Environment Agency

Ecological indicators for abandoned mines, Phase 1: Review of the literature

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Integrated catchment science programme

Evidence Directorate

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Authors:
Tipping, E.[1], Jarvis, A.P.[2], Kelly, M.G.[3], Lofts, S[1]., Merrix, F.L.[4], and Ormerod, S.J.[4].
[1]Centre for Ecology and Hydrology (Chapters 5, 6 & 7), [2]Newcastle University (Chapter 2), [3]Bowburn Consultancy (Chapter 3), [4]University of Wales, Cardiff (Chapter 4).

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Research Contractor:
Professor E. Tipping, Centre for Ecology & Hydrology Lancaster, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP
Tel: 44 (0)1524 595866. E-mail: et@ceh.ac.uk

Environment Agency's Project Manager:
Dr Hugh Potter, Evidence Directorate

Collaborator:
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Miranda Kavanagh

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Director of Evidence

Executive summary

Mine waters have been identified as a significant issue in the majority of Environment Agency draft River Basin Management Plans. They are one of the largest drivers for chemical pollution in the draft Impact Assessment for the Water Framework Directive (WFD), with significant failures of environmental quality standards (EQS) for metals (particularly Cd, Pb, Zn, Cu, Fe) in many rivers linked to abandoned mines. Existing EQS may be overprotective of aquatic life which may have adapted over centuries of exposure. This study forms part of a larger project to investigate the ecological impact of metals in rivers, to develop water quality targets (alternative objectives for the WFD) for aquatic ecosystems impacted by long-term mining pollution. The report reviews literature on EQS failures, metal effects on aquatic biota and effects of water chemistry, and uses this information to consider further work.

A preliminary assessment of water quality and biology data for 87 sites across Gwynedd and Ceredigion (Wales) shows that existing Environment Agency water quality and biology data could be used to establish statistical relations between chemical variables and metrics of ecological quality. Visual representation and preliminary statistical analyses show that invertebrate diversity declines with increasing zinc concentration. However, the situation is more complex because the effects of other metals are not readily apparent. Furthermore, pH and aluminium also affect streamwater invertebrates, making it difficult to tease out toxicity due to individual mine-derived metals.

The most characteristic feature of the plant communities of metal-impacted systems is a reduction in diversity, compared to that found in comparable unimpacted streams. Some species thrive in the presence of heavy metals, presumably because they are able to develop metal tolerance, whilst others consistently disappear. Effects are, however, confounded by water chemistry, particularly pH. Tolerant species are spread across a number of divisions of photosynthetic organisms, though green algae, diatoms and blue-green algae are usually most abundant, often thriving in the absence of competition and/or grazing. Current UK monitoring techniques focus on community composition and, whilst these provide a sampling and analytical framework for studies of metal impacts, the metrics are not sensitive to these impacts. There is scope for developing new metrics, based on community-level analyses and for looking at morphological variations common in some taxa at elevated metal concentrations. On the whole, community-based metrics are recommended, as these are easier to relate to ecological status definitions.

With respect to invertebrates and fish, metals affect individuals, population and communities but sensitivity varies among species, life stages, sexes, trophic groups and with body condition. Acclimation or adaptation may cause varying sensitivity even within species. Ecosystem-scale effects, for example on ecological function, are poorly understood. Effects vary between metals such as cadmium, copper, lead, chromium, zinc and nickel in order of decreasing toxicity. Aluminium is important in acidified headwaters. Biological effects depend on speciation, toxicity, availability, mixtures, complexation and exposure conditions, for example discharge (flow). Current water quality monitoring is unlikely to detect short-term episodic increases in metal concentrations or evaluate the bioavailability of elevated metal concentrations in sediments. These factors create uncertainty in detecting ecological impairment in metal-impacted ecosystems. Moreover, most widely used biological indicators for UK freshwaters were developed for other pressures and none distinguishes metal impacts from other causes of impairment. Key ecological needs for better regulation and management of metals in rivers include: i) models relating metal data to ecological data that better represent influences on metal toxicity; ii) biodiagnostic indices to reflect metal effects; iii) better methods to identify metal acclimation or adaptation among sensitive taxa; iv) better investigative procedures to isolate metal effects from other pressures.

Laboratory data on the effects of water chemistry on cationic metal toxicity and bioaccumulation show that a number of chemical parameters, particularly pH, dissolved organic carbon (DOC) and major cations (Na, Mg, K, Ca) exert a major influence on the toxicity and/or bioaccumulation of cationic metals. The biotic ligand model (BLM) provides a conceptual framework for understanding these water chemistry effects as a combination of the influence of chemical speciation, and metal uptake by organisms in competition with H^+ and other cations. In some cases where the BLM cannot describe effects, empirical bioavailable models have been successfully used. Laboratory data on the effects of metal mixtures across different water chemistries are sparse, with implications for transferring understanding to mining-impacted sites in the field where mixture effects are likely.

The available field data, although relatively sparse, indicate that water chemistry influences metal effects on aquatic ecosystems. This occurs through complexation reactions, notably involving dissolved organic matter and metals such as Al, Cu and Pb. Secondly, because bioaccumulation and toxicity are partly governed by complexation reactions, competition effects among metals, and between metals and H^+, give rise to dependences upon water chemistry. There is evidence that combinations of metals are active in the field; the main study conducted so far demonstrated the combined effects of Al and Zn, and suggested, less certainly, that Cu and H^+ can also contribute. Chemical speciation is essential to interpret and predict observed effects in the field. Speciation results need to be combined with a model that relates free ion concentrations to toxic effect. Understanding the toxic effects of heavy metals derived from abandoned mines requires the simultaneous consideration of the acidity-related components Al and H^+.

There are a number of reasons why organisms in waters affected by abandoned mines may experience different levels of metal toxicity than in the laboratory. This could lead to discrepancies between actual field behaviour and that predicted by EQS derived from laboratory experiments, as would be applied within the WFD. The main factors to consider are adaptation/acclimation, water chemistry, and the effects of combinations of metals. Secondary effects are metals in food, metals supplied by sediments, and variability in stream flows. Two of the most prominent factors, namely adaptation/acclimation and bioavailability, could justify changes in EQS or the adoption of an alternative measure of toxic effects in the field. Given that abandoned mines are widespread in England and Wales, and the high cost of their remediation to meet proposed WFD EQS criteria, further research into the question is clearly justified.

Although ecological communities of mine-affected streamwaters might be over-protected by proposed WFD EQS, there are some conditions under which metals emanating from abandoned mines definitely exert toxic effects on biota. The main issue is therefore the reliable identification of chemical conditions that are unacceptable and comparison of those conditions with those predicted by WFD EQS. If significant differences can convincingly be demonstrated, the argument could be made for alternative standards for waters affected by abandoned mines. Therefore in our view, the immediate research priority is to improve the quantification of metal effects under field circumstances. Demonstration of dose-response relationships, based on metal mixtures and their chemical speciation, and the use of better biological tools to detect and diagnose community-level impairment, would provide the necessary scientific information.

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1 Introduction

The Water Framework Directive (WFD) places new emphasis on the ecological characteristics of surface waters, by requiring the attainment of "good chemical and ecological status" in water bodies that lie within EU Member States. The five classes of ecological status are essentially determined by the degree of impairment to biological, physico-chemical and hydromorphological 'quality elements'. In the context of this proposal, physico-chemical quality elements are of particular importance, not only in supporting ecological status, but also intrinsically as they include the 'specific pollutants' arsenic, copper, iron, and zinc. Moreover, cadmium, lead, mercury and nickel are priority substances, and exceedance of environmental quality standards (EQS) affects chemical status. New EQS are being developed as part of the WFD, although some will remain unchanged. For Zn, Cu and Cd, these standards are hardness-related.

Mine waters have been identified as a significant issue in the majority of Environment Agency draft River Basin Management Plans. They are one of the largest drivers for chemical pollution under the WFD. There are significant EQS failures for Annex 8/10 metals ('specific pollutants' and 'priority substances') in many rivers due to abandoned mines (particularly Cd, Pb, Zn, Cu, Fe) with 94 per cent of Pb and 70 per cent of Cd EQS failures being located in metal mining areas. Existing EQS may be over-protective of aquatic life which may have adapted over centuries of exposure. This has implications for classification (Annex V), objective setting (Article 4), Programmes of Measures (Article 11), and the reduction in priority substances (Article 16, Priority Substances Daughter Directive).

Thus, there are important questions for mine-affected areas in England and Wales. Are proposed WFD EQS values, aimed at waters receiving relatively recent discharges of metals, appropriate for mine-affected waters with long-term chronic metal inputs? Do adaptation and bioavailability effects warrant the establishment of more relevant EQS? Are higher local background metal levels justified for streams draining areas of abandoned mines?

The Environment Agency project, which this review forms part of, aims to investigate the ecological impact of metals in rivers, and to develop water quality targets (alternative objectives for the WFD) for aquatic ecosystems that have adapted to long-term mining pollution.

Here we present the results of Phase 1 of the project, a literature review on the ecological impacts of metals in rivers. The review covers relationships of metal EQS failures to general quality assessment biology data, metal effects on plants (benthic algae and macrophtytes), invertebrates and fish, and the effects of water chemistry on metal impacts on biota. In the final chapter we summarise the available information and consider further research to address the problem. A proposal for further research in Phase 2 of the project is presented separately.

2 Sources and dynamics of metal mine water pollution and relationships between metal concentrations (EQS failures) and ecological quality metrics

2.1 Introduction

Pollution from abandoned mines is a major cause of degradation of surface water courses across many parts of England and Wales (Environment Agency, 2008a). Since 1994 discharges from abandoned deep coal mines have been addressed by the UK Coal Authority through a rolling programme of monitoring and remediation initiatives. To this end, the Coal Authority has so far constructed some 50 full-scale mine water treatment systems. The principal pollutant of concern in coal mine waters is iron, which is typically present in elevated concentrations due to the oxidative dissolution of pyrite (FeS_2). Untreated, iron from coal mine water discharges has a tendency to rapidly form a solid (oxy)hydroxide precipitate in surface watercourses. In some instances coal mine water discharges are also acidic, although most acidic discharges arise from abandoned spoil heaps, which are outside the environmental management remit of the Coal Authority. Sulphate concentrations are also typically elevated in coal mine water pollution, although reducing its concentrations is not, in itself, an objective of mine water treatment in the UK. This is partly because its impact on surface watercourses is usually limited, and partly because of the great difficulty of removing sulphate using conventional treatment technologies[1].

In order for the Coal Authority to address coal mine water pollution in a logical and cost-effective way, the Environment Agency (at the time the National Rivers Authority) undertook a national study of the impacts of coal mine water pollution to provide a prioritised list of coal mine water discharges for the Coal Authority to work from (those with the severest impact at the top of the list). The basis for this prioritisation exercise was the chemical and ecological impacts of these mine water discharges. The approach used is discussed in detail by Davies *et al.* (1997), and was critically evaluated by Jarvis and Younger (2000). Essentially the ecological impact was assessed using the biological monitoring working party (BMWP) score. Specifically the ecological impact below mine waters was assessed by measuring decreases in macroinvertebrate abundance and diversity compared to upstream locations, and the extent of ecological impact was measured by identifying the downstream point at which BMWP score returned to its upstream value.

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[1] Exactly because of its conservative behaviour, sulphate arising from mines can be a major concern in groundwater used for public supply. Sulphate may be transported over considerable distances down hydraulic gradients, potentially to abstraction boreholes, which may result in exceedances of sulphate concentrations for drinking water supply purposes (see Neymeyer *et al.*, 2007).

This approach worked well for coal mine water discharges. The deposition of iron hydroxide precipitates on stream beds is a major cause of faunal impoverishment, and a strong statistical inverse correlation between iron concentration and BMWP score in affected streams has already been shown (see Jarvis and Younger, 1997). Physical blanketing of stream beds with iron hydroxide, and reduced light penetration due to suspended (iron hydroxide) solids in the water column, appear to be the main causes of decreases in abundance and diversity. Acidity is also an important cause of faunal impoverishment, but in many instances around the UK deep coal mine water discharges are in fact net-alkaline (i.e. alkalinity > acidity), and therefore iron concentration is the predominant cause of reduction in macroinvertebrate abundance and diversity. Despite being developed to assess organic pollution, in the prioritisation exercise of abandoned deep coal mines the BMWP score was an effective metric for assessing ecological impacts.

Metal mine discharges arise from the oxidative dissolution of metal sulphide minerals such as galena (PbS), sphalerite (ZnS) and greenockite (CdS), and discharges from abandoned metal mines may consequently contain elevated concentrations of these metals. Pyrite may also be present in association with these minerals, and therefore iron concentrations may be elevated. However, at many sites this is not the case, and therefore the visible deposition of iron (oxy)hydroxides, which typifies coal mine drainage, is often absent below metal mine water discharges. The strong inverse relationship between iron concentration and BMWP score is therefore often not evident below metal mine water discharges. In the absence of this strong cause-effect relationship, discerning the impacts of metal mine water discharges on the ecology of receiving streams, at least using the BMWP score, is far more difficult. The relationship between metal ion concentrations, such as Zn, Pb, Cd, and BMWP scores in streams affected by metal mine water pollution are far more ambiguous than for coal mine drainage.

This ambiguity raises questions about the appropriateness of proposed environmental quality standards (EQS) in streams that have long been impacted by metal mine water discharges. In particular, some streams may have been impacted by metal mine water discharges for decades or even centuries, and therefore it is conceivable that acclimation and/or adaptation of organisms may have occurred. Thus, some streams may fail to meet EQS for one or more metals but without exhibiting signs of ecological stress, at least as indicated by metrics such as the BMWP score.

The purpose of this report is to review the effects of metals and other chemical factors that influence the behaviour of these metals, on aquatic flora and fauna (plants, invertebrates, fish), with a view to developing better ecological indicators of metal mine water pollution.

In this first section of the report, a preliminary assessment of the relationship between metal ion concentrations and BMWP scores is conducted for a large region of Wales known to be impacted by metal mine water pollution. Before moving to the first of these main areas of discussion, a brief outline of the dynamics of metal mine water discharges is provided, together with a commentary on contamination of sediments in mine-impacted river catchments. Discharges from abandoned mines may exhibit rather unique characteristics in terms of their temporal variability, which may influence ecological welfare in receiving watercourses, and adds a further layer of complexity to the interpretation of ecological data from impacted watercourses. Because of their long-standing nature, and the potential at many sites for ongoing inputs of contaminated sediment (from waste spoil heaps, for example), bed sediment in many streams affected by mine water pollution may contain elevated concentrations of metals. The precise impact such sediments may have on aquatic ecology is unclear, but may be an avenue worthy of future investigation. Therefore in the section below, examples of the characteristics of such sediments are provided.

2.2 Metal mine water pollution dynamics

Upon first emergence to the surface, discharges from abandoned deep mines are typically of very poor quality, due to initial ‘flushing’ of particularly contaminated waters from the abandoned mine void. Over time water quality tends to improve, not least because the potential for further oxidative dissolution of sulphide minerals is much reduced in the anoxic conditions of a flooded mine void. In contrast, dissolution of basic minerals such as calcite may continue under anoxic conditions, and therefore once a void is flooded there is greater potential for alkalinity-generating processes than acid-generating ones, further limiting the severity of long-term pollution to the surface environment.

For most abandoned mines across England and Wales this ‘first flush’ phenomenon has already occurred, and mine waters tend to have rather consistent quality, albeit remaining polluted. Superimposed on this long-term evolution of water quality are seasonal and diurnal fluctuations in quality, with implications for the aquatic ecology at downstream locations. The nature of these fluctuations depends on the former mine facilities from which these waters emerge, which can be divided into three categories:

(1) *Mine waters arising from deep mine workings*: Such discharges show the most consistent quality, principally because the influence of rainfall events and changes in groundwater tables are minimised by a large ‘reservoir’ of water within the abandoned workings.

(2) *Mine waters arising from shallow mine workings*: In unsaturated (or partially flooded) shallow workings the influence of meteoric water may be substantial. Infiltrating water may be transported rapidly through abandoned workings, particularly where mine features such as open stopes[2] are present. Mine water flow-rates to surface may therefore increase. Whilst concurrent decreases in metal ion concentrations may also result (from simple dilution), in some instances (particularly following protracted dry periods) residues of oxidised sulphide minerals may be flushed ahead of the additional recharge water, meaning that the relationship between flow-rate and metal concentration may not be linear, and absolute metal loads to surface streams may increase.

(3) *Mine spoil discharges*: Most susceptible to rainfall events, not only via subsurface drainage through the spoil heap, but also by surface runoff transporting both sediment and metals directly to surface watercourses. Suspended solids, metal concentrations and flow-rates may increase simultaneously.

Thus, there is considerable scope for variation of both metal concentrations and flow-rates of mine water discharges which, coupled with varying hydrological conditions in surface waters, may result in variations in metal ion concentrations in these receiving streams. In some streams affected by mine water discharges, diurnal fluctuations in metal ion concentrations (especially zinc) have been reported. These are thought to be due to the presence of zinc-tolerant algae (such as *Mougeotia*, *Stigeoclonium*), which often flourish in polluted watercourses because of the competitive advantage they gain precisely because of the presence of zinc. During daylight hours such algae are thought to take up zinc from the water column, whilst at night they do not, resulting in higher reported concentrations at night than during the day (see Gammons *et al.*, 2007).

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[2] An open stope is a vertical or near-vertical mine void which extends (not necessarily intentionally) all the way to the surface

Thus, there is a complex interaction of factors that may result in considerable variation in metal concentrations and flow-rates of mine water discharges and the streams into which they discharge. Detailed characterisation of such fluctuations is difficult due to the intensity of monitoring required, but it seems likely that such changes will inevitably have an impact on stream ecology, which is the reason the issue is raised here.

2.3 Stream sediment contamination in former mining districts

The Environment Agency (2008b) has recently reviewed the extent and implications of contaminated river sediment (and floodplain sediment) from former mining activities across England and Wales. There is evidence that stream sediments in formerly mined river catchments contain elevated metals concentrations. The Environment Agency (2008b) reports metal concentrations of up to 11,000 mg/kg for As, 29.5 mg/kg for Cd, 8,000 mg/kg for Cu, 20,310 mg/kg for Pb, and 12,203 mg/kg for Zn in channel sediments of rivers below mining districts. In a separate investigation Gozzard (2008) reported a staggering 73,925 mg/kg Zn in channel sediment of the River West Allen, Northumberland. In England and Wales there are currently no sediment quality guidelines, but to put these values into context the proposed values are shown in Table 2.1, below.

Table 2.1 Proposed sediment quality guideline values for selected metals and arsenic (from Environment Agency, 2008b).

Metal(loid)	Threshold effect level (TEL) (mg/kg)	Predicted effect level (PEL) (mg/kg)
As	5.9	17
Cd	0.596	3.53
Cu	36.7	197
Pb	35	91.3
Zn	123	315

Gozzard (2008) adopted the protocol of Tessier *et al.* (1979) to make a preliminary evaluation of the availability of zinc contained in the sediment of the River West Allen. For the sample cited above, zinc was distributed as follows:

Exchangeable (adsorption)	2%
Carbonate	28%
Fe/Mn oxides	13%
Organic	10%
Residual (crystalline structures)	47%

Although such data are informative, and imply that a major proportion of all sediment-associated zinc may be ‘available’, the actual bioavailability of these metals to benthic organisms, and exposure routes of such organisms to contaminants, remains unclear. The role of contaminated sediments in governing ecological quality in mining-impacted rivers therefore warrants further exploration, particularly if the impacts of such metal sources are to be discerned from those of metals in the water column.

2.4 Relationship between metal ion concentration(s), EQS limits, and BMWP score

To gain a broad indication of the relationship between commonly used biological quality metrics and water chemistry, and especially metals, selected Environment Agency Wales water quality and biology data were collated for the Gwynedd and Ceredigion regions of Wales. It should be noted that the BMWP and associated average score per taxon (ASPT) scoring systems were originally developed as indicators of organic pollution, and therefore might not be expected to reflect impacts of metal pollution. Despite this caveat, BMWP scores, number of taxa and ASPT values were compared with metal concentrations (Al, As, Cd, Cu, Fe, Mn, Ni, Pb) and other chemical variables such as pH, dissolved organic carbon (DOC), NO_3-N, hardness and dissolved oxygen concentration. Gwynedd and Ceredigion were selected on the basis that (a) they are well-known former mining districts, and (b) urban areas are limited, and therefore the influence of at least one confounding effect is minimised. The relevant area is shown in Figure 2.1.

In order to make such a comparison, data for locations monitored for both ecological and chemical quality are required. Environment Agency ecology and chemistry monitoring locations do not always coincide, and the chemical monitoring conducted at biology sites is in some cases very limited. Across Gwynedd and Ceredigion 87 locations were identified where biology and chemistry data are available. These data were merged into a single database to enable a preliminary assessment of the relationship between metal concentrations and macroinvertebrate health as indicated by the BMWP score, number of taxa and ASPT.

Historically, Environment Agency biology monitoring has taken place three times a year, corresponding to spring, summer and autumn, and such data were made available for this analysis. Multiple years were also provided. To simplify this preliminary analysis, spring 1990 was selected on the grounds that more sites were monitored in this season and year than any subsequent season and year combination (at least for the dataset provided here). Water quality monitoring has historically been undertaken monthly. For this assessment, annual mean values were calculated for 1990. Some 260 different chemical variables appear on the Environment Agency's water quality reports (although not all of them are determined for any given sample). This list was reduced to 13 variables for the purpose of this analysis. Clearly, there is scope for conducting a more in-depth interrogation of water quality and biology data, both within the region selected here, and across other regions of England and Wales. It would be useful to compare regions in which baseline water quality was different due to geology and lithology, such as the softer waters of the north Pennines compared to the harder waters of mid-Wales.

Even at locations which are both chemistry and biology monitoring points, water quality data can be limited, and/or of limited value for the purposes of statistical analyses. Zn, Pb and Cu are the only metals routinely analysed (out of 87 sites, 70 sites for Pb, 70 for Cu and 86 for Zn). Furthermore, routine measurements are for total concentration, and determination of dissolved concentrations happens only occasionally (28 sites for Pb, 42 sites for Cu, and 22 sites for Zn). A further restriction of the data when looking at any given region, as opposed to pre-selecting locations at which metal concentrations are known to be high, is that potentially large numbers of values are reported as below detection limits (or are assigned the value of the detection limit).

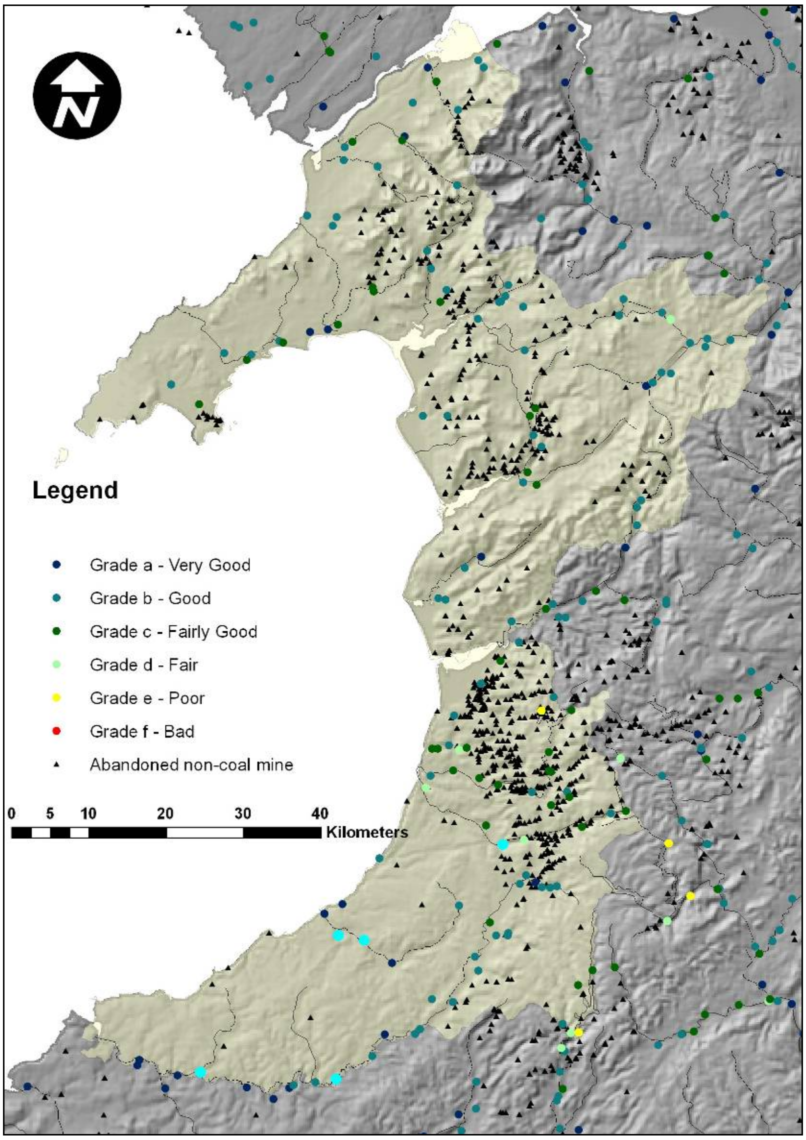

Figure 2.1 Gwynedd and Ceredigion (pale colour), with known mine sites (triangles) and biological quality grades shown.

Notwithstanding these limitations, the clearest relationship between BMWP score and any individual metal is that with total Zn concentration. The range of zinc concentrations at the 86 sites at which it has been monitored is shown in Figure 2.2, and its relationship with BMWP score and number of taxa recorded is shown in Figures 2.3 and 2.4 respectively. There is no relationship between total Zn and ASPT. Dissolved aluminium concentrations show a similar inverse relationship with BMWP score, although the dataset is smaller (n = 24) (Figure 2.5). No clear relationship between any other metal (total or dissolved fraction) and BMWP score, ASPT or number of taxa, is apparent from simple inspection of data alone. There may be a number of reasons for this, including:

- there is no cause-effect relationship;
- there are insufficient data to permit an assessment of any relationship between chemical and biological variables;
- there is insufficient spread of concentrations for some metals, where multiple results indicate below detection limits;
- adaptation/acclimation;
- confounding effects.

Neither is there any discernible relationship between the non-metal variables inspected and biological indices. This appears to be due largely to a paucity of data .

There is a relationship between total molar metal concentration and BMWP score (Figure 2.6), but this largely appears to be a consequence only of the comparatively strong inverse relationship between Zn and BMWP. Thus, the dominance of zinc appears to mask any influence on BMWP score exerted by other metals, though absolute concentrations of other metals are comparable to, or greater than, that of zinc (mean values of the data inspected are Zn = 68 μg/l (1.0 μM); Mn = 69 μg/l (1.3 μM); Al = 143 μg/l (5.3 μM); Fe = 155 μg/l (2.8 μM)).

Many mine waters contain at least one metal in excess of its EQS limit. In fact the most severe mine water discharges, which appear at the upper end of the new prioritised list of non-coal mine water discharges (Jarvis *et al.*, 2008) invariably contain multiple metal contaminants, particularly where pH is low. Since these will be transported into the receiving streams below them, making a distinction between the ecological impacts of individual metals will need to be addressed in future investigations.

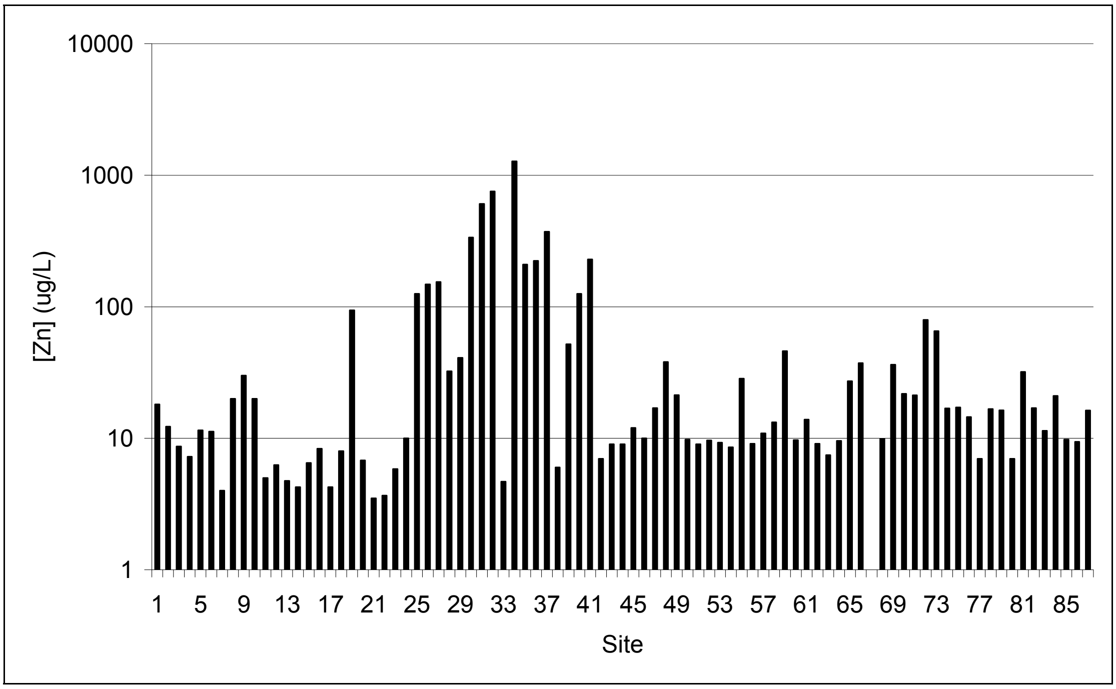

Figure 2.2 Range of total Zn concentrations at 86 sites across Gwynedd and Ceredigion (mean annual averages for 1990).

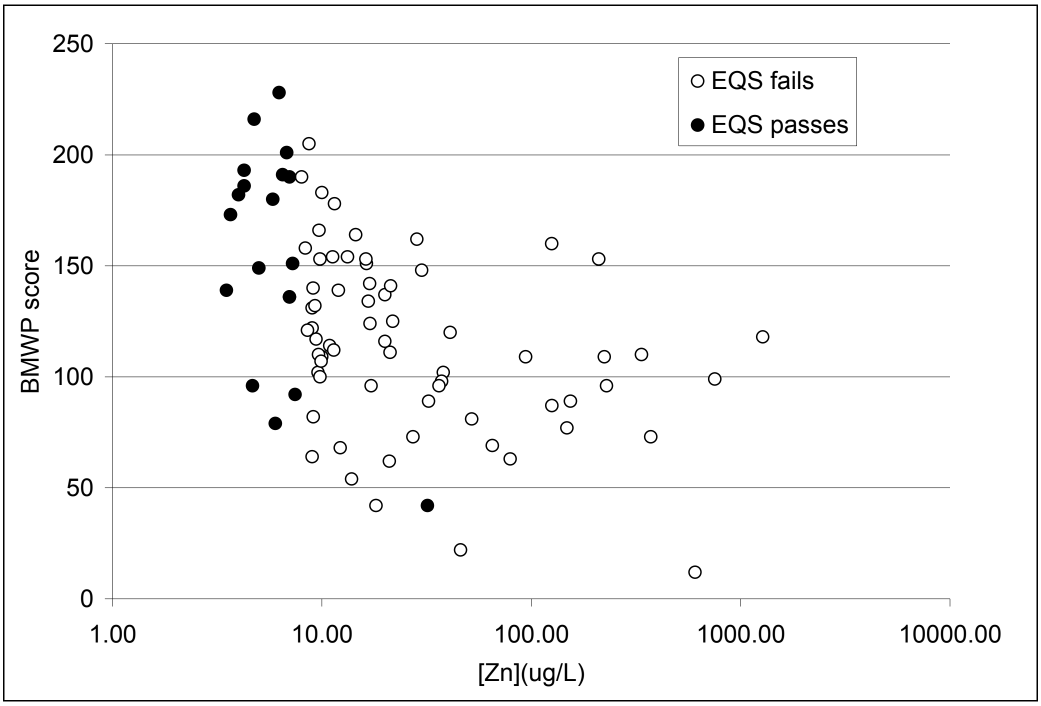

Figure 2.3 Relationship between total Zn concentration and BMWP score (n = 86). Hardness is accounted for in identifying sites at which Zn is less than EQS.

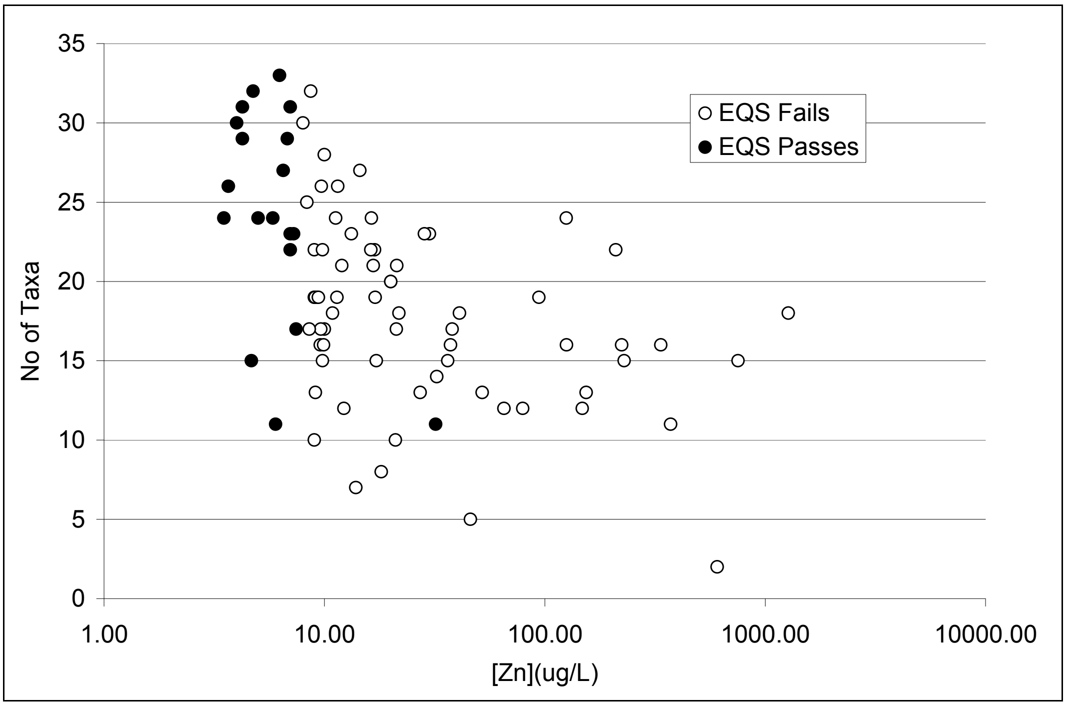

Figure 2.4 Relationship between total Zn concentration and number of taxa (n = 86). Hardness is accounted for in identifying sites at which Zn is less than EQS.

