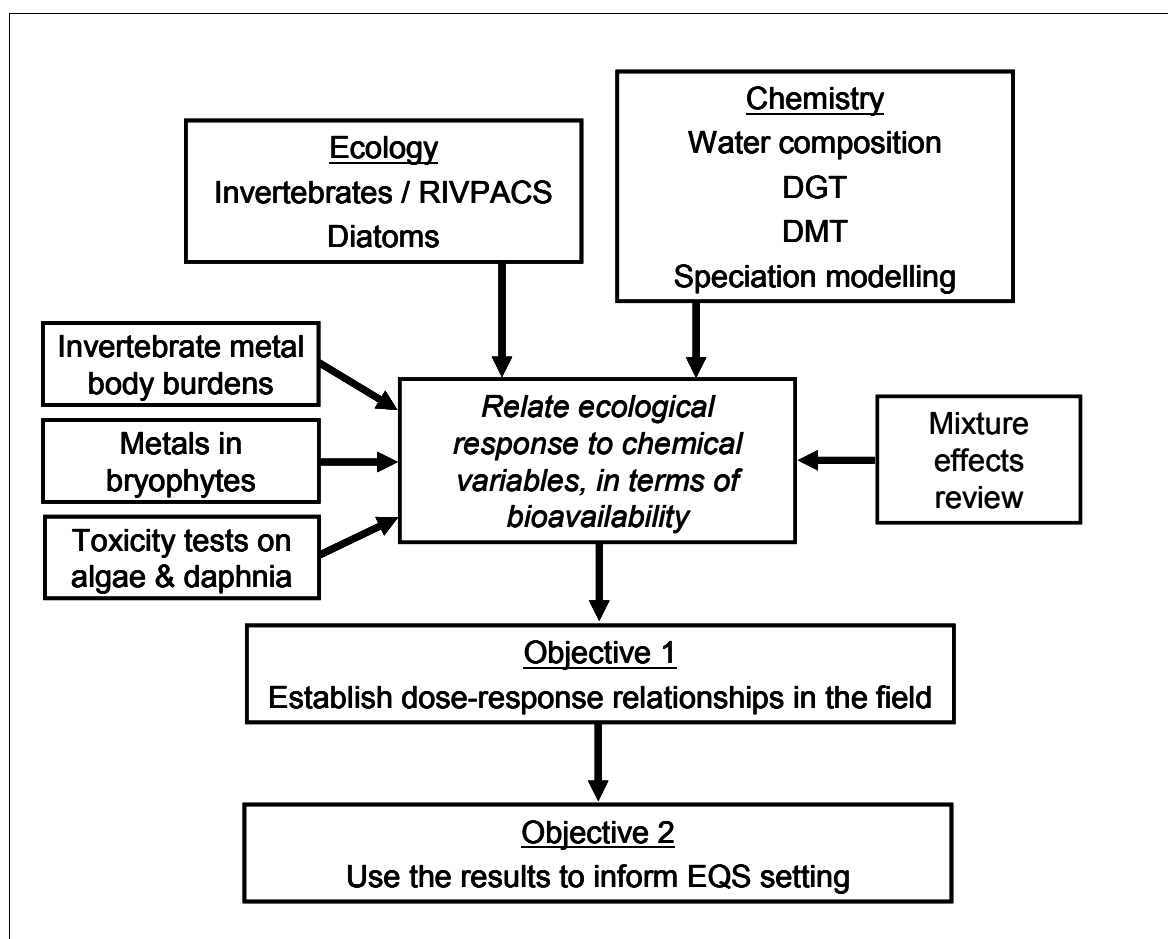


Figure 1.3 Schematic diagram of the structure of the project.

1.5.1 Objective 1 Dose–response relationships in the field

The first objective of the project was to test for dose–response relationships in the field. To this end, we carried out field studies of the ecology and chemistry of upland streams, influenced by past mining activity. We conducted field sampling and

performed analyses at carefully chosen field sites, and analysed the data to attempt to establish quantitative relationships between the chemical and ecological variables. Such field sites were perceived to have several advantages, notably similarity in their physical characteristics, appreciable ranges of water and metal chemistries, and the absence of other pollutants. Therefore they made it possible to explore the effects of differences in streamwater chemistry on ecological response, with few confounding factors.

The ecological part of the fieldwork comprised sampling of benthic invertebrates and diatoms, samples being collected for identification and enumeration of taxa. Chemical characterisation of the streamwaters was achieved by repeated sampling during the 6 weeks before the ecological fieldwork. As well as collecting water samples for conventional chemical analysis, we also deployed DGT and DMT devices (Section 1.2.1). We applied the WHAM chemical speciation model (Section 1.2.2) to calculate free metal ion activities and other variables.

As part of the invertebrate collection programme, separate samples of key taxa were collected to be analysed for metal body burdens. We also collected samples of stream bryophytes and analysed them for metals. In another supporting study, we collected water samples from the study sites and used them in laboratory toxicity experiments with an algal and a daphnid species.

We carried out a literature review of metal mixture effects, to aid interpretation of field data from those sites (the majority) where more than one metal may exert a toxic effect.

In order to establish dose–response relationships, the data were analysed by a variety of approaches, principally regression analysis and parameterisation of a BLM-type model, and in the case of diatoms Canonical Correspondence Analysis (CCA).

1.5.2 Objective 2 Use the results to inform EQS setting

The aim here was to use the results and analysis of the field data to review and assess current and alternative methods for setting EQSs, covering the entire range of freshwaters in England and Wales, and taking into consideration ecotoxicology and chemical speciation.

To this end we reviewed current methods and considered alternatives. A major issue was the choice of metric by which to express EQSs, bearing in mind the needs for scientific rigour, ease of application, and the availability of input data. Another important aspect was the toxicity of mixtures of metals. We also addressed the issues of background metal concentrations, and the Added Risk approach, which may be adopted in the Water Framework Directive's approach towards water quality (Lepper, 2002). We bore in mind the fact that the ultimate aim is to protect the whole ecosystem, even though the fieldwork conducted in the present project concerned only benthic macroinvertebrates and diatoms.

1.5.3 Expected benefits

The work was undertaken as part of the Environment Agency's R&D programme on environmental and human health standards, the client for the work being Air and Chemicals Policy. However, the need to assess the ecological impacts of trace metals spans a number of functions within the Environment Agency and the information will therefore be of use to staff in a variety of operational and policy roles. The project will

also develop knowledge for use in debates in Europe under the auspices of the Water Framework Directive.

The work brings together the latest scientific information about the aquatic toxicology of metals, and state-of-the-art chemical speciation, in the context of a toxicity occurring in the field, at the ecosystem level. The results will help to bring about better, more cost-effective, protection of freshwater ecosystems by:

- clarifying the relationship between EQSs and the underpinning science, permitting discussion and negotiation to be conducted more effectively between regulator and regulated;
- helping regulators to deal with 'problem sites', where for example current standards are exceeded but where there is no evidence of biological or ecological effect;
- informing consent setting, leading to better and more cost-effective protection, and permitting resources to be targeted more effectively.

1.5.4 Terminology

This report focuses mainly on the five metals nickel, copper, zinc, cadmium and lead, and refers to them collectively as 'trace metals', except where other workers' usage of the term 'heavy metals' is quoted directly.

2 Methods

2.1 Chemical sampling and analysis

2.1.1 Streamwaters

On each sampling trip, separate samples were taken at each site for major solutes (one-litre, high-density polyethylene bottle), pH (glass bottle with a ground glass stopper, completely filled), trace metals (500 cm³ acid-washed polyethylene bottles). The samples were kept in cool boxes during transport back to the laboratory, where they were stored cold and dark.

Table 2.1 Chemical determinands.

Determinand	Method	Unit	LOD
pH	glass electrode	N/A	N/A
Na, Mg, Al, K, Ca, Fe	ICP-OES	mg l ⁻¹	0.020
Mn	ICP-OES	mg l ⁻¹	0.01
Chloride	ion chromatography	mg l ⁻¹	0.1
Nitrate-N	ion chromatography	mg l ⁻¹	0.01
Sulphate-S	ion chromatography	mg l ⁻¹	0.01
Alkalinity	Gran titration	meq l ⁻¹	0.001
Conductivity	electrochemistry	µS cm ⁻¹	0.54
Suspended particulate matter	gravimetry	mg l ⁻¹	0.1
Ferrous Fe	colorimetry	µg l ⁻¹	5
Total phosphorus	colorimetry	µg l ⁻¹	3.7
Ammonium-N	colorimetry	mg l ⁻¹	0.01
Dissolved silica as SiO ₂	colorimetry	mg l ⁻¹	0.050
Dissolved organic carbon	combustion	mg l ⁻¹	0.5
Absorbance at 340 nm	spectrophotometry	AU	0.001
Dissolved (< 0.45 µm) Ti	ICP-MS	µg l ⁻¹	0.04
Dissolved (< 0.45 µm) V	ICP-MS	µg l ⁻¹	0.02
Dissolved (< 0.45 µm) Ni	ICP-MS	µg l ⁻¹	0.01
Dissolved (< 0.45 µm) Cu	ICP-MS	µg l ⁻¹	0.02
Dissolved (< 0.45 µm) Zn	ICP-MS	µg l ⁻¹	1.00
Dissolved (< 0.45 µm) Cd	ICP-MS	µg l ⁻¹	0.002
Dissolved (< 0.45 µm) As	ICP-MS	µg l ⁻¹	0.008
Dissolved (< 0.45 µm) Pb	ICP-MS	µg l ⁻¹	0.06

Chemical analytical methods are summarised in Table 2.1. They were conducted in accordance with ISO 17025 in the UKAS-accredited CEH Lancaster analytical facility. They made use of International Quality Control standards, with verification by the

Proficiency Testing scheme. Furthermore, Certified Reference Materials were used in the determination of trace metals in surface waters and aquatic moss.

2.1.2 DGT samplers

DGT samplers were deployed as a 'cluster' of six devices, three containing open pore diffusive gels and three containing restricted diffusive gels, which were tied together using Dyna Cable Teflon-coated fishing line. The DGT devices were cleaned by soaking them sequentially in the following four solutions: 2% Decon, 1.5 M HNO₃ (Anal-R), 1.5 M HNO₃ (Aristar), 4 M HCl (Aristar). The devices were allowed to cold-soak overnight with a complete rinse between each step. To remove all residual acid from the devices, they were allowed to soak in 18.2 MΩ water over several days until final assembly. All devices were assembled under Class-100 clean room conditions.

Once assembled, the device clusters were placed into an acid-cleaned plastic bag and a second outer uncleaned plastic bag for transport into the field. Upon arrival at the field site, the assembled device cluster was fixed within a short piece of plastic pipe (22 cm length and 15 cm diameter) using plastic cable ties. The pipe was placed on the bottom of the streambed and held in place using rocks that were gathered from the surrounding area. At the conclusion of the deployments, which were carried out for 1, 2 or 14 days, depending upon stream metal concentration, the cable ties were cut and the DGT devices retrieved and placed into an acid-washed plastic bag. Upon return to the laboratory, the devices were rinsed with deionised water and disassembled in a Class-100 environment.

Except for samples from the control sites (3, 4, 6, 27, 32, 34, 35), DGT samples were analysed for metals by CEH Lancaster using a Perkin-Elmer Elan DRC II ICP-MS Instrument. Metals in the control site samples were determined by the Department of Environmental Sciences, Lancaster University.

2.1.3 DMT samplers

Free metal ion concentrations were measured by means of the field Donnan Membrane Technique (field DMT). The field DMT consists of an acceptor chamber, on two sides separated from the sample solution (the donor) by negatively charged membranes. Cations enter the acceptor side at a rate much higher than complexed cations or anions, leading to equilibrium between free metal ion concentrations in the donor and the acceptor side. The acceptor side consisted of 5 mM Ca(NO₃)₂ and 30 mg l⁻¹ purified humic acid (Temminghoff *et al.*, 1997). The humic acid accumulates metal ions at the acceptor side, which decreases the detection limit of the DMT. Detailed description of the application of the DMT to natural waters can be found in Kalis *et al.* (2006).

At each of the 35 sampling locations DMTs were exposed to the streaming water in triplicate, by fixing the DMTs inside a PVC pipe. The exposure lasted 3 days and took place in the period from 13 to 27 April 2006. Concentrations of Cu, Cd, Zn, Ni and Pb were measured on ICP-MS (Perkin-Elmer; Elan 6000), Ca, K, Mg and Na concentrations on ICP-AES (Spectro, Spectroflame), and humic acid concentrations on a DOC/TOC analyser (Skalar).

At each location an average (and a standard deviation) value for the free metal ion concentration of Cu, Ni, Cd, Pb and Zn were calculated. For all measured metals equilibrium was reached within 3 days except for Cu. In those cases a kinetic approach was used to calculate the free copper concentration at equilibrium (Weng *et al.*, 2005; Kalis *et al.*, 2006). As well as the free metal ion concentration, the total metal

concentration was also determined. Triplicate sampling was achieved at all sites except numbers 12, 15, 16, 17 and 22, which were sampled only twice because of slight contamination of the DMTs by suspended sediment.

2.1.4 Invertebrate body burdens

For the metal analysis individual or pooled organisms were collected as part of the field invertebrate sampling programme. In total over 1000 samples were collected and analysed. They were stored in 1.5-ml polypropylene sampling vials and stored at 4°C after transfer to the laboratory during the campaign. For each 50 sample vials with invertebrates, five empty sample vials were also included to be used as process controls. After completion of the sampling campaign all samples were transferred to the University of Antwerp for further processing and analysis.

Samples were dried until constant weight at 60°C in a laboratory furnace. Invertebrate samples were weighed on a Sartorius SE2 Ultra Micro balance to the nearest 0.001 mg and transferred to 0.001 M HCl cleaned and pre-weighed 0.5 or 1.5-ml polypropylene vials. Invertebrate samples were microwave digested in a nitric acid–hydrogen peroxide solution by a stepwise method in which samples were microwave treated for four times, each time increasing the microwave power by 10%. For this purpose the vials were loaded in an air-tight polycarbonate box and placed in the microwave. For each series of 50 samples, five control samples were also processed and five samples of invertebrate reference material (mussel BCR-668) were included for quality control. After each 5-minute treatment the box was removed from the microwave and vented in a fume hood for 5 minutes and the microwave treatment repeated.

After the procedure the digest was diluted with ultra-pure water to obtain a solution of 5–10% acid and the vials were reweighed to accurately determine the final sample volume. Metals were analysed using a Varian quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

2.1.5 Bryophyte metal contents

Bryophytes, irrespective of species, were collected from as many streams as possible with care taken to ensure they were removed from places where the plants were fully submerged. After being thoroughly washed in streamwater to remove large particles and attached invertebrates they were placed in labelled plastic bags. These bags were then sealed and stored in cool boxes ready to be returned to the laboratory where each plant was washed separately in distilled water to remove any obvious sediment. This washing process continued until the rinse water ran clear with care being taken not to unduly damage the plant. The washed plant material was then dried to a constant weight for 48 hours at 105°C in an acid-washed snap-top glass vial.

After cooling in a desiccator for 1 hour, 0.5 g of the dried material was added to 5 cm³ of high-purity concentrated HNO₃ (Baker Ultrex) and 2.5 cm³ Milli-Q water in an acid-washed conical flask fitted with a loose glass stopper. Two identical flasks containing no plant material were also prepared to act as blanks. Eventually all the samples were gently refluxed for 3 hours at 140°C on a hotplate in a clean fume cupboard. The resultant digests were then allowed to cool, and then were filtered and made up to 50 cm³ with Milli-Q water.

Finally, the digests were analysed for all metal determinands using ICP-MS along with relevant blanks and quality control standards. The reliability of the digestion method was assessed by using a reference terrestrial moss purchased from the Finnish Forest

Research Institute. This reference moss was subjected to the same method of digestion and analysis as that employed for the field samples.

The bryophyte species were identified by Professor John Rodwell of Lancaster University.

2.1.6 Chemical speciation calculations

Calculations were performed with software that implements the two comprehensive models of ion binding by humic matter, Humic Ion-Binding Model VI (Tipping, 1998) and the NICA–Donnan model (Kinniburgh *et al.*, 1999). The former was incorporated within WHAM (Tipping, 1994), and the latter within ECOSAT (Keizer and Van Riemsdijk, 2002).

2.2 Ecological sampling

2.2.1 Macroinvertebrates

Monitoring of macroinvertebrate fauna and the associated assessment of community response was carried out with the River Invertebrate Prediction and Classification System (RIVPACS; Clarke *et al.*, 2003). The RIVPACS sampling approach (Wright *et al.*, 1984; Environment Agency, 1999) includes recovery of macroinvertebrates from all major habitats at a site, the 3-minute sampling activities being divided in proportion to habitat extent. In addition, a 1-minute hand search is conducted for taxa likely to be missed in the kick sample (e.g. those adhered to large stones and bedrock). In this manner the full range of macroinvertebrates at the site is incorporated in the sample and at their prevailing relative abundance levels.

In the laboratory, each entire sample was carefully sorted through for macroinvertebrates, with the aim of identifying and recording all the taxa within the sample, but using where appropriate varying degrees of subsampling (1/2, 1/4, 1/8 sample, etc.) purely to estimate the abundance of very common taxa (Wright, 2000). The procedure of sorting through the whole sample within the aim of finding all the taxa present is an important difference in the RIVPACS method compared to some other methods which only identify a 'random' subsample and/or a random fixed number of individuals in a sample. Samples were analysed at the species level, with the exception of the Hydracarina, Oligochaeta and Chironomidae. The numbers of each taxon were recorded.

An essential part of RIVPACS is the recording, at each macroinvertebrate sampling site, of the following physical, chemical and ecological characteristics.

Slope	Catchment vegetation
Surface geology	Vegetation within 50 m
Distance from source	Vegetation on bank top
Altitude	Bankside erosional features
Mean substrate size (phi value)	Mean channel width
Conductivity/alkalinity	Mean water depth

These were used to generate RIVPACS predictions (e.g. the probability of occurrence of individual macroinvertebrate families/species, RIVPACS scores and indices). Predictions also provide a warning if the site is a 'poor fit' to the UK dataset of running water sites and therefore unsuitable for measuring possible impacts.

In addition to sampling and analysing the study sites, we also selected sites from existing RIVPACS high quality reference sites and/or from sites within the Environment Agency's GQA surveys with 'high' or at least 'good' ecological status, to provide a set of control sites. We used our existing environmental data on these databases of RIVPACS and General Quality Audit (GQA) sites to select sites which are as similar as possible in physical type to the impacted sites (i.e. similar discharge categories, distance from source, substratum composition and alkalinity – all of which can influence the macroinvertebrate community composition in high quality streams). This made it possible, in looking for differences in macroinvertebrate community between the two groups of sites, to test for differences in overall community composition and/or differences in the proportional occurrence or average abundance of individual taxa (families or species).

2.2.2 Diatoms

Diatom samples were collected using the standard UK method (Kelly *et al.*, 2001), which is compatible with the European standard method (Kelly *et al.*, 1998; CEN, 2003). Diatoms were sampled during periods of stable stream flow, that is at least 4 weeks after a period of extreme conditions such as a major flood or drought. A minimum of five cobbles were collected from the stream and the upper part of the stone was rubbed with a toothbrush and rinsed into a tray with clear river water. The resulting suspension was decanted into a sample bottle and a few drops of formalin were added.

On returning to the laboratory, a portion of the sample was transferred to a centrifuge tube to which 5 ml of sulphuric acid was added followed by approximately 0.1 g potassium permanganate. Oxalic acid (10 ml) was then slowly added resulting in a suspension of bleached particles (mainly diatom valves). Distilled water was added and the suspension centrifuged. The centrifugation stage was repeated at least three times to ensure removal of all trace of acidity. Finally, the resulting diatom pellet was mixed into a small amount of distilled water.

A Pasteur pipette was used to remove some of the shaken suspension and a drop was placed on a cover slip. This was slowly evaporated leaving a whitish film. A small drop of mountant (Naphrax) was put on a warm slide and the cover slip placed face down on top of it. The slide was heated on a hotplate until the Naphrax spread and began to bubble. Gentle pressure was then applied to remove bubbles and then the assembly was allowed to cool.

At least 300 diatom valves were identified at 1000x magnification using an oil immersion lens, making sure that the examined fields were not all located in one small area. Taxa were identified to at least species level. Examples of diatom taxa were recorded photographically, and the lists of diatoms identified from each sampling site were checked by Martyn Kelly (Bowburn Consultancy) .

2.3 Toxicity experiments

Toxicity Identification Evaluations (TIEs) were carried out with two aquatic organisms, the green alga *Pseudokirchneriella subcapitata*, and the invertebrate *Daphnia magna*. In each case the growth of the organism was measured in water samples collected from the study sites. These measurements enabled us to investigate whether toxicity to

the organisms in contaminated samples was only due to metals present in the samples or whether other factors were possibly also involved.

2.3.1 Toxicity Identification Evaluation with *P. subcapitata*

All samples, including eight control samples (from uncontaminated surface waters) and 26 'contaminated' samples were investigated. Before testing MOPS (3-(N-morpholino)propanesulfonic acid) buffer (3.6 mmol l^{-1}) was added to all samples with a field pH between 6 and 8 and pH was adjusted to field pH with an appropriate amount of NaOH. No pH buffer was added to samples with $\text{pH} > 8$ or < 6 (since this is clearly outside the useful pH buffering range of MOPS). For these samples pH was initially adjusted to field pH by adding either HCl or NaOH if necessary. pH in all samples was maintained within 0.2 pH units during tests by daily manual addition of HCl or NaOH if necessary.

Algal growth experiments in all samples were conducted in triplicate according to OECD guideline No. 201 (OECD, 2006) at 25°C . Tests were initiated by introducing $104 \text{ cells ml}^{-1}$ of *P. subcapitata* into the samples. After 24, 48 and 72 hours, cell density was recorded by an electronic particle counter. Growth rate (μ) was calculated after 72 hours for all samples.

Samples showing toxicity were taken further into the TIE test phase to determine whether the toxicity was due to the metals present or due to another factor. Those eight samples, as well as the 'control' samples, were amended with a concentration of the complexing ligand EDTA at a molar concentration equal to three times the sum of the molar concentrations of all metals (Zn, Cu, Ni, Cd, Pb). As such, the metals that may have caused toxicity in the first testing phase were bound to EDTA, leaving very little free metal ion in the solution, and taking away the metals as the cause for the toxicity. When the earlier-observed toxicity disappears by this amendment, then the toxicity in the toxic raw samples can be attributed to metals only.

2.3.2 Toxicity Identification Evaluation with *D. magna*

Since *D. magna* has been shown to perform poorly (i.e. produce less offspring) in waters with hardness below $25 \text{ mg CaCO}_3 \text{ l}^{-1}$ and/or $\text{pH} < 6$, we decided to investigate the reproduction of *D. magna* only in those samples with higher hardness and pH. In a first phase eight 'contaminated' and four 'uncontaminated' raw samples were investigated. MOPS ($3.6 \text{ mmoles l}^{-1}$) was added as a pH buffer to samples with $\text{pH} < 8$, and the pH of the samples was adjusted to the pH measured in the field through addition of NaOH. Two 'uncontaminated' samples were slightly modified with respect to natural field conditions: the pH of water from Site 6 (see Table 3.3) was adjusted to 6 and the hardness values of samples from Sites 6 and 32 were adjusted to $25 \text{ mg CaCO}_3 \text{ l}^{-1}$ by adding the appropriate amount of CaCl_2 .

We prepared 5 litres of each of the 12 samples and let them equilibrate for 48 hours at 20°C . Next, per sample 10 juvenile *D. magna* (< 24 hours) were introduced into 10 separate test containers holding 50 cm^3 of sample. Survival and reproduction were monitored during 21 days following OECD test guideline No. 211 (OECD, 1998). Daphnids were fed daily with a mixture of *P. subcapitata* and *Chlamydonas reinhardtii* at a ration of 250, 500 or $750 \mu\text{g dry wt per day}$ in the first, second and third week of exposure, respectively. Three times a week, the daphnids were transferred to fresh medium.

2.4 Statistics and optimisation

Analysis of TIE testing results were performed with t-tests and analysis of variance, using Statistica 6.0 software (Statsoft, Tulsa, OK, USA). Model optimisation was performed using the 'SOLVER' function in Microsoft Excel, and parameter significance was assessed using the 'chi-squared' test. Canonical Correspondence Analysis was carried out with CANOCO software. Multiple regression was done in MINITAB Release 14 (Minitab Inc.).

3 Field programme

3.1 Preliminary survey, 2005

During 2005, a survey of upland stream sites was carried out in order to identify suitable locations for the main field programme, which was carried out in 2006. The survey aimed to establish a set of streams contaminated to different extents with trace metals (Ni, Cu, Zn, Cd, Pb), and possessing a range of water chemistries (pH, DOC, alkalinity). In the original project supported by the Environment Agency, 16 contaminated, and 8 uncontaminated controls, were required. With the additional funding that became available from the Metals Industry, this was extended to 11 more sites, making 35 in all.

3.1.1 Stream chemistry

We surveyed 46 sites (S1–S46) in the northern Pennines and the Lake District. These were low-order streams, with similar physical characteristics. In this preliminary survey, only sites associated with abandoned mine workings were examined, the aim being to establish that suitably high levels of metals could be found. In the main project other sites, not impacted by trace metals, were also examined.

Streamwater samples were collected and analysed for key chemical variables. Inspection of the results (Table 3.1) shows that reasonably wide ranges of trace metal levels and major-component water chemistries were established. The chemistry data are considered in detail in relation to choice of sites for the main study in Section 3.1.3, but to summarise briefly the results in Table 3.1, we found pH values in the range 4.2 to 8.4, [DOC] from near-zero to 25 mg l⁻¹, and alkalinity from near-zero to 226 mg CaCO₃ l⁻¹. Comparison of the metal concentrations with the simple 'flat standard' Dutch system of Maximum Permissible Concentrations showed five of the sites to exceed the MPC for Ni, 17 for Cu, 37 for Zn, 18 for Cd and 20 for Pb.

3.1.2 Ecological variables

Macroinvertebrate samples were collected at 24 sites from the April 2005 survey, and were sorted and identified to family level. For the five most contaminated sites (Numbered S2, S5, S13, S24 and S27 in Table 3.1), the identification was also completed at species level. The 24 macroinvertebrate samples yielded between 5 and 14 (RIVPACS-scoring) macroinvertebrate families. For all 24 macroinvertebrate sites, the RIVPACS predictions of faunal composition and probabilities of occurrence of each taxon were generated from standard physico-chemical site descriptors.

Nineteen of the 24 test sites yielded robust RIVPACS predictions, indicating that macroinvertebrate species can be reliably forecast in terms of percentage probability of occurrence for each species. As all test sites are located on small upland headwater streams in the same geographic region the mean percentage occurrence probability was calculated for all common (> 20% probability) macroinvertebrate species. The predictions are species occurrence probabilities in the absence of site stress. The species recorded at the five most contaminated sites were also compared with the data on occurrence rates at 10 unimpacted RIVPACS reference sites in adjacent NGRs.

Table 3.1 Preliminary stream survey 2005 – summary of chemistry data.

Site	pH	DOC ¹ mg l ⁻¹	Alk ² mg l ⁻¹	Al µg l ⁻¹	Ni µg l ⁻¹	Cu µg l ⁻¹	Zn µg l ⁻¹	As µg l ⁻¹	Cd µg l ⁻¹	Pb µg l ⁻¹
S1	7.68	3.3	70	19.1	5.39	1.58	590.0	0.19	1.18	21.80
S2	7.35	2.0	137	12.0	13.50	1.14	3000.0	0.30	6.48	12.20
S3	7.3	3.8	95	26.5	3.55	1.00	565.0	0.28	1.80	12.90
S4	7.37	4.1	34	24.9	2.51	1.74	768.0	0.22	2.67	44.30
S5	7.64	< 0.1	226	2.7	11.20	2.05	4400.0	0.18	3.61	0.77
S6	7.52	7.8	37	86.9	2.05	2.26	0.5	0.33	0.02	2.30
S7	6.98	13.5	10	157.0	3.20	1.35	5.3	0.39	0.04	1.71
S8	7.97	5.0	70	35.1	0.96	0.71	1.7	0.28	0.01	0.59
S9	8.25	4.6	80	39.8	1.03	0.50	33.4	0.22	0.09	0.94
S10	7.91	4.9	84	41.9	1.26	0.58	66.8	1.08	0.10	0.80
S11	7.9	5.2	67	19.4	0.86	0.55	76.5	0.24	0.28	2.23
S12	8.03	3.3	79	12.3	0.87	0.53	40.1	0.21	0.13	1.35
S13	4.67	0.3	6	42.9	0.93	0.77	45.9	0.42	0.26	142.00
S14	6.2	< 0.1	6	6.0	0.28	0.33	4.4	0.27	0.02	0.03
S15	6.27	< 0.1	7	7.0	0.33	0.61	7.8	0.27	0.05	6.15
S16	7.38	0.3	26	5.3	2.89	2.89	49.1	8.67	0.29	3.97
S17	7.24	< 0.1	33	2.2	4.87	3.28	64.2	14.00	0.43	4.38
S18	7	< 0.1	37	1.7	5.65	5.87	71.6	15.50	0.47	3.14
S19	7.19	1.5	11	11.1	0.39	0.86	30.5	1.04	0.10	1.34
S20	7.4	0.4	24	5.7	2.04	2.42	44.0	6.76	0.28	3.29
S21	7.31	< 0.1	22	0.8	0.26	4.66	297.0	2.04	2.43	23.40
S22	7.51	< 0.1	38	1.4	0.50	1.52	381.0	112.00	3.02	11.40
S23	7.89	< 0.1	34	3.7	0.43	2.55	275.0	91.40	2.51	19.60
S24	6.09	< 0.1	20	3.0	0.96	0.78	426.0	0.57	3.08	98.30
S25	6.31	< 0.1	20	0.6	0.99	0.67	451.0	0.53	3.24	116.00
S26	6.55	< 0.1	12	25.6	0.35	5.87	19.0	0.78	0.15	0.16
S27	6.72	< 0.1	9	19.6	0.22	8.57	14.0	0.42	0.10	0.07
S28	5.06	< 0.1	3	128.0	0.29	10.60	33.9	0.14	0.19	1.41
S29	7.58	12.4	37	81.2	3.53	1.10	25.0	0.47	0.58	7.90
S30	7.48	14.3	18	57.1	5.00	1.20	3.3	0.42	0.03	0.60
S31	8.07	3.0	68	58.4	2.44	0.67	78.0	0.35	0.41	14.00
S32	7.71	4.8	66	31.1	0.90	0.39	27.0	0.26	0.24	2.60
S33	8.21	9.7	50	71.4	1.48	0.42	11.0	0.35	0.06	2.80
S34	8.41	1.3	85	15.1	0.99	0.35	110.0	0.15	1.50	12.00
S35	7.43	8.2	29	75.9	2.33	0.96	16.0	0.32	0.07	14.00
S36	7.92	6.1	56	33.5	1.33	0.67	13.0	0.29	0.04	5.10
S37	7.82	4.5	34	35.6	1.82	1.00	130.0	0.24	0.52	21.00
S38	7.12	22.2	9	162.0	2.43	1.00	21.0	0.56	0.06	12.00
S39	8.22	12.8	73	77.2	3.20	5.50	470.0	0.33	0.64	28.00
S40	6.86	18.6	8	160.0	3.94	1.80	200.0	0.31	0.58	41.00
S41	6.57	24.9	3	246.0	2.94	0.57	16.0	0.41	0.05	3.00
S42	7.61	17.8	21	86.4	2.38	1.00	16.0	0.35	0.08	37.00
S43	4.24	0.3	47	1700.0	78.50	5.60	10000.0	0.50	20.00	180.00
S44	6.46	0.4	3	12.9	0.53	0.28	7.2	0.17	0.03	0.05
S45	7.11	0.1	7	7.1	0.16	0.14	3.3	0.59	0.01	0.03
S46	6.71	0.5	4	15.0	0.42	0.46	6.7	0.60	0.03	0.45

Notes: ¹Approximated from absorbance at 340 nm, using extinction coefficients given by Thacker *et al.* (2005).

²Estimated from conductivity. Note that this leads to incorrect values in highly acid waters (e.g. S43).

A total of 33 taxa were predicted to occur at rates > 20% at the study sites. Of these, nine were absent in the contaminated streams, and five were present in significantly reduced numbers. One species showed a higher than predicted occurrence rate. For comparison, data for the RIVPACS reference sites were also compared with predictions. In these cases, the observations and predictions were in acceptable agreement. Table 3.2 summarises the results for the metal-sensitive species. In broad terms, a range of caddisfly species and two mayflies displayed reductions in

occurrence, while all common stoneflies showed either no reduction, or minor reduction at the five most contaminated sites.

At least three hundred individual diatoms were identified from each of the five most contaminated sites. Three of these sites yielded a very low number of species, suggesting that trace metals have harmful effects.

Clear evidence was obtained in five selected instances for reduced macroinvertebrate and diatom diversity, due to trace metals. Therefore, it was considered that the feasibility of the project had been demonstrated, and we proceeded to select the field sites for the 2006 field programme.

Table 3.2 Occurrences of macroinvertebrate taxa.

Taxon		A ¹	B ²	C ³
Mayfly	<i>Rhithrogena</i> sp.	79.5	80	40
Beetle	<i>Elmis aenea</i> (Muller)	77.0	80	40
Caddisfly	<i>Hydropsyche siltalai</i> Dohler	59.5	20	0
Mayfly	<i>Heptagenia lateralis</i> (Curtis)	49.7	20	0
Mites	Hydracarina	47.0	50	20
Caddisfly	<i>Polycentropus flavomaculatus</i> (Pictet)	45.4	20	0
Caddisfly	<i>Sericostoma personatum</i> (Spence)	39.7	20	0
Beetle	<i>Hydraena gracilis</i> Germar	34.7	20	0
Caddisfly	<i>Halesus radiatus</i> (Curtis)	32.5	20	0
Caddisfly	<i>Drusus annulatus</i> (Stephens)	29.9	60	20
Caddisfly	<i>Agapetus</i> sp.	27.9	40	0
Caddisfly	<i>Potamophylax cingulatus</i> (Stephens)	27.4	60	20
Limpet	<i>Ancylus fluviatilis</i> Muller	26.2	10	0
Muscid fly	<i>Limnophila (Eloeophila)</i> sp.	20.9	30	0
<i>Taxon with enhanced occurrence at trace metal sites</i>				
Caddisfly	<i>Plectrocnemia conspersa</i> (Curtis)	21.3	20	80

Notes: ¹RIVPACS-predicted mean % probability of taxon occurrence at 15 study sites, assuming them not to be affected by trace metals.

²observed % occurrence at 10 RIVPACS reference sites in adjacent NGR squares (with similar altitude and site characteristics).

³observed % occurrence at five highly contaminated trace metal sites for which robust RIVPACS predictions could be made.

3.1.3 Choice of study sites

At this point in the project, it was anticipated that the available funding permitted a total of 34 sites to be used, of which eight were intended as 'control' sites, not significantly affected by trace metals. We considered that, for macroinvertebrates, eight control sites will be sufficient, because the RIVPACS database contains many other sites that are suitable references, for which data are already available. Given that (a) the macroinvertebrate approach was likely to provide the most reliable results, because of the RIVPACS background, and (b) the unfeasibility of sampling diatoms at different sites to the macroinvertebrates, eight control sites were applied to the diatoms also.

The following criteria were applied sequentially to choose metal-contaminated sites.

1. The streams were selected purely on physical and chemical grounds. Thus, although we have demonstrated ecological effects, this knowledge was not used to choose sites, to avoid bias towards those sites that would give 'positive' results.

2. The streams should have physical characteristics (distance from source, width, slope, substratum) that conform to RIVPACS requirements, to ensure that valid comparisons with reference sites can be made, thereby fully exploiting the large body of information held in the RIVPACS database.
3. The sites should be reasonably accessible, in order to ensure that all can be visited within the time available for the fieldwork, and to maximise the time available for the detailed sampling.
4. Duplication of sites, that is the location of two (or more) sites on the same stream, should be avoided.
5. The trace metal concentrations should exceed current EQSs. Here, we considered EQSs used by the Environment Agency and in the Netherlands, together with critical limit functions (CLFs) for soil solution (Lofts *et al.*, 2004; de Vries *et al.*, 2004). To take account of the combined effects of several trace metals, we used the cumulative criterion unit (CCU; US EPA, 1986; see also Clements *et al.*, 2000; Hirst *et al.*, 2002), defined as the sum of the ratios of metal concentration to the EQS. If CCU exceeds unity, then the stream is considered contaminated.

Application of Criteria [2] and [3] reduced the number of potential sites to 33. In fact, only four sites failed the accessibility test (reachable within 20 minutes from the vehicle), and in any case none of these sites met the RIVPACS criteria. Two sites were rejected because they were on the same stream as other sites (Criterion 4), leaving 31 sites. To apply Criterion [5], values of CCU for the 31 sites were computed, for each EQS system. In four cases, CCU did not exceed two for any of the EQS systems, and therefore these sites were rejected. This left 27 sites. As things turned out, the additional funding from the Metals Industry permitted a total of 35 sites (including 8 controls) to be sampled, and so the 27 sites remaining at this point were all selected for use in the study. However, during the initial stages of the 2006 field campaign, it became clear that one of the chosen streams (S41) was too narrow to be valid in the RIVPACS assessment, and so a new site was substituted. Eight control sites were identified on the basis of previous data and experience. They were located in the Lake District, Ribblesdale, Swaledale and the Howgill Fells, and therefore within convenient travelling distance of the CEH Lancaster base and the other sites. The names and locations of the 35 selected sites are listed in Table 3.3.

Additional comparative information on invertebrate assemblages was utilised from equivalent and geographically adjacent RIVPACS reference sites (initially selected as the best available and closest to stress-free) that have been previously sampled and analysed by CEH and its predecessor organisations. In northwest England (confined to NGR squares adjacent to the present study area) there are 98 RIVPACS reference sites. Of these 98 reference quality sites most are discharge category 3 or more, while 13 are discharge category 2 or less and directly comparable to the small streams used in the present study.

Table 3.3 The sites selected for the main field programme.

No.	Name	NGR	Area
1	Dell Beck	SD 291 985	Lake District
2	Levers Water Beck	SD 287 984	Lake District
3	Dale Head Gill	NY 242 007	Lake District
4	Mosedale Beck	NY 245 018	Lake District
5	Uzzicar stream	NY 236 218	Lake District
6	Newlands Beck above spoil	NY 231 182	Lake District
7	Newlands Beck below spoil	NY 231 185	Lake District
8	Eller Gill	NY 252 183	Lake District
9	Roughton Gill	NY 302 347	Lake District
10	Wood Head stream	NY 336 365	Lake District
11	Threlkeld stream	NY 325 259	Lake District
12	Brown Gill	NY 751 424	Tynedale
13	Rough Rigg stream	NY 823 343	Teesdale
14	Ashgill Head stream	NY 808 354	Teesdale
15	Reddycomb Sike	NY 808 339	Teesdale
16	Willyhole Sike	NY 812 334	Teesdale
17	Harwood Beck	NY 813 337	Teesdale
18	Langdon Beck	NY 849 334	Teesdale
19	Langdon Beck tributary	NY 848 336	Teesdale
20	Sedling Burn	NY 857 407	Weardale
21	Nenthead stream #1	NY 781 436	Tynedale
22	Nenthead stream #2	NY 784 434	Tynedale
23	Nenthead stream #3	NY 784 434	Tynedale
24	Nentsberry stream #1	NY 765 446	Tynedale
25	Nentsberry stream #2	NY 758 445	Tynedale
26	Killhope Burn	NY 830 429	Weardale
27	Long Gill	SD 795 823	Ribblesdale
28	Slei Gill	NY 018 021	Swaledale
29	Black Mires Gill	NY 993 036	Swaledale
30	Great Punchard Gill	NY 961 044	Swaledale
31	Hurr Gill	NY 011 063	Swaledale
32	Uldale Beck	NY 818 036	Swaledale
33	Upper Scandale Beck	NY 742 028	Howgill Fells
34	Gais Gill	NY 716 011	Howgill Fells
35	Cluntering Gill	SD 712 800	Whernside

3.2 The main field programme, 2006

Figure 3.1 summarises the timing of fieldwork activities. The bulk of the fieldwork was carried out between 3 March and 28 April 2006. Some additional DGT measurements were performed in July 2006. Four sampling trips for general chemistry were made, and one each to collect invertebrates and diatoms. Additional chemical analysis work was done with DGT and DMT (see Sections 2.1.2 and 2.1.3), in each case samples being taken over a single period. Selected invertebrate species were collected for

analysis of metal body burdens (Section 2.1.4). Samples of bryophytes (Section 2.1.5) were collected during one of the chemistry sampling trips, and large volume water samples were also collected for laboratory toxicity testing (Section 2.3).

We measured streamwater temperatures routinely, and made semi-quantitative estimates of flow (Table 3.4). During one sampling trip we measured streamwater oxygen levels, and found them to be close to saturation in all cases.

Week beginning	06 Mar	13 Mar	20 Mar	27 Mar	03 Apr	10 Apr	17 Apr	24 Apr		10 Jul	24 Jul
Chemistry sampling	•		•		•		•				
Ecology sampling							•	•			
Water samples for TIE					•						
DGT					•		•			•	•
DMT						•	•	•			

Figure 3.1 Schedule of fieldwork in 2006.

Table 3.4 Summary of streamwater temperatures and flows.

Date	Sites	Temp °C	Flow ¹
08 March	1–11	6.3	3–4
09 March	12–26	3.6	3–4
10 March	27–35	5.0	2–4
22 March	1–11	6.2	2
23 March	12–26	2.3	1–2
24 March	27–35	3.8	1–3
5 April	1–11	7.4	2–3
6 April	12–26	4.7	2–4
7 April	27–35	5.8	1–2
10 April	12–26	6.3	2–3
13 April	12–26	6.5	2–3
19 April	1–11	9.6	2
20 April	12–26	7.6	1–2
21 April	27–35	8.6	2–3
22 April	1–11	9.5	1–2
23 April	12–26	10.8	1–2
24 April	27–35	9.7	2–3
27 April	27–35	9.2	2–3

¹1 = low, 2 = low-to-moderate, 3 = moderate, 4 = moderate-to-high, 5 = high.

4 Chemistry results

4.1 Concentrations of major solutes and trace metals

The streamwaters were sampled four times during the course of the study, and analysed for major solutes and trace metals.

4.1.1 Major solutes

Table 4.1 summarises the major solute data. As anticipated from the preliminary survey measurements (Section 3.1.1), the data reveal wide ranges of chemical conditions. Sites 1 to 8 are in the southern and central Lake District and show relatively soft water chemistry, the highest Ca concentration being 4.82 mg l^{-1} , and pH less than 7. Of these eight sites, number 5 has unusually high concentrations of Na, Mg, K and Cl, indicating water from sources (springs) other than rainfall; note that the other seven sites have very similar Na and Cl for example. Sites 9 and 10 in the northern Lake District have more Ca and alkalinity and higher pH. Site 11 is strongly influenced by a local disused lead mine, and its high sulphate concentration and low pH suggest that pyrite oxidation is occurring.

The 15 sites 12 to 26 are in Teesdale, Tynedale and Weardale, and they exhibit quite complex major ion chemistries. Sites 12, 13, 15, 16, 20, 24 and 25 have Na and Cl concentrations consistent with rainfall being the main source of water to the streams, but the other sites have appreciably higher Cl concentrations, suggesting contributions from deeper groundwater. Site 21 is high in sulphate, again probably from pyrite oxidation; however, weathering of Mg, K and Ca prevents the water being acid. These sites are generally of higher alkalinity than those in the Lake District, reflecting the widespread occurrence of limestone in the catchments. The range of mean pH of Sites 12 to 26 is 6.45 to 7.84.

Site 27, in upper Ribblesdale, has higher Na and Cl than would be expected from rainfall, and this suggests a groundwater influence. The same comment applies to Site 29, in Swaledale. Sites 28 and 30–35 have Na and Cl concentrations consistent with rainfall being the predominant source of streamwater, although the sulphate concentration of Site 28 is greater than anticipated from rainfall alone. Except for Sites 31 and 32, the influence of limestone dissolution on the water chemistries is clear. Thus, there is a strong correlation ($r^2 = 0.94$) between Ca and alkalinity, and the slope of the regression of alkalinity against Ca, expressed in molar concentrations, is very close to 2.0.

4.1.2 Nutrients

Table 4.2 summarises data for nutrients. The SiO_2 concentrations are quite low, indicating the slow weathering of silicate minerals in all the catchments, and where pH is neutral or slightly alkaline this can be attributed to the weathering of carbonates rather than alkaline silicates. Total phosphorus levels are all less than $50 \text{ } \mu\text{g l}^{-1}$, and the average value of $15 \text{ } \mu\text{g l}^{-1}$ is low compared to values of the order of $1000 \text{ } \mu\text{g l}^{-1}$ or more found for eutrophic lowland rivers (Muscutt and Withers, 1996). Ammonium concentrations are all less than $35 \text{ } \mu\text{g l}^{-1}$, and the mean is $5 \text{ } \mu\text{g l}^{-1}$, again very low

compared to lowland rivers. Nitrate concentrations are in most cases explicable in terms of atmospheric deposition inputs, although Sites 28 and 33 have values sufficiently high to suggest that fertiliser applications may be having an influence. There are no significant correlations between the different nutrients.

Table 4.1 Summary of major solute concentrations. Each value is the mean from four samples.

Site	pH	Na mg l ⁻¹	Mg mg l ⁻¹	K mg l ⁻¹	Ca mg l ⁻¹	Cl mg l ⁻¹	NO ₃ mg l ⁻¹	SO ₄ mg l ⁻¹	Alk meq l ⁻¹
1	6.75	2.65	0.85	0.28	2.32	4.35	0.386	2.215	0.06
2	7.00	2.49	0.68	0.27	3.03	3.57	0.360	1.573	0.08
3	5.14	2.76	0.44	0.16	0.44	4.83	0.450	0.921	0.00
4	5.67	2.68	0.41	0.13	0.65	4.53	0.235	0.984	0.00
5	6.79	6.30	1.41	0.65	4.82	11.64	0.421	2.908	0.18
6	6.8	2.88	0.65	0.14	1.73	4.87	0.103	1.248	0.07
7	6.78	2.86	0.65	0.14	1.72	4.86	0.106	1.248	0.07
8	6.72	2.99	0.76	0.13	1.69	4.94	0.097	1.563	0.07
9	7.58	3.53	2.14	0.52	6.75	5.68	0.216	1.320	0.46
10	7.91	4.21	2.37	0.56	12.48	5.66	0.171	2.730	0.74
11	4.09	3.26	3.09	0.50	4.67	6.20	0.296	18.800	0.00
12	7.56	3.18	2.45	0.91	14.61	6.42	0.233	5.065	0.66
13	6.45	2.66	0.56	0.32	3.57	5.53	0.135	1.014	0.08
14	7.76	4.33	3.72	1.08	20.82	8.52	0.128	3.848	1.17
15	7.84	2.49	2.70	1.05	24.62	4.83	0.114	3.406	1.35
16	7.84	2.58	1.66	0.56	23.34	5.15	0.191	1.857	1.25
17	7.84	8.96	1.83	0.76	20.95	18.63	0.102	2.250	1.05
18	7.24	5.28	2.34	0.72	12.10	10.30	0.097	2.333	0.64
19	6.58	9.84	0.86	0.42	5.53	20.25	0.124	2.145	0.13
20	7.16	3.14	1.64	0.73	7.84	6.21	0.181	3.805	0.27
21	7.75	8.28	9.36	2.09	33.45	13.97	0.108	26.130	1.21
22	7.06	22.22	2.05	0.65	10.92	42.79	0.094	5.548	0.29
23	7.51	4.14	2.64	0.66	12.80	7.46	0.126	7.680	0.46
24	6.87	2.90	0.99	0.79	5.35	6.33	0.122	2.495	0.35
25	7.59	2.99	0.96	0.59	12.40	6.04	0.084	2.560	0.57
26	7.02	12.54	1.57	0.59	9.09	24.83	0.160	2.803	1.68
27	7.48	8.14	0.92	0.37	9.03	14.37	0.090	0.979	1.33
28	8.23	4.14	5.04	0.77	31.13	7.61	0.775	6.080	0.65
29	7.83	4.20	1.13	0.57	26.90	7.98	0.114	2.090	0.40
30	7.75	4.29	1.78	0.43	12.40	7.45	0.229	2.341	0.39
31	6.88	3.59	1.73	0.45	9.24	6.69	0.268	3.165	1.45
32	5.64	3.96	0.71	0.48	7.11	7.82	0.184	1.570	0.28
33	8.33	3.25	1.80	0.65	27.26	6.47	0.516	1.575	0.34
34	7.52	3.38	1.58	0.33	4.74	5.85	0.058	1.665	0.00
35	7.55	3.19	0.97	0.37	5.21	5.78	0.105	1.125	0.00

Table 4.2 Streamwater concentrations of nutrients, DOC and SPM. Each value is the mean from four samples.

Site	SiO ₂ mg l ⁻¹	P-tot µg l ⁻¹	NH ₄ µg l ⁻¹	NO ₃ N mg l ⁻¹	DOC mg l ⁻¹	SPM mg l ⁻¹
1	1.73	5.7	4.9	0.39	0.7	0.6
2	1.52	7.7	2.5	0.36	0.8	0.6
3	1.81	5.5	2.5	0.45	1.1	0.3
4	2.13	6.1	2.5	0.24	1.2	0.4
5	3.26	13.1	4.4	0.42	0.6	0.2
6	1.85	7.9	2.5	0.10	0.9	0.2
7	1.85	5.2	2.5	0.11	0.9	0.5
8	1.95	7.3	2.5	0.10	0.8	0.8
9	5.61	25.9	4.1	0.22	2.0	8.1
10	9.76	42.5	3.1	0.17	1.2	3.9
11	4.57	8.2	31.9	0.30	0.6	1.1
12	2.27	24.6	3.4	0.23	5.8	9.6
13	0.85	11.5	3.6	0.13	8.9	4.2
14	2.59	8.4	3.1	0.13	4.8	6.6
15	1.66	22.0	3.9	0.11	5.4	32.7
16	1.11	18.6	3.4	0.19	6.6	9.0
17	1.12	24.8	2.5	0.10	5.6	12.6
18	1.74	29.5	3.6	0.10	6.4	8.6
19	1.48	14.6	10.3	0.12	5.9	7.1
20	2.39	10.8	2.5	0.18	5.5	3.3
21	3.00	17.7	4.1	0.11	3.4	5.3
22	1.70	18.4	4.4	0.09	6.1	4.1
23	1.85	18.4	3.6	0.13	4.8	5.7
24	1.57	19.6	2.5	0.12	7.8	2.4
25	1.47	26.2	15.1	0.09	6.3	9.4
26	2.12	35.1	2.5	0.16	7.5	42.1
27	1.16	9.5	3.1	0.10	7.2	0.6
28	4.67	9.0	2.5	0.80	3.4	1.0
29	2.57	12.1	5.1	0.12	4.6	3.1
30	3.75	10.8	5.8	0.28	6.8	1.1
31	3.64	16.9	15.8	0.15	7.4	4.9
32	2.60	9.1	2.5	0.19	4.7	0.9
33	1.36	18.5	2.5	0.65	4.5	1.0
34	2.65	8.2	2.5	0.03	2.7	1.0
35	1.41	10.5	3.6	0.06	8.1	4.6

4.1.3 Dissolved organic carbon and suspended particulate matter

As shown in Table 4.2, the concentrations of DOC in the Lake District streamwaters (Sites 1 to 11) are low, with only one (Site 9) reaching even 2 mg l⁻¹. Average concentrations in the remaining streams are appreciably higher, ranging from 3 to almost 9 mg l⁻¹. As will be seen in Section 4.4, these concentrations are sufficient to produce substantial effects on copper and lead speciation.

The SPM concentrations in the Lake District sites (1–11) are low; the value for Site 9 is by far the highest at 8.1 mg l⁻¹. The concentrations of SPM in streams 12–35 varied between 0.6 and 42.1 mg l⁻¹. There are no obvious correlations between DOC and SPM concentrations.

4.1.4 Metals

Table 4.3 shows concentrations of metals in the different streams. Although the metal contamination only originates from copper and lead mines, the metal present at the highest concentration is, in nearly all cases, zinc. Site 5 is interesting because it has very low Al ($< 1 \mu\text{g l}^{-1}$), and this sets it aside from all the others, which have concentrations of at least $9 \mu\text{g l}^{-1}$, and up to several hundred and even in excess of $1000 \mu\text{g l}^{-1}$ at the highly acid Site 11. The sites are generally free of other obvious metallic contaminants, with the exception of Site 10, which has a high concentration of arsenic.

Correlations between pairs of metals were examined, using logarithms of average total dissolved concentrations because of the wide ranges of values. A number of significant relationships were found, the strongest ($r^2 > 0.5$) being between Cd and Zn ($r^2 = 0.93$), Pb and Zn (0.63), Pb and Cd (0.59) and Zn and Ni (0.58). There were no significant correlations between Al and any of the toxic trace metals.

Figure 4.1 compares the metal concentrations in the study sites with those in a range of other streams in the English uplands including sites that are being studied in connection with heavy metal critical loads (Tipping *et al.*, 2005a, 2005b; Hall *et al.*, 2006), and sites that have been characterised for hydrochemical and ecological research in the Eden basin (Simon *et al.*, 2004). In the critical loads work, the sites have been chosen to reflect only atmospheric inputs of trace metals, and catchment-scale dynamic modelling has been used, in conjunction with estimated past metal deposition and catchment weathering rates, to calculate concentrations of metals in streams under 'pristine' conditions (i.e. before significant anthropogenic inputs from atmospheric deposition), and results for these conditions are also shown.

It can be seen that the concentrations of all five trace metals tend to be higher in the study sites than in the 'current background' sites, and this especially applies to Zn, Cd and Pb. It is also seen that the 'current background' concentrations exceed those estimated for pristine conditions, again more noticeably for Zn, Cd and Pb. In the case of Al, there is not much difference between pristine and current background if pH is taken into account, but the concentrations in the study streams tend to be greater than those in the 'current background' streams.

4.1.5 Comparison of dissolved metal concentrations with EQS values

The metal concentrations of Table 4.3 can be compared with EQS values that are based on total dissolved concentrations. The simplest EQS systems use a single metal concentration irrespective of the water chemistry. An example is the Dutch system, which defines Maximum Permissible Concentrations (MPCs) for Ni, Cu, Zn, Cd and Pb of 5.1, 1.5, 9.4, 0.42 and $11 \mu\text{g l}^{-1}$, respectively (Crommentuijn *et al.*, 1997). Figure 4.2 compares the average values of Table 4.3 with these MPC values, in terms of the ratio, r_{EQS} (i.e. the ratio of the observed concentration to the MPC). Figure 4.3 does the same for current Environment Agency EQS values, which are hardness dependent. Table 4.4 summarises the exceedances of individual metals under the two systems. There is broad agreement, but the Dutch values are exceeded appreciably more for Cd, while the Environment Agency limits are exceeded somewhat more for Cu and Pb. Figure 4.4 shows values of the CCU, as defined in Section 3.1.3.

The greatest exceedance, and the most sites exceeding, are seen for Zn. This seems unavoidable, and reflects the geochemistry of the mine ores. The values of CCU cover three orders of magnitude, which suggests that a range of ecological responses is likely.

Table 4.3 Streamwater concentrations of metals. Each value is the mean from four samples.

