THE EFFECT OF SEWAGE EFFLUENT ON TRACE METAL SPECIATION:

IMPLICATIONS FOR THE BIOTIC LIGAND MODEL APPROACH

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ABSTRACT

This research examined the suitability of the biotic ligand model (BLM) approach for assessing environmental risk in surface waters consisting substantially of treated sewage effluent, and the implications of its use within a compliance-based regulatory framework aimed at controlling discharges of metals into the aquatic environment.

The results from a series of *Daphnia magna* acute copper toxicity assays conducted in an undiluted sewage effluent medium demonstrated that BLMs could predict an acute copper toxicity endpoint with suitable accuracy. This finding contributes further support for the use of BLMs for assessing risk and compliance, even in effluent impacted waters. Additional studies, however, also demonstrated that effluent derived organic matter contained a greater concentration of metal complexing ligands per milligram of dissolved organic carbon than organic matter derived from natural sources. This indicates that effluent derived organic matter offers greater protection against the potentially adverse effects from metals than the protection offered by organic matter derived from natural sources. These studies also demonstrated that improvements in the accuracy of BLM forecasts were achievable by taking these differences into account which, from a regulatory perspective, is also desirable since this enhances the environmental relevance of compliance criteria. These findings therefore justify that consideration for the influence of site-specific metal complexation characteristics should included as part of the regulatory framework within which BLMs will be applied.

The implication of the BLM approach for Severn Trent Water Limited is that, as currently proposed, the approach will require a reduction in the concentration of copper in the effluent discharges for a small number of wastewater treatment works (9), whereas for zinc, concentration reductions may be required for numerous treatment works (126).

EXECUTIVE SUMMARY

1. BACKGROUND

The Water Framework Directive (WFD) (European Parliament, 2000) is requiring more stringent water quality standards for numerous substances that will have a potentially profound effect on wastewater treatment works operators and the standard to which wastewaters are required to be treated. The prospect of more stringent water quality standards, for trace metals in particular, has however raised a number of specific concerns within the Water Industry.

In the first instance, whereas wastewater treatment works (WwTWs) can represent a significant source of trace metal inputs into the aquatic environment, WwTWs are not primarily designed to remove trace metals and consequently, removal is variable (Lester, 1987). Achieving metal removal rates in order to comply with more stringent standards may therefore not be achievable or require additional wastewater treatment processes that may be disproportionately expensive.

In the second instance, the Environment Agency (EA) has proposed specifying new standards for copper and zinc (and possibly for nickel at some point in future) in terms of a permissible bioavailable concentration, which represents the metal concentration that is in a form available to be taken up by aquatic organisms but at a level at which adverse ecotoxicological impacts are not expected to occur. Whereas the consideration of bioavailability improves the ecological relevance of water quality standards it does, however, introduce significant complexity into assessing compliance since the bioavailable concentration cannot be measured directly (e.g. with a probe) and requires the use of computational tool, known as a Biotic Ligand Model (BLM), to ‘convert’ the bioavailable concentration into a measureable dissolved metal concentration against which compliance may be assessed. Consequently, the relative complexity of the approach is such that the potential impact on compliance, and therefore the degree to which wastewater effluents may be required to be treated, is uncertain.

In the third instance, many parts of the UK are known to have low effluent dilution capacity (≤1:10) (Keller, Whelan and Rees, 2006) and the extent by which BLM predicted dissolved metal concentrations might accurately reflect a particular level of bioavailability in waters consisting substantially of sewage effluent is uncertain since the current suite of BLMs have been parameterised using data from natural and reconstituted waters only. For example, BLMs require water chemistry inputs such as pH, and the concentrations of dissolved organic carbon (DOC) and calcium as inputs, where the effect of DOC on metal bioavailability is simulated on the assumption that it is comprised of humic substances which form metal complexes of a particular type, whereas effluent derived DOC has been shown to contain non-humic and anthropogenic substances which display metal complexing characteristics which differ from that of humics (Sarathy and Allen, 2005). Furthermore, effluents are also complex mixtures containing multiple compounds that might also influence metal bioavailability in an unpredictable manner so that the extent by which the use of BLMs may be appropriate is uncertain. It is within this context that the aims of this research project were formulated.