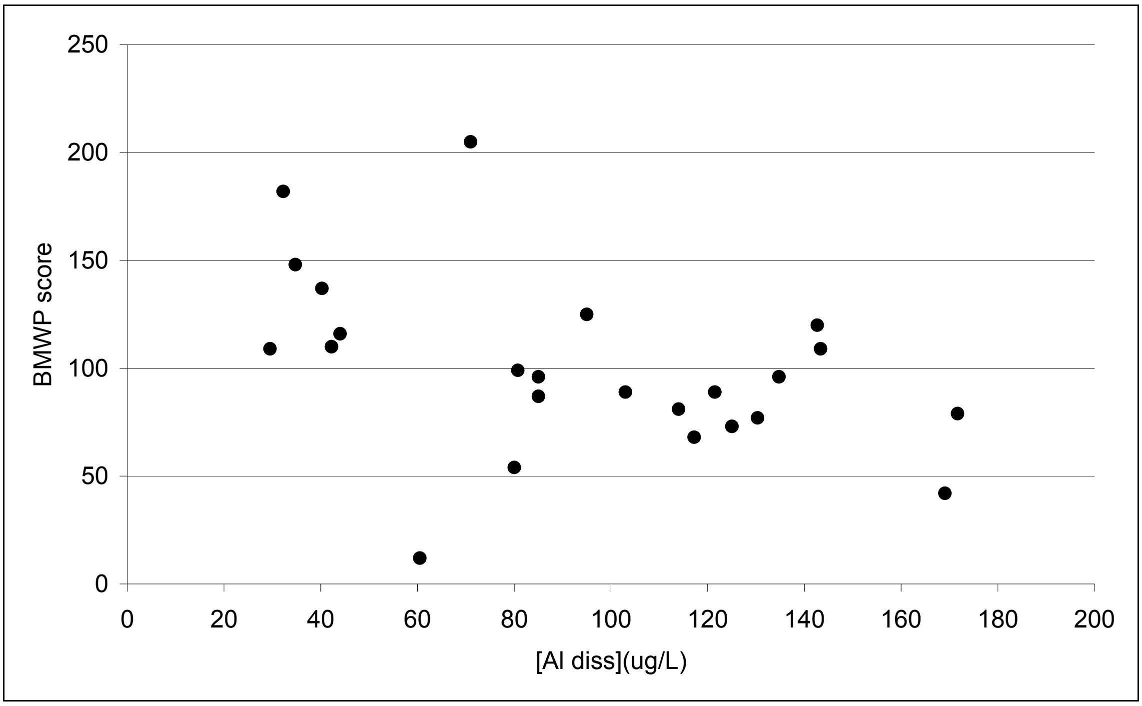

Figure 2.5 Relationship between dissolved aluminium concentration and BMWP score (n = 24).

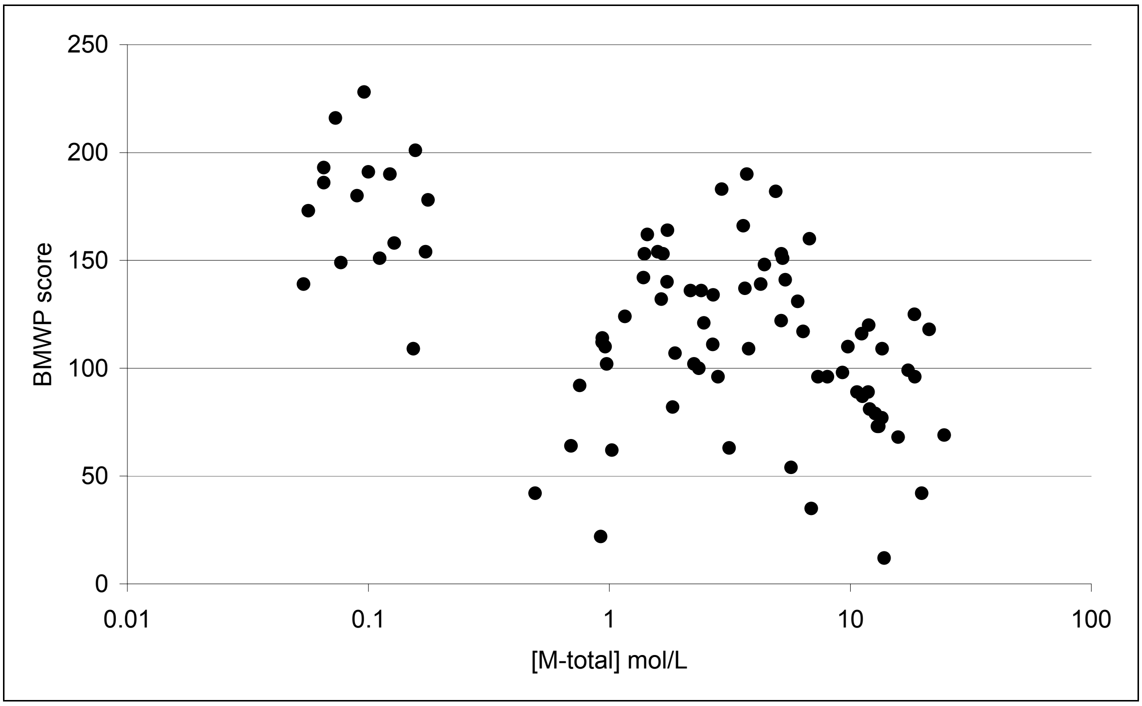

Figure 2.6 Relationship between total molar metal concentration (Al, Cd, Cu, Fe, Mn, Pb, Zn) and BMWP score.

Whilst zinc is less toxic than some other metals in mine water discharges (such as Cd, Cu, Pb), the prevalence of zinc in such waters may result in it being the single biggest metal of concern in absolute terms, and this is the implication from these data. Table 2.2 indicates the number of EQS failures due to selected metals at Environment Agency monitoring points across England and Wales, and also that proportion of each total for which abandoned metal mines have been identified as the most likely cause.

Table 2.2 Number of contaminant EQS failures across England and Wales, and percentage occurring in mining areas (Data courtesy of Environment Agency).

Contaminant	Number of failures	% in mining areas
Cd	133	71
Pb	69	94
Zn	470	30

It is clear from these data that zinc is a major contributor to overall failures. There is an inverse relationship between Zn and BMWP, and the BMWP scores of sites at which zinc is below the EQS limit are clearly higher than those at which it is exceeded. Nevertheless, there is considerable scatter in the data shown in Figures 2.3 and 2.4. For example, in Figure 2.3 BMWP scores ranging from 60 to over 200 are evident in waters in which Zn does not exceed its EQS (8 μg/l for waters of this hardness: under 50 mg/l), showing that other factors likely account for the variation in ecological quality. Similarly, even where Zn is an order of magnitude higher than its EQS, BMWP scores vary from around 60 to 160. The conclusion from this is that other factors influence macroinvertebrate abundance and diversity (at least insofar as it is reflected by the BMWP score), irrespective of whether Zn is substantially below or considerably above its EQS.

Numerous other factors may influence the BMWP score. One is the presence of mixtures of metals in metal mine water discharges. Such influences can be difficult to diagnose. For example, when concentrations of metals such as Pb and Cd are consistently below the detection limits of analytical equipment (as they often are at mine water discharges), it is not possible to undertake statistical analyses to demonstrate the causative influences of ecological degradation.

Another possible factor that is worth noting in specific relation to mining-related pollution is the impact of diffuse sources of pollution on absolute metal loading to rivers and streams. There is an ever-growing body of literature demonstrating the importance of diffuse sources of metal mining pollution (see Jarvis *et al.*, 2006; Mayes *et al.*, 2008; Mighanetara, 2008). Since these are most significant in high flow conditions (due, for example, to increased inputs via surface runoff from exposed waste spoil heaps) metal concentrations and flux may be substantially underestimated by routine water quality data collected by the Environment Agency (typically collected during baseflow conditions). Such sources of metal pollution, coupled with long-standing point source discharges, may also result in extremely elevated sediment-metal concentrations in impacted streams and rivers. Thus, there may not be a clear relationship between aqueous and sediment metal concentrations, with implications for the specific route by which metals result in stress effects on biota.

In terms of confounding effects, there is a strong inverse relationship between pH and BMWP score, as illustrated in Figure 2.7 (a similar relationship exists between pH and number of taxa). Although there are some notable exceptions, metal mine water discharges tend not to exhibit low pH, and the distribution of pH values are unlikely to be related to the locations of mine water discharges. There is no clear relationship between zinc concentration and pH for this dataset.

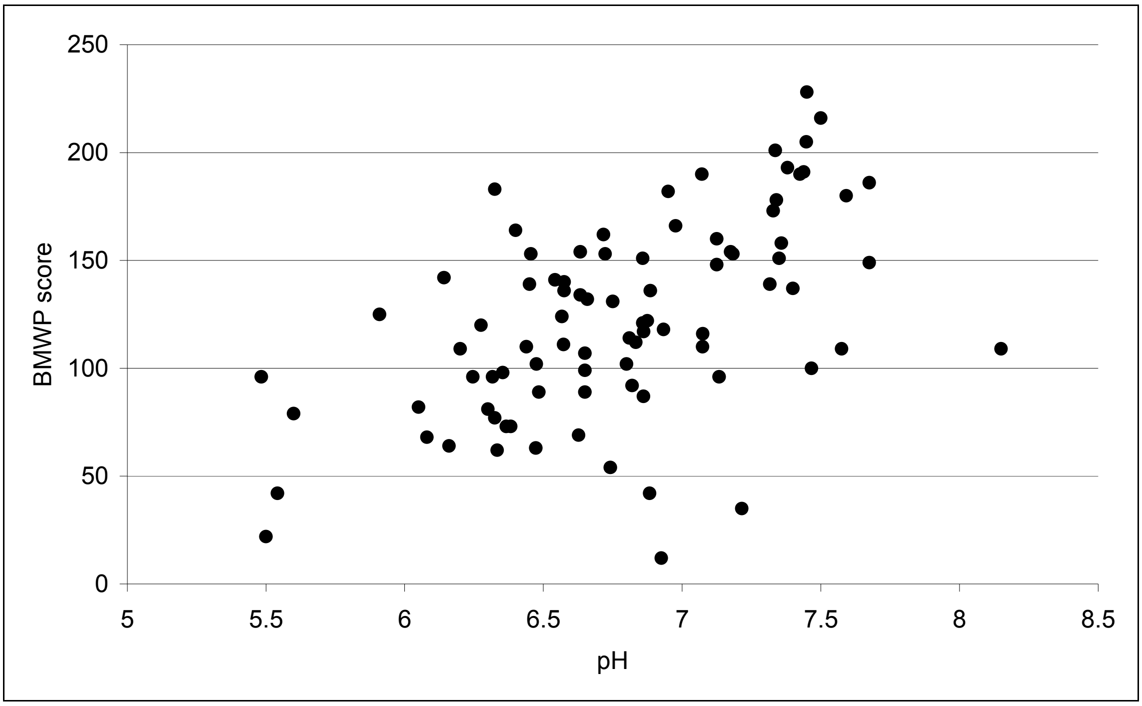

Figure 2.7 Relationship between pH and BMWP score across sites in Gwynedd and Ceredigion.

As expected, there is a strong relationship between pH and dissolved aluminium (Figure 2.8). The impacts of pH and aluminium individually are therefore likely to be difficult to discern. Aluminium is also a minor contaminant in most circumneutral metal mine water discharges, and therefore pH and aluminium impacts on invertebrate abundance and diversity are unlikely to be due to past mining activities, but will serve to complicate interpretation of the impacts of metals associated with these discharges.

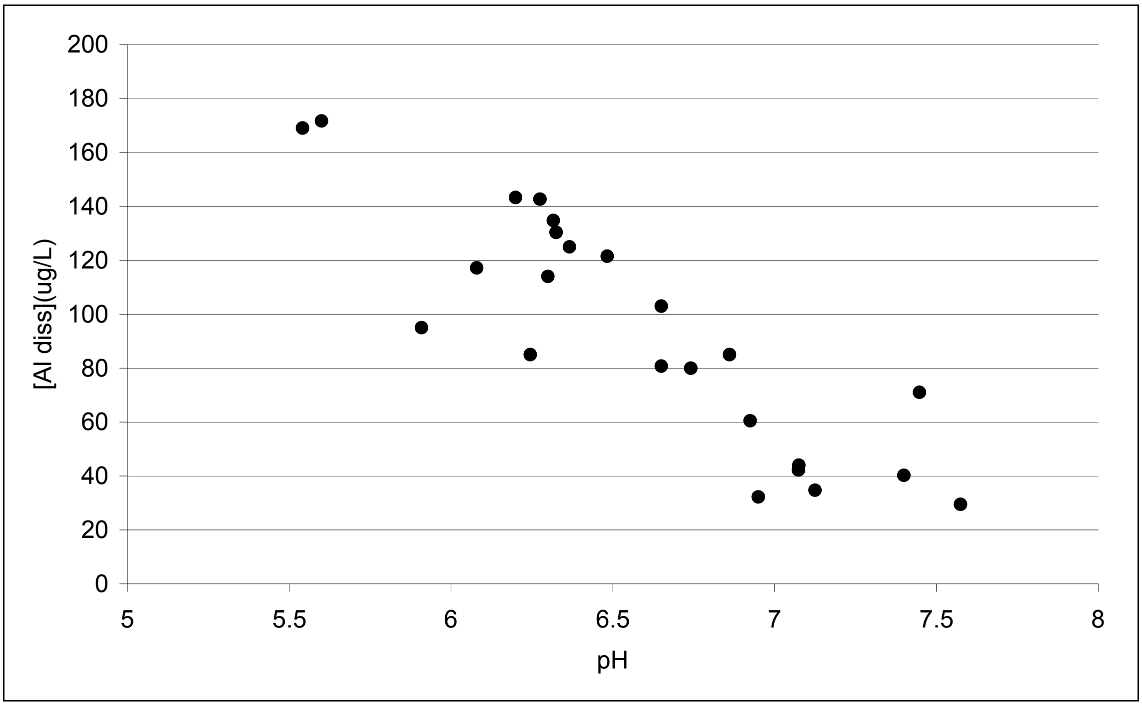

Figure 2.8 Relationship between pH and aluminium across sites in Gwynedd and Ceredigion.

Many studies of the use of biotic indices and other metrics to evaluate the impacts of mine water pollution, and the impacts of such waters in general, have targeted stream and river reaches in relatively close proximity to the source(s) of mine water pollution (see Armitage *et al*., 2007; García-Criado, 1999; Gray and Delaney, 2008). However, regulatory biological monitoring points are typically located some distance from mine water discharges (which are often in upland regions), in some cases tens of kilometres away. Therefore concentrations of key metal contaminants, whilst still above EQS limits in some cases, are substantially lower than those in the mixing zones downstream of mine water discharges. Regional-scale assessments of the degree to which metrics of biological welfare indicate mine water pollution will inevitably be more susceptible to confounding effects than investigations at a local scale. Even at a local scale, where metals associated with mine waters are likely the dominant cause of biotic stress, variation in the success of various types of biotic index are evident. We need to develop metrics to assess the ecological impacts of metals from abandoned mines. In addition, however, when considering ecological indicators of mining pollution there appears to be a need to identify the spatial loci for such investigations (such as an immediately impacted river reach or regulatory monitoring point), since dominant influences on biota are likely to change across spatial scales.

2.5 Summary and conclusions

- This section has briefly outlined the background to the assessment of ecological impacts due to mine water pollution, and in particular illustrated that the method used to assess the ecological impacts of abandoned coal mines is unlikely to be suitable for abandoned metal mines.

- Complications arising from the variability of flow-rates and metal concentrations of mine water discharges, and possible ecological impacts of sediment metals as well as those in the aqueous phase, should also be considered.

- To preliminarily assess the influence of metals on the ecological quality of streams and rivers affected by metal mine waters, water quality and biology data for 87 sites across Gwynedd and Ceredigion were used to evaluate the extent to which the BMWP score and related metrics reflect metal concentrations in surface waters. To some degree the utility of the data was limited by a paucity of coincident data for key variables at individual sites.

- Nevertheless, it may be possible to use Environment Agency water quality and biology data to establish statistical relations between chemical variables and metrics of ecological quality. For example, with larger datasets multivariate statistical techniques (such as partial canonical correspondence analysis; Beasley and Kneale, 2003) might shed light on key controls on ecological welfare. In cases where specific metals (or combinations of metals) associated with former mines are identified as important controls, such analyses may help to define goals for mine water remediation. If such an approach is to be pursued, consideration should be given to the spatial scale of investigation, since major controls on ecological quality below mine water discharges will change with distance downstream.

- Visual representation and preliminary statistical analyses in this study show that zinc has the most obvious relationship with BMWP score, specifically an inverse relationship. Although total molar metal concentration also exhibits an inverse relationship with BMWP score, this appears to be due to the zinc, despite the fact that its concentration in these waters is comparable to, or less, than the concentrations of other metals. There is considerable scatter in the relationship between zinc concentration and BMWP score, implying that other factors are also influential. This applies at sites at which Zn is below its EQS limit, but also at sites where Zn concentration is at least an order of magnitude above its EQS limit.

- Both pH and dissolved aluminium concentration have a relationship with BMWP (positive and negative respectively). However, pH and aluminium are themselves strongly inversely related. It seems unlikely that variation in either variable is driven by mine water discharges, which are largely circumneutral and low in aluminium. Nevertheless, this relationship will complicate further interpretation of the ecological impacts of mine waters.

- Many mine waters, especially the most heavily polluted, contain mixtures of metals, all of which may be above their EQS. There is thus potential for additive or inhibitory effects on the ecology.

- Further work should identify key mine water variables that result in statistically significant decreases in BMWP score and related metrics. Other areas of investigation include:

    1) The extent to which variations in mine water chemistry, especially episodic events, govern ecological abundance and diversity.

    2) The influence of contaminated stream sediments on ecological abundance and diversity, and specifically the availability of these metals to aquatic organisms.

    3) Regional-scale differences in the response of biota to metal inputs from abandoned mines, for example due to differences in geology and lithology.

3 Metal effects on plants, including algae and diatoms, in mine-impacted rivers

3.1 Underlying ecological principles

Central to an understanding of the ecological effects of pollution is the concept of “stress”, defined as “*external constraints limiting the rate of resource acquisition, growth or reproduction of organisms*” (Grime, 1989) or “*an environmental condition that, when first applied, impairs Darwinian fitness*” (Sibley and Calow, 1989). These definitions apply principally to individual organisms; however, the integrated effects on individuals may ultimately affect community structure. Although the concepts of succession (natural sequence of changes in an ecosystem over time), and climax (equilibrium community at the end of a succession) are difficult to apply in flowing waters, biologists recognise, implicitly or explicitly, an “expected” or “ideal” community for a given river type. Departures from this suggest that some form of external stress is acting on the system, although many forms of stress are natural (extremes of temperature, floods and so on) and, indeed, stress is the driving force behind natural selection and evolution (Hoffman and Parsons, 1993).

The “struggle for existence” occurs primarily within populations of the same species rather than between species. For any particular population exposed to heavy metal toxicity downstream of a mine, some individuals may prove more tolerant than others and will survive and possibly thrive in the absence of competition. It is a classic case of “natural selection”: successful (tolerant) individuals pass on their genes to the next generation which, therefore, contains a greater proportion of the genes responsible for tolerance. Over time, populations come to be composed almost entirely of tolerant individuals. This has been shown particularly clearly in the algae (see below) and some grasses (Bradshaw and McNeilly, 1981).

However, particular species within a community will also vary in the extent to which they are affected by a toxin. This may be due to physiological differences between species but also to ecological or behavioural factors: plants may vary in the proportion of surface area exposed to water, while invertebrates that ingest large quantities of sediment may accumulate higher concentrations of toxin than species that ingest leaf material. Thus, the relative composition of species within a stream may change. One that is particularly sensitive may decline in abundance whilst one that is more tolerant will increase. As the concentration of toxin increases, some species may disappear altogether while, at very high concentrations, conditions may approach those of biologically “extreme” environments such as saline lakes and thermal springs (Brock, 1969). When the level of environmental stress is particularly high, it becomes the principal determinant of species numbers at a site. At lower stress levels, however, other factors (such as diversity of habitats, nutrients) may also affect species number (Fryer, 1980). The relationship to stress can therefore be characterised as in Figure 3.1. Whitton and Diaz (1980) present a good example of this type of relationship, based on the number of photosynthetic organisms found in a large survey of Zn-polluted streams in UK, Europe and the USA.

The reduction in competition from less tolerant individuals at high concentrations of heavy metals, along with changes in grazing pressure, if grazing invertebrates are also metal sensitive, may enable the few species that are tolerant to thrive. One result can be especially lush growths of algae growing at the entrance to mine adits or in metal-rich streams downstream from these (Armitage, 1979; Patterson and Whitton, 1981). Such massive growths may themselves change conditions in the stream, by smothering the substratum (Armitage, 1979) or by removing oxygen from the water at night, so interpretation of these effects is not necessarily straightforward.

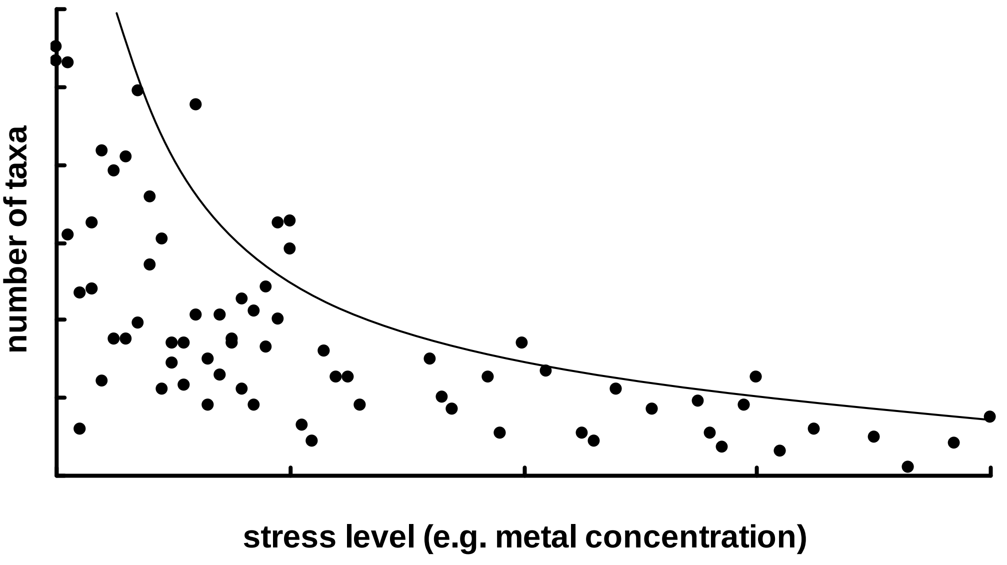

Figure 3.1 Generalised view of the relationship between environmental stress and number of plant species recorded. Stress sets an upper limit on the number of taxa found at a site, with the effect becoming more pronounced as the level of stress (such as concentration of metals) increases. The actual number of taxa found may be lower due to other reasons. A low number of taxa is not, of itself, indicative of a high level of stress; however, a high number of taxa may well be indicative of the absence of stress.

3.2 Historical perspective

Kathleen Carpenter, visiting the then active mining regions of West Wales in the 1920s, noted that the flora immediately downstream of mines was sparse – limited only to growths of the red algae *Batrachospermum* and *Lemanea* along with some mosses and liverworts (Carpenter, 1924). The greater the distance she travelled downstream from the mines, the more species she recorded and, visiting the site again after the mines closed, she recorded a gradual increase in species numbers. Her observations extended only to those plants which were visible to the naked eye and these streams are, at best, a harsh habitat for plants. But these 80-year old observations lay out some general principles still applicable today.

Most of the heavy metal mining activity in the UK ceased within two decades of Carpenter's studies but streams in these areas continued to receive inputs, both direct drainage from adits and leaching through and runoff from spoil heaps and contaminated land. Although a few workers continued to study the effects of metals on animals (such as Jones, 1940), Hynes's (1960) chapter on the "biological effects of poisons" records a single study (Butcher, 1955) with direct observations of the effects of heavy metals on algae. A rise in public concern during the 1970s led to more stringent legislation against heavy metal discharges (European Community, 1976) and more studies. The former mining regions of northern England and Wales, in particular, formed valuable outdoor laboratories.

An important body of work was performed by Whitton and co-workers, in the northern Pennine area, where Zn, Cd and Pb are the principal polluting metals. This area was underlain by Carboniferous limestone with high buffering capacity, which meant that pH was rarely a confounding factor in data interpretation. Their principal findings – which are largely supported by data and observations elsewhere in the temperate world – were:

1. A reduction in the number of taxa recorded as metal concentrations increased. Say and Whitton (1981) recorded a maximum of 61 photosynthetic species in an unpolluted stream close to Nenthead in the northern Pennines, but only 25 in a nearby stream with very high (mean = 25.6 mg/l) Zn. The metal-tolerant populations were drawn from most of the photosynthetic phyla present in unpolluted reaches, indicating that the ability to acquire tolerance is a widespread phenomenon amongst photosynthetic organisms. Similar results are available from elsewhere in the world (Besch *et al.,* 1972; Whitton, 1980; Moore, 1981). Exceptions do occur: Deniseger *et al.* (1986) found no pronounced effect on species number but this may reflect their experimental design (in particular, their use of artificial substrata), and Say and Whitton (1982) did not find a relationship between Zn concentrations and species numbers in streams in the Pyrénées, but this is still a useful generalisation. One consequence is less competition for those taxa that can survive elevated metal concentrations, leading to mass growths.

2. The most conspicuous of these mass growths in the northern Pennines were green algae such as *Mougeotia, Stigeoclonium* and *Klebsormidium* which form copious quantities of mucilage. Again, similar observations have been made elsewhere: Williams and Mount (1965), for example, recorded mats of Cyanobacteria and associated organisms (such as *Chlamydomonas snowii*) which produce copious amounts of mucilage when subjected to high Zn concentrations in experimental channels in the USA and Besch *et al.* (1972) noted that *Mougeotia* was frequent at the most polluted sites in the Northwest Miramichi river system in Canada, whilst assemblages dominated by *Microspora*, another mucilaginous alga, were abundant in streams in the copper and lead mining regions of Cornwall (Foster, 1982a) and in the River Drish, Ireland (author, unpublished observations). The association with mucilage production was also emphasised.

3. A related observation, again made by a number of workers, is the absence of *Cladophora* from metal-enriched streams (see reviews by Whitton, 1970; 1980; Dodds and Gudder, 1992). There are few records where Zn is above one mg/l; exceptions occur where water is hard and nutrient rich, and the possibility of the toxicity being reduced through complexation cannot be ruled out.

4. Plants that do survive often accumulate high concentrations of metals. This has been shown in algae (Harding and Whitton, 1981; Patterson and Whitton, 1981; Whitton *et al*., 1989; Kelly and Whitton, 1989a) and bryophytes (Whitton *et al.*, 1982; Say and Whitton, 1983; Wehr and Whitton, 1983). The mechanism is not always clear and shows interspecific differences (Kelly and Whitton, 1989b) although Tipping *et al.* (2008) suggest that chemical speciation may be a more important factor than bryophyte species. This accumulation occurs partly by passive adsorption onto the cell wall (Wehr *et al*., 1987) and partly by absorption into the cell (Matzku and Broda, 1970), followed by binding into low molecular weight proteins (Robinson, 1989; Jackson *et al*., 1991). Clarke *et al*. (1987) noted that production of siderophores, low molecular weight chelators associated

with Fe metabolism, could be induced in the cyanobacterium *Anabaena* in the presence of Cu.

5. Whilst most taxa found growing under high concentrations of metals are morphologically identical to populations growing in areas without elevated metal concentrations, there are a number of records of morphological changes. *Stigeoclonium tenue*, a common green alga, forms a basal "mat" of intertwined filaments from which "upright" filaments grow. However, Harding and Whitton (1976) recorded only basal growths at high Zn concentrations, noting that these populations started forming upright growths when grown in culture at lower Zn concentrations. Similarly, another green alga, *Klebsormidium rivulare* (as "*Hormidium rivulare*") forms linear, unbranched filaments, which have occasional acute bends, termed "geniculations". Say *et al.* (1977) noted a greater frequency of these geniculations in populations from high Zn environments. Sorentino (1985) recorded increased mucilage production in *Klebsormidium fluitans* (as "*Hormidium fluitans*") in the presence of Cu, and that this mucilage had a high proportion of the total Cu content of the alga.

   Another characteristic of metal-enriched streams is the presence of distorted diatoms, particularly representatives of the Fragilariophyceae (Lowe, 1973; McFarland *et al*., 1997; Falasco *et al*., 2009). There is a good relationship between numbers of 'twisted' cells of *Fragilaria capucina* and ambient metal concentrations in streams in the northern Pennines, with more than a tenth of distorted cells indicating metal enrichment (Figure 3.2; author, unpublished). However, a similar study in West Wales (Atkins Ltd, 2008) showed no similar effect; even when *Fragilaria capucina* was common, no sample had more than five per cent of distorted valves. The reason for this difference between northern Pennine and Welsh sites is not clear. One possibility is that there is a negative relationship between pH and metal concentrations in the Welsh dataset, which means that the most metal-rich sites are those with the lowest pH and which are, therefore, not an ideal habitat for *F. capucina* to thrive in the first place. Most of the sites where *F. capucina* was most abundant in West Wales had under 0.1 mg l^{-1} Zn, where the proportions of twisted valves tend not to be significantly greater than background.

6. Whitton and co-workers went on to demonstrate that populations that thrived in metal-enriched conditions were genetically adapted to high metal concentrations (Harding and Whitton, 1976; Say and Whitton, 1977). They used a technique developed by Stokes *et al.* (1973) who performed bioassays in which populations from field sites were exposed to a range of metal concentrations in the laboratory. Populations from sites with background metal concentrations died when exposed to elevated metal concentrations whilst those from metal-enriched conditions were able to grow more-or-less normally. A similar procedure was used by Foster (1982b) who tested isolates of 87 species from Cornish rivers influenced by mining. She confirmed that many species appear to acquire tolerance when grown in metal-rich environments but noted that some species appear to be tolerant to metals, even when isolated from non-polluted habitats. Foster (1982b) found that, in some cases, tolerance to one metal appeared to confer tolerance to other metals; however, Shehata and Whitton (1982) observed the opposite, with mutants of *Anacysistis nidulans* (*Synechococcus* sp.) tolerant to one metal showing no increase in tolerance to other metals. A subsequent development of this was pollution-induced community tolerance (PICT) by Blanck (1985; 2002), in which communities allowed to develop on glass discs submerged in a water body are assayed for ^{14}C uptake (indicating photosynthesis) or thymidine incorporation into bacterial DNA (indicating prokaryotic metabolism). Communities that have evolved tolerance show significantly less inhibition of ^{14}C uptake than communities from clean sites when exposed to comparable levels of a pollutant.

This approach has been applied to a wide variety of pollutants, including heavy metals (Lehmann *et al.,* 1996; Admiraal *et al.,* 1999), herbicides (Guasch *et al.,* 1997) and anti-fouling agents (Blanck and Dahl, 1996). PICT integrates the species shifts within the community with shifts in the genetic structure of populations of tolerant species. This is a mixed blessing – less so, perhaps, when combined with community-level approaches.

The process by which metal tolerance is acquired was studied in the laboratory by Shehata and Whitton (1982), who repeatedly subcultured the Cyanobacterium *Anacstis nidulans* into concentrations just sufficient to cause a marked reduction in growth rate. This is a classic case of natural selection (albeit on a clonal, rather than sexually-reproducing population) which, after each subculture, selected only tolerant cells from the population. Over the course of 75 subcultures, the strain changed from one that tolerated 1.45 mg/l Zn to one capable of growing in 16.5 mg/l Zn. Further studies demonstrated that the metal-tolerant mutants have multiple copies of the metallothionein genes and lack repressors which would normally limit expression of this gene (Gupta *et al*., 1992, 1993). Ivorra *et al*. (2002) found a Zn-tolerant strain of the diatom *Gomphonema parvulum* persisted two years after isolation.