Site	Al µg l ⁻¹	Mn µg l ⁻¹	Fe-tot µg l ⁻¹	Fell µg l ⁻¹	Ni µg l ⁻¹	Cu µg l ⁻¹	Zn µg l ⁻¹	As µg l ⁻¹	Cd µg l ⁻¹	Pb µg l ⁻¹
1	23.6	16.0	10.5	2.5	0.32	7.5	18.3	0.49	0.13	0.2
2	29.9	16.4	9.0	2.5	0.32	9.4	14.2	0.34	0.09	0.1
3	171.0	25.7	12.0	2.5	0.23	0.5	4.3	0.16	0.05	0.3
4	87.7	9.5	9.0	2.5	0.12	0.2	1.9	0.10	0.02	0.1
5	0.1	5.4	15.0	2.5	1.16	1.1	427.5	0.52	2.77	76.2
6	9.4	2.0	13.5	2.5	0.31	0.4	4.9	0.26	0.02	2.2
7	9.8	2.4	8.0	2.5	0.33	0.7	6.1	0.26	0.04	4.0
8	10.1	6.2	12.0	2.5	0.37	0.5	5.2	0.54	0.03	0.7
9	18.9	3.8	19.0	6.0	1.70	2.4	42.1	4.52	0.26	46.4
10	11.7	13.6	15.0	2.5	0.76	4.5	366.0	278	2.76	27.1
11	1565	1095	45.8	9.0	75.93	5.2	10965	0.39	19.13	155.5
12	62.6	19.9	133.8	39.0	4.12	2.5	404.8	0.20	0.69	26.4
13	117.7	26.5	207.0	87.8	1.05	1.0	25.6	0.25	0.06	7.9
14	64.1	50.6	158.5	44.3	2.05	1.3	273.8	0.16	0.36	17.2
15	68.7	37.3	82.5	29.3	1.06	0.5	57.7	0.48	0.09	1.1
16	63.1	5.1	83.8	60.0	1.11	0.7	31.4	0.20	0.12	4.8
17	55.5	12.6	76.5	37.0	0.96	0.6	12.0	0.30	0.04	1.5
18	103.0	65.8	165.3	90.0	1.56	1.0	26.4	0.29	0.08	6.4
19	131.5	82.6	154.0	52.5	1.95	0.9	29.4	0.27	0.08	10.0
20	101.6	55.0	101.8	53.0	2.47	0.9	125.4	0.19	0.42	12.4
21	34.0	190.2	105.5	48.0	12.09	1.6	2814.8	0.28	5.32	19.4
22	58.3	57.5	148.0	45.0	2.97	1.4	589.5	0.33	1.66	12.0
23	40.7	18.1	75.8	33.0	2.67	1.9	1111.8	0.18	3.07	36.1
24	61.7	14.7	115.0	47.3	2.30	1.5	176.3	0.16	0.41	16.2
25	114.2	9.0	73.5	35.0	0.96	0.6	8.6	0.55	0.04	10.7
26	123.3	46.6	202.3	85.0	2.18	1.1	123.6	0.28	0.29	19.2
27	37.8	7.6	131.8	61.0	0.74	0.8	2.5	0.32	0.02	0.6
28	36.1	3.1	81.7	33.0	1.84	1.0	129.5	1.70	1.30	12.9
29	98.5	33.3	66.8	18.0	2.30	1.2	113.6	0.23	0.44	8.5
30	82.8	11.7	157.0	68.0	1.52	0.5	14.2	0.31	0.08	4.0
31	98.5	55.4	203.3	57.8	3.45	1.2	9.0	0.29	0.06	0.9
32	87.2	46.4	151.0	45.8	1.27	0.4	8.8	0.20	0.05	0.2
33	36.8	16.3	53.3	19.0	1.28	0.7	1.3	0.24	0.01	0.2
34	19.7	2.1	21.8	11.0	0.44	0.4	1.1	0.21	0.00	0.1
35	76.8	4.9	149.0	57.0	0.82	0.6	2.7	0.27	0.02	0.4

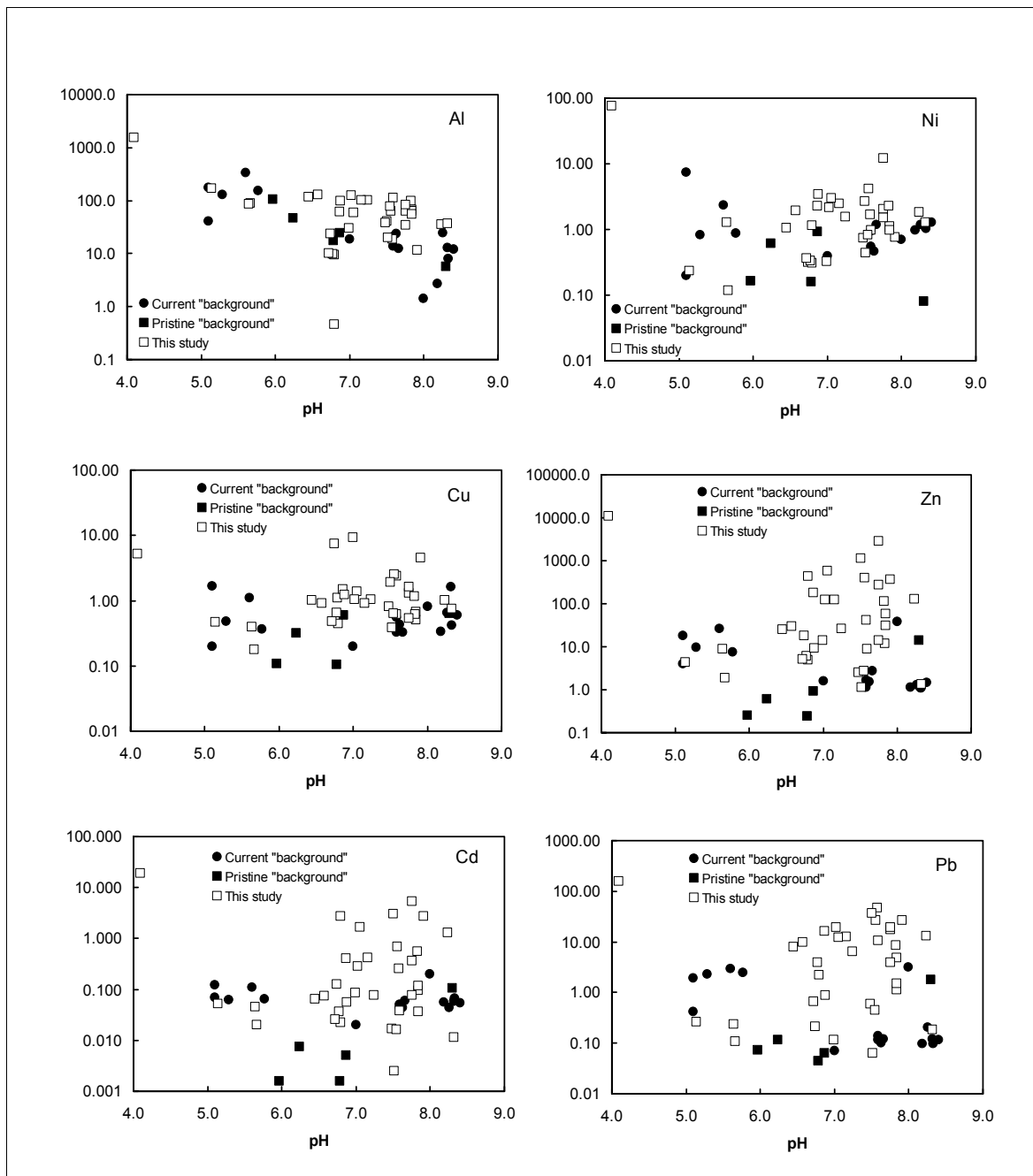


Figure 4.1 Dissolved metal concentrations ($\mu\text{g l}^{-1}$) as a function of pH, in the study streams, in streams representative of current background conditions, and estimated for streams under pristine conditions.

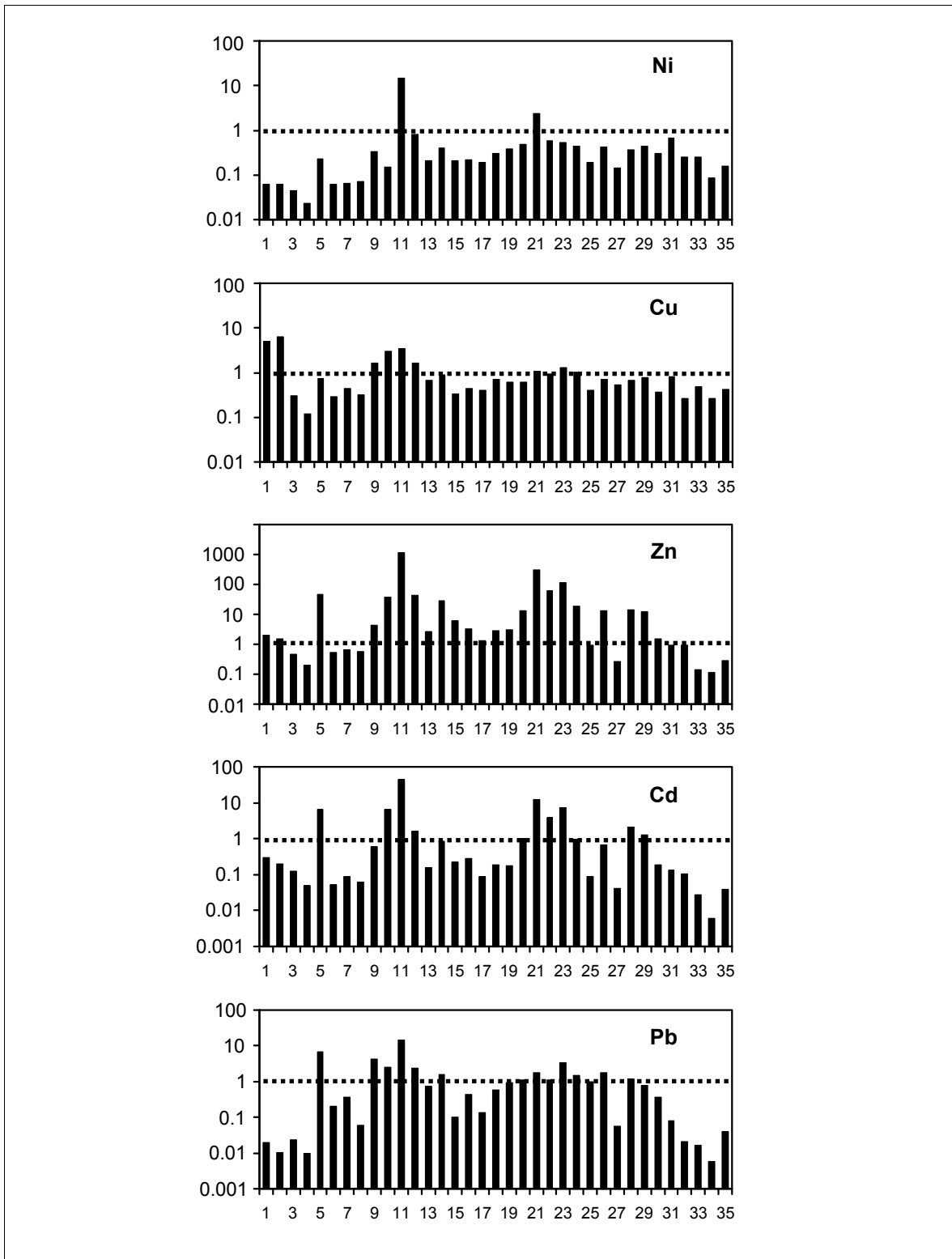


Figure 4.2 Values of r_{EQS} based on the Dutch MPC system.

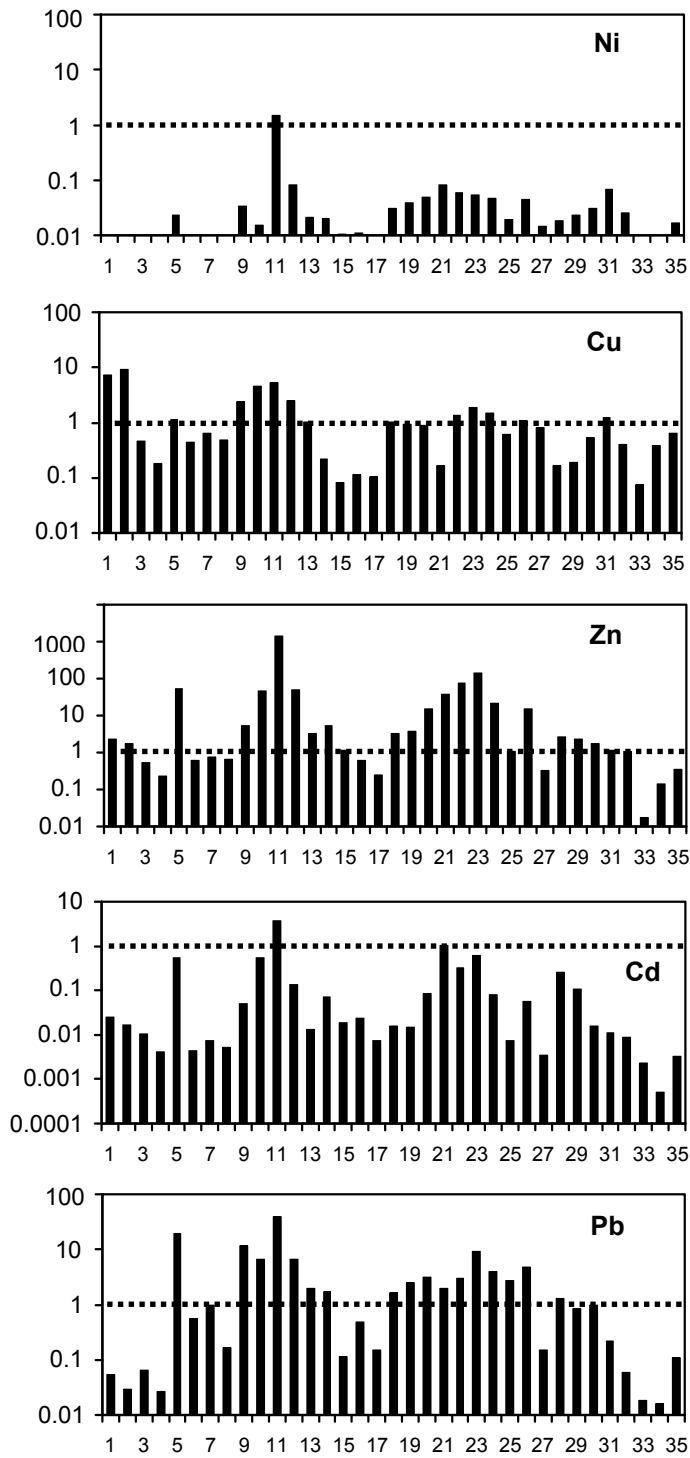


Figure 4.3 Values of r_{EQS} based on the Environment Agency EQS system.

Table 4.4 Numbers of sites showing EQS exceedances.

Metal	Dutch MPC	Environment Agency EQS
Ni	2	1
Cu	9	14
Zn	23	24
Cd	9	2
Pb	13	17

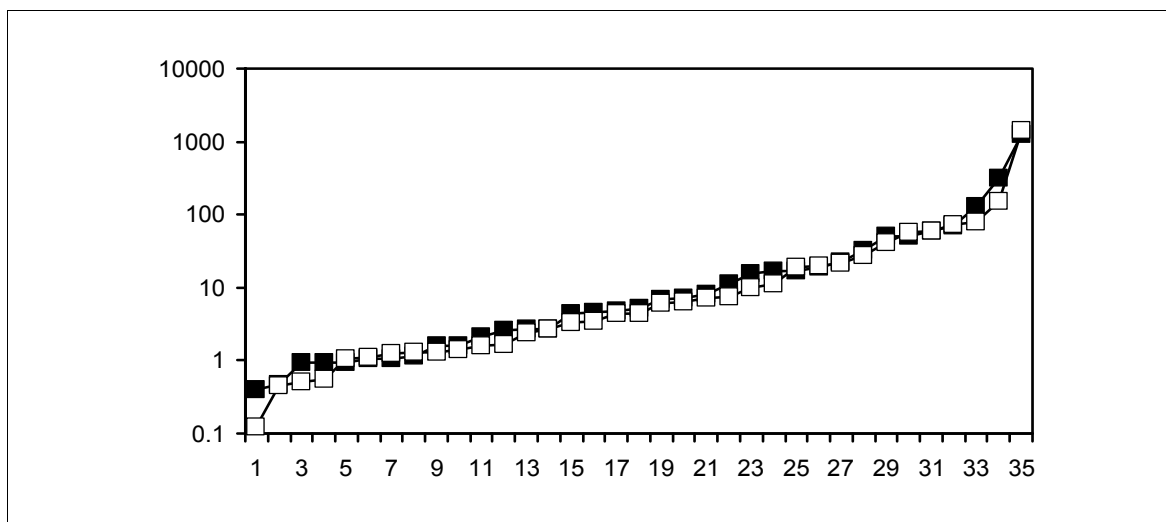


Figure 4.4 CCU values for the Dutch (filled) and Environment Agency (open) EQS systems. The values are ordered in terms of increasing CCU, so the numbers do not correspond to those in Table 3.3, nor is the ordering the same for each EQS system.

4.2 Measurements by Diffuse Gradients in Thin films

In total, 204 Diffuse Gradients in Thin films (DGT) devices were deployed: three devices of each type (open and restricted pore gels) at each of 34 sites. All the open pore gel devices deployed were successfully retrieved and analysed. Five restricted pore devices (two at Site 13 and one each at Sites 15, 21 and 28) were not successfully retrieved. The reproducibility of replicate analyses (expressed as percentage standard deviations of three replicates) was 9% overall for open pore gels (varying between 6 and 12% on a per metal basis) and 16% overall for restricted pore gels (between 12 and 23% on a per metal basis).

Concentrations of metals measured using open pore gels (denoted M_{ODGT}) are compared with spot samples of filtered ($< 0.45 \mu\text{m}$) metals in Figure 4.5. Some trends can be deduced from the plots:

1. Filterable concentrations of Al and Cu were almost exclusively higher than those measured by DGT. Values of Al_{ODGT} and Cu_{ODGT} were within a factor of two of the mean filterable concentrations at eight and two sites, respectively. The Al_{ODGT} was more than twice the mean filterable concentration at only two sites, and there were no sites where the Cu_{ODGT} was more than twice the mean filterable concentration. For both metals there is a general trend for the

difference between measurements to be larger with a smaller filterable concentration.

2. Filterable concentrations of Ni and Pb were occasionally higher than the DGT-measured values, but were frequently similar or lower. Values of Ni_{oDGT} and Pb_{oDGT} were within a factor of two of the mean filterable concentrations at 18 and 10 sites, respectively. The Ni_{oDGT} was more than double the mean filterable concentration at only one site and the Pb_{oDGT} was more than double the mean filterable concentration at four sites.
3. Filterable concentrations of Zn and Cd were largely similar to those measured by DGT, being within a factor of two at 25 and 28 sites, respectively, but there were some exceptions where filterable metal was either higher or lower than M_{oDGT} .

Since the concentration of DGT-labile metal may be affected by chemical speciation, we considered the possible effects of pH and dissolved organic carbon (DOC) by calculating the correlation coefficients between these variables, and the difference between filtered metal and M_{oDGT} . Differences between $\log(\text{mean filtered metal})$ and $\log(M_{oDGT})$ were calculated to avoid overemphasising outlying differences. Differences in Al and Ni concentrations were significantly ($p < 0.05$) correlated with pH (correlation coefficients -0.42 and -0.43, respectively). Inspection of these data showed that the correlations were driven by four sites with mean pH below 6; after removal of these sites the correlations were no longer significant. Differences in Al, Ni, Cu and Pb were significantly correlated with DOC concentration (coefficients were -0.62, -0.58, -0.58 and -0.50, respectively). The negative correlation coefficients indicate that M_{oDGT} tended to reduce relative to filtered metal as DOC increased. This is consistent with a reduction in DGT-labile metal relative to filtered metal, due to increased organic complexation of metal. In the case of Al it is possible that fine colloidal matter (e.g. aluminosilicates), if present, may pass a 0.45 μm filter and contribute to non-DGT-labile material within the filterable pool.

No significant correlations were observed for Zn and Cd. Differences between M_{oDGT} and mean filterable concentrations were sometimes large: up to a factor of 77 for Zn, at Site 16, and up to a factor of 10 for Cd, at Site 29. The reasons for these differences cannot be explained by the observed stream chemistries.

Concentrations of metals measured using restricted pore DGT (M_{rDGT}) are compared with concentrations measured using open pore devices in Figure 4.6. The metal concentrations measured using the two types of device were very similar: of the 204 measurements of M_{rDGT} made, 192 were within a factor of two of the corresponding measurement of M_{oDGT} .

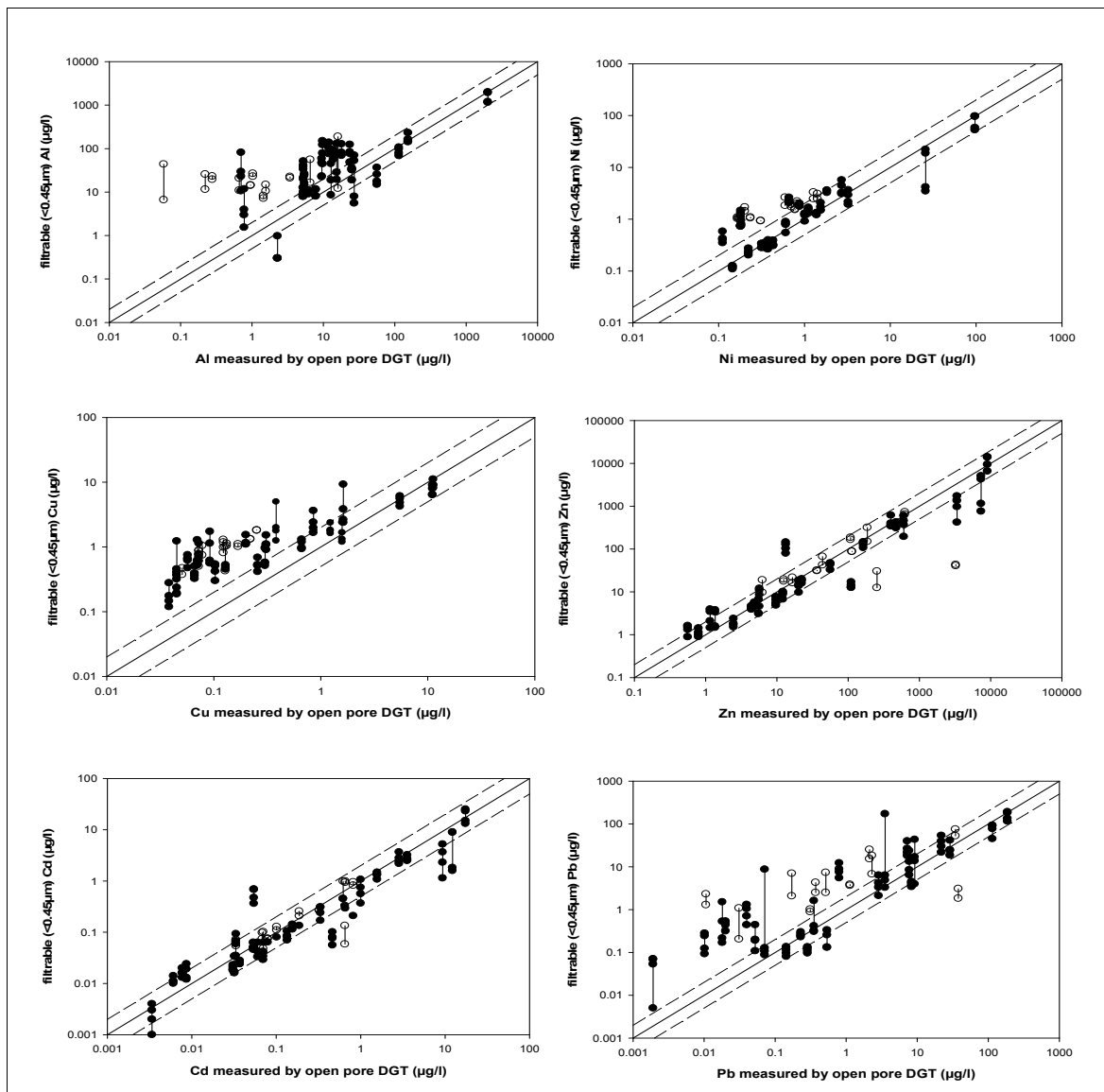


Figure 4.5 Concentrations of metals measured by open pore DGT deployment, compared to spot measurements of the metals in filterable form. Closed circles represent sites at which DGT was deployed in March–April 2006, open circles sites at which DGT was deployed in July 2006. March–April 2006 deployments are compared with four spot samples taken during this period. July 2006 deployments are compared with spot samples taken at the beginning and the end of the deployment period. The vertical lines indicate the ranges of concentrations measured in spot samples and are for guidance.

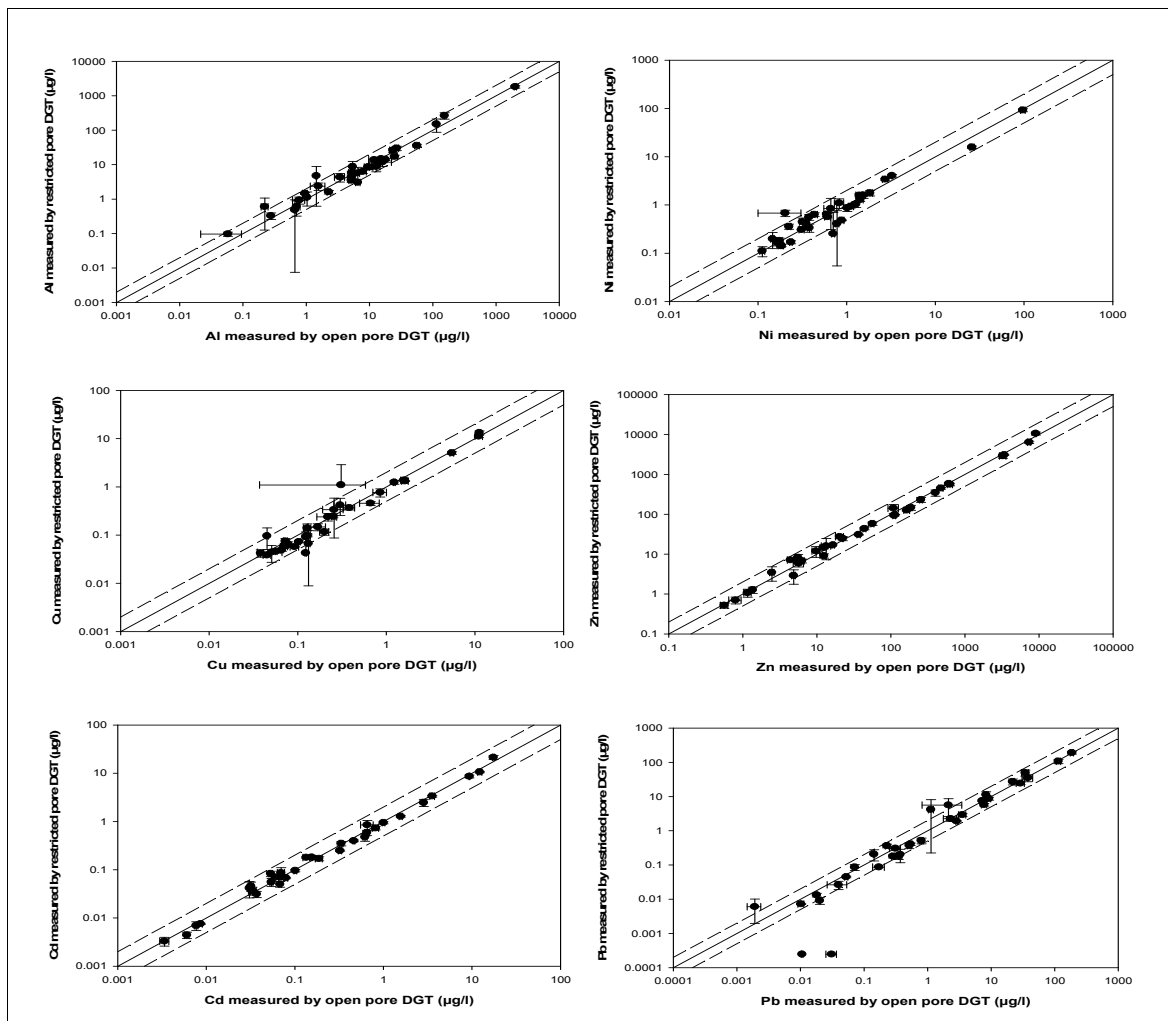


Figure 4.6 Concentrations of metals measured by restricted pore DGT devices, compared to concentrations measured using open pore devices. Means of three replicates, error bars indicate ± 1 standard deviation.

4.3 Measurements by Donnan Membrane Technique

Free ion concentrations were estimated using the Donnan Membrane Technique (DMT), deployed in the field for three days on the dates in April 2006 shown in Figure 3.1. The measured values are compared with total filterable metal concentrations in Figure 4.7. As would be expected, the free ion concentrations are lower than the total filterable concentrations, because some of the filterable metal is complexed with dissolved ligands (e.g. OH^- , HCO_3^- , DOM), or possibly bound to colloidal particles. Of the five trace metals under study, Zn is the least complexed, so that concentrations of Zn^{2+} come closest to the total filterable concentrations. The tendency for each of the metals to be complexed then increases in the order $\text{Zn} < \text{Cd} < \text{Ni} < \text{Pb} < \text{Cu}$. These findings are consistent with modelling results, as discussed below (Section 4.4).

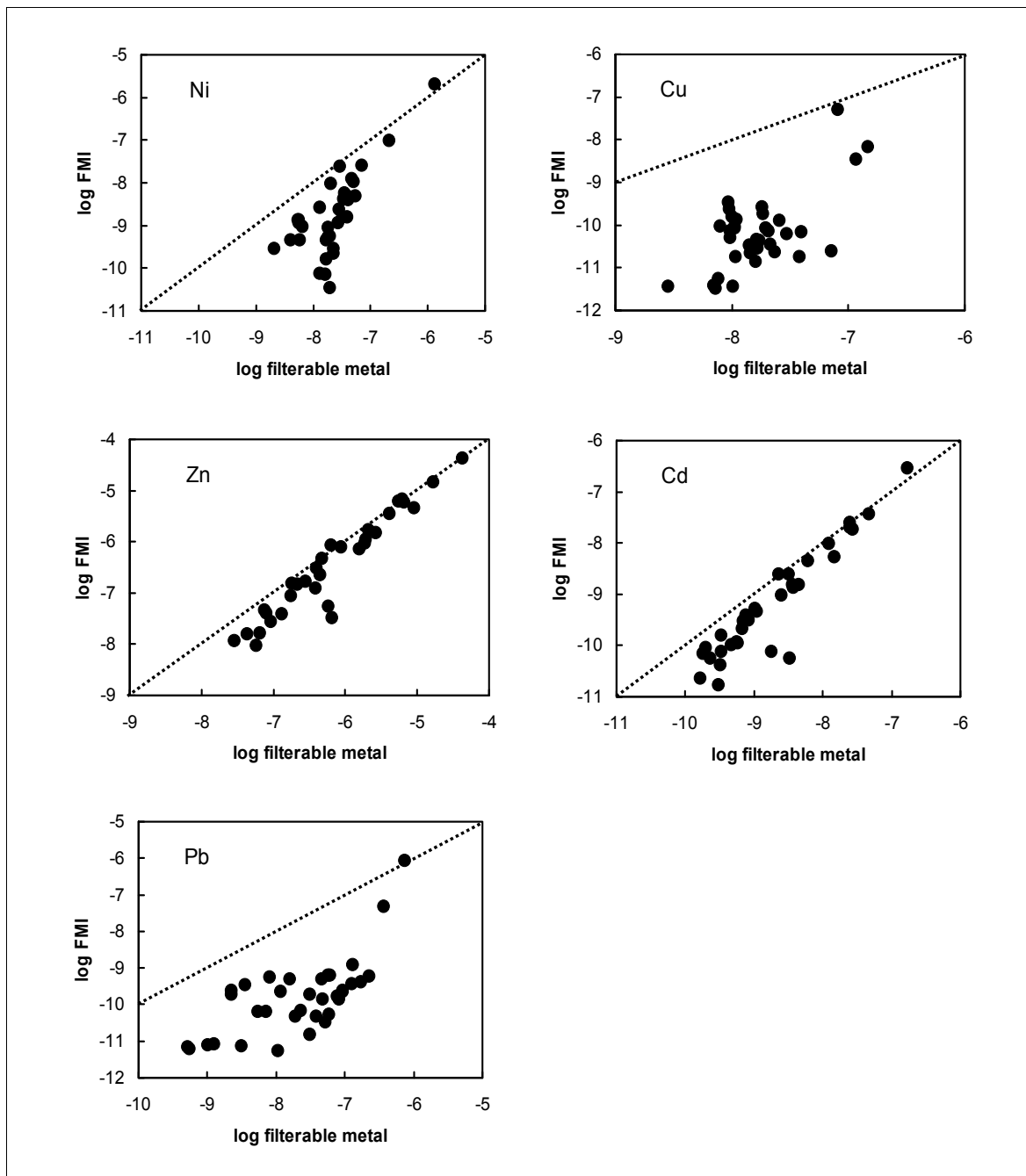


Figure 4.7 Free metal ion (FMI) concentrations estimated using DMT compared with total filterable concentrations. The units of both axes are \log_{10} molar concentration.

4.4 Chemical speciation modelling

4.4.1 Application of WHAM

We used WHAM (Tipping, 1994) incorporating Humic Ion-Binding Model VI (Tipping, 1998) to calculate the speciation of the trace metals. The calculations were performed assuming that the DOM was 65% active, as estimated in previous work (Bryan *et al.*, 2002) on the basis of copper binding. The activity of Fe^{3+} , which can be important for competition, was assumed to be controlled by $\text{Fe}(\text{OH})_3$ with a solubility product of $10^{4.0}$

at 25 °C, for the reaction $\text{Fe}(\text{OH})_3 + 3\text{H}^+ = \text{Fe}^{3+} + 3\text{H}_2\text{O}$. Two alternative assumptions were made for Al^{3+} activity ($a_{\text{Al}^{3+}}$). Firstly, it was assumed to be controlled by $\text{Al}(\text{OH})_3$ with a solubility product of $10^{8.5}$ at 25 °C, for the reaction $\text{Al}(\text{OH})_3 + 3\text{H}^+ = \text{Al}^{3+} + 3\text{H}_2\text{O}$, in solutions where that solubility product would otherwise be exceeded (pH greater than about 5.8). This provides a reasonable explanation of available field data for waters in the pH range 4 to 7 (Tipping, 2005), but it is by no means certain that a true solubility control is operating, and the relationship may simply be an empirical fit. Furthermore, extrapolation to higher pH is uncertain. Secondly, all measured filtered Al was assumed to be truly dissolved, so that Al^{3+} activity was determined simply by solution speciation. Of the two alternatives, the $\text{Al}(\text{OH})_3$ solubility control assumption probably gives more realistic estimates of Al^{3+} , but further work is definitely required on this topic. A temperature of 10 °C was assumed in all cases.

Figure 4.8 compares free ion concentrations with total dissolved metal concentrations. In the case of Al, assumptions about Al^{3+} activity (see previous paragraph) lead to an average difference of 0.6 in $\log_{10} a_{\text{Al}^{3+}}$, the values being higher when $\text{Al}(\text{OH})_3$ solubility control is not applied. The assumptions about Al^{3+} activity had little effect on the calculated concentrations of Ni^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} , but Pb^{2+} concentrations were greater by about a factor of two when all Al was assumed dissolved, reflecting greater competition between the two metals for binding to DOM.

Free ion metal concentrations calculated for the present study sites were also compared with values for other streams not affected by local mining activities. The values are plotted against pH in Figure 4.9. It is clear that the trace metal values are higher in the study streams, most noticeably for Cu and Zn, in some cases by several orders of magnitude. The concentrations of Al^{3+} also tend to be higher, but the pattern is different from those of the trace metals, because of the strong pH dependence of Al speciation, which leads to a more regular pH dependence.

4.4.2 NICA–Donnan speciation calculations

Free metal ion concentrations in the streams were calculated by means of the speciation program ECOSAT (Keizer and Van Riemsdijk, 2002), in which the relevant (in)organic ion complexes are accounted for using the reaction constants in the database of this program. As with the WHAM modelling, we assumed that 65% of the total DOC concentration consisted of fulvic acid and 35% is inert (Tipping *et al.*, 2003). For this calculation the NICA–Donnan model was used, with generic parameters (Kinniburgh *et al.*, 1999; Milne *et al.*, 2003).

4.4.3 Comparing measured and modelled free metal ion concentrations

Calculated values from Sections 4.4.1 and 4.4.2 are compared with each other and with DMT results (Section 4.3) in Figure 4.10. The agreement is reasonable for Zn and Cd at all concentrations, mainly because neither metal interacts strongly with DOM, so that the 'correction' for complexation is minor. Agreement is less good for Ni, especially at low concentrations, where DMT suggests that binding to DOM is stronger than predicted by the models. Agreement is satisfactory for Cu and Pb at high concentrations, but relatively poor at low concentrations. Moreover, there is considerable scatter around the 1:1 line, indicating that the models do not consistently underestimate or overestimate free ion concentrations. The two models perform very similarly, as judged by the deviations from the measured values.

4.4.4 Comparison of speciation output with DGT measurements

The DGT devices are known to collect both inorganic and organically complexed metal, but because of the larger molecular size of metal complexed to DOM, the rate of diffusion of the organically complexed forms is smaller than that of the inorganic forms. Therefore the DGT measurement effectively represents all the inorganic metal plus a fraction of the organic. According to Unsworth *et al.* (2006), if the organic complexes are assumed to undergo fast dissociation kinetics, the total DGT value is given by;

$$C_{\text{dyn}} = [\text{inorganic metal}] + 0.20 \times [\text{organically complexed metal}] \quad (4.1)$$

Values of C_{dyn} were computed with WHAM, and are compared with the measured open pore DGT values in Figure 4.11, together with total dissolved metal concentrations and computed inorganic concentrations. For Ni, Zn and Cd, there are only small differences among the three types of metal concentration, but for Al, Cu and Pb the C_{dyn} values are closer to the measured DGT concentrations than either total metal or inorganic metal. The application of C_{dyn} is especially successful for Cu. The lower value of Cu measured by DGT compared to the predicted C_{dyn} at very low Cu concentrations is consistent with increased kinetic limitation of complex dissociation when a greater proportion of the strong binding sites of humic material are occupied.

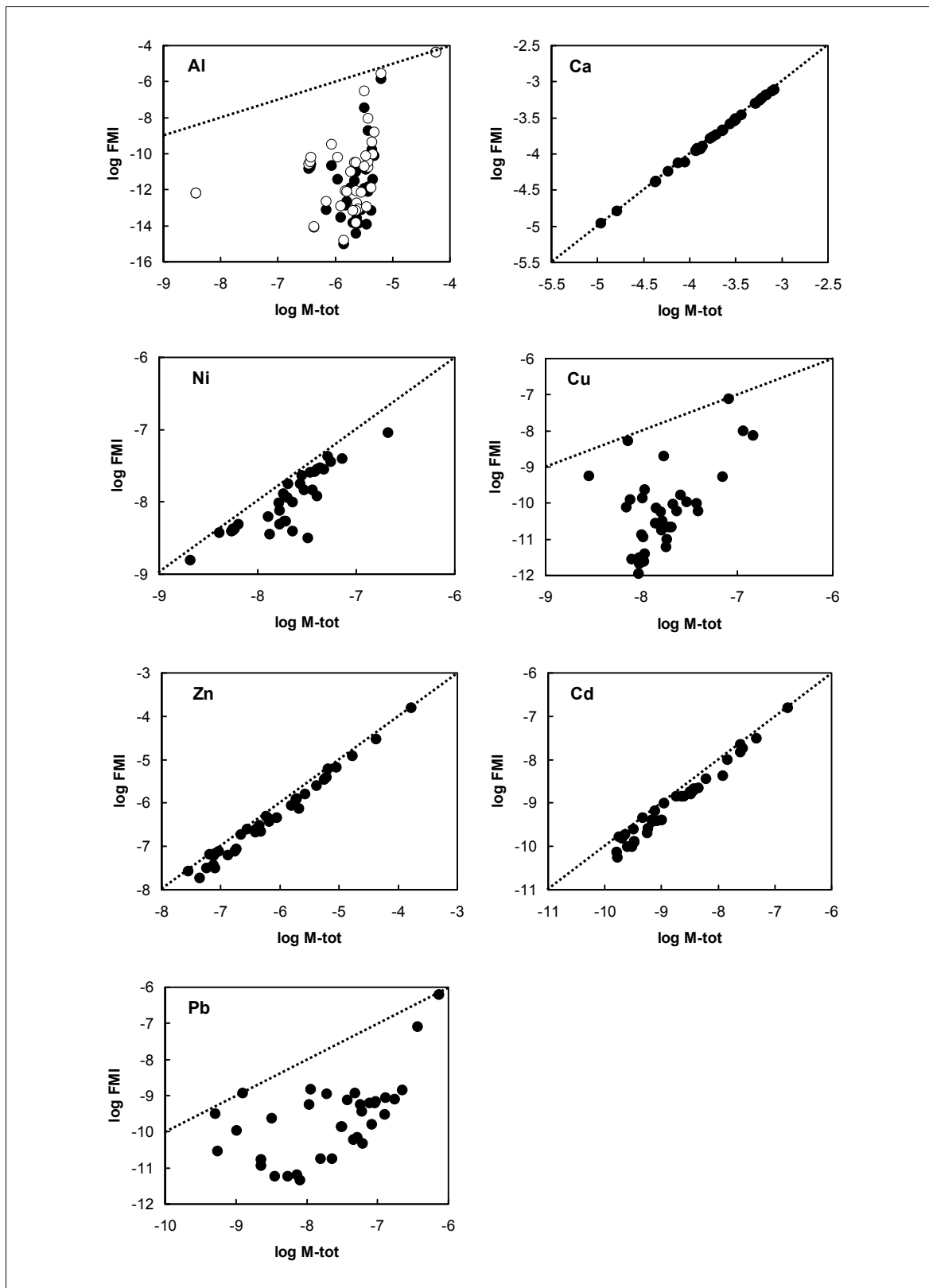


Figure 4.8 Free ion concentrations (mol l⁻¹), calculated with WHAM/Model VI, compared with total filterable metal concentrations. Except for Al, the results refer to the case where Al³⁺ activity solubility was assumed to be controlled by Al(OH)₃ with log K_{so} = 8.5 at 25°C. The Al panel compares this solubility control (filled circles) with results assuming all Al to be truly dissolved (open circles).

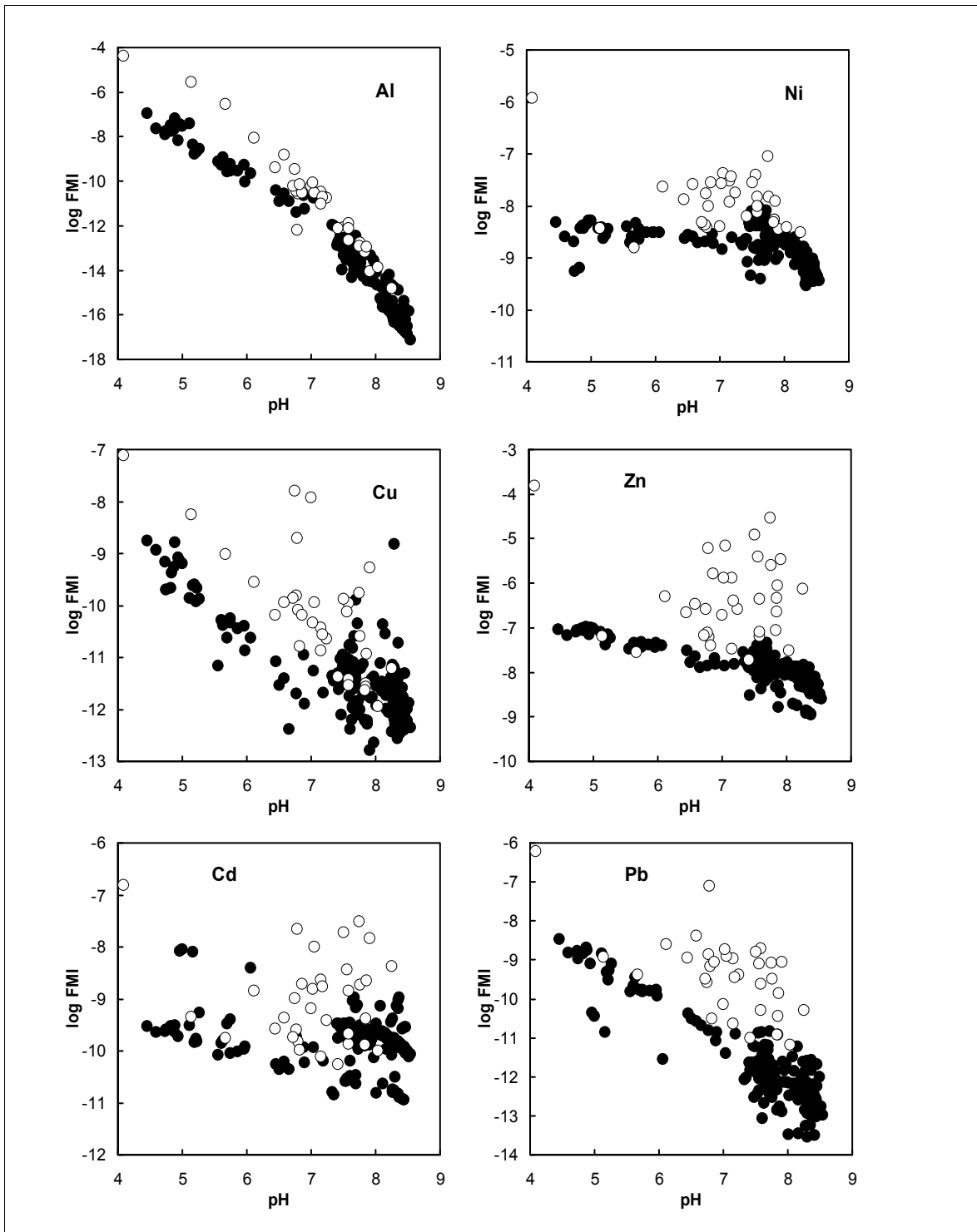


Figure 4.9 Calculated free ion concentrations (log values, mol l⁻¹) plotted against pH for sites from the present study (open circles), and for similar sites lacking catchment mining activities (closed circles).

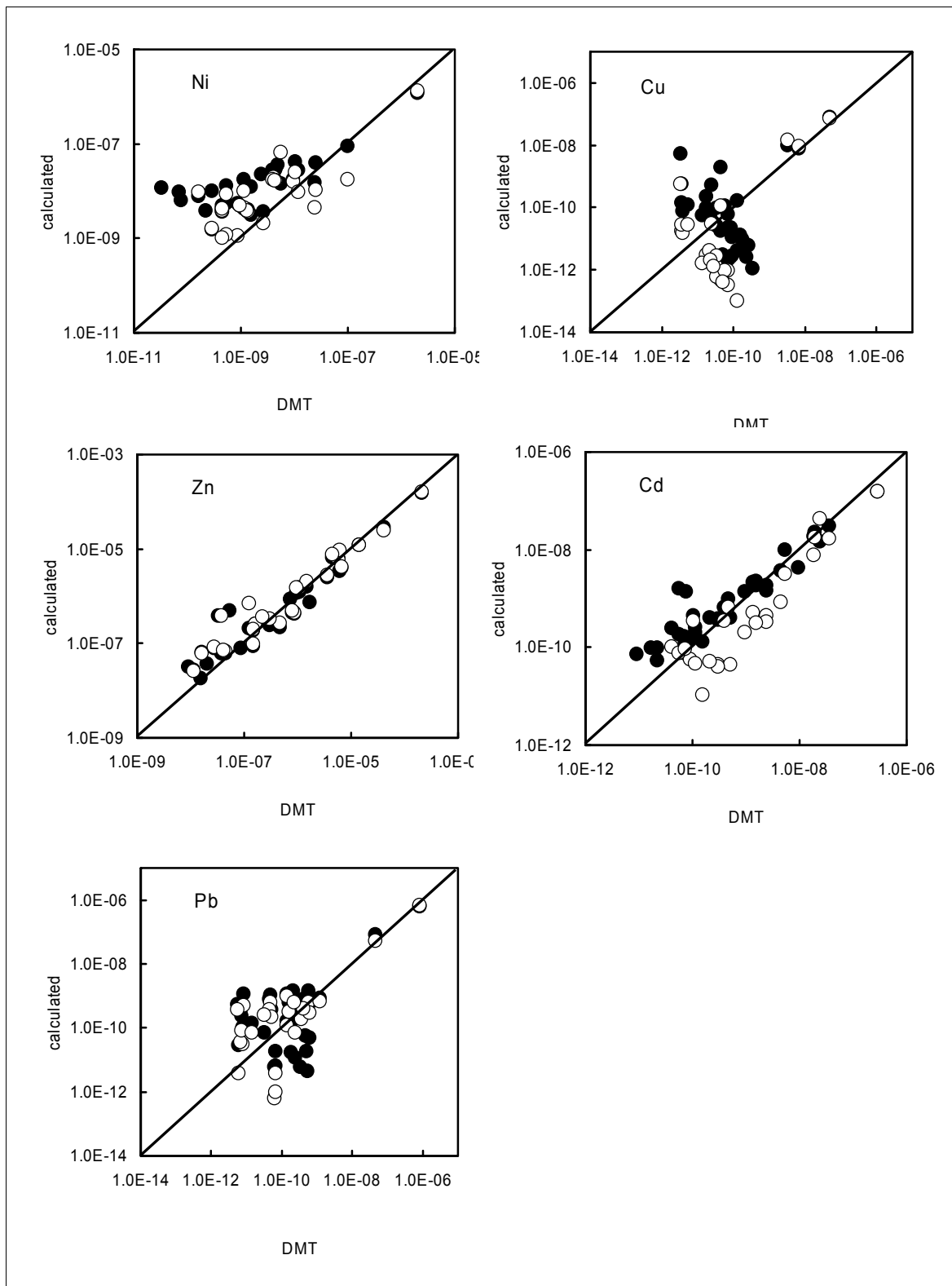


Figure 4.10 Free ion concentrations (mol l^{-1}) calculated with WHAM/Model VI (closed circles) and ECOSAT/NICA-Donnan (open circles), plotted against values measured using DMT.

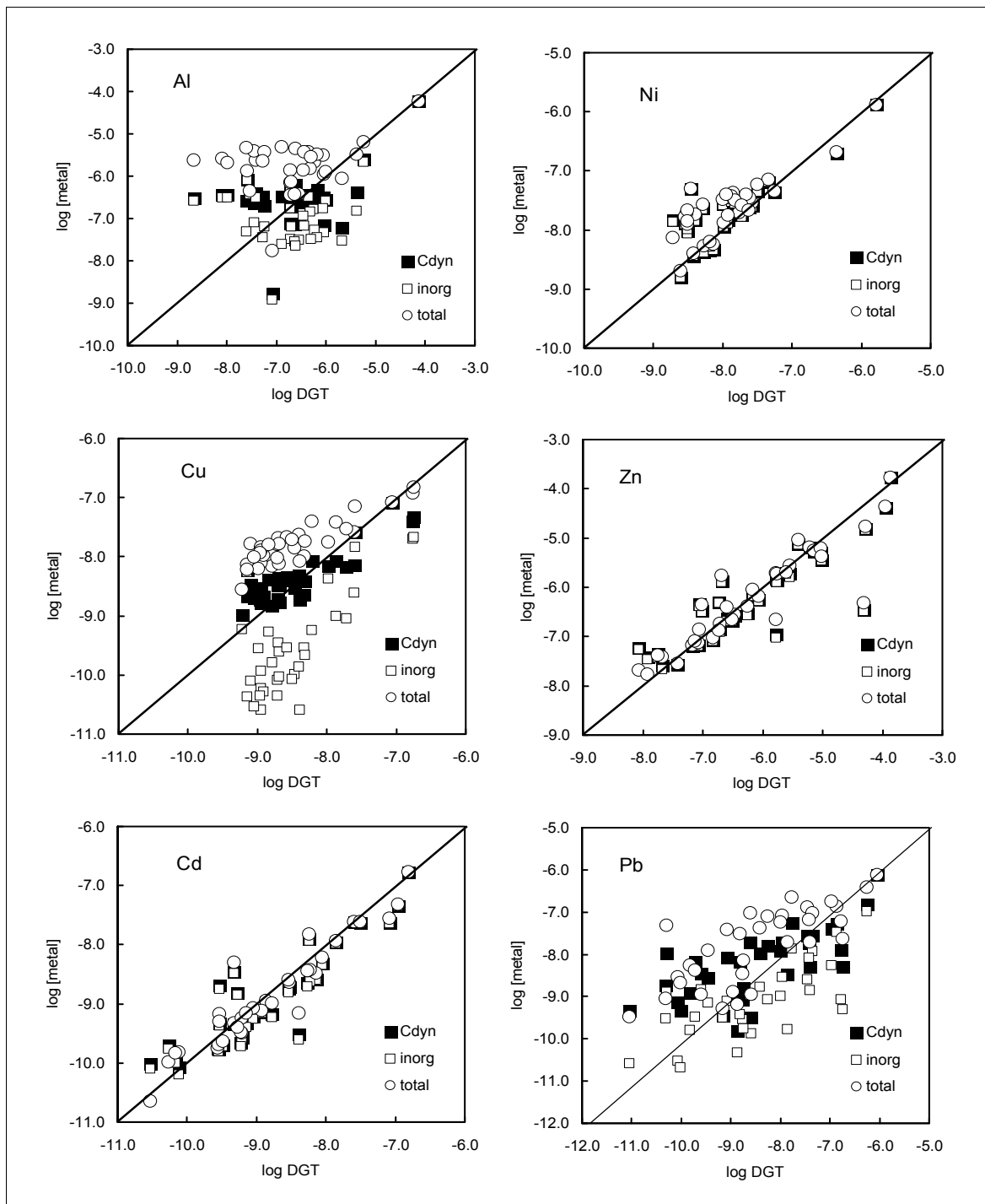


Figure 4.11 Metal concentration variables (total, inorganic, C_{dyn} , all in mol l^{-1}) plotted against open pore DGT data. Total metal is the measured filtered concentration, inorganic metal and C_{dyn} were calculated with WHAM (see Section 4.4.4).

4.5 Metals in bryophytes

Table 4.5 shows the 20 streams from which bryophyte samples could be obtained, and the species names. Table 4.6 shows the measured metal contents of the bryophytes, which cover large ranges, up to three orders of magnitude in several cases.

Regression of the logarithms of bryophyte metal contents against the logarithms of total

dissolved metal concentrations reveals highly significant correlations for Cu, Zn and Pb, significant correlations for Ni and Cd, but no correlation for Al (Table 4.7).

Table 4.5 Bryophyte samples.

Site	Species	Type
1	<i>Scapania undulata</i>	liverwort
2	<i>Scapania undulata</i>	liverwort
3	<i>Nardia compressa</i>	liverwort
4	<i>Nardia compressa</i>	liverwort
6	<i>Scapania undulata</i>	liverwort
7	<i>Hygrohypnum ochraceum</i>	moss
8	<i>Fontinalis squamosa</i>	moss
9	<i>Brachythecium rivulare</i>	moss
10	<i>Rhynchostegium riparioides</i>	moss
12	<i>Hygrohypnum</i> sp.	moss
13	<i>Racomitrium aciculare</i>	moss
15	<i>Rhynchostegium riparioides</i>	moss
17	<i>Orthotrichum rivulare</i>	moss
24	<i>Rhynchostegium riparioides</i>	moss
26	<i>Bryum pseudotriquetrum</i> and <i>Hygrohypnum luridum</i>	mosses
28	<i>Rhynchostegium riparioides</i>	moss
31	<i>Scapania undulata</i>	liverwort
32	<i>Scapania undulata</i>	liverwort
33	<i>Fontinalis antipyretica</i>	moss
35	<i>Hygrohypnum ochraceum</i>	moss

Previous studies (Vincent *et al.*, 2001) showed that metal incorporation into bryophytes was dependent not only upon the total metal concentration in streamwater but also on pH and metal speciation. Therefore, we examined the possibility that the bryophyte results of the present study could be interpreted in terms of metal chemistry, treating the bryophytes as 'biotic ligands'.

As a first attempt at describing the data, it was simply assumed that the proton and metal-binding properties of the bryophytes could be represented by humic acid, which has been parameterised as part of the WHAM software (Tipping, 1994, 1998). The WHAM model was run using fulvic acid (65% active) to represent DOM, as in Section 4.4.1, and with a very low concentration of humic acid (100% active) to represent the bryophytes. By using a low concentration of humic acid, we ensured that metal binding to this phase did not alter the solution speciation, thereby simulating a bryophyte in steady state with the solution phase. To compare measured and modelled values, the metal contents of the bryophytes (Table 4.6) were converted to units of moles g⁻¹.

Strong correlations between measured and modelled bryophyte metal contents were observed, with results for several of the metals showing near 1:1 correspondence. The fits were improved by optimising the values of the WHAM parameter LKMA, to yield the results shown in Figure 4.12.

Figure 4.12 shows that bryophytes have metal-binding properties close to that of humic acids, in terms of both affinity (expressed through LKMA) and capacity, as evidenced

by the similarities between the absolute values of bound metal per gram from WHAM and the measured bryophyte metal contents. The optimised LKMA values do not differ greatly from the WHAM default values.

The analysis assumed that all bryophytes behaved identically towards metals, and while this is probably an oversimplification, the success of the WHAM application suggests that there are not great differences (see also Kelly, 1988; Kelly and Whitton, 1989a). We attempted a simple analysis by comparing deviations from the model with bryophyte species. We found that the moss species consistently contained less Zn and Cd than the model predicted, by about a factor of two, but there were no other clear differences. Thus, there is no strong evidence that bryophytes need to be distinguished in order to explain their accumulation of metals; metal concentration and streamwater chemistry play the dominant roles.

Table 4.6 Metal contents of bryophyte samples.

Site	Al mg g ⁻¹	Ni µg g ⁻¹	Cu µg g ⁻¹	Zn µg g ⁻¹	As µg g ⁻¹	Cd µg g ⁻¹	Pb µg g ⁻¹
1	27.5	3.1	609	92	184	0.64	187
2	9.52	2.3	2020	111	163	0.43	160
3	7.58	0.7	6	13	5	0.09	13
4	20.8	2.2	4	16	5	0.10	25
6	9.1	21.4	43	228	36	2.72	258
7	11.1	14.8	104	159	45	0.76	5513
8	6.51	50.7	51	594	162	13.57	1055
9	5.62	22.5	199	877	110	4.47	4487
10	2.31	13.2	741	4825	1910	28.37	26400
12	5.88	49.5	36	4620	10	10.00	12497
13	5.36	7.6	9	248	6	0.69	1137
15	2.84	18.2	10	1045	6	4.95	847
17	4.96	16.2	29	946	6	3.21	985
24	3.68	17.8	15	1250	5	3.84	2817
26	6.12	21.4	17	1175	9	3.37	4437
28	2.93	28.5	11	1710	3	33.20	2443
31	3.4	15.8	12	109	15	0.48	144
32	4.84	8.5	8	47	9	0.14	42
33	0.922	6.6	6	104	2	0.59	22
35	4.82	14.6	9	202	10	1.50	121

Table 4.7 Results of regression analysis with bryophyte metals data. Logarithms of bryophyte metal contents were regressed against logarithms of total dissolved metal concentrations, and against values predicted with WHAM.

	Total [metal]		WHAM predictions	
	r ²	p	r ²	p
Al	0.03	> 0.1	0.47	< 0.01
Ni	0.37	< 0.005	0.45	< 0.01
Cu	0.65	< 0.001	0.87	< 0.001
Zn	0.58	< 0.001	0.82	< 0.001
Cd	0.27	< 0.05	0.67	< 0.001
Pb	0.57	< 0.001	0.92	< 0.001

Table 4.8 WHAM LKMA values, default and optimised.