2. RESEARCH AIMS

The aims of this research project were to:

- Investigate the extent by which the BLM approach may be appropriate for assessing bioavailability in waters consisting substantially of sewage effluent.
- Determine the implications of bioavailability based standards and the BLM approach for Severn Trent Water Limited.

The research aims were addressed by formulating a number of specific research problems, which formed the basis for a series of individual studies. The research problems were:

a) Can BLMs predict metal bioavailability with sufficient accuracy in effluent impacted waters?

b) Can the accuracy of BLM forecasting be improved through model optimisation?

c) To what extent do model simulations describe the influence of effluent derived organic matter on metal speciation?

d) To what extent do the metal complexing characteristics of surface waters vary?

e) Could the use of the BLM approach within a compliance based regulatory framework require Severn Trent Water to reduce the concentrations of trace metals in sewage effluent discharges?

3. STUDIES AND RESULTS

The research problems and their rationale, along with the approaches applied and the results from the individual studies which addressed the individual research problems are summarised.

a) Can BLMs predict metal bioavailability with sufficient accuracy in effluent impacted waters?

The most direct method for assessing the general suitability of the BLM approach for effluent impacted waters was to conduct toxicity assays in an effluent medium and compare an observed toxicity endpoint with that predicted by a BLM. For this purpose, eight acute copper toxicity assays were conducted in undiluted (filtered) sewage effluent obtained from four Severn Trent Water WwTWs, using *Daphnia magna* as the test organism. The predictive accuracy of two BLMs was assessed; the Refined acute copper toxicity BLM for *D. magna* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere and Janssen, 2002) and the HydroQual BLM (Di Toro *et al.*, 2001; Paquin *et al.*, 2000), which has been accepted as suitable for use to specify water quality criteria in the United States (United States Environmental Protection Agency, 2007a). BLM forecasts were considered sufficiently accurate if the predicted median effective concentration (EC50) was within a factor of two of the observed EC50 concentration, which is the range within which BLM forecasts are typically considered to be accurate.

The results from this study demonstrated that the EC50 values were predicted by within a factor of two for seven out of the eight assays using the Refined BLM, and for six out of the eight using the HydroQual BLM. In two of the assays (one of which the EC50 was not predicted by the Refined BLM by within a factor of two) the concentration of sodium exceeded the range within which the BLM parameter controlling the influence of sodium had been determined, and therefore the Refined BLM did not predict all EC50 values by within a factor of two rather as a consequence of the high sodium concentrations than due to the influence of some effluent specific characteristic. The superior predictive accuracy of the Refined BLM (in comparison with the HydroQual BLM) was attributable to the enhanced mechanistic basis upon which the Refined BLM toxicity forecasts had been based. These findings contribute support for the use of BLMs in waters which receive sewage effluent inputs, but reinforce the need for an understanding of the limitations of the model thermodynamic parameters. The findings from this study have been peer reviewed and presented in the journal *Environmental Toxicology and Chemistry* (Constantino *et al.*, 2011) (Appendix 1).

b) Can the accuracy of BLM forecasting be improved through model optimisation?

Improvements in the accuracy of BLM forecasting offer the opportunity to enhance the environmental relevance of compliance criteria determined using the BLM approach. To investigate opportunities to improve upon the forecast accuracy of the two current BLMs for *D. magna* (the Refined BLM and the HydroQual BLM), a Modified BLM was developed which used the WHAM VI speciation model to simulate ion interactions (the Refined and HydroQual BLMs use WHAM V), and which included a number of additional effluent specific water chemistry characteristics, such as the concentrations of ethylenediaminetetraacetic acid (EDTA), iron, and zinc, as model inputs.