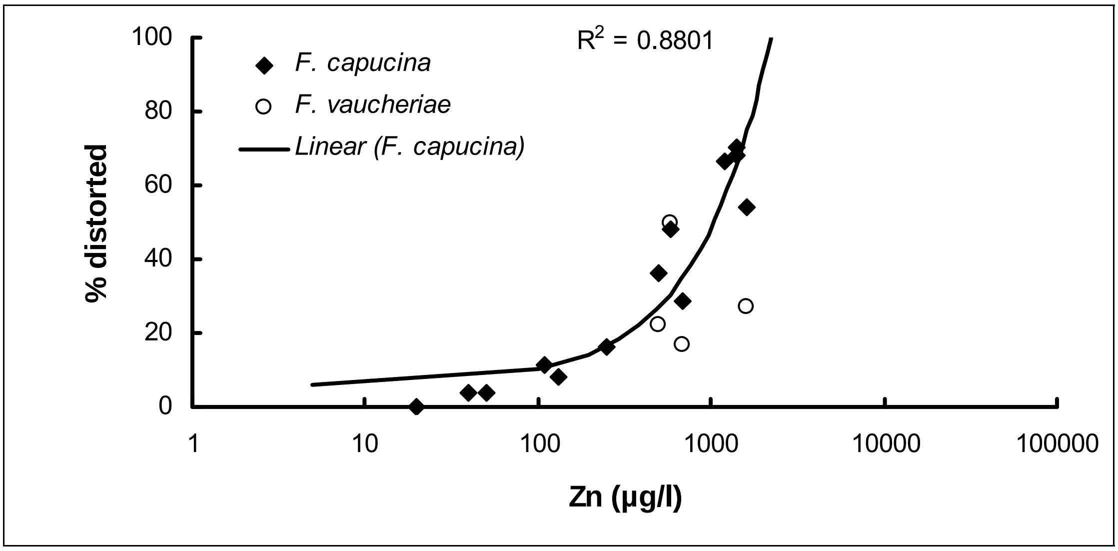

Figure 3.2 Relationship between total Zn concentration and the percentage of distorted valves in *Fragilaria* populations from the northern Pennines.

3.3 Confounding effects of water chemistry on metal effects

Water chemistry, in particular pH and hardness, has a significant effect on metal impacts in the field. Many mining discharges have low pH and this alone is a sufficiently strong factor to modify community composition independent of metals associated with the discharge. There is much literature on the effects of pH on diatoms (Battarbee *et al*., 1999; de Nicola, 2000) with some genera (such as *Eunotia*) characteristic of low pH waters. Teasing out effects of metals from effects of low pH can be difficult in areas where such effects are combined (Hirst *et al.,* 2002).

Both field and laboratory studies have shown low pH to result in lower concentrations of metals accumulated by plants (Harrison *et al.,* 1986; Satake *et al*., 1984; Whitton *et al.,* 1982; Wehr *et al*., 1987), presumably through competition for binding sites between

H^+ and metal ions. Water hardness has a similar effect, with high Ca reducing the amount of metal accumulated (Wehr and Whitton, 1983; Wehr *et al.,* 1987).

If less metal is accumulated at low pH, organisms should be able to tolerate higher concentrations in acid conditions, compared to neutral or alkaline conditions. This is largely borne out by evidence (Campbell and Stokes, 1985). However, if a decrease in pH causes an independent physiological stress on an organism, this will act synergistically with metals to give an apparent increase in toxicity (Starodub *et al*., 1987). One should expect, therefore, a decrease in toxicity close to the pH optimum of an organism, as was observed for *Klebsormidium rivulare* from an acid mine drainage, which was shown to be most tolerant to Zn and Cu at pH 3.5, the pH at which it grew best (Hargreaves and Whitton, 1976a, b). Campbell and Stokes (1985) summarise the role of pH in mediating metal toxicity to algae as follows:

1. Where there is little change in speciation and metal binding at the biological surface is weak, a decrease in pH will decrease toxicity due to competition for binding sites from hydrogen ions (“type 1 behaviour”);
2. Where there is a marked effect on speciation and strong binding of the metal at the biological surface, the dominant effect of acidification will be to increase metal availability (“type 2 behaviour”).

Water hardness behaves in a similar way to pH, with high concentrations of Ca and Mg ameliorating the effect of metals on toxicity, presumably through competition for binding sites (Stokes, 1983), an observation borne out by evidence for reduced uptake of metals at elevated Ca and Mg concentrations (Wehr and Whitton, 1983; Schecher and Driscoll, 1985; Wehr *et al*., 1987). Populations of *Stigeoclonium tenue* and *Klebsormidium rivulare* from sites with low Ca tended to have less genetic tolerance to a particular Zn concentration than populations from sites with high Ca (Harding and Whitton, 1976; Say *et al*., 1977).

Several studies document the role of phosphorus in mediating metal toxicity although interpretation of results is sometimes difficult as the phosphorus may stimulate growth independent of any interactions with metals. There is evidence of metals being stored as insoluble phosphate complexes within cells, particularly in the Cyanobacteria (Jensen *et al*., 1986) but also for inhibition of phosphate uptake mechanisms by metals (Singh and Yadava, 1984). In practice, most UK mining areas are naturally low in available P, so these interactions are probably less important than pH and hardness.

3.4 Detecting metal effects using the current monitoring toolkit

Monitoring of photosynthetic organisms in UK rivers is in the process of being revised to meet the needs of the Water Framework Directive. This has resulted in the development of two new tools, DARLEQ (Diatoms for Assessing River and Lake Ecological Quality; Kelly *et al*., 2008), which uses diatoms as proxies for phytobenthos, and LEAFPACS, which uses macrophytes. DARLEQ is currently based on a single metric, calibrated against a nutrient-organic gradient, whilst a second metric calibrated against an acidification gradient is under development. LEAFPACS is a multimetric tool designed to assess nutrient/organic, hydromorphological and general degradation effects via five metrics: river macrophyte nutrient index, river macrophyte hydromorophological index, number of functional groups, number of taxa and percentage of stream bed covered by filamentous algae.

Neither DARLEQ nor LEAFPACS has been designed specifically to detect toxic effects. Although some authors have claimed that established diatom metrics are responsive to toxic metals (Morin *et al*., 2008), these are almost certainly based on erroneous interpretation of datasets where metals co-vary with other environmental gradients. Of the LEAFPACS metrics, number of taxa shows potential although, as relatively few macrophyte taxa are expected in the upland areas where abandoned mines are usually located (and those that are common are often metal-tolerant mosses), this is unlikely to be especially sensitive. Although the macrophyte survey protocol includes options for recording filamentous algae to genus or beyond, this is not always carried out. Consequently, it can be difficult to distinguish between records of nutrient-tolerant filamentous algae such as *Cladophora* and those such as *Microspora* and *Mougeotia* which are often associated with elevated metal concentrations.

3.5 Fine-tuning DARLEQ and LEAFPACS to detect metal effects

Although neither DARLEQ nor LEAFPACS is likely to be especially sensitive to metal effects in their current forms, the presence of established sampling and analysis protocols (CEN, 2003a, b, 2004) should provide a foundation for future developments.

A second phase of DARLEQ has just started, one aim of which is the incorporation of a second metric (diatom acidification metric, DAM) alongside the trophic diatom index (TDI). This has already been tested on a large dataset of forested catchments in Ireland and is sensitive to both pH and acid-neutralising capacity. Though it has not yet been tested on mining sites, it is likely that it will also detect acid-related effects caused by sulphide oxidation.

Additional metrics to test for the reduction in diatom diversity observed at high metal concentrations could be added. Species diversity and richness metrics are available and have already been tested, though not always with positive results (Hirst *et al.,* 2002). Metrics such as these may be less sensitive as general surveillance tools, but could be useful for before-after-control-impact-type studies, where statistical power can be increased through temporal replication. Species number could be used, though the relationship is not straightforward and is better explained by threshold functions rather than linear regressions (Figures 3.3 and 3.4). A consistent effort would need to be applied to each sample and taxonomic conventions adhered to rigorously.

Some taxa (such as *Achnanthidium minutissimum, Navicula minima*) are commonly reported at sites with high metal concentrations whilst others (*Tabellaria flocculosa*) are rarely present, and there may be potential for developing a metal-sensitive metric to use alongside the TDI and DAM. However, the taxa characteristic of metal-enriched systems are often the ‘pioneer’ or ‘weedy’ species that would be found in that habitat anyway; taxa that thrive are, presumably, those whose populations are able to develop tolerance. No taxa “indicate” high metal concentrations, although *Amphora oligotraphanta* (syn: *A. libyca* var. *capitata*) is often associated with metal-enriched systems in northern England (author, unpublished observations).

Use of the proportion of distorted valves was discussed above (3.2). In practice, this would require a slight modification to the DARLEQ analytical protocol (to ensure that sufficient sensitive taxa such as *Fragilaria capucina* are counted to detect effects). Whilst the presence of distorted valves is a strong indication of toxic effects, their absence does not necessarily mean no effect.

All of these approaches (diversity, metal-sensitive metric, distorted valves) have a risk of false positives; a more statistically-powerful option would be to combine the three approaches into a multimetric approach.

LEAFPACS probably holds less potential than DARLEQ, for reasons stated above, although ensuring that filamentous green algae are recorded accurately to genus will provide useful supporting evidence. The absence of *Cladophora* is unlikely to be a strong indicator as this species is generally less common in upland streams anyway. A combination of evidence on filamentous algae and diatoms may well provide a stronger case than macrophytes or diatoms alone.

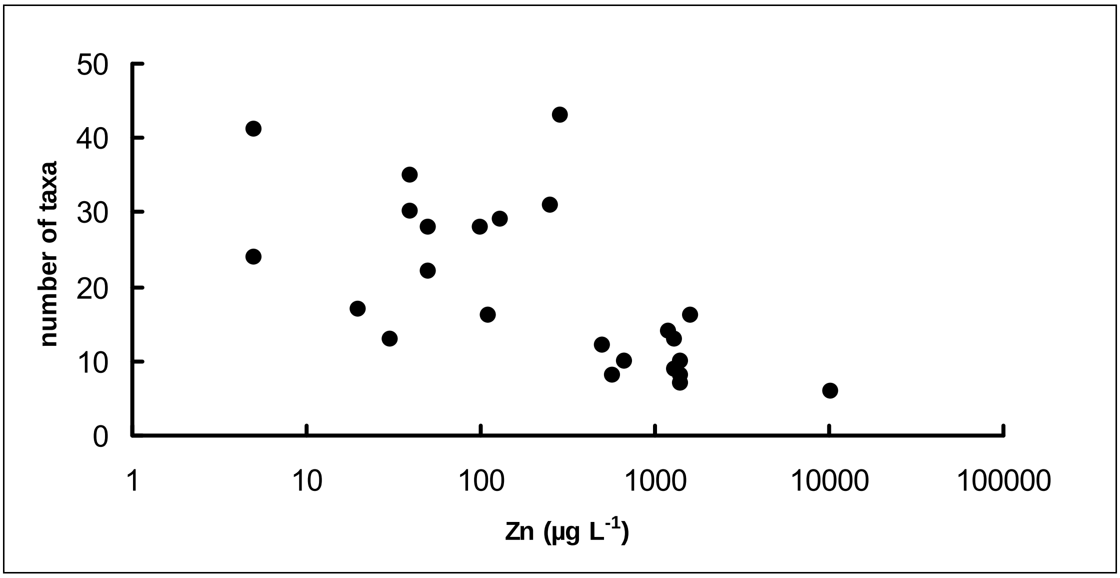

Figure 3.3 Relationship between number of diatom taxa and total Zn concentration for 18 stream sites draining the northern Pennines orefield plus nine DARLEQ "reference sites".

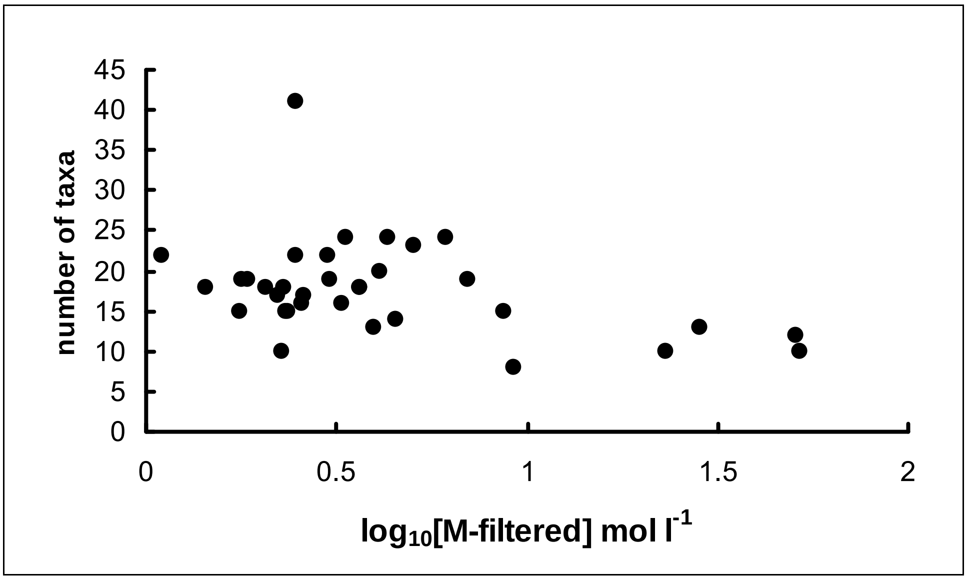

Figure 3.4 Relationship between number of diatom taxa and Zn concentration for sites in the Lake District and Northern Pennines.

3.6 Detecting metal effects: alternative approaches

The use of metal accumulation by bryophytes as monitoring tools was explored in the late 1970s and early 1980s but has not been adopted widely. The most useful application was for monitoring intermittent sources of pollution (Say and Whitton, 1983; Kelly *et al*., 1987) and this was used widely in some parts of the country (particularly NW Region) until 1989. Reasons for the decline were partly due to lack of demand: major industrial polluters were either regulated or were victims of industrial decline. The case for using metal accumulation was less strong in former mining regions where metal inputs were already known and fairly predictable. A further reason for the decline in these techniques was increasing separation of chemical and biological laboratories within the NRA and Environment Agency: biologists had less access to the facilities required to prepare and analyse bryophyte samples and chemistry laboratories worked increasingly under tight accreditation protocols which limited the scope for *ad hoc* analyses.

Metal accumulation should not be overlooked as an investigative monitoring tool, especially if intermittent sources of metals are suspected; however, the value for monitoring abandoned mines in light of current legislation is probably limited. There are two reasons for this: the pollution is mostly continuous and therefore detectable by routine chemical analyses; and the focus of the WFD is on community-level effects, making it difficult to relate bioaccumulation data to clauses in Annex V. Bioaccumulation studies give some indication of the fraction of total metal load that is bioavailable, so it may have a potential role as “supporting evidence”. Methods were developed and standardised in the 1980s (Whitton *et al.,* 1991), but would need to be calibrated for use with new EQS and ecological boundaries.

That populations of several cosmopolitan algae are able to develop tolerance to metals seems well-established (see 3.2). Whether methods for assessing tolerance should be a routine monitoring tool is less clear. Such methods are relatively expensive and time-consuming compared to community-based analyses. A further problem is that WFD goals are expressed in terms of community properties. Methods for assessing tolerance may have a useful role in validating EQS, rather than for routine monitoring.

3.7 Recommendations

- Further development should focus on community-level analyses, building on the foundations laid by DARLEQ and LEAFPACS. Of these, diatoms hold the most promise, as the macrophyte assemblages in mining areas is often sparse, even in the absence of metal stresses.
- It may, nonetheless, be worth exploring the effect of metals on LEAFPACS metrics, particularly number of taxa.
- A number of diatom-based metrics need to be explored. These include diversity (number of taxa, richness, evenness) and percentage of distorted valves, as well as the potential for a simple community-based metric, based on presence or absence of taxa with known tolerances or sensitivities to metals. Combining these into a multimetric should also be explored.
- The Diatom Acidification Metric (DAM), under development, should be tested on datasets from mining areas.
- Other methods may provide useful supporting evidence but will be harder to relate to WFD goals. Baseline values for metal accumulation in aquatic bryophytes and for tolerance could be established for “reference sites” and used as a baseline against which impacts or remediation measures assessed.

4 Detecting and diagnosing metal impacts on freshwater fish and invertebrates in impacted freshwaters

4.1 Introduction

Metals from mining activities are important to water quality in the UK. In particular, the South West of England, West/Central Wales and the North Pennine Orefields were formerly important mining areas which still contain a network of metal-polluted rivers. Metals are loaded into rivers also from acidified drainage from disused coal mines, from metal processing operations and where acid deposition on base-poor soils releases metals through buffering reactions (Licskó *et al*., 1999; Karageorgis *et al*., 2003; Krachler *et al*., 2005; Satapathy *et al*., 2009).

Metal effects on organisms in British rivers have been studied since the early twentieth century (see Carpenter, 1924; Brown, 1977; Armitage, 1980). However, identification of causal relationships between metals and field biological responses still presents difficulties because species distribution and community composition are affected by many other factors (Clements, 2004). Extrapolation of results from laboratory toxicology studies to natural systems is also difficult (Heugens *et al*., 2001; Harper *et al*., 2008). While modern regulation and management require practical tools to identify where metal problems are most acute, and most in need of mitigation, there is concern about some methods currently in use. For example, environmental quality standards do not always estimate accurately the risk posed to biota by priority substances, with failing water bodies sometimes supporting healthy ecosystems (see Crane *et al*., 2007; Comber *et al*., 2008). Similarly general biotic indices, such as BMWP and ASPT, do not distinguish metal effects from other sources of pollution, while widely used models such as RIVPACS are not currently configured to diagnose metal effects. Identifying locations most in need of management is therefore difficult.

This review outlines the complexities of metal pollution from abandoned mines and associated ecological impacts by answering the following key questions:

1. Do increases in metal concentrations lead unequivocally to ecological impairment?
2. What are the problems faced in detecting the presence and extent of metal pollution and its toxicological effects on aquatic biota?
3. How useful are widely used bioindicators and water quality indices for the detection of metal impacts on aquatic biota?
4. What could be done to improve the detection of metal impacts from field data?

The focus is on freshwater fish and invertebrates where particular problems arise with respect to (a) understanding how metal toxicity and ecological effects are best detected, evaluated and diagnosed from field surveys or field experiments; (b) understanding the consequences for water quality management in general; (c) guiding decisions about the management of any single source of metal discharge.

4.2 Metals in freshwater environments

Metals are present in rocks and soil as metal salts, which leach into water bodies through geochemical processes such as weathering or the buffering of acidity in deposition (see Karageorgis *et al*., 2003; Krachler *et al*., 2005; Satapathy *et al*., 2009). Anthropogenic inputs from past and present mining activities can significantly increase the metal loading to water bodies. Of the metals most commonly elevated in mine-affected streams, cadmium is considered to pose the greatest risk to aquatic biota, followed by copper, lead, chromium, zinc and nickel in order of decreasing toxicity (Directive 76/464/EEC). Aluminium is particularly important in acidified headwaters due to the increased occurrence of labile forms over the pH range 4.5-5.7 that are considered to have greater toxicological effect on aquatic biota (see Turnpenny *et al*. 1987; Ormerod *et al*., 1987; Weatherley *et al.,* 1988).

Metals in the body of an organism can be categorised into two forms: metabolically active or detoxified. Detoxified metals are metabolically unavailable to the organism and as such there is no limit to the amount that can be held in the body. Metals present in metabolically available forms have the potential to exert toxic effects above a given threshold concentration (Rainbow, 2002). Toxicity is therefore not a simple function of metal concentration (Hansen *et al*., 2002b) but is related to the bioavailability of a metal to aquatic organisms which is in turn dependent on a range of complex factors.

4.2.1 Metal mixtures

Mining effluents rarely contain a single metal but are often mixtures. Some metals have the potential to suppress (Musibono and Day, 1999; Stewart, 1999; Shuhaimi-Othman and Pascoe, 2007; Niyogi *et al*., 2008) or enhance (Roy and Campbell, 1995; Veltz-Balatre, 2000; Vosyliene *et al.,* 2003; Clements, 2004; Shuhaimi-Othman and Pascoe, 2007) the bioavailability and toxicity of other metals in a mixture. Whilst the toxicity of some mixtures results from simple additive effects of the toxicity of all metals present (Kamo and Nagai, 2008), in other cases antagonism and synergism can occur simultaneously in particular metal combinations (Shuhaimi-Othman and Pascoe, 2007). Consequently, assessing water quality from individual metals may not provide an accurate insight into the impact on aquatic organisms and ecosystems processes.

4.2.2 Metal speciation and complexation

The physico-chemical form in which a metal is present in aquatic environments affects its reactivity, bioavailability and toxicity (Tessier *et al*., 1979; Allen and Hansen, 1996; Erickson *et al*., 1996; Gagnon *et al*., 2008).

Complexation of metals with dissolved organic carbon (DOC) plays an important protective role in aquatic systems, reducing bioavailability, accumulation and toxicity (Erickson *et al*., 1996; Kramer *et al*., 2004; Doig and Liber, 2006; Niyogi *et al*., 2008; Rickwood *et al*., 2008). At particularly high metal concentrations, however, the capacity for complexation by organic carbon may be exceeded, leading to the formation of inorganic complexes in more available forms (Tessier *et al.,* 1979; Strauber *et al*., 2008). Despite evidence of its role in metal toxicity, and high concentrations in some metal-rich areas, DOC is not currently taken into account in EQS guidelines, although there are suggestions in the literature to adjust environmental risk assessments accordingly (see Kramer *et al*., 2004), and work is in progress to incorporate interactions with DOC for Cu and Zn (see Chapter 5).

Metal effluents from mine drainage are often accompanied by increases in suspended solids. This material can transport metals, but inert solids can also reduce the sensitivity of freshwater organisms to metals (Erickson *et al*., 1996; Pyle *et al*., 2002) up to a threshold concentration, after which physical irritation to the gills of aquatic organisms negates any protective effects (Pyle *et al*., 2002). As with DOC, metal contaminant EQS are not related to suspended solids, and may be at risk of overestimating the concentrations at which negative impacts on freshwater biota occur.

Tessier *et al*. (1979) identified five different forms in which a metal may be present in aquatic sediments, the bioavailability of the metal in each affected by different environmental conditions. The most reactive and biologically available forms are readily exchangeable and reducible, while the least reactive forms are oxidisable and inert.

Although the direct effects of hardness on metal toxicity are difficult to separate from effects of alkalinity, complexation of metals with calcium carbonate is one mechanism by which water hardness can reduce the toxicity of zinc (Hansen *et al*., 2002), copper (Howarth and Sprague, 1978; Miller and Mackay, 1980; Erickson *et al*., 1996) and nickel (Pyle *et al*., 2002) and cadmium (Canton and Sloof, 1982; Pascoe *et al*., 1986; Hansen *et al*., 2002) to aquatic organisms. Because of these effects, EQS maximum allowable concentrations for some metals in water courses are hardness-related.

This topic is dealt with in more detail in Chapters 5 and 6.

4.2.3 Metals and pH

Many water bodies affected by mine drainage are also affected by acidification, which in many areas has been a long-standing complication or confound in interpreting metal effects. In the copper- and zinc-affected Mawddach, Wen and Gain, in Wales, Howells *et al.* (1983) showed that ephemeropteran distribution tracked pH and water hardness better than copper and zinc, while salmonid distribution reflected a combination of Cu, Zn and low pH. Similarly, in broader surveys across mid and North Wales, absence or paucity of salmonids related equally well to high levels of labile monomeric aluminium (above 40 µg/l) or Cu-Zn-Pb toxicity, with both, in turn, linked to low pH waters (Turnpenny *et al*. 1987).

Difficulties of this type persist; for example, in New Zealand O'Halloran *et al.* (2008) found that the primary driver of toxicity of acid mine drainage to mayflies was low pH rather than metals. The effect of low pH on metal bioavailability and toxicity differs between metals (see Hickie *et al*., 1993; Campbell and Stokes, 1995; Gerhardt *et al*., 2005; Stillings *et al*., 2008) and whilst several attempts have been made to partition the effects of metals and pH (see Ormerod *et al*., 1987; Weatherley and Ormerod, 1987; Dsa *et al*., 2008; O'Halloran *et al*., 2008), the precise mechanisms behind the relationship remain unclear.

Interactions between metal toxicity and pH are not straightforward and are highly metal-specific. Low pH has been associated with increased toxicity in some metals and metal mixtures in acid-sensitive fish and invertebrates (Gerhardt, 1990; Hickie *et al*., 1993), but in other organisms low pH can reduce metal toxicity (Pyle *et al*., 2002). Similarly, high pH can increase the toxicity of some metals to aquatic biota (Niyogi *et al*., 2008) but reduce the toxicity of others (Erickson *et al*., 1996). While pH-mediated changes in the toxicity of metals are not always consistent with the effect of pH on bioavailability (Niyogi *et al.,* 2008; Gerhardt, 1990), the impact of pH on the form in which a metal is present in the aquatic environment is thought to be key to the interaction between the two. For example, decreases in pH lead to higher rates of desorption of copper and lead from ferrihydrite, changing the speciation in solution and transport mechanism of these metals and increasing their bioavailability (Stillings *et al*., 2008). Alkalinity can encourage the precipitation of dissolved metal ions in the form of ochreous minerals, which are unavailable for accumulation by aquatic organisms (Espana *et al*., 2006). Improved models to account for interactions between H^+, metals and toxicity are a priority.

4.2.4 Hydrological events and episodes

Episodes of heavy rainfall or intense snow-melt can reduce the pH of freshwaters through a combination of effects including base-cation dilution, mobilisation of organic acids, and acid-anion addition from soils (see Kowalik *et al.* 2007). Metal desorption from sediments or increasing mobilisation from soils and parent material then increases dissolved metal concentrations in runoff (Brown, 1977; Weatherley *et al*., 1988, 1990; Green *et al.,* 2006). These episodic increases in metal concentrations can affect organisms through brief toxic exposure (Ward *et al*., 2005, 2006). Alkalinity and calcium can protect freshwater organisms from the ecotoxic effects of metals but both decrease during high flow episodes due to dilution and a surge in hydrogen ions (Gundersen and Steinnes, 2001).

Recovery following an episode of elevated metal may be relatively rapid, making episodes difficult to detect through water quality monitoring alone. Conversely, the effects of short-term episodic increases in metal concentration may not be apparent immediately following exposure in some freshwater organisms (Salanki *et al*., 1991), but may remain detectable in the community structure for a considerable time following initial onset (MacCausland and McTammany, 2007). This provides an opportunity to use aquatic communities as indicators of episodic metal increases that would otherwise go undetected and highlights the risks involved in considering mean water quality parameters in long-term assessments of ecosystem quality.

4.2.5 Sediment contamination

Sediments in mine-affected water bodies are not currently included in assessments of water quality under the Water Framework Directive (WFD), yet can be important in mediating the exposure of benthic or interstitial organisms. The high affinity of some metals to clay-like minerals can lead to sediments holding much higher concentrations of metals than the overlying water (Beasley and Kneale, 2001) yet there is less literature on the effects of contaminated sediments than on contaminated waters on freshwater biota. Bioavailability of sediment metals depends on the physico-chemical characteristics of the sediment, with total metal concentration often a poor predictor of toxicity (Péry *et al*., 2008; Berry *et al*., 1996). The impact of sediments on aquatic organisms is closely linked to how their behaviour lifecycle interacts with the sediment. For example, sediments tend to be a more important source of metals to oligochaete worms, which ingest sediments for food, than benthic invertebrates that feed by filtering water (Hare *et al*., 2003). As such, the presence of metals in freshwater sediments are implicated in effects on the community structure of benthic ecosystems (Beasley and Kneale, 2003; Besser *et al*., 2008).

4.3 Biological response to metals in freshwaters

Metal toxicity occurs at all levels of biological organisation: cell, individual, population and community. However, the vast majority of research showing these effects has been derived from laboratory ecotoxicology, and extrapolation to natural environments is not straightforward (Hare, 1992).

The toxicity of metals arises from actions at the sub-cellular level, where changes in enzyme activities, membrane permeability and cell turnover can occur (Squibb and Fowler, 1981). Certain metals act as catalysts for the production of reactive oxygen species (ROS) including free radicals and peroxides. Normal anti-oxidant defences of a cell may be unable to compensate for excess ROS production, resulting in oxidative stress to an organism through lipid, protein and DNA damage (Ercal *et al*., 2001). It is thought that many of the toxic effects of metals occur through this mechanism.

There exist a wide variety of organism responses to metal pollutants, varying between species as well as between individuals of the same species, and affecting inter-individual interactions. Sub-lethal responses may be behavioural, locomotory, physiological, morphological or a combination of these and are highly dependent on the type of metal, its bioavailability and whether exposure is chronic or acute (Table 4.1). A complete understanding of ecosystem responses to metal pollution requires knowledge of the likely effects on the organisms it supports.

4.3.1 Individual responses

Organism responses to metals tend to be generalised and reported for taxonomic groups. However, even individuals of the same species may differ in their responses to a particular metal contaminant under the same environmental conditions. Individual body size, for example, is an important factor determining metal sensitivity. Smaller individuals are known to be more sensitive in invertebrates (Kiffney and Clements, 1994; Kiffney and Clements, 1996) and fishes (Alam and Maughan, 1995).

At the individual level, reduced survival, feeding rate, growth and development (Figure 4.1) are widely reported in organisms following exposure to metal pollution (Gerhardt, 1990; Hruska and Dubé, 2004; Besser *et al*., 2007; Faria *et al*., 2008). Metals often accumulate in high concentrations in the gills of aquatic organisms, due to the direct contact between water and the gill surface (Gill *et al*., 1992; Couillard *et al*., 1993; Baudrimont *et al*., 1997; Chowdhury *et al*., 2004). At the gill, metals can cause morphological deformities (Pandey *et al*., 2008) as well as physiological changes in the mechanism of ion binding and respiration (Reid *et al*., 1991). Over-secretion of mucus has also been reported in invertebrates (Bouche *et al*., 2000; Ward *et al*., 2006) and fish (McCahon *et al*., 1987), which can further impair gill function, rendering affected individuals vulnerable to infection (Ward *et al*., 2006). Due to its role in detoxification, the liver also accounts for a large proportion of the metal body burden of an organism (see Langston *et al*., 2002; Hogstrand *et al*., 2003; Lwanga *et al*., 2003; Chowdhury *et al*., 2004). Here, metals can cause histological changes such as hyalinisation, hepatic vacuolisation and cellular swelling (Van Dyk *et al*., 2007).

Table 4.1 Examples of sub-lethal effects of metals on freshwater fish and invertebrate species.

Metal	Taxon		Sublethal effect	References
Cd	Fish	*Oncorhynchus mykiss*	Reduced number of aggressive attacks	Sloman *et al.*, 2003 ; Brown *et al.*, 1994
			Subordination	
			Delayed oogenesis	
		Salvetinus namaycush	Reduced prey capture	Kislalioglu *et al.*, 1996
			Reduced number of attacks	
			Less prey consumed	
	Invertebrate	*Gammarus pulex*	Reduced feeding rate	Felten *et al.*, 2008
			Impaired locomotory activity	
			Impaired ventilatory activity	
		Hydropsyche contubernalis	Reduced amount of time entering competitors nets	Vuori, 1994
			Anal papillae deformities	
		Hydropsyche siltalai	Reduced amount of time entering competitors nets	Vuori, 1994
			Anal papillae deformities	
			Darkening of abdomen	
		Clyptotendipes pallens	Reduced development	Heinis *et al.*, 1990
			Reduced activity	
			Reduced food intake	
		Leptophlibia marginata	Reduced number of emergents	Gerhardt, 1990
		Baetis rhodani	Reduced number of emergents	Gerhardt, 1990
			Reduced locomotory activity	
		Tubifex tubifex	Autonomy of caudal region	Bouche *et al.*, 2000
			Increased mucus production	
		Hydropsyche betteni	Loss of weight	Balch *et al.*, 2000
			Reduced frequency of normal nets constructed	
Al	Fish	*O. mykiss*	Internal disruption	Reid *et al.*, 1991
	Invertebrate	*Chironomus riparius*	Chromosomal aberrations	Michailova *et al.*, 2003
		Pacifasticucus leniusculus	Reduced immuno-competency due to oversecretion of mucus	Ward *et al.*, 2006
Cu	Fish	*Oncorhynchus mykiss*	Reduced number of aggressive attacks	Sloman *et al*, 2003 ; Hansen *et al.*, 2002a
			Subordination	
			Reduced growth	
		Salvetinus	Reduced prey capture	Kislalioglu *et al.*, 1996

	namaycush	Reduced number of attacks	
		Less prey consumed	
Invertebrate	*Hydropsyche angustipennis*	Increased mesh size	
		Reduced number of nets	
	Chironomus tetans	Reduced total number of emergents	Hruska and Dubé, 2004
		Increased time to emerge	
		Reduced hatching success	
	Lubriculus variegatus	Delayed and atypical cephalic regeneration	Veltz-Balatre, 2000
	Hydropsychid larvae	Adverse net construction	Besch *et al*., 1979
		Net thread interruption	
		Reduced meshes per net	
	Baetis tricaudatus	Reduced feeding	Irving *et al*., 2003
		Inhibited growth	
	Chironomus riparius	Chromosomal aberrations	Michailova *et al*., 2006

In addition to physiological, morphological and histological toxic responses, organisms may display altered behaviour in response to metals. Case and net construction of some caddisfly larvae, for example, is inhibited by heavy metal exposure (Besch *et al*., 1979; Petersen and Petersen, 1983; Balch *et al*., 2000; Tessier *et al*., 2000) and is in fact highly sensitive compared with other effects on invertebrates (Tessier *et al*., 2000). Altered structure of these nets reduces feeding efficiency in affected individuals, which has a direct negative impact on growth, development and survival. Exposure to metals can also promote (Macedo-Sousa *et al*., 2008) or inhibit (Gerhardt, 1990; Felten *et al*., 2008) locomotory activity in aquatic organisms. Physical drift of sensitive invertebrates away from polluted sites has also been shown to occur in metal-affected water bodies (Groenendijk *et al*., 1998; Clements, 1999), while Vosyliene *et al*. (2003) determined that adult salmonids are able to detect and avoid metals in water. Such behaviour has consequences for detectable community composition, with only more tolerant individuals remaining at contaminated sites.

4.3.2 Inter-individual responses

Altered species interactions in response to metal inputs (Clements, 1999) can lead to the disturbance of important life cycle processes. Reproductive success is commonly adversely affected, with reproductive interactions (Poulton and Pascoe, 1990), egg production (Rickwood *et al*., 2008), emergence (Gerhardt, 1990; Hruska and Dubé, 2004), hatching success and survival of offspring (Hruska and Dubé, 2004) disrupted in invertebrates and fish species. Changes in competitive interactions (Vuori, 1994), social status (Sloman *et al*., 2003; 2005) and predator-prey interactions (Kislalioglu, 1996) have also been detected in fish and invertebrates following exposure to metals.