Metal	Default	Optimised
Al	2.6	2.6
Ni	1.1	1.1
Cu	2.0	1.7
Zn	1.5	1.3
Cd	1.3	1.3
Pb	2.0	2.2

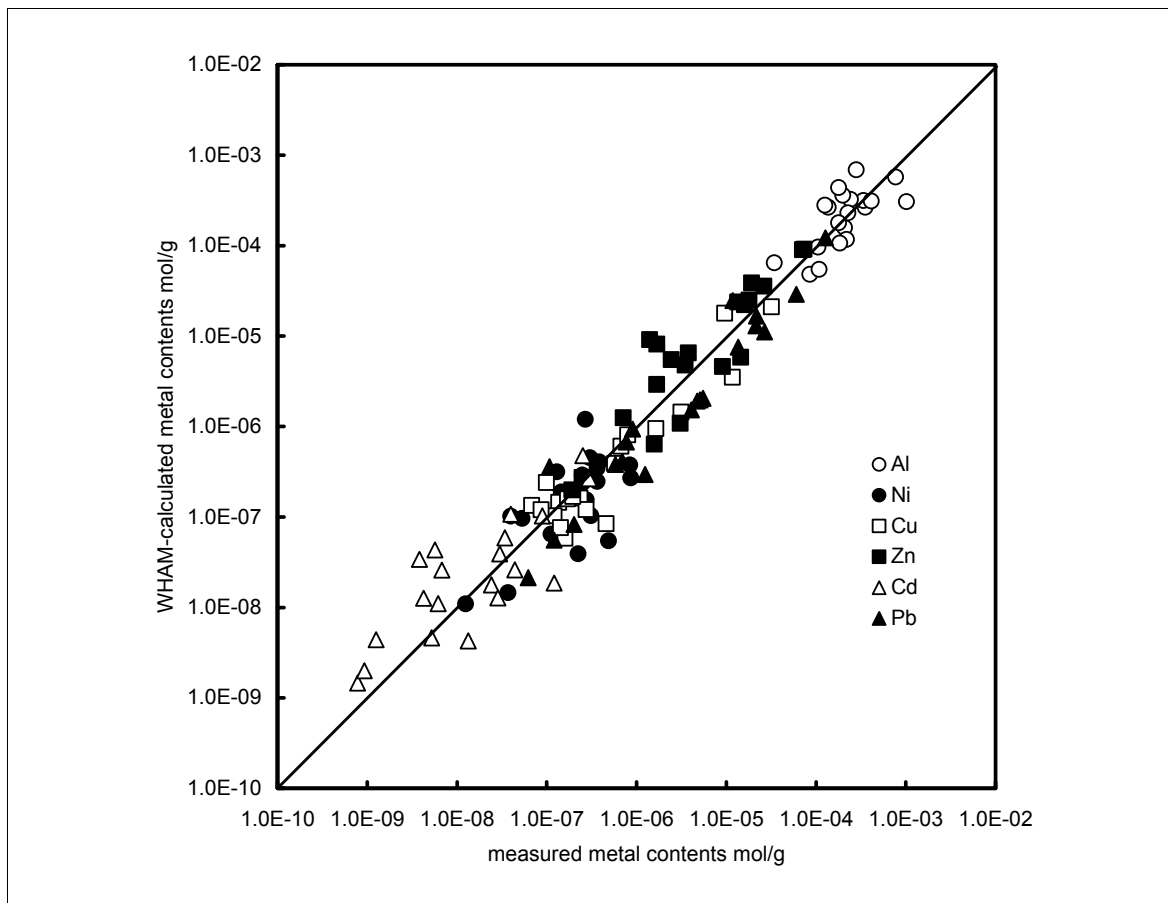


Figure 4.12 Metal contents of bryophytes from 20 of the study streams after optimising.

4.6 Macroinvertebrate metal body burdens

Metal body burdens were successfully estimated for six species (within Leuctra, Simuliidae, Rhythrogena, Nemouridae, Perlolidae, Chloroperlidae). The metal body concentrations measured covered several orders of magnitude across sites for each metal. Metal concentrations showed significant differences among taxa with some taxa clearly showing more accumulation than others from the same site. The logarithmic metal contents of the different species were well correlated for Cu, Zn, Cd and Pb, but less so for Al and Ni (Table 4.9).

The results of the metal analysis (Cd, Cu, Zn, Pb, Ni and Al) for the six best documented taxa (18 to 33 sites covered) are summarised in Table 4.9. Invertebrate metal concentrations (log scale) were regressed against metal exposure concentrations (log scale) expressed in four ways: total dissolved (filterable) metal, free metal ion estimated using WHAM, total dissolved metal divided by $[H^+]$, and free metal ion divided by $[H^+]$. Table 4.9 shows results for the first and last of these; the other two variables generally gave lower values of the correlation coefficient. The free ion concentration divided by $[H^+]$ provides the best correlation overall, although the correlations are not significant for Al and Ni.

Table 4.9 Regression coefficients for invertebrate metal data. Logarithms of invertebrate metal concentrations were regressed against each other for each metal. Key: L Leuctra, S Simulidae, R Rhythrogena, N Nemouridae, P Perlolidae, C Chloroperlidae.

Al							Ni						
	L	S	R	N	P	C		L	S	R	N	P	C
L	1.00						L	1.00					
S	0.63	1.00					S	0.51	1.00				
R	0.17	0.66	1.00				R	0.43	0.58	1.00			
N	0.57	0.30	0.61	1.00			N	0.41	-0.23	0.13	1.00		
P	0.42	0.55	0.17	0.80	1.00		P	0.35	0.22	0.21	0.79	1.00	
C	0.41	0.72	0.45	-0.02	0.61	1.00	C	0.37	0.67	0.50	0.27	0.54	1.00

Cu							Zn						
	L	S	R	N	P	C		L	S	R	N	P	C
L	1.00						L	1.00					
S	0.91	1.00					S	0.90	1.00				
R	0.71	0.74	1.00				R	0.84	0.93	1.00			
N	0.52	0.88	0.46	1.00			N	0.88	0.46	0.56	1.00		
P	0.81	0.85	0.85	0.38	1.00		P	0.81	0.27	0.51	0.93	1.00	
C	0.90	0.91	0.71	0.55	0.86	1.00	C	0.86	0.46	0.82	0.85	0.88	1.00

Cd							Pb						
	L	S	R	N	P	C		L	S	R	N	P	C
L	1.00						L	1.00					
S	0.94	1.00					S	0.65	1.00				
R	0.91	0.90	1.00				R	0.90	0.92	1.00			
N	0.90	0.74	0.87	1.00			N	0.90	0.53	0.71	1.00		
P	0.90	0.67	0.75	0.83	1.00		P	0.79	0.28	0.71	0.92	1.00	
C	0.91	0.75	0.91	0.89	0.86	1.00	C	0.92	0.80	0.95	0.87	0.79	1.00

Table 4.10 Correlation coefficients (logarithmic) of metal body burdens with solution chemistry variables. Key as for Table 4.9.

	L	S	R	N	P	C	
No. of samples	33	24	20	18	25	23	
Al filt		-0.25	-0.26	-0.40	-0.31	-0.27	0.04
[Al ³⁺] / [H ⁺]	0.18	0.19	0.29	-0.18	0.21	0.21	
Ni filt		0.21	0.62	0.36	0.10	0.37	0.62
[Ni ²⁺] / [H ⁺]	0.41	0.63	0.39	0.24	0.11	0.47	
Cu filt		0.53	0.89	0.33	0.49	0.64	0.58
[Cu ²⁺] / [H ⁺]	0.67	0.94	0.62	0.77	0.80	0.80	
Zn filt		0.76	0.85	0.94	0.66	0.79	0.58
[Zn ²⁺] / [H ⁺]	0.85	0.73	0.90	0.80	0.78	0.78	
Cd filt		0.77	0.81	0.88	0.68	0.81	0.70
[Cd ²⁺] / [H ⁺]	0.93	0.88	0.82	0.79	0.85	0.84	
Pb filt		0.83	0.77	0.88	0.81	0.71	0.91
[Pb ²⁺] / [H ⁺]	0.84	0.79	0.85	0.83	0.80	0.87	

Following the approach taken for bryophytes in Section 4.5, we compared invertebrate metal body burdens with metal loadings of humic acid, calculated with WHAM. No adjustments of LKMA values were performed in this case. As shown in Figure 4.13, there is a fair measure of agreement between the two sets of values. A notable difference is that the modelled Al contents are appreciably lower than the measured values, which may result from the smaller number of binding sites per gram in the invertebrates compared to humic acid.

Closer examination of the measured and modelled values reveals that for several metals the relationship is not linear. For Cu, Zn and Cd, and possibly also for Ni, at low loadings the measured values tend to exceed the modelled ones, and not to vary greatly. An example is shown for Cu in Figure 4.14. This phenomenon arises because the organisms exert homeostatic control over their contents of some metals, maintaining them at near-constant levels at relatively low loadings, but eventually becoming overwhelmed when the metal concentrations are high. Neither Al nor Pb exhibited such behaviour, however.

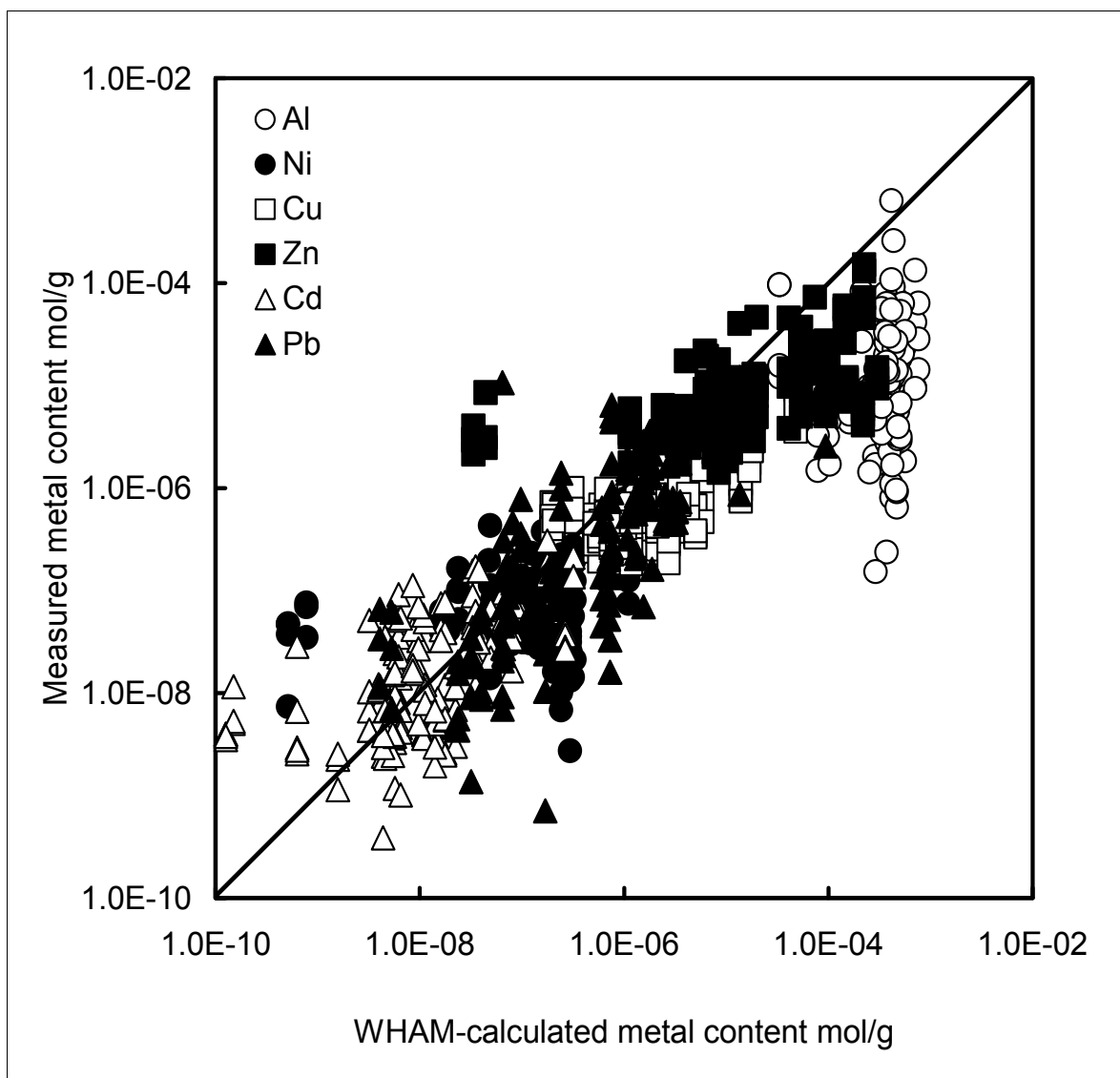


Figure 4.13 Measured metal contents of macroinvertebrates compared with the metal loading of humic acid, calculated with WHAM. The 1:1 line is shown for guidance.

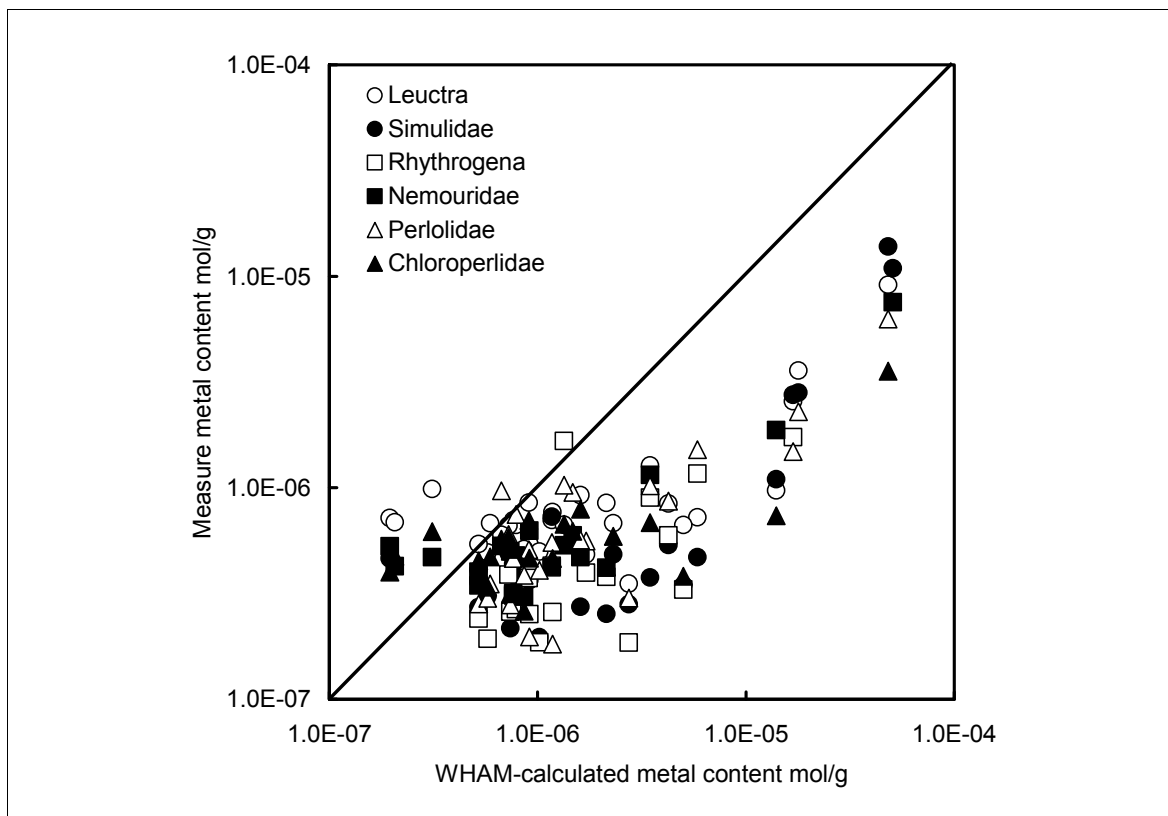


Figure 4.14 Measured Cu contents of macroinvertebrates compared with the metal loading of humic acid, calculated with WHAM. The 1:1 line is shown for guidance.

4.7 Chemistry results – conclusions

The main findings from the chemical measurements and calculations, relevant to the objectives of the project, are as follows.

4.7.1 Major solute concentrations

The major solute compositions of the study streams vary substantially, covering appreciable ranges of acidity, alkalinity, total ionic concentration and DOC concentration. However, the maximum alkalinity value of 2.0 meq l^{-1} was lower than values of 5 meq l^{-1} or greater reported for other waters in the north of England (Simon *et al.*, 2004). Also, the highest DOC concentration of 8.1 mg l^{-1} is lower than values from other sites, notably those draining peatlands for which concentrations up to 33 mg l^{-1} have been reported (Simon *et al.*, 2004).

The nutrient concentrations are in all cases low, although at some sites there are somewhat elevated levels attributable to agricultural runoff. The concentrations of nutrients in the study streams are unlikely to have affected either macroinvertebrates or diatoms.

Concentrations of suspended particulate matter were in the range $0\text{--}50 \text{ mg l}^{-1}$. The higher levels may have affected invertebrate communities (see Sections 5.2 and 5.4).

4.7.2 Total dissolved metal concentrations

As expected because of the historical mining activities, most of the streamwaters were contaminated with trace metals, at levels exceeding EQS values. Therefore they are suitable for attempting to establish dose–response relationships. However, the dominance among the trace metals of zinc hampers a full exploration of the effects of different individual metals, even though quality standards for Cu, Cd and Pb were each exceeded in several cases.

The study sites included several acid streamwaters, some of which were contaminated with trace metals, but all of which had high levels of aluminium. At the outset of the work, the effects of Al were not a prime focus of study, but the chemical and macroinvertebrate data strongly suggest that Al plays an important toxic role, possibly even in streams where its concentration is at the background level (see Chapter 5). Therefore the inclusion of the acid, high-Al sites within the study was a definite advantage.

One streamwater (number 10) had high concentrations of arsenic, and this was borne in mind when analysing the data for dose–response relationships with the 'target' metals.

4.7.3 Chemical speciation of metals

Comparison of the total filtered ('dissolved') metal concentrations with those measured by DGT suggests that Zn and Cd are present largely in inorganic forms in the streamwaters, whereas there was appreciable organic complexation of Al, Cu and Pb, and to a lesser extent Ni. These findings are borne out by the DMT estimates of free metal ion concentrations, although Al was not determined by DMT.

Calculations with WHAM and NICA–Donnan were in reasonable agreement with the speciation measurements at higher metal concentrations, but the matches were less convincing at lower concentrations. However, the discrepancies are reflected in data scatter rather than systematic deviations. In terms of dose–response relationships, the speciation information is most reliable under conditions where the greatest effect is anticipated.

It is not clear from the present data whether filterable Al concentrations at higher pH represent truly dissolved metal or whether colloidal particulates might be included. In view of the major effect of Al on macroinvertebrate toxicity (Chapter 5), this is a significant uncertainty, highlighted in Section 5.4.

4.7.4 Metal interactions with biota

The metal contents of both bryophytes and macroinvertebrates varied considerably depending upon streamwater chemistry. The greatest variability was shown by Pb in bryophytes, with a range of more than 1000-fold. These findings provide direct evidence that biota respond to the chemistry of the medium.

Furthermore, for both types of organism, the metal contents are approximately predictable using chemical speciation concepts. Specifically, bryophyte accumulation of metals follows quite closely the interactions of metals with humic acid, as described by WHAM. This applies not only to the relative affinities of the different metals, but also to the metal loadings per gram dry weight. The relationship is less good for macroinvertebrates, partly reflecting their ability to control the body burdens of some metals.

5 Macroinvertebrates and streamwater chemistry

Here we analyse the field data on macroinvertebrates, comparing indices of diversity with chemical variables. The overall purpose is to search for dose–response relationships. Because of the knowledge encapsulated in RIVPACS, macroinvertebrate diversity can be expressed in terms of observed divided by expected (O/E) occurrence of taxa, and this provides a powerful means to assess macroinvertebrate community response to pollution. Since few, if any, of the field sites are dominated by a single toxic metal, the analysis necessarily has to consider mixture effects, but the aim is to separate these (i.e. to partition the total toxic effect among all contributing metals).

5.1 Indicators of diversity and multiple regression analysis

The fauna expected at a site in the absence of any anthropogenic stress will depend on its physical type and environmental characteristics. These physical characteristics are also likely to continue to influence the fauna or flora present at a site when it is impacted by some form of stress (metal or otherwise). In the analysis of the macroinvertebrate community relationship with metals, we can allow for the effect of site type by using the RIVPACS bioassessment system to determine the site-specific expected fauna and expected values of macroinvertebrate metrics. By standardising observed metric values by dividing by site-specific expected values, we can make appropriate assessments of the relationship between the (environmentally standardised) biota at a site and its metal loadings.

The following four invertebrate metrics were used for multiple regressions:

1. As well as the ratio of observed to RIVPACS-expected number of BMWP scoring taxa at each site (denoted O/E taxa), values of three alternative metrics were calculated in order to investigate their use.
2. The ratio of observed to expected number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa at each site (O/E EPT).
3. The ratio of observed to expected number of the 67% most sensitive scoring taxa at each site (O/E 67% taxa).
4. The ratio of observed to expected number of the 50% most sensitive scoring taxa at each site (O/E 50% taxa).

The use of EPT taxa as a metric is based on the observed sensitivity of these organisms to trace metal pollution (e.g. Clements *et al.*, 1992). The 67% and 50% of the most sensitive taxa were chosen by calculating observed to expected ratios for each taxon across all the sites and screening out the taxa with the highest ratios, after first removing from consideration taxa predicted to be captured at two or fewer sites. Of the 59 scoring taxa, 19 were predicted to occur at two or fewer sites. Sets of 27 and 20 taxa, respectively, comprised the '67% most sensitive' and '50% most sensitive' group. By this method, we identified sensitive taxa in order to attempt to increase any response to metals, but without introducing bias by using metal data *per se*.

Relationships between O/E taxa and the other derived metrics are shown in Figure 5.1. Interestingly, O/E EPT values tend to be higher than O/E taxa rather than the other way

around as might have been expected; this suggests that in these sites at least, EPT taxa are not more sensitive than average. O/E 67% values are usually lower than O/E taxa values, with the exception of two sites (1 and 17); the range of O/E 67% values is also greater than that of O/E taxa. O/E 50% values are consistently lower than O/E taxa values, and at five sites none of the taxa are present.

We consider that the above metrics are the most logical descriptions of the macroinvertebrate community structure in the present context. However, it can be mentioned that the first of the above metrics (i.e. total scoring taxa) correlates very well ($r^2 = 0.94$) with the BMWP score that is widely used to assess streamwaters in terms of organic pollution. Therefore very similar results would be expected if the 'conventional' metric were employed.

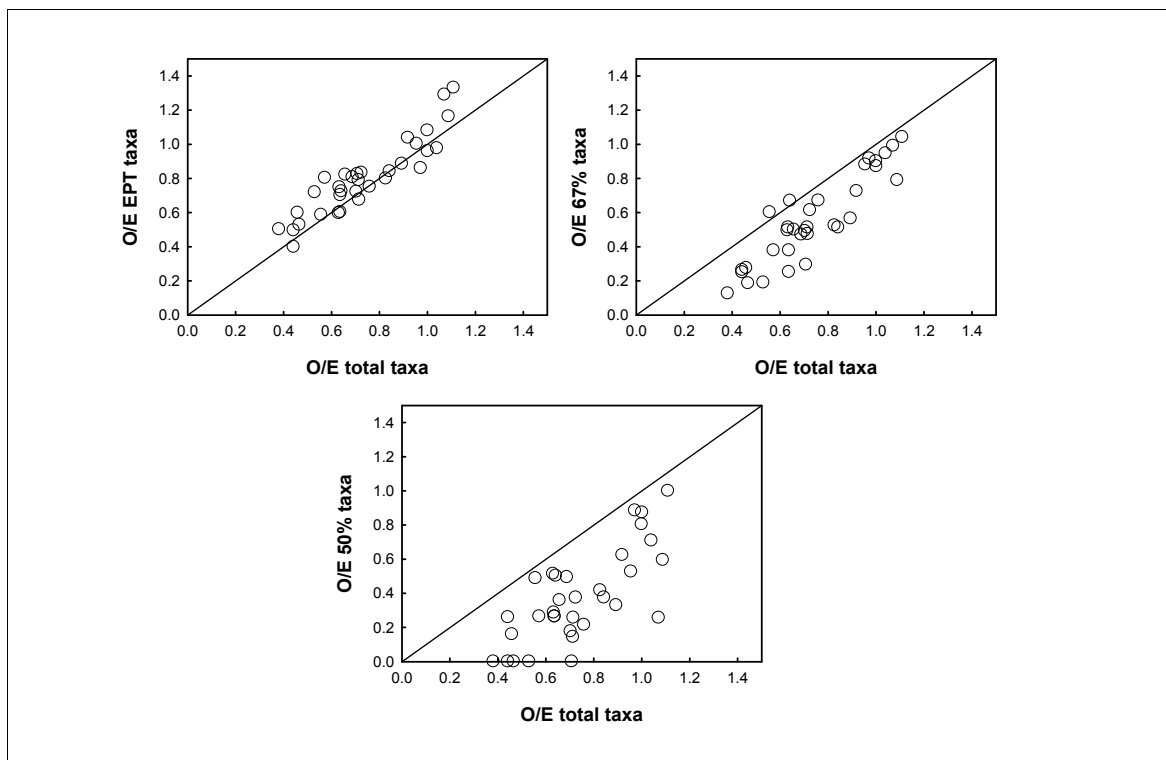


Figure 5.1 Relationships between invertebrate community metrics used in regressions: O/E for EPT taxa, 67% most sensitive taxa, and 50% most sensitive taxa, plotted against O/E for total scoring taxa.

Four metrics derived from measured metal concentrations were used in multiple regressions:

- (a) total filterable metal concentrations;
- (b) 'hardness functions' combining filterable metal concentration and water hardness;
- (c) open pore DGT measured metal concentrations;
- (d) 'free metal ion functions' combining free metal ion activities calculated using WHAM (Section 4.4.1) and water pH.

As a first step, stepwise regression including all six metals was done using the forward selection, backward elimination and stepwise methods, in order to tentatively identify the significant predictors of biological status. Variable selection in stepwise regression procedures was done on the basis of alpha values (i.e. significance level of an added or removed variable). Alpha levels used were 0.25 for forward selection, 0.1 for backward elimination and 0.15 for stepwise selection or elimination. Regressions were

done on two sets of sites: firstly all the sites, and secondly a set of sites without those where the pH was below 6 on average.

In order to gain further insight into the importance of individual metals on regression outcomes, forward stepwise regression was also done manually. This was done as follows:

1. Regression of the biological metric against each metal descriptor (filterable, free ion function, etc.) individually. Determination of significant individual metal descriptors at $\alpha = 0.05$.
2. For each significant metal descriptor, regression of the biological metric against all combinations of the significant metal and the other metals. Determination of significant pairs of metals by chi-tests of the significance of the additional metal, at $\alpha = 0.05$.
3. If any significant pairs of metals found, repeat the process for triads of metal, and so on until no increase in regression significance is found by adding additional descriptors.

5.2 Multiple regression analysis

5.2.1 Multiple regression with filterable metal

Average filterable metal measurements were calculated as the arithmetic mean of logged concentrations. Where measured concentrations were below the detection limit (Al on three occasions at Site 5 and Pb on one occasion at Site 34) a value of half the detection limit was used.

Results of the regressions are shown in Table 5.1. The metals most frequently selected as significant predictors of biology, when all the sites are considered, are Al and Zn. When only the non-acid sites ($\text{pH} > 6.0$) were considered, Al became somewhat less important as a predictor. Zn was selected in almost all circumstances, and was always statistically significant when selected. On only two occasions was Zn not selected: regression of O/E EPT using forward selection and using stepwise selection. The results of the backward selection procedure, however, showed that when Cd was removed from selection, the p-value of the Zn coefficient decreased from 0.163 to 0.001. This indicates strong co-linearity between $\log(\text{Cd})$ and $\log(\text{Zn})$, as was indicated in Chapter 4, meaning that both predictors may explain the observations well, but not independently of each other. The choice of Zn in the majority of cases, and its statistical significance, does not therefore indicate that Cd is a poor predictor in itself.

Another result of note is the fact that in many cases where predictors other than Al, Zn or Cd are selected, their coefficients are positive. In the context of this work this is a rather counterintuitive, since we would expect the value of the biological metric to decrease with increasing metal concentration, implying a negative coefficient. It is notable that where Al and Zn are selected in regressions they consistently have negative coefficients. A positive coefficient could indicate (but not confirm) a non-additive mixture effect or could be a result of the multi-co-linearity among the metal concentrations.

The R^2 values are reasonably similar (in the range 0.35–0.41) throughout with the exception of those where the biological metric is O/E 50% where they are consistently lower (0.21–0.24). There are no systematic differences in R^2 values when all the sites are used and when acid sites are excluded.

Table 5.1 Results of stepwise multiple regression of invertebrate metrics against log(filterable metal). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (, $p < 0.001$; *, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al* (Ni) Zn** (Pb) 0.41 (0.32)	Al** (Ni) Zn** 0.36 (0.29)	Al* (Ni) Zn** (Pb) 0.41 (0.32)
O/E EPT	Al Cd** (Pb*) 0.31 (0.24)	Al (Ni) Zn** (Pb) 0.38 (0.30)	Al Cd** (Pb*) 0.31 (0.24)
O/E 67%	Al* (Ni) Zn** 0.35 (0.28)	Al Zn** 0.30 (0.26)	Al Zn** 0.30 (0.26)
O/E 50%	Zn** 0.24 (0.21)	Zn** 0.24 (0.21)	Zn** 0.24 (0.21)

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al* (Ni) Zn** (Pb) 0.40 (0.31)	Al Zn** (Pb) 0.36 (0.28)	Al Zn** (Pb) 0.36 (0.28)
O/E EPT	Cu Zn** (Pb) 0.41 (0.33)	Zn** 0.28 (0.25)	Zn** 0.28 (0.25)
O/E 67%	Al Zn** Pb 0.36 (0.29)	Al Zn** 0.31 (0.26)	Al Zn** 0.31 (0.26)
O/E 50%	Zn** 0.21 (0.18)	Zn** 0.21 (0.18)	Zn** 0.21 (0.18)

5.2.2 Multiple regression with total metal and hardness

Functions to take account of water hardness were derived from the hardness functions used by US EPA (<http://www.epa.gov/waterscience/criteria/wqcriteria.html>) to set site-specific recommended water quality criteria. These functions have the form:

$$\ln[M]_{EQS} = m_c \ln H + b_c \quad (5.1)$$

where $[M]_{EQS}$ is the hardness-dependent criterion concentration ($\mu\text{g l}^{-1}$ dissolved), H is the water hardness in $\text{mg CaCO}_3 \text{ l}^{-1}$ and m_c and b_c are constants (Table 5.2). A hardness dependent metric for use in regressions was calculated as $\text{HF} = \ln[M]_{\text{filterable}} - \ln[M]_{EQS}$. As no hardness-dependent function for Al was available a value of m_c was estimated as unity. Note that estimation of b_c was not necessary as it does not influence the distribution of values of the hardness function HF.

Table 5.2 Values of m_c and b_c for US EPA hardness functions. The bracketed values for Al are estimates.

Metal	m_c	b_c
Al	(1.00)	(0.00)
Ni	0.846	0.0584
Cu	0.8545	-1.702
Zn	0.8473	0.884
Cd	0.7409	-4.719
Pb	1.273	-4.705

Table 5.3 Results of stepwise multiple regression of invertebrate metrics against hardness functions. Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (**, $p < 0.001$; *, $p < 0.01$; , $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al* Zn** (Pb) 0.42 (0.36)	Al* Zn** (Pb) 0.42 (0.36)	Al* Zn** (Pb) 0.42 (0.36)
O/E EPT	Al* (Ni) Zn** (Pb) 0.39 (0.30)	Al Zn** (Pb*) 0.35 (0.29)	Al Zn** (Pb*) 0.35 (0.29)
O/E 67%	Al* Zn** 0.42 (0.38)	Al* Zn** 0.42 (0.38)	Al* Zn** 0.42 (0.38)
O/E 50%	Al Zn*** 0.39 (0.35)	Zn*** 0.36 (0.34)	Zn*** 0.36 (0.34)

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al** Zn*** (Pb) 0.46 (0.40)	Al** Zn*** (Pb) 0.46 (0.40)	Al** Zn*** (Pb) 0.46 (0.40)
O/E EPT	Al* Zn** (Pb*) 0.40 (0.33)	Al* Zn** (Pb*) 0.40 (0.33)	Al* Zn** (Pb*) 0.40 (0.33)
O/E 67%	Al** Zn*** (Pb) 0.53 (0.48)	Al** Zn*** 0.51 (0.47)	Al** Zn*** 0.51 (0.47)
O/E 50%	Al Zn** (Cd) 0.48 (0.42)	Al Zn*** 0.44 (0.42)	Al Zn*** 0.44 (0.42)

Regression results are shown in Table 5.3. Again, Al and Zn are most frequently selected as predictors. The R² values are generally similar to those for regressions on filterable metal but are higher when O/E 50% is the biological metric.

5.2.3 Multiple regression with DGT-determined metal

The chemical metric used was log(open pore DGT metal). Results (Table 5.4) show broadly similar results as for filterable metal although R² values for O/E taxa are notably lower. The R² for O/E EPT when considering all sites is also notably lower. When acid sites are not considered, Cu becomes a significant predictor and R² increases substantially.

Table 5.4 Results of stepwise multiple regression of invertebrate metrics against log(open pore DGT metal). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, p < 0.001; **, p < 0.01; *, p < 0.05). Metals in brackets have positive regression coefficients. The numbers shown are the R² and (in brackets) the R² adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al Zn* 0.21 (0.15)	Zn* 0.16 (0.13)	Zn* 0.16 (0.13)
O/E EPT	Al Cd* 0.22 (0.17)	Cd* 0.15 (0.12)	Al Cd* 0.22 (0.17)
O/E 67%	Al (Cu) Cd* 0.36 (0.29)	Al Zn** 0.35 (0.30)	Al Cd** 0.32 (0.26)
O/E 50%	Al Zn*** 0.38 (0.34)	Zn** 0.32 (0.30)	Al Zn*** 0.38 (0.34)

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al Zn** 0.26 (0.21)	Al Zn** 0.26 (0.21)	Al Zn** 0.26 (0.21)
O/E EPT	(Ni) Cu** Zn** (Pb) 0.50 (0.41)	Cu* Zn* 0.43 (0.38)	Cu* Zn* 0.43 (0.38)
O/E 67%	Al (Cu) Cd** 0.36 (0.29)	Al Zn** 0.35 (0.30)	Al Cd** 0.31 (0.26)
O/E 50%	Zn*** 0.41 (0.38)	Zn*** 0.41 (0.38)	Zn*** 0.41 (0.38)

5.2.4 Multiple regression with free ion functions

Free metal ion functions were constructed following the theory of Lofts *et al.* (2004), whereby toxic effects are considered to be mediated by the free metal ion and the 'protective' effects of other cations, such that:

$$\log M_{\text{free, toxic}} = \alpha \cdot \text{pH} + \gamma \quad (5.2)$$

where α and γ are constants and $M_{\text{free, toxic}}$ is a metal free ion activity corresponding to a defined constant level of toxicity. According to this theory, γ is dependent on the defined toxicity (α is independent); thus, the level of toxicity (γ_{field}) associated with a free ion activity measured in the field or computed by speciation can be written as:

$$\gamma_{\text{field}} = \log M_{\text{free, field}} - \alpha \cdot \text{pH} \quad (5.3)$$

Values of α for Ni, Cu, Zn, Cd and Pb have been computed for soils using toxicological data. For this work, values of α were rounded to values similar to those computed in order to avoid overinterpretation. Values of α chosen were -0.5, -1.0, -0.25, -0.25 and -1.0, respectively. No soil value of α was computed for Al. A value of -1.0 was chosen, on the basis that α values computed for soils tended to be smaller for metals having a greater reactivity (i.e. a tendency to form complexes) (Zn ~ Cd < Ni < Cu ~ Pb), and that Al has a reactivity broadly comparable to Cu and Pb. In principle it would be possible to extend the functions to include terms for other 'protective' cations (e.g. Na⁺, Ca²⁺), but there is no data available for *a priori* estimation of α terms for these ions.

As shown in Table 5.5, the results were again broadly similar to those seen previously, although the 'best' R² values are on average slightly higher. Both Al and Zn were consistently found to be statistically significant as predictors, which was not the case with any of the other sets of predictors.

Table 5.5 Results of stepwise multiple regression of invertebrate metrics against free metal ion functions (γ_{field}). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R² and (in brackets) the R² adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al** Zn** (Pb) 0.39 (0.33)	Al** Zn** 0.35 (0.31)	Al** Zn** 0.35 (0.31)
O/E EPT	Al* Cu Zn** (Pb) 0.36 (0.27)	(Ni**) Zn** (Pb) 0.34 (0.27)	Al* Zn* 0.27 (0.23)
O/E 67%	Al** Zn** 0.41 (0.36)	Al** Zn** 0.41 (0.36)	Al** Zn** 0.41 (0.36)
O/E 50%	Al** Zn** 0.38 (0.33)	Al** Zn** 0.38 (0.33)	Al** Zn** 0.38 (0.33)

All sites	Forward selection	Backward elimination	Stepwise
O/E taxa	Al** Zn** (Pb*) 0.46 (0.40)	Al** Zn** (Pb*) 0.46 (0.40)	Al** Zn** (Pb*) 0.46 (0.40)
O/E EPT	Al** Cu Zn** (Pb) 0.39 (0.30)	Al** Zn** (Pb) 0.37 (0.29)	Al** Zn** (Pb) 0.37 (0.29)
O/E 67%	Al*** Zn** (Pb) 0.52 (0.47)	Al*** Zn** (Pb) 0.52 (0.47)	Al*** Zn** (Pb) 0.52 (0.47)
O/E 50%	Al** Zn** (Cd) 0.46 (0.40)	Al** Zn** 0.41 (0.36)	Al** Zn** (Cd) 0.46 (0.40)

5.2.5 Manual stepwise regression

Manual stepwise regression was done to follow more closely the effects of adding metal descriptor terms to the regressions. Manual regression was done for the OE67 metric only, as this generally provided the best results in automatic stepwise regression.

For filterable metal, Zn ($p = 0.01$, $R^2 = 0.21$) and Cd ($p = 0.019$, $R^2 = 0.18$) gave significant fits. Only addition of Al to Zn produced a significant improvement to the fit ($p = 0.048$, $R^2 = 0.31$). Similarly, only Al significantly improved the fit when added to Cd ($p = 0.037$, $R^2 = 0.29$). Further additions did not significantly improve either fit. Combining Zn and Cd did not produce significant improvements in fits over either single metal, and the p-values for the coefficients were both inflated (p for Zn = 0.266 and p for Cd = 0.739), demonstrating the relatively high degree of correlation between the filterable concentrations of these metals.

Four hardness functions – those of Ni, Zn, Cd and Pb – gave significant regressions. Of these, Zn gave the highest R^2 of 0.35. Addition of Al to Zn improved the fit significantly ($p < 0.001$, $R^2 = 0.51$). The next best fits were Cd and Al, and Cd and Ni, both with R^2 values of 0.42. Adding more terms did not significantly improve fits.

For DGT-determined metals, Ni, Zn and Cd gave significant regressions. Significant improvement ($p = 0.048$) was obtained by adding Al to Zn, with R^2 increasing from 0.25 to 0.35. No further improvement was possible by adding additional metal terms.

For the free ion functions, only Zn was significant ($p = 0.025$, $R^2 = 0.17$) alone, although Cd was almost significant ($p = 0.051$, $R^2 = 0.13$). Addition of Al to Zn produced a significant improvement in fit ($p < 0.001$, $R^2 = 0.46$), which was notably superior to the fit obtained using filterable Zn and Al. Addition of Al to Cd also produced a significant improvement in the fit ($p = 0.001$, $R^2 = 0.38$). Further additions of metal terms did not produce any significant improvements.

Regression fits for each metric are shown in Figure 5.2. The regression coefficients for both Zn and Al are consistently negative.

The regression residuals for the regression using Al and Zn were themselves regressed against other water quality variables in order to detect any confounding influences on predictions. The concentration of filterable Ti was found to correlate significantly ($R = -0.49$, $p = 0.007$) with residuals (expressed as observed OE67 – calculated OE67) when the free metal ion functions were used, although not for the

other metal terms. Regression including Ti as an additional variable gave the following expression:

$$OE67 = -0.123FMIF_Al - 0.191FMIF_Zn - 0.239 [Ti] \quad (5.4)$$

where [Ti] has units of $\mu\text{g l}^{-1}$. The R^2 was 0.61 and the addition of the Ti term was significant ($p = 0.003$). This relationship is plotted in Figure 5.3 after correcting OE67 for the Ti term, $\% OE67_{\text{corr}} = OE67 + 0.239[Ti]$. (See also Section 5.4.3 below.)

Inspection of the data, and the theoretical fact that OE67 should equal unity for unimpacted sites, suggested that a threshold model might improve the fit. Such a model was constructed as follows:

$$FMIF_Al + q.FMIF_Zn \leq T, OE67 = 1 \quad (5.5)$$

$$FMIF_Al + q.FMIF_Zn > T, OE67 = 1 + \alpha(FMIF_Al + q.FMIF_Zn) - T.\alpha \quad (5.6)$$

Where q , T and α are fitting parameters. The fit and associated parameters are shown in Figure 5.3. A small improvement on the standard linear regression fit is seen.

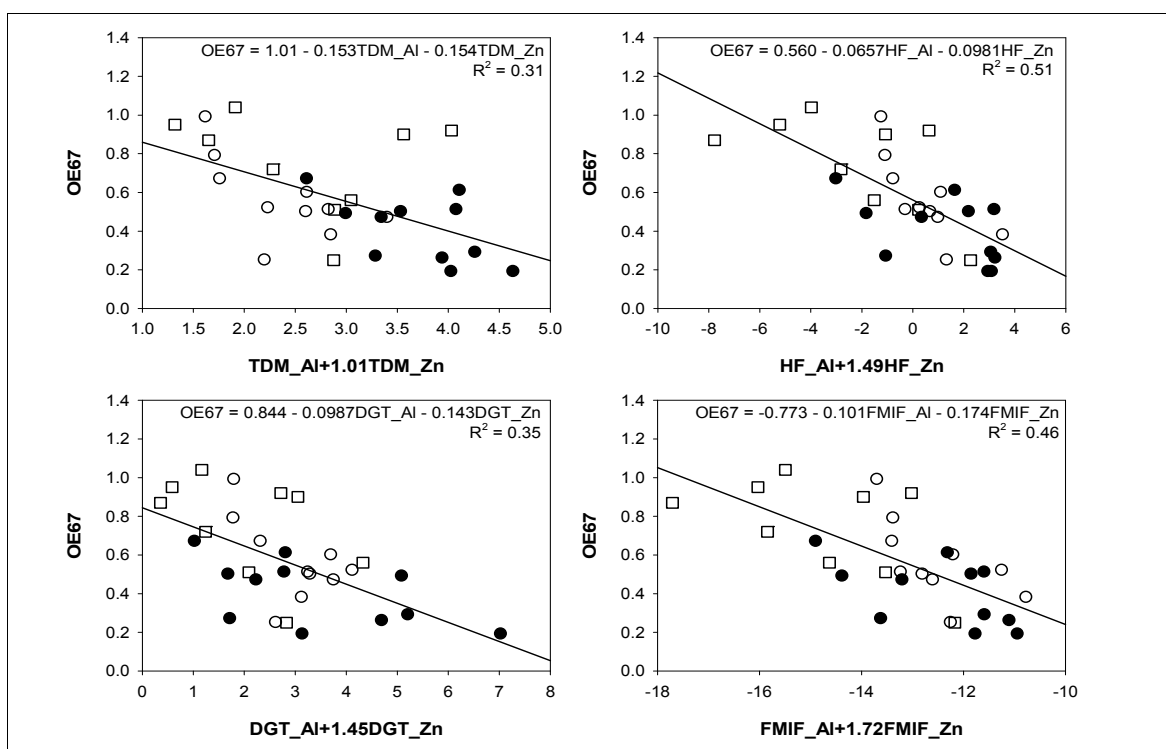


Figure 5.2 Regression of the OE67 metric against Al and Zn terms: total dissolved metal (TDM) , hardness functions (HF), open pore DGT (DGT) and free metal ion functions (FMIF). The open circles are the Lake District sites (1–10), the closed circles are the Teesdale, Tynedale and Weardale sites (12, 14–22 and 26), and the open squares are the Howgills and Swaledale sites (27–35).

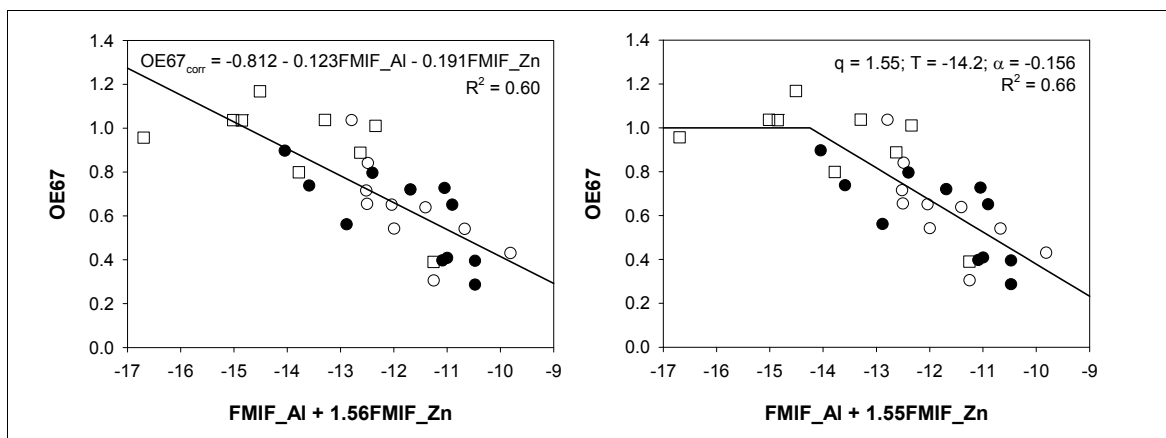


Figure 5.3 Regressions of free ion functions of Al and Zn against OE67, after correction for the relationship between OE67 and filterable Ti. Standard linear model (left) and threshold model (right).

5.2.6 Discussion of multiple regression results

The regression results clearly indicate significant associations between biological effects and increased metal concentrations. Of particular interest is the fact that Al tends not to be a significant predictor alone, but does become significant in combination with Zn, while Zn tends to be significant alone. This would be consistent with Zn contributing to toxicity at most sites where effects are seen, and Al not being a significant contributor at most sites, but somehow contributing to the overall toxic effect. Of the other metals, Cd is potentially important given that it correlates well with Zn, regardless of the metric taken. Although it is not possible from these data to make a definitive case, and both Cd and Zn may be important stressors, Zn appears to be somewhat more important. Cu and Pb are not significant predictors in the context of the regressions. This does not, however, mean that they are not significant stressors on the system, simply that such effects are not apparent from simple linear regressions. Effects may not be apparent due to the metal only exerting a stress at a few sites, or where the stress correlates well with another metal or metals.

Of note is the observation that Al improved the regression of the free metal ion functions more than it did the regression of filterable metal; this supports the contention that the bioavailability of Al is important in influencing its effects. On the other hand, such an effect was not seen for Zn or Cd. This is probably because bioavailability effects on Zn and Cd are expected to be less obvious than those for Al. Bioavailability ought to be more important also for Cu and Pb, so in datasets where these metals have more noticeable effects the influence of bioavailability may become statistically significant.

5.3 Application of Biotic Ligand Models

5.3.1 Zinc BLM for *Daphnia magna*

It was considered instructive to compare a field biological metric with the predictions of a laboratory-based Biotic Ligand Model. For this purpose, the chronic zinc BLM developed by Heijerick *et al.* (2005) for *Daphnia magna* was used to calculate site-specific values of a 'toxicity index' $[Zn^{2+}]_{\text{observed}}/[Zn^{2+}]_{\text{NOEC}}$ at sampling sites. Since the water composition at all but 12 of the sites would not be suitable for *D. magna* in the

absence of metal stress (Section 2.3.2, water hardness below 25 mg CaCO₃ l⁻¹ and/or pH below 6.0), the relationship between OE67 and the 'toxicity index' (Figure 5.4) was calculated only for these suitable sites. The regression of OE67 against the toxicity ratio is not significant at the 95% confidence level (p = 0.11).

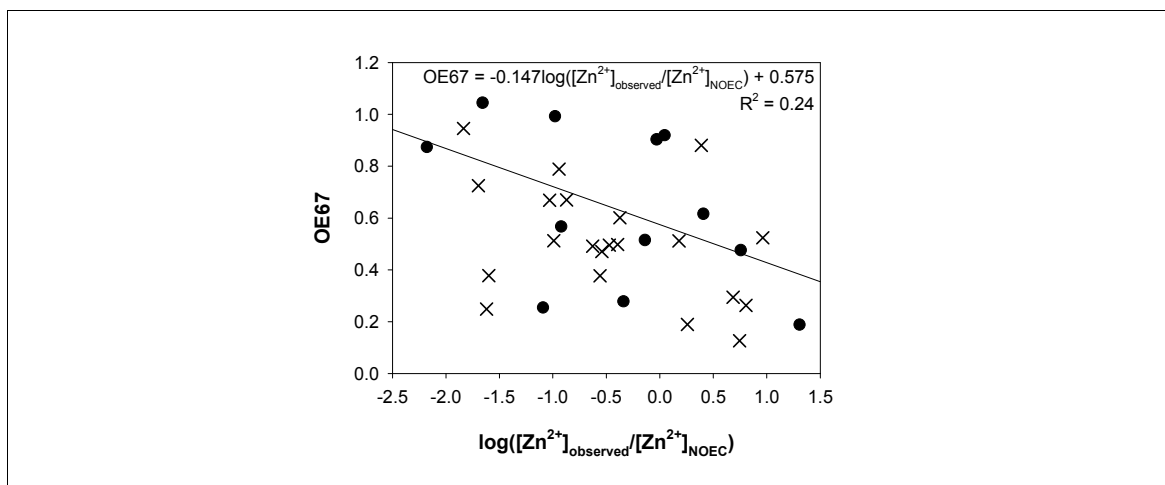


Figure 5.4 Relationship between OE67 and $[Zn^{2+}]_{observed}/[Zn^{2+}]_{NOEC}$ calculated using the zinc BLM for *Daphnia magna*. The regression line is only for sites with suitable water composition for *D. magna* (closed circles). Sites not suitable for *D. magna* are shown as crosses and were not used for computing the regression.

5.3.2 Copper BLM

The performance of the copper BLM was assessed with the method used in the Voluntary Risk Assessment (VRA) to predict PNECs (Predicted No Effect Concentrations) for Cu on a site-specific basis. Briefly, chronic Cu toxicity test data (No Observed Effect Concentrations, NOECs) for plant, invertebrate and fish are converted (normalised) to NOECs at the target water composition, using chronic BLMs derived from toxicity data for *Pseudokirchneriella supracapitata*, *Daphnia magna* and a combination of *Pimephales promelas* and *Oncorhynchus mykiss*, respectively. The PNEC is calculated as the 5th percentile of the lognormal probability distribution of species mean NOECs at the 50% confidence level. In calculating site-specific PNECs it was noted that the mean chemistry at six sites (3, 4, 6, 7, 8 and 32) was outside the range found in the toxicity dataset for at least one of pH, hardness and DOC concentration; thus the calculated PNECs for these sites must be considered extrapolations and less reliable than the PNECs for the other 24 sites.

The predicted PNEC was above the geometric mean observed Cu at Sites 1 and 2 only. Of the remaining reliable sites, the ratio of PNEC to observed Cu was above 0.5 only at Sites 5 and 10, and was below 0.1 at all but one of the remaining sites. Of the six sites for which the PNEC was considered an extrapolation, the ratio was 0.9 at Site 3 and between 0.1 and 0.5 at the other sites. It is notable that Site 3 was originally chosen as a control site low in metals, and had an average Cu in this analysis of 0.32 µg l⁻¹, which is within the range of 'pristine' background concentrations given in Figure 4.1. The PNEC for this site was 0.35 µg l⁻¹, reflecting the fact that the site had low pH (5.1), DOC (1.1 mg l⁻¹) and hardness (2.9 mg CaCO₃ l⁻¹); thus, there is ample reason to treat the calculated PNEC with caution.

According to the multiple regression results copper does not generally affect macroinvertebrate communities at these sites; therefore, a blanket comparison of the site-specific PNECs with OE67 is not likely to generate useful knowledge because effects at many sites appear to be due to other metals, particularly Al and Zn.

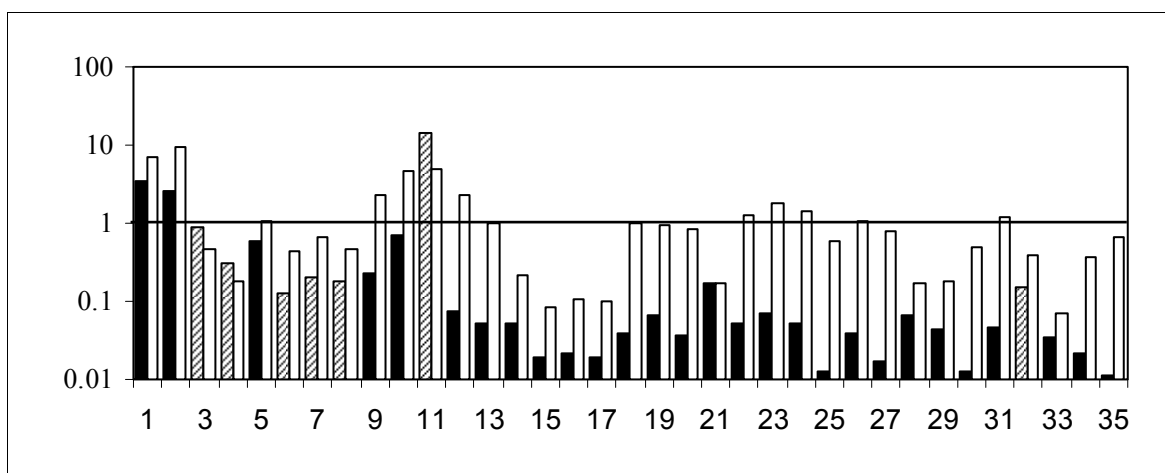


Figure 5.5 Comparison of r_{EQS} values for Cu, based on the Environment Agency EQS system (open bars), with those based on the PNEC calculated using the copper BLM (filled bars). Hatched bars are r_{EQS} values based on PNECs, that are extrapolations outside the water chemistry range found in toxicity tests.

On the other hand, comparison of r_{EQS} values based on the derived PNECs with those based on the Environment Agency EQS system (Figure 5.5) offers insight into how the predicted PNECs differ from current practice in EQSs. With the exception of Sites 3, 4 and 11, r_{EQS} based on the BLM are consistently lower than those based on Environment Agency EQS values. Sites 3, 4 and 11 are likely exceptions to this rule due to their low pH and DOC concentrations. While under the Environment Agency EQS system 14 of the 35 sites exceed the EQS, only three (Sites 1, 2 and 11) do so under the BLM. This implies that site-specific EQS concentrations, based on the BLM, would typically be higher than current EQSs. The difference would largely depend upon the pH, hardness and DOC concentration of the system in question.

5.4 Toxicity Binding Model

Application of the Biotic Ligand Model (BLM) to the present data was limited because a BLM for Al is not yet available, nor does the BLM take multiple metals into account. Moreover, BLM applications to date have focused on single organisms in simplified laboratory solutions. Therefore we adopted a modified approach, still based on the BLM principle, but using WHAM to estimate metal binding to sites in the organisms that elicit a toxic response. This calculation is given some credence by the results for bryophytes (Section 4.5) and for invertebrate body burdens (Section 4.6), both of which provide evidence that the accumulation of metals can be explained to some extent by 'conventional' chemical speciation.

As is the case for the BLM, the Toxicity Binding Model (TBM) comprises two central ideas:

- A. Metals bind to sites on the organism, in competition with H^+ and other cationic metals (i.e. including Na, Mg, K, Ca, Fe, etc.), and in equilibrium with chemical reactions taking place in the surrounding aqueous medium.
- B. The toxic effects exerted by bound metal are metal-specific (i.e. occupancy of the binding site is not the only determinant of toxicity – some metals are more intrinsically toxic than others).

5.4.1 Metal binding to TBM sites

We assumed that binding sites on the macroinvertebrates could be represented by the array of binding sites in humic acid, as described by WHAM. Amounts of metal bound, expressed in mol g⁻¹, and denoted by v_M were computed by assuming the invertebrate binding sites to be in equilibrium with the streamwaters. For completeness, hydrogen ion binding was included (i.e. the possibility was accepted that H⁺ binding *per se* can cause toxicity). The total concentration of invertebrate binding sites in the system was set to a low level for the calculations, to ensure that their presence did not affect the solution speciation when performing the calculation.

The computed values of v_M for the seven potentially toxic metals (including H⁺) at the 30 sites that conform to RIVPACS criteria are shown in Tables 5.6 and 5.7. It can be seen that Al has the highest values in general, although Zn dominates in a number of cases, while Cu is prominent at Sites 1 and 2, and Pb at Site 5. The higher solution activity of Al³⁺ when all filterable Al is assumed to be dissolved leads to higher values of v_{Al} and, because of competition, lower v_M for the other metals. Other metals also bind and compete, notably Mg and Ca, but they are assumed not to be toxic, and so are excluded from Tables 5.6 and 5.7.

Table 5.6 Values of v_M in the TBM, in $\mu\text{mol g}^{-1}$, calculated assuming that Al³⁺ activity is controlled by Al(OH)₃ with $\log_{10} K_{so} = 8.5$, when that solubility product would be exceeded.

Site	H	Al	Ni	Cu	Zn	Cd	Pb
1	1258	507	0.1	96.7	36.2	0.06	0.3
2	1199	426	0.1	121.8	36.2	0.05	0.2
3	1453	1094	0.0	0.8	0.1	0.00	0.0
4	1441	974	0.0	0.9	0.3	0.00	0.0
5	1282	100	0.2	26.5	390.8	0.54	251.0
6	1278	547	0.3	6.4	20.4	0.03	2.4
7	1281	551	0.3	9.3	23.8	0.04	4.4
8	1292	566	0.3	7.8	19.2	0.03	0.8
9	1091	253	0.5	19.7	148.0	0.14	43.7
10	893	130	0.0	38.7	526.3	0.57	18.2
12	995	187	0.4	5.4	461.2	0.12	3.7
14	972	165	0.2	3.6	390.3	0.07	3.1
15	1032	192	0.1	1.6	166.2	0.03	0.3
16	1050	204	0.2	2.0	105.9	0.05	1.1
17	1073	221	0.2	2.5	54.8	0.02	0.5
18	1148	358	0.5	3.2	48.6	0.03	1.7
19	1283	555	0.7	2.3	35.7	0.02	1.8
20	1146	361	0.6	2.6	179.2	0.11	3.0
21	779	112	0.2	5.0	853.6	0.24	3.1
22	1104	310	0.3	2.9	403.6	0.20	2.0
26	1168	397	0.5	2.2	135.6	0.06	3.2
27	1150	355	0.5	2.3	8.6	0.01	0.2
28	904	132	0.1	4.4	274.8	0.31	3.8
29	1006	176	0.3	3.5	246.2	0.15	2.3
30	1121	293	0.5	1.9	36.9	0.03	0.8
31	1160	382	0.1	3.2	71.7	0.12	1.4
32	1375	726	0.3	1.8	22.7	0.04	0.6
33	1034	190	0.2	2.6	29.0	0.02	0.5
34	1182	422	0.8	2.9	10.4	0.01	0.1
35	1240	504	0.6	1.4	8.1	0.01	0.1

Table 5.7 Values of v_M in the TBM, in $\mu\text{mol g}^{-1}$, calculated assuming that all filterable Al is dissolved and Al^{3+} activity is controlled by solution speciation.