For the purposes of speciation modelling, DOC is traditionally considered to be comprised of an ‘active’ fraction to which ions bind, and another fraction which is inert. The active fraction determines the DOC concentration that is used as the model input and therefore has a significant effect on the BLM forecast. Since, typically, the actual active fraction values are not known, a default value is applied; however, the extent by which the default active fraction values, which are assumed to be representative of the ion binding characteristics of naturally derived DOC, may be representative of sewage effluent derived DOC was uncertain. In order to identify an active fraction value that would be more appropriate for sewage effluents, the active fraction was also treated as an adjustable parameter and optimised to that which resulted in perfect agreement between observed and predicted toxicity. This approach implies using the test organism as a type of biological speciation probe, which has become well established practise for estimating active fraction values in the context of biotic ligand modelling (De Schamphelaere *et al.*, 2004; De Schamphelaere, Lofts, and Janssen, 2005). Since numerous studies have also found active fraction values to be variable (Bryan, Tipping and Hamilton-Taylor, 2002; Cheng *et. al*., 2005), and since the methods used to quantify these directly are complex and not amenable for use as part of routine water quality monitoring, an additional investigation was undertaken to examine whether a Chelex column ion exchange technique could be used to obtain a more precise determination of the active fraction that could be used as part of a routine monitoring programme to quantify site-specific active fraction values.

The results from this study found that the Modified BLM produced toxicity forecasts which were more accurate than those of either the Refined or HydroQual BLMs; however, this was dependent on optimising the DOC fraction considered active, rather than as a consequence of the inclusion of effluent specific chemical characteristics in the speciation modelling, or the use of a more advanced speciation model. The study also found that the active fraction values for effluent derived DOC were, on average, greater than the default values, and suggests that sewage effluent derived DOC may therefore be more protective towards adverse effects from metals in comparison with DOC derived from natural sources. The findings have also been peer reviewed and were also presented in the article published in *Environmental Toxicology and Chemistry* (Constantino *et al.*, 2011).

In addition, a set of regression parameters were derived whereby the Chelex column ion exchange method could be used to estimate site specific active fraction values, which reduces dependency on default values. In summary, these findings offer the opportunity to improve the accuracy of BLM forecasts in effluent impacted waters, and therefore, also the opportunity to enhance the environmental relevance of compliance criteria determined using the BLM approach.

c) To what extent do model simulations describe the influence of effluent derived organic matter on metal speciation?

Although adjusting the DOC fraction considered active provides a plausible and straightforward method that allows variability in complexation characteristics to be taken into account, differences between simulated and observed complexation may rather be attributable to differences in complexing ligands within a specific binding affinity range rather than across the entire affinity distribution (which is assumed in adjusting the active fraction). Furthermore, the effect of differences between the actual distribution of complexing ligands and that represented in the speciation model are also likely to be most significant where these relate to high affinity ligands since ligand affinity (complex stability) determines the extent by which a reaction between a metal and a ligand goes to completion, and therefore the proportion of metal that might otherwise become bioavailable.

This study aimed at addressing this research problem therefore assessed differences between measured concentrations of high affinity ligands and those predicted by WHAM VI in order to identify further opportunities for improving BLM forecast accuracy. The WHAM VI speciation model was selected for this comparison since it has been demonstrated to provide the most accurate representation of metal complexation for copper, nickel and zinc. Nickel was included in this study since there is anecdotal evidence that the BLM approach may be applied in future, as described for copper and zinc. An additional study, which also used the Chelex column ion exchange technique, was conducted to assess the extent by which effluent derived ligands might retain their influence upon mixing with river water, and the extent by which this may be predicted on the basis of dilution. The second study focused on nickel and zinc since studies by other researchers (van Veen *et al.* 2002) had already determined that copper complexation characteristics could be determined with good accuracy on the basis of dilution.