4.3.3 The importance of life stage

There is evidence that within species, sensitivity to metal pollutants varies between individuals of different life stages due to their different biological requirements (McCahon and Pascoe, 1990; Vosyliene *et al*., 2003). Salmonid fry, for example, show a higher sensitivity than juveniles of the same species (Marr *et al*., 1995), while larval fish stages are more sensitive than adults or embryonic stages, with adults more able to avoid low metal concentrations (Vosyliene *et al*., 2003) and embryos protected by egg membranes which provide a physical barrier to poor water quality (Finn, 2007).

Invertebrate life stages are also subject to differences in metal sensitivity. Differences in sensitivity have been detected between sexually mature males and females of the freshwater shrimp *Gammarus pulex* (McCahon and Pascoe, 1988; Maltby and Naylor, 1990), while juveniles are more sensitive to metals than adults (McCahon and Pascoe, 1989a). The higher sensitivity of juveniles than adults has been reported in other invertebrate species (see Green *et al*., 1986; Williams *et al*., 1986; McCahon *et al*., 1989; Gerhardt, 1990; Musibono and Day, 1999; Clark and Clements, 2006). Invertebrates commonly undergo a series of moults throughout their life cycle, immediately following which recalcification occurs to restore the exoskeleton. Metal pollution impedes this process, reducing the amount of dissolved calcium carbonate in water, rendering moulting individuals particularly sensitive to metal pollution (McCahon and Pascoe, 1988; McCahon and Pascoe, 1989a; Musibono and Day, 1999).

Discrepancies between life stage sensitivities can bring about a change in the age composition of a population (Maund *et al*., 1992). Authors such as McCahon *et al*. (1989) have highlighted the need to consider life cycle information when designing laboratory-based toxicity tests, while Clark and Clements (2006) advocate field assessments coinciding with the occurrence of the most sensitive life stages to detect minimum concentrations that have a potential environmental impact.

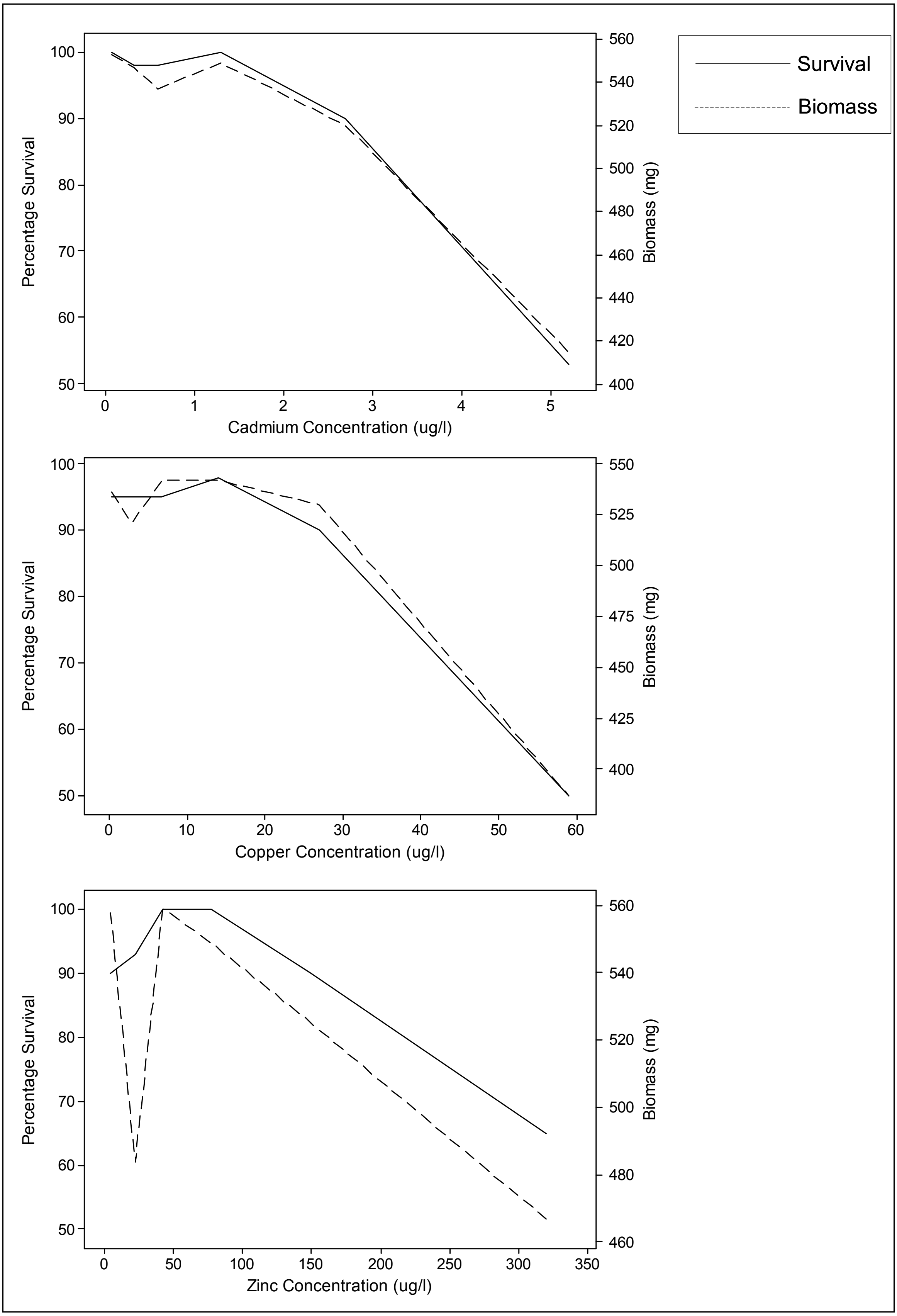

Figure 4.1 Percentage survival and biomass of rainbow trout (*Onchorhynchus mykiss*) following 28-day exposure to cadmium, copper and zinc at different concentrations (from Besser *et al*., 2007).

4.3.4 Trophic transfer

Determining the exposure route is important in estimating the ecotoxic response of a particular organism to metals. Some fish and invertebrate species accumulate the majority of their metal body burdens from the surrounding water (see Xu and Pascoe, 1993) and in conventional ecological assessments, only metal concentrations in water are considered. However, many species obtain greater metal body burdens from ingested food (Hare *et al*., 2003; Borgmann *et al*., 2007; Tsui and Wang, 2007; Croteau and Luoma, 2008; Dumas and Hare, 2008). In some species, the toxic response to dietary metals is more sensitive than that to water-borne exposure (Irving *et al*., 2003). Food and water exposure together has been shown to have both an additive (Sofya *et al*., 2007; Geffard *et al*., 2008) and an antagonistic effect on the toxicity of metals compared to exposure through water alone (Rickwood *et al*., 2008). By considering only metal concentrations in water, environmental assessments are potentially overlooking an important source of metal exposure to aquatic invertebrates in the fraction taken up in food (Hare *et al*., 2003; Geffard *et al*., 2008).

Evidence that organisms obtain significant amounts of metal from diet has important implications for the potential transfer of metals between trophic levels. The ability of prey organisms to accumulate large metal body burdens may pose a toxicological risk to predators if the metal is present in a bioavailable form (Bouche *et al*., 2000). Indeed, some studies have shown that mercury (Watras *et al*., 1998), zinc (Quinn *et al*., 2003), cadmium (Croteau *et al*., 2005), nickel and thallium (Dumas and Hare, 2008) can be transferred to higher trophic levels. Others have shown no evidence of biomagnification (Winterbourn *et al*., 2000; Powell and Powell, 2001) and that transfer to higher trophic levels is highly metal-specific (Quinn *et al*, 2003). Clearly, there are implications for biological water quality assessment if metals are not accumulated equally at all trophic levels. Further study is needed to establish whether biomagnification occurs within an aquatic ecosystem and if so, the trophic levels to consider in biological appraisals of water quality.

4.3.5 Adaptation and acclimation

In some freshwater invertebrate and fish species, a reduction in the toxic impacts of metals has been observed under prolonged or historical metal exposure (see Reid *et al*., 1991; Gill *et al*., 1992; Mersch *et al*., 1996; Balch *et al*., 2000; Bouche *et al*., 2000; Knapen *et al*., 2004; Ward *et al*., 2006). There is ongoing debate as to whether this is primarily due to genetically evolved adaptation over several generations, or acclimation to sub-lethal metal concentrations through induction of protective metal-binding proteins. A number of studies have provided evidence for, or inferred the role of, genetic adaptation in the increased tolerance of previously exposed populations (Postma *et al*., 1995; Groenendijk *et al*., 2002; Knapen *et al*., 2004). Similarly, maternal pre-exposure has been seen in some species to increase tolerance to metal pollutants (e.g. Pynnönen, 1995, Tsui and Wang, 2007). Conversely, other authors have argued that acclimation of individuals is more important than genetically determined sensitivity in explaining toxicological effects of metals (Lopes *et al*., 2005; Hansen *et al*., 2006) and that maternal exposure is unimportant in determining offspring sensitivity or physiological performance (García *et al*., 1999; Tsui and Wang, 2005).

Regardless of the mechanism, it is widely accepted that low level chronic exposure has the potential to enhance an organism's tolerance to a specific metal (Mersch *et al*., 1996; Balch *et al*., 2000; Harper *et al*., 2008) and in some cases to other metals (Hansen *et al*., 2002; Ward and Robinson, 2005). This has important ramifications in the assessment of water quality and determination of EQS, which may overestimate concentrations of metals at which environmental damage is caused if consideration is not given to the adaptive abilities of freshwater organisms.

4.3.6 Detoxification and sequestration

Metal concentration in water is a poor indicator of toxicity. Better predictors of a metal's toxicity are its bioavailability, how rapidly and in what form it is accumulated by an organism. All living organisms employ defence systems to eliminate or neutralise potentially harmful substances from the body and fall into one of two categories in their management of accumulated metals in the body. Regulators store metals in a soluble form, bound to storage proteins, which can be transferred to higher trophic levels (see Langston *et al*., 2002; Amiard *et al*., 2006; Fraysse *et al*., 2006). Non-regulators store metals in insoluble granules, which can be relocated and eventually excreted (see. Everard and Denny, 1984; Elangovan *et al*., 2000; Campbell *et al*., 2005).

Metallothioneins (MTs) are metal-binding storage proteins which are thought to have a protective role in the body (Roesijadi, 1992) and are induced by elevated metal concentrations (Couillard *et al*., 1993; Baudrimont *et al*., 1997; Van Campenhout *et al*., 2008). The precise biological function of MTs is unclear, despite an abundance of literature on the subject. It is generally accepted that they are involved in the homeostasis of essential metals in the body (Amiard *et al*., 2006). Additionally, the ability of non-essential metals to displace MT-bound essential metals (Roesijadi, 2000; Amiard *et al*., 2006) and the increased metal tolerance observed with MT induction (Baudrimont *et al*., 2003) has led to the conclusion that they have a potential role in metal detoxification. MTs have been identified for around 80 species of fish and invertebrate, with some species producing multiple forms, each with a potentially different role in the body (Roesijadi, 1992). Consequently, their potential use as biomarkers in detecting metal pollution has been widely researched and is discussed further in Section 4.4.1.

In organisms that are non-regulators, the primary metal detoxification mechanism is through storage in insoluble granules, the number of which increases with increasing exposure time (Elangovan *et al*., 2000). In freshwater molluscs, these storage granules take the form of calcium concretions, which are laid down inexchangeably in the shells (Everard and Denny, 1984; Campbell *et al*., 2005). Similarly, other invertebrates are able to store metals in the exoskeleton, which will be lost as the organism undergoes moulting (Groenendijk *et al*., 1999). In addition to sequestration in hard structural material, storage granules have been found in the digestive cells of some aquatic organisms, implicating the digestive system in excretory mechanisms of non-regulators (Everard and Denny, 1984; Elangovan *et al*., 2000).

While complex and requiring further study, organism responses to metal pollution could be used to indicate ecosystem quality in metal-impacted freshwaters.

4.4 Biological indicators of ecosystem quality

Freshwater biota can provide an important insight into the effects of metals and other pollutants on freshwater ecosystems. Fundamental to this are the close links between water chemistry and community structure and the abilities of organisms to respond adaptively to pollution in a taxon- and contaminant-specific manner. This has stimulated studies into harnessing the natural response of organisms and communities to detect metal contamination. Bonada *et al* (2006) have suggested that ideal characters for biological indicators include:

1. Derived from sound theoretical concepts
2. *A priori* predictive
3. Potential to assess ecological functions
4. Potential to detect overall human impact
5. Potential to discriminate specific types of human impact
6. Inexpensive sampling and sorting or standardised experimental approach
7. Simple sampling protocol
8. Inexpensive identification
9. Large-scale applicability across geographic regions
10. Reliable indication of changes in overall human impact
11. Reliable indication of changes in specific types of human impact
12. Human impact identifiable on a linear scale

4.4.1 Biomarkers

Several authors advocate the use of metallothioneins as biomarkers due to their metal-specific induction in fish and invertebrates (Couillard *et al*., 1993; Baudrimont *et al*., 1997; Van Campenhout *et al*., 2008), indicating exposure to particular metal stressors. Several authors have highlighted the limitations of using MTs as biomarkers, including the dependence of MT induction on environmental variables (see Langston *et al*., 2002; Baykan *et al*., 2007); the lack of MT induction despite elevated metal concentrations in the tissues of some species (Pedersen and Lundenbye, 1996; Berthet *et al*., 2003; Bae *et al*., 2005); and the discrepancies between laboratory and field-based studies of MT induction (Amiard *et al*., 2006). Nevertheless, MTs remain potential biomarkers for the detection of metal stress in aquatic invertebrates, the development of which is ongoing.

Other proposed biomarkers provide sensitive and reproducible early warning indicators of trace metal contamination. These include physiological responses, such as whole-body sodium flux in fish (Grippo and Dunson, 1991, Grippo and Dunson, 1996a and 1996b) and oxidative stress biomarkers (Benassi *et al*., 2006), as well as behavioural responses, such as changes in locomotory activity in invertebrates (Petrauskiene, 2003; Macedo-Sousa *et al*., 2008).

4.4.2 Bioindicators and water quality metrics

Bioindicators are species or groups of species with known sensitivity to a particular pollutant, whose presence in a system is a strong indication that the pollutant is absent or not bioavailable. Field-based bioindicators are a practical way of avoiding the uncertainties associated with extrapolating from laboratory data to the field. Macroinvertebrate communities are commonly favoured as bioindicators due to their relatively ubiquitous distribution in running freshwaters; spectrum of species responses to pollutants; relatively sedentary nature and long life cycles, enabling spatial and temporal analysis of pollution; inexpensive equipment needed for sampling; well-described taxonomy; and known sensitivity of many common species to a range of pollutants (Bonada *et al*., 2006).

A number of studies have shown that changes in macroinvertebrate assemblages closely reflect changes in metal pollution (see Hirst *et al*., 2002; Cain *et al*., 2004; Clements, 2004; Buchwalter *et al*., 2008; Gray and Delaney, 2008). Chironomid midges are one of the most tolerant taxa, representing large proportions of assemblages in heavily polluted rivers (Winner *et al*., 1980; Gower *et al*., 1994; Groenendijk *et al*., 2002). Conversely, mayfly (Ephemeroptera), stonefly (Plecoptera) and caddisfly (Trichoptera) larvae are numerically important at the most uncontaminated sites but rare or absent from highly contaminated reaches (Winner *et al*., 1980; Gower *et al*., 1994; Malmqvist and Hoffeten, 1999; Courtney and Clements, 2002; Beasley and Kneale, 2003; Cain *et al*., 2004; Clements, 2004). These species-specific responses to metal contamination are complex, however (Beltman *et al*., 1999; Courtney and Clements, 2002) and the mechanisms behind them are not well understood (Cain *et al*., 2004). Additionally, proving causal links between community impairment and metal pollution is difficult due to the lack of knowledge of reference conditions (Clements, 2004) and the close relationship between metal bioavailability and pH.

Despite these complexities, the careful use of indicator species may be a far more useful method of detecting metal contamination than conventional water chemistry testing, due to their ability to detect episodic metal pollution (MacCausland and McTammany, 2007) and subtle concentration changes (Clark and Clements, 2006) that might otherwise be overlooked with water quality assessments. Further research is needed to develop indices which accurately reflect ecosystem quality based on the response of key indicator species (Gray and Delaney, 2008).

A number of water quality metrics are employed in the UK to assess the state of water bodies with respect to the biota supported, all of which are based on the differential sensitivities of freshwater macroinvertebrates to poor water qualty. The biological monitoring working party (BMWP) and associated average score per taxon (ASPT) indices of water quality are widely used in the UK to detect eutrophication and organic pollution. The BMWP allocates scores to each taxon based on tolerance to poor water quality and is used as the basis of the Environment Agency's N-taxa (Number of BMWP-scoring taxa). RIVPACS compares observed macroinvertebrate communities to site-specific predictions of those which would be expected under pristine conditions (Wright *et al*., 1993). Among the available indices, BMWP and ASPT tend to be used for the generic detection of poor water quality and as such have been widely used in metal-impacted waters (García-Criado *et al*., 1999; Gerhardt *et al*., 2004; Bradley, 2008). Moreover, these indicators were developed largely to detect sanitary effects on waters receiving organic waste. There are limitations in the BMWP with respect to sensitivity to sampling variation, site selection, seasonality (Armitage *et al*., 1983). Gray and Delaney (2008) found that ASPT is not an accurate index of acid mine drainage.

In general, each of these metrics is limited in its ability to diagnose the causes of poor water quality and none have been shown to accurately detect the impact of metal contamination on freshwater systems. Several authors have highlighted the need to develop indices that better reflect the response of indicator species to specific pollutants (Hirst *et al*., 2002; Clews and Ormerod, 2008; Gray and Delaney, 2008). There are increasing calls for reconfiguration of Environment Agency tools such as RIVPACS (or RICT) to improve diagnostic capabilities, but this would require pressure-sensitive metrics, such as those which exist for the detection and diagnosis of other important perturbations. The acid water indicator community (AWIC) (Davy-Bowker *et al*., 2005), for example, uses macroinvertebrate assemblage composition to detect acidification, while the trophic diatom index (TDI) (Kelly and Whitton, 1995) uses diatom assemblages to detect eutrophication in freshwaters.

Currently no such metrics are available for metal pollution, but several approaches already used to develop biodiagnostic indicators of other pressures have a potential role in the development of metal-specific tools. One is the identification of specific relationships between metal toxicity and individual species responses. This approach was successfully used by Larsen *et al*. (2009) to identify species-specific macroinvertebrate responses to fine-sediment load. Another potential method is the elimination of sources of variation other than metal concentration. The latter approach relies on statistical methods such as partial ordination, as used by Murphy *et al*. (under review) to develop a species-specific AWIC index. Additionally, the use of metrics, such as number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (Wallace *et al*., 1996) or measures of trait diversity (see Statzner *et al*., 2005; Dolédec *et al*., 2006; Tullos *et al*., 2009; Larsen and Ormerod, unpubl.) have also been investigated for use in river biomonitoring but while these have the advantage of being transferable across large geographical regions, they potentially lack the diagnostic capabilities of other developmental approaches.

4.4.3 Biotic ligand models

Researchers have attempted to boost the accuracy of water quality assessments by developing biotic ligand models (BLMs) (see Heijerick *et al*., 2002; DeSchamphelaere and Janssen, 2004; DeSchamphelaere *et al*., 2005). BLMs give a more accurate representation of the biological responses to metal pollution by predicting interactions between metal species and competitive protective anions, such as calcium and sodium at the biotic ligand (Heijerick *et al*., 2002, Kamo and Nagai, 2008; Niyogi *et al*., 2008). This accuracy, however, is achieved by considering a larger range of physico-chemical predictors, often leading to complex models that are less practical for use as regulatory tools (Comber *et al*., 2008). BLMs are widely used in the US by the Environmental Protection Agency (EPA) but have yet to be employed in the UK (see Chapter 5).

4.5 Conclusions

- There is much literature on metals and toxicological responses of freshwater biota. However, many of the mechanisms behind biological responses remain unclear. Confounding factors include dependence of metal bioavailability and toxicity on environmental variables, chemical properties of metals and different responses of organisms both between and within taxa. These complexities have led to difficulties in developing sensitive and easily reproducible indices that accurately detect metal pollution in freshwaters with respect to biotic responses to such contaminants.

- Biotic indices currently available to water quality assessors have been developed mainly to detect eutrophication, organic pollution and acidification. Even within the context for which they were developed, limitations have been identified. Thus, they are of restricted suitability to detect the presence and magnitude of metal pollution and several authors have advocated development of a more reliable suite of pressure-specific indices. Until these are created, there remains a risk of overestimation of harmful metal concentrations, which could lead to over-spending on ameliorative techniques or for elevated metal concentrations to go undetected, with the potential to undermine remediation strategies.

- The need for more reliable metal-impairment indicators is clear but the difficulties of appraising ecosystem responses to metals must be balanced against practicalities of detecting and diagnosing field effects for regulation and management. There is a need to develop models relating metal data to ecological data that better represent influences on metal toxicity; biodiagnostic indices to reflect metal effects; better methods to identify the extent of metal acclimation or adaptation in sensitive taxa; and better investigative procedures to isolate metal effects at supposedly impacted sites from other pressures.

5 Effects of water chemistry on the bioaccumulation and toxicity of metals in the laboratory

5.1 Introduction

The influence of chemistry on the toxicity of metals to aquatic organisms under laboratory conditions has long been recognised. Grande (1966) showed that the acute toxicity of copper to salmonid fish was reduced in the presence of humic acid. Studies in the 1970s and 1980s (see Zitko *et al*., 1973; Pagenkopf *et al*., 1974; Zitko *et al*., 1976; Campbell and Stokes, 1985; Cusimano *et al*., 1986) established that the free metal ion activity was a better indicator of acute toxicity to fish than the total dissolved metal concentration, and demonstrated that the toxic effect of the free ion was itself modified by varying levels of hardness and acidity in the test water. Concurrently, studies on aquatic invertebrates (like Andrew *et al*., 1977; Dodge and Theis, 1979) and phytoplankton (see Sunda and Guillard, 1977) also demonstrated that the free metal ion was better correlated to toxicity than the total metal. In sediments, the role of both solid and solution phase chemistry has been recognised as important in controlling metal bioavailability and toxicity (see Swartz *et al*., 1995).

Pagenkopf (1983) developed a model (the gill surface accumulation model, GSIM) for the acute toxicity of cationic metals to fish based on the competitive interactions of the metal and solution cations (such as H^+, Na^+, Mg^{2+}, K^+, Ca^{2+}) at uptake sites on the gills, considered to be the main target organ for the toxic action of the metals. This model was linked to an equilibrium chemical speciation model which was used to calculate the activities of these solution ions from the bulk chemical composition. Under the assumption that toxicity was directly related to the extent of gill metal binding, the model allowed the toxic effect to be predicted as a function of gill metal accumulation, itself a function of the bulk solution chemistry.

GSIM provided the theoretical basis for subsequent development of the biotic ligand model (BLM) for the acute toxicity of metals. Key in the development of BLM was the creation of chemical speciation models able to model the binding of metal to natural organic matter (NOM) in addition to small ligands (such as Cl^-, SO_4^{2-}). While GSIM could model gill accumulation of metal in reconstituted waters lacking NOM, for field prediction the significant interactions of metals with these moieties could not be ignored. The development of Humic Ion Binding Model V (Tipping and Hurley, 1992) and the WHAM model (Tipping, 1994) provided a means of modelling metal-NOM interactions and provided a spur to the development of the BLM as a toxicity model applicable to complex water chemistries. The key chemical and biological interactions of a toxic metal, including interaction with the biotic ligand, are summarised in Figure 5.1.

Initial development of the BLM focused on the acute toxicity of metals to fish, since this was found to occur by inhibition of ion transport functions at the gill membrane (see Lauren and McDonald, 1985; Verbost *et al*.,1987; Spry and Wood, 1989). Key to the development of the model was the determination of (i) the amounts of gill-bound metal following short-term (two to three hour) exposure of live fish to metals (see Playle *et al*., 1992) and (ii) the relationship between this metal accumulation and eventual toxicity (see Playle *et al*, 1993). The scope of the acute BLM has subsequently been extended to aquatic invertebrates (see De Schamphelaere and Janssen, 2002a) although in this application the accumulation of metal at the biotic ligand was not measured and the model was applied directly to the toxic endpoint (LC_{50}), assuming that this level of effect was directly related to a certain amount of metal-BL binding.

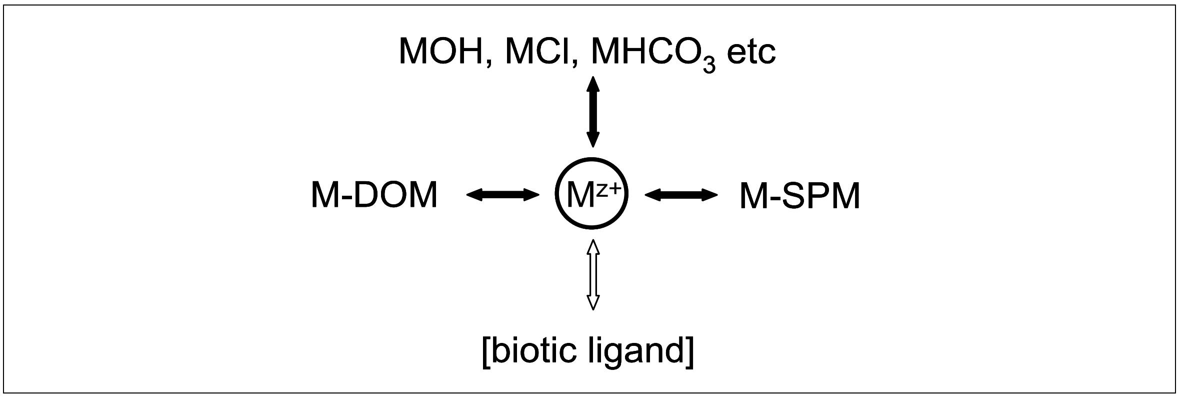

Figure 5.1 Schematic diagram of metal speciation, including binding to a biotic ligand. Note that all the reactions are subject to competition by other metal cations and H^+. DOM = dissolved organic matter, SPM = suspended particulate matter.

More recently, the BLM has been used to quantify the effects of water chemistry on metal toxicity to aquatic organisms in chronic exposures. This work is of particular relevance to the setting of EQS since long-term exposure to metals is more characteristic of organism exposure under field conditions than is short-term exposure.

For this reason, this review will include the outcomes of chronic BLM development in considering the implications of site-specific water chemistry variations for EQS setting. Firstly, the results of chronic exposure of organisms in reconstituted laboratory waters will be considered. Then, the effects of exposing organisms to natural waters under laboratory conditions will be reviewed. If available, information on the effects of mixtures of more than one metal will be considered, since under field conditions exposure of organisms is likely to comprise multiple rather than single metals. UK freshwaters contaminated by drainage from metal mines typically exhibit higher concentrations of multiple metals, depending upon the mineralogy of the mine (such as Cu, Zn from copper mines; Zn, Cd, Pb from lead-zinc mines). Finally, information on the reliability of BLM and associated models in generating site-specific EQS will be reviewed.

The role of bottom sediments as a control on metal bioavailability and toxicity has long been recognised. Since many of the organisms inhabiting UK streams and rivers affected by mine drainage are bottom or sediment-dwelling (particularly invertebrates), consideration of the role of sediment in influencing metal toxicity is necessary. We review current understanding of how sediments influence bioavailability and toxicity, in the context of sediment and porewater chemistry, and the role of organism traits (such as mode of feeding) on metal exposure routes.

5.2 Chemistry effects on metal toxicity in the water column

We first consider studies carried out in reconstituted laboratory waters. The advantage of using such waters is that chemical variables such as pH and hardness may be independently manipulated to study their effects on toxicity in isolation; this is not always possible in natural waters due to co-variance between variables. We consider studies according to organism type: algae, invertebrates and fish. The studies considered are summarised in Table 5.1 and listed in detail in Table 5.2.

Twenty-three studies on algae were identified. Copper was the most studied metal, followed by nickel. Zinc and cadmium were much less studied, while cobalt and lead were studied only once each. The pH was the most frequently studied water chemistry parameter in studies on algae (18 studies). In all studies where the pH was changed, an effect on metal toxicity was seen. A clear trend was seen, in that 15 of the 18 studies found that increasing pH increased the toxicity of the metal to the algae. The exceptions were the studies on Starodub *et al*., 1987 on the toxicity of Cu, Zn and Pb to *Scendesmus quadricauda*. Here, increasing pH from 4.5 to 6.5 and 8.5 decreased the toxicity of Cu and Zn, while for Pb a maximum in toxicity at pH 6.5 was seen.

The effects of hardness cations (Mg and Ca) were investigated in nine studies. With the exception of the study of De Schamphelaere *et al*. (2003), increasing the concentrations of hardness cations decreased metal toxicity. This is consistent with the theory of competitive binding to uptake sites postulated in BLM. The study of De Schamphelaere *et al*. (2003) is of note since the findings in respect of hardness appear to contradict those of a later study from the same laboratory (Heijerick *et al*., 2005a) using the same organism and toxic metal. In the later study, concentrations of Mg and Ca were varied at a single constant pH while in the earlier study pH, hardness and DOC were varied together. De Schamphelaere *et al*. (2003) theorised that effects of hardness at a given pH might not be seen at other pHs, resulting in the lack of a hardness effect observed in their experiments.

Table 5.1 Summary of toxicity tests in reconstituted waters.

Organism type	Metals used	Species tested	Significant effects
Algae	Co, Ni, Cu, Zn, Cd, Pb	*Pseudokirchneriella subcapitata* *Scendesmus quadricauda* *Chlamydomonas reinhardtii* *Chlorella* sp. *Ankistrodesmus falcatus* *Pediastrum duplex*	pH, Na, Hardness, DOC
Invertebrates	Co, Ni, Cu, Zn, Cd, Pb	*Hyalella azteca* *Ceriodaphnia dubia* *Daphnia magna* *Daphnia pulex*	pH, Na, Hardness, DOC
Fish	Ni, Cu, Zn, Cd, Pb	*Oncorhynchus mykiss* *Pimephales promelas*	pH, Na, Hardness, DOC

Effects of other cations (Na and K) were investigated in a single study on zinc. Increasing Na was found to decrease toxicity while no effect of K was seen. The effect of DOC was studied by De Schamphelaere *et al*. (2003) who observed an ameliorative effect.

Thirteen studies on invertebrates were identified. Copper was the most studied metal while cobalt and lead were the least studied. Hardness cations were varied in 12 studies. Amelioration of toxicity by increased hardness was observed in 11 of these studies. De Schamphelaere and Janssen (2002; 2004) observed no significant effects of Mg or Ca on copper toxicity to *Daphnia magna*, while Schwartz and Vigneault (2007) observed an ameliorative effect of Mg but not Ca on copper toxicity to *Ceriodaphnia dubia*. With the exception of one study (Winner, 1986) a consistent ameliorative effect of DOC was seen. Winner (1986) observed no effect of DOC (as 1.5 mg/l humic acid) on cadmium accumulation by *Daphnia magna* despite observing an ameliorative effect of the same concentration and type of DOC on the survival and reproduction of *Daphnia pulex* exposed to the same metal. The effect of pH was less consistent: of the five studies investigating pH, two showed an ameliorative effect, one showed a deleterious effect, one showed a bimodal effect with a maximum in toxicity, and one showed no significant effect. This variability in the nature of the pH effect can be related to the dual role that the H+ ion appears to play in controlling metal toxicity: its effect on the chemical speciation of the metal (reduced H+ competition for binding to many ligands, such as humic substances, at higher pH) and its competitive interaction with the toxic metal ion at the biotic ligand. The influence of pH on metal speciation can be accounted for by expressing toxicity as the free metal ion; competition at the biotic ligand would then result in an increase in free ion toxicity (decreasing endpoint concentrations) as pH increased, if other water chemistry variables were kept constant. In three of the five studies the effect of pH alone was studied and endpoints were expressed as the free metal ion; in all cases, the toxicity of the free ion increased. In the studies of de Schamphelaere and Janssen (2002; 2004) on copper toxicity to *Daphnia magna*, the effect of pH was not studied in isolation, but the subsequent derivation of a binding constant for protons at the biotic ligand infers that free copper ion toxicity would be ameliorated by increasing pH.

The effect of Na was investigated in three studies. De Schamphelaere and Janssen (2002) observed an ameliorative effect on copper toxicity to *Daphnia magna* and Heijerick *et al*. (2005b) observed an ameliorative effect on zinc toxicity to *Daphnia magna*. Schwartz and Vigneault (2007) observed no significant effect of Na on copper toxicity to *Ceriodaphnia dubia*.

Eight studies on fish were identified. Copper and lead were the most studied metals. Dissolved organic carbon was investigated in four studies and a consistent ameliorative effect on toxicity seen. Hardness cations were investigated in seven studies and with one exception ameliorative effects were seen. The exception was the lack of an effect of Ca on the accumulation of Pb by fathead minnows (*Pimephales promelas*) over 150 days (Mager *et al*., 2008). This contrasts with the ameliorative effect of Ca on Pb toxicity to the same species observed by Grosell *et al*. (2006) and shows that some caution may be needed in interpreting the results of accumulation and toxicity testing together since the total accumulated metal may not all be taken up in the same manner as the metal that exerts toxicity.