Site	H	Al	Ni	Cu	Zn	Cd	Pb
1	940	860	0.1	67.2	20.6	0.04	0.2
2	924	738	0.1	84.4	22.8	0.04	0.1
3	1320	1178	0.0	0.6	0.0	0.00	0.0
4	1060	1237	0.0	0.7	0.1	0.00	0.0
5	1282	100	0.2	26.5	390.8	0.54	251.0
6	1210	610	0.3	6.1	18.7	0.03	2.3
7	1198	629	0.3	8.7	21.3	0.04	4.2
8	1188	665	0.3	7.1	16.6	0.02	0.8
9	1020	356	0.5	17.2	116.6	0.13	37.7
10	890	135	0.0	38.3	524.1	0.57	18.0
12	896	375	0.4	4.5	350.9	0.10	4.9
14	894	301	0.2	3.1	316.4	0.06	3.9
15	943	334	0.1	1.3	119.4	0.03	0.3
16	963	337	0.2	1.6	75.9	0.04	1.2
17	983	351	0.2	2.0	37.4	0.02	0.5
18	895	642	0.5	1.7	27.1	0.02	0.9
19	908	965	0.4	1.6	18.8	0.01	1.9
20	878	687	0.5	1.4	108.4	0.09	1.7
21	741	167	0.2	4.1	820.7	0.23	2.8
22	941	556	0.3	2.1	288.6	0.17	1.5
26	882	724	0.4	1.2	86.4	0.05	2.0
27	1051	457	0.5	1.7	6.4	0.01	0.1
28	887	152	0.1	4.1	267.2	0.31	3.6
29	908	344	0.2	2.9	172.8	0.12	2.6
30	960	489	0.4	1.2	19.6	0.02	0.6
31	927	638	1.0	1.9	45.6	0.10	0.8
32	1140	961	0.2	1.4	12.7	0.02	0.5
33	969	282	0.2	2.2	22.8	0.02	0.5
34	1000	604	0.7	2.1	7.8	0.01	0.1
35	1056	683	0.5	1.2	6.7	0.01	0.1

5.4.2 Toxicity coefficients

The values of v_M (conveniently expressed in mmol g^{-1}) were used to define a toxicity function, F_{tox} , according to the equation:

$$F_{\text{tox}} = \sum \alpha_M v_M \quad (5.7)$$

where α_M is the toxicity coefficient and M refers to each metal (including H^+). The toxicity coefficients relate the amount of bound metal to its individual toxic effect. Referring to Tables 5.6 and 5.7, it is evident that, because values of v_M are low in all cases for Ni and Cd, for these metals to exert comparable toxic effects to the other metals, their toxicity coefficients would have to be correspondingly greater (i.e. they would have to exert a greater toxic effect per amount bound).

5.4.3 Linear model

In the first attempt to fit the TBM, the values of α_M were adjusted to provide the best linear fit of observed/expected values for the macroinvertebrates, using the most sensitive 67% of the taxa, referred to as OE67. Thus, the overall model took the form:

$$OE67 = A F_{tox} + B \quad (5.8)$$

The term F_{tox} can be expanded from equation 5.7 so that there are up to seven values of α_M although only six of these have to be optimised. The total number of possible parameters is therefore eight (six α_M values, A and B).

Figure 5.6 shows the results of applying the model to the 30 data that conform to RIVPACS criteria. Fitting was done using Excel SOLVER, by minimising the sum of the squared errors between observed and calculated OE67. A slightly better fit is achieved if all filterable Al is assumed to be dissolved (right panel of Figure 5.6). At this stage, no attempt is made either to interpret the parameter values, or to reduce the number of metals that have to be included to achieve the fit.

Inspection of the plots in Figure 5.6 suggests that some of the sites in Teesdale, Tynedale and Weardale (closed circles) give lower OE67 values at a given F_{tox} than the other sites. This suggests that additional factors may affect OE67. To explore the possibility that these might be physico-chemical, the residuals from the plots in Figure 5.6 were regressed against all the individual physico-chemical variables determined during the project. For two determinands, the value of r^2 from these regressions exceeded 0.1, these being (a) suspended particulate matter (SPM), with $r^2 = 0.168$ (Al controlled by $Al(OH)_3$) and 0.160 (all Al dissolved), and (b) filtered titanium, with $r^2 = 0.332$ for both assumptions about Al. The Ti regression for one of the Al assumptions is shown in Figure 5.7.

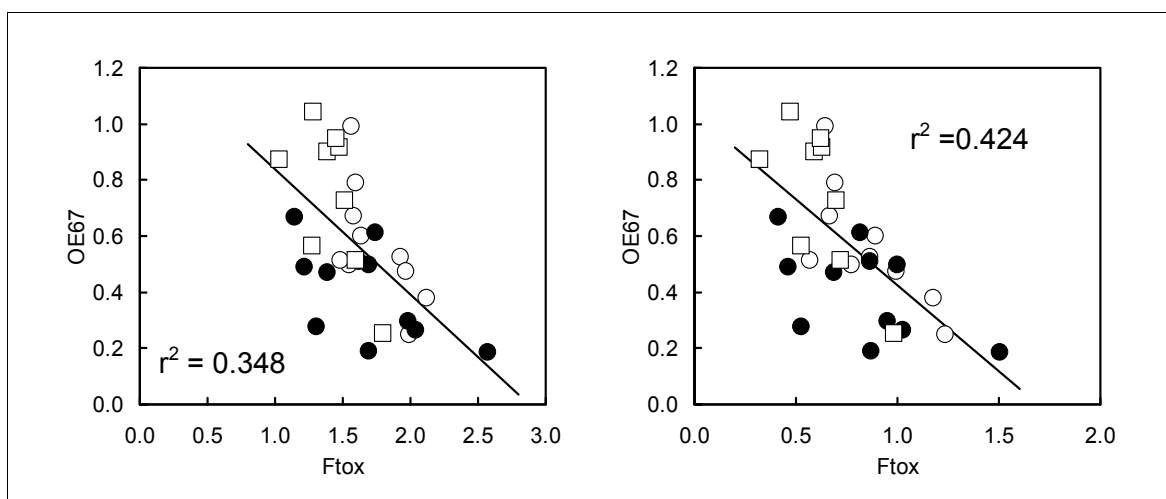


Figure 5.6 Linear modeling of macroinvertebrate occurrence, using equation 5.8. For the left panel, $Al(OH)_3$ control was assumed when the solubility product would be exceeded; for the right all filterable Al is assumed to be truly dissolved. The open circles refer to sites in the Lake District (1–10), closed circles to sites in Teesdale, Tynedale, and Weardale (Sites 12, 14–22, 26) and open squares to sites in Swaledale and the Howgill Fells (Sites 27–35).

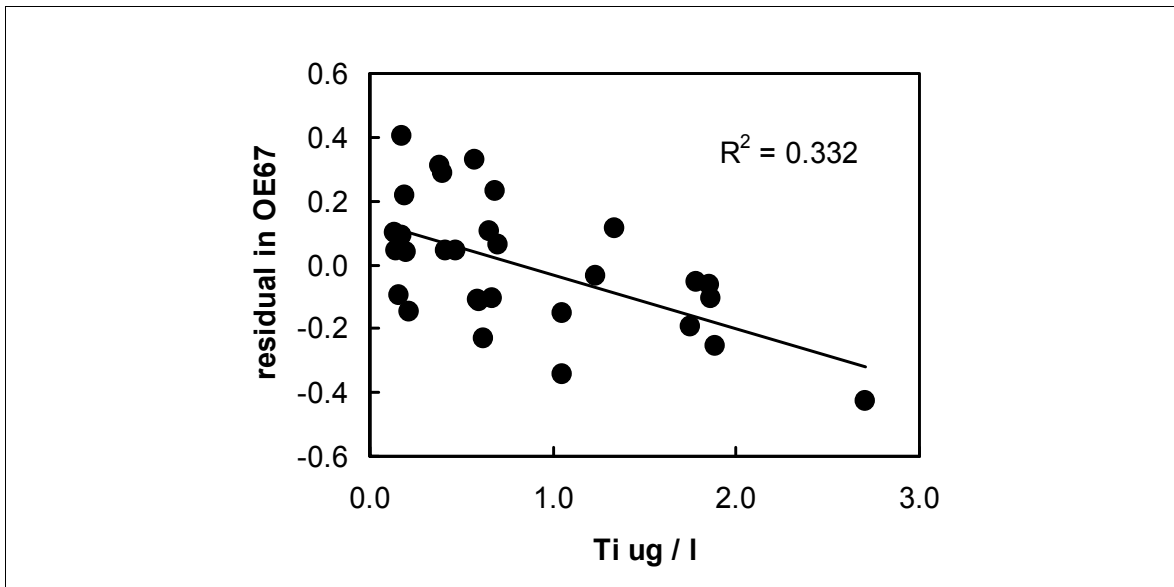


Figure 5.7 Residuals from the left plot of Figure 5.6, plotted against filterable Ti concentration.

The appreciable correlation between the residuals and Ti is not readily explained. Titanium is *not* known for its toxicity, and is a highly insoluble element, extensively hydrolysed in the pH range of most streamwaters. It is unlikely that the measured concentrations of filterable Ti reflect the truly dissolved element and they may be an indicator of fine colloidal material. A deleterious effect on the invertebrates of such material would be consistent with the (weaker) dependence on SPM, since both involve the mobilisation of particulates. Thus, if fine particulate material in streamwaters interferes with invertebrate feeding for example, reduced diversity might arise. However, we have been unable to find literature reports to support this contention about colloidal effects.

If the correlations with Ti and SPM indicate a physical rather than a chemical effect on the macroinvertebrates, it can be assumed that it occurs independently of metal toxicity. Therefore we propose the following modified equation:

$$OE67 = A F_{tox} + B + C [Ti] \quad (5.9)$$

Which can be rearranged to give:

$$OE67 - C [Ti] = A F_{tox} + B \quad (5.10)$$

The left side of the equation can be considered to represent a 'corrected' OE67, leaving the right side to describe the effects of toxic metals, that is:

$$OE67_{corr} = A F_{tox} + B \quad (5.11)$$

Figure 5.8 shows that application of equation 5.9 to the data, with optimisation of the additional parameter (the constant C) substantially improved the model fit, principally by relocating the points symbolised by closed circles and open squares (i.e. the non-Lake District sites where suspended particulates tend to be present at higher concentrations).

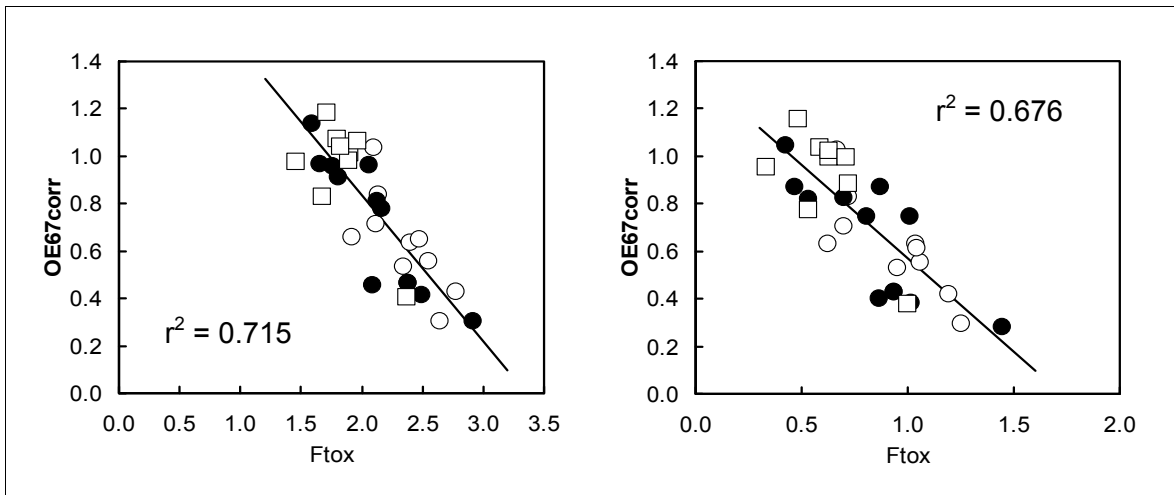


Figure 5.8 Linear modeling of macroinvertebrate occurrence, fitted using equation 5.9, and plotted according to equation 5.11 (i.e. with $OE67_{corr}$ on the y-axis). The symbols are the same as in Figure 5.6.

5.4.4 Threshold model

The plots in Figure 5.8, together with the theoretical idea that the maximum value of OE67 should be 1.0, suggest that a preferable way to describe the data is with a model that identifies a value of F_{tox} below which no toxic effects are observed, and another value above which $OE67 = 0$. Therefore the data were fitted to the following equations:

$$F_{tox} \leq F_{tox,min} \quad OE67 = 1.0 \quad (5.12)$$

$$F_{tox} > F_{tox,min} \quad OE67 = (F_{tox,max} - F_{tox}) / (F_{tox,max} - F_{tox,min}) \quad (5.13)$$

$$F_{tox} > F_{tox,max} \quad OE67 = 0.0 \quad (5.14)$$

where $F_{tox,min}$ is the lower threshold and $F_{tox,max}$ is the upper threshold. See Figure 5.9.

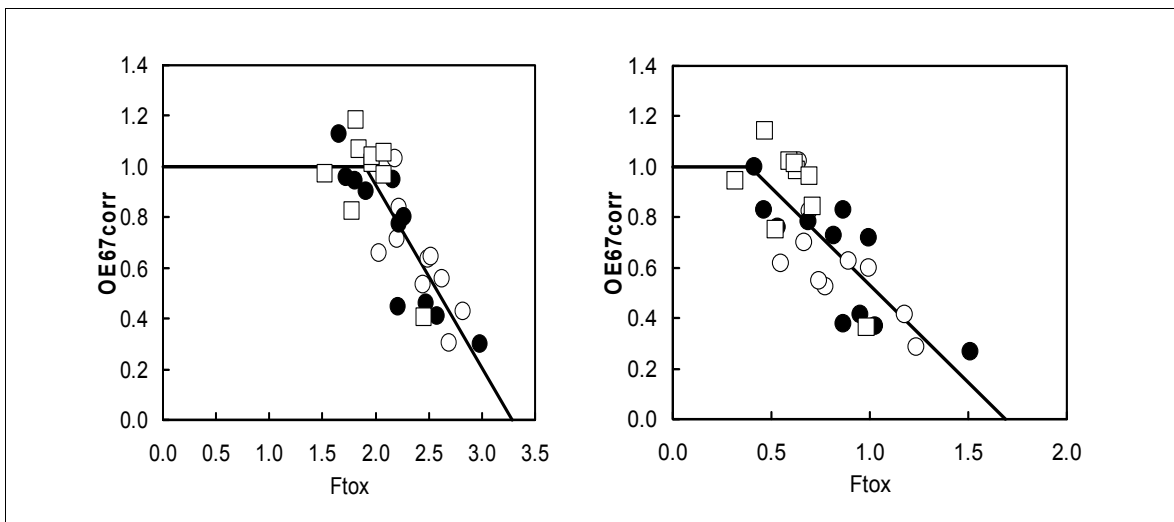


Figure 5.9 Threshold modelling of macroinvertebrate occurrence, fitted using equation 5.9, and plotted according to equations 5.12–5.14. The symbols are the same as in Figure 5.6. Left panel: Al solubility controlled by $Al(OH)_3$. Right panel: all filterable Al assumed in true solution.

The significance of the toxicity coefficients was determined using the chi-squared test. For both assumptions about Al solubility, Al and Zn were clearly the most important metals. With Al(OH)₃ solubility control, Cu and H made the fits significantly better, but Ni, Cd and Pb did not. If all filterable Al is assumed to be dissolved, Cu, Pb and H do not significantly improve the fit. Cadmium does not exert a statistically significant effect in either case, which may partly be explained by its strong correlation with Zn (Section 4.1.4). Table 5.8 shows the model parameters.

Table 5.8 Macroinvertebrate threshold model parameters for the two assumptions about Al³⁺ activity control. The value of α_{Al} is fixed at 1.0, because only relative metal toxicities need be defined. NS = not significant ($p > 0.05$).

Parameter	Al(OH) ₃	All Al dissolved
α_{Al}	(1.00)	(1.00)
α_{Ni}	NS	NS
α_{Cu}	3.97	NS
α_{Zn}	2.28	1.58
α_{Cd}	NS	NS
α_{Pb}	NS	NS
α_H	1.27	NS
$F_{tox,min}$	1.92	0.39
$F_{tox,max}$	3.43	1.69

5.4.5 Contributions of different metals to the TBM toxicity function

The toxicity function F_{tox} is defined by equation 5.7. It expresses the combined toxicities of different metals by the products of the amounts bound, v_M , and the toxicity coefficient, α_M . By considering the products of these two terms for each metal, the separate contributions of the different toxic metals to the overall value of F_{tox} can be obtained. These are plotted for each of the 30 field sites in Figure 5.10.

Considering first the case where Al³⁺ activity is controlled by Al(OH)₃, the upper plot in Figure 5.10 shows that the combined contribution of bound H and Al to F_{tox} varies appreciably among the sites. As would be expected, the acid 'control' sites 3, 4 and 32 are especially affected by H and Al, while alkaline sites (10, 21, 28) are relatively unaffected. Trace metals contribute most to F_{tox} at Sites 5, 10, 12, 14, 21 and 22. The upper panel of Figure 5.10 shows that the parameter values derived for Cu come only from Sites 1 and 2.

When all filterable Al is assumed to be in true solution, the toxicity pattern is simpler (Figure 5.10, lower panel) because only Al and Zn are significant (Table 5.8).

One way to consider the interplay among the different metals is to say that the different sites are 'poised' differently by the Al and H. Cases can be identified where in the absence of toxic trace metals the value of F_{tox} would be much lower than the minimum threshold, $F_{tox,min}$, and in such cases a relatively high degree of binding of a toxic trace metal would be required for the threshold to be exceeded. Sites 10, 21 and 28 fit into this category. On the other hand, at an acid site, only a small amount of trace metal binding would be needed to pass $F_{tox,min}$, as for Sites 1, 2 and 31. However, metal binding is weaker under acid conditions and so to achieve the required trace metal site occupancy might require a comparatively high solution concentration.

When identifying metals that significantly affect the model fit, Al was found to be highly significant, even if acid sites were omitted from the dataset. At first sight, this was a surprise, because it was expected that any toxic effects of Al would be taken into account when deriving the expected macroinvertebrate occurrences in the original RIVPACS parameterisation. Since RIVPACS does not take chemistry into account, except through alkalinity, any toxic effects of Al would be subsumed within the observed macroinvertebrate occurrences. However, the TBM allows the possibility that streams can differ in the extents of binding of H and Al, while still having $F_{\text{tox}} < F_{\text{tox,min}}$. Thus, the macroinvertebrates at most or all non-acid sites will not be affected by H and Al, but differences in the binding of H and Al can cause differences in their susceptibilities to toxic trace metals. Hence, H and Al come into play in fitting the data, even though they do not cause toxicity by themselves.

Consideration of both Figures 5.9 and 5.10 shows that few sites have an F_{tox} value substantially below the threshold. To explore how typical this might be, we applied the parameterised models to 12 other upland streams for which full chemical analyses were available, but which were unaffected by past mining activities. Nine of the 12 streamwaters had $F_{\text{tox}} < F_{\text{tox,min}}$. With $\text{Al}(\text{OH})_3$ control of Al^{3+} activity, the lowest F_{tox} was 1.34, only slightly less than the lowest value found for the study sites. With all Al assumed to be in solution, the lowest F_{tox} was 0.35, again not very much lower than the lowest value for the study sites. It therefore appears that streamwaters with F_{tox} values near to zero may be rare, or non-existent, and the toxicity-eliciting binding sites are always occupied to some extent, although with $F_{\text{tox}} < F_{\text{tox,min}}$.

The TBM model does not include As, because this metal is present in anionic forms in freshwaters, and currently is assumed not to bind directly to ligands for cations. The site (number 9) at which As is present in high concentrations fits reasonably well within the analysis; however, it does have a relatively low OE67 (~ 0.65) for its F_{tox} values (1.92 or 0.63 depending on assumptions about Al). Therefore it may be that at this site As is exerting an additional toxic effect (cf. Section 7.2).

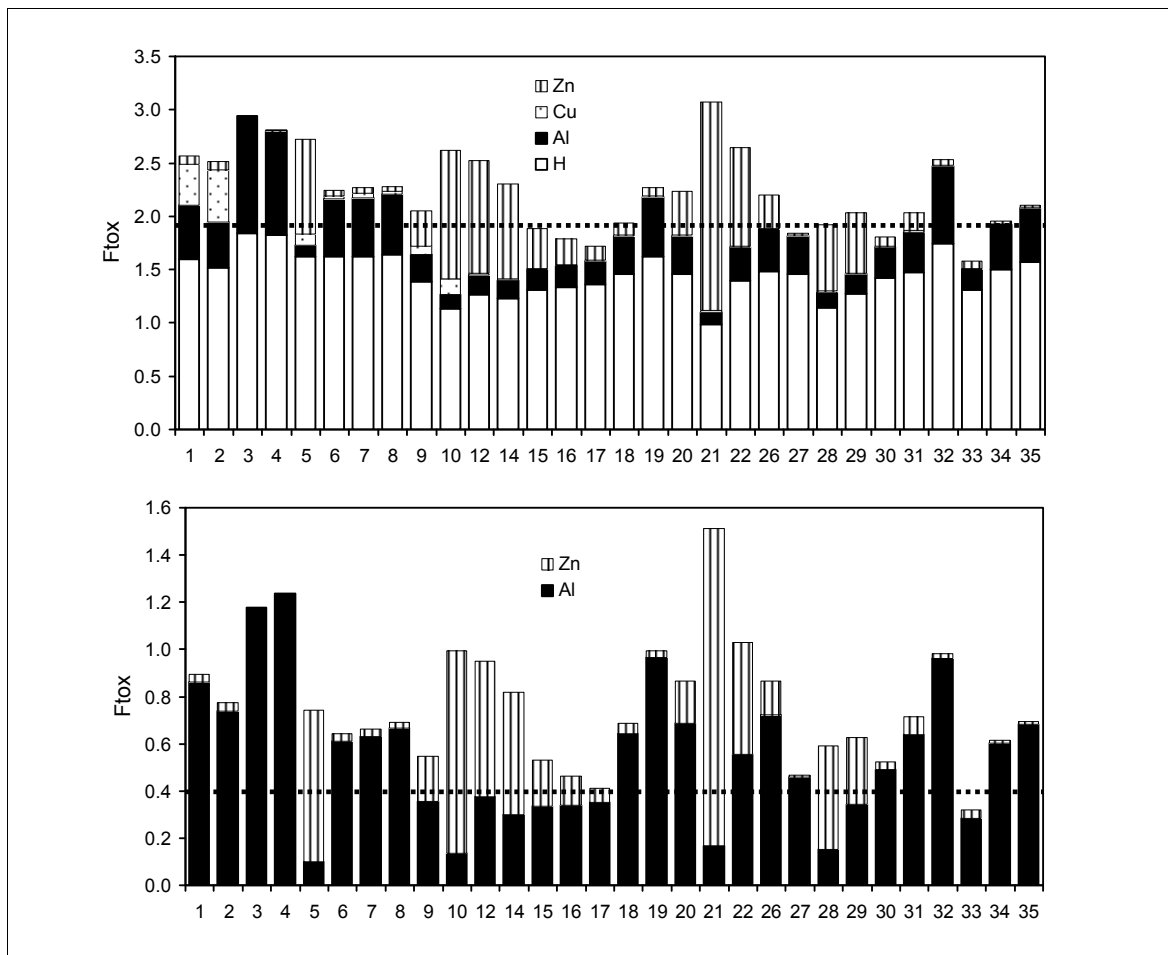


Figure 5.10 Contributions of different metals to the toxicity function F_{tox} at the study sites. Upper panel: Al solubility controlled by $Al(OH)_3$. Lower panel: all filterable Al assumed in true solution. The lower toxicity thresholds are shown by the dotted lines.

5.4.6 Predictive calculations for Cu and Zn

We used the parameterised model to explore the effects of Cu and Zn on stream macroinvertebrates, by calculating F_{tox} as a function of a hypothetical titration with a single metal (Cu or Zn), but with Al present with Al^{3+} activity controlled by $Al(OH)_3$. We chose three contrasting sites, numbers 1, 10 and 33. Reference to Tables 4.1 and 4.2 shows that Site 1 has pH slightly below 7, a low DOC concentration and relatively high Al. Site 10 has a higher pH, appreciably higher alkalinity, a low DOC concentration and a lower Al concentration. Site 33 has a pH above 8, high alkalinity and relatively high DOC (4.5 mg l^{-1}).

At Site 1, F_{tox} already exceeds $F_{tox, min}$ even in the absence of Cu and Zn (top left panel in Figure 5.11). Increasing concentrations of the two metals cause F_{tox} to rise, with $F_{tox, max}$ being reached at a total Cu concentration of ca. $5 \times 10^{-7} \text{ M}$, or a total Zn concentration of ca. $2 \times 10^{-5} \text{ M}$. The different concentrations required to reach $F_{tox, max}$ reflect (a) the stronger binding of Cu, so that v_M values are achieved at lower free metal concentrations, (b) the slightly greater toxicity coefficient of Cu (Table 5.8), and (c) the low DOC concentration, which means that there is relatively little competitive complexation of Cu in solution.

At Site 10, F_{tox} is less than $F_{tox, min}$ in the absence of added Cu or Zn, which means that the invertebrates are not affected by toxic metals. Total Cu and Zn concentrations of

ca. 10^{-7} and 10^{-6} M, respectively are required to reach $F_{\text{tox}, \text{min}}$, and about 10^{-6} M and 3×10^{-5} M, respectively to reach $F_{\text{tox}, \text{max}}$. The latter two concentrations are similar to those required to reach $F_{\text{tox}, \text{max}}$ at Site 1, and this reflects opposing trends associated with the lower Al activity and higher pH at Site 10; more bound Cu and Zn are needed to reach a given F_{tox} , but binding of the two metals is stronger because of the higher pH.

At Site 33, F_{tox} is again less than $F_{\text{tox}, \text{min}}$ in the absence of added Cu or Zn. However, this site differs from Site 10 in that rather similar total concentrations of Cu and Zn (5×10^{-7} to 10^{-6} M) are needed to reach $F_{\text{tox}, \text{min}}$. This is because DOM competes with the organisms' binding sites for Cu, but not so much for Zn. For the same reason, the concentration of Zn needed for F_{tox} to reach $F_{\text{tox}, \text{max}}$ at Site 33 is only slightly greater than at Sites 1 and 10, whereas about five times the total copper concentration is required.

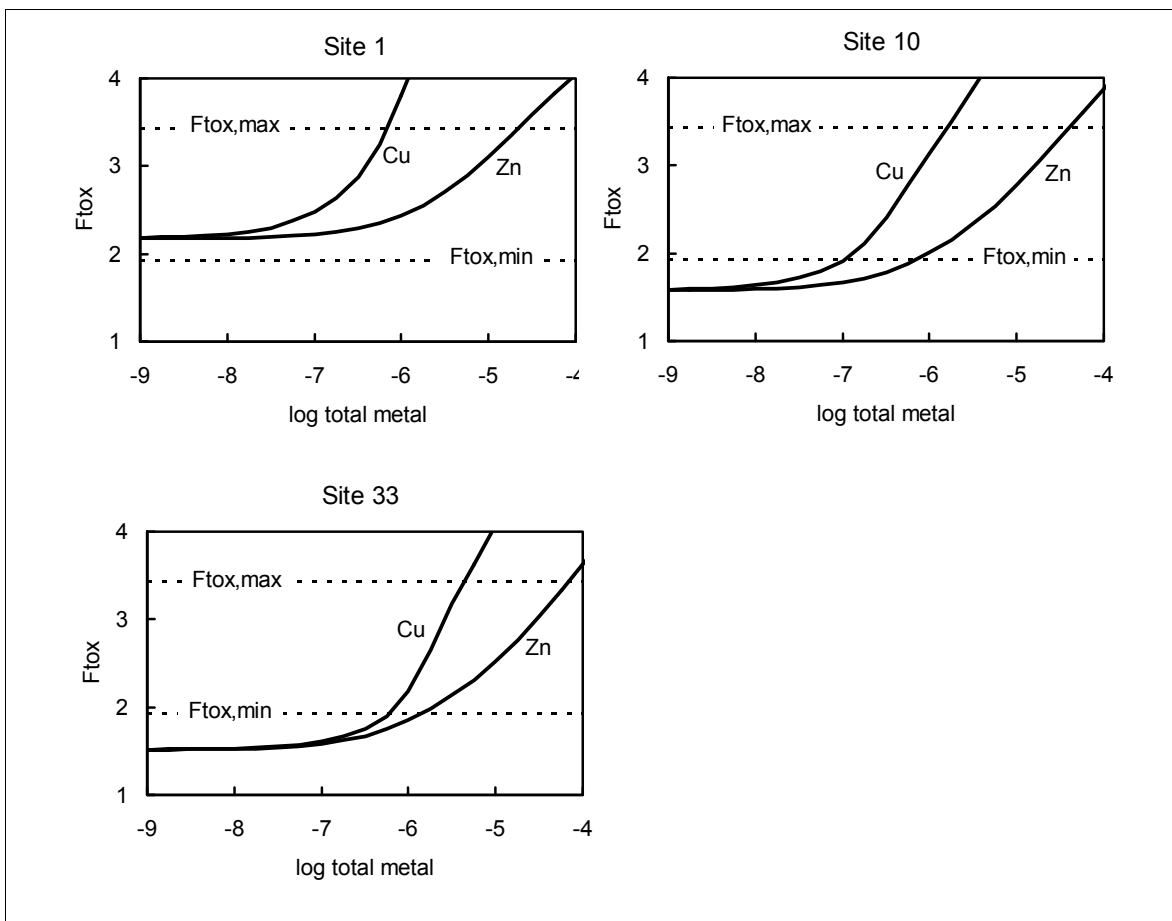


Figure 5.11 Calculated F_{tox} as a function of total metal (Cu or Zn) concentration for Sites 1, 10 and 33. The dotted lines show the values of $F_{\text{tox}, \text{min}}$ (the value of F_{tox} below which there are no toxic effects) and $F_{\text{tox}, \text{max}}$ (the value of F_{tox} required to cause complete absence of responsive macroinvertebrate species).

5.4.7 TBM: concluding remarks

Operating on the same principle as the Biotic Ligand Model, the threshold model condenses complex water chemistries into a single linear variable, F_{tox} . This provides an assessment of the combined effects of toxic metals, in terms of lower and upper threshold values. Not only does the model describe toxicity due to trace metals, but it

also readily incorporates the effects of Al and H, which are especially important in acid waters, but cannot be neglected in neutral waters.

The results obtained by application of the BLM to macroinvertebrates can be regarded as highly promising, providing a better quantitative explanation of variability in macroinvertebrate taxa than any of the other approaches tried in this chapter. However, the results with the TBM are by no means conclusive, principally for the following reasons:

- (a) The modelling is based on the assumption that the biotic ligand(s) possessed by the macroinvertebrates can be represented by the collection of binding sites postulated for humic acid. Although it is reasonable to suppose that similar types of ligand atoms (oxygen, nitrogen and possibly sulphur) are involved in both cases, it is clearly a gross approximation to assume that they are arranged in the same ways. However, the results of applying WHAM to describe the metal contents of bryophytes (Section 4.5) and invertebrates (Section 4.6) support the approximation.
- (b) The good model fits of Figures 5.7 and 5.8 depend to a considerable extent on the correction applied, based on Ti concentrations. Clearly, a full explanation of the correlation with Ti is required in order to make fitting of the corrected data convincing.
- (c) The streamwaters do not provide a full range of concentrations of all the metals of interest. Only Al and Zn are adequately represented. The parameter value derived for Cu depends on only a few sites.
- (d) Although we consider Al^{3+} to be better predicted by the solubility control assumption (when the calculated solubility product would be exceeded), the aluminium chemistry of neutral and alkaline waters is uncertain, and because of the ubiquity of Al in the streamwaters, this affects the parameterisation for all other metals.
- (e) Even after applying the Ti-based correction, the model only explains about 70% of the variance in OE67. Inspection of the plots in Figure 5.8 suggests that there may be a 'roof' formed by the higher points, reminiscent of boundaries produced by quantile regression, which implies that additional factors may need to be identified and quantified in order to isolate the effects purely due to metals. One such additional factor, in the case of Site 9, could be arsenic.

Therefore, the development of the model, and the conclusions drawn, must be regarded as speculative at present. To resolve the uncertainties, further fieldwork is needed, which should extend the approach used in the present study. In particular, the different toxic metals need to be covered more completely, aluminium chemistry needs to be better defined, and the postulated effects of colloidal material should be investigated.

The TBM is applied to field diatom data in Chapter 6 and to laboratory algal toxicity results in Chapter 7.

6 Diatoms and streamwater chemistry

This chapter describes analyses of diatom data in terms of streamwater chemistry, the aim being to search for dose–response relationships with toxic metals.

As explained in Section 5.1, for macroinvertebrates we can allow for differences in the physical characteristics among the field sites by the use of RIVPACS, and this enables the observed macroinvertebrate occurrences to be standardised by comparison with the expected taxa. Unfortunately, no such equivalent system is yet available for diatoms. For diatoms, the Diatoms for Assessing River and Lake Ecological Quality (DARLEQ) system (Kelly *et al.*, 2006) gives estimates of the Trophic Diatom Index (TDI) expected in the absence of anthropogenic stress; however, this assumes a nutrient/organic pressure gradient and is not sensitive to toxic stresses. Therefore, in order to analyse the diatom data, we applied statistical community analysis in an attempt to separate out physical factors. Regression analysis and the Toxicity Binding Model (TBM) were also applied, simply using the number of species to describe the diatom communities.

6.1 Diatom community analyses

As a preliminary step, we looked for possible relationships between diatom taxon richness and macroinvertebrate taxon richness, but there is no apparent overall correlation ($r = -0.075$, $p = 0.68$) between the number of diatom taxa recorded at a site and the number of macroinvertebrate BMWP families observed (Figure 6.1). There are a variety of potential factors leading to this lack of inter-correlation: differing requirements and response to physical conditions, differing responses to metals, predator–prey type interactions, and macroinvertebrate grazing impacts on relative abundance of diatoms and thus probability of capture in a limited diatom sample count (total count of 300 individuals per sample – see Section 2.2.2).

We then proceeded to ordination analyses. All diatom community ordination analyses were based on the full dataset of 34 field sites and 104 diatom taxa (unidentifiable 'unknown' individuals were ignored, as was *Pinnularia capitata* which was present only at HS6 but in numbers too low to be recorded in the count of 300 valves). All ordination analyses and randomisation tests for relationships between diatoms, environmental variables and metals were performed using the CANOCO for Windows software (ter Braak and Šmilauer, 2002).

6.1.1 Detrended Correspondence Analysis (DCA) of diatom community

In a preliminary analysis we examined the overall variation in the diatom community composition without reference to either the physical/environmental characteristics of the sites or their metal loadings. This was done using Detrended Correspondence Analysis (DCA) ordinations to provide a representation of the major axes of variation between the 34 sites in their diatom community composition (Figure 6.2). The DCA was based on the CANOCO options of detrending by segments, using $\log(x+1)$ transformed abundances to avoid overdependence on the very common taxa and on any outlier of unusually high individual abundances, and with CANOCO's standard down-weighting of rare species. With this form of analysis no single site dominates and distorts the

DCA ordinations through having a completely different 'outlier' diatom community from the other sites. This suggests at this stage that we do not need to consider omitting any of the sites from further analyses.

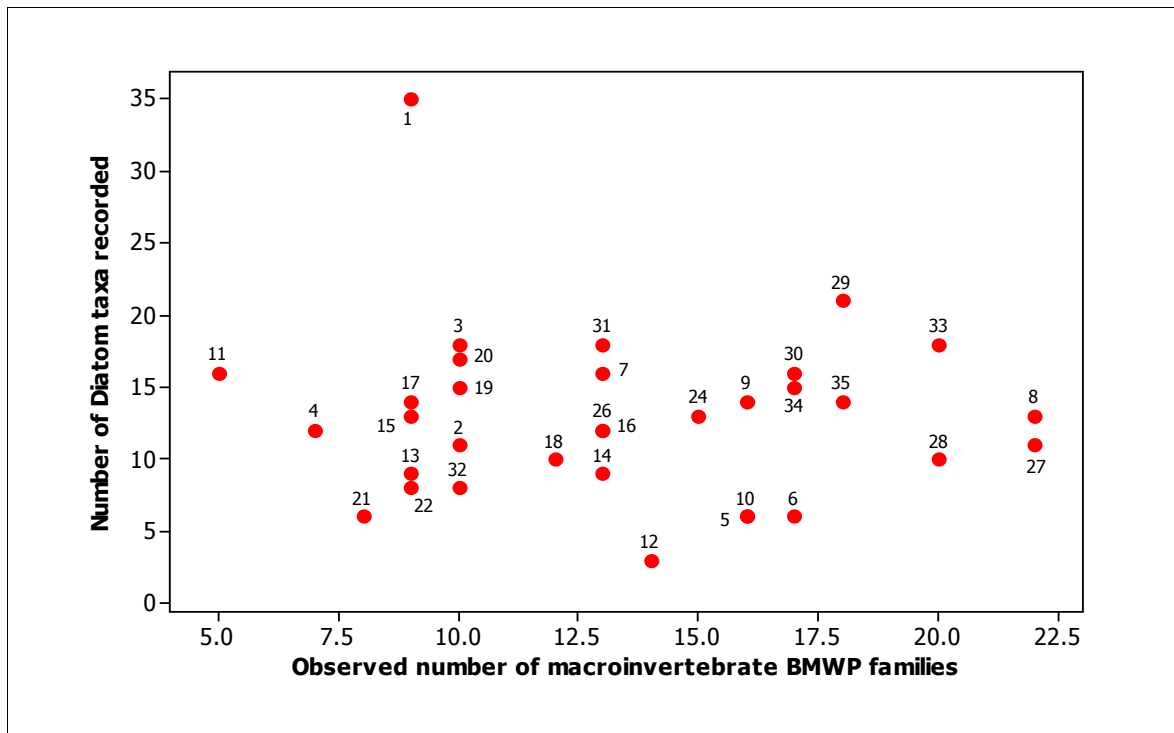


Figure 6.1 Relationship between the number of diatom taxa recorded at a site and the number of macroinvertebrate BMWP families observed.

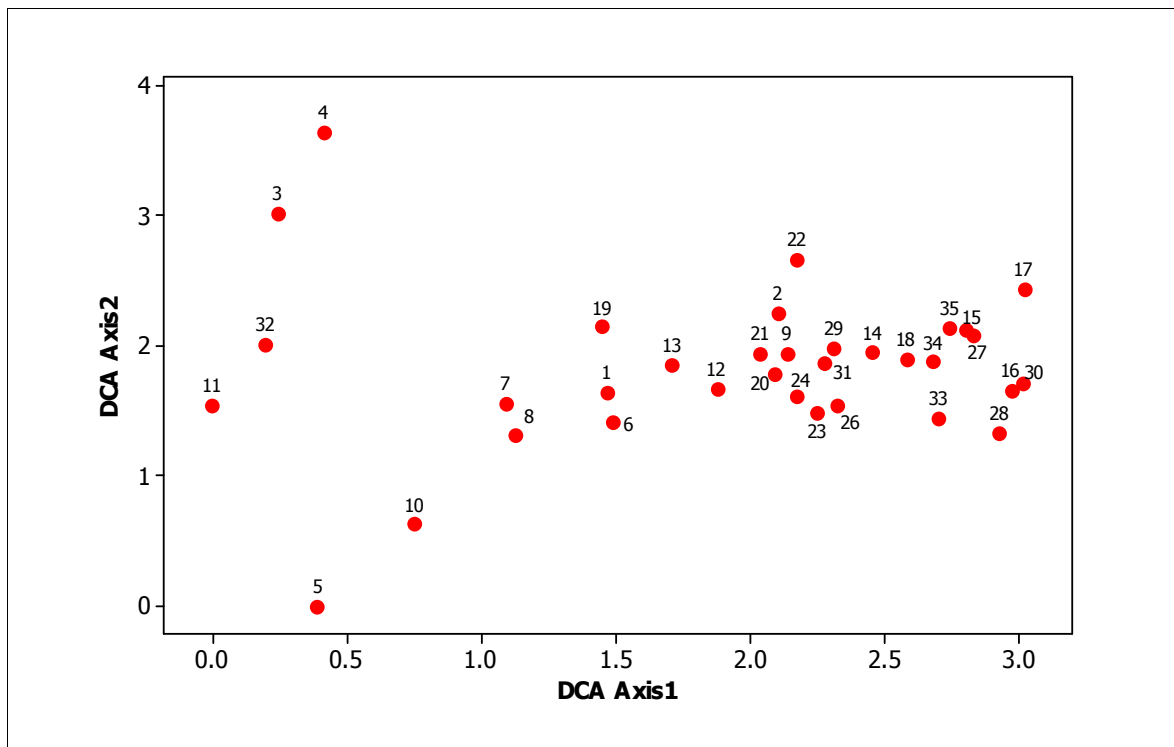


Figure 6.2 Plot of the distribution of the 34 sites on the first two ordination axes of a Detrended Correspondence Analysis (DCA) of the diatom community composition.

6.1.2 CCA of the relationship between diatom community and the environmental characteristics of sites (excluding metal effects)

The diatom community composition and abundances to be expected at a site in the absence of any anthropogenic stress will depend on its physical type and environmental characteristics. These physical characteristics are also likely to continue to influence the fauna present at a site when it is impacted by some form of stress (metal or otherwise). To address the problem of the absence of expected values for diatom species abundance and community composition, our solution was to use the RIVPACS environmental predictor variables in a partial Canonical Correspondence Analysis (pCCA), using CANOCO, to 'partial out' (i.e. hopefully remove) the effects of differences in the physical characteristics of the sites before assessing the relationship between the residual (i.e. remaining) diatom community variation and the metal concentrations. This is similar to the approach used by Davy-Bowker *et al.* (2005) in their analysis of macroinvertebrate community composition in relation to site acidity (i.e. pH) that led to the development of the AWIC metric (Acid Waters Indicator Community).

Specifically, we first carried out a Canonical Correspondence Analysis (CCA) of the diatom composition in relation to the RIVPACS environmental predictor variables listed in Table 6.1. We used the same $\log(x+1)$ transformation of diatom species abundances. Variables were manually selected one-by-one in a stepwise manner by carrying out sequences of Monte Carlo randomisation tests (999 randomisations) of the statistical significance of the partial relationship between the currently unexplained component of the inter-site variation in diatom community and each variable in turn (Table 6.1). These Monte Carlo tests repeatedly randomise the (residual) values of the environmental variable under consideration between the sites to estimate the null (i.e. chance) distribution of the strength of relationship measure with the remaining diatom community variation.

Table 6.1 Statistical significance (*p* values) of Monte Carlo randomisation tests in stepwise selection of environmental variables for maximising the relationship with diatom community composition among the 34 sites (p-value of variable selected in each step highlighted in bold).

Environmental variable	CCA variable selection step				
	1	2	3	4	5
pH	0.001				
Altitude	0.001	0.003			
Alkalinity	0.005	0.010	0.009		
Streamwater width	0.259	0.173	0.045	0.021	
Slope at site	0.167	0.076	0.103	0.083	0.071
Distance from source	0.284	0.229	0.105	0.085	0.370
Streamwater depth	0.651	0.531	0.600	0.483	0.686

When each environmental variable was considered on its own (Step 1), only pH ($p = 0.001$), altitude ($p = 0.001$) and alkalinity ($p = 0.005$) showed statistically significant relationships with the diatom community composition. In the stepwise selection of variables, these three variables all gave significant partial improvements in the strength of biota–environment relationship (Table 6.1). In addition, after adjusting for the effects

of these three variables, streamwater width (at the time of sampling) gave a further minor improvement in fit ($p = 0.021$). No other variables gave any further improvement.

These results concur with observations made during the development of DARLEQ (Kelly *et al.*, 2006). These analyses focused on a dataset of circumneutral sites, so pH was not a major factor, although there is good evidence of a strong pH-related effect on diatom assemblages (e.g. Battarbee *et al.*, 1999). Altitude and alkalinity both explained a significant part of the variation in assemblage composition when multivariate regression trees (De'ath, 2002) were constructed for reference sites in the dataset. The final predictive equation proposed by Kelly *et al.* (2006) is a quadratic function based on alkalinity and season; altitude was also tested but did not improve the fit significantly.

The CCA bi-plot ordination (derived using the CANOCO extension graphics package CANODRAW) relating the sites' diatom compositions to the four selected environmental predictor variables is shown in Figure 6.3. The direction and length of arrows indicate the inter-relationships and strength of effects of the variables on the diatom fauna; this highlights the strong correlation between pH and alkalinity and that pH has the slightly stronger relationship with diatom community composition. Altitude also has a strong association with aspects of the diatom composition, which is largely independent of the pH/alkalinity component, indicated by the different directions of the arrows in Figure 6.3.

These four 'best-fit' environmental variables (pH, altitude, alkalinity and streamwater width) were therefore selected to partial out the major effects of stream type and physical characteristics on the 'natural' variation in diatoms between the study sites, before assessing remaining relationships with metal concentrations (Section 6.1.3).

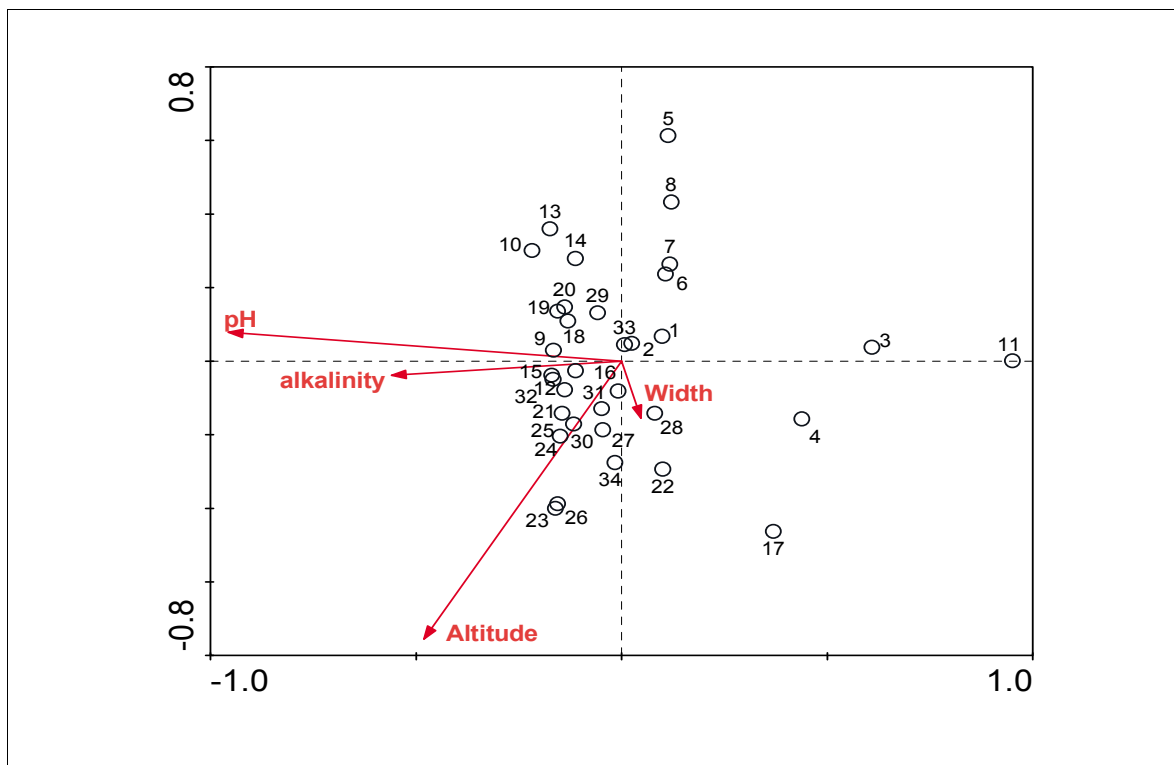


Figure 6.3 Bi-plot of the distribution of the 34 sites on the first two ordination axes of a CCA of the relationship between their diatom community composition and four selected environmental variables (direction and length of arrows indicate the inter-relationships and strengths of effects of the variables on the diatom fauna of the sites).

6.1.3 pCCA of the relationship between diatom community composition and metal concentrations allowing for the environmental characteristics of sites

The next stage of the analysis was to use partial CCA (pCCA) to eliminate the joint effect of the four environmental variables (pH, altitude, alkalinity and streamwater width) selected in the stepwise CCA (Section 6.1.2) from the overall diatom community variation among the sites, prior to assessing the relationship between the metal concentrations and the residual diatom community variation.

This attempts to adjust for 'natural' variability in the diatom community composition among the study sites before assessing the community relationship with metal concentrations. This should help reduce spurious correlations between the diatom community and metal concentrations.

The metal concentrations have highly skewed distributions with a few sites having much higher values than the other sites. Although these highly contaminated sites might be expected to have the highest impacts on the diatom communities, any ordination analyses involving metals will be overly influenced by the values for these one or few sites. Also it may be expected that ecological response of individual taxa to stress could operate on a logarithmic scale, in that, although taxa may have different preferences and tolerances to metals, they may be more likely to respond more equitably to a percentage increase in metal concentrations (e.g. Kelly and Whitton, 1989a; Whitton *et al.*, 1989). For this reason, it was decided to assess the relationship between diatom community and metals using metal concentrations in their logarithmic form as predictor environmental variables in the partial CCA (pCCA).

The analyses were carried out on the five metals individually and together, using the metals in one of three forms: total dissolved concentration (TD), free ion concentration (FI) or DGT. As noted in Chapter 4, and summarised in Table 6.2, the metal concentrations are correlated, which made it difficult in the pCCA to disentangle the effects of each metal and even which metal was the cause of the impact.

Initially, the pCCA analyses were carried out using all five metals together, without attempting to disentangle their individual effects. Monte Carlo permutation tests in CANOCO were used to assess the statistical significance of the observed strength of the overall relationship between diatom species and metals based on all canonical ordination axes (i.e. based on all aspects of the species–metals partial relationship). These overall tests were most highly statistically significant for metals in the FI form ($p = 0.0023$ based on 9999 permutations), also significant for metals in their TD form ($p = 0.0146$), but not for metals in their DGT form ($p = 0.3401$).

However, if some metals show associations with diatoms species and others do not, then the overall effect (which is a form of average effect) may be diluted or be non-significant. Therefore the pCCA analyses were repeated using stepwise selection of individual metals based on appropriate randomisation tests of their association with diatom community composition; results based on the different forms of metal concentration are summarised in Table 6.3 and Figures 6.4–6.6.

The column headed 'Step 1' in Table 6.3 gives the randomisation test p-values for assessing the statistical significance of any relationship between the diatom community (adjusted for environmental type) and each of the five metals in turn. The column headed 'Step 2' shows the results of the randomisation tests for each remaining variable once the effect of the most significant metal variable has already been included (as per stepwise multiple regression).

Table 6.2 Correlations between log metal concentrations among the 34 study lakes in (a) TD form, (b) FI form and (c) DGT form.

(a)	log TD Ni	log TD Cu	log TD Zn	log TD Cd
log TD Cu	0.392			
log TD Zn	0.776	0.568		
log TD Cd	0.703	0.594	0.968	
log TD Pb	0.690	0.384	0.847	0.824

(b)	log FI Ni	log FI Cu	log FI Zn	log FI Cd
log FI Cu	0.404			
log FI Zn	0.756	0.524		
log FI Cd	0.656	0.602	0.962	
log FI Pb	0.651	0.765	0.743	0.758

(c)	log DGT Ni	log DGT Cu	log DGT Zn	log DGT Cd
log DGT Cu	0.544			
log DGT Zn	0.694	0.500		
log DGT Cd	0.751	0.602	0.937	
log DGT Pb	0.562	0.531	0.847	0.871

For the total dissolved (TD) form of metal concentrations, there were statistically significant partial relationships of diatom community with each metal (Step 1 column in Table 6.3(a)). Zinc was the most significant ($p = 0.013$), but once zinc was included in the ordination, only nickel added any significant ($p = 0.032$) improvement to the relationship with the diatom community. This was because of the inter-correlations between the TD forms of metal among these study sites. The pCCA bi-plot of diatom species and (log) TD metal concentrations in Figure 6.4 shows that nickel can explain a mostly independent aspect of the diatom community to that of zinc and the other metals.

The positions of the various diatom taxa along each pCCA axis are shown in Figures 6.4–6.6. It should be remembered that the directions of the axes from positive to negative are meaningless in their own right and can change between analyses. The interpretation of axes should only be based on the directions of the metals along these axes (arrows on figures indicate direction of increasing metal concentrations).

For the free ion (FI) form of metal concentrations, there were statistically significant partial relationships of diatom community with each individual metal except copper (Table 6.3(b)). Lead was most highly significant ($p = 0.002$), but once lead was included in the ordination, only nickel added any significant ($p = 0.008$) improvement to the relationship with diatom community. The pCCA bi-plot of diatom species and (log) FI metal concentrations in Figure 6.5 shows that nickel explains a largely independent aspect of the diatom community to that of lead and the other metals.

Table 6.3 Statistical significance (p-values) of Monte Carlo randomisation tests in stepwise selection of metal variables for maximising the relationship with diatom community composition among the 34 sites after partialling out the effect of significant environmental covariables (pH, altitude, alkalinity and streamwater width). Metals are present in one of three forms: (a) total dissolved, (b) free ion and (c) DGT. Statistically significant p-values (i.e. ≤ 0.05) are highlighted in italics, and the p-value of the variable selected in each step is highlighted in bold.

Metal	pCCA variable selection step		
	Step 1	Step2	Step 3
(a) Total dissolved (TD) form			
Cadmium	<i>0.050</i>	0.098	0.165
Zinc	<i>0.013</i>		
Copper	0.056	0.421	0.499
Nickel	<i>0.028</i>	<i>0.032</i>	
Lead	<i>0.050</i>	0.605	0.456
(b) Free ion (FI) form			
Cadmium	<i>0.030</i>	0.417	0.445
Zinc	<i>0.014</i>	0.186	0.642
Copper	0.053	0.403	0.394
Nickel	<i>0.014</i>	<i>0.008</i>	
Lead	<i>0.002</i>		
(c) DGT form			
Cadmium	<i>0.028</i>		
Zinc	<i>0.043</i>	0.826	0.766
Copper	0.071	0.191	0.446
Nickel	0.070	<i>0.036</i>	
Lead	0.129	0.989	0.975

Table 6.4 shows the score (i.e. position) of each diatom species along the ordination axis of a pCCA involving only TD lead, the most individually statistically significant metal. As TD lead concentrations are positively correlated with axis scores, species with negative scores have some overall preference for sites with relatively low lead concentrations. However, it is important to take account of the frequency of occurrence of each species as measured by N2, a form of Simpson's diversity index which indicates the effective number of sites over which each species was found (i.e. taking account of their relative abundance on each site). The species with the very large negative scores tend to have only occurred on one or two sites; in fact many species only occurred on a few sites. Thus, individually, each taxon does not contain much information about the effect of lead (or more generally any other metal), but collectively the diatom species community does show significant associations with metal concentrations. This is part of the statistical power of community analyses.

When based on the DGT form of metal concentrations, there were statistically significant partial relationships of diatom community with each of two metals, cadmium ($p = 0.028$) and zinc ($p = 0.043$) (Step 1 column in Table 6.3(c)). However, once cadmium was included in the ordination, only nickel added any significant ($p = 0.036$) improvement to the relationship with diatom community. The pCCA bi-plot of diatom

species and (log) FI metal concentrations in Figure 6.4 highlights how nickel explains the most independent aspect of the diatom community to that of cadmium.

Diatom species positioned on the ordination axes away from the directions of the arrows are those which tend to have the lower (relative) abundances when the metal(s) concentrations are higher.

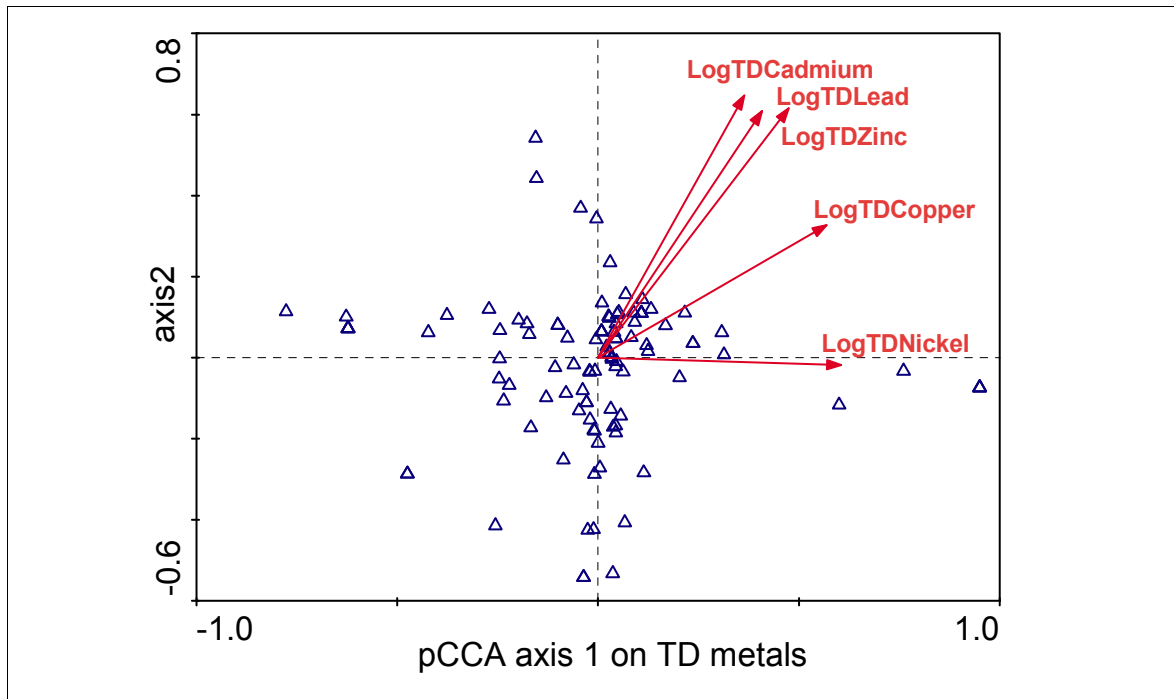


Figure 6.4 The position of diatom taxa (triangles) along the first two ordination axes of a partial CCA (pCCA) with the \log_{10} total dissolved (TD) metal concentrations for lead, cadmium, copper, zinc and nickel as explanatory variables after partialling out the effects of significant environmental covariables (pH, altitude, alkalinity and streamwater width). Arrows indicate direction and strength of increasing metal concentration.