The results from this study suggested that concentrations of high affinity ligands were not well predicted by WHAM VI (only 2 out of the 24 simulated values were within the 95% confidence interval range of the measured values). In addition, whereas certain effluents were found to contain high affinity ligands in excess of that predicted by WHAM VI, all the effluents were found to contain concentrations of ligands with a high affinity for zinc that were well in excess of that predicted by WHAM VI. Furthermore, these also exceeded the concentrations of zinc detected in 50% of the effluents and therefore that the zinc contained within those effluents was effectively inert. This finding, however, also suggested that effluents may contribute ligands which further reduce the bioavailability of zinc in the aquatic environment. The dilution experiment provided evidence that effluent derived ligands were likely to retain their influence once discharged into surface waters. In summary, these findings argue that the influence of site specific complexation characteristics should be included as a factor in assessing compliance criteria.

d) To what extent do the metal complexing characteristics of surface waters vary?

This research question was posed in order to examine the variability in the capacity of ligands present in surface waters, with a focus on zinc following the results from the previous study. This assessment was useful to further assess the extent by which a standardised characterisation of complexation (as applied using the BLM approach) may be appropriate for assessing risk and compliance with bioavailability expressed standards. Complexation characteristics were determined using a chelex column ion exchange method. River water samples were collected from rivers in two river catchments:

- The River Tame, in the Tame-Anker-Mease river catchment, follows a course through the Birmingham-Wolverhampton metropolitan area and receives inputs from a diverse range of sources, including landfill leachate, and discharges from numerous large WwTWs. The capacity of the River Tame samples to complex nickel was also assessed since the River Tame had been reported (by Severn Trent Water) to contain high concentrations of nickel (>20µg/L) so that variability in complexation characteristics may be of relevance should the BLM approach be applied in future for assessing compliance.

- The Smestow Brook, in the Mid-Severn river catchment, flows to the west of Dudley town centre and receives inputs from only one large WwTW. The Smestow Brook is impacted by anthropogenic inputs to a far lesser extent than the River Tame.

- The River Stour, also in the Mid-Severn river catchment, bisects the Dudley and Stourbridge urban areas, and does not receive any direct sewage effluent inputs. The River Stour converges with the Smestow Brook to the west of Dudley prior to its confluence with the River Severn. The River Stour is also impacted by anthropogenic inputs to a far lesser extent than the River Tame.

The results from this study demonstrated that nickel and zinc complexation capacity varied over a 3 and 6 fold range, respectively, and for zinc was not well predicted on the basis of the DOC concentration. Complexation capacity attributable to high affinity ligands was detected at locations immediately downstream of WwTWs, but also in samples obtained from rural locations, suggesting there may sources of high affinity ligands other than WwTWs. These findings further support the consideration of site-specific complexation characteristics within the compliance framework that been proposed by the Regulator.

An additional finding from this study has been that landfill leachate, such as that derived from the Slacky Lane landfill, may represent a significant source of metal inputs in to the River Tame.

e) Could new water quality standards require Severn Trent Water to reduce the concentrations of trace metals in sewage effluent discharges?

This research problem was intended to identify the Severn Trent Water WwTWs for which a reduction in the concentration of metal in effluent discharges may be required as a consequence of bioavailability based water quality standards for copper and zinc, and as a consequence of the 20µg/L standard for nickel specified by the WFD. The ‘at risk’ WwTWs were identified using a three stage process. The first stage entailed determining the water quality standards for 1,636 EA water quality monitoring locations within the Severn Trent Water region. A database containing water quality monitoring data (BLM input criteria and metals concentration data) was obtained for the period 01/09/2005 to 31/08/2006. Effluent quality data for 705 Severn Trent Water WwTWs was also obtained covering the same period. The chronic toxicity BLMs for copper and zinc developed as part of the EU risk assessments (European Copper Institute, 2006; De Schamphelaere, Lofts and Janssen, 2005) were used to predict the dissolved metal concentration at which no adverse ecotoxicological impacts were expected to occur for each of the monitoring locations which, for the purposes of this assessment, were considered to be analogous to values that would be applied as statutory standards. The second stage entailed determining the compliance status of each of the monitoring locations whereby the reported metal concentration was compared at face value with the standard. The third stage entailed identifying the WwTWs that were ‘at risk’, which were the WwTWs identified as upstream of a location at which the water quality standard is exceeded.