The effect of changing pH varied across the four studies in which it was investigated. Deleebeeck *et al*. (2007) and De Schamphelaere *et al*. (2004) found that increasing pH increased the toxicity of nickel and zinc, respectively, to rainbow trout (*Oncorhynchus mykiss*). Waiwood and Beamish (1978) observed an ameliorative effect of pH on copper toxicity to rainbow trout, while Grosell *et al*. (2006) observed a bimodal response of lead toxicity to fathead minnow to pH using exposures at pH 6.7, 7.4 and 8.1, with the lowest toxicity at pH 7.4. Deleebeeck *et al*. (2007) showed that toxicity expressed as free nickel ion increased with pH. De Schamphelaere and Janssen (2004b) did not investigate the effect of pH on zinc toxicity to rainbow trout in isolation, but developed a BLM from their data containing a proton-BL binding constant, thus inferring an increase in free zinc toxicity with increasing pH. The bimodal response to pH observed by Grosell *et al*. (2006) was investigated by calculating the free toxic lead ion using WHAM. A bimodal trend with pH was observed, which cannot be explained by the BLM theory in the absence of variations in concentrations of other BL-binding ions. The potassium concentration was higher at pH 6.7 and 8.1 than at pH 7.4, but this cannot explain the observations in the context of the BLM since this would require potassium to exert an additional toxic effect. A greater number of studies would be needed to better evaluate the pH effect in this instance.

Only a small number of studies have looked at the effects of water chemistry on the toxicity of metal mixtures to aquatic organisms. Starodub *et al*. (1987) looked at the effect of pH on the toxicity of binary and ternary combinations of copper, zinc and lead to the green alga *Scenedesmus quadricauda*. The results of the mixture analysis were ambiguous. The authors used three methods to assess mixture behaviour at each pH, and each approach gave different results. Generally, a greater degree of antagonistic behaviour was seen at higher pH and a greater degree of additive or synergistic behaviour at lower pH. Hatano and Shoji (2008) studied the response of duckweed (*Lemna paucicostata*) to combinations of copper and cadmium, and analysed the results using a BLM invoking a single biotic ligand at which Cu^{2+}, Cd^{2+} and H^+ bound competitively. The mixture toxicity was then predicted on the basis of the 'toxic units' of both metals bound to the biotic ligand and assuming additive behaviour of the two metals. The BLM approach was able to better describe the results than an approach based on consideration of the metal dose in terms of toxic units of the free ion in solution. However, the authors did not explicitly assess whether including a deviation from additive mixture behaviour in the model would have improved the fit.

The number of studies looking at water chemistry effects in natural waters is relatively small, and the available data are contained within studies that also investigate the effects of water chemistry on toxicity in reconstituted waters. De Schamphelaere and Janssen (2004a) presented results of exposing *Daphnia magna* to copper in ten natural surface waters and compared derived toxicity endpoints (no observed effect concentrations and 50 per cent effect concentrations) to those predicted using two chronic BLMs derived from tests using reconstituted waters not containing any added DOC. The models predicted most of the observations to within a factor of two, indicating that the water chemistry effects modelled by the BLMs were effectively predicting the toxicity in natural waters.

Heijerick *et al*. (2005a) exposed the alga *Pseudokirchneriella subcapitata* to copper in 13 natural waters from Europe, covering wide ranges of pH, hardness and DOC. They did not analyse the data using a BLM or bioavailability model but showed that in the dataset DOC was significantly correlated to toxicity, while pH and hardness were not. However, pH and hardness co-varied in the surface waters and the authors concluded that effects on toxicity were thus cancelled out in the dataset. De Schamphelaere and Janssen (2006) applied an empirical bioavailability model, derived from tests in reconstituted waters, to ten of these waters. Nine toxicity endpoints were predicted to within a factor of two and 94 per cent of the variability in endpoints was accounted for by the model.

Kallqvist (2008) exposed *Pseudokirchneriella subcapitata* to cadmium in three soft lake waters of Norway. The lake having the lowest hardness and DOC concentration gave the greatest toxicity of Cd. Adding calcium to the waters to artificially increase the hardness reduced the toxicity of Cd.

De Schamphelaere (2007, in Bass *et al*., 2008) exposed the alga *Pseudokirchneriella subcapitata* to Ni, Cu, Zn and Cd both singly and in quaternary mixtures, in two natural waters of northwest England. One sample was a soft, acidic water (pH 6.0 and hardness 5.8 mg $CaCO_3$/l) and the other was a hard, alkaline water (pH 8.1 and hardness 96 mg $CaCO_3$/l). The mixture results were analysed according to the method of Jonker *et al*. (2005) using two reference models: concentration addition and independent action. These two models assume similar and different modes of action of the mixture constituents respectively. However, toxicant modes of action cannot be inferred from toxicity data alone and so there was no a priori reason to exclude either model from consideration. In hard alkaline water both models fitted the results equally well assuming additive behaviour of the metals. In soft acidic water the independent action model fitted best assuming additive behaviour, while the concentration addition model fitted best assuming a synergistic effect. From the predictive viewpoint, the observed effect of the mixtures on the algae was predicted to within 10 per cent regardless of the reference model used and whether mixture interactions were accounted for. Therefore, although from a statistical viewpoint interactions among the metals might be significant, De Schamphelaere (2007) concluded that the predictive capacity of the reference models was similar. From this, it may be inferred that the choice of reference mixture model may not be significant for predicting mixture effects, although a larger data set would be required to test this hypothesis more fully.

The development of BLMs for risk assessment purposes has been a driver behind the generation of much of the toxicity test data described previously. BLMs or equivalent empirical bioavailability models have been developed for nickel (Deleebeeck *et al*., 2005 for *Pseudokirchneriella subcapitata*; Deleebeeck *et al*., 2007 for *Daphnia magna* and De Schamphelaere *et al*. 2006 for *Ceriodaphnia dubia*; Deleebeeck *et al*., 2008 for rainbow trout and fathead minnow), copper (De Schamphelaere and Janssen, 2006 for *Pseudokirchneriella subcapitata*; De Schamphelaere and Janssen, 2004a for *D. magna*; De Schamphelaere and Janssen, 2005 for rainbow trout and fathead minnow) and zinc (De Schamphelaere *et al*., 2005 for *Pseudokirchneriella subcapitata*, *Daphnia magna* and rainbow trout). In some cases, the effect of H^+ on the toxicity of the free ion cannot be described by the BLM. In this situation researchers have described toxicity (the endpoint concentration of the free metal ion) using an empirical regression of the toxic free metal ion concentration against pH.

These models have been used in EU risk assessments to derive water-specific predicted no-effect concentrations (PNECs), which may be used as the basis for EQS. In all three risk assessments, the species sensitivity distribution (SSD) approach was used to derive PNEC values from literature chronic toxicity endpoints. The key step in deriving a dataset of toxic endpoints for a site-specific SSD is to take the available set of toxic endpoints for the metal and to estimate, for each point, what the endpoint concentration would be if the toxicity test had been carried out in a water identical in composition to the water for which the SSD is to be derived. In the nickel and copper risk assessments this was done by a method termed 'read across'. Depending on the nature of the organism used in the test (plant/invertebrate/fish), a BLM was used to 'normalise' the toxicity data to the target water composition. A PNEC for the target water was then calculated using the SSD approach. The choice of bioavailability model varied but was based primarily on ecological relevance (such as the use of an algal model to process algal and plant endpoints). The approach was tested by considering, where possible, groups of toxicity endpoints for the same effect on the same organism, and considering whether variability in the endpoints was reduced when endpoint concentrations were 'normalised' to a common water composition. For nickel endpoint variability was reduced in 13 of 14 species for which endpoints were available in different water compositions, and for copper in 14 of 15 species. A distinct approach was taken in the zinc risk assessment whereby a non site-specific 'generic' PNEC was first calculated. Site-specific bioavailability effects were considered by adjusting the zinc concentration at the site using a bioavailability factor. This factor was found by calculating, for each of *Pseudokirchneriella subcapitata, Daphnia magna* and *Oncorhynchus mykiss*, the ratio of the predicted no observed effect concentration (NOEC) in the site water (using a bioavailability model) to the NOEC for a reference water. The smallest of the three ratios was used as the bioavailability factor.

BLM development for cobalt, cadmium and lead in freshwaters is currently at a much less advanced stage. EU risk assessments for cadmium and lead have proposed a hardness-dependent PNEC and a single generic PNEC respectively.

5.3 Chemistry effects on toxicity of metals in sediments

Exposure of sediment-dwelling organisms to metals has been a longstanding topic of interest for metal risk assessment. Sediments present a somewhat complex chemical environment compared with freshwaters due to possible extensive binding of metals to sediment solids. This raises the possibilities of (a) control of interstitial porewater metal concentrations by reactions with the solid phase, and (b) significant dietary uptake of metals by organisms following ingestion (deliberate or incidental) of sediments. A key aspect of sediment metal chemistry is associated with the development of anoxic conditions resulting in the formation of sulphide and the consequent precipitation of metals as insoluble sulphide precipitates. Much of the previous work on the relationship between sediment metal concentrations and toxicity has focused on the degree to which metals are sequestered as sulphides. In contrast to the work on the bioavailability of metals within the water column, this work has focused on measurement, particularly acid-volatile sulphide (AVS) and simultaneously extracted metals (SEM).

Acid-volatile sulphide is measured by extraction of sediment using cold dilute acid; SEM comprises the pool(s) of metals that are simultaneously released from sediment solids during this measurement. The SEM is assumed to comprise any metal originally bound to the sulphide released by extraction[1], along with any metal bound to other solid phases in the sediment. A number of studies (such as Di Toro *et al*., 1990; Ankley, 1996; Hansen *et al*., 1996) have shown that the ratio of SEM to AVS is an excellent predictor of lack of toxicity in sediments, where molar concentration of AVS exceeds the sum of molar concentrations of SEM. The resulting lack of toxicity is considered to arise from sequestration of metals into insoluble sulphides resulting in low porewater concentrations, independent of other variations in sediment chemistry such as pH and organic matter content. In sediments where SEM exceeds AVS, toxicity may or may not be observed. In this situation, there must be a significant pool of solid-phase metal(s) that is not in the form of sulphides but is bound to other solid phases such as organic matter. Here, metal porewater concentrations may be controlled by complexation with these solid phases and will depend on conditions such as solid phase concentration and porewater pH, giving rise to variations in metal bioavailability and toxicity. A corollary of the SEM/AVS approach is that the oxygen status of sediments is a key parameter since it will influence AVS concentrations.

The SEM/AVS approach has proved attractive for the risk assessment of metal-contaminated sediments since it is highly effective at predicting lack of bioavailability and toxicity when SEM is less than AVS. There have also been efforts to extend the approach to consider the situation when SEM is greater than AVS. Burton *et al*. (2005) proposed normalising sediment metal concentrations to take account of AVS and organic matter in considering likely toxic effects using the expression $(SEM - AVS)/f_{OC}$. In a field study on zinc-spiked systems of contrasting SEM and AVS, this normalised concentration was shown to be useful alongside SEM and AVS in predicting effects. Di Toro *et al*. (2005) formulated a sediment BLM based on the principle that AVS and organic matter are the main metal-binding phases and that when SEM is higher than AVS, porewater free metal ion activity is controlled by binding of metal to sediment organic matter. The model was able to explain the presence or absence of Cd and Zn toxicity to single and multiple organisms in chronic exposures and should form a good basis for developing our knowledge of how sediment chemistry affects metal toxicity.

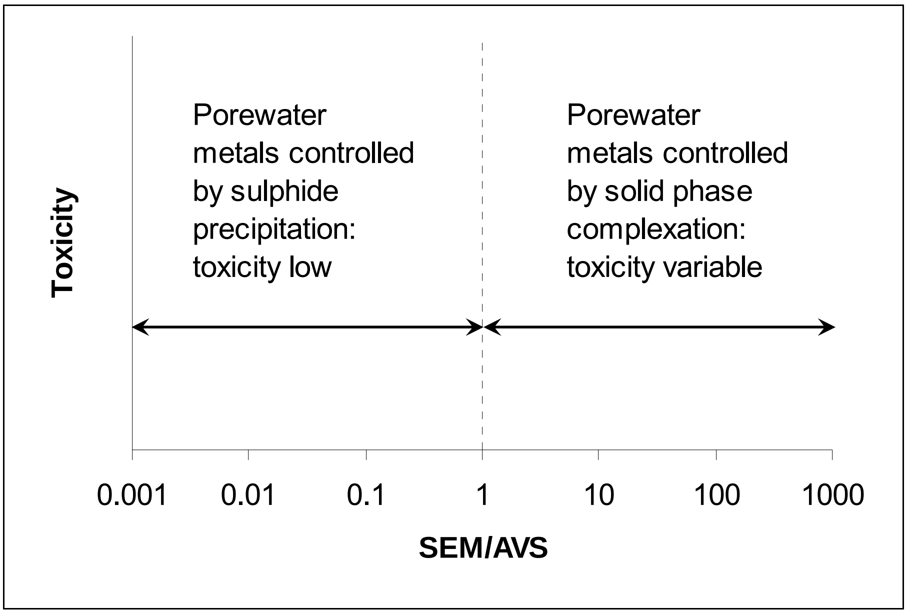

Figure 5.2 Conceptual picture of sediment metal toxicity as a function of the SEM/AVS ratio.

A notable assumption of SEM/AVS theory is that metal toxicity results from direct exposure in the pore water and that exposure via ingestion of sediment and/or food items within the sediment is negligible. The success of the theory, in many studies, in predicting absence of toxicity when SEM is less than AVS and porewater metal concentrations are minimal supports this contention. However, some workers (such as Lee *et al*., 2000a; 2000b) have obtained contradictory results where bioaccumulation and/or uptake has been observed when SEM is less than AVS. Lee *et al*. (2000a, 2000b) suggested that in their study dietary uptake of sediment was a vector for metal uptake and considered the biological attributes of test species, particularly mode and depth of feeding, to be key in controlling routes of metal exposure, uptake and toxicity. They also considered their experimental design to be more realistic than that used by other workers, particularly in terms of metal concentrations and stratification of oxygen concentrations within the sediment column. Clearly, however, organism traits are likely to be key in determining exposure routes and the consequent influence of sediment and/or porewater chemistry on metal toxicity. Some studies (such as Eimers *et al*., 2002 on Cd uptake by the benthic detritivore *Asellus*; Sibley *et al*. on Zn uptake by the detritivore *Chironomus tentans*) indicate that the porewater or overlying water is the main source of metal. On the other hand, Faria *et al*. (2007) suggested that metal uptake by *Chironomus riparius* was mainly due to sediment ingestion, based on a study where the ingestible sediment fraction was removed prior to testing. Experimental design and choice of organism are likely to be key in determining conclusions on exposure route. More research is needed to generate a robust picture of how organism traits influence exposure routes.

Although research on sediment metal toxicity has focused largely on anaerobic systems and the role of sulphide in sequestering metals, the potential role of this in UK ex-mining areas needs to be examined. Former mining areas of the UK are largely located in upland areas characterised by fast-flowing highly oxygenated streams and rivers. River beds are largely composed of relatively coarse material although finer material such as sand and fine sand may accumulate in patches of lower flow velocity (see Larsen *et al*., 2009). Given these conditions, generation of anaerobic zones within sediments, and the associated formation of metal sulphides, is likely to be small compared with lowland and lacustrine systems, although research in this area is lacking. Where sediments remain aerobic, metal complexation and direct organism exposure will be controlled by sorption reactions to chemically active phases on the sediment and to dissolved ligands present (particularly organic ligands such as humic substances). Important solid phases controlling sorption are likely to be organic matter and metal oxides, particularly those of iron and manganese (Lofts and Tipping, 1998; 2000). The amount of these materials within sediments will be controlled by factors such as catchment geology and land use.

5.4 Conclusions

- Available data on the effects of water chemistry on cationic metal toxicity and bioaccumulation in the water column, under laboratory conditions, strongly suggest that a number of chemical parameters, particularly pH, DOC and major cations (Na, Mg, K, Ca) exert a significant influence on the toxicity and/or bioaccumulation of cationic metals.
- Data on the effects of metal mixtures across different water chemistries are sparse, with implications for transferring understanding to mining-impacted sites in the field where mixture effects are likely.
- The BLM provides a conceptual framework for understanding these water chemistry effects as a combination of the influence of chemical speciation, and metal uptake by organisms in competition with H^+ and other cations. In some cases where the BLM cannot describe effects, empirical bioavailable models have been successfully used.
- BLM and empirical bioavailable models have performed well in reducing the variability in toxic endpoint concentrations of metals across waters of different compositions, even when applied to organisms for which they were not developed. This has allowed methods to be developed to derive site-specific measures of toxicity for nickel and copper. A simpler method has been implemented to perform the same task for zinc.
- Chronic BLMs for cobalt, cadmium and lead have not yet been developed, at least in the open literature. Development of BLMs, particularly for cadmium and lead, could be a step towards an integrated assessment of metal effects in UK mine-impacted systems.
- The effects of sediment chemistry on metal toxicity are relatively complex and not currently as well understood as the effects of water column

chemistry. It is clear that knowledge of the routes of metal exposure for bottom- or sediment-dwelling organisms is important in elucidating likely effects of chemistry on metal toxicity. Knowledge in this area is developing, but is somewhat patchy at the moment.

6 Effects of water chemistry on the bioaccumulation and toxicity of metals in mine-impacted systems in the field

6.1 Chemical speciation concepts

Natural waters are complex mixtures of numerous interacting components. From the viewpoint of the ecological effects of metals, the crucial issue is the bioavailability of a given metal, in terms of exerting toxic effects. The distribution of the metal among the different forms is referred to as its chemical speciation (Figure 5.1).

The "master species" is the free metal ion, for example Cu^{2+} in the case of copper, or Zn^{2+} for zinc, which can be used to express reactions with other components. All chemical speciation models use free ions as the master species in computing the (equilibrium) distribution of metals amongst their various chemical forms. The most useful speciation techniques produce estimates of free ion concentrations.

A widely-accepted paradigm in metal ecotoxicity is that the free metal ion concentration is a good indicator of bioavailability. The biotic ligand model (BLM) (Section 5.1) expresses this idea in the most straightforward way, by incorporating the biotic ligand into the speciation scheme, as shown in Figure 5.1. The toxic effect is taken to be proportional to the extent of occupation of the BL. However, this depends not only on the free ion concentration, but also on the concentrations of any ions that can compete for binding to the BL. In natural waters, the main competitors for the BL are H^+ (toxicity is pH-dependent), major cations such as Mg^{2+} and Ca^{2+}, and possibly Al(III) and Fe(III) species.

Thus, the free ion concentration is only a direct measure of bioavailability or toxicity if concentrations of potential competitors are kept constant, as might occur in controlled laboratory tests, or in seawater. In freshwaters, all interactions need to be factored in. This general idea applies whether or not the BLM is used to describe toxicity.

In summary, for metal toxicity in natural waters, we should take into account;

(a) distribution of the metal among different "non-biotic" forms;

(b) competition effects in metal interactions with organisms.

6.2 Metal speciation in freshwaters

Recent years have seen major advances in 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.

Tipping (2002) compared measured and modelled metal speciation in freshwaters and other media. Bass *et al.* (2008) compared the outputs of the WHAM6 (Tipping, 1994; 1998) model with measurements of speciation by 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). DMT is especially useful in the current context, because it provides estimates of the free metal ion. Quite good agreement was found between DMT estimates and WHAM6 predictions at the higher free ion concentrations that are most relevant for possible toxic effects in waters influenced by abandoned mines.

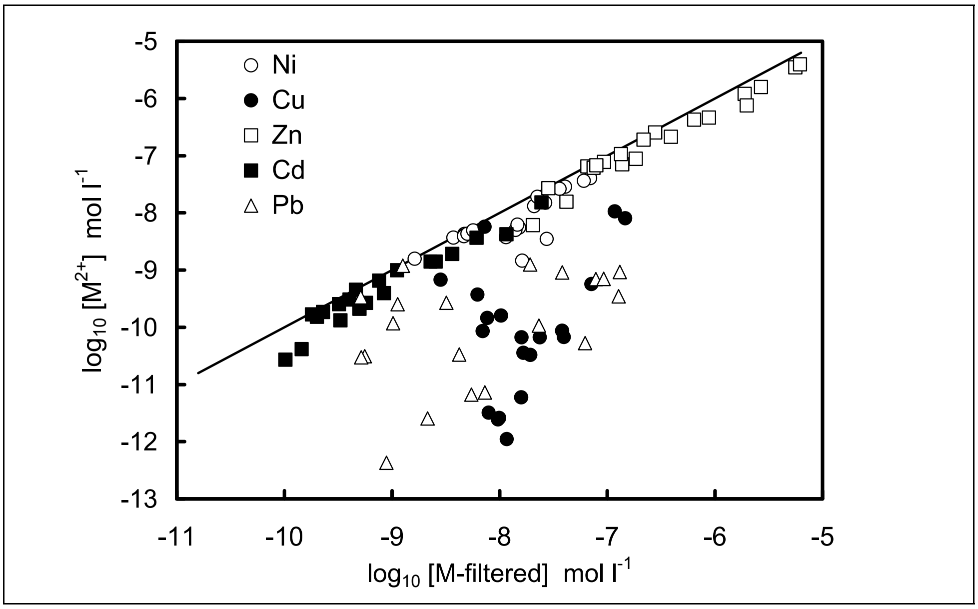

Figure 6.1 Free ion concentrations (logarithmic values) of Ni, Cu, Zn, Cd and Pb calculated using WHAM/Model VI, compared with the total filtered concentrations. The line is the 1:1 relation.

The significance of chemical speciation is shown by Figure 6.1 in which the computed free ion concentrations of Ni, Cu, Zn, Cd and Pb are plotted against the total filterable concentrations for 20 waters with ranges of pH and concentrations of DOC, major cations and heavy metals (Tipping *et al.*, 2008). The concentrations of Cu^{2+} and Pb^{2+} are strongly affected by complexation with DOM, and therefore differ substantially from the total concentrations, while complexation by DOM has a much smaller effect on Ni, Zn and Cd. In low-alkalinity waters these three metals are present largely as the free ions, but at high alkalinity interactions with carbonate also comes into play, and more than half of Ni, Zn and Cd is complexed. Aluminium trends (not shown in Figure 6.1) are broadly similar to those of Cu and Pb.

6.3 Bioaccumulation

Bioaccumulation is not necessarily related to toxicity, since some organisms respond to high metal levels by accumulating them in non-toxic forms (Chapter 4). However, measured bioaccumulation provides a useful means of evaluating relationships between chemical speciation and bioavailability.

In the context of metals in freshwaters, the most studied organisms are bryophytes, which accumulate metals roughly in proportion to total or filtered metal concentrations (Kelly and Whitton, 1989; Sergio *et al*., 2000; Whitton, 2003). Tipping *et al*. (2008) used the WHAM speciation model for humic acid to describe metal accumulation in bryophytes from stream sites, most of which were high in metals due to abandoned copper or lead mines (the same waters as in Figure 6.1). The WHAM modelling was based on metal free ion concentrations, and took into account competition effects for binding to the plants. The improved agreement between observed and calculated bryophyte metal contents (Figure 6.2 right-hand panel) is good evidence that speciation concepts apply to bioaccumulation in the field.

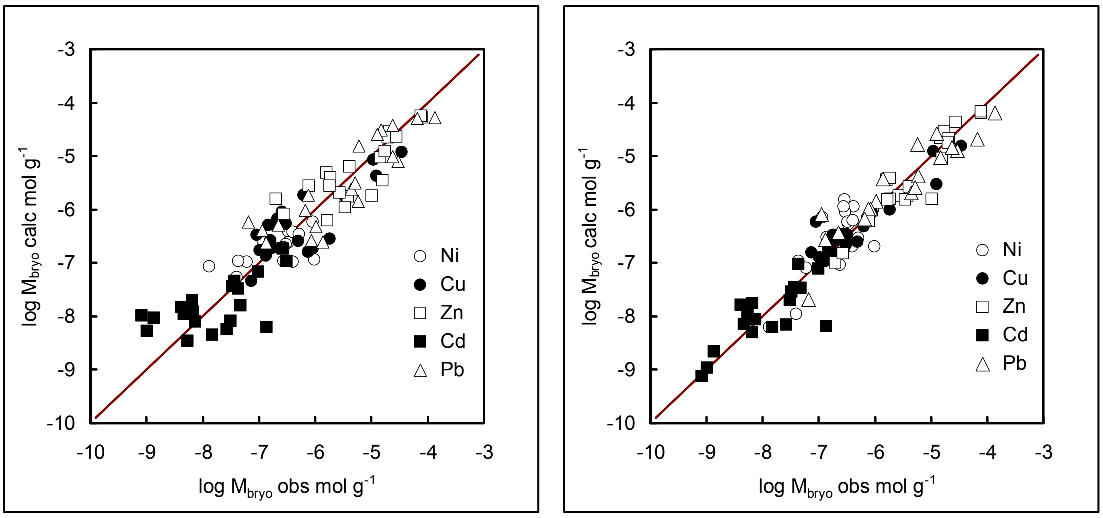

Figure 6.2 Observed and calculated log M_{bryo}. Panel A, calculated from regression with total filterable metal. Panel B calculated from Model VI.

Metal accumulation in six species of stream invertebrates was measured for the same streams of the bryophytes study, by Professor R Blust (University of Antwerp) as part of the Bass *et al*. (2008) project. Measured metal body burdens were compared with metal loadings of humic acid, calculated with WHAM. A fair measure of agreement between the two sets of values was obtained. However, non-linear relationships were observed for Ni, Cu, Zn and Cd, so that at low metal loadings, measured values tended not to vary greatly compared with modelled ones, whereas at higher loadings a slope nearer to 1:1 was observed. This suggests that organisms exert homeostatic control over metal contents at low loadings, but become overwhelmed when metal concentrations are high.

6.4 Field evidence of water chemistry effects

Chapters 3 and 4 reviewed the evidence of “heavy” metal effects in the field, but with interpretations based on total or filterable metal concentrations, and simple representations of other water chemistry factors, for example through alkalinity or pH. Chapter 5 considered investigations of water chemistry or speciation effects on metal toxicity through toxicity testing on natural water samples. In neither case was water chemistry or speciation related directly to observable biological or ecological effects, attributable to metal toxicity, in the field. A thorough review of the literature suggests that the only study in which this has been done is Bass *et al*. (2008), carried out for the Environment Agency, Scottish Environmental Protection Agency (SEPA), and the metals industry (Environment Agency Science Project SC030194).

Bass *et al*. (2008) carried out field studies of the ecology (macroinvertebrates and diatoms) and chemistry of 35 upland streams, influenced by past mining activity, and contaminated to different extents with trace metals (nickel, copper, zinc, cadmium, lead). Conventional EQS were exceeded in many cases, and there were a number of control sites. Different chemical variables were used to express metal concentrations and bioavailability. Data that enabled apparent toxic effect to be related to water chemistry were most readily-interpretable for invertebrates, partly because the River Invertebrate Prediction and Classification System (RIVPACS; Wright, 2000) could be used to provide expected numbers of taxa, against which observations could be compared.

The field data for invertebrates was used to calculate the variable OE67 for each stream. This is the ratio of the number of observed taxa to the expected number, but ignoring the most common 33 per cent of all taxa, in order to increase the sensitivity of the response variable. Using free metal ion concentrations estimated by WHAM modelling, empirical variables were used to attempt to explain the OE67 values. The variables, called free metal ion functions (FMIF_M), were based on results from soil toxicity studies (Lofts *et al*., 2004), and were of the form $\log_{10} \{[M^{z+}] / [H^+]^{\alpha}\}$ where α was 1.0, 0.5, 1.0, 0.25, 0.25 and 1.0 for Al, Ni, Cu, Zn, Cd and Pb respectively. By multiple regression analysis, the best explanation of the data was found to be a linear combination of FMIF_Al and FMIF_Zn (Figure 6.3).

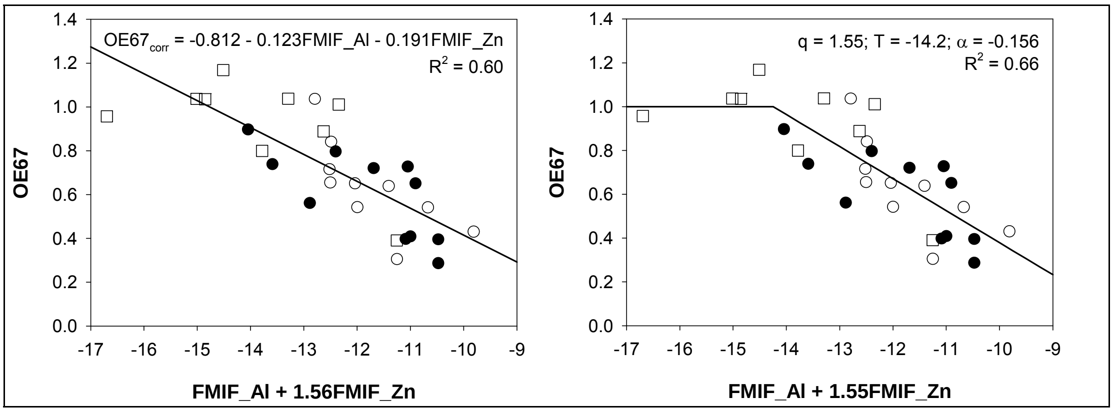

Figure 6.3 Regressions of free ion functions of Al and Zn against OE67, after correction for the relationship between OE67 and filterable Ti. Linear model (left), threshold model (right). Symbols refer to different site localities.

Note that the plots in Figure 6.3 include an additional empirical correction to the OE67 based on a correlation with filterable titanium, the reason for which we do not have a convincing explanation. If the Ti correction is not made, the multiple linear regression gives an r^2 of 0.46, comparable to a combination of hardness-corrected Al and Zn, and superior to linear combinations of filtered Al and Zn concentrations and of Al and Zn concentrations determined by DGT (r^2 around 0.3 in each case). The inclusion of the other metals did not improve the fits significantly.

Bass *et al*. (2008) used the BLM for Zn developed by Heijerick *et al*. (2005) for *Daphnia magna* to calculate site-specific values of a 'toxicity index' $[Zn^{2+}]_{observed}/[Zn^{2+}]_{NOEC}$. This application was limited by the fact that the water composition at only 12 of the study sites would be suitable for *D. magna* in the absence of metal stress. No significant relationship between OE67 and the toxicity index was found. Given that the multiple regression approach demonstrated the need to take both Al and Zn into account when dealing with field data, lack of success of the Zn BLM was attributed partly to the fact that it does not predict mixture effects. Theoretical work on the use of BLM for metal mixtures has been reported by Playle (2004) and Kamo and Nagai (2008). Practical applications to laboratory toxicity results with Cu, Cd and duckweed were carried out by Hatano and Shoji (2008). Promising results have been obtained, and it seems likely that a full mixture BLM could be developed. However, this will probably take some time, and it is a major and difficult step to transfer parameterisations obtained from laboratory studies with test organisms to the field.

Therefore we developed a model related to the BLM, but suitable for parameterisation with field data. The toxicity binding model (TBM) postulates a toxicity function F_{tox} given by the equation

$$F_{tox} = \Sigma \alpha_M v_M + \alpha_H v_H \qquad (6.1)$$

where v_M and v_H are the amounts of metals or protons non-specifically bound by the organism (mol/g) and α_M and α_H are toxicity coefficients that relate binding to a common toxic effect. The values of v_M and v_H are estimated by assuming that metal binding by the organisms can be represented by metal binding to humic acid, an assumption that is partly justified by the results with bryophytes and from direct measurements on invertebrates (see above). The model is fitted to field data by adjusting the values of α_M and α_H (Figure 6.4). TBM provides a slightly better fit of the field data than the empirical functions of Figure 6.3. Moreover, it attaches statistical significance to the toxic effects of H^+ and Cu, as well as Al and Zn.

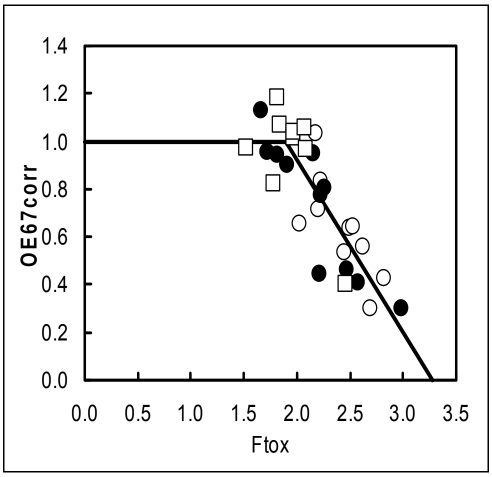

Figure 6.4 Threshold modelling of Ti-corrected macroinvertebrate occurrence, fitted using Equation 6.1.

TBM is still at a preliminary stage, and needs to be further tested Ongoing work with the model shows promise, with applications to data from other locations affected by heavy metals, including Cornwall and Wales (Hirst *et al*., 2002), Colorado (Deacon *et* *al*., 2001) and acid waters where acidity and Al are the principal concerns (Weatherley *et al*., 1987). An advantage of the model as it currently stands is that it provides a framework for considering mixture effects, not only among mine-derived metals, but also for H^+ and Al. This can even be extended to explore the effects of changing water conditions (such as acid pulses at high flow). TBM is potentially useful to the Environment Agency because it provides a single combined measure of the toxic effects of protons and metals, and incorporates a clear threshold above which macroinvertebrates are deleteriously affected. This idea is developed in Section 7.4.2.

Although TBM seems promising for macroinvertebrates in the field, it was less successful for diatoms, mainly because of the lack of a suitable response variable (the equivalent of OE67 in Figures 6.3 and 6.4). In principle, the model could be extended to fish, again given a suitable variable to use for fitting.