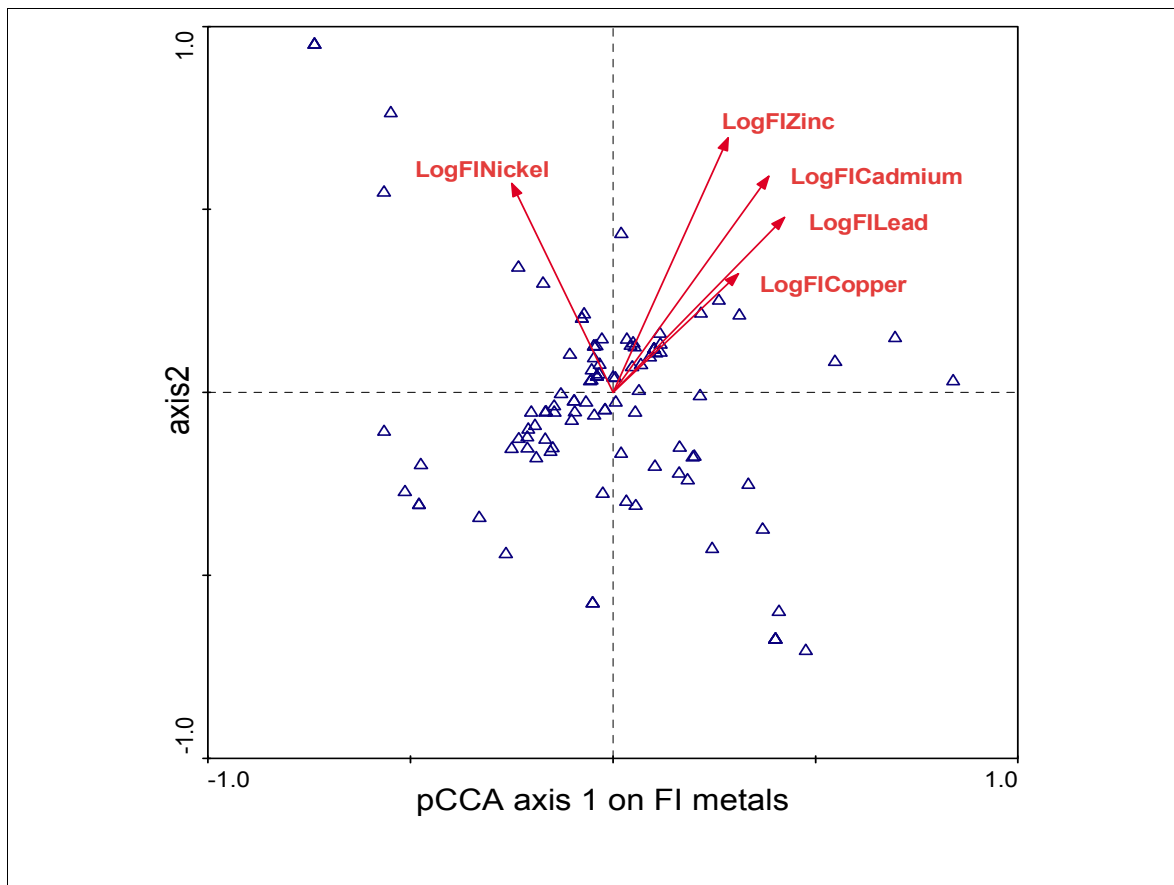


Figure 6.5 The position of diatom taxa (triangles) along the first two ordination axes of a partial CCA (pCCA) with the \log_{10} free ion (FI) metal concentrations for lead, cadmium, copper, zinc and nickel as explanatory variables after partialling out the effects of significant environmental covariables (pH, altitude, alkalinity and streamwater width). Arrows indicate direction and strength of increasing metal concentration.

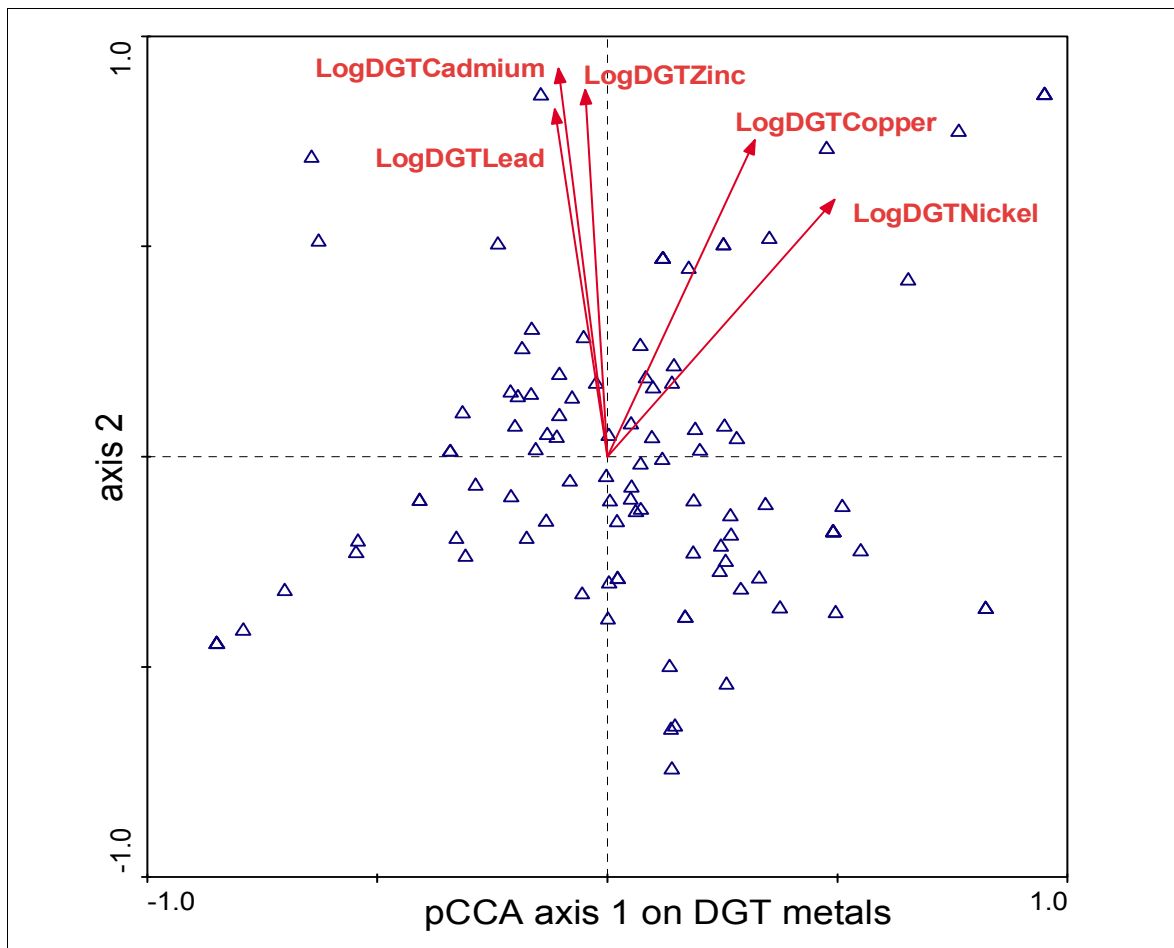


Figure 6.6 The position of diatom taxa (triangles) along the first two ordination axes of a partial CCA (pCCA) with the \log_{10} DGT metal concentrations for lead, cadmium, copper, zinc and nickel as explanatory variables after partialling out the effects of significant environmental covariables (pH, altitude, alkalinity and streamwater width). Arrows indicate direction and strength of increasing metal concentration.

Table 6.4 Ordination score of each diatom species along the pCCA axis based on TD lead concentrations; negative species scores suggest preference for sites with relative low lead concentrations; N2 = effective number of occurrences of each species.

Diatom taxon	Axis score	N2
<i>Cymbella descripta</i>	-0.73	1.0
<i>Cterophora pulchella</i>	-0.62	1.9
<i>Synedra ulna</i>	-0.60	1.9
<i>Cymbella amphicephala?</i>	-0.44	1.0
<i>Gomphonema clavatum</i>	-0.44	1.0
<i>Pinnularia appendiculata/silvatica</i>	-0.42	2.0
<i>Frustulia rhomboides</i> var. <i>viridula</i>	-0.40	1.0
<i>Cymbella</i> sp. cf. <i>laevis</i>	-0.39	1.0
<i>Nitzschia</i> sp. cf. <i>bryophila</i>	-0.33	1.0
<i>Eunotia bilunaris</i> v. <i>mucophila</i>	-0.30	1.0
<i>Eunotia paludosa?</i>	-0.30	1.0

Diatom taxon	Axis score	N2
<i>Eunotia rhomboidea?</i>	-0.30	1.0
<i>Navicula cincta</i>	-0.30	1.0
<i>Gomphonema</i> sp. cf. <i>pumilum</i>	-0.26	8.4
<i>Gomphonema</i> girdle	-0.25	7.0
<i>Denticula tenuis</i>	-0.25	4.1
<i>Navicula gregaria</i>	-0.25	1.7
<i>Nitzschia dissipata</i>	-0.23	1.9
<i>Achnanthes</i> sp. cf. <i>laevis</i>	-0.21	1.0
<i>Fragilaria tenera</i>	-0.21	1.0
<i>Reimeria sinuata</i>	-0.21	2.8
<i>Achnanthes</i> sp. cf. <i>marginulata</i>	-0.19	4.1
<i>Nitzschia</i> sp.	-0.18	2.9
<i>Cocconeis placentula</i>	-0.18	3.0
<i>Gomphonema olivaceum</i>	-0.16	4.5
<i>Diatoma monoliformis</i>	-0.16	6.1
<i>Fragilaria perminuta</i>	-0.16	1.0
<i>Gomphonema acuminatum</i>	-0.16	1.0
<i>Peronia fibula</i>	-0.15	2.3
<i>Nitzschia supralitorea</i>	-0.15	2.0
<i>Eunotia incisa</i>	-0.15	2.5
<i>Cymbella affinis</i>	-0.13	3.6
<i>Eunotia exigua</i>	-0.12	4.5
<i>Cocconeis placentula</i> var. <i>pseudo</i>	-0.12	1.0
<i>Fragilaria capucina</i> v. <i>rumpens?</i>	-0.11	1.9
<i>Fragilaria</i> girdle	-0.11	4.2
<i>Pinnularia</i> sp.	-0.11	3.0
<i>Meridion circulare</i> var. <i>constrictum</i>	-0.09	3.9
<i>Tabellaria flocculosa</i>	-0.09	5.1
<i>Achnantheidium biasoletiana</i>	-0.09	5.2
<i>Brachysira vitrea</i>	-0.07	4.3
<i>Achnanthes</i> sp.	-0.07	2.9
<i>Gomphonema olivaceoides</i>	-0.07	14.9
<i>Navicula miniscula</i>	-0.06	1.0
<i>Eunotia</i> sp.	-0.06	2.7
<i>Frustulia rhomboides</i>	-0.04	2.9
<i>Eunotia subarcuatoides</i>	-0.03	4.0
<i>Amphora?</i> girdle	-0.03	1.0
<i>Cyclotella</i> sp.	-0.03	1.0
<i>Gomphonema parvulum</i>	-0.03	15.2
<i>Fragilaria capucina/vaucheria</i> spp. complex	-0.03	7.0
<i>Fragilaria ulna</i> v. <i>ulna</i>	-0.02	2.0
<i>Gomphonema angustatum</i>	-0.01	3.9
<i>Hannaea arcus</i>	0.00	13.2
<i>Eunotia bilunaris</i>	0.00	1.9
<i>Encyonema minutum</i>	0.01	6.4
<i>Achnanthes</i> girdle	0.01	3.0
<i>Fragilaria capucina</i> var. <i>perminuta</i>	0.02	1.0
<i>Eunotia</i> girdle	0.02	3.5
<i>Eunotia tenella</i>	0.02	3.0
<i>Cocconeis pediculus</i>	0.02	1.0
<i>Eunotia</i> sp. cf. <i>pectinalis</i>	0.02	1.0
<i>Gomphonema affine</i>	0.02	1.0
<i>Rhoicosphenia abbreviata</i>	0.02	1.0

Diatom taxon	Axis score	N2
<i>Eunotia praerupta</i>	0.02	2.0
<i>Achnantheidium minutissimum</i>	0.03	31.0
<i>Cymbella cystula</i>	0.03	1.0
<i>Fragilaria construens</i> f. <i>venter</i>	0.03	1.0
<i>Nitzschia</i> cf. <i>frustrulum</i>	0.03	1.0
<i>Staurosirella pinnata</i> / <i>Puactastriata ovalis</i>	0.03	1.0
<i>Fragilaria capucina</i> var. <i>gracilis</i>	0.03	14.1
<i>Fragilaria vaucheriae</i>	0.03	7.3
<i>Achnanthes lanceolata</i>	0.04	4.7
<i>Cymbella microcephala</i>	0.06	1.9
<i>Fragilaria capucina</i> sensu lato	0.06	3.2
<i>Nitzschia</i> cf. <i>paleacea</i>	0.06	2.0
<i>Nitzschia palea</i>	0.07	5.5
<i>Cymbella delicatula</i>	0.08	4.7
<i>Navicula cryptotenella</i>	0.09	3.0
<i>Achnanthes helvetica</i>	0.09	2.0
<i>Anomoeoneis brachysira</i>	0.09	2.4
<i>Encyonema silesiacum</i>	0.10	13.7
<i>Navicula</i> sp. cf. <i>atomus</i>	0.13	2.9
<i>Achnanthes oblongella</i>	0.14	2.7
<i>Achnanthes hungarica</i>	0.15	1.0
<i>Diatoma</i> girdle	0.15	1.0
<i>Meridion circulare</i>	0.16	14.9
<i>Psammothidium subatomoides</i>	0.17	2.6
<i>Navicula cryptocephala</i>	0.17	2.9
<i>Psammothidium abundans</i>	0.17	2.0
<i>Navicula</i> sp. cf. <i>minima</i>	0.19	5.4
<i>Fragilaria capucina</i> var. <i>capucina</i>	0.20	5.2
<i>Surirella brebissoni</i>	0.22	1.8
<i>Eunotia bilunaris/subarcuatooides</i>	0.23	1.5
<i>Surirella minuta</i>	0.29	2.0
<i>Achnanthes lanceolata</i> sp. <i>frequilissima</i>	0.29	1.0
<i>Hantzchia amphioxys</i>	0.29	1.0
<i>Navicula tripunctata</i>	0.29	1.0
<i>Diatoma mesodon</i>	0.35	5.8
<i>Eunotia</i> cf. <i>minor</i>	0.40	1.0
Naviculoid girdle view	0.42	1.0
<i>Planothidium lanceolata</i>	0.43	2.3
<i>Navicula saprophila</i>	0.55	1.0
<i>Navicula</i> sp. <i>seminulum?</i>	0.56	2.4

6.1.4 Conclusions from diatom community ordination analyses

Diatom species community composition can be partly explained by four significant non-metal environmental variables. These are pH, altitude, alkalinity and streamwater width. After the 'natural' effect of these stream-type factors was allowed, there were still statistically significant relationships between diatom community composition and metal concentrations. Overall tests based on all five metals were most highly statistically significant for metals in their FI form, but were also significant in TD form. However, the metals with the strongest relationships differed depending on the concentration form used. In the case of total dissolved metal, all metals showed effects, but Zn was the

most significant. For free metal ion concentration, all except copper showed some significant association with diatom community type and lead was most significant statistically. For the DGT metal form, only cadmium ($p = 0.028$) and zinc ($p = 0.043$) individually showed statistical significance associated with diatom community, although nickel had a subsidiary 'influence' adjusted for cadmium levels.

The FI form of metals appears to show the greatest overall level of association with diatom community composition, potentially most strongly with lead, but it is especially difficult to separate the potential effects of lead, cadmium, zinc and copper among this set of study sites.

It is conceivable that the inclusion of additional water quality variables might be helpful—especially, in the light of findings with macroinvertebrates and aluminium concentrations. However, it was felt that the analysis reported above was already quite complex, and that the addition of further variables was not justified. Moreover, in the analyses described below, Al did not emerge as a significant predictor of diatom species variability.

6.2 Multiple regression analysis

Four metrics of diatom community were used in regressions:

1. The total number of taxa identified.
2. The number of taxa with a relative abundance ≥ 6 valves ($\geq 2\%$).
3. The total number of taxa identified, at sites with mean pH above 6.
4. The total number of taxa with more than 6 valves identified, at sites with mean pH above 6.

The removal of taxa with less than 6 valves gets rid of the long 'tail' of uncommon and rare taxa in the sample. This assumes that the probability of sampling any diatom species follows a binomial distribution. For taxa with less than 6 valves, the lower 95% confidence limit on numbers is < 1 and there is, therefore, a strong likelihood that these will not be present in a replicate analysis of the same sample. Removal of acid ($pH < 6$) sites serves to constrain the community by eliminating sites expected to have a bias towards acidophilic diatom species.

The multiple regression results are shown in Tables 6.5 to 6.8. In general, only weak relationships were found, and no improvement was achieved by considering only diatom species for which more than 6 valves were present.

If all sites are considered, Zn and Cd give the most significant negative correlations with diatom taxon numbers, when expressed as $\log(\text{total dissolved metal})$ or $\log(\text{DGT metal})$. Since for these two metals, DGT metal is highly correlated with total dissolved metal (Figure 4.5) the similar results are to be expected. Moreover, the total dissolved concentrations of Zn and Cd are highly correlated (Section 4.1.4 and Table 6.2), which means that it is difficult to attribute the assumed toxic effect to either metal. Similar conclusions are drawn if acid sites are excluded from consideration. The inclusion of hardness functions does not improve predictions.

Therefore, from this analysis, we conclude that Zn and/or Cd have the most effect on diatom numbers, explaining about 30% of the variability.

Manual stepwise regression, as per Section 5.2.5, was done using the number of taxa with more than 6 valves as a predictor. No single metal term gave a statistically

significant ($\alpha = 0.05$) fit against the response variable, regardless of the formulation of the metal term. Further combinations of metal terms were therefore not tested.

Table 6.5 Results of stepwise multiple regression of diatom metrics against log(filterable metal). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Al) (Cu*) Zn Pb 0.30 (0.20)	(Al) (Cu*) Zn** 0.30 (0.23)	(Al) (Cu) Pb 0.25 (0.17)
No. taxa > 6 valves	(Al*) Ni Zn (Pb*) 0.30 (0.20)	(Al*) Zn* (Pb) 0.27 (0.19)	(Al*) Ni 0.18 (0.13)
Non-acid sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Al) (Cu) Pb* 0.27 (0.19)	(Cu) Zn** 0.25 (0.19)	Pb* 0.18 (0.15)
No. taxa > 6 valves	(Al*) 0.17 (0.14)	(Al*) 0.17 (0.14)	(Al*) 0.17 (0.14)

Table 6.6 Results of stepwise multiple regression of diatom metrics against hardness functions. Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Al) (Cu) Pb* 0.24 (0.16)	(Cu) Pb* 0.18 (0.13)	(Cu) Pb* 0.18 (0.13)
No. taxa > 6 valves	(Al) Zn* (Pb) 0.25 (0.17)	(Al) 0.11 (0.09)	(Al) Zn* (Pb) 0.25 (0.17)
Non-acid sites	Forward selection	Backward elimination	Stepwise
no. taxa	(Cu) Zn** 0.26 (0.20)	(Al*) Zn* (Cd) 0.31 (0.22)	(Cu) Zn** 0.26 (0.20)
no. taxa > 6 valves	(Al*) 0.18 (0.15)	(Al*) 0.18 (0.15)	(Al*) 0.18 (0.15)

Table 6.7 Results of stepwise multiple regression of diatom metrics against log(open pore DGT metal). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Cu*) Zn** 0.29 (0.25)	(Cu*) Cd** 0.32 (0.28)	(Cu*) Zn** 0.29 (0.25)
No. taxa > 6 valves	Cd 0.11 (0.08)	Cd 0.11 (0.08)	Cd 0.11 (0.08)
Non-acid sites	Forward selection	Backward elimination	Stepwise
no. taxa	(Cu*) Zn Cd 0.37 (0.29)	(Cu**) Cd** 0.36 (0.32)	(Cu*) Zn** 0.32 (0.27)
no. taxa > 6 valves	Cd 0.12 (0.08)	Cd 0.12 (0.08)	Cd 0.12 (0.08)

Table 6.8 Results of stepwise multiple regression of diatom metrics against free metal ion functions (γ_{field}). Metal species listed are those selected by the stepwise procedure; those in bold have statistically significant coefficients (*, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Metals in brackets have positive regression coefficients. The numbers shown are the R^2 and (in brackets) the R^2 adjusted for the number of predictor variables.**

All sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Cu) Pb* 0.19 (0.13)	Zn 0.10 (0.07)	(Cu) Pb* 0.19 (0.13)
No. taxa > 6 valves	Zn 0.10 (0.07)	Zn 0.10 (0.07)	Zn 0.10 (0.07)
Non-acid sites	Forward selection	Backward elimination	Stepwise
No. taxa	(Cu) Pb** 0.23 (0.17)	Zn* 0.16 (0.13)	Pb* 0.17 (0.14)
No. taxa > 6 valves	Cu 0.10 (0.07)	Cu 0.10 (0.07)	Cu 0.10 (0.07)

6.3 Application of Biotic Ligand Models

Application of the copper BLM was described in Section 5.3.1.

The chronic zinc BLM for *Pseudokirchneriella subcapitata* (De Schampelaere *et al.*, 2005) was applied to the data to obtain ratios of the observed free zinc to the free predicted EC10 zinc for the alga. Figure 6.7 shows the results. There was no significant trend between the toxicity ratio and the biological metric ($p = 0.091$).

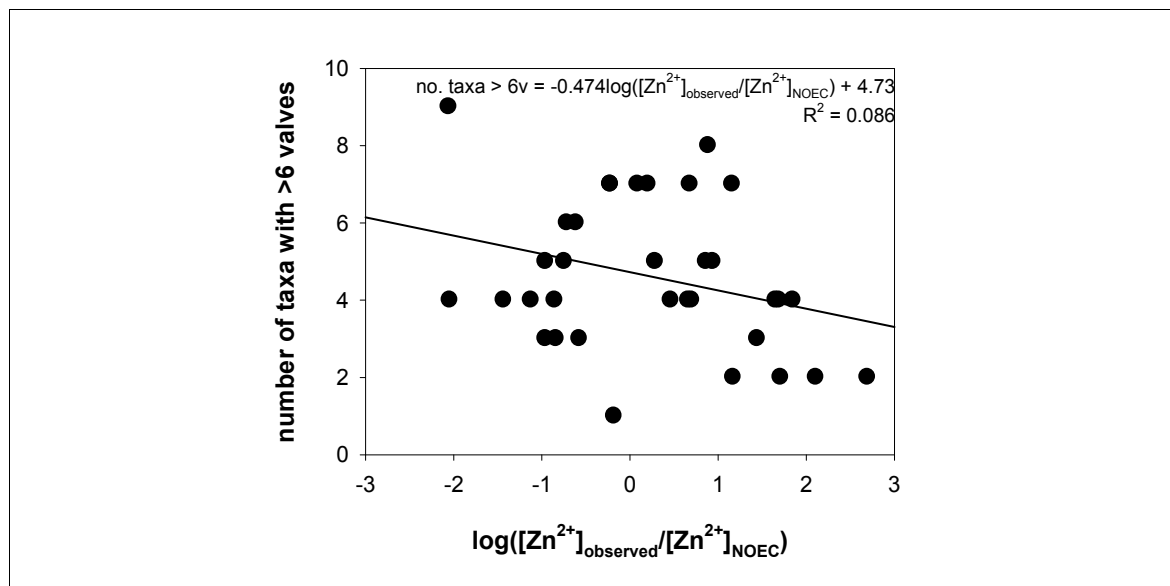


Figure 6.7 Relationship between OE67 and $[\text{Zn}^{2+}]_{\text{observed}}/[\text{Zn}^{2+}]_{\text{NOEC}}$ calculated from the BLM for *Pseudokirchneriella subcapitata*.

6.4 Toxicity Binding Model

We applied the TBM, described in Section 5.4, to the diatom data, assuming that binding sites on the diatoms could be represented by the array of binding sites in humic acid, as described by WHAM. For the diatoms, data were available for 34 sites. We attempted to explain variations in diatom species numbers (n_{DS}) with the model.

6.4.1 Linear regression

The model was applied by optimising the values of v_{M} in order to explain as much of the variance in n_{DS} as possible by linear regression. The result with all 34 sites included is shown in Figure 6.8. The model explains 25% of the variance, suggesting that metal toxicity does reduce the number of diatom species at a site. The plot identifies a clear outlier (Site 1), which has many more diatom species than the other sites. Rejection of this site increased the value of R^2 to 0.334. Further analyses were performed using only the 33 sites that appeared to form a consistent set.

The residuals from the model applied to 33 sites were plotted against physical and chemical variables in order to test for explanatory variables. No strong correlations were found with individual variables, the greatest being with NH_4 ($R^2 = 0.075$), Ca ($R^2 = 0.058$) and Fe(II) ($R^2 = 0.032$). When these three were combined in a multiple regression, the R^2 was 0.201. Following the results from Section 6.1, we also tested the

effects of altitude and stream width. A small dependency on altitude was found ($R^2 = 0.07$) but addition of altitude as a variable in the multiple regression did not bring about an improvement.

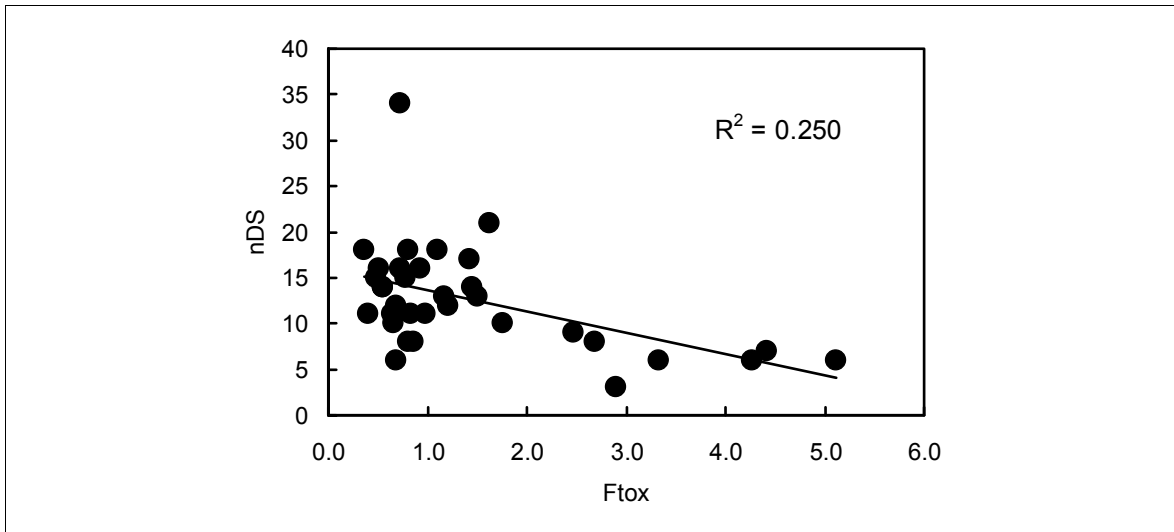


Figure 6.8 Dependence of n_{DS} on F_{tox} after optimising the TBM for all 34 sites.

Although the incorporation of $[NH_4]$, $[Ca]$, $[Fe(II)]$ and altitude into the model improved the statistical fit, the new parameter values did not appear physically realistic. It was found that of the potentially toxic metals, including H^+ , only Zn and Cd had significant influences on the diatom species numbers, whether or not the modifications by NH_4 , Ca or Fe(II) were applied. Of these two metals, Zn gave somewhat better results. Therefore, the best linear version of the TBM can be described by the equation:

$$n_{DS} = 13.8 - 11.0 v_{Zn} \quad (6.1)$$

which yields an R^2 of 0.302. Figure 6.9 shows the diatom species numbers plotted against F_{tox} . There seems little point in attempting to derive a threshold model, but inspection of the plot suggests that zinc toxicity begins at $F_{tox} \sim 0.25$. The eight sites with $F_{tox} > 0.25$ are numbers 5, 10, 12, 14, 21, 22, 23 and 28; five of these were also found to have water that elicited a toxic response in laboratory tests (see Section 7.1).

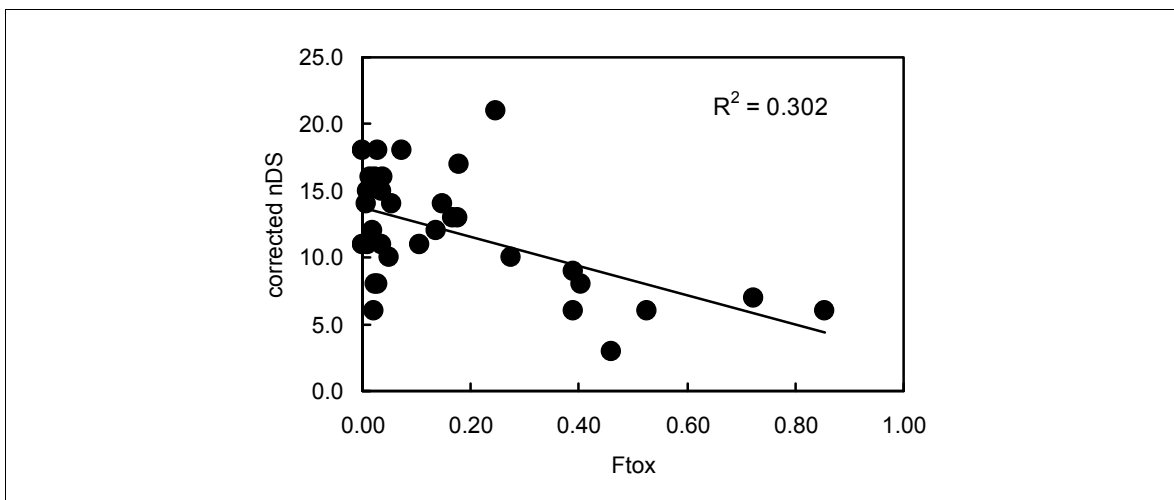


Figure 6.9 Diatom species numbers for 33 sites plotted against F_{tox} , due only to Zn.

6.5 Summary

These data are limited firstly by their relatively small number and, secondly, by the absence of a measure which will enable the number of taxa expected in the absence of toxic effects to be calculated. Thus, none of the attempts to explain diatom variability in terms of metal variables was very successful, nor could any effects of pH or hardness be discerned.

There are a number of good reasons why diatoms, rather than other groups of algae, are so widely used for environmental monitoring. However, the evidence here is that the conventional approach of using community analyses of diatoms alone is not especially sensitive. Other algae, particularly green algae (Chlorophyta) are often conspicuous in the stream flora; sometimes thriving due to the absence of grazers and incorporating these into monitoring systems might increase sensitivity. The nature of trace metal tolerance is such that there are almost no taxa that are characteristic of metal-rich streams and the taxa that do thrive are often cosmopolitan species with fast growth rates that are capable of developing tolerance via metallothioneins (Ivorra *et al.*, 2002). *Amphora veneta* var. *capitata* is the only species that does seem to be more common in metal-rich streams of the northern Pennines than elsewhere (M.G. Kelly, pers. comm.); otherwise, the most tolerant taxa are *Achnanthydium minutissimum*, *Meridion circulare* and *Fragilaria capucina*, all of which are common in circumneutral upland streams at high and good status.

Some diatoms – particularly the Fragilariophyceae – show characteristic distortions in the presence of metals (McFarland *et al.*, 1997). This was not examined in this study, nor in Hirst *et al.* (2002), but unpublished data from the northern Pennines suggests that the rate of distorted to normal valves of *Fragilaria capucina* is typically 2–3% at 'reference sites' (i.e. those free from any known human impacts) but that this rate is linearly related to Zn concentration ($r^2 = 0.88$). This is a fairly straightforward characteristic to record and provides a sensitive means for assessing the effect of metal contamination not just in streams where there are continuous metal inputs but also in situations where metal inputs are intermittent and missed by conventional water chemistry analyses (M.G. Kelly, pers. comm.)

With regard to the TBM, the decline in species numbers at elevated F_{tox} values is significant; however, it is important to note that samples with low numbers of taxa can also occur when F_{tox} is low. Similar relationships have been noted elsewhere (e.g. Whitton and Diaz, 1981) but are easier to discern in large datasets. Hirst *et al.* (2002) also noted a stronger effect of metals on macroinvertebrate species richness than on diatom species richness and that diatom ordination scores were more effectively predicted using a mixture of physico-chemical and macroinvertebrate predictors than using physio-chemical predictors alone. In general, sites with elevated metals are likely to have fewer than 10 diatom taxa and those sites with impoverished flora overlap substantially with the sites identified as toxic to algae (Chapter 7).

The results suggest that diatoms could be useful in assessing the toxic effects of metals in the field, but there is a clear need to understand more fully the factors controlling their variations in the absence of toxic metals, in order to provide the necessary expected species numbers.

7 Laboratory toxicity testing

Laboratory ecotoxicity tests were carried out in order to support the field programme. Growth rates were determined of two 'standard' freshwater test organisms (an alga and a daphnid), inoculated into water samples from the field sites. The specific aims of this part of the research were:

(a) to assess the possible ecotoxicity of raw (i.e. non-amended) samples from all field sites, by means of bioassays with standard test organisms *Pseudokirchneriella subcapitata* (alga) and *Daphnia magna* (invertebrate, crustacean);

(b) to assess whether ecotoxicity of contaminated samples was only due to metals present in the samples or possibly also to other factors, by means of Toxicity Identification Evaluation (TIE).

7.1 Toxicity testing of raw samples with *P. subcapitata*

Streamwater samples from all 35 field sites, divided for the purposes of this part of the study into 27 'contaminated' samples and 8 'control' samples (from uncontaminated surface waters) were investigated. For eight contaminated sites, significant decreases in growth rate were found, and these were taken further into the TIE test phase to determine whether the toxicity was due to the metals present or due to another factor.

The eight responding samples, as well as the eight control samples, were amended with the complexing ligand EDTA at a molar concentration equal to three times the sum of the molar concentrations of Zn, Cu, Ni, Cd and Pb. As such, the metals that may have caused toxicity in the first testing phase were chelated to EDTA, leaving very little free metal ion in the solution, and taking away the metals as the cause for the toxicity. If the earlier-observed toxicity disappears by this amendment, then – according to the TIE concept the toxicity in the original raw field samples can be attributed to metals only. In cases where toxicity does not (entirely) disappear, other factors may have contributed to the toxicity in the raw field samples.

Figure 7.1 gives an overview of the growth rate of all samples as a function of the pH of the sample. The data from the first testing phase clearly illustrate that at $\text{pH} < 5.6$, *P. subcapitata* growth becomes limited, even in uncontaminated samples. The growth rate data in the seven remaining 'uncontaminated' samples with $\text{pH} > 5.6$ was between 0.96 and 1.14 d^{-1} , which is above the control growth rate required by OECD (2006) for a valid test (i.e. 0.9 d^{-1}). The growth rate in each of the 26 contaminated samples with $\text{pH} > 5.6$ (three replicates) was statistically compared with the growth rate in the 'uncontaminated' samples (seven values of average growth rate, one value for each sample) by means of a Student t-test at a significance level of 0.05.

Only seven 'contaminated' samples were observed to be toxic to *P. subcapitata*, that is, they exhibited a lower growth rate than in 'uncontaminated' samples (in order of decreasing toxicity or increasing growth rate): Site 5 > 10 > 21 > 23 > 28 > 29 > 12. The growth rate in water from Site 11 was the lowest of all samples, but this sample was at the same time extremely low in pH (4.2) and extremely high in metal concentration $\sum(\text{Zn,Cu,Ni,Cd,Pb}) = 102 \mu\text{mol l}^{-1}$ (98% Zn or 6.6 mg Zn l^{-1}).

The second test phase demonstrated that the addition of EDTA resulted in the disappearance of the toxic response in all samples with $\text{pH} > 5.6$ (Figure 7.1). This indicates that the toxicity in all samples was due to the metals present only. In the Site

11 sample (pH 4.2), growth rate was enhanced by the addition of EDTA to 0.27 d^{-1} , which is below the range of growth rates in uncontaminated samples (0.84 to 1.23 d^{-1}) but higher than the growth rate at pH 5.2 (i.e. 0.19 d^{-1}). This may suggest that the EDTA addition made the metal toxicity disappear while the toxicity due to the acidity of the sample remained. Hence, the raw Site 11 sample appears to be toxic due to both the metals present and the acidity, but probably without any other factor being involved.

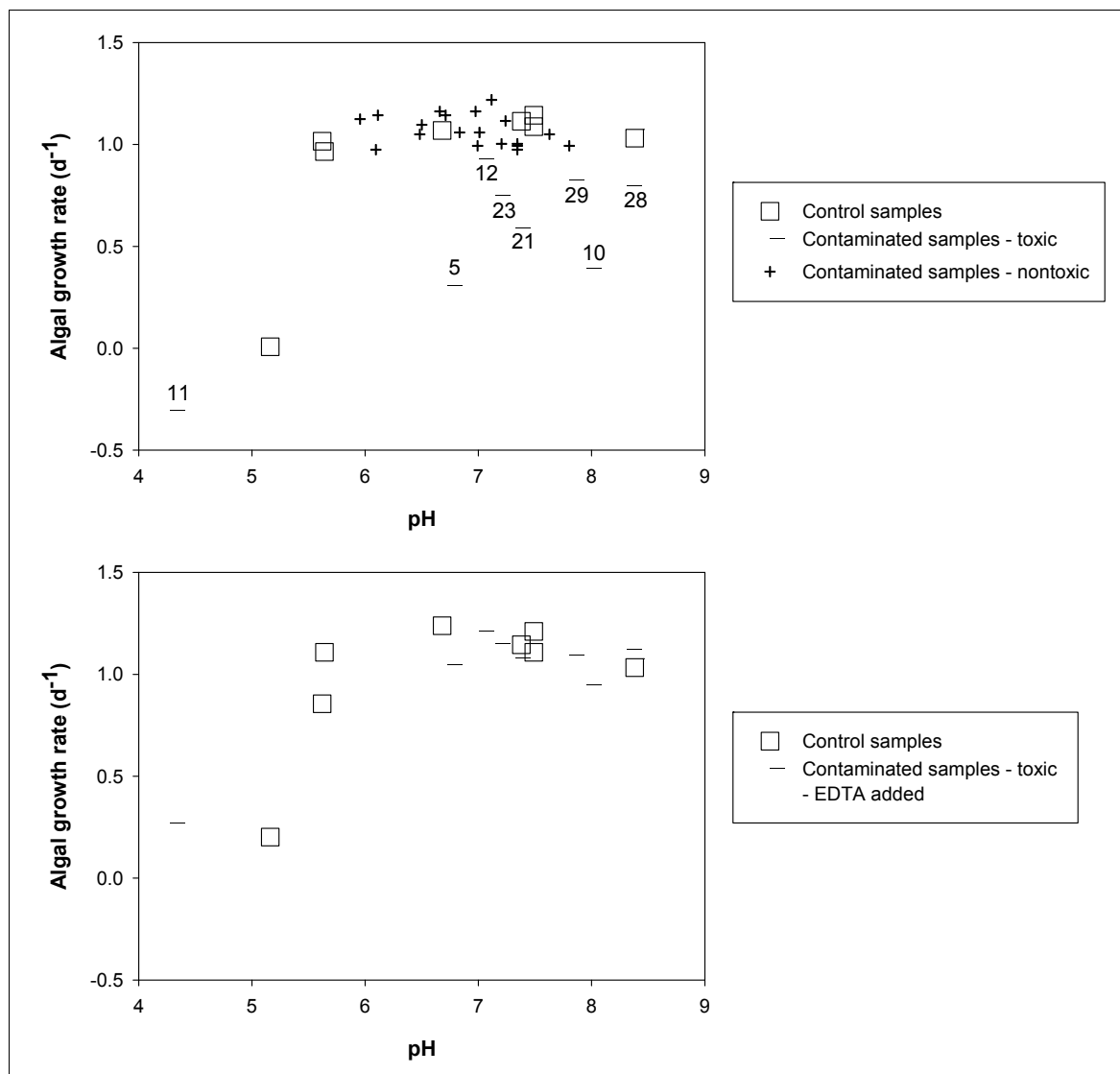


Figure 7.1 Growth rate of *P. subcapitata* in raw samples (upper panel) and in samples to which EDTA was added (lower). The numbers indicate sampling sites that gave toxicity.

7.2 Toxicity testing of raw samples with *D. magna*

As discussed in Section 2.3.2, *D. magna* has been shown not to perform very well (i.e. produce less offspring) in waters with hardness below $25 \text{ mg CaCO}_3 \text{ l}^{-1}$ and/or $\text{pH} < 6$. We therefore decided to investigate the reproduction of *D. magna* only in those raw samples with higher hardness and pH. After determining survival and reproduction, the two samples that were designated to be toxic (Sites 10 and 21) in the first phase, as well as the 'uncontaminated' samples 6, 27 and 33, were taken to the TIE phase.

Reproduction in each of the samples is presented in Figure 7.2. Two samples appeared to be clearly toxic, i.e. Sites 10 and 21, as demonstrated by 100% mortality within three days of exposure. Those two samples were those with the highest trace metal concentrations among the 12 investigated samples: $\sum(\text{Zn,Cu,Ni,Cd,Pb})$ at Site 10 = $6.7 \mu\text{mol l}^{-1}$ (97% Zn); $\sum(\text{Zn,Cu,Ni,Cd,Pb})$ at Site 21 = $12 \mu\text{mol l}^{-1}$ (97% Zn). Reproduction in the 10 other samples was between 64.1 and 83.9 juveniles per female. Analysis of variance (ANOVA) demonstrated that there were no significant differences in reproduction among the other 10 samples (F-test, $p = 0.394$). Hence, only the two above-mentioned samples, 10 and 21, were designated to be toxic to *D. magna* reproduction.

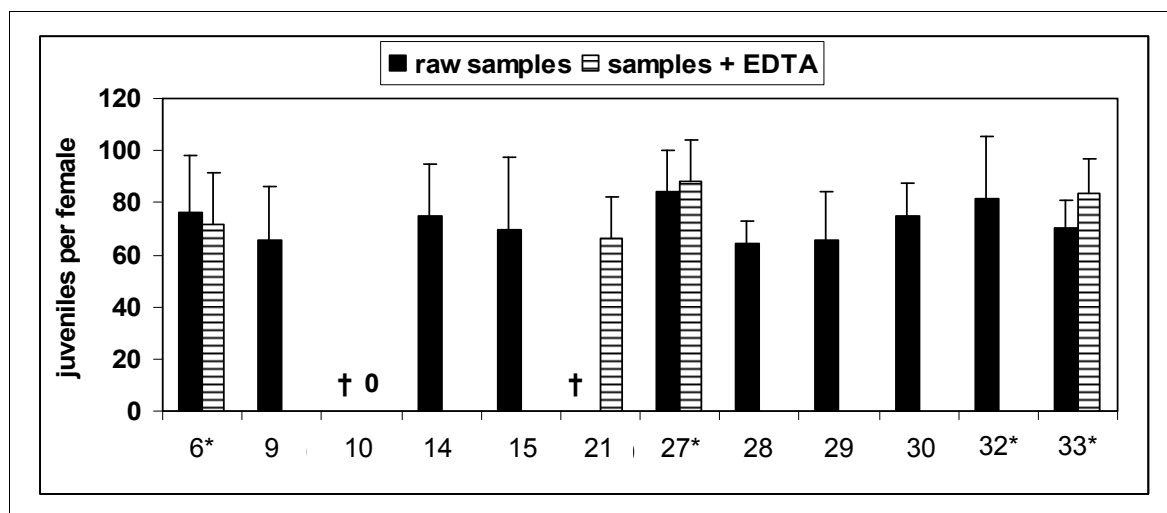


Figure 7.2 Reproduction of *D. magna* in raw samples and in samples to which EDTA was added; error bars denote one standard deviation; a '0' indicates that no reproduction was observed; a '†' indicates that 100% mortality was observed; sample codes marked with a * indicate 'uncontaminated' samples.

In the second test phase, the two toxic samples (10 and 21), as well as the 'uncontaminated' samples 6, 27 and 33 were amended with a concentration of the complexing ligand EDTA at a molar concentration equal to three times the sum of the molar concentrations of all metals (Zn, Cu, Ni, Cd, Pb). As such, the metals that may have caused toxicity in the first testing phase were bound to EDTA, leaving very little free metal ion in the solution, and taking away the metals as the cause for the toxicity. When earlier-observed toxicity disappears by this amendment, then the toxicity in the toxic raw samples (i.e. Sites 10 and 21) can be attributed to those metals only. This procedure is called Toxicity Identification Evaluation (TIE). The results of this test phase are also given in Figure 7.2.

ANOVA indicated that there were no significant reproduction differences among the samples 6, 27, 33 and 21 (F-test, $p = 0.066$), suggesting that the EDTA addition resulted in the disappearance of the toxicity in Site 21 and that in the raw sample toxicity was indeed due to the metals present and not to any other factor.

In contrast, the Site 10 sample remained toxic even after the addition of EDTA. Although effects on survival disappeared, the daphnids in the EDTA amended sample were still not able to reproduce. This suggests the presence of another factor in the raw sample that contributes to reproductive impairment. Upon inspection of the trace metal contents of the Site 10 sample, we found a high concentration of arsenic (As) (i.e. $889 \mu\text{g l}^{-1}$) (note that this is higher than the average value; see Table 4.3). In this sample (near neutral pH, low DOC) arsenic most likely occurs in an anionic form (i.e. H_2AsO_4^- or HAsO_4^{2-}) and can thus not form complexes with EDTA. Hence, the addition of EDTA

to a sample cannot alleviate As-induced toxicity. For *D. magna*, Biesinger and Christensen (1972) found reproductive EC10 and EC50 values of 520 and 1400 $\mu\text{g As l}^{-1}$, respectively. This strongly suggests that the toxicity to *D. magna* in the sample from Site 10 was due to the degree of contamination with As.

7.3 Application of the Toxicity Binding Model

We applied the threshold TBM, introduced in Section 5.4 to the algal toxicity results. We again assumed that parameters for metal binding by humic acid would represent the interactions of cationic metals with the biotic ligands of *P. subcapitata*, and adjusted the toxicity coefficients, α_M , to fit the model to the data. Again, two assumptions about Al chemistry were adopted, control of Al^{3+} by $\text{Al}(\text{OH})_3$ and complete solubility of all filterable Al. In both cases, the data could be explained well. As an example, Figure 7.3 shows the results for the complete solubility assumption.

The significant parameters required to fit the data were the toxicity coefficients of Zn, Cd and H (the value of α_{Al} was fixed at 1.0). The full model parameters are shown in Table 7.1.

The assumptions about Al chemistry have little effect on the parameter values (Table 7.1). The test alga appears insensitive to Cu, and because of the likely precipitation of lead phosphate in the test medium, any effects of Pb are not tested. According to the model, the toxicity is accounted for by Al, Zn, Cd and H. The value of α_{Cd} is ca. 500 times that of the other toxicity coefficients, implying a much greater toxic effect per mole of bound metal. The values of $F_{\text{tox,min}}$ and $F_{\text{tox,max}}$ are closer than found when the model was applied to the macroinvertebrate field community data (Table 5.8), which means that the alga, a single organism, shows a sharper toxic response.

As was found when applying the TBM to the macroinvertebrate data, the results here are promising, and appear to provide a good explanation not only of the effects of toxic trace metals, but also of Al and H. However, the model fitting is based mostly on the rather few (6–8) data points for which a toxic effect operates, and the results cannot be considered at all conclusive.

Figure 7.4 shows the calculated contributions of the different toxic cations to F_{tox} . Of the eight streamwaters for which $F_{\text{tox,min}}$ is calculated to be exceeded, the toxic effect is attributed to Al and H in four cases (Sites 3, 4, 11 and 32) and to the combination of Al, Zn, Cd and H in the other four (Sites 5, 10, 21 and 23).

It is interesting that at Site 11 the trace metal concentrations are very high (Table 4.3), but they are not calculated to exert much toxic effect, because the high acidity and Al prevent their binding to the putative biotic ligands. Thus, the model attributes the toxic response almost entirely to Al and H. This is consistent with the result from the TIE measurements (Section 7.1), which showed that the toxic response at this site could not be fully alleviated by the addition of EDTA. It is probable that the amount of EDTA added in the TIE test was sufficient to complex not only the trace metals but also aluminium, which would mean that after EDTA addition the only toxic 'metal' present would have been H^+ . Inspection of Figure 7.4 may give the impression that F_{tox} might not be exceeded in the absence of Al and the other metals, but removal of those metals by complexation with EDTA would reduce their competition for H^+ binding to the biotic ligand(s), which would be more highly loaded with H^+ , and this could explain the continuing toxicity of the water sample.

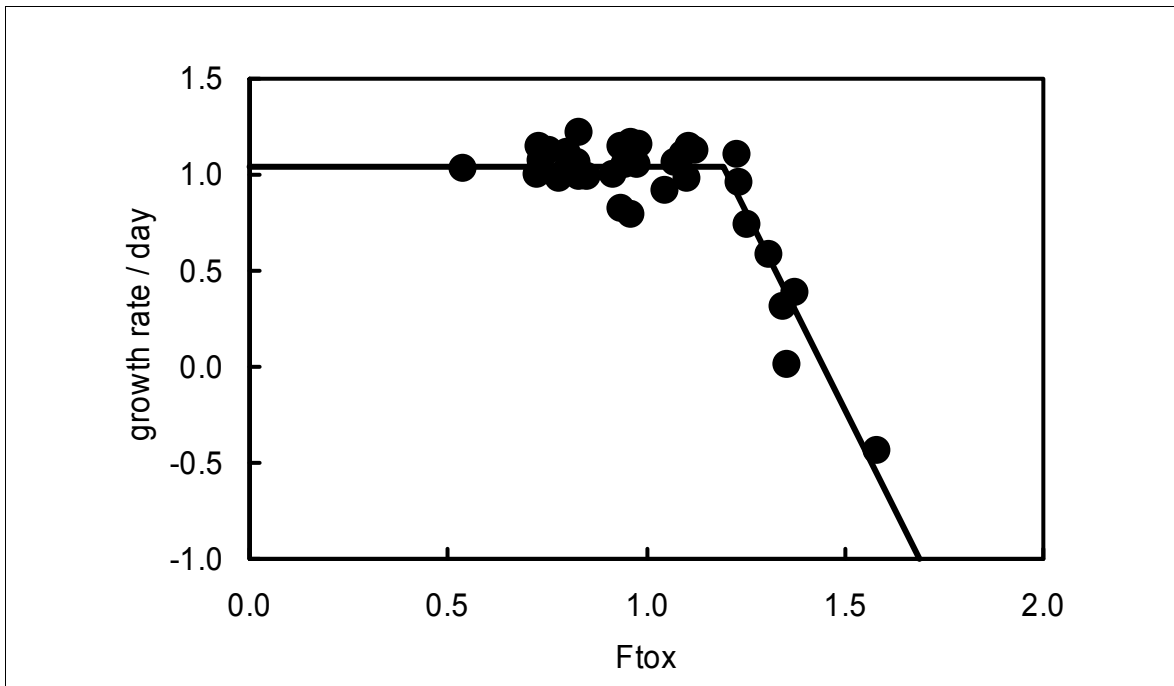


Figure 7.3 Application of the threshold TBM to the algal toxicity data, with the assumption that all the filterable Al is in true solution.

Table 7.1 Algal toxicity threshold model parameters for the two assumptions about Al^{3+} activity control. NS = not significant ($p > 0.05$), NT= not tested.

Parameter	$Al(OH)_3$	All Al dissolved
α_{Al}	(1.00)	(1.00)
α_{Ni}	NS	NS
α_{Cu}	NS	NS
α_{Zn}	1.99	1.84
α_{Cd}	811	984
α_{Pb}	NT	NT
α_H	0.66	0.72
$F_{tox, min}$	1.15	1.20
$F_{tox, max}$	1.38	1.45

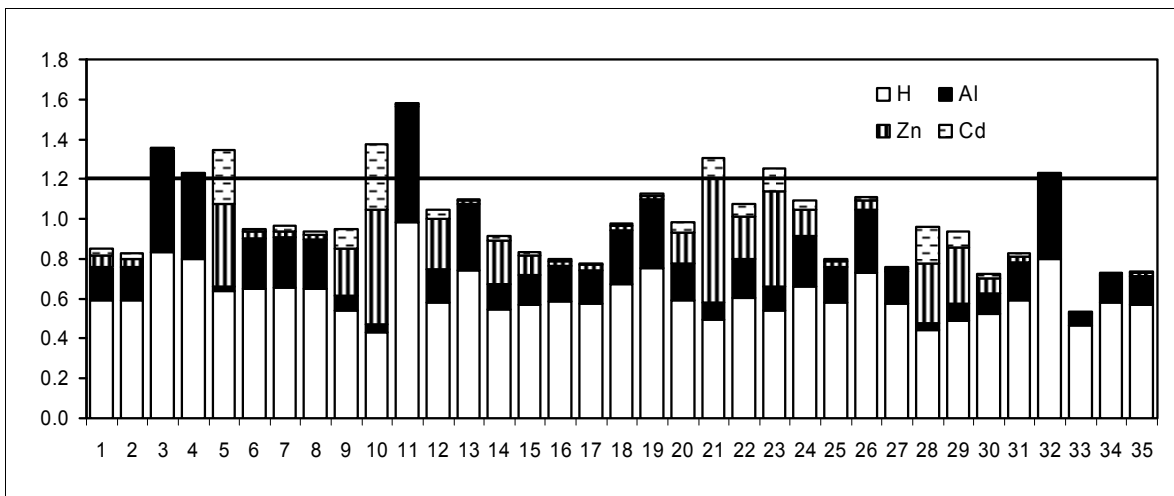


Figure 7.4 Contributions of different metals to the toxicity function F_{tox} . The solid line shows the lower toxicity threshold, $F_{tox, min}$.

7.4 Toxicity testing results: conclusions

The main result from these studies is that several of the streamwaters contain cationic trace metals at concentrations sufficient to cause toxic effects on the alga *Pseudokirchneriella subcapitata* and one streamwater causes toxicity towards the invertebrate crustacean *Daphnia magna*.

As already noted in Chapter 6, five of the eight streamwaters for which toxic effects on diatoms could be demonstrated were also found to be toxic towards *P. subcapitata*. Furthermore, at seven of the sites that gave a toxic response with *P. subcapitata*, the toxicity threshold ($F_{\text{tox,min}}$) for invertebrates was exceeded (Section 5.4); the remaining two sites were not used in the macroinvertebrate analysis.

The results with *D. magna* identified a possible confusing factor at Site 10, which is the high concentration of arsenic in the streamwater. This is evidently responsible for most of the toxicity of that streamwater towards *D. magna*, but this does not appear to apply to *P. subcapitata*, because the toxicity of streamwater from Site 10 towards the alga can be relieved by the addition of EDTA, which would not be expected to interact significantly with the predominant anionic forms of As. Moreover, inspection of the results reported in Chapters 5 and 6 did not reveal anomalous results for this site, which suggests that As is not exerting any additional toxic effects.

The toxicity binding model accounts very well for the observed toxic effects towards *P. subcapitata*, and indicates that Al and H, as well as Zn and Cd, may be principal toxic cations. This result supports the findings from application of the TBM to the macroinvertebrate data. However, further testing of the model against laboratory data is clearly required.

8 Summary and conclusions

8.1 Dose–response relationships

Here we summarise the findings of Chapters 5, 6 and 7, and discuss them in relation to the first objective of the project, namely to test for dose–response relationships in the field. This required the demonstration that there are indeed measurable effects on stream biota that can be attributed to metals, and the assessment of different measures of metal concentration.

8.1.1 Background: chemistry and metal mixtures

It is important to recognise that the metals under study – Al, Ni, Cu, Zn, Cd and Pb – differ in their solution chemistries and toxicities, and these differences must be borne in mind in interpreting the results. The properties of the metals are summarised in Table 8.1. Complexation by carbonate species parallels DOM except for Al, which prefers hydrolysis to carbonate complexation.

Table 8.1 Characteristics of the metals.

Metal	Hydrolysis	DOM complexation	Dependence of free ion toxicity on pH
Al	strong	strong	probably high
Ni	negligible	moderate	low
Cu	moderate	strong	high
Zn	negligible	weak	low
Cd	negligible	weak	low
Pb	moderate	strong	high

For each metal, the interplay among the properties shown in Table 8.1 influences solution chemistry and the toxic response. The metal source is also important in distinguishing Al (mobilised under acid conditions) from the other metals. Thus, Al tends to be present at high concentrations in acid waters, whereas the total dissolved concentrations of the other metals are highly variable, due to the presence or absence of mine spoil and its susceptibility to leaching. However, within the neutral and alkaline waters, there are appreciable variations in dissolved Al (Figure 4.1).

Consideration of Table 8.1 shows that Ni, Zn and Cd have similar chemistries. For each of these metals, large variations of chemical speciation with pH and [DOC] are not expected. On the other hand, the hydrolysis and DOM complexation tendencies of Al, Cu and Pb mean that their free ion concentrations will decrease substantially with pH, and will depend strongly upon [DOC].

There is a general tendency for metal toxicity, expressed in terms of the free metal ion concentration, to increase with pH; that is the free ion concentration required to elicit a given response decreases with pH. This follows from the biotic ligand idea, and arises because competition by H^+ for binding decreases with pH. This pH dependence of toxicity is comparatively high for Cu and Pb, and probably also for Al, and low for Ni, Zn and Cd.

A review of mixture effects based on laboratory observations is presented in Appendix 2. At the outset of the project, it was hoped that streams could be found that were dominated by a single metal, and that this would permit the effects of individual metals to be quantified. This turned out not to be the case, except possibly for Zn. The reasons for this were firstly that Zn was nearly always present at appreciable concentrations when other toxic trace metals displayed elevated concentrations, and secondly that aluminium plays a significant role in toxic response, even for the neutral and alkaline waters in which its toxicity *per se* is not apparent.

Therefore, we are forced to attempt interpretation of the results of the field and laboratory toxicity studies in terms of mixture effects. However, this does not rule out the possibility of considering the toxic effects of single metals. Neither does it imply that EQSs need be set in terms of mixtures.

Another point regarding mixture effects is that, once Al is accepted as a toxic metal, the distinction between 'contaminated' and 'control' sites becomes less clear, since the original selection of sites was based only on the trace metals.

The need to take metal mixtures into account, and the 'Al effect', makes it difficult to identify threshold solution concentrations or threshold solution chemical variables. However, a possible way to derive thresholds has been found, through the TBM.