The results from this study indicated different impacts for the considered metals. For copper, a small number of WwTWs (9) appeared likely to contribute to an exceedance of BLM derived water quality standards and for which reduction in discharge concentrations may be required, whereas for zinc a large number of WwTWs (126) appear likely to contribute to an exceedance and for which a reduction in discharge concentrations may be required. For nickel, the number of WwTWs that have been predicted to contribute to an exceedance of the new standard was small (13), although impacted WwTWs that discharge into the River Tame, or tributaries thereof, and included some of the largest WwTWs in the Severn Trent Water network.

4. CONCLUSIONS

The aims of this research project were, in the first instance, to investigate the extent by which the BLM approach may be appropriate for assessing bioavailability in waters which receive sewage effluent inputs, and in the second instance, to determine the implications of bioavailability based standards and the BLM approach for Severn Trent Water Limited.

In terms of the first aim, the studies conducted as part of this research have demonstrated that BLMs were able to predict acute toxicity with an acceptable degree of accuracy, and have contributed evidence supporting the use of BLMs in effluent impacted waters. The studies have, however, also demonstrated sewage effluent derived organic matter to contain a greater concentration of complexing ligands per milligram of DOC compared to organic matter derived from natural sources and therefore, that effluents may offer greater protection from the potentially adverse effects of metals, and therefore that BLM forecasts may be precautionary in effluent impacted waters. In combination with the finding that the complexation characteristics of surface water may also vary substantially, these findings argue in favour of considering site-specific complexation characteristics as a factor in assessing compliance. This would also enhance the environmental relevance of compliance criteria, so that where wastewater treatment works operators are required to reduce metal concentrations in effluent discharges, there can be good confidence that this will result in an improvement in environmental quality.

For the second aim, the study which examined the compliance implications for Severn Trent Water Limited found that bioavailability based standards, and the BLM approach, may require reductions in the concentration of copper in effluent discharges for a small number of WwTWs, whereas for zinc, reductions are likely to be required at a substantial number WwTWs. The result from this assessment further underscores the importance of taking into account factors which influence the environmental relevance of compliance criteria.

5. FUTURE OPPORTUNITIES

In addition to assessing the impact of metals in the aquatic environment, a number of BLMs have also been developed to predict metal impacts to plants and soil based organisms in the terrestrial environment (Wang *et al*., 2010; Li *et al.*, 2009; Luo and Zhou, 2008; Lock *et al.*, 2007; Koster *et al*.; 2006), which highlights the general suitability of the BLM approach as a platform for quantifying ecotoxicological impacts. Whereas at present, the BLM approach has been applied only for metals, there may also be opportunities to apply this approach for assessing risk for non-metal toxicants, perhaps in particular for substances where the chemical form (i.e.

speciation) is of particular significance to its bioavailability. Although at present there do not appear to have been any attempts to do so, the scientific literature suggests a number of plausible opportunities which may also be of particular interest to the environmental regulator and the Water Industry alike. For example, a study by Akkanen and Kukkonen (2003) demonstrated that the toxicity of benzo(a)pyrene, a Priority Hazardous Substance under the WFD, was dependent on a freely dissolved fraction, which was also reduced in the presence of organic matter. Similarly, a study by Neale, Escher and Schäfer (2009) demonstrated the significance of pH to the adsorption of steroid hormones to organic matter (including EE2 and E2, which currently under consideration for classification as Priority or Priority Hazardous Substances). The studies by Akkanen and Kukkonen (2003) and Neale, Escher and Schäfer (2009) to some extent resemble some of the early studies within which the significance of the chemical form of a metal (i.e. the FIAM) (Campbell, 1995; Morel, 1983), and the significance of metal complexation (Di Toro *et al.*, 2001) had been proposed, which suggests an opportunity for the BLM approach to be applied for developing models to predict ecotoxicological impacts for substances other than metals. There is therefore an opportunity to extend the BLM approach to non-metal toxicants, and to further enhance the environmental relevance of water quality standards and compliance criteria for non-metal toxicants.