6.5 Conclusions

- The available data, although sparse, indicate that water chemistry influences metal effects on the ecosystem. This occurs firstly through complexation reactions, notably involving dissolved organic matter and metals such as Al, Cu and Pb.
- Secondly, because bioaccumulation and toxicity are partly governed by complexation-type reactions, competition effects among metals, and between metals and H^+, give rise to dependences upon water chemistry.
- There is evidence that combinations of metals are active in the field. In particular, Bass *et al*. (2008) demonstrated the combined effects of Al and Zn, and suggested that Cu and H^+ can also contribute.
- Chemical speciation is essential to interpret and predict observed effects in the field. Speciation results need to be combined with a model that relates free ion concentrations to toxic effect. This could be a multiple-regression model, BLM adapted to deal with mixtures, or TBM, which can be fitted to field data. BLM and TBM are attractive because they provide frameworks for considering metal interactions and competition effects.
- Toxic effects of heavy metals in the field should be considered together with those of Al and H^+.

7 Implications for waters affected by abandoned mines

The foregoing chapters cover the biological effects and chemistry of metals in freshwaters in the field and laboratory. Considerable knowledge has built up, which should be helpful in understanding the ecological effects of metals released from abandoned mines. However, the complexities of biological and ecological responses, and of water chemistry, mean that there is no direct answer to the question:

Are environmental quality standards for metals, developed for surface waters in general, applicable to the special case of waters that receive metal discharges from abandoned mines?

The literature review suggests that a number of factors need to be considered when addressing this question. Firstly, the extent to which organisms in mine-affected waters are adapted or acclimated to high levels of metals. Secondly, whether metal bioavailability in mine-affected waters differs significantly from that implied by the simplified approach in setting EQS. Several "secondary" factors should be mentioned.

7.1 Adaptation and acclimation

In several of the groups that figure in the WFD, specifically phytobenthos, invertebrates and fish species, the toxic impacts of metals can drop under prolonged metal exposure. Much of this evidence has been gathered from laboratory studies rather than in the field. There is ongoing debate about whether effects reflect genetically evolved adaptation over several generations (selection), or acclimation to sub-lethal metal concentrations through the induction of protective metal-binding proteins.

In smaller, rapidly growing organisms such as algae, genetic adaptation is better known than acclimation because of the difficulties of assessing acclimation in short-lived organisms. In invertebrates and fish there is evidence for genetic adaptation to metal exposure and for increased tolerance following maternal exposure, but individual acclimation is at least as important in reducing sensitivity, for example through the production of metal-binding proteins (metallothioneins). Either process - adaptation or acclimation - could affect the biological assessment of water quality or determination of EQS leading to overestimation of the metal concentrations at which ecological impairment occurs.

7.2 Bioavailability

Dissolved metal concentration alone is an inadequate predictor of toxic effect. Laboratory studies show clear effects of complexation by DOC, effects of pH on toxicity, and the protective action of major cations. In the field, bioaccumulation by aquatic plants and invertebrates depends on water chemistry. If chemical speciation is applied to field water samples, dose-response relationships can be derived (but taking mixtures into account). This kind of information has already prompted the application of bioavailability effects in setting EQS (Table 7.1).

Table 7.1 Water chemistry modifications to environmental quality standards proposed for the Water Framework Directive.

Metal	Modifications
Nickel	none[1]
Copper	pH, DOC, Na, Mg, Ca[2]
Zinc	pH, DOC, Na, Mg, Ca[2]
Cadmium	Hardness (five bands)
Lead	none[3]

[1] Subject to review based on the outcome of the nickel risk assessment.
[2] Under proposed tiered assessment scheme.
[3] Subject to review based on the outcome of the lead risk assessment.

In two cases (Cu and Zn), account will be taken of variations in bioavailability due to differences in water chemistry. Because there is nothing chemically unusual about waters around abandoned mines, relative variations due to chemistry in derived EQS should be reasonable from a bioavailability perspective. The only question is whether absolute values of EQS are appropriate for the organisms in the affected streams.

For Cd, EQS values should reflect bioavailability to some extent, by factoring in the protective effects of Mg and Ca. They will not take into account complexation by DOM (as measured by DOC), but this is probably not a big effect (see Figure 6.1). Variability in toxicity with pH would not be included, and this is potentially important.

No account is taken of bioavailability in the cases of Ni and Pb. Since Ni appears not to be an important metal in mine-affected areas of England and Wales, lack of bioavailability consideration is probably of minor importance. However, it is a serious deficiency for Pb, which is strongly complexed by DOM in all but the most acid streamwaters (Figure 6.1).

Table 2.2 was compiled using EQS values that do not take bioavailability into account, other than hardness effects. Application of the new WFD EQS of Table 7.1 would lead to fewer exceedances of Zn. However, there would only be a change for Pb if the absolute (single) concentration were altered.

At present, full bioavailability corrections are not proposed for Ni, Cd and Pb, that is, chemistry is not taken into account as well as it might be. Therefore, there will be a tendency to overprotect waters with elevated total dissolved concentrations of these three metals. But this could not be argued for Cu and Zn. In terms of water chemistry, new EQS proposed for the WFD are unsatisfactory from a bioavailability viewpoint for Ni, Cd and Pb, but probably acceptable for Cu and Zn.

In the field, organisms in streams that receive inputs of metals from abandoned mines are subject to potential toxic effects from mixtures of mine-derived metals, and also from the effects of aluminium and acidity. The only practical way to take this into account through the use of EQS is by the US EPA's Cumulative Criterion Unit (CCU; see Clements *et al*., 2000; Hirst *et al*., 2002). This takes the ratios of dissolved metal concentration to the EQS value and adds them together to generate an overall toxicity indicator, the CCU. The procedure implicitly assumes some kind of additivity of toxic effects. If the CCU exceeds unity, the "combined EQS" is exceeded. If applied to mine-affected waters, this would inevitably tend to increase the number of failures. It could, however, be argued that the approach would give a better indication of the deleterious effects of metals. Mixture toxicity criteria are difficult to employ when regulating point-source discharges resulting from multiple industrial or domestic processes. This might be less of a problem when, for example, setting discharge consents for a mine remediation scheme attempting to deal with the effects of several metals emanating from a single abandoned mine, or mine area.

7.3 Other factors: food, sediments, flow, flocs

Exposure of freshwater organisms to metals via the solution phase is undisputed, and widely regarded as the principal route. Accordingly, EQS values are derived from experiments in which toxic effects are induced by dissolved metals. However, metals may also be taken up in food (Section 4.3.4), in which case the characterisation of bioavailability by solution chemistry is less obviously applicable. The food uptake route would apply to invertebrates and fish, but not to algae and other plants. If it is quantitatively significant, susceptible organisms are actually exposed to greater amounts of metal than taken account of by EQS values.

Sediments can contain large quantities of metals (Section 2.2), and when they are soft and prone to anoxia, rather different chemical speciation reactions have to be taken into account to assess exposure (Section 5.3). Even in coarse sediment with relatively high water flows, benthic organisms may be exposed to greater amounts of metal than would be expected from the bulk streamwater (Section 4.2.5). Again, the implication is that greater exposure may occur in the field than anticipated by EQS values based on laboratory studies not involving sediments.

Streams affected by metal mine drainage are usually headwaters with large variations in water flow, driven by rainfall patterns. Assessment of a site by application of an EQS will involve the averaging of chemical composition, and this tends to underestimate exposure under high flow conditions. For streams affected by acidification, high flow conditions are generally the most dangerous with higher levels of acidity and aluminium, and less protective calcium (Section 4.2.4). This may also apply to heavy metals from mines, but on the other hand metal concentrations could be reduced by dilution under high-flow conditions. Consequently there is not an obvious general rule regarding the effects of flow.

Metal mines with sulphidic ores can release ferrous iron from pyrite, the subsequent oxidation of which leads to the precipitation of iron hydroxide and the formation of blanket flocs on stream beds. Such flocs are physically damaging to benthic invertebrates, and may increase stress and make organisms more susceptible to dissolved toxic metals.

7.4 Evaluation of the true effects of mine-derived metals

The effects discussed in Sections 7.1-7.3 are summarised in Table 7.2. There are a number of ways in which organisms in the field may experience different levels of metal toxicity than in the laboratory. Furthermore, the effects can be in opposite directions, with the majority expected to lead to greater toxicity in the field than in the laboratory. It is therefore difficult to argue that evidence favours the use of higher EQS values in the case of streams affected by abandoned mines. But it is certainly the case that the two most prominent factors, namely adaptation/acclimation and bioavailability, could justify changes in EQS, or the adoption of an alternative measure of toxic effects in the field.

Given that abandoned mines are widespread in England and Wales, and the high cost of their remediation to meet proposed WFD EQS criteria, further research is justified.

Table 7.2 Field factors that may affect toxic response.

Factor	Effect on toxic response
Adaptation/acclimation	Reduced
Chemical speciation (including bioavailability)	Reduced (mainly)
Metal mixtures	Increased
Metals in food	Increased
Exposure from sediment	Increased
Variations in flow	Increased or Reduced
Formation of flocs	Increased or Reduced

7.4.1 Can adaptation/acclimation effectively be proved?

We considered possibilities for research into adaptation/acclimation, aimed at producing results that could justify quantitative changes in EQS values. We judge this to be difficult. Investigations using transplantation experiments, protein expression and gene sequencing would be expensive. Any conclusions would be restricted to the array of test organisms investigated, which would likely be a limited sub-set of those contributing to quality assessment. Identifying acclimation or adaptation would not address more pressing issues with respect to predicting accurately metal concentrations likely to be toxic to any organism under varying exposure conditions.

For these reasons, we suggest that work on this topic is probably not the best way to proceed in Phase 2 of the project.

7.4.2 Quantifying relationships between chemistry and ecological communities

Although ecological communities in mine-affected streamwaters might be overprotected by the proposed WFD EQS, there are clearly some conditions under which metals emanating from abandoned mines exert toxic effects on biota, as evidenced by the reviews of Chapters 2 to 6. The main issue is therefore the reliable identification of unacceptable chemical conditions, and comparison of those conditions with those predicted by WFD EQS. If significant differences can convincingly be demonstrated, the argument could be made for alternative standards for waters affected by abandoned mines, and this could reduce the areas or numbers of watercourses deemed to fall beneath an acceptable standard.

Therefore in our view, the immediate research priority is to improve the quantification of metal effects under field circumstances. Demonstration of dose-response relationships, based on metal mixtures and their chemical speciation, and employing better biological tools to detect and diagnose community-level impairment, would provide the necessary scientific information to identify conditions at which metal effects on ecological communities (not just individual taxa) become unacceptable. Essentially, field data from areas that include abandoned mines would be used directly to quantify metal effects. Not only would the effects of mine-derived heavy metals be described, but also those of H^+ and Al^{3+}, enabling more diffuse acidification influences to be separated from the mines *per se*.

This is an ambitious goal, but the results of the recently completed Environment Agency/SEPA/metals industry project *Environmental quality standards for trace metals in the aquatic environment* (Bass *et al*., 2008) suggest that it may be achievable, and this is supported by the preliminary analysis of data from Wales presented in Chapter 2. In particular, in the Bass *et al*. study it was possible to demonstrate dose-response relationships in a relatively small data set, but only if Al^{3+} and Zn^{2+} were combined in predictive variables. This approach can be taken substantially further by combining several field data sets that already exist for the UK, and drawing in data from other countries. This would produce a sufficiently large number (around 200) of matched chemical and ecological (invertebrates and diatoms) data to provide the necessary strong constraints for the analysis. The most obvious method would involve parameterisation of the TBM described in Section 6.4, but other ways of combining the data could be considered.

Although this approach is potentially powerful, it would only explicitly take into account two of the factors (bioavailability and mixture effects) listed in Table 7.2. However, the effects of adaptation and/or acclimation would be implicit in the results, since these would be included in ecological relationships with metal concentrations and availability. Furthermore, metal contents of both invertebrate food and sediments are determined by essentially the same solution chemical reactions that govern the metal levels of susceptible biota. Indeed, evidence for this with respect to bryophytes is shown in Figure 6.2. Thus proportionality might be expected between food and sediments and the same water chemistry used to explain toxic effects.

Therefore, an effective way for the Environment Agency to assess the applicability of WFD EQS would be to compare their exceedances with those predicted by the field-based parameterised model(s) outlined above. This would be done using the same measured water chemistry. Without carrying out research to parameterise and prove the field-based model(s), we cannot yet forecast the outcome of this comparison. However, it would provide a strong and unbiased line of evidence in any proposal to amend or replace the general WFD EQS values.

References

ADMIRAAL, W., BLANCK, H., BUCKERT-DE JONG, M., GUASCH, H., IVORRA, N., LEHMANN, V., NYSTRÖM, B.A.H., PAULSSON, M., SABATER, S., 1999. Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. *Water Research*, 33, 1989-1996.

ALAM, M. K., MAUGHAN, O. E. 1995. Acute toxicity of heavy metals to common carp (Cypricus carpio). *Journal of Environmental Science and Health* – Part A Environmental Science and Engineering and Toxic and Hazardous Substance Control, 30, 1807-1816.

ALLEN, H. E., HANSEN, D. J. 1996. The importance of trace metal speciation to water quality criteria. *Water Environment Research*, 68, 42-54.

AMIARD, J. C., AMIARD-TRIQUET, C., BARKA, S., PELLERIN, J., RAINBOW, P. S. 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquatic Toxicology*, 76, 160-202.

ANDREW, R.W, BIESINGER, K.E., GLASS, G.E., 1977. Effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. *Water Research*, 11, 309-315.

ANKLEY, G.T., 1996. Evaluation of metal/acid-volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. *Environmental Toxicology and Chemistry*, 15, 2138-2146.

ARMITAGE, P. D. 1980. The effects of mine drainage and organic enrichment on benthos in the river Nent system, Northern Pennines. *Hydrobiologia*, 74, 119-128.

ARMITAGE, P. D., MOSS, D., WRIGHT, J. F., FURSE, M. T. 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running water sites. *Water Research*, 17, 333-347.

ARMITAGE, P.D., BOWES, M.J. AND VINCENT, H.M. (2007) Long-term changes in macroinvertebrate communities of a heavy metal polluted stream: the River Nent (Cumbria, UK) after 28 years. *River Research and Applications*, 23, 997-1015.

ATKINS LTD, 2008. Report on the impact of metal mine pollution from the Cwmystwyth, Frongoch-Wemyss and Dylife mines on macroinvertebrates, diatoms and fish Report to Environment Agency, Wales. 76pp.

BAE, H., NAM, S. S., PARK, H. S., Park, K. 2005. Metallothionein mRNA sequencing and induction by cadmium in gills of the crucian carp, Carassius auratus. *Journal of Health Science*, 51, 284-290.

BALCH, G. C., EVANS, R. D., WELBOURNE, P. Prairie, R 2000. Weight loss and net abnormalities of *Hydropsyche betteni* (Caddisfly) larvae exposed to aqueous zinc. *Environmental Toxicology and Chemistry*, 19, 3036-3043.

BASS, J.A.B., BLUST, R., CLARKE, R.T., CORBIN, T.A., DAVISON, W., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R., KALIS, E.J.J., KELLY, M.G., KNEEBONE, N.T., A.J. LAWLOR, A.J., LOFTS, S., TEMMINGHOFF, E.J.M., THACKER, S.A., TIPPING, E. VINCENT, C.D., WARNKEN, K.W., ZHANG. H., 2008. Environmental Quality Standards for trace metals in the aquatic environment. Environment Agency Science Report – SC030194

BATTARBEE, R.W., CHARLES, D.F., DIXIT, S.S., RENBERG, I., 1999. Diatoms as indicators of surface water acidity, In: *The Diatoms: Applications for the Environmental and Earth Sciences*, edited by E.F. Stoermer and J.P. Smol). Cambridge University Press, Cambridge.

BAUDRIMONT, M., ANDRES, S., DURRIEU, G., BOUDOU, A. 2003. The key role of metallothioneins in the bivalve *Corbicula fluminea* during the depuration phase, after in situ exposure to Cd and Zn. *Aquatic Toxicology*, 63, 89-102.

BAUDRIMONT, M., METIVAUD, J., MAURY BRACHET, R., RIBEYRE, F., BOUDOU, A. 1997. Bioaccumulation metallothionein response in the Asiatic clam (Corbicula fluminea) after experimental exposure to cadmium and inorganic mercury. *Environmental Toxicology and Chemistry*, 16, 2096-2105.

BAYKAN, U., ATLI, G., CANLI M. 2007. The effects of temperature and metal exposure on the profiles of metallothionein-like proteins in *Oreochromis niloticus. Environmental Toxicology and Pharmacology*, 23, 33-38.

BEASLEY, G. KNEALE P. 2001. Macroinvertebrates, heavy metals and PAHs in urban watercourses. Working Paper of the University of Leeds, School of Geography, 1, 3-58.

BEASLEY, G. KNEALE P. 2003. Investigating the influence of heavy metals on macroinvertebrate assemblages using Partial Canonical Correspondence Analysis (pCCA). *Hydrology and Earth Systems Sciences*, 7, 221-233.

BELTMAN, D. J., CLEMENTS, W. H., LIPTON, J., CACELA, D. 1999. Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. *Environmental Toxicology and Chemistry*, 18, 299-307.

BENASSI, J. C., LAUS, R., GEREMIAS, R., LIMA, P. L., MENEZES, C. T. B., LARANJEIRA, M. C. M., WILHELM, D., FAVERE, V. T., PEDROSA, R. C. 2006. Evaluation of remediation of coal mining wastewater by chitosan microspheres using biomarkers. *Archives of Environmental Contamination and Toxicology*, 51, 633-640.

BERRY, W. J., HANSEN, D. J., MAHONY, J. D., ROBSON, D. L., DITORRO, D. M., SHIPLEY, B. P., ROGERS, B., CORBIN, J. M., BOOTHMAN, W. S. 1996. Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulphide and interstitial water normalizations. *Environmental Toxicology and Chemistry*, 15, 2067-2079.

BERTHET, B., MOUNEYRAC, C., AMIARD, J. C., AMIARD-TRIQUET, C., BERTHELOT, Y., LE HAN, A., MASTAIN, O., RAINBOW, P. S., SMITH, B. D. 2003. Response to metals of the polychaete annelid *Hediste diversicolor*, a key species in estuarine and coastal sediments. *Archives of Environmental Contamination and Ecology*, 45, 468-478.

BESCH, K.W., RICHARD, M., CANTIN, R., 1972. Benthic diatoms as indicators of mining pollution in the Northwest Miramachi River System, New Brunswick, Canada. *Internationale Revue der Gesamten Hydrobiologie*, 57, 39-74.

BESCH, W. K., SCHNEIBER, I., MAGNIN, E. 1979. Influence of copper sulphate on the structure of the net of larvae of *Hydropsyche (Insecta, Trichoptera*). *Annales de Limnologie*, 15, 123-138

BESSER, J. M., BRUMBAUGH, W. G., ALLERT, A. L., POULTON, B. C., SCHMIDT, C. J., INGERSOLL, C. G. 2008. Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water. *Ecotoxicology and Environmental Safety*, 72, 516-526.

BESSER, J. M., MEBANE, C. A., MOUNT, D. R., IVEY, D., KUNZ, J. L. 2007. Sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Onchorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper, and zinc. *Environmental Toxicity and Chemistry*, 26, 1657-1665.

BLANCK, H., 1985. A simple, community-level, ecotoxicological test system using samples of periphyton. *Hydrobiologia*, 124, 251-261.

BLANCK, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. *Human and Ecological Risk Assessmen*t, 8, 1003-1024.

BLANCK, H., Dahl, B. 1996. Pollution-induced community tolerance (PICT) in marine periphyton in a gradient of tri-n-butyltin (TBT) contamination. *Aquatic Toxicology*, 35, 59-77.

BONADA, N., PRAT, N., RESH, V. H., STATZNER, B. 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, 51, 495-523.

BORGMANN, U, COUILLARD, Y., GRAPENTINE, L. C. 2007. Relative contribution of food and water to 27 metals and metalloids accumulated by caged *Hyalella azteca* in two rivers affected by metal mining. *Environmental Pollution*, 145. 753-765.

BOUCHE, M.L., HABETS, F., BIAGIANTI-RISBOURG, S., VERNET, G. 2000. Toxic effects and bioaccumulation of cadmium in the aquatic Oligochaete *Tubifex tubifex*. *Ecotoxicology and Environmental Safety*, 46, 246-251.

BRADLEY, H. A., 2008. Sediment chemistry and benthic macroinvertebrate communities within an acid mine drainage impacted stream. *Earth and Environment*, 3, 1-31.

BRADSHAW, A.D., MCNEILLY, T. 1981. Evolution and Pollution. Edward Arnold, London.

BROCK, T.D., 1969. Microbial growth under extreme conditions. *Symposia of the Society for General Microbiology*, 19, 15-41.

BROWN, B. E., 1977. Effects of mine drainage on the River Hayle, Cornwall a) factors affecting concentrations of copper, zinc and iron in water, sediments and invertebrate fauna. *Hydrobiologia*, 52, 221-233.

BROWN, V., SHURBEN, D., MILLER, W., CRANE, M. 1994. Cadmium toxicity to rainbow trout *Oncorhynchus mykiss walbaum* and brown trout *Salmo trutta L.* over extended exposure periods. *Ecotoxicology and Environmental Safety*, 29, 38-46.

BUCHWALTER, D. B., CAIN, D. J., MARTIN, C. A., XIE, L., LUOMA, S. N. GARLAND, T. 2008. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 8321-8326.

BURTON, G.A., NGUYEN, L.T.H., JANSSEN, C., BAUDO, R., MCWILLIAM, R., BOSSUYT, B., BELTRAMI, M., GREEN, A., 2005. Field validation of sediment zinc toxicity. *Environmental Toxicology and Chemistry*, 24, 541-553.

BUTCHER, R.W., 1955. Relation between the biology and the polluted condition of the Trent. *Verhandlungen, Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 12, 823-827.

CAIN, D. J., LUOMA, S. N., WALLACE, W. G. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry*, 23, 1463-1473.

CAMPBELL, P. G. C., GIGUÈRE, A., BONNERIS, E., HARE, L. 2005. Cadmium-handling strategies in two chronically exposed indigenous freshwater organisms – The yellow perch (*Perca flavescens*) and the floater mollusc (*Pyganodon grandis*). *Aquatic Toxicology*, 72, 83-97.

CAMPBELL, P. G. C., STOKES, P. M. 1985. Acidification and toxicity of metals in aquatic biota. *Canadian Journal of Fisheries and Aquatic Science*, 42, 2034-2049.

CANTON, J. H., SLOOF, W. 1982. Toxicity and accumulation studies on cadmium (Cd^{2+}) with freshwater organisms of different trophic levels. *Ecotoxicity and Environmental Safety*, 6, 113-128.

CARPENTER, K.E., 1924. A study of the fauna of rivers polluted by lead mining in the Aberystwyth District of Cardiganshire. *Annals of Applied Biology*, 11, 1-23.

CEN, 2003a. Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. EN 13946: 2003. Comité European de Normalisation, Geneva.

CEN, 2003b. Water quality - Guidance standard for the surveying of aquatic macrophytes in running waters. EN 14184: 2003. Comité European de Normalisation, Geneva.

CEN, 2004. Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. EN 14407:2004. Comité European de Normalisation, Geneva.

CHOWDHRY, M. J., MCDONALD, D. G., WOOD, C. M. 2004. Gastrointestinal uptake and fate of cadmium in rainbow trout acclimated to sublethal dietary cadmium. *Aquatic Toxicology*, 69, 149-163.

CLARK, J. L., CLEMENTS, W. H. 2006. The use of in situ and stream microcosm experiments to assess population- and community-level responses to metals. *Environmental Toxicology and Chemistry*, 25, 2306-2312.

CLARKE, S.E., STUART, J., SANDERS-LOEHR, J., 1987. Induction of siderophore activity in *Anabanea spp*. and its moderation of copper toxicity. *Applied and Environmental Microbiology*, 53, 917-922.

CLEMENTS, W. H. 1999. Metal tolerance and predator-prey interactions in benthic macroinvertebrate stream communities. *Ecological Applications*, 9, 1073-1084.

CLEMENTS, W. H. 2004. Small-scale experiments support causal relationships between metal contaminants and macroinvertebrate community responses. *Ecological Applications*, 14, 954-967.

CLEWS, E., ORMEROD, S. J. 2008. Improving biodiagnostic monitoring using simple combinations of standard biotic indices. *River Research and Applications*, 25, 348-361.

COMBER, S. D. W., MERRINGTON, G., STURDY, L., DELBEKE, K. and VAN ASSCHE, F. 2008. Copper and zinc water quality standards under the EU Water Framework Directive: The use of tiered approach to estimate the levels of failure. *Science of the Total Environment*, 403, 12-22.

COUILLARD, Y., CAMPBELL, P. G. C., TESSIER, A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. *Limnology and Oceanography*, 38, 299-313.

COURTNEY, L. A., CLEMENTS, W. H. 2002. Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: An experimental approach. *Freshwater Biology*, 47, 1766-1778.

CRANE, M., KWOK, K. W. H., WELLS, C., WHITEHOUSE, P., LUI, G. C. S. 2007. Use of field data to support European Water Framework Directive Quality Standards for dissolved metals. *Environmental Science and Technology*, 41, 5014-5021.

CROTEAU, M.-N., LUOMA, S. N. 2008. A biodynamic understanding of diet-borne metal uptake by a freshwater invertebrate. *Environmental Science and Technology*, 42, 1801-1806.

CROTEAU, M.-N., LUOMA, S. N., STEWART, A. R. 2005. Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. *Limnology and Oceanography*, 50, 1511-1519.

CUSIMANO, E.F., BRAKKE. D.F., CHAPMAN, G.A., 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*, 43, 1497-1503.

DAVIES, G., BUTLER, D., MILLS, M., WILLIAMS, D. 1997. A survey of ferruginous minewater impacts in the Welsh coalfields. *Journal of the Chartered Institute of Water and Environmental Management*, 11(2), 140-146.

DAVISON, W. AND ZHANG, H., 1994. In situ speciation measurements of trace components in natural waters using thin-film gels. *Nature*, 367, 545-548.

DAVY-BOWKER, J., MURPHY, J. F., RUTT, G. P., STEEL, J. E. C., FURSE, M. T. 2005. The development and testing of a macroinvertebrate biotic index for detecting the impact of acidity on streams. *Archiv für Hydrobiologie*, 163, 383-403.

DE SCHAMPHELAERE K.,VAN LAER L., DELEEBEECK N., MUYSSEN B., DEGRYSE F., SMOLDERS E., AND JANSSEN, C., 2006. Nickel speciation and ecotoxicity in European natural surface waters: development, refinement and validation of bioavailability models. University of Ghent. 125 p.

DE SCHAMPHELAERE, K. A. C., LOFTS, S., JANSSEN, C. R. 2005. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. *Environmental Toxicology and Chemistry*, 24, 1190-1197.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R., 2002a. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: The effects of calcium, magnesium, sodium, potassium, and pH. *Environmental Science and Technology*, 36(1), 48-54.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R., 2002b. Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to *Daphnia magna*. *Environmental Toxicology and Chemistry*, 23(5), 1115-1122.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2004a. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry*, 23, 1365-1375.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2004b. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. *Environmental Science and Technology*, 38, 6201-6209.

DE SCHAMPHELAERE, K.A.C., VASCONCELOS, F.M., HEIJERICK, D.G., TACK, F.M.G., DELBEKE, K., ALLEN, H.E., JANSSEN, C.R. 2003. Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata*. *Environmental Toxicology and Chemistry*, 22, 2454-2465.

DEACON JR, SPAHR NE, MIZE SV, BOULGER RW, 2001. Using water, bryophytes, and macroinvertebrates to assess trace element concentrations in the Upper Colorado River Basin. *Hydrobiologia* 455, 29-39.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C. HEIJERICK, D.J., BOSSUYT B., JANSSEN, C.R., 2005. Development and validation of biotic ligand models for predicting nickel toxicity to fish, daphnids and algae. University of Ghent.150 p.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2007. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. *Ecotoxicology and Environmental Safety*, 67, 1-13.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters. *Environmental Toxicology and Chemistry*, 27, 2097-2107.

DENICOLA, D.M., 2000. A review of diatoms found in highly acidic environments. *Hydrobiologia*, 433, 111-122.

DENISEGER, J., AUSTIN, A., LUCEY, W.P., 1986. Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. *Freshwater Biology*, 16, 209-218.

DESCHAMPHELAERE, K. C. A., JANSSEN, C. R. 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry*, 23, 1365-1375.

DESCHAMPHELAERE, K. C. A., LOFTS, S., JANSSEN, C. R. 2005. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids and fish in natural surface waters. *Environmental Toxicology and Chemistry*, 24, 1190-1197.

DI TORO, D.M., MAHONY, J.D., HANSEN, D.J., SCOTT, K.J., HICKS, M.B., MAYR, S.M., REDMOND, M.S., 1990. Toxicity of cadmium in sediments: the role of acid-volatile sulphide. *Environmental Toxicology and Chemistry*, 9, 1487-1502.

DI TORO, D.M., MCGRATH, J.A., HANSEN, .D.J., BERRY, W.J., PAQUIN, P.R., MATHEW, R., WU, K.B., SANTORE, R.C., 2005. Predicting sediment metal toxicity using a sediment biotic ligand model: methodology and initial application. *Environmental Toxicology and Chemistry*, 24, 2410-2427.

DIRECTIVE 76/464/EEC. 1976. Council Directive of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community. Official Journal of the European Communities L129/23.

DODDS, W.K., GUDDER, D.A., 1992. The ecology of Cladophora. *Journal of Phycology*, 28, 415-427.

DODGE, E.E., THEIS, T.L., 1979. Effect of chemical speciation on the uptake of copper by *Chironomus tentans*. *Environmental Science and Technology*, 13, 1287-1288.

DOIG, L. E., LIBER, K. 2006. Influence of dissolved organic matter on nickel bioavailability and toxicity to *Hyalella azteca* in water-only exposures. *Aquatic Toxicology*, 76, 203-216.

DOLÉDEC, S, PHILLIPS, N., SCARSBROOK, M., RILEY, R. H., TOWNSEND, C. R. 2006. Comparison of structural and functional approaches to determining land use effects on grassland stream invertebrate communities. *Journal of the North American Benthological Society*, 25, 44-60.

DSA, J. V., JOHNSON, K. S., LOPEZ, D., KANUCKEL, C., TUMLINSON, J. 2008. Residual toxicity of acid mine drainage – contaminated sediment to stream macroinvertebrates: Relative contribution of acidity vs. metals. *Water, Air and Soil Pollution*, 194, 185-197.

DUMAS, J., HARE, L. 2008. The internal distribution of nickel and thallium in two freshwater invertebrates and its relevance to trophic transfer. *Environmental Science and Technology*, 42, 5144-5149.

EIMERS, M.C., EVANS, R.D., WELBOURN, P.M., 2002. Partitioning and bioaccumulation of cadmium in artificial sediment systems: application of a stable isotope tracer technique. *Chemosphere*, 46, 543-551.

ELANGOVAN, R., MCROHAN, C. R., BALANCE, S., POWELL, J. J., WHITE, K. N. 2000. Localisation and fate of aluminium in the digestive gland of the freshwater snail *Lymnaea stagnalis. Tissue and Cell*, 32, 79-87.

ENVIRONMENT AGENCY 2008a. Abandoned Mines and the Water Environment. Environment Agency Science Project report SC030136-41.

ENVIRONMENT AGENCY 2008b. Assessment of metal mining-contaminated river sediments in England and Wales. Environment Agency Science Project report SC030136/SR4.

ERCAL, N., GURER-ORHAN, H., ARIKIN-BURNS, N. 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current Topics in Medicinal Chemistry*, 1, 529-239

ERICKSON, R. J., BENOIT, D. A., MATTSON, V. R., NELSON Jr., H. P., LEONARD, E. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. *Environmental Toxicology and Chemistry*, 15, 181-193.

ESPANA, J. S., PAMO, E. L., PASTOR, E. S., ANDRES, J. R., RUBI, J. A. M. 2006. The impact of acid mine drainage on the water quality of the Odiel River (Huelva, Spain): Evolution of precipitate mineralogy and aqueous geochemistry along the Concepcion-Tintillo segment. *Water, Air and Soil Pollution*, 173, 121-149.

EUROPEAN COMMUNITY, 1976. Council directive of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community (76/464/EEC). Official Journal of the European Community Series L 129.

EVERARD, M., DENNY, P. 1984. The transfer of lead by freshwater snails in Ullswater, Cumbria. *Environmental Pollution Series A: Ecological and Biological*, 35, 299-314.

FALASCO, E., BONA, F., BADINO, G., HOFFMAN, L., ECTOR, L., 2009. Diatom teratological forms and environmental alterations: a review. *Hydrobiologia*, 623, 1-35.

FARIA, M.S., LOPES, R.J., NOGUEIRA, A.J.A., SOARES, A.M.V.M., 2007. In situ and laboratory bioassays with *Chironomus riparius* larvae to assess toxicity of metal contamination in rivers: the relative toxic effect of sediment versus water contamination. *Environmental Toxicology and Chemistry*, 26, 1968-1977.

FARIA, M. S., LOPES, R. J., MALCATO, J., NOGUEIRO, A. J. A., SOARES, A. M. V. M. 2008. In situ bioassays with *Chiromomus riparius* larvae to biomonitor metal pollution in rivers and to evaluate the efficiency of restoration measures in mine areas. *Environmental Pollution*, 151, 213-221.

FELTEN, V., CHARMANTIER, G., MONS, R., GEFFARD, A., ROUSELLES, P., COQUERY, M., GARRIC, J., GEFFARD, O. 2008. Physiological and behavioural responses of *Gammarus pulex* (Crustacea: Amphipoda) exposed to cadmium. *Aquatic Toxicology*, 86, 413-425.

FINN, R. N. 2007. The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. *Aquatic Toxicology*, 81, 337-354.

FOSTER, P.L., 1982a. Metal resistances of Chlorophyta from rivers polluted by heavy metals. *Freshwater Biology*, 12, 41-61.

FOSTER, P.L., 1982b. Species associations and metal content of algae from rivers polluted by heavy metals. *Freshwater Biology*, 12, 17-39.