8.1.2 Macroinvertebrates

The automated multiple regression results with the macroinvertebrates presented and discussed in Section 5.2 establish that toxic effects are seen in the field, and that they can be attributed nearly entirely to Al and Zn. If the analysis is restricted to non-acid sites, the importance of Al diminishes somewhat for some of the four investigated variables. None of the four variables provides a clearly superior explanation of the toxic effects, but the total metal + hardness variable, and the free ion function, are slightly superior to the other two variables (total filterable metal and DGT metal).

More insight was obtained through manual multiple regression analyses (Section 5.2.5). The results show that Zn and Al are the main toxicants, and that the free ion functions give the best explanation of the invertebrate data (Figures 5.2 and 5.3). The main reason for the superiority of the free ion function approach is that it describes the effects of Al better than, for example, total dissolved metal. This is much less important for Zn, because of the chemistry of the metal (Table 8.1), which is rather insensitive to pH or [DOC], that is its free ion concentration is well-correlated with the total concentration (Figures 4.7 and 4.8). Moreover, the pH dependence of Zn toxicity is low, and so the use of the free ion function does not provide much improvement to the explanation.

Application of the laboratory-derived BLM for an invertebrate test organism (*Daphnia magna*) produced a significant dependence of O/E macroinvertebrate taxa on the BLM Zn function. The unexplained variance in these applications is readily attributed to other factors, such as multiple metal effects and biological variability ('noise') within the relatively small dataset.

The TBM explains 35–42% of the variation in macroinvertebrate diversity, and this increases to about 70% after applying the 'correction' based on Ti concentrations. The results again show that Al and Zn are exerting significant toxic effects. If the Al activity is assumed to be controlled by $\text{Al}(\text{OH})_3$ then it is found that Cu and H^+ also contribute significantly.

The model can be configured in terms of two threshold values of the key variable F_{tox} , and these explicitly identify (a) the highest F_{tox} that can occur without toxic effects, and (b) conditions under which all sensitive taxa are absent.

The TBM relies upon full chemical speciation of each streamwater, not only with respect to the dissolved metals, but also their interactions with the 'biotic ligand' represented by humic acid. Therefore, the importance of chemical speciation is assumed from the outset, and this assumption is supported by the reasonably good descriptions that the model can provide.

8.1.3 Diatoms

The diatom results are more difficult to interpret than those for the macroinvertebrates because we do not yet have quantified expectations of diatom community variations in terms of stream physical and 'background' chemical properties. Therefore metal effects cannot readily be isolated.

Community analyses were performed in order to attempt to identify trace metals (i.e. Ni, Cu, Zn, Cd, Pb) that exerted toxic effects on diatoms, but Al was not included. Canonical correspondence analysis revealed four factors, other than toxic metals, that influence the number of diatom species at a site. Two of these were chemical (pH and alkalinity) and the other two were altitude and stream width.

Variations in diatom species numbers can be partly explained by four significant non-metal environmental variables. These are pH, altitude, alkalinity and streamwater width. After the 'natural' effect of these stream-type factors was allowed, there were still statistically significant relationships between diatom community composition and metal concentrations.

The most highly statistically significant results were obtained for metals in their free ion form. All the metals except copper showed some significant association with diatom community type, lead being the most significant. In the case of total dissolved metal, all metals showed effects, but Zn was the most significant. For the DGT metal form, only cadmium ($p = 0.028$) and zinc ($p = 0.043$) individually showed statistically significant association with diatom community, although nickel had a subsidiary 'influence' adjusted for cadmium levels.

The free ion form of metals appears to show the greatest overall level of association with diatom community composition, potentially most strongly with lead, but it is especially difficult to separate the potential effects of lead, cadmium, zinc and copper among this set of study sites.

We conclude that diatoms are influenced by trace metals, and that there is potential to separate toxic metal effects from 'natural' environmental effects.

The results from multiple regression analysis show that diatom numbers depend most significantly ($r^2 \sim 0.3$) upon the total dissolved concentrations of Zn and Cd (or equally well on DGT concentrations). No significant effects of hardness were evident. The Toxicity Binding Model did not give any better explanation than the multiple regression approach, with only Zn (or possibly Cd) providing any explanation of the variation in diatom numbers. Application of a zinc BLM for a standard laboratory algal species (*Pseudokirchneriella subcapitata*) showed no significant relationship to the number of taxa found.

8.1.4 Laboratory toxicity testing with streamwater samples

Toxicity testing with the green alga *Pseudokirchneriella subcapitata* confirmed that about eight of the streamwaters contain cationic trace metals at concentrations sufficient to cause toxic effects. Five of these were the same as the eight streamwaters for which diatom species numbers were reduced. The invertebrate crustacean *Daphnia magna* was less sensitive than the alga.

The TBM was successfully applied to the algal toxicity data, and H, Al, Zn and Cd were all found to contribute significantly to the variation in algal growth rate. The toxicity coefficient for Cd was found to be two to three orders of magnitude greater than the coefficients of the other metals, implying that the bound metal is exceptionally toxic.

For diatoms, the TBM identified eight sites with $F_{\text{tox}} > 0.25$ (i.e. above a likely toxicity threshold), and five of these were also found to have water that elicited a toxic response in laboratory toxicity tests with the green alga *Pseudokirchneriella subcapitata*. Similarly, seven of the streamwaters that were toxic towards *P. subcapitata* also produced a toxic response from macroinvertebrates in the field.

8.1.5 Dose–response relationships: conclusions

1. Toxic metals were demonstrated to reduce species numbers of both macroinvertebrates and diatoms in the field.
2. Laboratory tests confirmed that eight of the streamwaters in which field effects were observed contained metals at toxic concentrations.
3. The macroinvertebrate community provides an effective and sensitive tool for detecting metal toxicity. In the study sites, toxicity could be attributed definitely to Al and Zn, and there was some evidence of toxic effects due to H^+ and Cu.
4. For diatoms, which were less sensitive than macroinvertebrates, the most likely toxicant was Zn, but Cd may also have been active.
5. The free ion function, that is a combination of $\log(\text{free ion concentration})$ and $\log(\text{H}^+)$, provided the best solution-based measure of metal toxicity towards macroinvertebrates, because it accounted best for Al effects.
6. Toxicity towards diatoms was expressed best, in terms of solution concentrations, by $\log(\text{total dissolved metal concentration})$ or $\log(\text{DGT metal concentration})$.
7. The field data permitted the formulation and partial parameterisation of the Toxicity Binding Model, which is based on Biotic Ligand Model principles, but applicable to mixtures of metals. The model permits clear toxicity thresholds to be identified, and quantifies the contributions of different metals to the overall toxicity.
8. Because of the dominant effect of zinc, among the trace metals, the available data did not permit conclusions to be drawn about the effect of chemical speciation on copper or lead toxicity. However, the finding that aluminium effects are best expressed in terms of the free ion, and the promising results from the TBM, imply that speciation-based measures provide the best way to describe the toxic effects of metals in the field.

8.2 Implications for Environmental Quality Standards setting

In this section we consider the implications of the findings of the present work for EQS setting, drawing upon the detailed review of EQS derivation methods presented in Appendix 1.

8.2.1 Bioavailability

Bioavailability is firmly established as a significant factor influencing the toxicity of metals in laboratory tests. The Biotic Ligand Model has significantly improved the conceptual understanding of bioavailability, with a new emphasis on chemical speciation, particularly the effects of pH and dissolved organic matter, and the competitive uptake of cations by the biotic ligand. As Table A1.1 of Appendix 1 shows, bioavailability models (either BLMs or empirical models) have been published for four of the five trace metals studied in the present work, with applications to organisms at a variety of taxonomic levels – plants, invertebrates and vertebrates (fish).

The calibrated bioavailability models improve the prediction of metal toxicity across different water compositions, in toxicity tests that are used to derive EQS/PNEC values. From the point of view of risk assessment based upon laboratory toxicity data, this is a compelling reason for incorporating bioavailability considerations into EQS setting.

Section 8.1 identified several results from the present study that show the value of the bioavailability approach. These are the improved description of the macroinvertebrate data when free ion functions (especially of Al) are used in multiple regression, the partially successful application of the Zn BLM, and the promising applications of the TBM. We have not made an unequivocal case for Cu and Pb, but one would expect that these 'speciation-sensitive' metals (Table 8.1) would behave analogously to Al. The interesting agreements between observed and WHAM-predicted bryophyte and invertebrate metal contents provide evidence that biota respond to the chemistry of the medium. Therefore, our findings add to the case for the use of bioavailability in setting EQSs.

8.2.2 Metal background concentrations

If the Added Risk methodology is to be applied, or if freshwater conditions are more generally to be compared with 'pristine' circumstances, then a major issue is the estimation of background metal levels at different locations. Estimation methods can be broadly categorised into two groups, those based on field measurements of current metal concentrations, and those based on conceptual models of metal (bio)geochemistry. The latter include, for example, models of rock weathering and metal accumulation in sediments.

In work related to heavy metal critical loads, Tipping *et al.* (2005b, 2007) used a combination of assumed congruent mineral weathering and estimates of background atmospheric deposition to estimate 'pristine' steady-state background metal concentrations in systems similar to those studied here. Results for several sites are compared with Al, Ni, Cu, Zn, Cd and Pb concentrations in the streamwaters of the present study in Figure 4.1.

As might be expected, the 'pristine' concentrations of Al do not differ greatly from present-day values, while those of the trace metals are generally considerably lower (by 10 to 1000 times) than the levels in the highly contaminated sites. The plots in

Figure 4.1 also show concentrations for present-day sites not contaminated by old mining activities, but contaminated as a result of atmospheric deposition. These concentrations are generally significantly greater than the pristine values, although direct comparison is hampered by differences in streamwater pH. Nonetheless, the results give some indication of the metal levels under 'pristine', 'present-day background' and 'contaminated' conditions.

A potentially useful way to compare background and contaminated conditions is through the TBM variable F_{tox} (Section 5.4) which could give a direct measure of combined metal toxicity, perhaps more readily apprehended than solution concentrations. The calculations for 'present-day background' sites using the TBM reported in Section 5.4.5 suggest that even in streamwaters where toxicity appears highly unlikely the biotic ligand(s) may still be appreciably loaded with potentially toxic metals.

8.2.3 Methods to express EQSs

Given that we consider the present findings to support the expression of EQSs in terms of bioavailability, we recommend the use of the BLM philosophy. In our view, this provides the optimum means by which scientific understanding can be used by regulators, with the implication that this will lead to the best-possible regulation.

Application of BLM-type models of course requires the use of appropriate software, and the necessary input data. In principle, it could be usefully supported by DMT measurements of metal free ion concentrations, but the DMT technique probably requires more development and evaluation before it can be used routinely.

The alternative bioavailability-based metric considered in this work was the direct use of DGT-metal, but for the field sites studied this does not seem to offer any clear advantages in terms of EQS setting, nor is it readily linked to an underlying theory. However, DGT provides useful information for the assessment of chemical speciation models, by measuring a quantity, C_{dyn} , that can also be predicted.

In order to interpret the somewhat limited results of the present study, we developed a 'field BLM', the TBM, that could be optimised with field data, considering mixture effects directly. The issue of mixtures has not yet been considered in 'conventional' BLM work, and to develop a mixtures description from laboratory data would be a considerable task. Thought should perhaps be given to whether the ability to handle mixtures is desirable within an EQS context. There are clear regulatory advantages in keeping things simple, and dealing with one metal at a time in consent setting for example. However, the conclusion from the present results that 'natural' Al plays a significant toxic role even in neutral waters suggests that mixture effects always operate for trace metals, that is the effect of the trace metal will always depend upon the contribution of aluminium. Therefore, the need to address the mixture issue assumes greater importance. The TBM approach provides a potential means of doing this, because the model predicts the combined effects of the mixture, and also quantifies the individual toxic effects of the different metals. In principle, BLMs could also be combined to make this assessment.

8.2.4 Field assessment of EQSs

The Water Framework Directive requires EQSs derived from toxicity tests to be assessed against field effects data if possible, and for the EQS derivation to be reviewed to address any anomalies that arise. Field assessment of EQSs should

ideally account for the fact that multiple contaminants (metals and others) are likely to be present and contributing to effects.

The quantile regression approach with a large body of field observations was used by Crane *et al.* (2007) to evaluate possible toxic effects in terms of a series of metals, considered individually. This provides a ready comparison with EQSs derived from laboratory toxicity data, including BLM-based EQSs, but inevitably cannot distinguish cases where several metals combine to exert a toxic effect.

In the present work, we have not evaluated EQSs as such, but the findings have demonstrated that metals do affect freshwater ecosystems, and that these effects can be at least partially quantified in terms of bioavailability and chemical speciation. In principle, this methodology could be extended, within the TBM framework, to derive EQSs from field data, but linking field and laboratory observations is perhaps a better option.

8.2.5 Tiered application of EQSs

The different types of EQSs require different data inputs for their application, and this raises the possibility of using a hierarchical assessment approach. This might avoid the collection of unnecessary data, and permit effort to be focused on 'problem' sites, for example where the concentration of a given metal exceeds an EQS expressed in terms of total dissolved concentration, but where there is no evidence for deleterious effects.

Perhaps the simplest next step is to establish that metal concentration is well-represented by the sampling programme (e.g. based on 12 samples per year). The use of DGT as a continuous monitoring device would give a more reliable measure of exposure to the metal.

It is becoming increasingly clear that metal toxicity depends upon pH, a phenomenon interpreted in terms of competition between the metal and protons for key binding sites. Thus, a given concentration of zinc at pH 5 may be more toxic than the same concentration at pH 7. This pH-dependence is included in Biotic Ligand Models, and could be assessed without the need for DOC data, although information about solutes such as Na, Mg and Ca would be needed, as well as a BLM for each appropriate organism.

Another possible complication is the binding of metals by dissolved organic matter and the consequent reduction in bioavailability. This would apply mainly to Cu and Pb, both of which interact strongly with organic matter. Again, this could be assessed using the BLM, but such an application would require DOC data.

8.3 Further work

The present study was successful in demonstrating metal dose–response relationships in the field, showing that metal accumulation in stream biota is related to the chemical composition and speciation of the streamwater, and in formulating a potentially valuable model for the interpretation of field metal toxicity. However, the chemical characteristics of the field sites were such that toxic effects could only be quantified for Al and Zn. In hindsight, it was perhaps over-ambitious to expect more information from a relatively small number of sites, and with the toxic response expressed in terms of field community variables. Clearly, therefore, in order to progress understanding in this area, we need information on a wider range of sites, including examples where Ni, Cu, Cd and Pb exert greater toxic effects. Some such sites may exist in other areas of the UK where headwater streams are affected by past mining activities such as Wales and

Cornwall (Hirst *et al.*, 2002), but it may also be necessary to extend the range of field sites.

The present results suggest that Al is a significant contributor to toxicity even in neutral waters. This needs to be explored more fully, and valuable insights could be obtained by improving our understanding of Al chemistry in natural waters, and by carrying out targeted laboratory toxicity studies.

The Toxicity Binding Model offers a means to apply BLM principles directly to the field, and it has the potential for development into a useful tool to assess waters for metal toxicity. Given its promising application to the laboratory toxicity data (Chapter 7), it may be possible to derive useful parameters from the wealth of existing toxicity data. The existing TBM could be tested by applying it to the Environment Agency's macroinvertebrate data for stream and river waters that were employed in the study of Crane *et al.* (2007). It would be expected that the OE scores provided by this dataset would fall under the 'roof' defined by the model (Figure 5.4), in a mechanistic equivalent of the quantile regression method.

A question that could be addressed, even with the TBM in its present form, is how quantitatively different EQSs would be if a BLM approach were used. The speculative exercise for Cu and Zn at three of the study sites described in Section 5.4.6 makes an attempt to do this, and the results suggest that total concentrations of Cu required for toxicity would vary more than those of Zn. It would be informative to carry out this exercise for a wider range of waters.

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List of abbreviations (main text)

ANOVA	Analysis of variance
AWIC	Acid Waters Indicator Community
BLM	Biotic Ligand Model
BMWP	Biological Monitoring Working Party
CCA	Canonical Correspondence Analysis
CCU	Cumulative criterion unit
CEH	Centre for Ecology and Hydrology
CHUM-AM	Chemistry of the Uplands Model – Annual, Metals
CLF	Critical limit function
DARLEQ	Diatoms for Assessing River and Lake Ecological Quality
DCA	Detrended correspondence analysis
DGT	Diffusive Gradients in Thin films
DMT	Donnan Membrane Technique
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EDTA	Ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency (US)
EPT	Ephemeroptera, Plecoptera and Trichoptera
EQS	Environmental Quality Standard
FI	Free ion
FMIF	Free metal ion (function)
GQA	General Quality Audit
HF	Hardness function
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LOD	Limit Of Detection
MERAG	Metal Risk Assessment Guidance
MOPS	3-(N-morpholino)propanesulfonic acid
MPC	Maximum Permissible Concentration
NGR	National Grid Reference
NICA	Non-Ideal Competitive Adsorption
NOEC	No Observed Effect Concentration
NS	Not significant
NT	Not tested
O/E	Observed (divided by) expected
OECD	Organisation for Economic Cooperation and Development
pCCA	partial Canonical Correspondence Analysis

PNEC	Predicted No Effect Concentration
RIVPACS	River Invertebrate Prediction and Classification System
RAR	(Existing Substances Regulations) Risk Assessment Report
SCAMP	Surface Complexation Assemblage Model for Particles
SPM	Suspended particulate matter
TBM	Toxicity Binding Model
TD	Total dissolved
TDI	Trophic Diatom Index
TIE	Toxicity Identification Evaluation
TOC	Total organic carbon
VRA	Voluntary Risk Assessment
WFD	Water Framework Directive
WHAM	Windermere Humic Aqueous Model

Symbols

α_M	Toxicity coefficient in the TBM
F_{tox}	Toxicity function
$F_{tox,max}$	Upper threshold of F_{tox}
$F_{tox,min}$	Lower threshold of F_{tox}
LKMA	Log equilibrium constant for metal binding to type A sites (WHAM)
n_{DS}	Number of diatom species
OE67	The 67% most sensitive RIVPACS taxa
r_{EQS}	Ratio of metal concentration to EQS value
V_M	Moles bound per gram

Appendix 1 Review of EQS derivation methodologies

A1.1 Introduction

This appendix presents a detailed review of Environmental Quality Standard (EQS) derivation methods drawn on in the main body of this report and discusses EQS setting in the context of the work done within this research project.

An EQS is a policy tool for management of the state of the environment. Broadly, an EQS represents a concentration of a given substance or group of substances that, if exceeded, triggers a particular management action designed either to further assess the resulting environmental consequences and/or to ultimately reduce the environmental concentration of the chemical(s) below the EQS. The EQS therefore provides a policy benchmark against which to assess the state of the environment, and provides a target for control and limitation of anthropogenic emissions to the environment. EQS values may be set for different compartments of the environment (e.g. air, soil, freshwater, sediment, groundwater) and for different protection purposes (e.g. protection of ecosystems, human health). The environmental compartment under consideration, and the purpose for which the EQS is defined, are key starting points that determine the data to be used in calculations and the methodology to be used.

The purpose of this review is to evaluate a set of current methods for setting EQS values, for the ecological protection of freshwaters against the toxic effects of the metals Ni, Cu, Zn, Cd and Pb. We shall first discuss considerations to be made when setting EQS values specifically for the freshwater environment, and the considerations to be made specifically for the metals under investigation, which arise from their chemical behaviour in relation to their toxicity and to their presence as natural constituents of freshwaters.

Many of the methods to be reviewed are related in that they involve the scientific evaluation of data on the toxicity of solutions of a single metal to freshwater organisms under laboratory conditions. Beyond this, these methods differ in the principles upon which laboratory data are chosen and evaluated, and the methods by which the whole collection of toxicity data is used to derive a final EQS.

Most freshwaters are dynamic environments, where the chemical composition of the system, including the concentrations of potentially toxic substances, evolves over a timescale of days or hours in response to precipitation. Rivers are more temporally dynamic than lakes, while the dynamic characteristics of an individual lake will depend upon its mean residence (flushing) time. Given this dynamic behaviour with respect to chemistry, ecological protection of freshwaters should ideally encompass both short and longer term impacts of toxicants. Short-term impacts on UK freshwaters are most likely to arise either from release of the toxicant from another environmental compartment (e.g. soil) during a period of high flow, or the instantaneous release of toxicant from a point source. Longer term impacts are most likely to arise from continuous release of the toxicant from one or more point sources, or from diffuse sources.

A1.1.1 Scope of this review

There are a number of generic considerations to be made when deriving EQS values, and a number of considerations specific to EQSs for the cationic metals considered here.

Nature of the toxicity data used

Broadly speaking, toxicity tests and the resulting data may be categorised into two classes: acute tests, where the organism is exposed over a short time period to a range of concentrations of the test substance intended to cause lethal effects, and chronic tests, where the organism is exposed, over a time period that is at least a significant proportion of its expected single lifetime, to concentrations of the test substance expected to significantly impair one or more aspects of the organism's well-being, such as its behaviour, biochemistry, physiology, growth or reproductive capabilities. The methodologies evaluated here may make use of either or both types of toxicity data; the use to which each type of data is put in the derivation will be a key aspect of the evaluation.

The specific derivation of an EQS within a method may be dependent upon the amount of suitable data available. Methodologies may require the consideration of data according to species or genus, depending upon the specific protection aim. The requirements of the method in terms of numbers of species and taxa will be evaluated for completeness and for the alternative calculation methods available where available data are restrictive. While in theory it would be desirable to have toxicity data for as wide a range of species as possible, in practice literature toxicity tests tend to be biased towards organisms that are easy to culture and maintain in the laboratory, and particularly to organisms for which standard test procedures have been published.

Chemical speciation and biouptake effects

Metals in freshwaters can exist in several phases; dissolved, and associated with colloidal and particulate material either in 'reactive' or 'non-reactive' forms. 'Reactive' here means metals that can potentially exchange with the solution on a short timescale (e.g. metal bound to surface functional groups of colloids or particulate material); 'non-reactive' means metal that cannot exchange on a short timescale (e.g. metal found within the lattices of particulate minerals). This phase may also be considered to include anthropogenically discharged metal that is in a chemically inert form. The chemical speciation of the metal is its distribution among these different phases and among different dissolved forms. Measurement of chemical speciation, in the laboratory or the field, is technically complex, and models that predict the speciation of the dissolved and 'reactive' metal at thermodynamic equilibrium are commonly used as an alternative. Speciation is considered important since it has been long known (e.g. Campbell, 1995) that toxic effects on an aquatic organism are commonly proportional to the activity or concentration of the free ion, the uncomplexed form of dissolved metal, if other water chemistry parameters (e.g. pH) are held constant. The Free Ion Activity Model (FIAM) explains this finding theoretically by postulating that toxicity is caused by metal binding to a receptor site on the organism (e.g. on the surface of a fish gill or on the cell wall of an alga) followed by transfer into the organism to the site of toxic action (internalisation). The main receptor-binding metal species is considered to be the free metal ion, although the theory does not preclude the binding of complexed forms, the key concept being that not all forms of dissolved metal can interact with the receptor.

Another key aspect of the theory is that equilibrium between dissolved and receptor-bound metal is rapid in comparison with the internalisation rate. The theory also allows for the binding of other dissolved metals, including major solution ions such as sodium and calcium, at the same receptor. Thus, changes in the activities of these major ions can modify the toxic effect. Pagenkopf used FIAM theory to develop a Gill Surface Interaction Model (GSIM) describing the effects of varying water chemistry (pH and hardness) on metal binding at receptor sites on fish gills. This work has been further developed into the Biotic Ligand Model (BLM), which allows the receptor-bound metal concentration to be calculated as a function of the free ion activities of the metal of interest and of all the competing solution ions, using expressions analogous to those describing thermodynamic equilibria between cations and a binding ligand in solution.

Parameterised BLMs have been derived for acute and chronic metal effects on a number of species (Table A1.1 in Section A1.3.3), and extension to previously unconsidered metals and organisms continues. Use of the BLM in deriving EQS values is encountered in some the methods evaluated here; detailed discussion of its use and the implications will be made later.

Essentiality and the natural background

Some metals are essential to the health of aquatic organisms. Of the metals looked at here, Cu and Zn are considered essential for all organisms and Ni has been shown to be essential for some. An organism will therefore have a minimum requirement for these metals, corresponding to the lowest level in the water column at which the organism can take up sufficient metal to meet its essential physiological needs. For a given organism this level may vary spatially, particularly if uptake is via an external receptor site, for the same reasons as toxic effect varies (see above). Given that an EQS is designed to protect the health of the aquatic ecosystem it would be logical to protect against metal deficiency as well as toxicity, if practically possible.

All the metals under consideration are likely to be present in freshwater due to natural sources. The most significant natural sources are likely to be weathering of catchment rocks and soils, and long-range atmospheric transport and deposition. The ecosystem that will have developed in the presence of the natural metal must be considered by definition the natural ecosystem that must be protected. This is not to say that ecosystems developed in the presence of differing natural metal concentrations will not show effects due to the metal; on the contrary, the presence of natural metal, if it influences the ecosystem, can be considered to be a driver of biodiversity.

EQS derivation methods to be reviewed

This document is based in part around a number of prominent current methods for deriving EQSs for the protection of freshwater ecology. These methods are:

1. The methodology used to derive Environmental Quality Standards in the United Kingdom (under the provisions of the EU Dangerous Substances Directive (76/464/EEC)).
2. The methodology used to derive Water Quality Criteria in the USA.
3. The methodology used to derive Environmental Risk Limits and Environmental Quality Standards in the Netherlands.
4. Methodologies used in the European Union to derive Predicted No Effect Concentrations for derivation of Quality Standards under the provisions of the EU Water Framework Directive (Lepper, 2002).

5. Methodologies used to derive and apply Water Quality Guidelines in Australia and New Zealand.

A1.2 Metal essentiality and the natural background

All the metals under consideration will be present in freshwaters due to natural sources, regardless of the extent of anthropogenic contamination present. The most significant natural sources are likely to be weathering of catchment rocks and soils, and possibly atmospheric deposition. Some metals are also essential trace minerals for aquatic life and so must be present for the ecosystem to function in an optimal way. Copper and zinc are well known to be essential. The essentiality of nickel for aquatic life has been inferred by study of its bioconcentration behaviour and analogies with other essential cations. Cadmium has been shown to be essential in certain circumstances for marine phytoplankton but a general role as an essential element has not been established. Lead does not appear to be essential at all.

Essentiality and the natural background are issues that have not had great emphasis in EQS derivation schemes despite the knowledge possessed. Recently, however, the consideration of the natural background in the EQS derivation scheme for the Netherlands, and its intended application to the metal EQS values proposed for application under the Water Framework Directive, have increased interest in the incorporation of the natural background into EQS values (the Added Risk approach) rather than deriving standards from the total metal to which organisms are exposed in tests (the Total Risk approach). At the same time, the metalloregion concept has been developed as a means of classifying systems according to common ecological and geochemical characteristics in order to delineate areas for regional risk assessment.

The Added Risk approach and current recommendations for its use will be discussed first, followed by the metalloregion concept and methods for estimating the natural background metal.

A1.2.1 The Added Risk approach

The Added Risk approach is intended to provide a robust method of quantifying risk due to anthropogenic elevation of field concentrations of a substance that also occurs naturally (and indeed may be essential to organisms in small amounts). All the metals considered in this report fall into this category of substances. As the name implies, the aim is to consider only the risk due to the added amount of substance, and to prevent as far as possible the derivation of an EQS that is below the natural background in some places. An EQS derived using the Added Risk approach would be considered to represent the highest acceptable field concentration above the natural background.

Consideration of the Added Risk Approach raises a number of questions regarding its theoretical validity and practical applicability, in particular when considered against the alternative 'Total Risk' approach where risk is considered on the basis of the total concentration of potentially toxic substance present.

In evaluating the usefulness of the Added Risk approach for risk assessment and EQS derivation a number of key points must be addressed:

1. Whether it is in fact more ecologically realistic and robust to apply the Added Risk approach rather than the Total Risk approach.
2. How laboratory toxicity test results intended for use with the Added Risk approach should be handled.

3. How the estimation of (the) natural background concentration(s) of the metal can be done robustly.
4. Whether any benefits of the Added Risk approach (from the point of view of practical management of freshwater contamination) outweigh any additional complexities in its use.

In considering these issues, we shall first look at the past development and application of the Added Risk approach and then discuss the theory and application of the approach in detail, before discussing the above key points.

A1.2.2 Use of Added Risk in calculating EQSs/PNECs

The Dutch Added Risk approach

Peijnenburg *et al.* (1996) proposed two methods for added risk. In both cases the approach is based around the use of a Species Sensitivity Distribution (SSD) of laboratory derived toxic endpoints (e.g. no observed effect concentrations, NOECs). The proportion of species with NOECs below a given metal concentration is termed the Potentially Affected Fraction (PAF) for that concentration.

The effect limitation approach is presented conceptually in Figure A1.1. The background concentration of metal, C_B , is considered to comprise bioavailable and inactive fractions. The bioavailable fraction (denoted ϕ) is considered to exert an effect on the ecosystem which is quantified as a 'background PAF' (PAF_B). The maximum acceptable PAF (PAF_{MAX}) is then taken to be the background PAF plus 5% of the fraction of species unaffected by the background:

$$PAF_{MAX} = PAF_B + 0.05 \cdot (1 - PAF_B) \quad (A1.1)$$

The Maximum Permissible Concentration (MPC) is defined as the metal concentration corresponding to the PAF_{MAX} , plus the inactive background, $MPC = C_B + C_A$. The Maximum Permissible Addition (MPA) is the MPC minus the total background. This compares with the Total Risk approach where the maximum acceptable total metal concentration would be taken as that affecting 5% of the species (i.e. $PAF_{MAX} = 0.05$).

The concentration limitation approach sets the MPC equal to the sum of the background and the concentration of metal equivalent to a PAF of 0.05 (i.e. affecting 5% of species, denoted HC5). Essentially, concentration limitation sets the acceptable metal addition independently of variation in the bioavailable background, while the effect limitation approach accounts for bioavailable background variation in setting an acceptable addition.

The effect limitation approach was applied in developing aquatic MPCs for nickel, copper, zinc, cadmium and lead in a 1997 report (Crommentuijn *et al.*, 1997). A fuller description of the policy background is given in Section A1.4.3. Since values of ϕ were not available, calculations were done with values of 0, 0.5 and 1. It is worth noting that when $\phi = 0$ the approach gives the same results as the concentration limitation approach. MPAs were calculated using a species sensitivity distribution approach, using NOECs calculated as the metal concentration added to the toxicity test medium (i.e. discounting any metal present in the control medium). It was considered that varying ϕ had, in practice, little impact upon the magnitude of the MPC.

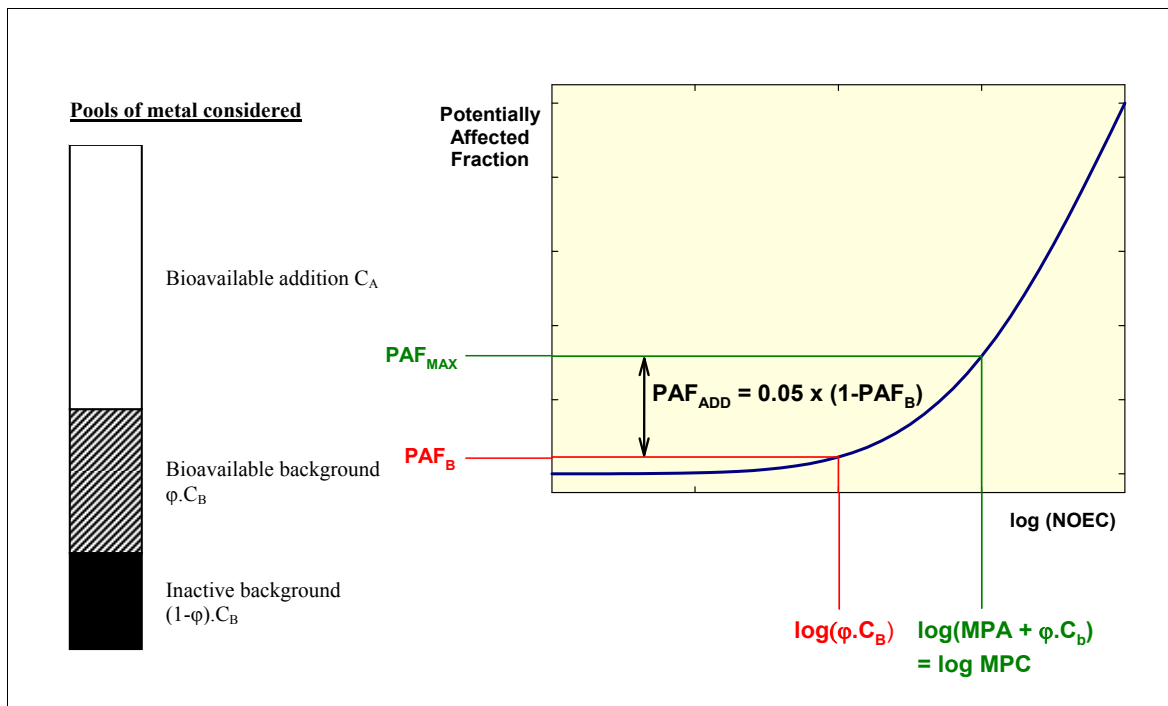


Figure A1.1 The effect limitation approach.

The natural background metal C_B comprises a fraction ϕ that is bioavailable. The maximum acceptable Potentially Affected Fraction (PAF) of species is calculated taking into account the fraction affected by the bioavailable natural background.

Values for background concentrations of metals were calculated assuming that a single background concentration would be representative of surface waters in the Netherlands. Concentrations estimated by Zuurdeeg *et al.* (1992) were converted to total metals using a model proposed by De Bruijn and Denneman (1992), and were converted to dissolved concentrations using metal-specific partition coefficients assuming a suspended particulate matter concentration of 30 mg l^{-1} .

Use of Added Risk in deriving EQSs under the Water Framework Directive

Derivation methods for EQSs for substances designated as Priority Substances under the Water Framework Directive were proposed in a 2002 report (Lepper, 2002). The Added Risk method was recommended for use with the concentration limitation approach (see also Section A1.4.4).

A1.2.3 Estimation of background metal concentrations

This section reviews the work of the Analysis and Monitoring of Priority Substances (AMPS) Expert Group in recommending methods for the estimation of natural metal concentrations, here termed background reference concentrations (BRCs), in the context of the proposed use of Added Risk to set standards for Priority Substances under the Water Framework Directive. Of the metals under consideration here cadmium and its compounds are Priority Hazardous Substances and nickel and lead and their compounds are Priority Substances. Methodologies for estimating BRCs will also be applicable to copper and zinc, which are classed as Specific Pollutants.

Several important methodological issues must be addressed when considering how to estimate natural metal concentrations in aquatic environments:

1. The scientific method by which natural metals should be estimated.
2. The degree to which spatial variability in natural metals should be considered.

The AMPS Export Group provided a formal definition of background metal concentration: 'The background concentration of target metals (Pb, Cd, Ni, Hg) in the aquatic ecosystems of a river basin, river sub-basin or river basin management area is that concentration in the past or present corresponding to very low anthropogenic pressure.'

On a practical level this will relate to current concentrations in systems where point sources of metals are absent and diffuse sources are insignificant, and provides a theoretical basis for estimating background concentrations for such systems on the basis of current measurements of metal concentrations. In systems where point discharges of metals (e.g. factories, working or abandoned mines) are absent contamination may still occur via diffuse sources such as leaching from biosolids application or the deposition of atmospheric contamination. Metals from such sources must be leached from the soil; evidence suggests that due to the strong binding of metals to soil solids this leaching is an extremely slow process and so the resulting concentrations in surface water would be expected to be quite low. Such sources would in many circumstances not be expected to be as significant as point sources.

Sources of natural metal to aquatic systems can be summarised as:

1. Leaching from soil of natural metal deposited from the atmosphere. The major source of such metal is expected to be volcanic activity.
2. Leaching from soil, and seepage from groundwater, of metal naturally present due to weathering. The flux and aquatic concentrations of this metal will be dependent on local geology. This source of natural metal is expected to be the most spatially variable.

The AMPS Expert Group proposed three options for application of BRCs: assumption of a zero background, use of a single default BRC, or use of a BRC calculated for local conditions. This would allow the general use of a default value except in cases where a local BRC or BRCs was felt justified, thus minimising the effort involved in deriving values. The justification for setting a local BRC would be based upon monitoring or other evidence of a significant deviation from the default BRC.

Suggested BRCs for Ni, Cd and Pb were presented by a drafting group on Background Concentrations, based on percentiles of streamwater concentrations of the metals at relatively unimpacted sites across Europe (807 samples) Suggested procedures for calculating local BRCs were:

1. The use of local concentrations from 'pristine' areas (i.e. not affected by local activities). Lake sediment concentration profiles were suggested as a means of evaluating whether an area could be defined as 'pristine'. Where sediment enrichment is found a BRC could be estimated by taking ratios of the current dissolved metal concentration against the current and pre-industrial sediment concentrations:

$$\frac{[M]_{dissolved,preindustrial}}{[M]_{dissolved,current}} = \frac{\{M\}_{sed,preindustrial}}{\{M\}_{sed,current}} \quad (A1.2)$$

or by considering element ratios in pre-industrial and current sediment, soil or wetland profiles (including peat). Aluminium was suggested as a possible reference element.

2. The use of metal concentrations in groundwater. It was suggested that because of the likely close relationship between geology and groundwater metal concentrations, and the limitation of diffuse anthropogenic contamination, groundwater concentrations in pristine areas were likely to reflect natural metal levels well.
3. Evaluation of long-term spatial or temporal datasets on metal concentrations in surface waters.
4. Estimation of dissolved BRCs from metal contents of sediment or suspended particulate matter (SPM) by application of partition coefficients.
5. Use of geochemical models.
6. Estimation of BRCs by spatial correlation against other geochemical characteristics, for example a conservative element such as Ti, in areas subject to very low anthropogenic pressure.

The use of the calculated default BRCs was not recommended by the AMPS Expert Group, based on the fact that spatial variation in actual natural concentrations appeared to be significant across Europe. A preference was expressed for local BRCs calculated by one of the methods listed above. These will be commented on in turn:

1. This is effectively a partition coefficient approach. The use of lake sediments is potentially very useful although careful consideration would need to be given to the relevance of the data. Use of the total (e.g. hydrofluoric acid digested) metal concentrations in the sediment would tend to underestimate anthropogenic enrichment since it would include metal physically transported into the lake in minerals: this metal would not influence the dissolved concentration, and less harsh extraction methods should ideally be used. Possible effects of other changes in water chemistry (e.g. acidification) might also affect the partitioning over time. It is worth noting that even in remote areas of the UK (e.g. Lochnagar, northeast Scotland) lake sediment profiles show measurable metal enrichment due to long-term deposition from the atmosphere. In larger lakes dissolved metal might be removed by particle binding and settling, resulting in concentrations lower than in surrounding rivers, although the relevance of this to the UK situation is not known.

Some work has been done on calculating anthropogenic enrichment factors from UK lake sediments and comparing them with factors calculated from soils and from modelling, for Lochnagar (Tipping *et al.*, 2007). Sediment factors ranged from 1.8 to 2.7, while soil factors ranged from 3.6 to 14.3 and model-calculated factors from 1.6 to 19.0. Clearly there is considerable variability in factors and more work is needed to resolve these differences. A pragmatic possibility would be to take the lowest factor, thus giving the highest (most conservative) estimated background.

The use of element ratios does not seem promising. If deep sediment chemistry is available then the use of enrichment factors would probably be more robust. Ratios of metal to other elements in soil profiles would need to be considered carefully. If iron is not considered suitable then it is difficult to understand how aluminium could be; although it is true that clay minerals are rich in Al, not all UK soils are rich in clay (e.g. peats and upland organic soils). Neither is it necessarily true that in such soils the anthropogenic input of Al would be unimportant, since atmospheric deposition may be a significant input to the

uplands. For example, Smith *et al.* (2005) measured total Al concentrations between 170 and 42,000 mg kg⁻¹ for a transect of peats from northern England to central Scotland. It is possible that this approach has value for clay-rich soils but further investigation would be needed.

2. Where groundwater is a major source to surface waters the use of metal concentrations is potentially useful if reliable monitoring data are available. It would be necessary to define areas where groundwater is a major source of surface water, including a definition of what is meant by a 'major source'. The nature of the source aquifer would also need to be considered, particularly if susceptible to recharge from contaminated surface waters.
3. This is an attractive approach from the practical point of view, provided that data are available at wide spatial scales (e.g. British Geological Survey G-BASE dataset). However, it is worth noting that lake sediment studies show that even remote areas have been and remain enriched with metals, largely from atmospheric deposition. The data obtained from large-scale surveys may well be useful in rapidly delineating areas of high metal concentration for further investigation.
4. This approach has many potential drawbacks that are recognised in the report. The recommendation that partition coefficients could be used with lake sediments is effectively the same methodology as in Item 1.
5. It is not clear from the text exactly what is meant by a 'geo-chemical model', which is a term that can encompass different types of models including chemical speciation, weathering and whole catchment geochemistry. A catchment geochemistry model has been used, for example to simulate historical sediment enrichment in Lochnagar, Scotland (Tipping *et al.*, 2007), and work in this area is continuing. A key input in such models is the weathering rate of the catchment minerals. At the moment the general approach is to scale metal weathering rates for particular rock types against weathering rates for major ions (e.g. Si, Ca). More work would be required to apply this approach across differing geologies.
6. This is a potentially useful approach, although in practice its application in the UK may well be limited to semi-pristine upland areas having different geologies to lowland areas.

A1.2.4 The metalloregion concept

Metalloregions are a concept developed in response to the growing awareness of the importance of natural metal concentrations in risk assessment, particularly for essential metals. Metalloregions are a development of the concept of ecoregions, which can be defined as 'a relatively large area of land or water that contains a geographically distinct assemblage of natural communities that share a large majority of their species dynamics and environmental conditions and whose ecological interactions are critical for long-term persistence'. Ecoregions are broadly defined based upon factors such as climate, latitude and elevation. Metalloregions are subdivisions of an ecoregion defined on the basis of those factors that influence the response of the community to contaminant metal (i.e. for aquatic systems pH, hardness, dissolved organic carbon and the background metal would be among the delineating chemical factors). The value of metalloregions in risk assessment is that PNECs specific to each can be defined, for example by correcting for bioavailability based on the metalloregion's average surface water chemistry.

A1.2.5 Metalloregions and natural metal

Natural background metal concentrations are a crucial factor in prospective delineation of metalloregions. Within a metalloregion, species that are performing optimally must by definition be exposed to natural levels of each essential metal high enough not to cause deficiency yet low enough not to cause toxicity, and to natural levels of each non-essential metal low enough not to cause toxicity. For essential metals there will be a concentration range within which a particular species' metabolic requirements for that metal are satisfied, and within which the organism can regulate its internal concentration of the metal. This is termed the species' Optimal Concentration of Essential Element (OCEE) range (Figure A1.2). For non-essential metals there will be a concentration range within which significant toxic effects are not seen, analogous to the OCEE. The OCEE ranges of species living in the same ecosystem are not necessarily equal, but by definition they must overlap to a certain degree (Figure A1.2, left). Organisms will not have minimum requirements for a non-essential metal but if they exist in the presence of a certain natural concentration they must by definition be naturally acclimated to it.

The range of metal concentrations indicated by the shaded area is that range at which the requirements of the organisms in the system for the essential metal are simultaneously met. Van Assche et al. defined this common range as the No Risk Area, that is where no organism suffers from toxicity or deficiency of any metal.

OCEEs can differ greatly among different organisms, depending upon the natural metal concentrations characteristic of each organism's habitat. For example, according to Waeterschoot *et al.* (2003) the zinc OCEEs for three species (marine diatom *Thalassiosira weissflogii*, freshwater sponge *Ephydatia fluviatilis* and cladoceran *Daphnia magna*) from environments (metalloregions) having natural zinc concentrations of $\sim 0.0065 \mu\text{g l}^{-1}$, $< 1 \mu\text{g l}^{-1}$ and $4\text{--}35 \mu\text{g l}^{-1}$, respectively, were $\sim 0.0004\text{--}\sim 0.06 \mu\text{g l}^{-1}$, $\sim 0.04\text{--}\sim 3 \mu\text{g l}^{-1}$ and $\sim 1\text{--}\sim 2000 \mu\text{g l}^{-1}$, respectively. A metalloregion therefore has a distinct community that is adapted to the natural concentrations of both essential and non-essential metals encountered there. The potential for OCEEs for different organisms to differ greatly from each other is a likely reason why use of the Total Risk approach can result in PNECs/EQs below background.

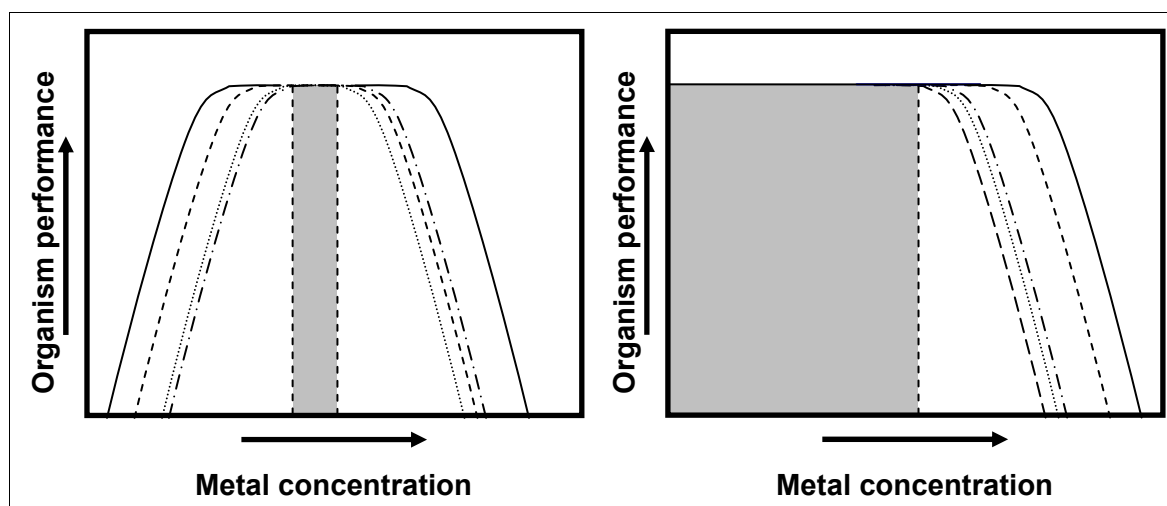


Figure A1.2 Concept of multiple OCEEs for an essential metal (left) and a non-essential metal (right) in a given environment, and of the No Risk Area (shaded).

Metalloregion theory can be applied to the calculation of region- or site-specific PNECs based on the theory that a toxicity test using a culture and control metal concentration within a certain defined range can be used to define a regional or site-specific PNEC

for locations where the natural metal is within this defined range. This has been done for toxicity tests using zinc, in an early draft of the zinc RAR. An HC5 of $17.2 \mu\text{g l}^{-1}$ total was calculated using a complete chronic toxicity dataset for zinc. This dataset was then subdivided into three categories based on the culture zinc concentration: $<2 \mu\text{g l}^{-1}$ ('Great Lakes' conditions), $2\text{--}50 \mu\text{g l}^{-1}$ (typical lowland EU river) and $>50 \mu\text{g l}^{-1}$ (mineralised areas), and HC5s were calculated for each group. Values of 2.5, 17 and $654 \mu\text{g l}^{-1}$ were calculated for the low, typical and high culture zinc concentrations. From this example it would seem that subdivision of toxicity data according to culture metal concentration could be a promising way to account for metalloregion variability in natural field metal concentrations. Non-essential metals would be expected to exhibit similar trends in OCEE to essential metals, but the No Risk Area would have no lower limit. Knowledge of natural metal concentrations would still be needed to establish the spatial extent of metalloregions.

A1.2.6 Tiered approach to Added Risk

As part of research aimed at assessing efficient and practical ways of applying the Added Risk approach, the Environment Agency has considered the use of a tiered approach to assessing site compliance with proposed quality standards for copper and zinc (Comber and Weir, 2006). The framework of the approach is presented in Figure A1.3. It should be noted that the approach is intended to provide a flexible approach to consider metal bioavailability (Section A1.3) as well as the background.

The approach provides a reflexive and potentially efficient approach to assessing EQS compliance at a given site, by considering the metal background and bioavailability only where necessary. There are three stages: firstly, the PEC (Predicted Environmental Concentration) for the location is obtained from monitoring data and compared with a 'default' PNEC (Predicted No Effect Concentration). A 'default' PNEC is one that has been calculated for a water chemistry resulting in either average or high ('worst case') metal bioavailability. The PNEC may have been computed using either Total or Added Risk. If the PEC does not exceed the PNEC then the location is considered compliant. If the PEC exceeds the PNEC then an adjustment of the PEC for the natural background at the location is made and the comparison repeated. If the PEC still exceeds the PNEC then a further correction is made for bioavailability and the comparison is done a third time to determine the final pass or fail status for the location.

An instant attraction of the approach is the quick removal from consideration of sites where neither bioavailability nor background need to be invoked to demonstrate non-exceedance of the EQS/PNEC. This in principle allows resources to be focused particularly on estimating the background only for locations where it is necessary.

In the Comber and Weir (2006) report, PNECs for copper and zinc were taken from the most up to date versions of the EU Risk Assessments for these metals in progress at the time. For copper, the available 'default' PNEC of $8.2 \mu\text{g l}^{-1}$ dissolved was calculated according to the Total Risk approach for a 'reasonable worst case' bioavailability scenario. For zinc, the available PNECs were $7.8 \mu\text{g l}^{-1}$ dissolved for waters with a hardness above $24 \text{mg CaCO}_3 \text{l}^{-1}$ and $3.1 \mu\text{g l}^{-1}$ for softer waters. Background concentrations used in the assessment were $1.6 \mu\text{g l}^{-1}$ for copper and $3.4 \mu\text{g l}^{-1}$ for zinc, based on a preliminary assessment by the Environment Agency (2006). Despite the fact that the copper PNEC was based on Total Risk the same approach was adopted for both metals, on the basis that background copper in toxicity tests used to derive the PNEC was low (assumed to be $0.5 \mu\text{g l}^{-1}$ if not measured) relative to what might be locally expected as background in mineralised areas.

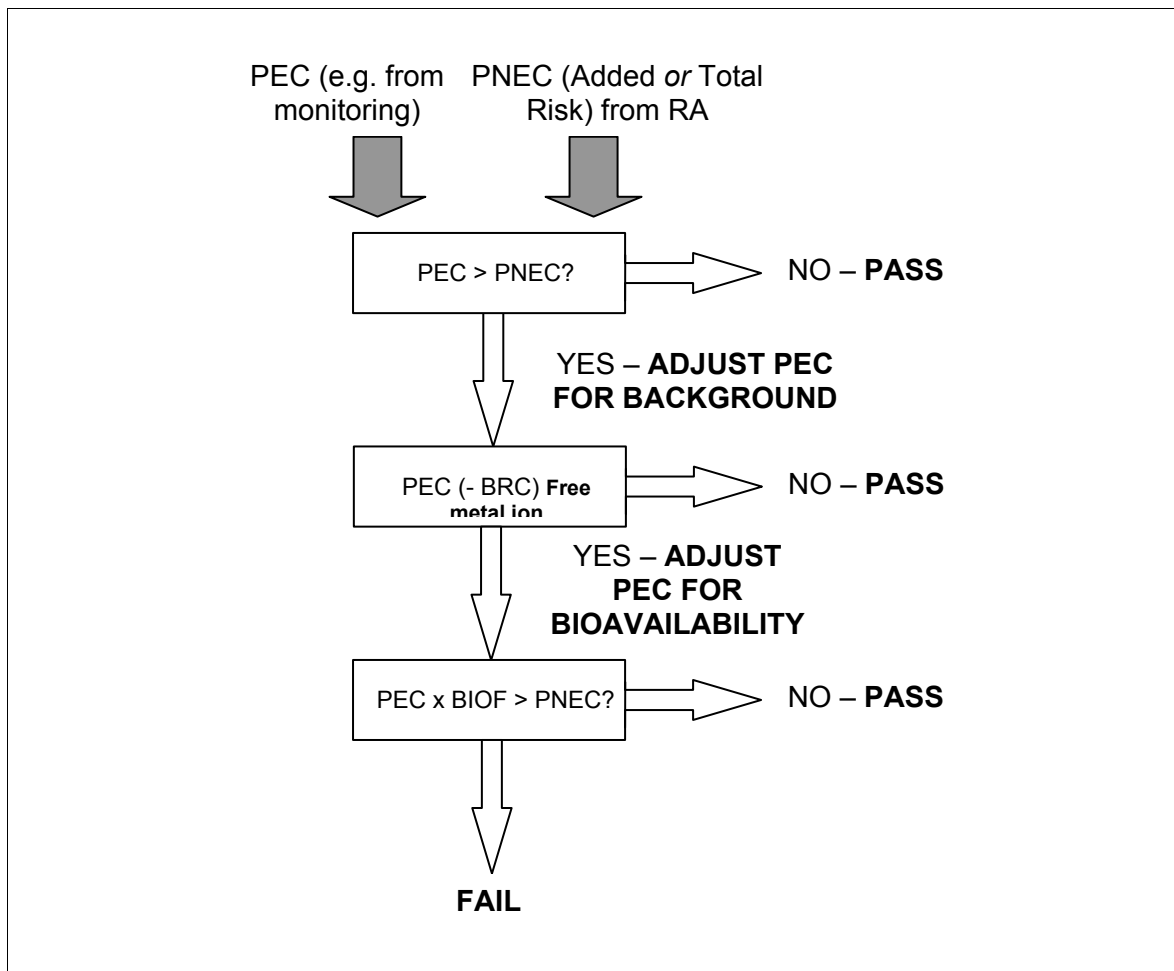


Figure A1.3 Proposed tiered assessment scheme, redrawn from Comber and Weir (2006).

The method was assessed using Environment Agency monitoring data. The first two tiers alone were assessed against the entire available monitoring dataset, while a more limited dataset, containing sufficiently detailed information, was used to assess all three tiers. The results of the Tier 1 and 2 assessment showed that, without correction for background, a significant proportion (72%) of monitoring samples had zinc exceeding the Added Risk PNEC. Addition of the estimated background removed some samples but 60% still 'failed'. When a higher zinc background of $11.1 \mu\text{g l}^{-1}$ was used, 40% of samples 'failed'. By contrast only 5.7% of samples failed for copper before background correction and this dropped to 4.2% after background correction.

Assessment of all three tiers using the limited dataset showed that application of the zinc BLM significantly reduced the number of sample failures for rivers of known good ecological quality. Some of the remaining failures were considered due to locally high backgrounds although others were associated with adverse ecological effects. Application of the copper BLM to the data did not greatly alter the number of failures, and it was also notable that in some cases the 'reasonable worst case' PNEC used in Tier 1 was greater than the PNEC after adjustment with the BLM; in other words, copper was more bioavailable than the reasonable worst case. This was found in sites showing low pH, hardness and/or dissolved organic carbon, all factors contributing to increased copper bioavailability.

A1.2.7 Discussion

Environmental Quality Standards are intended to protect aquatic systems from harm due to anthropogenic chemical contamination. Where the contaminating substance is also present naturally, as in the case of metals, it is necessary to avoid as far as possible a situation where natural metal is mistaken for contamination, leading to wasteful and needless management efforts.

The Added Risk approach is a relatively simple way (at least in concept) to deal with the issue of natural metals. As currently constructed, the approach implies three central assumptions:

1. The natural metal concentrations characteristic of an aquatic system do not, by definition, cause deleterious effects to the ecosystem. Additionally, by definition, adaptation and/or acclimation of ecosystems to different natural metal concentrations means that the tolerance of ecosystems to additional metal ought to be uniform. It should be noted that this assumption does not apply to the effects limitation approach as originally proposed by Struijs *et al.* (1997) since this allows for the 'toxic' effect of the background metal to be accounted for.
2. Toxic endpoints in tests, when expressed as the added metal ($\text{NOEC}_{\text{added}}$), are independent of the test background (control concentration) or the acclimation (culture) conditions used prior to the test. Thus, $\text{NOEC}_{\text{added}}$ values from tests on different organisms with different requirements for an essential metal or different sensitivities to non-essential metals can be combined for $\text{PNEC}_{\text{added}}$ derivation. For example, a test of zinc effects that gave a $\text{NOEC}_{\text{added}}$ of $50 \mu\text{g l}^{-1}$ in the presence of a background of $10 \mu\text{g l}^{-1}$ (a $\text{NOEC}_{\text{total}}$ of $60 \mu\text{g l}^{-1}$), would be assumed to have a similar $\text{NOEC}_{\text{added}}$ in the presence of a background of $50 \mu\text{g l}^{-1}$, that is a $\text{NOEC}_{\text{total}}$ of $100 \mu\text{g l}^{-1}$.
3. A background metal concentration for a given location/watershed/region can be robustly estimated.

Evidence exists that aquatic communities can show increased tolerance for trace metals under conditions of chronic exposure (e.g. Klerks and Weis, 1987) through either physiological and/or genetic changes. Adaptation of aquatic organisms to different natural metal concentrations in the field over many generations is demonstrated by the zinc OCEEs for different organisms given by Waeterschoot *et al.* (2003) (Section A1.2.5). Laboratory studies of acclimation generally show that the higher the concentrations, the greater the tolerance of the organism to acute toxic effects. LeBlanc (1982) studied the survival of *Daphnia magna* exposed for 12 generations to $30 \mu\text{g l}^{-1}$ copper and found increased tolerance, but this was rapidly lost following exposure to a control (low copper) medium. Münzinger (1990) studied multi-generation acclimation of *Daphnia magna* to nickel on population-level characteristics (e.g. intrinsic rate of population growth). More recently, Janssen and co-workers (Muyssen and Janssen, 2001, 2004; Bossuyt and Janssen, 2005; Bossuyt *et al.*, 2005) have studied the effects of long-term acclimation of copper, zinc and cadmium to *Daphnia magna*, and of copper to *Pseudokirchneriella subcapitata*, and Taylor *et al.* (2000) have studied the effects of copper acclimation on rainbow trout chronic response. Postma and Davids (1995) studied the development of tolerance to Cd by the benthic invertebrate *Chironomus riparius* over nine generations, and found that, although some tolerance was induced, mortality rates remained high, and that toxicity thresholds derived from a single generation exposure might not be protective of populations in the longer term. Researchers have also studied the effects of field metal exposure to organisms on the subsequent effects of laboratory exposure. For example, Muyssen *et al.* (2002) compared acute and chronic responses of three *D. magna*

populations (two from a contaminated pond containing $\sim 80 \mu\text{g l}^{-1}$ zinc and a laboratory clone maintained in $\sim 13 \mu\text{g l}^{-1}$ zinc). The field animals (from the contaminated pond) were up to four times as tolerant to acute toxicity as the laboratory clones and also showed higher reproduction rates and growth in chronic testing. However, the field animals gradually lost their zinc tolerance when cultured in its absence. Lopes *et al.* (2006) exposed populations of *Daphnia longispina* from contaminated and uncontaminated locations to acid mine drainage (AMD) and found decreased susceptibility in organisms from populations previously exposed to field stress from AMD.