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1. INTRODUCTION

As part of an initiative to improve the protection of human health and the environment, the Water Framework Directive (WFD) (European Parliament, 2000) requires European Union Member States to achieve ‘good’ status for designated waterbodies (including rivers, lakes, estuarine, sea, and ground waters), where ‘good’ is determined on the basis of quantifiable chemical and ecological criteria. The WFD supersedes the Dangerous Substances Directive (DSD) (European Commission, 2007), and appears likely to require more stringent water quality standards, with a potentially profound impact on wastewater treatment works (WwTW) operators and the standard to which wastewater effluents are required to be treated.

The prospect of more stringent water quality standards has, however, raised a number of specific concerns regarding the effect this may have on standards for trace metals such as copper, nickel, and zinc. In the first instance, although WwTWs can represent a significant source of trace metal inputs into the aquatic environment, WwTWs are not primarily designed to remove metals, and as a consequence, removal is variable (Lester, 1987). Achieving metal removal rates in order to comply with more stringent standards may therefore require additional wastewater treatment processes, which may be either unfeasible or disproportionately expensive. In the second instance, numerous studies have demonstrated that neither the total nor the dissolved concentrations of the aforementioned trace metals are good indicators of toxicity (risk) (Deleebeeck *et al.*, 2007; De Schamphelaere, Lofts and Janssen, 2005; De Schamphelaere and Janssen, 2004a; De Schamphelaere and Janssen, 2002; Di Toro *et al.*, 2001; Allen and Hansen, 1996). Rather, toxicity has been found to be dependent on a metal fraction that can be taken up by aquatic organisms (i.e. bioavailable), but which varies on the basis of waterbody physico-chemical conditions, and is not directly measureable. Whereas from a regulatory perspective the consideration of bioavailability in the setting and implementation of water quality standards is advantageous since this could ensure their environmental relevance, the factors that influence bioavailability are complex, and consequently, this had not been considered practicable.

Within the last decade, however, computer modelling approaches, such as the Biotic Ligand Model (BLM), have demonstrated good accuracy in predicting metal toxicity to a range of aquatic organisms. BLMs simulate the interactions between a dissolved metal and an aquatic organism with consideration for the influence of key physico-chemical characteristics such pH, water hardness, alkalinity, and dissolved organic carbon (DOC), and predict toxicity on the basis of the simulated accumulation of bioavailable metal at some biologically sensitive receptor (Di Toro *et al.*, 2001). BLMs effectively enable a determination of the toxicity potential of a specific dissolved metal concentration and therefore, from a practical perspective, represent a paradigm shift opportunity to integrate bioavailability into the formulation of environmentally relevant water quality standards for trace metals. Indeed, there are strong indications that regulatory authorities in England and Wales (E&W) are in the process of developing a framework for the implementation of BLM based water quality standards (Peters, Merrington and Brown, 2009), and in the United States the use of a BLM to determine water quality criteria for copper has already been approved by the U.S. Environmental Protection Agency (US EPA) (U.S. Environmental Protection Agency, 2007a).

The prospect of the application of BLMs for regulatory purposes has, nonetheless, generated concern; within Severn Trent Water in particular, but also the UK Water Industry in general. The relative complexity of the BLM approach is such that the impact on water quality standards, and consequently, the standard to which wastewater effluents may be required to be treated, is uncertain. Furthermore, whereas the predictive accuracy of BLM toxicity forecasts has been validated in a wide range of natural and reconstituted waters, waters which receive inputs from WwTWs may consist substantially of treated wastewater effluent, for which the accuracy of BLM forecasts is less certain. For example, BLMs simulate the effect of DOC on metal speciation and toxicity on the assumption that DOC is comprised of humic substances (Santore *et al.*, 2001) whereas effluent derived organic matter has been shown to contain non-humic and anthropogenic substances (Baken *et al.*, 2011; Sarathy and Allen, 2005) that display metal complexing characteristics which differ from those of humic substances. Consequently, the suitability of a standardised characterisation of complexation, as applied by BLMs, is uncertain. This element of uncertainty was of particular interest to Severn Trent Water since the Severn Trent Water region is known to have low effluent dilution capacity but also since, as a consequence of this region’s industrial legacy, numerous Severn Trent Water wastewater treatment works receive substantial inputs of copper, nickel and zinc that may be difficult to control. Indeed, it was on this basis, following discussion with Severn Trent Water, that these metals were chosen for this study. This concern is, however, perhaps also of significance elsewhere in the UK, in