FRAYSEE, B., GEFFARD, O., BERTHET, B., QUEAU, H, BIAGIANTI-RISBOURG, S., GEFFARD, A. 2006. Importance of metallothioneins in the cadmium detoxification process in *Daphnia magna*. *Comparative Biochemistry and Physiology C – Toxicology and Pharmacology*, 144, 286-293.

FRYER, G., 1980. Acidity and species diversity in freshwater crustacean faunas. *Freshwater Biology*, 10, 41-45.

GAGNON, C., TURCOTTE, P., VIGNEAULT, B. 2008. Comparative study of the fate and mobility of metals discharged in mining and urban effluents using sequential extractions on suspended solids. *Environmental Geochemistry and Health* (In press), http://www.springerlink.com/content/h5214k4412182912/

GAMMONS, C.H., MILODRAGOVICH, L., BELANGER-WOODS, J. 2007. Influence of diurnal cycles on metal concentrations and loads in streams draining abandoned mined lands: an example from High Ore Creek, Montana. *Environmental Geology*, 53(3), 611-622.

GARCíA, M. E., CAPPELLETTI, C. A., SALIBIÁN, A. 1999. Sublethal maternal pre-exposure of fish to cadmium. Effect on the survival of the newly hatched alevins. *Archives of Physiology and Biochemistry*, 107, 152-158.

GARCíA-CRIADO, F., TOMÈ, A., VEGA, F. J., ANTOLíN, C. 1999. Performance of some diversity and biotic indices in rivers affected by coal mining in north western Spain. *Hydrobiologia*, 394, 209-217.

GEFFARD, O., GEFFARD, A., CHAUMOT, A., VOLLAT, B., ALVAREZ, C., TUSSEAU-VUILLEMIN, M.-H., GARRIC, J. 2008. Effects of chronic dietary and waterborne cadmium exposures on the contamination level and reproduction of *Daphnia magna. Environmental Toxicology and Chemistry*, 27, 1128-1134.

GERHARDT, A. 1990. Effects of subacute doses of cadmium on pH-stressed *Leptophlibia marginata* (L.) and *Baetis rhodani pictet* (Insecta: Ephemeroptera). *Environmental Pollution*, 67, 29-42.

GERHARDT, A., DE BISTOVEN, L. J., SOARES, A. M. V. M. 2004. Macroinvertebrate response to acid mine drainage: Community metrics and on-line behavioural toxicity bioassay. *Environmental Pollution*, 130, 263-274.

GERHARDT, A., DE BISTOVEN, L. J., SOARES, A. M. V. M. 2005. Effects of acid mine drainage and acidity on the activity of *Choroterpes picteti* (*Ephemeroptera: Leptophlibiidae*). *Archives of Environmental Contamination and Toxicology*, 48, 450-458.

GILL, T. S., BIANCHI, C. P., EPPLE, A. 1992. Trace-metal (copper and zinc) adaptation of organ systems of the American Eel, *Anguilla rostrata*, to external concentrations of cadmium. *Comparative Biochemistry and Physiology C – Pharmacology, Toxicology and Endocrinology*, 102, 361-371.

GOWER, A. M., MYERS, G., KENT, M., FOULKES, M. E. 1994. Relationship between macroinvertebrate communities and environmental variables in metal-contaminated streams in south-west England. *Freshwater Biology*, 32, 199-221.

GOZZARD, E. 2008. The sources, fate and dynamics of abandoned metal mine water pollutants: A catchment scale investigation of the River West Allen, Northumberland, UK. Unpublished PhD thesis, Newcastle University, UK. 184 pp plus appendices.

GRANDE, M., 1966. Effect of copper and zinc on salmonid fishes. Third International Conference on Water Pollution Research, Munich, 1, 97-111.

GRAY, N. F., DELANEY, E. 2008. Comparison of benthic macroinvertebrate indices for the assessment of the impact of acid mine drainage on an Irish river below an abandoned Cu-S mine. *Environmental Pollution*, 155, 31-40.

GREEN, D. W. J., WILLIAMS, K. A., PASCOE, D. 1986. The acute and chronic toxicity of cadmium to different life history stages of the freshwater crustacean *Asellus aquaticus* (L.). *Archives of Environmental Contamination and Toxicology*, 15, 465-471.

GREEN, R., MACDONALD, B. C. T., MELVILLE, M. D., WAITE, T. D. 2006. Hydrochemistry of episodic drainage waters discharged from an acid sulphate soil affected catchment. *Journal of Hydrology*, 325, 356-375.

GRIME, J.P., 1989. The stress debate: symptoms of impending synthesis? *Biological Journal of the Linnaean Society*, 37, 3-17.

GRIPPO, R. S., DUNSON, W. A. 1992. Use of whole-body sodium loss from the fathead minnow (*Pimephales promelas*) as an indicator of acid and metal toxicity. *Archives of Contamination and Toxicology*, 21, 289-296.

GRIPPO, R. S., DUNSON, W. A. 1996a. The body ion loss biomarker 1. Interactions between trace metals and low pH in reconstituted coal mine polluted water. *Environmental Toxicology and Chemistry*, 15, 1955-1963.

GRIPPO, R. S., DUNSON, W. A. 1996b. The body ion loss biomarker 2. Field validation in coal mine-polluted streams. *Environmental Toxicology and Chemistry*, 15, 1964-1972.

GROENENDIJK, D., KRAAK, M. H. S., ADMIRAAL, W 1999. Efficient shedding of accumulated metals during metamorphosis in metal-adapted populations of the midge *Chironomus riparius. Environmental Toxicology and Chemistry*, 18, 1225-1231.

GROENENDIJK, D., LUCKER, S. M. G., PLANS, M., KRAAK, M. H. S., ADMIRAAL, W 2002. Dynamics of metal adaptation in riverine Chironomids. *Environmental Pollution*, 117, 101-109.

GROENENDIJK, D., POSTMA, J. F., KRAAK, M. H. S., ADMIRAAL, W. 1998. Seasonal dynamics and larval drift of *Chironomus riparius* (Diptera) in a metal contaminated lowland river. *Aquatic Ecology*, 32, 341-351.

GROSELL, M., GERDES, R., BRIX, K. V. 2006. Influence of Ca, humic acid and pH on lead accumulation and toxicity in the fathead minnow during prolonged waterborne lead exposure. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 143, 473-483.

GUASCH, H., MUNOZ, I., ROSÉS, N., SABATER, S., 1997. Changes in atrazine toxicity throughout succession of stream periphyton communities. *Journal of Applied Phycology*, 9, 137-146.

GUNDERSEN, P., STEINNES, E. 2001. Influence of temporal variations in river discharge, pH, alkalinity and Ca on the speciation and concentration of heavy metals in some mining polluted rivers. *Aquatic Geochemistry*, 7, 173-193.

GUPTA, A., MORBY, A.P., TURNER, J.S., WHITTON, B.A., ROBINSON, N.J., 1993. Deletion within the metallothionein locus of cadmium-tolerant Synechococcus PCC 6301 involving a highly iterated palindrome (HIP1). *Molecular microbiology*, 7, 189-195.

GUPTA, A., WHITTON, B.A., MORBY, A.P., HUCKLE, J.W., ROBINSON, N.J., 1992. Amplification and rearrangement of a prokaryotic metallothionein locus smt in Synechococcus pcc-6301 selected for tolerance to cadmium. *Proceedings of the Royal Society of London Series B*, 248, 273-281.

HANSEN, B. H., RØMMA, S., GARMO, Ø. A., OLSVIK P. A., ANDERSEN, R. A. 2006. Antioxidative stress proteins and their gene expression in brown trout (*Salmo trutta*) from three rivers with different heavy metals. *Comparative Biochemistry and Physiology C - Toxicology and Pharmacology*, 143, 263-274.

HANSEN, D.J., BERRY, W.J., MAHONY, J.D., BOOTHMAN, W.S., DI TORO, D.M., ROBSON, D.L., ANKLEY, G.T., MA, D., YAN, Q., PESCH, C.E., 1996.Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations, Environmental Toxicology and Chemistry, 15, 2080–2094.

HANSEN, J. A., LIPTON, J., WELSH, P. G., MORRIS, J., CACELA, D., SUEDKAMP, M. J. 2002b. Relationship between exposure duration, tissue residues, growth and mortality in rainbow trout (*Oncorhynchus mykiss*) juveniles sub-chronically exposed to copper. *Aquatic Toxicology*, 58, 175-188.

HANSEN, J. A., WELSH, P. G., LIPTON, J., CACELA, D. 2002a. Effects of copper exposure on growth and survival of juvenile Bull Trout. *Transactions of the American Fisheries Society*, 131, 690-697.

HARDING, J.P.C., WHITTON, B.A., 1976. Resistance to zinc of *Stigeoclonium tenue* in the field and the laboratory. *British Phycological Journal*, 11, 417-426.

HARDING, J.P.C., WHITTON, B.A., 1981. Accumulation of Zn, Cd and Pb by field populations of Lemanea. *Water Research*, 15, 301-319.

HARE, L. 1992. Aquatic insects and trace metals: Bioavailability, bioaccumulation, and toxicity. *Critical Reviews in Toxicology*, 22, 327-369.

HARE, L., TESSIER, A. BORGMANN, U. 2003. Metal sources for freshwater invertebrates: Pertinence for risk assessment. *Human and Ecological Risk Assessment*, 9, 779-793.

HARGREAVES, J.W., WHITTON, B.A., 1976a. Effect of pH on growth of acid stream algae. *British Phycological Journal*, 11, 215-223.

HARGREAVES, J.W., WHITTON, B.A., 1976b. Effect of pH on tolerance of *Hormidium rivulare* to zinc and copper. *Oecologia* (Berlin), 26, 235-243.

HARPER, D. D., FARAG, A. M., BRUMBAUGH, W. G. 2008. Effects of acclimation on the toxicity of stream water contaminated with zinc and cadmium to juvenile cutthroat trout. *Archives of Environmental Contamination and Toxicology*, 54, 697-704.

HARRISON, G.I., CAMPBELL, P.G.C., TESSIER, A., 1982. Effects of pH changes on zinc uptake by *Chlamydomonas variabilis* grown in batch culture. *Canadian Journal of Fisheries and Aquatic Science*, 43, 687-693.

HATANO, A., SHOJI, R. 2008. Toxicity of copper and cadmium in combinations to duckweed analyzed by the Biotic Ligand Model. *Environmental Toxicology*, DOI 10.1002/tox.20348.

HEIJERICK, D. G., DESCHAMPHELAERE, K. A. C., JANSSEN, C. R. 2002. Predicting acute toxicity for Daphnia magna as a function of key water chemistry characteristics: Development and validation of a biotic ligand model. *Environmental Toxicology and Chemistry*, 21, 1309-1315.

HEIJERICK, D.G., BOSSUYT, B.T. A., DE SCHAMPHELAERE, K.A.C., INDEHERBERG, M., MINGAZZINI, M., JANSSEN, C.R. 2005a. Effect of varying physicochemistry of European surface waters on the copper toxicity to the green alga *Pseudokirchneriella subcapitata. Ecotoxicology*, 14, 661-670.

HEIJERICK, D.G., DE SCHAMPHELAERE, K.A.C., VAN SPRANG, P.A. AND JANSSEN, C.R., 2005b. Development of a chronic zinc Biotic Ligand Model for *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 62, 1-10.

HEINIS, F., TIMMERMANS, K. R., SWAIN, W. R. 1990. Short-term sublethal effects of cadmium on the filter feeding chironomid larvae *Glyptotendipes pallens* (Meigen) (Diptera). *Aquatic Toxicology*, 16, 73-86.

HEUGENS, E. H. W., HENDRICKS, A J., DEKKER, T., VAN STRAALEN, N. M., ADMIRAAL, W. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty of factors for use in risk assessment. *Critical Reviews in Toxicology*, 31, 247-284.

HICKIE, B. E., HUTCHINSON, N. J., DIXON, D. G., HODSON, P. V. 1993. Toxicity of trace metal mixtures to Alevin Rainbow Trout (*Oncorhynchus mykiss*) and larval Fathead Minnows (*Pimephales promelas*) in soft acidic water. *Canadian Journal of Fisheries and Aquatic Science*, 50, 1348-1355.

HIRST, H., JÜTTNER, I. AND ORMEROD, S.J., 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology*, 47, 1752-1765.

HOFFMAN, A.A., PARSONS, P.A., 1993. Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford.

HOGSTRAND, C., GROSELL, M., WOOD, C. M., HANSEN, H. 2003. Internal redistribution of radiolabelled silver among tissues of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): The influence of silver speciation. *Aquatic Toxicology*, 63, 139-159.

HOWARTH, R. S., SPRAGUE, J. B. 1978. Copper lethality to rainbow trout in waters of various hardness and pH. *Water Research*, 12, 455-462.

HOWELLS, E. J., HOWELLS, M. E., ALABASTER, J. S.1983. A field investigation of water quality, fish and invertebrates in the Mawddach river system, Wales. *Journal of Fish Biology*, 22, 447-469.

HRUSKA, K. A., DUBÉ, M. G. 2004. Using artificial streams to assess the effects of metal mining effluent on the life cycle of the freshwater midge *Chironomus tetans* in situ. *Environmental Toxicology and Chemistry*, 23, 2709-2718.

HYNES, H.B.N., 1960. The Biology of Polluted Waters. Liverpool University Press, Liverpool.

IRVING, E C., BAIRD, D. J., CULP, J. M. 2003. Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: Implications for toxicity testing. *Environmental Toxicology and Chemistry*, 22, 1058-1064.

IVORRA, N., BARRANGUET, C., JONKER, M., KRAAK, M.H.S., ADMIRAAL, W., 2002. Metal-induced tolerance in the freshwater microbenthic diatom *Gomphonema parvulum. Environmental Pollution*, 116, 147-157.

JACKSON, P.P., ROBINSON, N.J., WHITTON, B.A., 1991. Low molecular weight metal complexes in the freshwater moss *Rhynchostegium riparioides* exposed to elevated concentrations of Zn, Cu, Cd and Pb in the laboratory and field. *Environmental and Experimental Botany*, 31, 359-366.

JARVIS, A.P., GOZZARD, E., MAYES, W.M. AND POTTER, H.A.B. 2006. The importance of point and diffuse pollution in developing management strategies for long-abandoned deep coal and metal mines in the UK. Proceedings of the 13th Annual British Columbia – MEND ML/ARD Workshop: Challenges with open pits and underground workings. Vancouver, Canada, 29-30 November 2007.

JARVIS, A.P., YOUNGER, P.L. 1997. Dominating chemical factors in mine water induced impoverishment of the invertebrate fauna of two streams in the Durham Coalfield, UK. *Chemistry and Ecology*, 13, 249-270.

JARVIS, A.P., YOUNGER, P.L. 2000. Broadening the scope of mine water impact assessment - a UK perspective. *Environmental Impact Assessment Reviews*, 20(1), 85-96.

JARVIS, A.P., JOHNSTON, D., MAYES, W.M. AND POTTER, H.A.B. 2008. Application of a new methodology for assessing the priorities for abandoned mine water pollution remediation at a national scale: Results and implications from a study across England and Wales. Proceedings of the 10th International Mine Water Association Congress, Karlovy Vary, Czech Republic, 2-5 June 2008, pp 525-528.

JENSEN, T.E., RACHLIN, J.W., JANI, V., WARKENTINE, B.E. 1986. Heavy metal uptake in relation to phosphorus nutrition in *Anabaena variabilis* (Cyanophyceae). *Environmental Pollution Series A*, 42, 261-271.

JONES, J.R.E. 1940. A study of the zinc-polluted River Ystwyth in North Cardiganshire, Wales. *Journal of Animal Ecology*, 18, 67-88.

KALIS, E.J.J., WENG, L., TEMMINGHOFF, E.J.M. AND VAN RIEMSDIJK, W.H. 2006. Measuring free metal ion concentrations in situ in natural waters using the Donnan Membrane Technique. *Environmental Science and Technology*, 40, 955-961.

KALLQVIST, T. 2008. Effect of water hardness on the toxicity of cadmium to the green alga *Pseudokirchneriella subcapitata* in an artificial growth medium and nutrient-spiked natural lake waters. *Journal of Environmental Toxicology and Health – Part A* – Current Issues, 72(3-4), 277-283.

KAMO, M., NAGAI, T. 2008. An application of the Biotic Ligand Model to predict the toxic effects of metal mixtures. *Environmental Toxicology and Chemistry*, 27, 1479-1487.

KARAGEORGIS, A. P., NIKOLAIDIS, N. P., KARAMANOS, H., SKOULIKIDIS, N. 2003. Water and sediment quality assessment of the Axios River and its coastal environment. *Continental Shelf Research*, 23, 1929-1944.

KELLY, M.G. and WHITTON, B.A., 1989. Interspecific differences in Zn, Cd and Pb accumulation by freshwater algae and bryophytes. *Hydrobiologia*, 175, 1-11.

KELLY, M. G., WHITTON, B. A. 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7, 433-444.

KELLY, M.G., GIRTON, C., WHITTON, B.A., 1987. Use of moss-bags for monitoring heavy metals in rivers. *Water Research*, 111, 1429-1435.

KELLY, M.G., JUGGINS, S., GUTHRIE, R., PRITCHARD, S., JAMIESON, J., RIPPEY, B, HIRST, H., YALLOP, M, 2008. Assessment of ecological status in UK rivers using diatoms. *Freshwater Biology*, 53, 403-422.

KELLY, M.G., WHITTON, B.A., 1989a. Relationship between accumulation and toxicity of zinc in Stigeoclonium (*Chaetophorales, Chlorophyta*). *Phycologia*, 28, 512-517.

KELLY, M.G., WHITTON, B.A., 1989b. Interspecific differences in Zn, Cd and Pb accumulation by freshwater algae and bryophytes. *Hydrobiologia*, 175, 1-11.

KIFFNEY, P. M., CLEMENTS, W. H. 1994. Effects of heavy metals on a macroinvertebrate assemblage from a Rocky Mountain stream in experimental mesocosms. *Journal of the North American Benthological Society*, 13, 511-523.

KIFFNEY, P. M., CLEMENTS, W. H. 1995. Size-dependent response of macroinvertebrates to metals in experimental streams. *Environmental Toxicology and Chemistry*,15, 1352-1356.

KISLALIOGLU, M., SCHRERER, E., MCNICOL, R. E. 1996. Effects of cadmium on foraging behaviour of lake charr (*Salvelinus namaycush*). *Environmental Biology of Fishes*, 46, 75-82.

KNAPEN, D., BERVOETS, L., VERHEVEN, E., BLUST, R. 2004. Resistance to water pollution in natural gudgeon (*Gobio gobio*) populations may be due to genetic adaptation. *Aquatic Toxicology*, 67, 155-165.

KOWALILK, R. A., COOPER, D.M., EVANS, C. M., ORMEROD, S. J. 2007. Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology*, 13, 2439-2452.

KRACHLER, R., JISRA, F., AYROMLOU, S. 2005. Factors influencing the dissolved iron input by river water to the open ocean. *Biogeosciences Discussion*, 2, 537-549.

KRAMER, K. J. M., JAK, R. G., VAN HATTUM, B., HOOFTMAN, R. N., ZWOLSMAN, J. J. G. 2004. Copper toxicity in relation to surface water-dissolved organic matter: Biological effects to *Daphnia magna. Environmental Toxicity and Chemistry*, 23, 2971-2980.

KWANDRANS, J., 2007. Diversity and ecology of benthic diatom communities in relation to acidity, acidification and recovery of lakes and rivers. Diatom Monogarphs 9. A.R.G. Gantner Verlag K.G., Ruggell. 169pp.

LANGSTON, W. J., CHESMAN, B. S., BURT, G. R., POPE, N. D., MCEVOY, J. 2002. Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: An indicator of environmental quality? *Marine Environmental Research*, 53, 263-293.

LARSEN, S., VAUGHAN, I. P.,ORMEROD, S.J. 2009. Scale-dependent effects of fine sediments on temperate headwater invertebrates. *Freshwater Biology*, 54, 203-219.

LAURÉN, D.J., MCDONALD, D.G. 1985. Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo-gairdneri Richardson* - modulation by water hardness and pH. *Journal of Comparative Physiology B – Biochemical Systemic and Environmental Physiology*, 155(5), 636–644.

LEE, B.–G., GRISCOM, S.B., LEE, J–S., CHOI, H.J., KOH, C.–H.,LUOMA, S.N., FISHER, N.S., 2000b. Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments. *Science*, 287, 282-284.

LEE, B.–G., LEE, J–S., LUOMA, S.N., CHOI, H.J., KOH, C.–H., 2000a. Influence of acid volatile sulfide and metal concentrations on metal bioavailability to marine invertebrates in contaminated sediments. *Environmental Science and Technology*, 34, 4517-4523.

LEHMANN, V., TUBBING, G.M.J., ADMIRAAL, W., 1999. Induced metal tolerance in microbenthic communities from three lowland rivers with different metal loads. *Archives of Environmental Contamination and Toxicology*, 36, 384-391.

LICSKÓ, I., LOIS, L., SZEBÉNYI, G. 1999. Tailings as a source of environmental pollution. *Water Science and Technology*, 39, 333-336.

LOFTS, S., SPURGEON, D. J., SVENDSEN, C., TIPPING, E., 2004. Deriving soil critical limits for Cu, Zn, Cd, and Pb: A method based on free ion concentrations. *Environmental Science and Technology*, 38(13), 3623-3631.

LOPES, I., BAIRD, D. J., RIBEIRO, R. Ribeiro, 2005. Resistance to metal contamination by historically-stressed populations of *Ceriodaphnia pulchella*: Environmental influence versus genetic determination. *Chemosphere*, 61, 1189-1197.

LOWE, R.L., 1972. Notes on Iowa diatoms X: new and rare diatoms from Iowa. *Proceedings of the Iowa Academy of Science*, 79, 66-69.

LWANGA, M. S., KANSIIME, F., DENNY, P., SCULLION, J. 2003. Heavy metals in Lake George, Uganda, with relation to metal concentrations in tissue of common fish species. *Hydrobiologia*, 499, 83-93.

MACCAUSLAND, A., MCTAMMANY, M. E. 2007. The impact of episodic coal mine drainage pollution on benthic macroinvertebrates in streams in the Anthracite region of Pennsylvania. *Environmental Pollution*, 149, 216-226.

MACEDO-SOUSA, J. A., GERHARDT, A., BRETT, C. M. A., NOGUEIRA, A. J. A., SOARES, A. 2008. Behavioural response of indigenous benthic invertebrates (*Echinogammarus meridionalis, Hydropsyche pellucidula and Choroterpes picteti*) to a pulse of acid mine drainage: A laboratorial study. *Environmental Pollution*, 156, 966-973.

MAGER, E. M., WINTZ, H., VULPE, C. D., BRIX, K. V., GROSELL, M. 2008. Toxicogenomics of water chemistry influence on chronic lead exposure to the fathead minnow (*Pimephales promelas*). *Aquatic Toxicology*, 87, 200-209.

MALMQVIST, B., HOFFSTEN, P-O. 1999. Influence of drainage from old mine deposits on benthic macroinvertebrate communities in central Swedish streams. *Water Research*, 33, 2415-2423.

MALTBY, L., NAYLOR, C. 1990. Preliminary observations on the ecological relevance of the Gammarus "scope for growth" assay: effect of zinc on reproduction. *Functional Ecology*, 4, 393-397.

MARR, J. C. A., BERGMAN, H. L., PARKER, M., LIPTON, J., CACELA, D., ERICKSON, W., PHILLIPS, G. R. 1995. Relative sensitivity of brown and rainbow trout to pulsed exposures of an acutely lethal mixture of metals typical of the Clark Fork River, Montana. *Canadian Journal of Fisheries and Aquatic Sciences*, 52, 2005-2015.

MATZKU, S., BRODA, E., 1970. Die Zinkaufnahme in das Innere von Chlorella. *Planta* (Berlin), 92, 29-40.

MAUND, S. J., TAYLOR, E. J., PASCOE, D. 1992. Population responses of the freshwater amphipod crustacean *Gammarus pulex* (L.) to copper. *Freshwater Biology*, 28, 29-36.

MAYES, W.M., GOZZARD, E., POTTER, H.A.B. AND JARVIS, A.P. 2008. Quantifying the impacts of diffuse minewater pollution in a historically heavily coal mined catchment. *Environmental Pollution*, 151, 165-175.

MCCAHON, C. P., PASCOE, D. 1988. Increased sensitivity to cadmium of the freshwater amphipod *Gammarus pulex* (L.) during the reproductive cycle. *Aquatic Toxicology*, 13, 183-194.

MCCAHON, C. P., PASCOE, D. 1989a. Cadmium toxicity to the freshwater amphipod *Gammarus pulex* (L.) during the moult cycle. *Freshwater Biology*, 19, 197-205.

MCCAHON, C. P., PASCOE, D. 1989b. Short-term experimental acidification of a welsh stream: Toxicity of different forms of aluminium at low pH to fish and invertebrates. *Archives of Environmental Contamination and Toxicology*, 18, 233-242.

MCCAHON, C. P., PASCOE, D. 1990. Episodic pollution: causes, toxicological effects and ecological significance. *Functional Ecology*, 4, 375-383.

MCCAHON, C. P., PASCOE, D., MCKAVANAGH, C. 1987. Histochemical observations on the salmonids *Salmo salar* L. and *Salmo trutta* L. and the Ephemeropterans *Baetis rhodani* (Pict.) *and Ecdyonurus venosus* (Fabr.) following a simulated episode of acidity in an upland stream. *Hydrobiologia*, 153, 3-12.

MCCAHON, C. P., WHILES, A. J., PASCOE, D. 1989. The toxicity of cadmium to different larval instars of the trichopteran larvae *Agapetus fuscipes Curtis* and the importance of life cycle information to the design of toxicity tests. *Hydrobiologia*, 185, 153-162.

MCFARLAND, B.H., HILL, B.H., WILLINGHAM, W.T., 1997. Abnormal Fragilaria spp. (Bacillariophyceae) in streams impacted by mine drainage. *Journal of Freshwater Ecology*, 12, 141-149.

MERSCH, J., WAGNER, P., PIHAN, J. C. 1996. Copper in indigenous and transplanted Zebra Mussels in relation to changing water concentrations and body weight. *Environmental Toxicology and Chemistry*, 15, 886-893.

MICHAILOVA, P., ILKOVA, J., WHITE, K. N. 2003. Functional and structural rearrangements of salivary gland polytene chromosomes of *Chironomus riparius* Mg. (Diptera, Chironomidae) in response to freshly neutralised aluminium. *Environmental Pollution*, 123, 193-207.

MICHAILOVA, P., PETROVA, N., ILKOVA, J., BOVERO, S., BRUNETTI, S., WHITE, K. 2006. Genotoxic effect of copper on salivary gland polytene chromosomes of *Chironomus riparius Meigen* 1804 (Diptera, Chironomidae). *Environmental Pollution*, 144, 647-654.

MIGHANETARA, K., BRAUNGARDT, C., RIEUWERTS, J.S. AND AZIZI, F. 2008. Contaminant fluxes from point and diffuse sources from abandoned mines in the River Tamar catchment, UK, *Journal of Geochemical Exploration*, doi:10.1016/j.gexplo.2008.03.003.

MILLER, T. G., MACKAY, W. C. 1980. The effects of hardness, alkalinity and pH of test water on the toxicity of copper to rainbow trout (*Salmo gairdneri*). *Water Research*, 14, 129-133.

MOORE, J.W., 1981. Epipelic algal communities in a eutrophic northern lake contaminated with mine wastes. *Water Research*, 15, 97-105.

MORIN, S., DUONG, T.T., DABRIN, A., COYNEL, A., HERLORY, O., BAUDRIMONT, M., DELMAS, F., DURRIEU, G., SCHÄFER, J., WINTERTON, P., BLANC, G., COSTE, M., 2007. Long-term survey of heavy metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. *Environmental Pollution*, 151, 532-542.

MURPHY, J., DAVY-BOWKER, J., ORMEROD, S. J. In press. Developing bio-diagnostic indices for assessing acidity in sensitive freshwaters. *Journal of Applied Biology*.

MUSIBONO, D. E., DAY, J. A. 1999. The effect of Mn on mortality and growth in the freshwater amphipod *Paramelita nigroculus* (Barnard) exposed to a mixture of Al and Cu in acidic waters. *Water Research*, 33, 207-213.

NEYMEYER, A., WILLIAMS, R. T., YOUNGER, P.L. 2007. Migration of polluted mine water in a public supply aquifer. *Quarterly Journal of Engineering Geology and Hydrogeology*, 40: 75-84.

NIYOGI, S., KENT, R., WOOD, C. M. 2008. Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (*Onchorychus mykiss*): A biotic ligand model (BLM) approach. *Comparative Biochemistry and Physiology C – Pharmacology, Toxicology and Endocrinology*, 148, 305-314.

O'HALLORAN, K., CAVANAGH, J-A., HARDING, J. S. 2008. Response of a New Zealand mayfly (Deleatidium spp.) to acid mine drainage: Implications for mine remediation. *Environmental Toxicity and Chemistry*, 27, 1135-1140.

ORMEROD, S. J., BOOLE, P., MCCAHON, C. P., WEATHERLEY, N. S., PASCOE, D., EDWARDS, R. W. 1987. Short-term experimental acidification of a Welsh stream: Comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biology*, 17, 341-356.

PAGENKOPF, G.K., 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH, and water hardness. *Environmental Science and Technology*, 17, 342-347.

PAGENKOPF, G.K., RUSSO, R.C., THURSTON ,R.V., 1974. Effect of complexation on toxicity of copper to fishes. *Journal of the Fisheries Research Board of Canada*, 31, 462-465.

PANDEY, S., PARVEZ, S., ANSARI, R. A., ALI, M., KAUR, M., HAYAT, F., AHMAD, F., RAISUDDIN, S. 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata Bloch*. *Chemico-Biological Interactions*, 174, 183-192.

PASCOE, D., EVANS, S. A., WOODWORTH, J. 1986. Heavy metal toxicity to fish and the influence of water hardness. *Archive of Environmental Contamination and Toxicology,* 15, 481-487.

PATTERSON, G., WHITTON, B.A., 1981. Chemistry of waters, sediments and algal filaments in groundwater draining an old lead-zinc mine. In: *Heavy Metals in Northern England: Environmental and Biological Aspects*, edited by P.J. Say and B.A. Whitton. Department of Botany, University of Durham. pp. 65-81.

PEDERSEN, S. N., LUNDEBYE, A. K. 1996. Metallothionein and stress protein levels in shore crabs (*Carcinus maenas*) along a trace metal gradient in the Fal estuary (UK). *Marine Environmental Research*, 42, 241-246.

PÉRY, A. R. R., GEFFARD, A., CONRAD, A., MONS, R., GARRIC, J. 2008. Assessing the risk of metal mixtures in contaminated sediments on *Chironomus riparius* based on cytosolic accumulation. *Ecotoxicology and Environmental Safety*, 71, 869-873.

PETERSEN, L. B. M., PETERSEN Jr., R. C. 1983. Anomalies in Hydropsychid capture nets from polluted streams. *Freshwater Biology*, 13, 185-191.

PETRAUSKIENE, L. 2003. Water and sediment toxicity assessment by use of behavioural responses of medicinal leeches. *Environment International*, 28, 729-236.

PLAYLE, R.C., 2004. Using multiple metal-gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. *Aquatic Toxicology*, 67, 359-370.

PLAYLE, R.C, DIXON, D.G, BURNISON, K., 1993. Copper and cadmium-binding to fish gills - estimates of metal gill stability-constants and modelling of metal accumulation. *Canadian Journal of fisheries and Aquatic Sciences*, 50, 2678-2687.

PLAYLE, R.C, GENSEMER, R.W, DIXON, D.G., 1992. Copper accumulation on gills of fathead minnows - influence of water hardness, complexation and pH of the gill microenvironment. *Environmental Toxicology and Chemistry*, 11, 381-391.

POSTMA, J. F., KYED, M., ADMIRAAL, W. 1995. Site-specific differentiation in metal tolerance in the midge *Chironomus riparius* (Diptera: Chironomidae). *Hydrobiologia*, 315, 159-165.

POULTON, M., PASCOE, D. 1990. Disruption of precopula in Gammarus pulex (L.) – developments of a behavioural bioassay for evaluating pollutant and parasite induced stress. *Chemosphere*, 20, 403-415.

POWELL, J. H., POWELL, R. E. 2001. Trace elements in fish overlying subaqueous tailings in the tropical west Pacific. *Water, Air and Soil Pollution*, 125, 81-104.

PYLE, G. G., SWANSON, S. M., LEMKUHL, D. M. 2002. The influence of water hardness, pH, and suspended solids on nickel toxicity to larval fathead minnows (*Pimephales promelas*). *Water, Air and Soil Pollution*, 133, 215-226.

PYNNÖNEN, K. 1995. Effect of pH, hardness and maternal preexposure in the toxicity of Cd, Cu and Zn to the Glochidal larvae of a freshwater clam *Anodonta cygnaea*. *Water Research*, 29, 247-254.

QUINN, M. R., FENG, X. H., FOLT, C. L., CHAMBERLAIN, C. P. 2003. Analyzing trophic transfer of metals in steam food webs using nitrogen isotopes. *Science of the Total Environment*, 317, 73-89.

RAINBOW, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution*, 120, 497-507.

REID, S. D., MCDONALD, D. G., RHEM, R. R. 1991. Acclimation to sub-lethal aluminium - Modifications of metal-gill surface interactions of juvenile rainbow trout (*Oncorhyncus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 1996-2005.

RICKWOOD, C. J., DUBÉ, M. G., WEBER, L. P., LUX, S., JANZ, D. M. 2008. Assessing the effects of a mining and municipal sewage effluent mixture on fathead minnow (*Pimephales promelas*) reproduction using a novel field-based trophic-transfer artificial stream. *Aquatic Toxicology*, 86, 272-286.