In the case of essential metals, an additional factor to consider in laboratory tests is that the organisms may have been cultured in a metal-deficient medium, thereby increasing their sensitivity to toxic concentrations of the metal and overestimating its 'true' toxicity. This may result in NOECs biased to low values and may partly explain why the Total Risk approach can result in EQS values within the natural background range. Muysen and Janssen (2005) have summarised the implications of this for Zn: '...toxicity test results obtained with organisms cultured in media containing Zn concentrations outside the optimal range must be evaluated carefully before using them for risk assessments and derivation of water-quality criteria. Culture media containing no added Zn should be avoided.'

There is clearly a need to consider carefully the effects of organism acclimation and adaptation on toxic effects, both in the field and in the laboratory. In the field, acclimation/adaptation to a relatively high metal background would suggest that a relatively high added metal EQS may be acceptable. However, considerable uncertainties remain as to how organisms in a 'high background' system might respond to metal contamination; for example, it is possible that they may become more susceptible to other environmental stressors. There is also the issue that such ecosystems may well have special ecological and biodiversity value as a direct result of the metal background, and thus require more, rather than less, protection against contamination. Given the current level of knowledge on this subject, the current Added Risk approach, while appearing reasonable in principle, suffers from a lack of detailed knowledge and data against which its robustness may be tested.

The effects of culture conditions on organism sensitivity in laboratory tests has not been considered or studied greatly, yet the available information indicates that for essential metals it may be a crucial influence on sensitivity and hence toxic endpoints. Awareness of the issue has been growing and risk assessment methods for metals (e.g. International Council on Mining and Metals, 2007) are now making recommendations for ensuring that organisms used in tests with essential elements are not cultured in metal-deficient conditions.

The third and final assumption of the Added Risk approach, that a robust natural (or at least close to natural) background concentration can be estimated, is probably the most important from the point of view of implementation. As noted in Section A1.2.3, a number of methods have been proposed for estimating background concentrations. From a purely practical point of view, these all have advantages and disadvantages. Particularly obvious is the need as far as possible for robust validation of whichever method or methods were chosen, implying further (possibly extensive) research prior to application.

A1.3 Chemical speciation and bioavailability

A1.3.1 The importance of chemical speciation and biouptake in cationic metal toxicity

Cationic metals can exist in a number of forms in freshwaters; dissolved, and associated with colloidal and particulate material either in 'reactive' or 'non-reactive' forms. 'Reactive' here means metals that can potentially exchange with the solution on a short timescale (e.g. metal bound to surface functional groups of colloids or particulate material); 'non-reactive' means metal that cannot exchange on a short timescale (e.g. metal found within the lattices of particulate minerals). (This phase may also be considered to include anthropogenically discharged metal that is in a chemically inert form.) The chemical speciation of the metal is its distribution among these different phases and among different dissolved forms. Since the early 1970s, evidence has been accumulating for the role of metal speciation in controlling toxicity to aquatic organisms, including effects on algal growth (e.g. Lewis *et al.*, 1972; Allen *et al.*, 1980), invertebrate mortality (e.g. Daly *et al.*, 1990), and fish mortality (e.g. Howarth and Sprague, 1978; Cusimano *et al.*, 1986). The key piece of evidence from these studies was that the toxicity of the metal was proportional to the activity of the free ion (the uncomplexed form of the metal) rather than to the total or dissolved metal, if other chemical conditions were held constant. The Free Ion Activity Model (FIAM) explains this finding theoretically by postulating that toxicity is caused by rapid metal binding to a receptor site on the organism (e.g. on the surface of a fish gill or on the cell wall of an alga) followed by slow transfer into the organism to the site of toxic action (internalisation). The main receptor-binding metal species is considered to be the free metal ion, although the theory does not preclude the binding of complexed forms, the key concept being that not all forms of dissolved metal can interact with the receptor. The key aspect of the theory is that, since internalisation is slow in relation to binding to the receptor site, then, under controlled conditions where organisms are exposed to a constant concentration of the metal for a given time, the effect is proportional to the amount of metal bound to the receptor site. Under constant chemical conditions, the receptor-bound metal is proportional to the free ion activity, so long as the proportion of receptor sites that binds the metal is small compared to the total number of receptor sites. A full description and critique of the FIAM is given by Campbell (1995).

FIAM theory also allows for the binding of other dissolved metals, including major solution ions such as sodium and calcium, at the same receptor. Thus, variations in the activities of these major ions can modify the toxic effect. Pagenkopf (1983) used FIAM theory to develop a Gill Surface Interaction Model (GSIM) describing the effects of varying water chemistry (pH and hardness) on metal binding at receptor sites on fish gills. Since then, much work on developing the theory of the Biotic Ligand Model has focused on fish with emphasis on the effects of metals on the disruption of ionoregulation by binding to ion channels on the gill surface. Playle and co-workers (Playle *et al.*, 1993; Janes and Playle, 1995) and Macrae and co-workers (Macrae, 1994; Macrae *et al.*, 1999) showed that toxic effects of metals could be related to the amount bound to the gill surface, and Playle's work developed the use of chemical speciation modelling to predict the amount of gill-bound metal as a function of the exposure water chemistry. This work eventually developed into the Biotic Ligand Model, that in principle predicts the toxicity of a water of given chemical composition given a set of binding constants (analogous to equilibrium constants for metal–ligand binding in water) for the toxic metal, and for major cations that can also bind to the receptor (Di Toro *et al.*, 2001). The BLM will be discussed in more detail in the next section.

In considering the inclusion of chemistry effects in EQS derivation we shall first summarise the current state of knowledge with respect to available data and models (both the BLM and empirical models) and consider current and/or proposed methods for incorporating chemistry effects into EQSs and equivalent.

A1.3.2 The Biotic Ligand Model: application to Ni, Cu, Zn, Cd and Pb toxicity to freshwater organisms

The conceptual structure of the BLM is given in Figure A1.4. Equilibrium chemical speciation of the toxic metal and major ions, including the binding of the toxic metals and other cations (including H^+) to natural organic matter, is done using a version of the CHESSE chemical speciation model (Santore and Driscoll, 1995) that for organic matter complexation includes the algorithm of Humic Ion-Binding Model V (Tipping and Hurley, 1992), a multi-site complexation model for the binding of protons and metals. The binding of the toxic metal and competing solution cations (e.g. H^+ , Na^+ , Mg^{2+} , K^+ , Ca^{2+}) to the biotic ligand (BL) is modelled in a manner analogous to that done for binding to a solution ligand such as EDTA. In the available version of the BLM, only 1:1 reactions between the binding ion and the BL are allowed, such as $Cu^{2+} + BL \rightarrow Cu-BL$, although does not necessarily preclude the possibility of allowing for more complex interactions, as has been proposed by Borgmann *et al.* (2005). It is also worth noting, as shown in Figure A1.4, that the BLM can be adapted to allow toxic metal species other than the free ion to bind. The key assumption of the FIAM that internalisation of toxic metal following binding to the BL is a relatively slow process is also central to the BLM; as it follows from this that variations in the amount of metal bound to the BL relate to variations in toxic effect.

Application of the BLM to describing the variation in toxicity of a metal under varying water chemistry consists of measuring or estimating the concentration of BL-bound metal corresponding to a given toxic effect. For example, the concentration of gill-bound metal resulting in the mortality of 50% of fish in an acute test is termed the LA (lethal accumulation)₅₀. Constants for the association of the toxic metal and competing cations are then calculated by fitting the BLM to the results of toxicity tests (e.g. LC50 values) done in waters of varying chemical composition. Then, for other water chemistries, the solution metal concentration corresponding to the LA50 (or other endpoint) can be found, firstly by back-calculating from the LA50 (or other endpoint) to the free metal ion using the constants for ion-BL binding, then by using the solution speciation model component to calculate the dissolved toxic metal from the free ion. This concentration of dissolved metal is, in our example, the estimated LC50 for the water composition in question. Thus the BLM in principle allows the toxicity endpoint for a test done in a particular water composition to be translated to other water compositions. Currently, the BLM will only predict the toxic dissolved metal concentration although the chemical speciation component could be extended to consider binding of metals to suspended particulate matter.

Table A1.1 lists currently developed BLMs and species-specific empirical toxicity models published in the peer-reviewed literature. Niyogi and Wood (2004) provide a comprehensive overview of the state of BLM development for all the metals considered here. In addition to the studies presented here, currently unpublished work has been done to develop BLMs for cadmium, and work continues to develop a chronic BLM for nickel.

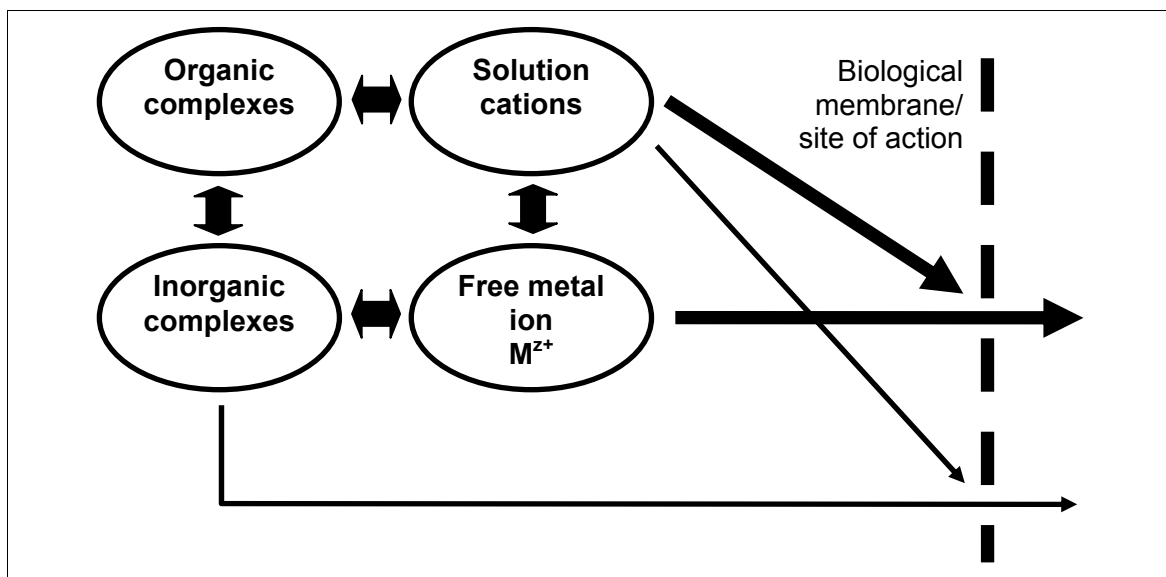


Figure A1.4 Conceptual structure of the Biotic Ligand Model. Double arrows represent chemical equilibrium. Solution cations include H^+ and other cation including Na^+ , Mg^{2+} , K^+ and Ca^{2+} , depending upon the organism and toxic effect type (i.e. acute or chronic effects). Single arrows represent binding of chemical species to the biological membrane or site of action.

A1.3.3 Empirical bioavailability models for individual aquatic species

Parallel to the development of BLMs for freshwater species, some authors have proposed empirical models to relate single organism toxicity to water chemistry, for certain organisms where further work is currently needed to allow full BLM development. De Schamphelaere and co-workers have proposed empirical expressions relating the effects of Cu and Zn on growth of the alga *Pseudokirchneriella subcapitata* to exposure water pH (De Schamphelaere *et al.* (2003, 2005b). Application of the current BLM algorithm to this organism was not successful, probably due to the presence of different types of binding site for the metals on algal surfaces. However, empirical relationships of metal effect concentration (as the free ion activities) and pH were highly significant:

$$\log(EC50_{Cu^{2+}}) = -1.431pH + 2.050 \quad (R^2 = 0.95); \quad (A1.3)$$

$$\log(EC10_{Cu^{2+}}) = -1.140pH - 0.812 \quad (R^2 = 0.91); \quad (A1.4)$$

$$\log(EC50_{Zn^{2+}}) = -0.652pH - 1.197 \quad (R^2 = 0.86); \quad (A1.5)$$

$$\log(EC10_{Zn^{2+}}) = -0.754pH - 1.294 \quad (R^2 = 0.96). \quad (A1.6)$$

De Schamphelaere and Janssen (2006) have extended this approach to a number of other freshwater algae. Borgmann and co-workers have investigated the toxicity of copper *Hyalella azteca* (a freshwater invertebrate) using empirical models consistent with the BLM algorithm, but allowing for a more flexible approach to the description of ion binding at the BLM.

Table A1.1 Peer-reviewed literature papers describing the development of BLMs and empirical species-specific toxicity models.

Type of model	Metal	Organism	Test type	Reference
BLM	Ni	<i>Ceriodaphnia dubia</i> (cladoceran)	acute	Keithly <i>et al.</i> (2004)
BLM	Ni	<i>Hyalella azteca</i> (scud)	acute	Keithly <i>et al.</i> (2004)
BLM	Cu	<i>Hyalella azteca</i> (scud)	acute	Borgmann <i>et al.</i> (2005)
BLM	Cu	<i>Daphnia pulex</i> (water flea)	acute	Santore <i>et al.</i> (2001)
BLM	Cu	<i>Pimephales promelas</i> (fathead minnow)	acute	Santore <i>et al.</i> (2001)
BLM	Cu	<i>Daphnia magna</i> (water flea)	acute	De Schamphelaere and Janssen (2002), De Schamphelaere <i>et al.</i> (2002)
BLM	Pb	<i>Oncorhynchus mykiss</i> (rainbow trout)	acute	MacDonald <i>et al.</i> (2002)
BLM	Zn	<i>Daphnia magna</i> (water flea)	acute	Heijerick <i>et al.</i> (2002), Santore <i>et al.</i> (2002)
BLM	Zn	<i>Oncorhynchus mykiss</i> (rainbow trout)	acute	Santore <i>et al.</i> (2002)
BLM	Zn	<i>Pimephales promelas</i> (fathead minnow)	acute	Santore <i>et al.</i> (2002)
BLM	Cu	<i>Daphnia magna</i> (water flea)	chronic	De Schamphelaere and Janseen (2004a)
BLM	Cu	<i>Brachionus calyciflorus</i> (rotifer)	chronic	De Schamphelaere <i>et al.</i> (2005a)
BLM	Zn	<i>Oncorhynchus mykiss</i> (rainbow trout)	chronic	De Schamphelaere and Janssen (2004b)
BLM	Cu	<i>Daphnia magna</i> (water flea)	chronic	De Schamphelaere and Janssen (2004c)
Empirical	Cu	<i>Pseudokirchneriella subcapitata</i> (alga)	chronic	De Schamphelaere <i>et al.</i> (2003)
Empirical	Cu	<i>Chlorella vulgaris</i>	chronic	De Schamphelaere and Janssen (2006)
Empirical	Cu	<i>Chlamydomonas reinhardtii</i>	chronic	De Schamphelaere and Janssen (2006)
Empirical	Cu	<i>Scenedesmus quadricauda</i>	chronic	De Schamphelaere and Janssen (2006)
Empirical	Zn	<i>Pseudokirchneriella subcapitata</i> (alga)	chronic	De Schamphelaere <i>et al.</i> (2005b)

While not explicitly making the mechanistic link between toxic effect and the binding of the toxic metal at the biotic ligand, these models are equally useful in predicting toxic effects on the organisms concerned within the range of chemistry to which they have been parameterised.

A1.3.4 The free ion approach and its application to freshwater toxicity

The free ion approach (Lofts *et al.*, 2004) was developed as a means of incorporating chemical influences on toxicity to existing soil ecotoxicology test data, thus allowing spatial variability in soil chemistry to be included in ecological risk assessment of metals. The approach is empirical and draws upon theory gained in the study of chemical effects on freshwater organism toxicity. The main points of the theory are:

Many soil organisms are exposed to toxic metals primarily via direct uptake from the soil solution.

Empirically, the effects of soil chemistry can be expressed as a function of the free metal ion concentration in the exposure solution, and the concentrations of the free ionic forms of 'protective' cations (H^+ , Ca^{2+} , Na^+ , etc.).

Concentrations of H^+ and other 'protective' cations in soil solution should co-vary across different soils (since the soil pH is controlled by the concentrations of major ions in the soil solution). Therefore the chemistry–toxicity relationship may be expressed solely as a function of H^+ :

$$\log(EC)_{Mz^+} = a \cdot pH_{ss} + b \quad (A1.7)$$

where $(EC)_{Mz^+}$ is the concentration of free metal ion exerting a given level of toxic effect upon the organism, pH_{ss} is the soil solution pH and a and b are empirical constants.

Currently, toxicity data for individual soil species across different soil types are not sufficient to allow parameterisation of equation A1.7. Therefore, Lofts *et al.* (2004) applied the theory to lumped ecotoxicity data comprising NOEC and EC10 endpoints for chronic effects on soil invertebrates, plants and microbial processes. Free metal concentrations at the endpoints were calculated using the empirical relationship of the free ion against the key soil properties pH, % organic matter and the 'reactive' soil metal concentration. Equation A1.7 was applied to the whole dataset for each metal to derive global values of a and b by regression. The species sensitivity distribution approach was then applied to the residuals of the regression to obtain a 95th percentile, ϵ . This was then used to calculate a function for the critical limits of the metals:

$$\log(EC)_{Mz^+, CRIT} = a \cdot pH_{ss} + b + \epsilon \quad (A1.8)$$

The approach therefore combines the use of a species sensitivity distribution with theoretical considerations of the effects of chemistry on toxicity. It has the advantage of resulting in a simple equation for the critical level of metal requiring knowledge of only one field variable, namely the soil solution pH.

The proposal that these critical limit functions (CLFs) for soils be also applied to surface waters is based on the comparison of free ion–pH relationships (Figure A1.5).

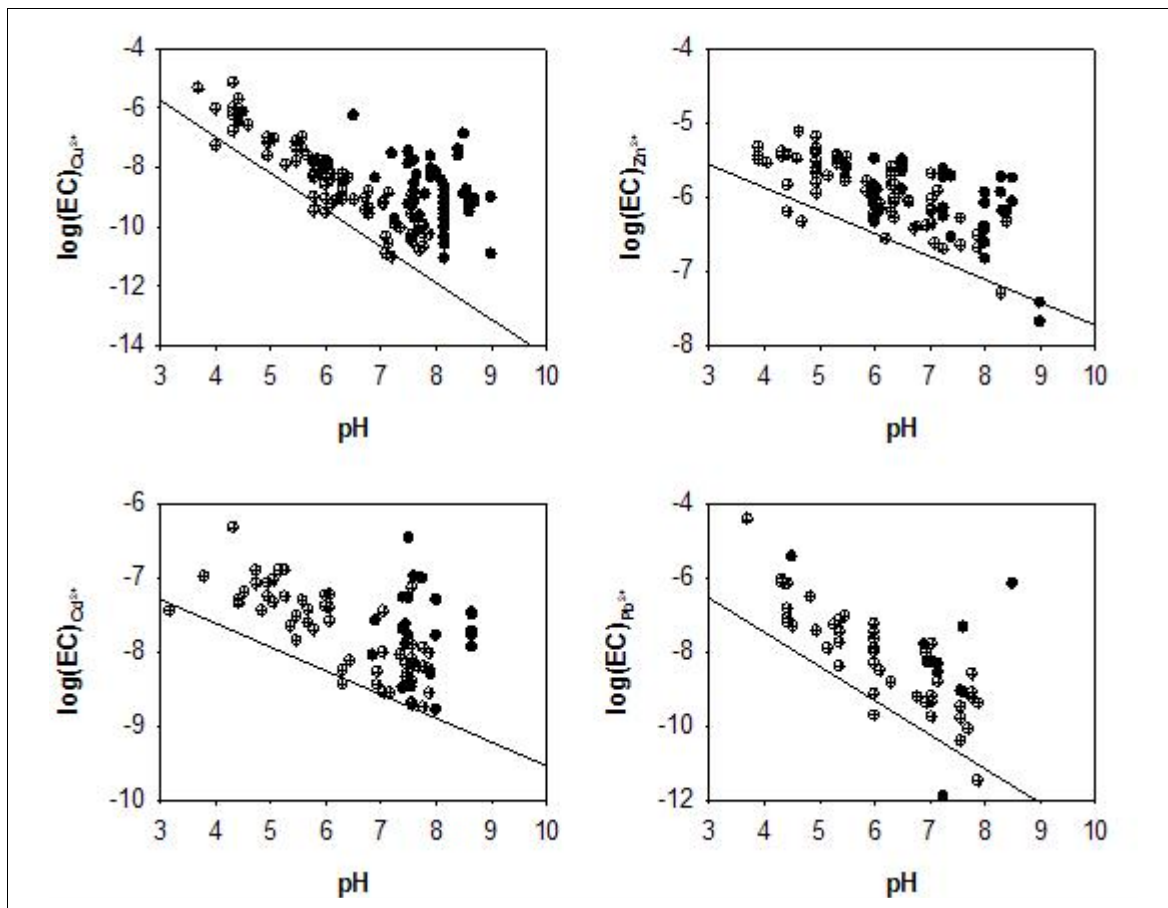


Figure A1.5 Comparison of terrestrial and aquatic toxicity data for Cu, Zn, Cd and Pb, shown as the free metal ion concentration at the toxic endpoint (NOEC or EC10) plotted against pH in soil solution or test water. Crossed circles are terrestrial toxicity data and the solid lines are the critical limit functions calculated from these. The solid circles are the aquatic toxicity data.

A1.3.5 Current/proposed uses of speciation-based approaches in EQS setting and risk assessment

USA

Much of the BLM work in the USA has had as its goal the development of a methodology to calculate site-specific Water Quality Criteria (WQCs). The Clean Water Act requires the US Environmental Protection Agency (US EPA) to recommend WQCs for the protection of aquatic life in surface waters, and to provide guidance to States and Tribes for the implementation of WQCs. Protection of aquatic organisms and their uses is defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally and other important species; (2) fish and benthic invertebrate assemblages in rivers and streams; (3) fish, benthic invertebrates and zooplankton assemblages in lakes, reservoirs, estuaries and oceans. Each WQC is expressed as two values. These values represent the short-term and continuous permitted maxima in concentration and are termed the Criterion Continuous Concentration (CCC) and Criterion Maximum Concentration (CMC).

The steps in calculating WQCs can be briefly summarised as follows:

1. Collation of suitable toxicity data. The approach is noteworthy in that plant toxicity data (e.g. for algae) are not considered here; instead, they are considered separately and expert judgement is used to determine whether the CMC and CCC are protective.
2. Calculation of Species Mean Acute Values and Genus Mean Acute Values from acute toxicity data.
3. Derivation of a Final Acute Value from the Genus Mean Acute Values.
4. Derivation of the CMC from the Final Acute Value.
5. Calculation of Species Mean Chronic Values, Genus Mean Chronic Values, a Final Chronic Value and a CCC as for acute data in steps 1–4 above, if sufficient suitable chronic data are available.
6. If sufficient suitable chronic data are not available, calculation of the CCC may be done by calculating a Final Acute–Chronic Ratio from comparison of acute and chronic data for the same species, and using it to calculate the Final Chronic Value and the CCC from the Final Acute Value.
7. If sufficient evidence for dependence of toxicity on one or more water quality parameters is available, and the available data allow it, the CMC and/or CCC may be expressed as functions of this parameter or parameters.

Currently, both CMCs and CCCs for Ni, Cu, Zn, Cd and Pb may be expressed as hardness functions. Alternatively, the US EPA allows for the water-effect ratio (WER) methodology (Carlson *et al.*, 1984) to be used to calculate site-specific WQCs. The WER approach involves testing at least two sensitive aquatic species in the site water and in standard laboratory water. The WER is the ratio of the toxic endpoint in site water to that in laboratory water. A site-specific WQC is obtained by multiplying the generic CMC and/or CCC by the WER. Acute toxic endpoints are considered suitable for modifying either the CMC or the CCC, although the option of conducting chronic testing to modify the CCC is allowed. In practice it may be necessary to calculate a number of individual WERs to cover seasonality and flow variations; these are summarised into a final WER for calculation purposes.

WERs are useful for modifying generic WQCs, particularly for metals where hardness–toxicity relationships are available and the generic WQC can be modified to the hardness of the site water. Welsh *et al.* (2000) have reviewed the derivation of WERs and indicated several areas where the methodology requires attention: the consideration of total hardness as a modifying factor not the separate effects of Ca and Mg; the effects of alkalinity and pH; the influence of organism acclimation; the small number of species required to be tested; the effects of multiple metals in the site water; the use of WERs from acute data for correcting the CCC; and the incorporation of spatial and temporal variability in WERs. These are all issues that can in principle be addressed by updating the methodology. But the generic drawback of WERs is the need to conduct time-consuming and expensive laboratory tests. Originally the method was intended for use only in exceptional circumstances; however, when its use became widespread its cumbersome nature became obvious (Paquin *et al.*, 2002). Use of the BLM is intended as a versatile and robust alternative to WER calculations (US EPA, 2000).

US EPA is currently reviewing its approach to developing WQCs for metals. The review process has been done for copper and has resulted in guidelines for incorporating the BLM into WQC derivation (specifically the CMC) for this metal (US EPA, 2007). The document proposes updates of the recommended WQCs for copper to include a

consideration of site-specific variability in acute toxicity based around the Biotic Ligand Model. The document gives the advantages of this approach over empirical relationships of toxicity to water hardness as being:

- an explicit accounting for individual water quality variables;
- no link to particular correlations among water quality variables;
- the ability to address variables not accounted for in the hardness–toxicity relationship.

The use of the Biotic Ligand Model in considering acute toxicity and the derivation of the CMC entailed some significant changes to previous data acceptability and screening procedures. In particular, requirements for water chemistry parameters are more stringent due to the requirements of the BLM. Some tests previously considered suitable are no longer so due to a lack of sufficiently detailed water chemistry. Some previously considered unsuitable due to extreme water chemistry (e.g. high DOC concentrations in the test waters) are now considered acceptable.

An important feature of the recommended process is that the BLM parameters are taken to be invariant for different organisms. The actual parameters are those based on the results of acute toxicity studies using fathead minnows (*Pimephales promelas*), which have been shown to adequately predict toxicity of Cu to other organisms.

The steps for calculating a WQC are essentially the same as those given above; the concepts of Species and Genus Mean Acute (Chronic) Values and the Final Acute (Chronic) Value are retained. The steps to calculate the Final Acute Value are as follows:

1. Water chemistry information for the available toxicity data is collated and suitable data selected.
2. The endpoint Cu concentration is converted from total recoverable to dissolved if necessary, using a conversion factor.
3. The reported L(E)C50 and water chemistry for each suitable toxicity test are input to the BLM to calculate the BL-bound Cu at the endpoint (the L(E)A50).
4. The L(E)C50 at a standard water composition (Table A1.2) is calculated, by predicting the dissolved copper concentration in equilibrium with this BL-bound amount.
5. The Final Acute Value is calculated for the standard water composition using the normalised toxicity data, following steps 2 and 3 above.
6. The L(E)A50 corresponding to the standard water Final Acute Value is calculated using the BLM. This criterion is termed the L(E)A₅₀ and is the single invariant toxicity parameter across different water chemistries.
7. Site-specific Final Acute Values are calculated as the dissolved copper in equilibrium with the criterion L(E)A₅₀. The site-specific CMC is set to half the Final Acute Value.

Chronic toxicity data were calculated as EC₂₀ values. The chronic toxicity dataset was not considered sufficient for direct calculation of a Final Chronic Value. Therefore an Acute–Chronic Ratio was calculated and this is used to calculate a site-specific Final Chronic Value from the site-specific Final Acute Value. The site-specific CCC is equal to the Final Chronic Value.

Table A1.2 Standard test water composition for BLM-normalisation of acute Cu toxicity data.

Temp	pH	DOC	Na	Mg	K	Ca	Cl	SO ₄	HCO ₃	S
20.0	7.50	0.5	26.3	12.1	2.1	14.0	81.4	1.9	65	0.0003

Notes: Temperature in °C, DOC and solution ions in mg l⁻¹, DOC modelled as 90% fulvic acid and 10% humic acid.

European Union risk assessments

European Union Council Regulation (EEC) no. 793/93 requires the assessment of risk for humans and environment of certain Priority Substances, using risk assessment principles laid down in Commission Regulation no. 1488/94 (risk assessment of existing substances). The framework for carrying out the assessment is laid out in a Technical Guidance Document (TGD) (European Commission, 1996) published in 1996. The assessment entails a series of actions:

1. Assessment of the adverse effects that a substance has an intrinsic likelihood to cause;
2. Assessment of exposure, resulting in derivation of a Predicted Environmental Concentration (PEC).
3. Assessment of effects, resulting in derivation of a Predicted No Effect Concentration (PNEC).
4. Characterisation of the incidence and severity of likely adverse effects due to exposure.

Here we are interested in the effects assessment (PNEC derivation) for the aquatic compartment. The TGD lays out guidelines for the selection of information to be gathered for PNEC derivation, and the methods to be used to derive it. The PNEC is analogous to the Final Chronic Value in the US approach (Section A1.4.2), and is derived in a broadly similar way, from analysis of single species toxicity tests. The use of safety extrapolation factors is stated by the TGD to be the preferred approach, with the use of statistical extrapolation (e.g. a species sensitivity distribution) recommended as a supplementary approach. The TGD makes mention of accounting for metal bioavailability in the aquatic compartment, with the recommendation that the PNEC be calculated at the same level of availability as the PEC (e.g. as dissolved metal). No further recommendations are made regarding the use of speciation in determining bioavailability; indeed the TGD states that this is not possible. Since the publication of the TGD, however, scientific developments have made incorporation of speciation and bioavailability more feasible and this is proposed in some assessments for metals that are currently in development.

Environmental risk assessments for forms of Ni, Cu, Zn, Cd and Pb are currently [July 2007] in various stages of development. The most developed assessments from the point of view of bioavailability incorporation are those for Cu, Zn and Cd; these will be considered first.

Copper

Consideration of the effects of copper speciation on its toxicity is thoroughly covered in the copper risk assessment. It is proposed that models describing these effects on

specific organisms be used in the assessment. The proposed methodology is as follows:

1. NOEC data were collected, assessed and selected according to the guidelines in the EU Technical Guidance Document for Risk Assessment. In the latest version of the draft report [dated July 2007] the Total Risk approach only is applied.
2. For the generic case (large-scale risk assessment), two scenarios were considered, 'reasonable worst case' and 'typical'. Reasonable worst case refers to chemical conditions of high bioavailability, typical refers to median chemical conditions of EU waters. For each scenario, the individual NOECs were normalised accordingly. Normalisation was done using two models: for invertebrates a chronic BLM for *Daphnia magna* (De Schamphelaere and Janssen, 2004) was used, for algae an empirical model for *Pseudokirchneriella subcapitata* (De Schamphelaere *et al.*, 2003), and for fish a chronic BLM based on data for *Pimephales promelas* and *Oncorhynchus mykiss*. NOECs were normalised to the chosen conditions, aggregated by taking species geometric means, and PNECs were calculated by statistical extrapolation using the best fit from a large number of tested statistical distributions.
3. It was suggested that for site-specific risk assessment, normalisation to a site-specific chemistry could be carried out on the same principles outlined in Step 2.

The power of this approach was shown by the reduction in the variability of NOECs for the same species after normalisation to standard water chemistry. Of 13 species (four invertebrate, six fish and three algae) with multiple NOECs in the test database, normalisation reduced the inter-test variability by up to 83%, and variability was reduced by 40% or more in nine cases. Only for one species, *Onchrrhynchus kitusch* (coho salmon), did inter-test variability increase.

Zinc

The inclusion of bioavailability considerations in the draft zinc assessment is based on the recent development of chronic toxicity models for key species representing their species groups: *Oncorhynchus mykiss* (rainbow trout) for fish (Macrae *et al.*, 1999) *Daphnia magna* for invertebrates and *Pseudokirchneriella subcapitata* for algae and higher plants (De Schamphelaere *et al.*, 2005b). The first two examples are BLMs while the algal model is empirical. The methodology for considering bioavailability effects differs from that for copper:

1. NOEC data were collected, assessed and selected according to the guidelines in the EU Technical Guidance Document for Risk Assessment (European Commission, 1996). Added risk only was considered, so only tests for which the added zinc NOEC (NOEC_{add}) could be found or estimated, were used. Only tests with water composition within defined ranges were accepted: pH 6–9, hardness 24–250 mg l⁻¹ as CaCO₃, background zinc > 1 µg l⁻¹. Criteria for the estimation of background zinc.
2. Species mean NOEC_{add}s were calculated and used to work out the PNEC_{add} by statistical extrapolation. An assessment factor of two was applied to the HC5 calculated by statistical extrapolation in order to give the PNEC_{add}.
3. Consideration of bioavailability was done by a correction of the exposure concentration, by use of bioavailability factors calculated by applying the three

models noted above to both a reasonable worst case scenario and a typical scenario for the region being assessed.

Cadmium

Correction for speciation/bioavailability effects on toxicity was not considered for cadmium due to the lack of any models for chronic exposure. No bioavailability correction was therefore applied in calculating the generic PNEC. For regional PNECs, correction for water hardness was done as follows:

1. Following the procedure done by the US EPA to derive a hardness–toxicity relationship for cadmium, hardness–toxicity relationships for *Daphnia magna*, *Salmo trutta* (brown trout) and *Pimephales promelas* (fathead minnow) were quantified and used to generate an expression for relating the NOEC in the hardness range 44–209 mg CaCO₃ l⁻¹ to a hardness of 50 mg CaCO₃ l⁻¹:

$$\text{NOEC}_{50} = \text{NOECH} \cdot (50/H)^{0.7409} \quad (\text{A1.9})$$

where H is the hardness of the test water in mg CaCO₃ l⁻¹.

2. All NOECs were corrected to a hardness of 50 mg CaCO₃ l⁻¹ using equation A1.9.
3. The HC₅ for this reference hardness was calculated by dividing by an assessment factor of two to give a PNEC for the reference hardness of 0.09 µg l⁻¹.
4. The expression for the PNEC at a hardness H is then

$$\text{PNEC}_H = 0.09 \cdot (H/50)^{0.7409} \quad (\text{A1.10})$$

It was proposed that this expression was not used to correct to hardness values below 40 mg CaCO₃ l⁻¹ due to the hardness range on which it was effectively parameterised. The PNEC₄₀ was 0.08 µg l⁻¹. On the basis that (a) NOECs from tests with softer water were all greater than 0.08 µg l⁻¹, and (b) multispecies studies in soft water lakes found adverse effects at about 0.20 µg l⁻¹, it was concluded that the PNEC₄₀ was protective of softer waters down to a hardness of about 10 mg CaCO₃ l⁻¹. However, no data were available for very soft waters below 10 mg CaCO₃ l⁻¹ hardness and it was admitted that the PNEC₄₀ might not prove protective for such environments.

Lead

The final draft assessment [December 2004] concluded that there was no current basis for incorporating bioavailability consideration into derivation of PNECs for lead, given the lack (at the time) of suitable data (including chronic BLMs) for bioavailability normalisation of NOECs.

Nickel

The effects assessment for nickel is currently ongoing. Bioavailability will be considered in setting PNECs. Biotic Ligand Models have been developed (e.g. Deleebeeck *et al.*, 2007) for this purpose.

A1.4 Current methods for the derivation of EQS values

A1.4.1 United Kingdom

Policy background

Definitions of an Environmental Quality Objective (EQO) and an Environmental Quality Standard (EQS) under the UK system are given as follows:

EQO: The requirement that a body of water should be suitable for those uses identified by the controlling authority.

EQS: That concentration of a substance which must not be exceeded if a specified use of the aquatic environment is to be maintained.

The Dangerous Substances Directive (76/464/EEC) laid down measures for the control of the discharge of designated dangerous substances to waters, including for the purposes of protection of the aquatic environment. Dangerous Substances were listed in two groups, List I and II:

List I: pollutants whose discharge to the environment should be eliminated.

List II: pollutants whose discharge to the environment should be reduced.

The Directive made provision for future directives setting limiting concentrations of List I substances in industrial discharges, and for setting EQOs for surface waters receiving discharges of List I and/or List II substances. The UK has opted to control both List I and List II substances by the setting of EQOs.

The Freshwater Fish Directive (78/659/EEC) specified 95th percentile EQO values for Cu and Zn in designated salmonid and cyprinid waters. There is therefore a requirement upon the UK that national-set EQS values for these waters be no higher than set out in the Directive.

Cd and its compounds are designated as List I substances. EQOs for cadmium in surface freshwaters are set out in a Directive on limit values and quality objectives for cadmium discharges (83/513/EEC).

Ni, Cu, Zn and Pb, and their compounds, are designated as List II substances. Recommended EQS values for these metals in surface waters were given in a series of reports to the Department of the Environment in August 1984 (Brown *et al.*, 1984; Mance and Yates, 1984a, 1984b; Mance *et al.*, 1984).

Derivation methodology

General

Two types of EQS may be defined:

- An annual average concentration EQS (AA), defined as the highest concentration to which an aquatic ecosystem may be exposed without any likely adverse effects.

- A maximum allowable concentration EQS (MAC), defined as the highest transient concentration that would be expected to cause adverse effects.

The MAC is designed to protect against short-term episodic pollution events, while the AA is designed to protect against long-term continuous pollution.

The process of deriving an EQS can be summarised in the following steps:

1. **Gather data** – from published texts and journals, commercial databases and unpublished data where available. Information on other aspects of the substance's behaviour (e.g. persistence, bioaccumulation) is also gathered as this will be evaluated alongside the toxicity data.
2. **Assess data** – evaluate quality and relevance (e.g. test procedures, appropriate species and endpoint).
3. **Select lowest reliable and relevant effect concentration** – this should be appropriate to whether the EQS is an AA or a MAC.
4. **Apply appropriate extrapolation factor to derive preliminary EQS** – the lowest reliable and relevant effect concentration is divided by an appropriate extrapolation factor. The extrapolation factor may be varied on the basis of the evaluation of persistence and bioaccumulation data.
5. **Compare with field data** – data on the responses of natural populations or outdoor mesocosms are reviewed against the preliminary EQS and the derivation is reviewed if anomalies are found.
6. **Recommend EQS** – on the basis of the previous steps.
7. **Peer review** – by the relevant government department (currently Defra), the regulatory authorities, and industry. Peer review may recommend a review of the proposed EQS including an assessment of any new data.
8. **Public consultation** – for statutory EQSs only. On the basis of the consultation the EQS may be reviewed as in Step 7.
9. **Adoption and monitoring**

If the quantity, quality and relevance of the available data are not considered sufficient to derive an EQS, a 'tentative' standard may be set as an alternative. In this case recommendations for expansion of the existing dataset may be made. Depending on the chemical behaviour of the substance, aquatic EQSs may be set as dissolved or total concentrations of the substance.

Data requirements

Toxicity test data are collected from the published scientific literature, commercial databases and unpublished sources. Data are critically assessed for:

1. The relevance of the tested species and of the studied effect.
2. The reliability of the test procedure used.
3. The establishment of a dose–response relationship in the test.

Data are categorised into two categories: primary data, for which the toxicity test is considered reliable and relevant, and secondary data, for which the provided details

are inadequate to perform the critical assessment. Primary data are used to derive the EQS while secondary data are used to provide supporting information.

Ideally the dataset should encompass both acute and chronic effects data for the following taxa:

1. Algae and/or macrophytes.
2. Arthropods (crustaceans, insects).
3. Non-arthropod invertebrates (e.g. molluscs).
4. Fish.

If data are available for other taxa this can be taken into account. The absence of any data for any one of these taxa would be considered significant in determining whether an EQS, as opposed to a tentative standard, could be set.

Standard derivation

As stated above, the gathered data are screened to find the lowest reliable and relevant effect concentration. While reliability is assessed during the critical evaluation of the data, relevance may be assessed against the intended purpose of the EQS, particularly whether it is an AA or an MAC. This concentration is then extrapolated by the use of a safety or extrapolation factor. The intended purpose of the extrapolation factor is to account for the uncertainty in translating a limited dataset of single-species toxicity test data to a standard intended to protect the entire ecosystem from harm. The application of safety factors is based in large degree upon scientific expert judgement; no fixed criteria exist for applying a given safety factor on the basis of factors such as number of data points. Some general guidance on safety factors is provided, but this is considered guidance which may be modified by expert judgement rather than a fixed scheme. If an adequate dataset, as defined in the previous section, is not available, then a larger extrapolation factor may be considered (e.g. a factor of 200 to 1000 may be considered for extrapolation of limited short-term acute data to no effect concentrations).

Chronic data are preferred over acute data for the derivation of both MAC and AA EQSs.

A1.4.2 USA

Policy background

The legal framework for management of the ecological state of surface waters in the USA is the Clean Water Act (1972, amended 1977). The Act established the basic structure for regulating discharges into surface waters. Water Quality Standards are the foundation of the water-quality-based pollution control programme mandated by the Clean Water Act. A Water Quality Standard has four aspects: it specifies appropriate uses of a water body to be achieved and protected (designated uses); it specifies Water Quality Criteria (WQC) for contaminants, appropriate to the designated uses; it specifies policies to prevent degradation of water quality; and it specifies general policies regarding the application of WQCs in relation to mixing zones, low flows and variance of WQCs.

The Clean Water Act requires the US EPA to recommend WQCs for the protection of aquatic life in surface waters, and to provide guidance to States and Tribes for the implementation of WQCs.

The current general guidelines for deriving WQCs date from 1985 and are described below. The derivation of the most recent version of WQCs for the individual metals will then be presented.

The 1985 Guidelines (Stephan et al., 1985)

This document describes the basic methodology for deriving national WQCs as recommendation to States and Tribes. Many basic aspects of the methodology presented are preserved in more recent and scientifically advanced guidelines. The Guidelines set out some points that can be listed as forming the basis for the method presented:

- Theoretically, the best method to derive a national WQC would be to conduct toxicity tests using a wide variety of unpolluted waters; however, testing of this scope is not practically feasible. The method described is intended to result in WQCs that would as far as possible provide the same level of protection as a WQC derived from the extensive testing procedure described above.
- Protection of all species at all times and in all places is not feasible or necessary; if acceptable toxicity data are available from an appropriate variety of taxonomic and functional groups, a reasonable level of protection will probably be provided if all except a small fraction of taxa are protected, unless a commercially or recreationally important species is very sensitive.
- Protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally and other important species; (2) fish and benthic invertebrate assemblages in rivers and streams; (3) fish, benthic invertebrates and zooplankton assemblages in lakes, reservoirs, estuaries and oceans.
- Effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.
- National criteria are intended to protect all or almost all bodies of water, in order to prevent the assumption that at the local level a concentration below a criterion indicates that no unacceptable effects will occur.
- Each derived WQC is expressed as two values. These values represent the short-term and continuous permitted maxima in concentration and are termed the Criterion Continuous Concentration (CCC) and Criterion Maximum Concentration (CMC).
- The CCC is intended to be a good estimate of the threshold of unacceptable effect on a commercially or recreationally important species. If maintained continuously, any concentration above the CCC is expected to cause an unacceptable effect. The concentration may exceed the CCC and not cause an unacceptable effect if the magnitude and duration of the exceedance is appropriately limited and if there are compensating periods of time for which the concentration is below the CCC. The higher the concentration is above the CCC the lower the time for which it can be acceptably tolerated. The most feasible way to determine exceedance of a CCC is to allow exceedance only if the variability around the CCC would be expected to have the same effect as continuous exposure to the CCC. Since fluctuating exposure concentrations

under laboratory conditions (over 20–30 days) tend to have greater adverse effects than a continuous exposure to an average concentration, a short averaging time is appropriate for the CCC. A period of four days is given, partly for the reasons stated above, and also in order to protect sensitive life stages of aquatic organisms.

- The averaging period for the CMC should similarly be short in comparison to the duration of the toxicity tests from which it is derived (typically 48–96 hours). A period of one hour is given.
- Since criteria are used to design waste treatment plants, which must operate on the basis of probability and cannot be designed to give a zero probability of exceeding a certain concentration of a waste substance in their output stream. Therefore it is appropriate to additionally build an element of exceedance probability into the criteria. This should be based upon the ability of an aquatic ecosystem to recover from the effects of exceedances. Since by definition such exceedances are due to the output of extreme concentrations of the material during the normal course of plant operations, they are expected to be mostly small. Most aquatic systems will probably recover from most exceedances within three years.

Data requirements

All available data on the substance under investigation concerning (1) its toxicity to, and bioaccumulation by, aquatic animals and plants; (2) US Food and Drug Administration Action Levels; (3) chronic feeding and long-term field studies on wildlife that regularly consume aquatic organisms, is first collected. Data should be accompanied by sufficient supporting information indicating that acceptable test procedures were used and that the results are probably reliable. Questionable data should not be used directly in the criterion derivation but may be used to provide supporting information. Neither tests on species not having reproducing wild populations in North America, nor tests on species previously exposed to substantial concentrations of the test substance or other contaminants, should be used.

Toxicity data requirements across the range of aquatic organisms are as follows; these requirements apply to both acute and chronic tests separately.

1. Acceptable tests with at least one species of freshwater animal in at least eight different families such that all the following are included:
 - a. a member of the family Salmonidae;
 - b. a member of a family in the class Osteichthyes (bony fishes) other than the Salmonidae, preferably a commercially or recreationally important warmwater species such as bluegill or channel catfish;
 - c. a third family in the phylum Chordata (fishes, amphibians, birds, reptiles, mammals);
 - d. a planktonic crustacean;
 - e. a benthic crustacean;
 - f. an insect;
 - g. a member of a family in a phylum not already represented (e.g. a rotifer, annelid or mollusc);

- h. a member of a family in any order of insect, or in any phylum not already represented.
2. Acute–chronic ratios in at least three different families provided that:
 - a. at least one species represented is a fish;
 - b. at least one species represented is an invertebrate;
 - c. at least one species represented is an acutely sensitive freshwater species.

The species can be saltwater or freshwater (provided condition c. is kept).

3. Results of at least one acceptable test with a freshwater alga or vascular plant, together with the results of an acceptable test with a plant in another phylum, if plants are among the most sensitive organisms to the substance.
4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available.

Processing of acute data for animals

The determination of whether an acute toxicity test can be considered acceptable should cover the following points:

1. Tests must be conducted using acceptable procedures (e.g. American Society for Testing and Materials – ASTM, 2002, 2004).
2. Tests during which the organisms were fed should not be included, unless data indicate that the food did not affect the toxicity of the test substance.
3. Tests conducted in unusual dilution water (e.g. where distilled or deionised water was used, or where particulate matter or dissolved organic carbon exceeded 5 mg l^{-1}) should not be used unless the data show that the presence of material such as particulates did not affect toxicity.
4. Tests must be based on endpoints covering severe acute adverse impacts of the test substance. Specifically, for daphnids and other cladocerans tests should be initiated with organisms less than 24 hours old. For midges, tests should be started with second- and third-instar larvae. The result should be the 48-hour EC50 based on the percentage of organisms killed or immobilised. If such an EC50 is not available then the 48-hour LC50 should be used if available. An EC50 or LC50 of longer than 48 hours may be used if the animals were not fed and control effects were acceptable at the end of the test.
5. Tests with the embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp and abalone should be the 96-hour EC50 based on the percentage of organisms killed or with incompletely developed shells. If such an EC50 is not available then the lower of the 96-hour EC50 for the percentage of organisms with incompletely developed shells or the 96-hour LC50, should be used. Endpoints from tests of duration between 48 and 96 hours may be used if none of the above endpoints are available.

6. Tests on all other organisms, including tests on old life stages of the organisms listed in ii., should be the 96-hour EC50 based on the organisms killed, immobilised or suffering loss of equilibrium. If such an EC50 is not available then the 96-hour LC50 may be used if available.
7. Tests on single-celled organisms are never considered to be acute regardless of duration.
8. Acute values reported as 'greater than' and those which exceed the solubility of the test material should not be used.

Appropriate measures of the acute toxicity of the substance are used to determine the Final Acute Value (FAV). Several points regarding the derivation are made:

1. If the acute toxicity of the substance is dependent upon a water quality characteristic such as hardness or particulate matter concentration then this dependence should be accounted for by derivation of a Final Acute Equation (see later).
2. Where acute data for two or more life stages of the organism are available and it is indicated that one or more of these life stages is at least a factor of two more resistant to the substances than the other life stages, then that data should not be used.
3. Where multiple acceptable endpoints exist for the same species, outliers should be rejected if they are considered to be suspect in comparison with the other values.

For each species for which at least one acute value is available, a Species Mean Acute Value (SMAV) is calculated as the geometric mean of the results of flow-through tests where the concentration of the substance was measured during the test. If no such results are available for a particular species then the SMAV should be calculated as the geometric mean of all tests. Then, for all genera for which multiple SMAVs are available, the Genus Mean Acute Value (GMAV) should be calculated as the geometric mean of the SMAVs for species within that genus.

The cumulative probability of the ranked GMAVs or GMCVs is calculated and the four GMAVs or GMCVs with cumulative probabilities closest to 0.05 are taken. Then the FAV or FCV is calculated using the following sequence of equations:

$$S^2 = [\Sigma(\ln \text{GMV})^2 - ((\Sigma \ln \text{GMV})^2 / 4)] / [\Sigma p - ((\Sigma p^{0.5})^2 / 4)] \quad (\text{A1.11})$$

$$L = (\Sigma \ln \text{GAV} - S(\Sigma p^{0.5})) / 4 \quad (\text{A1.12})$$

$$A = S(0.05^{0.5}) + L \quad (\text{A1.13})$$

$$\text{FV} = e^A \quad (\text{A1.14})$$

where GMV represents the GMAVs or GMCVs and p represents the cumulative probability.

If a commercially or recreationally sensitive species has a geometric mean of acute values from flow-through tests that is lower than the calculated FAV, then this geometric mean is used as the FAV.

Processing of chronic data for animals

The Final Chronic Value (FCV), from which the Criterion Continuous Concentration (CCC) is derived, is obtained either by calculating a Final Acute–Chronic Ratio (FACR)

and dividing the FAV by it, or in the same manner as the FAV (i.e. based upon toxicity data), depending upon the amount of relevant acceptable toxicity data available.

There are several criteria that a chronic test must conform to, to be considered an acceptable source of data:

1. Chronic Values must be based upon the results of flow-through tests during which the concentration of the test substance was properly measured at regular intervals throughout the test. For daphnids, renewal tests are acceptable.
2. A test must have acceptably high control survival, growth or reproduction; the limits of acceptability depend upon the test species.
3. Tests conducted in unusual dilution water, for example, where distilled or deionised water was used, or where particulate matter or dissolved organic carbon exceeded 5 mg l^{-1} , should not be used, unless the data show that the presence of material such as particulates did not affect toxicity.
4. Tests must be based upon endpoints and exposure durations appropriate to the species. Acceptable types of test are:
 - a. Life-cycle tests involving exposure of two or more groups of individuals to different concentrations of the test substance throughout a life cycle. If the test organism is a fish, it should begin with embryos or hatched young less than 48 hours old and should end not less than 24 days after the hatching of the next generation (90 days for salmonids). If the test organism is a daphnid, it should begin with young less than 24 hours old and last for not less than 21 days. If the test organism is a mysid, it should begin with young less than 24 hours old and continue until seven days past the median time of first brood release in the control.
 - b. Partial life-cycle tests for fish involving exposure of two or more groups of individuals to different concentrations of the test substance through most portions of the life cycle. These tests are acceptable for fish species that require more than one year to reach sexual maturity. Exposure should begin with immature juveniles at least two months before active gonad development and should end not less than 24 days after the hatching of the next generation (90 days for salmonids).
 - c. Early life-stage toxicity tests for fish consisting of 28–32 day exposures of the early life stages from fertilisation through to early juvenile development. For salmonids the test duration should be 60 days post-hatch. Since early life-stage tests are intended as predictors of the results of full and partial life-cycle tests, if such tests are available as well as an early life-stage test then the latter should not be used. Neither should such tests be used if mortality or the incidence of abnormalities increased substantially towards the end of the test.
5. In life-cycle tests and partial life-cycle tests on fish, data should be given on survival and growth of adults and young, maturation of both sexes, eggs spawned per female and egg hatchability. For salmonids data on embryo viability should also be given. For daphnids, data should be given on survival and young per female. For mysids, data should be given on survival, growth and young per female. In early life-stage tests, data should be given on survival and growth.

A Chronic Value should be calculated from the results of each acceptable test. This value is calculated as the geometric mean of the lower and upper chronic limits. The

lower chronic limit is the highest tested concentration that did not cause an unacceptable adverse effect on any of the measured biological parameters, provided that no lower concentration(s) caused unacceptable effects. The upper chronic limit is the lowest tested concentration causing an unacceptable adverse effect, provided that no higher concentration(s) caused acceptable effects.

If the chronic toxicity of the substance is dependent upon a water quality characteristic such as hardness or particulate matter concentration then this dependence should be accounted for by derivation of a Final Chronic Equation (see later).

If Chronic Values can be obtained for species in eight families, a Species Mean Chronic Value (SMCV) for each species should be calculated as the geometric mean of all Chronic Values for that species. Genus Mean Chronic Values (GMCV) should then be calculated as the geometric mean of each SMCV relating to a species in the genus. The FCV is then calculated by statistical means as is described for the FAV in the previous section.

If sufficient Chronic Values are not available as described above, then the FCV must be estimated by the use of acute–chronic ratios. An acute–chronic ratio may be calculated for each Chronic Value by dividing the geometric mean of all acute values from flow-through tests on the species (static tests are acceptable for daphnids) by the Chronic Value. For fish, the acute tests must have been conducted with juveniles. In all cases the acute test must have been done in the same study as the chronic test. If no such tests are available then the results of tests done in the same laboratory and the same dilution water may be used. If neither type of test is available then results of tests done in a different laboratory but in the same dilution water may be used. Then for each species the Species Mean Acute–Chronic Ratio (SMACR) is calculated as the geometric mean of the available ratios.

The Final Acute–Chronic Ratio (FACR) is calculated in a manner dependent upon the data:

1. If the SMACR changes as the SMAV increases, the FACR is the geometric mean of the ratios for species with SMAVs close to the FAV.
2. If no major trend in SMACR is apparent and ratios for a number of species are within a factor of ten, the FACR is the geometric mean of all the SMACRs for both freshwater and saltwater species.
3. If the lowest available SMAVs were determined with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp or abalones then the FACR may be assumed to be two.
4. If the most appropriate SMACRs are less than two and particularly if they are less than one, acclimation has probably occurred during testing. In this situation an FACR of two should be assumed.

If the SMACRs do not fit any of these cases then an FACR should not be calculated.

The FCV is calculated by dividing the FAV by the FACR. If a commercially or recreationally sensitive species has a geometric mean of Chronic Values from flow-through tests that is lower than the calculated FCV, then this geometric mean should be used as the FCV.

Final Acute/Chronic Equation

As described above, where a relationship between acute or chronic toxicity and one or more water quality characteristics is found then the derivation of a Final Acute/Chronic

Equation instead of an FAV or FCV may be considered. Relationships must be established for two or more species before an equation can be considered. The procedure for processing toxicity data to obtain an equation is dependent on whether a Final Acute Equation or a Final Chronic Equation is being derived. For a Final Acute Equation a statistical method outlined below is used. For a Final Chronic Equation the statistical method may be used, or alternatively a method based on acute–chronic ratios.

The statistical method is as follows:

1. For each species having comparable acute toxicity values at two or more different levels of the water quality characteristic in question, perform linear least squares regression of the toxicity values on the water quality characteristic to obtain a slope value and its 95% confidence limits for each species. If the data provide a better fit if one or more variables is first log-transformed then this may be done.
2. Determine whether the derived relationship is useful, using expert judgement. Useful criteria include the degree to which a relationship agrees with any others derived, whether the variability in the toxicity is significantly reduced by the relationship, and the degree of variability in the water quality characteristic covered by the relationship. Relationships may be altered and improved by removing outlying values in the regression, if this is deemed appropriate.
3. For acute data, if useful slopes are not available for at least one fish and one invertebrate, or if the regression slopes are too dissimilar, or if too few data are available to adequately define the relationship, then the standard method of deriving the FAV should be used. For chronic data, if useful slopes are not available for at least one species, or if the regression slopes are too dissimilar, or if too few data are available to adequately define the relationship, then the chronic slope can be assumed to be the same as the acute slope (if it exists), or the standard method of deriving the FCV should be used.
4. Normalise the individual toxicity values for each species by dividing each by the geometric mean of all the values for that species. Normalise the values of the water quality characteristic in the same way. As in Step 1, if a better regression relationship is found by log-transforming one or more of the variables this should be done prior to normalising.
5. Repeat Step 1 using the normalised data on a species by species basis, then repeat again taking all the toxicity data together, to give an overall slope v and its 95% confidence limits.
6. Calculate the logarithms of the individual SMAVs or SMCVs at a single selected value of the water quality characteristic:

$$SMV' = SMV - v[x - z];$$

where x is the geometric mean of the water quality characteristic in tests on the species, z is the selected value of the water quality characteristic, SMV is the Species Mean (Acute/Chronic) Value calculated from the toxicity values and SMV' is the calculated Species Mean (Acute/Chronic) Value at the chosen water quality characteristic value. These values should be replaced by their log-transformed equivalents if appropriate (see Step 1).

7. Calculate the normalised Final Acute/Chronic Value (FV) from the normalised SMVs by following the calculation steps for calculating and processing GMVs.
8. If a commercially or recreationally important species has a normalised SMV lower than the normalised FV then that SMV should be taken as the FV.
9. The FV is given by:

$$FV = v.Q + a - vz$$

where a is the FV when the water quality characteristic has value z , and Q is the value of the water quality characteristic for which the FV is required. Again, variables should be replaced by their log-transformed equivalents if appropriate.

Processing of data for plants

A Final Plant Value (FPV) is derived from the results of either 96-hour tests on an alga or a chronic test on an aquatic vascular plant. Tests done in media containing large concentrations of chelating agents such as EDTA should not be used (for EDTA, $200 \mu\text{g l}^{-1}$ is considered to be the lowest significant concentration). The FPV is taken as the lowest result in tests where the substance concentrations were measured and the endpoint was biologically important

Consideration of other data

Pertinent information not usable in previous sections may be considered. Examples include data on cumulative and delayed toxicity, data on behavioural or biochemical effects, and data from field studies. Where such data are the only data available for a particular species they assume special importance.

Calculation of the CMC and CCC

The CMC is set to one-half of the FAV.