Europe, and in the United States of America where regulators have also expressed interest in using the BLM approach, and which are also known to have low effluent dilution capacity (≤1:10) (Keller, Whelan and Rees, 2006).

It is within this context that the aims and objectives of this research project have been formulated.

1.1 Aims and Objectives

The aims of the presented research were to:

1. Investigate the extent by which the BLM approach may be appropriate for assessing risk in surface waters consisting substantially of sewage effluent.
2. Determine the implications of the BLM approach for Severn Trent Water Limited.

A number of specific research requirements were identified which formed the basis for the investigations conducted as part of this research. These were:

1. To what extent might sewage effluent impact the accuracy of BLM estimations of metal bioavailability?
2. Can BLM forecast accuracy be improved?
3. To what extent does the complexation capacity of sewage effluent derived organic matter differ from that predicted by the WHAM VI speciation model?
4. How variable are the metal complexing characteristics of organic matter present in surface waters?
5. Could new water quality standards require Severn Trent Water to reduce the concentrations of trace metals in sewage effluent discharges?

1.2 Guide to the thesis

This thesis consists of three volumes. Volume 1 presents the thesis for examination and contains all pertinent analyses, results, discussion, conclusions, and publications. Volume 2 and 3 contain the mandatory six-monthly progress reports produced throughout the EngD programme, which chronicle the research development. Volume 2 and 3 do not need to be read in order to examine the thesis presented in Volume 1. Volume 1 also includes a cd-rom which contains various computational models that have been applied and developed as part of this research, and other data that could not be practicably provided in print form.

Volume 1 is structured into 12 chapters.

Chapter 1 introduces the research topic, and outline the aims and objectives of the studies described in this thesis.

Chapter 2 describes the BLM approach, its origins, and the regulatory context within which the research has been conducted. More detailed reviews of background literature have been incorporated within relevant chapters.

Chapter 3 provides a technical description of the specific BLMs that have been applied in the studies described in this thesis.

Chapter 4 provides a description of the experimental and analytical methods that have been applied in the course of the research, and the rationale for their selection.

Chapter 5 describes the research undertaken in order to address the first research question, which entailed conducting a series of acute copper toxicity assays with the aquatic invertebrate *Daphnia magna* as the test organism in a wastewater effluent medium, and for which the predictive accuracy of two separate BLMs was assessed.

Chapter 6 addresses the second research question and describes the predictive accuracy of a Modified BLM that was constructed as part of this study, which utilised a more advanced chemical equilibrium model (WHAM VI), and incorporated additional effluent specific chemical characteristics in the speciation modelling.

Chapter 7 builds on the approach described in the Chapter 6, and improves the accuracy of toxicity forecasts via the development of a method for allowing an estimation of the dissolved organic carbon (DOC) fraction considered ‘active’ with regard to metal binding that reduces reliance on default values.

Chapter 8 addresses the third research question and describes the metal complexing characteristics of sewage effluent derived organic matter, the extent by which these differ from the complexation characteristics of organic matter derived from river water, and the extent by which effluent derived organic matter might retain its metal complexing characteristics once discharged into the aquatic environment.

Chapter 9 addresses the fourth research question and provides a comparison of zinc and nickel complexation characteristics for river waters in an around the Birmingham-Wolverhampton metropolitan area.