ROBINSON, N.J., 1989. Algal metallothioneins: secondary metabolites and proteins. *Journal of Applied Phycology,* 1, 5-18.

ROESIJADI, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquatic Toxicology*, 22, 81-114.

ROESIJADI, G. 2000. Metal transfer as a mechanism for metallothionein-mediated metal detoxification. *Cellular and Molecular Biology*, 46, 393-405.

ROY, R., CAMPBELL, P. G. C. 1995. Survival-time modelling of exposure of juvenile Atlantic Salmon (*Salmo salar*) to mixtures of aluminium and zinc in soft water at low pH. *Aquatic Toxicology*, 33, 155-176.

SALÁNKI, J., TURPAEY, T. M., NICHAEYA, M. 1991. Mussel as a test animal for assessing environmental pollution and the sub-lethal effects of pollutants. In: *Bioindicators and Environmental Management*, editors Jeffrey, D. W. and B. Madden. London, Academic Press pp 235-244.

SATAKE, K., SHIMIZU, H., NISHIKAWA, M., 1984. Elemental composition of the aquatic liverwort *Jungermannia vulcanicola Steph* in acid streams. *Journal of the Hattori Botanical Laboratory*, 56, 241-248.

SATAPATHY, D. R., SALVE, P. R., KATPATAL, Y. B. 2009. Spatial distribution of metals in ground/surface waters in the Chandrapur district (Central India) and their plausible sources. *Environmental Geology*, 56, 1323-1352.

SAY, P.J., DIAZ, B.M., WHITTON, B.A., 1977. Influence of zinc on lotic plants. I. Tolerance of Hormidium species to zinc. *Freshwater Biology*, 7, 357-376.

SAY, P.J., WHITTON, B.A., 1977. Influence of zinc on lotic plants. II. Environmental effects of toxicity of Zn to Hormidium rivulare. *Freshwater Biology*, 7, 377-384.

SAY, P.J., WHITTON, B.A., 1981. Changes in flora down a stream showing a zinc gradient. *Hydrobiologia*, 76, 255-262.

SAY, P.J., WHITTON, B.A. 1982 Chemistry and plant ecology of zinc-rich streams in France. 2. The Pyrénées. *Annales de Limnologie*, 18, 19-31.

SAY, P.J., WHITTON, B.A., 1983. Accumulation of heavy metals by aquatic mosses. I. *Fontinalis antipyretica*. *Hydrobiologia*, 100. 245-260.

SCHECHER, W.D., DRISCOLL, C.T., 1985. Interactions of copper and lead with *Nostoc muscorum. Water, Air and Soil Pollution*, 24, 85-101.

SERGIO, C., FIGUEIRA, R AND CRESPO, A.M.V., 2000. Observations of heavy metal accumulation in the cell walls of *Fontinalis antipyretica*, in a Portuguese stream affected by mine effluent. *J. Bryol*. 22, 252-255.

SHEHATA, F.H.A., WHITTON, B.A., 1982. Zinc tolerance in strains of the blue green alga *Anacystis nidulans*. *British Phycological Journal*, 17, 5-12.

SHUHAIMI-OTHMAN, M., PASCOE, D. 2007. Bioconcentration and depuration of copper, cadmium and zinc mixtures by the freshwater amphipod *Hyalella azteca. Ecotoxicology and Environmental Safety*, 66, 29-35.

SIBLEY, P.K., ANKLEY, G.T., COTTER, A.M., LEONARD, E.N., 1996. Predicting chronic toxicity of sediments spiked with zinc: an evaluation of the acid-volatile sulfide model using a life-cycle test with the midge *Chironomus tentans. Environmental Toxicology and Chemistry*, 15, 2102-2112.

SIBLEY, R.M., CALOW, P., 1989. A life-cycle theory of responses to stress. *Biological Journal of the Linnaean Society*, 37, 101-116.

SINGH, S.P., YADAVA, V., 1984. Cadmium induced inhibition of ammonium and phosphate uptake in *Anacystis nidulans*: interaction with other divalent cations. *Journal of General and Applied Microbiology*, 30, 79-86.

SLOMAN, K. A., BAKER, D. W., HO, C. G., MCDONALD, D. G., WOOD, C. M. 2003. The effect of trace metal exposure on agonistic encounters in juvenile rainbow trout, *Oncorhychus mykiss. Aquatic Toxicology*, 63, 187-196.

SLOMAN, K. A., LEPAGE, O., ROGERS, J. T., WOOD, C. M., WINBERG, S. 2005. Socially-mediated differences in brain monoamines in rainbow trout: Effects of trace metal contaminants. *Aquatic Toxicology*, 71, 237-247.

SOFYA, A., ROSITA, G., PRICE, D. J., BIRGE, W. J. 2007. Cadmium uptake by *Ceriodaphnia dubia* from different exposures: Relevance to body burden and toxicity. *Environmental Toxicity and Chemistry*, 26, 470-477.

SORENTINO, C., 1985. Copper resistance in Hormidium fluitans (Gay) Heering (Ulotrichaceae, Chlrophyceae). *Phycologia*, 24, 366-368.

SPRY, D. J., WOOD, C. M., 1989. A kinetic method for the measurement of zinc influx in vivo in the rainbow trout, and the effects of waterborne calcium on flux rates. *Canadian Journal of Experimental Biology*, 142, 425-446.

SQUIBB, K. S., FOWLER, B. A. 1981. Relationship between metal toxicity to subcellular systems and the carcinogenic response. *Environmental Health Perspectives*, 40, 181-188.

STARODUB, M.E., WONG, M.T.S., MAYFIELD, C.I., CHAU, Y.K., 1987. Influence of complexation and pH on individual and combined heavy metal toxicity to a freshwater green alga. *Canadian Journal of Fisheries and Aquatic Science*, 44, 1173-1180.

STATZNER, B., BADY, P., DOLÉDEC, S., SCHÖLL, F. 2005. Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river systems. *Freshwater Biology*, 50, 2136-2161.

STEWART, A. R. 1999. Accumulation of Cd by a freshwater mussel (*Pyganodon grandis*) is decreased in the presence of Cu, Zn, Pb and Ni. *Canadian Journal of Fisheries and Aquatic Sciences*, 56, 467-478.

STILLINGS, L. L., FOSTER, A. L., KOSKI, R. A., MUNK, L., SHANKS, W. C. 2008. Temporal variation and the effect of rainfall on metals flux from the historic Beatson mine, Prince William Sound, Alaska, USA. *Applied Geochemistry*, 23, 255-278.

STOKES, P.M., 1983. Responses of freshwater algae to metals. *Progress in Phycological Research*, 2, 87-112.

STOKES, P.M., HUTCHINSON, T.C., KRAUTER, K., 1973. Heavy-metal tolerance in algae isolated from contaminated lakes near Sudbury, Ontario. *Canadian Journal of Botany*, 51, 2155-2168.

SUNDA, W., GUILLARD, R.R.L., 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *Journal of Marine Research*, 34, 511-529.

SWARTZ, R.C., DITSWORTH, G.R., SCHULTS, D.W., LAMBERSON, J.O. 1985. Sediment toxicity to a marine infaunal amphipod: Cadmium and its interaction with sewage sludge. *Marine Environmental Research*, 18, 133-153.

TESSIER, A., CAMPBELL, P. G. C., BISSON, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 51, 844-850.

TESSIER, L., BOISVERT, J. L., VOUGHT, L. B-M., LACOURSIÈRE, J. O. 2000. Anomolies on capture nets of *Hydropsyche slossonae* larvae (Trichoptera; Hydropsychidae) following a sublethal chronic exposure to cadmium*. Environmental Pollution*, 108, 425-43.8

TIPPING, E., VINCENT, C.D., LAWLOR, A.J., LOFTS, S. 2008. Metal accumulation by stream bryophytes, related to chemical speciation. *Environmental Pollution,* 156, 936-943.

TIPPING, E., 1994. WHAM - A chemical equilibrium model and computer code for waters, sediments and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Computers and Geosciences,* 20, 973-1023.

TIPPING, E., 1998. Humic Ion-Binding Model VI: an improved description of the interactions of protons and metal ions with humic substances. *Aquatic Geochemistry*, 4, 3-48.

TIPPING, E., 2002. Cation binding by humic substances. Cambridge University Press, Cambridge.

TIPPING, E., HURLEY, M.A., 1992. A unifying model of cation binding by humic substances. *Geochimica et Cosmochimica Acta*, 56(10), 3627-3641.

TIPPING, E., VINCENT, C.D., LAWLOR, A.J., LOFTS, S., 2008. Metal accumulation by stream bryophytes, related to chemical speciation. *Environmental Pollution*, 156, 936-943.

TSUI, M. T. K., WANG, W. X. 2005. Influences of maternal exposure on the tolerance and physiological performance of *Daphnia magna* under mercury stress. *Environmental Toxicology and Chemistry*, 24, 1228-1234.

TSUI, M. T. K., WANG, W. X. 2007. Biokinetics and tolerance development of toxic metals in *Daphnia magna. Environmental Toxicology and Chemistry*, 26, 1023-1032.

TULLOS, D. D., PENROSE, D. L., JENNINGS, G. D., COPE, G. 2009. Analysis of functional traits in reconfigured channels: implications for the bioassessment and disturbance of river restoration. *Journal of the North American Benthological Society*, 28, 80-92.

TURNPENNY, A. W., SADLER, K., ASTON, R. J., MILNER, A. G. P. LYNAM, S. 1987. The fish populations of some streams in Wales and northern England in relation to acidity and associated factors. *Journal of Fish Biology*, 31, 415-434.

VAN CAMPENHOUT, K., INFANTE, H. G., GOEMANS, G., BELPAIRE, C., ADAMS, F., BLUST, R., BERVOETS, L. 2008. A field survey of metal binding to metallothionein and other cytosolic ligands in liver of eels using an on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS). *Science of the Total Environment*, 394, 379-389.

VAN DYK, J. C., PIETERSEN, G. M., VAN VUREN, J. H. J. 2007. Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. *Ecotoxicology and Environmental Safety*, 66, 432-440.

VELTZ-BALATRE, I. 2000. Toxicity studies of some pollutants from the Champagne-Ardene area on a freshwater invertebrate. *Bulletin de la Societe Zoologique de France*, 125, 333-341.

VERBOST, P., FLIK, G., LOCK, R.A.C., WENDELAAR BONGA, S.E., 1987. Cadmium inhibition of Ca^{2+} uptake in rainbow trout gills. *American Journal of Physiology*, 253, R216–R221.

VOSYLIENE, M. Z., KAZLAUSKIENE, N. SYECEVICIUS, G., 2003. Effect of a heavy metal mixture model on biological parameters of a rainbow trout (*Oncorhynchus mykiss*). *Environmental Science and Pollution Research*, 10, 103-107.

VUORI, K-M. 1994. Rapid behavioural and morphological response of Hydropsychid larvae (Trichoptera, Hydropsychidae) to sublethal cadmium exposure. *Environmental Pollution*, 84, 291-299.

WAIWOOD, K.G., BEAMISH, F.W.H. 1978. Effect of copper, hardness and pH on growth of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology*, 13, 591-598.

WALLACE, J.B., GRUBAUGH, J. W., WHILES, M. R. 1996. Biotic indices and stream ecosystem processes: Results from an experimental study. *Ecological Applications*, 6, 140-151.

WARD, R. J. S., MCCROHAN, C. R., WHITE, K. N. 2006. Influence of aqueous aluminium on the immune system of the freshwater crayfish *Pacifasticus leniusculus*. *Aquatic Toxicology*, 77, 222-228.

WARD, R. J. S., ROBINSON, W. E. 2005. Evolution of cadmium resistance in *Daphnia magna. Environmental Toxicology*, 24, 2341-2349.

WATRAS, C. J., BACK, R. C., HALVORSEN, S., HUDSON, R. J. M., MORRISON, K. A., WENTE, S. P. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, 219, 183-208.

WEATHERLEY, N.S. AND ORMEROD, S.J. 1987. The impact of acidification on macroinvertebrate assemblages in Welsh streams: towards an empirical model. *Environmental Pollution*, 46, 223-240.

WEATHERLEY, N. S., ORMEROD, S. J., THOMAS, S. P., EDWARDS, R. W. 1988. The response of macroinvertebrates to experimental episodes of low pH with different forms of aluminium, during a natural spate. *Hydrobiologia*, 169, 225-232.

WEATHERLEY, N. S., ROGERS, N. P., GOENAGA, X., ORMEROD, S. J. 1990. The survival of early life stages of brown trout (*salmo trutta* L.) in relation to aluminium speciation in upland Welsh streams. *Aquatic Toxicology*, 17, 213-230.

WEHR, J.D., WHITTON, B.A., 1983. Accumulation of heavy metals by aquatic mosses. 2. *Rhynchostegium riparioides. Hydrobiologia*, 100, 261-284.

WEHR, J.D., KELLY, M.G., WHITTON, B.A., 1987. Factors affecting accumulation and loss of zinc by the aquatic moss *Rhynchostegium riparioides. Aquatic Botany*, 29, 261-274.

WHITTON, B.A., 1970. Biology of Cladophora in freshwaters. *Water Research*, 4, 457-476.

WHITTON, B.A., 1980. Zinc and plants in rivers and streams. In: *Zinc in the Environment Part II: Health Effects*, edited by J.O. Nriagu. Wiley Interscience, New York, 363-400.

WHITTON, B.A., 2003. Use of plants in monitoring heavy metals in freshwaters. In: *Modern Trends in Applied Aquatic Ecology*, editors R.S Ambasht and N.K. Ambasht, Kluwer, New York, 43-63.

WHITTON, B.A., BURROWS, I.G., KELLY, M.G., 1989. Use of *Cladophora glomerata* to monitor heavy metals in rivers. *Journal of Applied Phycology*, 1, 293-299.

WHITTON, B.A., DIAZ, B.M., 1980. Chemistry and plants of streams and rivers with elevated Zn. In: *Trace Substances in Environmental Health - XIV*. University of Missouri, Columbia. pp. 457-463.

WHITTON B.A., KELLY M.G., HARDING J.P.C., SAY P.J., 1991. Use of Plants as Monitors of Heavy Metals in Rivers. Methods for the Assessment of Waters and Wastewaters. Her Majesty's Stationary Office, London. 43pp.

WHITTON, B.A., SAY, P.J., JUPP, B.P., 1982. Accumulation of zinc, cadmium and lead by the aquatic liverwort Scapania. *Environmental Pollution Series B*, 3, 299-316.

WHITTON, B.A., SHEHATA, F.H.A. 1982. Influence of cobalt, nickel, copper and cadmium on the blue-green algae *Anacystis nidulans. Environmental Pollution Series A* 27, 275-281.

WILLIAMS, K. A., GREEN, D. W. J., PASCOE, D., GOWER, D. E. 1986. The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera: Chironomidae) and its ecological significance for pollution regulation. *Oecologia*, 70, 362-366.

WILLIAMS, L.G., MOUNT, D.I., 1965. Influence of zinc on periphytic communities. *American Journal of Botany*, 52, 26-34.

WINNER, R. W., BOESL, M. W., FARRELL, M. P. 1980. Insect community structure as an index of heavy metal pollution in lotic ecosystems. *Canadian Journal of Fisheries and Aquatic Science*, 37, 647-655.

WINNER, R.W. 1986. Interactive effects of water hardness and humic acid on the chronic toxicity of cadmium to *Daphnia pulex*. *Aquatic Toxicology*, 8, 281-293.

WINTERBOURN, M. J., MCDIFFETT, W. F., EPPLEY, S. J. 2000. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: Effects of trophic level. *Science of the Total Environment*, 254, 45-54.

WRIGHT, J. F., FURSE, M. T., ARMITAGE, P. D. 1993. RIVPACS – a technique for evaluating the biological quality of rivers in the UK. *European Water Pollution Control*, 3, 15-25.

WRIGHT, J.F., 2000. An introduction to RIVPACS. In: *Assessing the Biological Quality of Freshwaters: RIVPACS and Similar Techniques,* editors J.F. Wright, D.W. Sutcliffe and M.T. Furse, Freshwater Biological Association, Cumbria.

XU, Q., PASCOE, D. 1993. The importance of food and water as sources of zinc during exposure of *Gammarus pulex. Archives of Environmental Contamination and Toxicology*, 26, 459-465.

ZITKO, V., 1976. Structure-activity relations and the toxicity of trace elements to aquatic biota. In: Proceedings of Toxicity to Biota of Metal Forms in Natural Water, International Joint Commission, 9–32.

ZITKO, V., CARSON, W.V., CARSON, W.G., 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. *Bulletin of Environmental Contamination and Toxicology*, 10, 265-271.

Appendix 1: Water chemistry effects on metal toxicity: summary of laboratory studies

Table A1a Effects of varying water chemistry on chronic toxicity and accumulation of Co, Ni, Cu, Zn, Cd and Pb to aquatic algae.

Metal	Organism	Endpoint	Water chemistry parameters varied[3]	Reference
Cobalt	*Chlamydomonas reinhardtii*	Growth, 5 days	**pH(+)**	Macfie *et al*., 1994
Nickel	*Chlamydomonas reinhardtii*	Growth, 5 days	**pH(+)**	Macfie *et al*., 1994
Nickel	*Pseudokirchneriella subcapitata*	Growth, 3 days	**pH(+)**, **Mg(-)**, **Ca(-)**	Deleebeeck *et al*., 2005
Nickel	*Chlamydomonas* sp.	Growth, 3 days	**Hardness(-)**	Deleebeeck *et al*., 2006
Nickel	*Ankistrodesmus falcatus*	Growth, 3 days	**Hardness(-)**	Deleebeeck *et al*., 2006
Nickel	*Pediastrum duplex*	Growth, 3 days	**Hardness(-)**	Deleebeeck *et al*., 2006
Nickel	*Coelastrum microporum*	Growth, 3 days	**Hardness(-)**	Deleebeeck *et al*., 2006

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[3] Parameters listed in bold had a significant influence on toxicity or accumulation. A (+) means that toxicity or accumulation increases with increase in the parameter, a (-) means that toxicity or accumulation declines with increase in the parameter.

Table A1a continued

Metal	*Organism*	Endpoint	**Water chemistry parameters varied**[4]	Reference
Copper	*Pseudokirchneriella subcapitata*	Growth, 3 days	**pH(+)**, **DOC(-)**[5], Hardness[6]	De Schamphelaere *et al*., 2003
Copper	*Pseudokirchneriella subcapitata*	Growth, 3 days	**pH(+)**, **Mg(-)**, **Ca(-)**	Heijerick *et al*., 2005a
Copper	*Pseudokirchneriella subcapitata*	Growth, 3 days	**pH(+)**	De Schamphelaere *et al*., 2005
Copper	*Chlorella sp.*	Growth, 3 days	**pH(+)**	Franklin *et al*., 2000
Copper	*Chlorella sp.*	Growth, 2 days	**pH(+)**	De Schamphelaere *et al*., 2005
Copper	*Chlorella* sp.	Growth, 2 days	**pH(+)**	Wilde *et al*., 2006
Copper	*Scenedesmus quadricauda*	P uptake	**pH(+)**	Peterson *et al*., 1984
Copper	*Scenedesmus quadricauda*	Growth, 15 days	**pH(-)**	Starodub *et al*., 1987
Copper	*Chlamydomonas reinhardtii*	Growth, 5 days	**pH(+)**	Macfie *et al*., 1994
Zinc	*Chlorella* sp.	Growth, 2 days	**pH(+)**	Wilde *et al*., 2006

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[4] Parameters listed in bold had a significant influence on toxicity or accumulation: (-) indicates ameliorative influence; (+) indicates toxic influence.

[5] Aquatic organic matter collected from natural waters using reverse osmosis.

[6] Constant Ca:Mg ratio of 4:1.

Table A1a continued.

Metal	*Organism*	Endpoint	**Water chemistry parameters varied**[7]	Reference
Zinc	*Pseudokirchneriella subcapitata*	Growth, 3 days	**pH(+)**, **Na(-)**, **Mg(-)**, **Ca(-)**, K	Heijerick *et al.*, 2002
Zinc	*Scenedesmus quadricauda*	Growth, 15 days	**pH(-)**	Starodub *et al.*, 1987
Cadmium	*Scenedesmus quadricauda*	P uptake	**pH(+)**	Peterson *et al.*, 1984
Cadmium	*Chlamydomonas reinhardtii*	Growth, 5 days	**pH(+)**	Macfie *et al.*, 1994
Cadmium	*Pseudokirchneriella subcapitata*	Growth, 3 days	**Hardness(-)**	Kallqvist, 2008
Lead	*Scenedesmus quadricauda*	Growth, 15 days	**pH([8])**	Starodub *et al.*, 1987

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[7] Parameters listed in bold had a significant influence on toxicity or accumulation: (-) indicates ameliorative influence; (+) indicates toxic influence.

[8] Bimodal response with minimum toxicity at pH 6.5.

Table A1b Effects of varying water chemistry on the chronic toxicity and accumulation of Co, Ni, Cu, Zn, Cd and Pb to aquatic invertebrates.

Metal	Organism	Endpoint	Water chemistry parameters varied[9]	Reference
Cobalt	*Hyalella azteca*	Survival, 7 days	**Hardness(-)**	Borgmann *et al*., 2005
Nickel	*Ceriodaphnia dubia*	Reproduction, 10 days	**pH(+)**, **Mg(-)**, **Ca(-)**	De Schamphelaere *et al*., 2006
Nickel	*Ceriodaphnia dubia*	Survival and reproduction, 7 days	**Hardness(-)**	Keithly *et al*., 2004
Nickel	*Daphnia magna*	Reproduction, 21 days	pH[10], **Mg(-)**, **Ca(-)**	Deleebeeck *et al*., 2008
Copper	*Daphnia magna*	Reproduction, 21 days	**pH(-)**[11], **DOC**[12]**(-)**, **Na(-)**, Mg, Ca	De Schamphelaere and Janssen, 2002; De Schamphelaere and Janssen, 2004a.
Copper	*Daphnia magna*	Bioaccumulation, 7 days	**DOC**[13]**(-)**, **Hardness(-)**	Winner, 1985
Copper	*Ceriodaphnia dubia*	Reproduction, 6–7 days	pH[10], **DOC**[14]**(-)**, Na, **Mg(-)**, Ca	Schwartz and Vigneault, 2007.

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[9] Parameters listed in bold had a significant influence on toxicity or accumulation: (-) indicates ameliorative influence; (+) indicates toxic influence.

[10] A deleterious effect of increasing pH was observed when the toxicity was expressed as the free metal ion.

[11] A deleterious effect of increasing pH when the toxicity was expressed as the free metal ion was inferred from the derivation of a significant proton competition effect at the biotic ligand.

[12] Aquatic organic matter collected from natural waters using reverse osmosis.

[13] Aldritch humic acid.

[14] Suwannee River and Nordic Reservoir natural organic matter.

Table A1b continued.

Metal	Organism	Endpoint	Water chemistry parameters varied[15]	Reference
Copper	*Daphnia pulex*	Survival, 42 days	**DOC**[13Error! Bookmark not defined.]**(-)**, **Hardness(-)**	Winner, 1985
Zinc	*Daphnia magna*	Reproduction, 21 days	pH(16)[10], Na(-), Mg(-), Ca(-)	Heijerick *et al*., 2005b
Zinc	*Daphnia magna*	Survival and reproduction, 50 days	Hardness[17](-), DOC[13](-)	Paulaskis and Winner, 1988
Cadmium	*Daphnia pulex*	Survival and reproduction, 42 days	DOC[18](19), Hardness(-)	Winner, 1986
Cadmium	*Daphnia magna*	Bioaccumulation, 20 days	DOC[18]	Winner, 1986
Lead	*Hyalella azteca*	Survival, 7 days	Hardness(-)	Borgmann *et al*., 2005

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[15] Parameters listed in bold had a significant influence on toxicity or accumulation: (-) indicates ameliorative influence; (+) indicates toxic influence.

[16] Bimodal response with maximum toxicity at pH 7.

[17] Approximate Ca:Mg ratio of 2:1

[18] Aldritch humic acid.

[19] Effects of the humic acid were inconsistent: at hardnesses of 100 and 200 mg/l $CaCO_3$, addition of 0.75 mg/l humic acid increased the effect of Cd, while at a hardness of 50 mg/l $CaCO_3$ and/or addition of 1.50 mg/l humic acid, the effect of Cd was ameliorated.

Table A1c Effects of varying water chemistry on the chronic toxicity and accumulation of Ni, Cu, Zn, Cd and Pb to fish.

Metal	Organism	Endpoint	Water chemistry parameters varied[20]	Reference
Nickel	*Oncorhynchus mykiss*	Survival, 17 days	**pH(+)**, **Mg(-)**, **Ca(-)**	Deleebeeck *et al*., 2007
Copper	*Oncorhynchus mykiss*	Growth, 30 days	**pH(-)**, **Hardness(-)**	Waiwood and Beamish, 1978
Copper	*Oncorhynchus mykiss*	Na regulation, 30 days	**DOC[21](-)**	McGeer *et al*., 2002
Zinc	*Oncorhynchus mykiss*	Survival and growth, 30 days	**pH(+)**, **Na(-)**, **Mg(-)**, **Ca(-)**	De Schamphelaere and Janssen, 2004b
Cadmium	*Oncorhynchus mykiss*	Survival, 30 days	**Ca(-)**	Hollis *et al*., 2000
Lead	*Oncorhynchus mykiss*	Life-cycle analysis	**Hardness(-)**	Davies *et al*., 1976
Lead	*Pimephales promelas*	Survival, 30 days	**pH([22])**, **DOC[21](-)**, **Ca(-)**	Grosell *et al*., 2006
Lead	*Pimephales promelas*	Accumulation, 150 days	**DOC[21](-)**, Ca	Mager *et al*., 2008

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[20] Parameters listed in bold had a significant influence on toxicity or accumulation: (-) indicates ameliorative influence; (+) indicates toxic influence.

[21] Aldritch humic acid

[22] Bimodal response with minimum toxicity at pH 7.4.

References – Appendix 1

BORGMANN, U., COUILLARD, Y., DOYLE, P., DIXON, D.G. 2005. Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness. *Environmental Toxicology and Chemistry*, 24, 641-652.

DAVIES, P.H., GOETTL, J.P., SINLEY, J.R., SMITH, N.F. 1976. Acute and chronic toxicity of lead to rainbow trout *Salmo gairdneri*, in hard and soft-water. *Water Research*, 10, 199-206.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C. HEIJERICK, D.J., BOSSUYT B., JANSSEN, C.R. 2005. Development and validation of biotic ligand models for predicting nickel toxicity to fish, daphnids and algae. University of Ghent.150 p.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C., MUYSSEN, B.T.A., DE LAENDER, F., JANSSEN, C.R. 2006. Ni toxicity to soft versus hard water organisms: a comparison of sensitivity and bioavailability. University of Ghent.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2007. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. *Ecotoxicology and Environmental Safety*, 67, 1-13.

DELEEBEECK, N.M.E., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters. *Environmental Toxicology and Chemistry*, 27, 2097-2107.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R., 2002. Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to *Daphnia magna. Environmental Toxicology and Chemistry*, 23(5), 1115-1122.

DE SCHAMPHELAERE, K.A.C., VASCONCELOS, F.M., HEIJERICK, D.G., TACK, F.M.G., DELBEKE, K., ALLEN, H.E., JANSSEN, C.R. 2003. Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry*, 22, 2454-2465.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2004a. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna. Environmental Toxicology and Chemistry*, 23, 1365-1375.

DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2004b. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. *Environmental Science and Technology*, 38, 6201-6209.

DE SCHAMPHELAERE, K.A.C., STAUBER, J.L., WILDE, K.L., MARKICH, S.J., BROWN, P.L., FRANKLIN, N.M., CREIGHTON, N.M., JANSSEN, C.R. 2005. Toward a biotic ligand model for freshwater green algae: Surface-bound and internal copper are better predictors of toxicity than free Cu^{2+} ion activity when pH is varied. *Environmental Science and Technology*, 39, 2067-2072.

DE SCHAMPHELAERE K.,VAN LAER L., DELEEBEECK N., MUYSSEN B., DEGRYSE F., SMOLDERS E., AND JANSSEN, C., 2006. Nickel speciation and ecotoxicity in European natural surface waters: development, refinement and validation of bioavailability models. University of Ghent. 125 p.

FRANKLIN, N.M., STAUBER, J.L., MARKICH, S.J., LIM, R.P. 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Chlorella sp.). *Aquatic Toxicology*, 48, 275-289.

GROSELL, M., GERDES, R., BRIX, K. V. 2006. Influence of Ca, humic acid and pH on lead accumulation and toxicity in the fathead minnow during prolonged water-borne lead exposure. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 143, 473-483.

HEIJERICK, D.G., DE SCHAMPHELAERE, K.A.C., JANSSEN, C.R. 2002. Biotic ligand model development predicting Zn toxicity to the alga Pseudokirchneriella subcapitata: possibilities and limitations. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 133, 207-218.

HEIJERICK, D.G., BOSSUYT, B.T. A., DE SCHAMPHELAERE, K.A.C., INDEHERBERG, M., MINGAZZINI, M., JANSSEN, C.R. 2005a. Effect of varying physicochemistry of European surface waters on the copper toxicity to the green alga *Pseudokirchneriella subcapitata. Ecotoxicology*, 14, 661-670.

HEIJERICK, D.G., DE SCHAMPHELAERE, K.A.C., VAN SPRANG, P.A., JANSSEN, C.R. 2005b. Development of a chronic zinc biotic ligand model for *Daphnia magna. Ecotoxicology and Environmental Safety*, 62, 1-10.

HOLLIS, L., MCGEER, J. C., MCDONALD, D. G., WOOD, C. M. 2000. Protective effects of calcium against chronic waterborne cadmium exposure to juvenile rainbow trout. *Environmental Toxicology and Chemistry*, 19, 2725-2734.

KALLQVIST, T. 2008. Effect of water hardness on the toxicity of cadmium to the green alga *pseudokirchneriella subcapitata* in an artificial growth medium and nutrient-spiked natural lake waters. *Journal of Environmental Toxicology and Health – Part A* – Current Issues, 72(3-4), 277-283.

MACFIE, S. M., TARMOHAMED, Y., WELBOURN, P. M. 1994. Effects of cadmium, cobalt, copper, and nickel on growth of the green alga *Chlamydomonas reinhardtii*: The influences of the cell wall and pH. *Archives of Environmental Contamination and Toxicology*, 27, 454-458.

MAGER, E. M., WINTZ, H., VULPE, C. D., BRIX, K. V., GROSELL, M. 2008. Toxicogenomics of water chemistry influence on chronic lead exposure to the fathead minnow (*Pimephales promelas*). *Aquatic Toxicology*, 87, 200-209.

MCGEER, J. C., SZEBEDINSZKY, C., MCDONALD, D. G., WOOD, C. M. 2002. The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 133, 147-160.

PAULAUSKIS, J. D., WINNER, R. W. 1988. Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna Straus. Aquatic Toxicology*, 12, 273-290.

PETERSON, H.G., HEALEY, F.P., WAGEMANN, R. 1984. Metal Toxicity to Algae: A Highly pH Dependent Phenomenon. *Canadian Journal of Fisheries and Aquatic Sciences*, 41, 974-979.

SCHWARTZ, M. L., VIGNEAULT, B. 2007. Development and validation of a chronic copper biotic ligand model for *Ceriodaphnia dubia. Aquatic Toxicology*, 84, 247-254.

STARODUB, M. E., WONG, P. T. S., MAYFIELD, C. I. 1987. Short-term and long-term studies on individual and combined toxicities of copper, zinc and lead to *Scenedesmus quadricauda. Science of the Total Environment*, 63, 101-110.

WAIWOOD, K.G., BEAMISH, F.W.H. 1978. Effect of copper, hardness and pH on growth of *rainbow trout, Salmo gairdneri*. *Journal of Fish Biology*, 13, 591-598.

WILDE, K.L., STAUBER, J.L., MARKICH, S.J., FRANKLIN, N.M., BROWN, P.L., 2006. The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (Chlorella sp.). *Archives of Environmental Contamination and Toxicology*, 51, 174-185.

WINNER, R.W., 1985. Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness. *Water Research*, 19, 449-455.

WINNER, R.W. 1986. Interactive effects of water hardness and humic acid on the chronic toxicity of cadmium to *Daphnia pulex. Aquatic Toxicology*, 8, 281-293.

MAGER, E. M., WINTZ, H., VULPE, C. D., BRIX, K. V., GROSELL, M. 2008. Toxicogenomics of water chemistry influence on chronic lead exposure to the fathead minnow (*Pimephales promelas*). *Aquatic Toxicology*, 87, 200-209.

List of abbreviations

ASPT	Average Score Per Taxon
AWIC	Acid Water Indicator Community
AVS	Acid-Volatile Sulphide
BLM	Biotic Ligand Model
BMWP	Biological Monitoring Working Party
CCU	Cumulative Criterion Unit
DAM	Diatom Acidification Metric
DARLEQ	Diatoms for Assessing River and Lake Ecological Quality
DGT	Diffusive Gradients in Thin films
DMT	Donnan Membrane Technique
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EEC	European Economic Community
EPA	Environmental Protection Agency
EQS	Environmental Quality Standard
GQA	General Quality Assessment
GSIM	Gill Surface Interaction Model
LEAFPACS	Prediction and Classification System
MT	Metallothionein
NOEC	No Observed Effect Concentration
OE67	Observed/Effective for 67% sensitive organisms
PEL	Predicted Effect Level
PNEC	Predicted No-Effect Concentration
RICT	River Invertebrate Classification Tool
RIVPACS	River Invertebrate Prediction and Classification System
ROS	Reactive Oxygen Species
SEPA	Scottish Environment Protection Agency
SEM	Simultaneously-Extracted Metals
SSD	Species Sensitivity Distribution
TBM	Toxicity Binding Model
TDI	Trophic Diatom Index
TEL	Threshold Effect Level
WFD	Water Framework Directive
WHAM	Windermere Aqueous Humic Model

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