The CCC is set to the lowest of the FCV, the FPV and the Final Residue Value, unless other data suggest a different criterion. Where a Final Chronic Equation has been derived the CCC is derived by selecting the one value or the combination of values that result in the lowest concentrations in the usual range of the water quality characteristic used to derive the Final Chronic Equation, unless other data indicate that a lower value should be used.

A1.4.3 The Netherlands

General

The Netherlands approach is based around the initial derivation of scientifically underpinned advisory values termed Environmental Risk Limits (ERLs), which are subsequently used to set Environmental Quality Standards. ERLs must be derived according to standard procedures (Traas, 2001) most recently updated in 2001. Three

types of ERLs may be derived: the Negligible Concentration (NC), the Maximum Permissible Concentration (MPC) and the Ecotoxicological Serious Risk Concentration (SRC_{ECO}). These types may be used to set EQSs as Target Values (TVs), Maximum Permissible Concentrations and Intervention Values (IVs). These values and their corresponding EQSs represent, respectively, a concentration of the substance causing negligible effect to the aquatic ecosystem (to take account possible mixture effects), a concentration of the substance that should protect the aquatic ecosystem from adverse effects, and a concentration at which ecological function in the aquatic ecosystem will either be seriously affected or threatened to be seriously affected.

Two extrapolation methods are defined for calculating ERLs, depending on the amounts of toxicity data available. Preliminary effect assessment is used where chronic toxicity data for less than four taxonomic groups are available or where chronic data are absent. Refined effects assessment is used where chronic toxicity data for four or more taxonomic groups are available. Since for all the metals considered here the refined effects assessment has already been applied (Crommentuijn *et al.*, 1997), we shall not consider the preliminary effects assessment further.

A notable feature of the Netherlands approach is that a single ERL for both freshwater and saltwater ecosystems is derived if statistical differences among both types of toxicity data are not found. This feature will be discussed in detail later.

The process of deriving NCs, MPCs and SRC_{ECO}s using the refined effects assessment can be summarised as follows:

1. **Gather data** – from the published literature, including grey literature where possible. Information on food chain bioaccumulation is also gathered if this is considered to be a significant potential route of toxic effects.
2. **Assess data** – for quality and relevance.
3. **Calculate a single reliable toxicity value for each species in the database.**
4. **Calculate ERLs by statistical extrapolation** – using the Species Sensitivity Distribution (SSD) approach. A Maximum Permissible Addition (MPA) is derived for the purposes of calculating the MPC; this will be discussed in detail later.
5. **Estimate a natural background concentration of the substance.**
6. **Calculate MPC and NC incorporating the natural background, and the SRC_{ECO}**
7. **Verification of advisory standards** – including assessment of the standards against available filed studies, if appropriate.
8. **Consultation on the translation of advisory standards into EQSs** – with relevant public and advisory bodies.
9. **Adoption of EQSs.**

Data requirements

It is recommended to collect toxicity data from a number of public literature sources including Current Contents, Toxline, Biosis and relevant technical libraries. Only primary literature may be used in deriving ERLs.

Required information from toxicity test reports comprises the species used, the age, weight, size and life stage of the organisms, the purity of the test substance, whether test concentrations were measured or assumed on the basis of dose (nominal concentrations), the duration of the test, the type of endpoint monitored, and the expression of the endpoint (LC_x, EC_x, NOEC, etc.). Information on the species taxon, strain and scientific name, the type of test water (natural, tapwater, reconstituted, etc.) and the experimental set-up (flow-through, static, etc.) are noted if given.

Acceptable ecotoxicological endpoints are those that affect the species at the population level, typically survival, growth and reproductive effects. The purity of the test substance must exceed 80% and recovery from the test system must exceed 80%.

Both chronic and acute data are collected, although in the case of these metals sufficient chronic data was available that detailed analysis of the acute data was not required. The distinction between chronic and acute data is dependent upon the test species and the test duration in relation to its life cycle. OECD guidelines on standard fixed test durations are used to determine whether data should be considered chronic or acute, along with several additional criteria:

1. NOECs from tests on Algae, Bacteriophyta and Protozoa of 3–4 days duration are considered chronic.
2. Tests of 48 or 96 hours duration on Crustacea or Insecta are considered acute.
3. The standard acute toxicity test for Pisces, Mollusca and Amphibia has a 96-hour duration. Early life-stage tests, egg and sac fry stage tests and 28-day growth tests are considered chronic. The OECD prolonged toxicity test is considered acute.
4. Tests with deviating durations are noted.

Data processing

The selected toxicity data are processed to obtain one reliable value for each species tested, if possible. The available chronic toxicity data are converted into NOECs by the following method:

1. The highest reported concentration giving an effect not statistically different from the control at $p < 0.05$ is regarded as the NOEC if it is not the highest tested concentration.
2. If no statistical evaluation of the dose–response relationship is possible then the highest concentration producing a 10% or lower effect relative to the control is taken as approximating the NOEC.
3. If a LOEC is reported but a NOEC is not, then the LOEC is used to approximate the NOEC according to the following guidelines:
 - a. If the LOEC produces an effect relative to the control of between 10% and 20%, then the NOEC is taken to be half the LOEC.
 - b. If the LOEC produces an effect relative to the control of greater than 20% then the EC₁₀ should be calculated if possible and taken as the NOEC. It is recommended that the EC₁₀ is calculated by fitting a log-logistic dose–response curve to the toxicity data, and that two or more data points with associated effects of less than 70% are needed to perform the fitting.

- c. If the LOEC produces an effect relative to the control of greater than 20% and no distinct concentration–effect relationship is available then:
 - i. If the LOEC produces an effect relative to the control of between 20 and 50%, the NOEC is taken to be one-third of the LOEC.
 - ii. If the LOEC produces an effect relative to the control of between 50 and 80%, the NOEC is taken to be one-tenth of the LOEC.
- d. If other test results within the same taxonomic group can be used to derive alternative NOEC approximation methods this should be done. The issue of whether alternative approaches can be derived is left to expert judgement.
- e. If the NOEC is the highest tested concentration (unbounded), then this is noted and the data can only be used for evaluation if bounded NOECs (those that are not the highest applied dose) are not available.
- f. Toxic Threshold endpoints are regarded as NOECs.
- g. Maximum Acceptable Toxicant Concentration endpoints are used to approximate a NOEC by dividing by two.
- h. If data are expressed as the total metal and there is reason to believe that dissolved concentrations deviate from these values (e.g. due to the presence of particulate matter) the dissolved concentration may be estimated by applying a partition coefficient (K_d).

A single toxicity value for each species is calculated using the following method:

1. If multiple endpoints are available for the same effect on the same species the geometric mean is taken.
2. If multiple endpoints are available for different effects on the same species the lowest value is taken. Where the set includes multiple endpoints for multiple effects these are first averaged according to 1. This criterion applies to tests on different life stages of the same species.

In addition, the following test information is collected if available: water pH, hardness, salinity (for saltwater organism tests).

Data on field studies, including those using microcosms, mesocosms, macrocosms, enclosures or experimental streams, are to be gathered for comparison with ERLs.

ERL derivation by refined effects assessment

The basis of the refined effects assessment is the derivation of a Hazardous Concentration (HC) by the use of a Species Sensitivity Distribution (Stephan *et al.*, 1985; Kooijman, 1987) (Figure A1.6). Briefly, the sensitivities of the set of single species toxicity data are assumed to be a representative sample of the sensitivity range of organisms in the ecosystem. Taking a chosen percentile x of the sensitivity distribution is then considered to be protective of $(1 - x)\%$ of the species in the ecosystem. The method of statistical analysis of the distribution, and the percentage of ecosystem species to be protected, must be chosen. The Netherlands method uses the 5th percentile (for the MPA) and the 50th percentile (for the SRC_{ECO}) of a normal distribution fit to the data. The derived concentration is termed the HC_5 or HC_{50} and is a function of the mean and standard distribution of the data, and of an extrapolation factor which is dependent on the data population size and the protection level desired.

The extrapolation factor is chosen to give the median (50% confidence) estimate of the HC5 or HC50; 90% confidence intervals on the value are also calculated.

For aquatic data, the possibility of combining freshwater and saltwater data is tested by first analysing the variances of the separate datasets using an F-test. If significant differences in variance are seen then datasets are subject to an unpaired t-test with Welch correction as a further analysis of variance. If no statistically significant difference is found then the differences in sensitivity are compared using an unpaired, two-sided t-test. If no differences in sensitivity are found then freshwater and seawater data are combined to derive the MPA and SR_{ECO} .

In the refined risk assessment, ERLs are calculated from the collated NOEC data by using a Species Sensitivity Distribution (SSD). An SSD is a statistical distribution model fitted to the probability distribution of $\log(\text{NOEC})$ values (Figure A1.6). The SSD then represents the relationship between the concentration of the substance and the proportion of species assumed to be directly affected at that concentration. The proportion of affected species is termed the Potentially Affected Fraction (PAF). ERLs are then taken as being the substance concentration corresponding to a given PAF. MPCs are taken as the concentration for which $PAF = 0.05$ (5%) and SR_{ECO} s are taken as the concentration for which $PAF = 0.5$ (50%). The fitting of a normal distribution is recommended as this allows confidence limits to be calculated on the ERL.

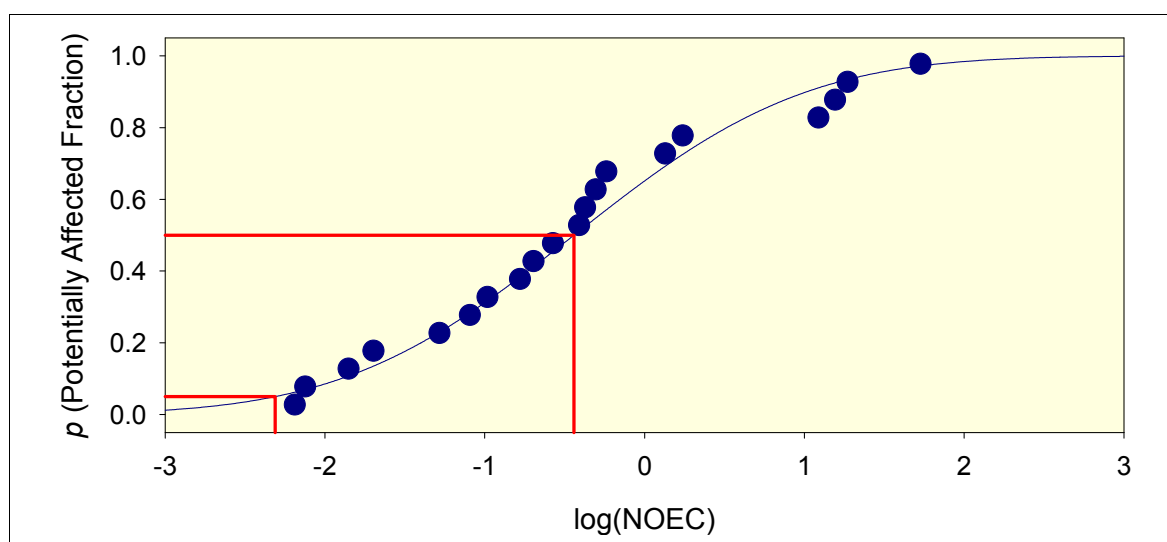


Figure A1.6 A species sensitivity distribution for a set of NOEC values (randomly generated for this example). Horizontal and vertical lines indicate the 5th and 50th percentiles of the distribution.

A notable feature of the approach is the use of the concept of Added Risk (AR), which is discussed briefly in the standard procedures. In applying the AR concept a natural background concentration of the substance (C_b) must be estimated. This is then added to the Maximum Permissible Addition (MPA) concentration which is calculated from toxicity data via the assessment approach described below, to give the MPC. The standard procedures document does not discuss the application of AR in detail, so application to metals will be discussed in the context of the derivation of their advisory standards below, as this involves a modification of the SSD approach discussed above.

Application to Ni, Cu, Zn, Cd and Pb

MPCs and Negligible Concentrations (NCs) for Ni, Cu, Zn, Cd and Pb were published in 1997 (Crommentuijn *et al.*, 1997), and SRC_{ECOS} for the same metals were published in 2001 (Verbruggen *et al.*, 2001). Here we shall discuss the specific derivation of advisory standards for these metals.

The Added Risk approach is central to the derivation of MPCs and NCs in the 1997 report. The technical details of the approach, and the method used for estimating background concentrations, are both discussed in detail. The Added Risk concept considers that the concentration of the substance naturally present in the ecosystem is not by definition a risk factor, since the natural state of the ecosystem will reflect the natural presence of the substance, and spatial variation in the natural background will be a driver of biodiversity.

Values for background concentrations of metals were calculated as follows. It was assumed that a single background concentration would be representative of surface waters in the Netherlands. These concentrations were calculated as total metals using a model proposed by De Bruijn and Denneman (1992), and were converted to dissolved concentrations using metal-specific partition coefficients assuming a suspended particulate matter concentration of 30 mg l⁻¹.

The Added Risk approach was used to calculate MPCs as described in Section A1.2.2.

A1.4.4 European Union (Water Framework Directive)

This section summarises the parts of the report on derivation of Quality Standards for Priority Substances under the Water Framework Directive (Lepper, 2002), that deals with the details of EQS derivation.

The overall objective of the Quality Standards set is firstly to protect the structure and function of aquatic ecosystems from any significant alteration. Preserving community structure is considered to be a means of protective ecosystem function, hence data on behaviour (e.g. avoidance) must be considered alongside data on growth, reproduction, etc. The Quality Standard must also protect humans against adverse effects resulting from consuming contaminated food or water from aquatic environments. Thus, pre-defined trigger criteria are used to define whether a substance should be considered a risk to the achievement of a given environmental or human health objective. For each objective considered to be at risk, a Quality Standard is derived. Where there are then multiple Quality Standards for a single contaminant, the lowest is used. The recommended procedures for deriving Quality Standards are those recommended for the derivation of PNEC in EU Risk Assessment Procedures (European Commission, 1996).

General procedures for setting standards will be discussed here as they apply to metals. Risk assessments for the metals are currently [July 2007] under differing stages of development and application. Of the metals, the copper and zinc assessments are most advanced. Specific procedures, particularly for dealing with bioavailability, have been derived in these assessments and are described in Section A1.3.5. Assessments for the other metals are ongoing, partly to incorporate bioavailability considerations as per the copper and zinc documents.

Quality Standards for metals in the water column are based on Added Risk, and for data-rich substances such as Ni, Cu, Zn, Cd and Pb are calculated using an SSD approach. The Added Risk approach is that used in the Netherlands (Section A1.2.2).

The general input data requirements for the SSD approach are:

1. At least 10 chronic NOECs (preferably full life-cycle or multi-generation studies) and preferably more than 15, for different species covering at least eight taxonomic groups:
 - a. fish;
 - b. a second family in the phylum Chordata;
 - c. a crustacean;
 - d. an insect;
 - e. a family in a phylum other than Arthropoda or Chordata;
 - f. a family in any phylum or order of insect not already represented;
 - g. algae;
 - h. higher plants.
2. Tests to be assessed for quality and relevance on a case-by-case basis, particularly if the test is non-standard.
3. NOECs to be expressed as the added metal (the report does not appear to make it explicit whether this should represent the addition above the control or the culture concentration).

Where multiple data are available for more than one species, the geometric mean of values is to be taken. Where there is then more than one endpoint for the same species, the lower value should be used. The report recommends the use of the SSD approach as described by Aldenberg and Jaworska (2000) but allows for the exploration of alternative methods, particularly in the choice of distribution to use. The 5th percentile cutoff of the SSD, at the 50% confidence interval, is calculated. This is then converted to a Maximum Permissible Addition (MPA, cf. Section A1.2.2) by dividing by an assessment factor. The assessment factor takes a value between one and five, based on expert judgement of:

1. the overall reliability and representativeness of the test data used;
2. the diversity of the taxonomic groups in the test dataset;
3. the mode of action of the chemical;
4. statistical uncertainties around the 5th percentile cutoff of the SSD;
5. comparisons of the 5th percentile/MPA with any available mesocosm and field studies.

The report makes no concrete long-term recommendations regarding incorporation of bioavailability in metals Quality Standards, due to the likely development of biotic ligand models for Ni, Cd and Pb in the near future (from 2002) as part of the risk assessment process. It recommended careful consideration of the bioavailability approaches when available. For cadmium, it considered that since the long-term policy objective is to cease discharges to the aquatic environment, consideration of bioavailability in Quality Standards might not be justified, although at the time of publication the ongoing Cd RAR was incorporating hardness dependence of the PNEC via the US EPA method (Section A1.4.2).

A1.4.5 Australia and New Zealand

General

The Australia and New Zealand Water Quality Guidelines (Australia and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, 2000) set out a methodology for calculating 'Trigger Values' (TVs) for toxicants including trace metals. The Guidelines are intended to provide a flexible framework for water quality and aquatic ecosystem management and the framework within which the TVs are applied reflects this. The Guidelines define three categories of ecosystem condition: *high conservation/ecological value systems*, *slightly to moderately disturbed ecosystems*, and *highly disturbed ecosystems*. High conservation/ecological value systems are intended to be managed such that there is no change in their ecological status. Trigger values are intended to be used to support the management of the other two ecosystem categories. Flexibility in the application of TVs is central to the recommended management systems, and the decision support framework in which TVs are recommended for use will be briefly described later. Adaptation of generic TVs to site-specific conditions (including accounting for the natural background and for bioavailability factors) is an important part of this system and will be considered here as an aspect of the derivation of trigger values.

Derivation of generic trigger values

Generic trigger values are calculated by extrapolation (statistical or by assessment factor) from experimental toxicity data. The requirements for test results to be acceptable for use are broadly similar to those in other methodologies, and basic guidelines (OECD, 1981; ASTM, 1994) were required to be followed in tests. Primary tests were retrieved from existing databases including those of the US EPA (the AQUIRE database). Where data did not previously exist in a database, the system used in AQUIRE to categorise tests by reliability was used.

A few distinctive features of the approach are worthy of note:

1. The main emphasis is on using chronic data (mortality, growth, reproduction endpoints) but if insufficient chronic data are available then acute L(E)C50 data may be used in conjunction with acute–chronic ratios. The chronic endpoint of preference is the NOEC. In the case of metals, the presence of many different endpoints in the test data forced the use of Maximum Acceptable Toxic Concentration (MATC), LOEC and chronic L(E)C50 endpoints also. Factors of 0.5, 0.4 and 0.2, respectively were used to adjust these values before use (Adjusted endpoint = Factor × unadjusted endpoint). Multiple NOECs for a species were averaged by taking the geometric mean.
2. Multiple species NOECs (i.e. microcosm/mesocosm community studies) are used if possible, even to the extent of not using any single species test data. Multiple species tests were assessed according to OECD (1992) guidelines as far as possible.
3. There is no requirement for tests to be carried out on geographically relevant species when generating generic TVs; local species test data may be used later to adjust TVs for site-specific conditions.
4. TVs obtained from SSDs are calculated for four different levels of protection: 99, 95, 90 and 80%.

When test data have been collated the TV is generated as follows:

1. If three or more reliable multiple species test NOECs are available then the lowest is taken and divided by an assessment factor of 10 to give a *high reliability* TV.
2. If five or more reliable chronic NOECs (or other adjusted endpoints in the case of metals) are available then an SSD is used to give a *high reliability* TV. A flexible approach was taken to SSD fitting allowing the data to be fitted to any of a family of distribution types. If the data were not considered sufficiently reliable for the SSD approach, a TV was calculated from the lowest value and an assessment factor of 10. Sufficient data were available for all the metals to apply an SSD. TVs so calculated are *high reliability*.
3. If only acute test results were available in sufficient numbers (over four species) for use then a similar approach to Step 2 was taken to give an 'acute TV' which was then adjusted using an acute–chronic ratio to give a *medium reliability* TV.
4. If neither acute nor chronic data were available in sufficient numbers, a *low reliability* TV was derived by dividing either the lowest NOEC or L(E)C₅₀ (after adjustment with an acute–chronic ratio) by an assessment factor.

Endpoint data for metals was screened for pH values below 6.5 and above 9. Additionally, endpoints were adjusted to a hardness of 30 mg CaCO₃ l⁻¹ prior to TV calculation, using hardness conversion algorithms.

The Guidelines provide detailed advice on how to assess the calculated TVs in relation to the spread of toxicity test endpoints. Of relevance for the metals was the advice that a high reliability TV should normally be more than three times less than the lowest acute L(E)C₅₀ value and should not be greater than any experimental chronic NOEC from 'species representative of important trophic levels'. Neither of these conditions was violated by any of the metal TVs. The cadmium TV was above the geometric mean of *Daphnia magna* NOECs, but individual NOECs for this species that were below the TV were in fact all adjusted acute endpoints. It was recommended that, if there was site-specific concern regarding similar species in slightly moderately disturbed ecosystems, the 99% protection level TV for Cd be used for management purposes. Otherwise, the standard approach was to recommend the 95% protection level for slightly moderately disturbed ecosystems. The protection level for disturbed ecosystems is recommended to be chosen on a site-specific basis, either at 95, 90 or 80% protection.

Decision tree for assessment and site-specific adjustment of TVs

This section summarises the procedure for assessment of toxicant effects in waters, in particular the site-specific adjustment of TVs. The assessment procedure is summarised as:

1. Define primary management aims.
2. Define appropriate TVs.
3. Compare total toxicant concentrations against adjusted TVs.
4. If concentration < TVs, consider risk to be low and exit the procedure. If not, consider site-specific adjustment of TVs, based on, for example,

background, analytical limits, locally important species, bioavailability modifiers, mixture interactions.

5. Compare toxicant concentrations against adjusted TVs.
6. If concentration < adjusted TVs, consider risk to be low and exit the procedure. If not, perform site-specific biological effects assessment to determine whether to initiate remedial action.

We will focus on the aspects of the approach that relate to the value of the TV: consideration of the background, bioavailability and mixture effects. The emphasis throughout the Guidelines is on flexibility and providing options for action.

Suggested actions regarding the background are:

1. Measure the background either at the site or at a geologically similar, unimpacted site. If the background exceeds the TV, take the 80th percentile of the background as the TV for subsequent steps and continue the assessment process.
2. Consider Direct Toxicity Assessment (DTA) using locally adapted species and consider further diagnostic tests if the results indicate an effect.

The suggested action regarding metal bioavailability is to first adjust the TV for site-specific hardness. If the adjusted value is still exceeded by the contaminant concentration, consider filtered (0.45 µm) rather than total concentrations. If exceedance still exists, consider either:

1. Making more sophisticated measurements or predictions of the bioavailable concentration (e.g. using speciation modelling and measurement). It is recommended that expert advice be sought.
2. Proceed to DTA.
3. Institute remedial actions.

If option 1 is taken and the bioavailable metal concentration still exceeds the hardness-adjusted TV then DTA may be used to confirm the results or develop a site-specific TV, or remedial actions may be instituted.

Possible effects of mixtures can be assessed by considering the sum of the ratios of contaminant concentrations to their TVs (generic or site-specific). If this ratio (sometimes termed the Cumulative Criterion Unit or CCU) is greater than unity, then mixture effects are possible (assuming additive effects of individual contaminants). DTA is recommended as the best way to confirm effects in this situation.

Appendix 2 Metal mixture toxicity

A2.1 Review of literature mixture studies

References to literature mixture toxicity studies were taken from the review of Norwood *et al.* (2003) and from a review of the literature for more recent papers. In all, 115 papers were considered for inclusion in the review. Papers were screened according to the following criteria:

1. Mixtures had to comprise only Ni, Cu, Zn, Cd and Pb.
2. The test organism had to be a freshwater species.
3. Complete dose–response curves for both the individual metals and the mixtures had to be available.
4. The tests using individual metals and the mixture(s) had to be conducted either at the same time or immediately consecutively, in order to minimise the effects of temporal variations in organism response on the interpretation of the results.
5. Sufficient numbers of single metal toxicity data were required in order to calculate reliable dose–response curves and effect concentrations (EC50s) for each single metal, in order to properly quantify the effect of mixtures relative to the single metals.
6. The type of test (acute or chronic) and the nature of the toxic endpoint were not used to screen the data.

Initially, it was intended to consider the mixture interactions in the context of metal speciation and if possible binding to a biotic ligand. In practice, no test data were found that were suitable for consideration and had single metal and mixture test results for multiple water compositions. In these circumstances the consideration of speciation would not influence the diagnosis of mixture interactions, so we have worked with dissolved metal concentrations instead.

The test data reviewed are shown in Table A2.1. The most frequent metals appearing were Cu, Cd and Zn. Ni appeared in four tests. There were no suitable tests using Pb. Most of the tests for fish (three of four) and invertebrates (all nine datasets) were acute. All the acceptable data were for binary mixtures only.

A2.1.1 Mixture interaction theory

Two primary reference models of toxicant mixture interaction exist: the concentration addition (CA) and independent action (IA) models. The concentration addition model predicts the effects of two or more toxicants in a mixture on the basis of their relative single toxicities, while the independent action model predicts on the basis of combined probabilities of response. Deviations from these reference models can be broadly categorised into synergism (observed effect greater than predicted effect) and antagonism (observed effect less than predicted effect).

Mixture interactions were quantified using the MIXTOX model framework of Jonker *et al.* (2005). This essentially entails firstly fitting models for concentration and effects addition without interaction, and then fitting extended models that quantify synergism/antagonism. Comparing models then allows a statistical assessment of

whether synergism or antagonism are seen to a significant degree. Given the relatively small amount of data in the studies considered acceptable (103 data points in all), it was decided to look for general trends in the data rather than study each dataset separately.

Table A2.1 Peer-reviewed literature papers on metal mixture effects used in this review.

Species	Endpoint	Metal 1	Metal 2	Reference
Invertebrates				
<i>Daphnia pulex</i>	48h survival	Zn	Cd	Shaw <i>et al.</i> (2006)
<i>Daphnia ambigua</i>	48h survival	Zn	Cd	Shaw <i>et al.</i> (2006)
<i>Ceriodaphnia dubia</i>	48h survival	Zn	Cd	Shaw <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h survival	Zn	Cd	Shaw <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h survival	Cu	Cd	Barata <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h feeding	Cu	Cd	Barata <i>et al.</i> (2006)
<i>Dreisena polymorpha</i>	48h filtration	Cu	Cd	Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisena polymorpha</i>	48h filtration	Zn	Cu	Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisena polymorpha</i>	48h filtration	Zn	Cd	Kraak <i>et al.</i> (1994a, 1994b)
Fish				
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	Khangarot <i>et al.</i> (1984)
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	Khangarot <i>et al.</i> (1984)
<i>Lepomis macrochirus</i>	96h survival	Cu	Zn	Thompson <i>et al.</i> (1980)
<i>Poecilia reticulata</i>	7d growth	Ni	Cu	Muska (1978)
Plants				
<i>Anabaena inaequalis</i>	acetylene reduction	Ni	Cd	Stratton and Corke (1979)
<i>Anabaena inaequalis</i>	CO ₂ fixation	Ni	Cd	Stratton and Corke (1979)
<i>Lemna trisulca</i>	14d growth	Zn	Cd	Huebert and Shay (1992)

If a mixture of n chemicals affects an organism according to the concentration addition reference model, then:

$$\sum_{1}^{n} \frac{c_i}{EC_{x,i}} = 1 \quad (\text{A2.1})$$

where c_i is the concentration of each chemical in the mixture, and $EC_{x,i}$ is the concentration of that chemical giving the same effect when acting alone. The ratio $c_i/EC_{x,i}$ is the toxic unit. Values of $EC_{x,i}$ are calculated from dose–response curves for the individual toxicants. To quantify deviations from concentration addition, equation A2.1 is extended:

$$\sum_{1}^{n} \frac{c_i}{EC_{x,i}} = e^G \quad (\text{A2.2})$$

where G is a deviation function, the form of which depends upon the nature of the deviation being investigated.

A similar principle is used with the independent action model. The general expression for the reference model is obtained by multiplying the probabilities of non-response for single toxicant exposure:

$$R = R_0 \cdot \prod_{1}^{n} q_{i(c_i)} \quad (\text{A2.3})$$

where R is the predicted response to the mixture, R_0 is the response in the absence of toxicants, and $q_i(c_i)$ is the non-response rate or probability, that is the proportion of organisms not responding to toxicant i when exposed to concentration c_i singly (e.g. if in a test the mortality rate is 40% then the non-response (survival) rate is 60%). Deviations from effect addition are quantified by:

$$R = R_0 \cdot \Phi \left[\Phi^{-1} \left(\prod_1^n q_{i(c_i)} \right) + G \right] \quad (\text{A2.4})$$

where $\Phi(x)$ and $\Phi^{-1}(x)$ respectively represent applying the standard cumulative normal distribution and the inverse standard cumulative normal distribution to the term x , and G again represents the deviation function.

Regardless of the reference model, quantification of synergism or antagonism is done with the same deviation function. The function includes a fitting parameter a . The statistical significance of synergism or antagonism was assessed by a chi-squared test using the fitting errors of the reference models (A2.1, A2.3) and the corresponding extended model (A2.2, A2.4, respectively). Data fitting was done using Microsoft Excel SOLVER (2002 version) and chi-tests were done using Microsoft Excel.

It is important to realise that the process of fitting the toxicity data to the models does not tell us whether one or other of the basic reference models is more appropriate. Mixture toxicity effects are fundamentally dependent upon the modes of action of the individual toxicants and these cannot be elucidated from toxicity testing. This fitting can tell us whether (i) the data do or do not comply with either or both of the reference models, and (ii) whether the choice of reference model is important for these data.

A2.1.2 Consideration of individual datasets

Results of fitting to individual datasets are shown in Tables A2.2 and A2.3. Most of the datasets (10 of 16) show highly significant ($p < 0.001$) deviations from either the CA or IA reference model. In most cases the deviations from each reference model were similar in nature and significance.

Mixtures of Zn and Cd consistently exhibited antagonism with respect to one or both reference models, with the exception of the dataset of Shaw *et al.* (2006) for *Daphnia magna*. Other mixtures show less consistent behaviour; Cu and Cd behave antagonistically towards *D. magna* in the tests of Barata *et al.* (2006) but synergistically towards *Dreissena polymorpha* in the tests of Kraak *et al.* (1994a) and Kraak *et al.* (1994b). Mixtures of Cu and Zn behaved synergistically towards *Cyprinus carpio* (common carp) (Khangarot *et al.*, 1984) but antagonistically towards *Lepomis macrochirus* (bluegill) (Thompson *et al.*, 1980) and towards *D. polymorpha*. Ni and Cu behaved antagonistically towards *Poecilia reticulata* (guppy) (Muska, 1978). Ni and Cd showed interesting behaviour, in that a strong synergism was seen in effects on CO₂ fixation by the cyanobacterium *Anabaena inaequalis* (Stratton and Corke, 1979), but none was seen for effects on rate of reduction of acetylene by the bacterium in the same tests.

Table A2.2 Results of model fitting to individual mixture toxicity datasets – detection of statistically significant synergism and antagonism. Column CA–SA shows significant deviations from the CA reference model, Column IA–SA shows significant deviations from the IA reference model. A indicates significant antagonism found, S indicates significant synergism found. Significance levels: *: $p < 0.05$; **: $p < 0.01$; *: $p < 0.001$.**

Species	Endpoint	Metal 1	Metal 2	Design ¹	CA-SA ²	IA-SA ⁵	Reference
<i>Daphnia magna</i>	48h survival	Zn	Cd	LR			Shaw <i>et al.</i> (2006)
<i>Daphnia pulex</i>	48h survival	Zn	Cd	LR	A***	A***	Shaw <i>et al.</i> (2006)
<i>Daphnia ambigua</i>	48h survival	Zn	Cd	LR	A***	A***	Shaw <i>et al.</i> (2006)
<i>Ceriodaphnia dubia</i>	48h survival	Zn	Cd	LR	A***	A***	Shaw <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h survival	Cu	Cd	LR	A***	A***	Barata <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h feeding	Cu	Cd	LR	A**		Barata <i>et al.</i> (2006)
<i>Dreisenna polymorpha</i>	48h filtration	Cd	Cu	L		S**	Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisenna polymorpha</i>	48h filtration	Zn	Cu	L		S**	Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisenna polymorpha</i>	48h filtration	Cd	Zn	L	A***		Kraak <i>et al.</i> (1994a, 1994b)
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	L	S***	S***	Khangarot <i>et al.</i> (1984)
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	L	S**	S***	Khangarot <i>et al.</i> (1984)
<i>Lepomis macrochirus</i>	96h survival	Cu	Zn	L	A**		Thompson <i>et al.</i> (1980)
<i>Poecilia reticulata</i>	7d growth	Cu	Ni	L	S***	S***	Muska (1978)
<i>Anabaena inaequalis</i>	acetylene reduction	Ni	Cd	LR			Stratton and Corke (1979)
<i>Anabaena inaequalis</i>	CO ₂ fixation	Ni	Cd	LR	S***	S***	Stratton and Corke (1979)
<i>Lemna trisulca</i>	14d growth	Cd	Zn	LR	A***	A**	Huebert and Shay (1992)

Notes: ¹ L: only dose level varied; R: only dose ratio varied LR: dose level and dose ratio varied.

² Concentration addition synergism/antagonism.

³ Independent action synergism/antagonism.

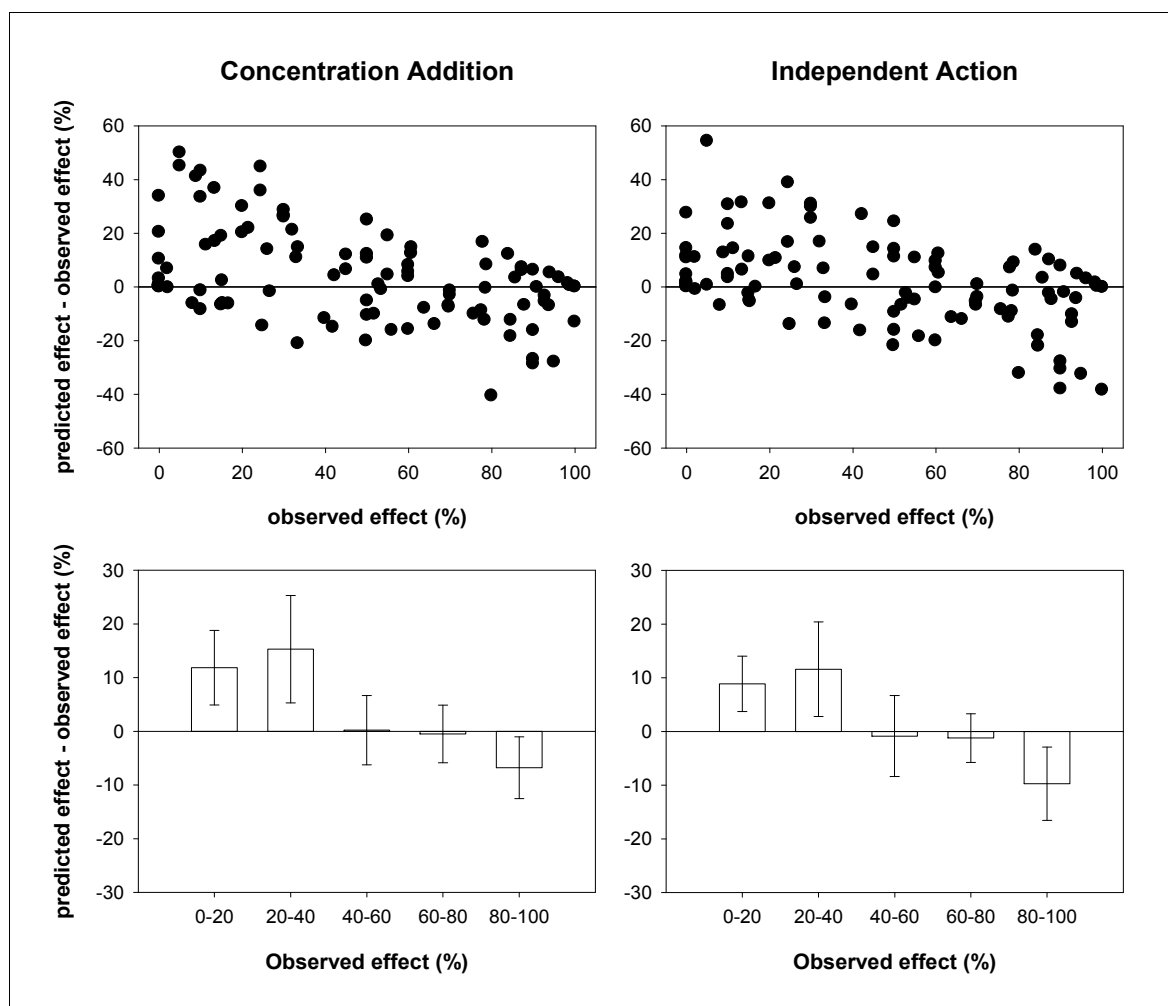


Figure A2.1 Plots of model residuals (predicted effect – observed effect) for both CA and IA models. Top panes show all individual data points, lower panes show means and standard deviations following categorisation according to observed effect.

A2.1.3 General trends in mixture interactions

Plotting the difference between observed and predicted effect against the observed effect, across all the datasets and for both reference models, revealed a distinctive trend (Figure A2.1) towards antagonism at low effect levels and synergism at high effect levels. Interestingly, this trend is the opposite of that predicted by Playle (2004) in a theoretical study of how competitive uptake of multiple metals at a Biotic Ligand would be expected to influence the mixture toxicity pattern. This suggests that other considerations, probably related to the specific mode(s) of action of individual metals, might require consideration in mixture modeling of metals.

A2.1.4 Summary

The number of reliable datasets for the mixture toxicity review proved disappointingly small. In addition, most of the datasets were of acute effects, and thus not necessarily suitable for extrapolation to the consideration of long-term effects under field conditions. Nonetheless, the general observation of antagonism at low effects and

synergism at high effects, across several studies involving invertebrates, plants and animals, suggests a common mechanism controlling or at least partially controlling the interactions of the metals.

Table A2.3 Results of model fitting to individual mixture toxicity datasets – detection of statistically significant dose-ratio and dose-level dependent synergism. Significance levels: *: p < 0.05; **: p < 0.01; *: p < 0.001. Significances are relative to the basic synergism/antagonism model unless underlined, in which case they are relative to the basic concentration addition or independent action model.**

Species	Endpoint	Metal 1	Metal 2	Design ¹	CA-DR ²	CA-DL ³	IA-DR ⁴	IA-DL ⁵	Reference
<i>Daphnia magna</i>	48h survival	Zn	Cd	LR					Shaw <i>et al.</i> (2006)
<i>Daphnia pulex</i>	48h survival	Zn	Cd	LR					Shaw <i>et al.</i> (2006)
<i>Daphnia ambigua</i>	48h survival	Zn	Cd	LR					Shaw <i>et al.</i> (2006)
<i>Ceriodaphnia dubia</i>	48h survival	Zn	Cd	LR	*		*		Shaw <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h survival	Cu	Cd	LR				*	Barata <i>et al.</i> (2006)
<i>Daphnia magna</i>	48h feeding	Cu	Cd	LR	***		*	**	Barata <i>et al.</i> (2006)
<i>Dreisenna polymorpha</i>	48h filtration	Cd	Cu	L		*		—	Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisenna polymorpha</i>	48h filtration	Zn	Cu	L		—			Kraak <i>et al.</i> (1994a, 1994b)
<i>Dreisenna polymorpha</i>	48h filtration	Cd	Zn	L					Kraak <i>et al.</i> (1994a, 1994b)
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	L					Khangarot <i>et al.</i> (1984)
<i>Cyprinus carpio</i>	48h survival	Cu	Zn	L				*	Khangarot <i>et al.</i> (1984)
<i>Lepomis macrochirus</i>	96h survival	Cu	Zn	L					Thompson <i>et al.</i> (1980)
<i>Poecilia reticulata</i>	7d growth	Cu	Ni	L					Muska (1978)
<i>Anabaena inaequalis</i>	acetylene reduction	Ni	Cd	LR		**		**	Stratton and Corke (1979)
<i>Anabaena inaequalis</i>	CO ₂ fixation	Ni	Cd	LR					Stratton and Corke (1979)
<i>Lemna trisulca</i>	14d growth	Cd	Zn	LR	***	***	***	***	Huebert and Shay (1992)

Notes: ¹ L: only dose level varied; R: only dose ratio varied; LR: dose level and dose ratio varied.

² Concentration addition dose ratio-dependent synergism/antagonism.

³ Concentration addition dose level-dependent synergism/antagonism.

⁴ Independent action dose ratio-dependent synergism/antagonism.

⁵ Independent action dose level-dependent synergism/antagonism.

A2.2 Mixture toxicity testing with algae

The aims were (1) to investigate if the same mixture toxicity model (i.e. concentration addition versus independent action) could explain the responses of the algae to metal mixtures in waters with different water characteristics (= different bioavailability levels), (2) to investigate if deviations of the standard model (i.e. synergism or antagonism) occurred and, if so, whether or not these deviations were the same in both waters, and (3) to determine to which extent different mixture models differed in their ability to accurately predict toxic effects.

To that end we investigated the toxicity of single metals and mixtures of metals spiked into two natural water samples with different chemistry, thereby encompassing as much as possible the pH and hardness ranges of all 35 samples. The two 'uncontaminated' water samples chosen for this purpose were from Sites 32 (Uldale Beck) and 33 (Scandale Beck). Site 33 was characterised by a pH of 8.4 and a hardness of 95 mg CaCO₃ l⁻¹. Site 32 was characterised by a pH of 5.5 and a hardness of 5.8 mg CaCO₃ l⁻¹. Since such a low pH is at the lower tolerance boundary of *Pseudokirchneriella subcapitata*, pH of this sample was buffered at pH 6 through addition of MOPS (3.6 mmol l⁻¹) and the appropriate amount of NaOH. No pH buffer was added to the Site 33 sample, but pH was kept within 0.2 pH units during tests by daily manual addition of HCl or NaOH if necessary. Nutrients were added to all test solutions according to the concentrations recommended for the 'OECD-medium' in the OECD test guideline No. 201 (OECD, 2006). The components CaCl₂, MgSO₄, MgCl₂, EDTA and NaHCO₃ listed in this guideline were not added.

In a first test phase, we wanted to determine the single metal toxicity of five metals (Cu, Zn, Ni, Cd, Pb) in both waters. Each metal was spiked as its chloride salt at six different concentrations into both test waters. Solutions were left to equilibrate for 24 hours at 25°C. All tests ($n = 10$, 2 waters x 5 metals) were conducted simultaneously according to OECD guideline No. 201 (OECD, 2006) in a test chamber at 25°C. Tests were initiated by introducing 104 cells ml⁻¹ of *P. subcapitata* into the test solutions. After 24, 48 and 72 hours, cell density was recorded by an electronic particle counter. Growth rate (μ) was calculated after 48 and 72 hours. However, since there were indications of non-exponential growth between the 48th and the 72nd hour of exposure, only the 48-hour growth rates were used for further data treatment. This decision was in line with the OECD criteria for a valid test (i.e. factor cell increase > 16 in two days). Dissolved metal concentrations measured using AAS (at Ghent University) at test initiation were used for data treatment. Concentration response curves were fitted to the data of the individual metals exposure using the logistic equation:

$$\mu_{[Me]} = \mu_0 / \{1 + ([Me] / EC50_{Me})^\beta\} \quad (A2.5)$$

where $\mu_{[Me]}$ = the estimated growth rate at metal concentration [Me], μ_0 = the mean growth rate in the unspiked control sample, [Me] = the metal concentration, $EC50_{Me}$ = the median effective concentration, and β = the slope of the concentration response curve. An alternative form of this equation (see De Schamphelaere and Janssen, 2004b) was used to estimate the EC10. Parameter estimation was carried out with the Levenberg-Marquardt method in Statistica 6.0 software (Statsoft, Tulsa, OK). The EC50 and EC10 values obtained for all metals in both waters are presented in Figure A2.2. Data of Pb are not presented because of precipitation of Pb as Pb₅(PO₄)₃Cl (chloropyromorphite) – phosphate was added to test solutions to ensure algal growth – which only left less than 5% of the Pb in the dissolved form, making the test results unreliable. For this reason, Pb was also omitted in the second test phase (mixed metal exposure).

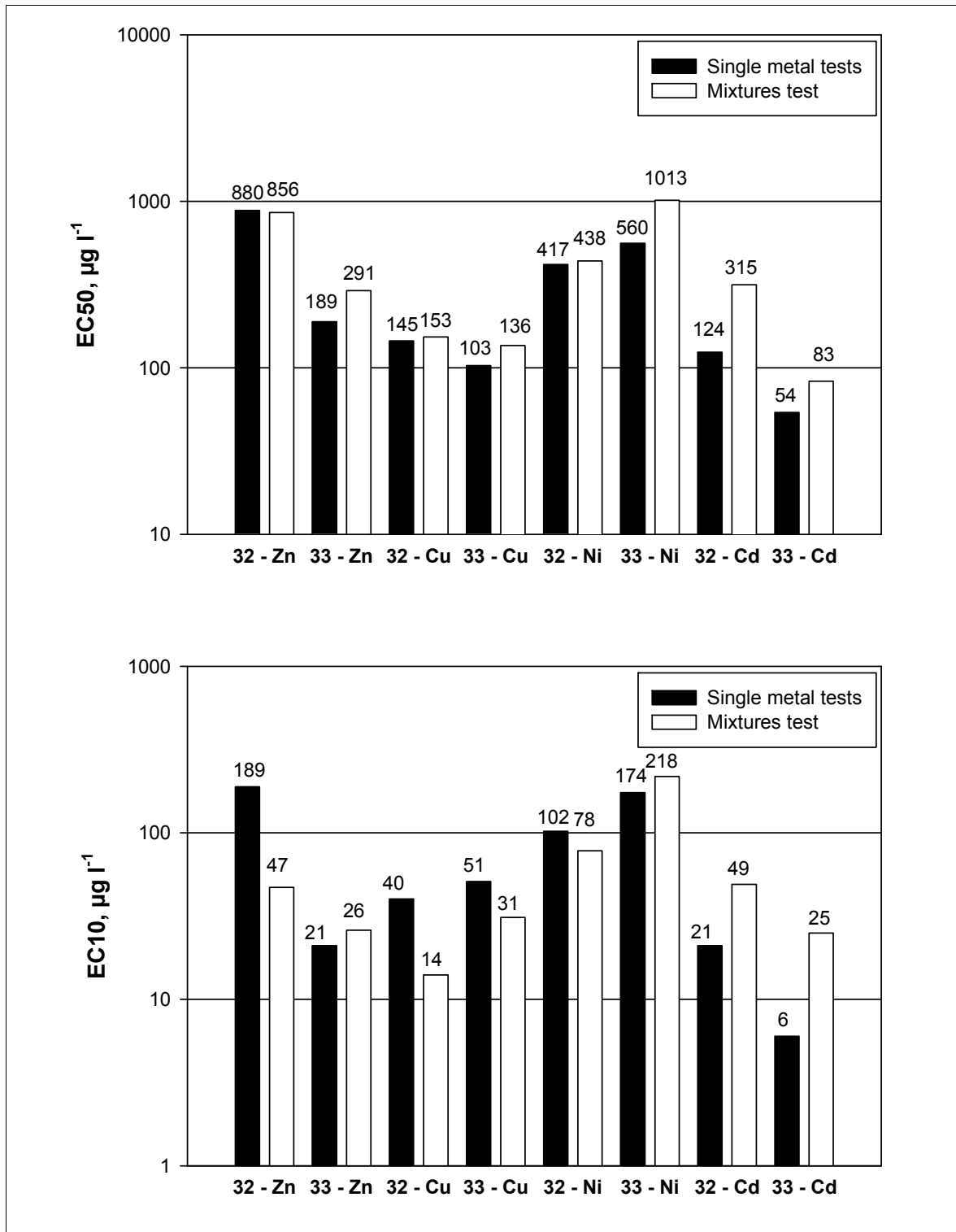


Figure A2.2 Single compound toxicity (EC50 and EC10) of four metals in two natural waters, No. 32 (low pH, low hardness) and No. 33 (high pH, high hardness). Black bars are the results from the first test phase (single metal experiments only), white bars are the results from the second test phase (single metal assays and mixed metal assay simultaneously). Values above the bars are the EC50 and EC10 values.

The results of this first test phase were then used to design the mixture toxicity experiment conducted in the second phase. In this experiment, next to the toxicity of metal mixture treatments, each metal was simultaneously tested individually for its toxic effect. The estimated EC10 of each metal (from test phase 1) was used to choose the concentrations to be tested. Next to a control, individual metals were tested at concentrations of EC10/2, EC10, 2×EC10, 4×EC10, 8×EC10 and 16×EC10, and quaternary mixtures (four metals together) of Zn, Cu, Ni and Cd were tested in which each metal was present at concentrations of EC10/8, EC10/4, EC10/2, EC10, 2×EC10 and 4×EC10. This resulted in a total number of 31 treatments that were investigated.

The results from the single metal exposure treatments were used to calculate again EC50 and EC10 values and those results are also plotted in Figure A2.2. The toxicity data resulting from the single metal exposures allow an assessment of the variability of metal toxicity to the algae over time. Although EC10 and EC50 values in most cases differed by less than a factor of two between the first and second test phase, the EC10 values for Cu and Zn in Site 32 were about a factor of 3–4 lower in the mixture experiment. The EC50 and the EC10 values for Cd were consistently higher (factor 2–4) in the mixture experiment in both water samples, suggesting a generally higher tolerance to Cd in this test phase. ***This indicates the importance of simultaneously testing the effects of single metal and metal mixture toxicity.***

Table A2.4 Statistical analysis (according to Jonker *et al.*, 2005) of the metal mixture toxicity experiment with *Pseudokirchneriella subcapitata*; the sum of squared errors (SSE) resulting from the reference models concentration addition (CA) or independent action (IA) is compared with the SSE resulting from an extended model including synergistic interaction (antagonistic interactions were not found); the reported *p*-value is the probability that the SSE with the reference model is the same as the SSE from the synergistic interaction model; if $p < 0.05$ the synergistic model performs significantly better than the reference model.

	32 CA	32 IA	33 CA	33 IA
SSE reference model	0.239	0.217	0.260	0.249
SSE synergism	0.183	0.209	0.239	0.242
<i>n</i>	31	31	31	31
<i>p</i>	0.005**	0.290	0.110	0.375
Decision	Synergism	Simple IA	Simple CA	Simple IA

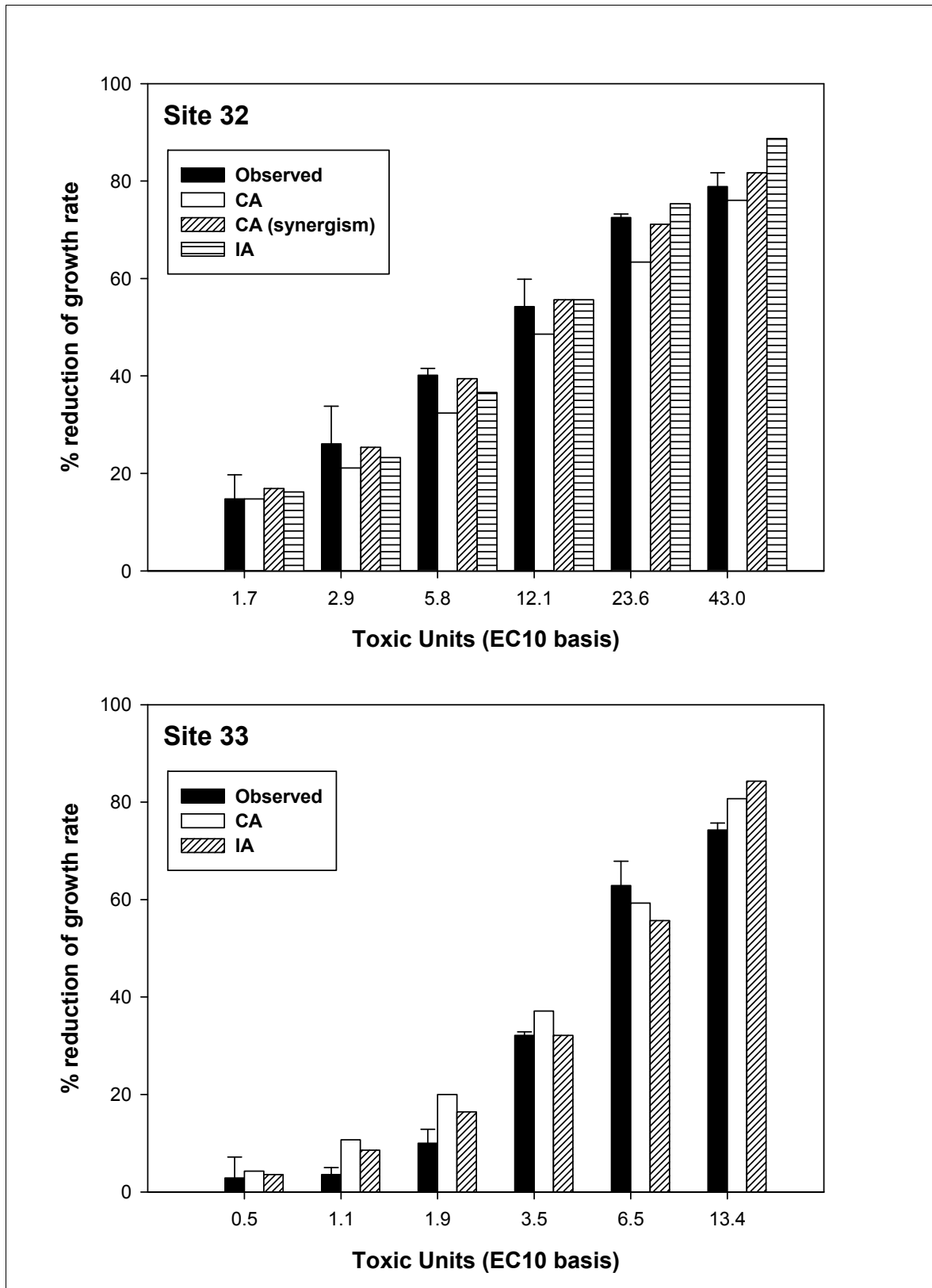


Figure A2.3 Effects of metal mixtures at different concentrations – expressed as toxic units on the basis of the EC10 values – on growth rate of *P. subcapitata*; observed effects as well as effects predicted with different mixture models are plotted, i.e. concentration addition (CA), independent action (IA), and CA with a synergistic interaction component.

The complete set of dose–response data from the second test phase was now used to statistically test, using the methodology of Jonker *et al.* (2005), whether significant deviations from the two standard models (i.e. concentration addition and independent action) occurred. It must be emphasised upfront that, as already argued by Jonker *et al.* (2005), this type of statistical dose–response analysis only sheds light on which models best represent the observations. It cannot be used to distinguish mechanisms of toxic action of and interactions between different metals in mixed metal exposures. Such information should rather come from physiological studies.

Nonetheless, a summary of the statistical dose–response analysis is given in Table A2.4. When IA was assumed, no significant synergism or antagonism was detected in either of the waters. However, based on the statistical analysis following Jonker *et al.* (2005), significant synergistic interaction among the metals was detected in the Site 32 water. This was not the case in the Site 33 water. ***This suggests that, from a statistical point of view, mixture toxicity interactions may not be identical in soft acid versus hard alkaline waters.***

Obviously, there is a difference in interpreting the data from a statistical significance perspective to interpreting it from a perspective of prediction errors, the latter of which may at least be of equal interest to those wanting to incorporate metal mixture toxicity effects into ecological risk assessment. Indeed, the same dose–response analysis demonstrated that both the reference CA and IA models, as well as the models including a synergistic component, yielded comparable sum of squared errors (SSE) in both Site 32 and Site 33 natural waters (Table A2.4). Thus, ***the predictive capacity of the response to the mixed metals exposures, obtained with the reference CA and IA models, as well as with the models including the synergistic component, is broadly comparable.***

Finally, as shown in Figure A2.3, both the CA and IA reference models predicted percentage of growth rate reduction following mixed metal exposure that were always within 10% of the observed effects, even in Site 32 water where a significant synergism was detected. Interestingly, at the lowest effect levels (i.e. lower than 20% reduction of growth rate), the reference CA and IA models exhibited very similar predictive capacities and effects were either accurately predicted (i.e. within the 95% confidence interval of the observed response) or were slightly overestimated (but always less than 10% prediction error).

The main conclusions of this study are:

- from a statistical point of view, interactive effects among metals in a mixture may be significant;
- from a statistical point of view, interactive effects (e.g., synergism) among metals in a mixture may be different in waters with different composition and may depend on the reference model used (CA versus IA);
- but from a perspective of predictive capacity of toxic effects: ***the differences in predicted responses among the different mixture models used remain limited.***

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List of abbreviations (appendices)

AMD	Acid mine drainage
AMPS	Analysis and Monitoring of Priority Substances (Expert Group)
BL	Biotic ligand
BLM	Biotic Ligand Model
BRC	Background reference concentration
CA	Concentration addition
CCC	Criterion Continuous Concentration
CCU	Cumulative Criterion Unit
CLF	Critical limit function
CMC	Criterion Maximum Concentration
DOC	Dissolved organic carbon
DTA	Direct Toxicity Assessment
EC _x	Effect Concentration affecting x percent of test organisms
EDTA	Ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency (US)
EQO	Environmental Quality Objective
EQS	Environmental Quality Standard
EQS (AA)	Environmental Quality Standard annual average concentration
EQS (MAC)	Environmental Quality Standard maximum allowable concentration
ERL	Environmental Risk Limit
FACR	Final Acute–Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FIAM	Free Ion Activity Model
FPV	Final Plant Value
FV	Final Acute/Chronic Value
GMAV	Genus Mean Acute Value
GMCV	Genus Mean Chronic Value
GMV	
GSIM	Gill Surface Interaction Model
HC	Hazardous Concentration
IA	Independent action
IV	Intervention Value
LA	Lethal accumulation
LC _x	Lethal Concentration affecting x percent of test organisms
LOEC	Lowest Observed Effect Concentration
MATC	Maximum Acceptable Toxic Concentration
MPA	Maximum Permissible Addition
MPC	Maximum Permissible Concentration
NC	Negligible Concentration
NOEC	No Observed Effect Concentration
OCEE	Optimal Concentration of Essential Element
PAF	Potentially Affected Fraction
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
RAR	(Existing Substances Regulations) Risk Assessment Report
SMACR	Species Mean Acute–Chronic Value
SMAV	Species Mean Acute Value
SMCV	Species Mean Chronic Value
SPM	Suspended particulate matter

SRC _{ECO}	Ecotoxicological Serious Risk Concentration
SSD	Species sensitivity distribution
SSE	Sum of squared errors
TGD	Technical Guidance Document
TV	Target Value
WER	Water-effect ratio
WQC	Water Quality Criteria

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