Chapter 10 addresses the fifth research question and evaluates the potential impact of the BLM approach on Severn Trent Water, and the extent by which it may require reductions in the concentrations of metals discharged from Severn Trent Water WwTWs.

Chapter 11 forms the main discussion which relates the themes from the individual chapters and considers the opportunities for applying the BLM approach to non-metals and chemical mixtures.

Chapter 12 summarises the conclusions of the research in relation to the research aims and research questions.

2. THE BIOTIC LIGAND MODEL APPROACH

This chapter consists of two parts. The first part provides an overview of the origins of the Biotic Ligand Model approach, a description of what BLMs are, how they work, and the issues related to simulating metal complexation. This component draws substantively on the Society for Environmental Toxicology and Chemistry (SETAC) publication “Effects of Water Chemistry on Bioavailability and Toxicity of Waterborne Cadmium, Copper, Nickel, Lead, and Zinc to Freshwater Organisms” (Meyer *et al.*, 2007), and the original journal articles in which the BLM concept was originally proposed (Di Toro *et al.*, 2001; Santore *et al.*, 2001; Paquin *et al.*, 2000). The second part describes the regulatory context within which the BLM approach has developed, and how the Regulator intends to apply BLM approach to set environmental quality standards in England and Wales.

2.1 The Biotic Ligand Model

2.1.1 Origin and development of the BLM approach

The BLM concept is a product of two of the classic theories of metal toxicity in the aquatic environment; the free ion activity model (FIAM) set out by Morel (Campbell, 1995; Morel, 1983), and the gill surface interaction model (GSIM) set out by Pagenkopf (1983). These theories provide separate, although similar descriptions of the chemical and physiological mechanisms that are responsible for metal toxicity. Both are based on the concept that toxicity correlates with the amount of metal that binds to some biologically sensitive receptor (i.e. the biotic ligand), and that specific toxicity endpoints can be predicted on the basis of the amount of metal that accumulates at the biologically sensitive receptor. Both approaches recognise the significance of the speciation of a metal, which refers to the different chemical forms in which the metal can exist (Neubecker and Allen, 1983), and that different metal species do not interact equally with the biologically sensitive receptor (Meyer *et al.*, 2007). Both approaches characterise the relationship between metal species, the various interacting ligands, and the biologically sensitive receptor using stability constant values, and as such, enable a quantitative determination of the distribution of metal species that, when associated with the biologically sensitive receptor, forms the basis for predicting toxicity. The two approaches differ in that the FIAM proposes that only the free ion accumulates at the biologically sensitive receptor, whereas the GSIM proposes that other metal species, such as metal hydroxides, also accumulate at the biologically sensitive receptor. In addition, the GSIM recognises that other metal ions, such as calcium, compete for binding at the biologically sensitive receptor and could thus reduce the accumulation of toxic metal at the biologically sensitive receptor.

The BLM was first presented in 2000 (Di Toro *et al.*, 2001; Paquin *et al.*, 2000) as an approach for assessing environmental risk from metals, and combined elements from both the GSIM and FIAM. For example, the BLM approach regarded the free ion as the toxic metal species (as in the FIAM) but also considered the influence of competition for binding at the biologically sensitive receptor from other metals (as in the GSIM). Conceptually, the BLM approach differed from the earlier approaches only marginally, although most notably in that it assumed a generic biologically sensitive receptor (i.e. the biotic ligand), emphasising its applicability to a range of aquatic organisms and, indeed, metals (Di Toro *et al.*, 2001). A conceptual schematic of the BLM approach, adapted from that provided by Di Toro *et al.* (2001) is provided in Figure 2.1 which demonstrates the relationship between the free metal ion (M^{2+}), the aquatic organism (biotic ligand), the organic and inorganic complexing ligands, and other ions which compete for binding at the biotic ligand and the other complexing ligands. Whereas the original BLM regarded only the free ion as the toxic metal species, this was later adapted to consider toxicity arising from hydroxide metal species, reflected by the dashed line in Figure 2.1.

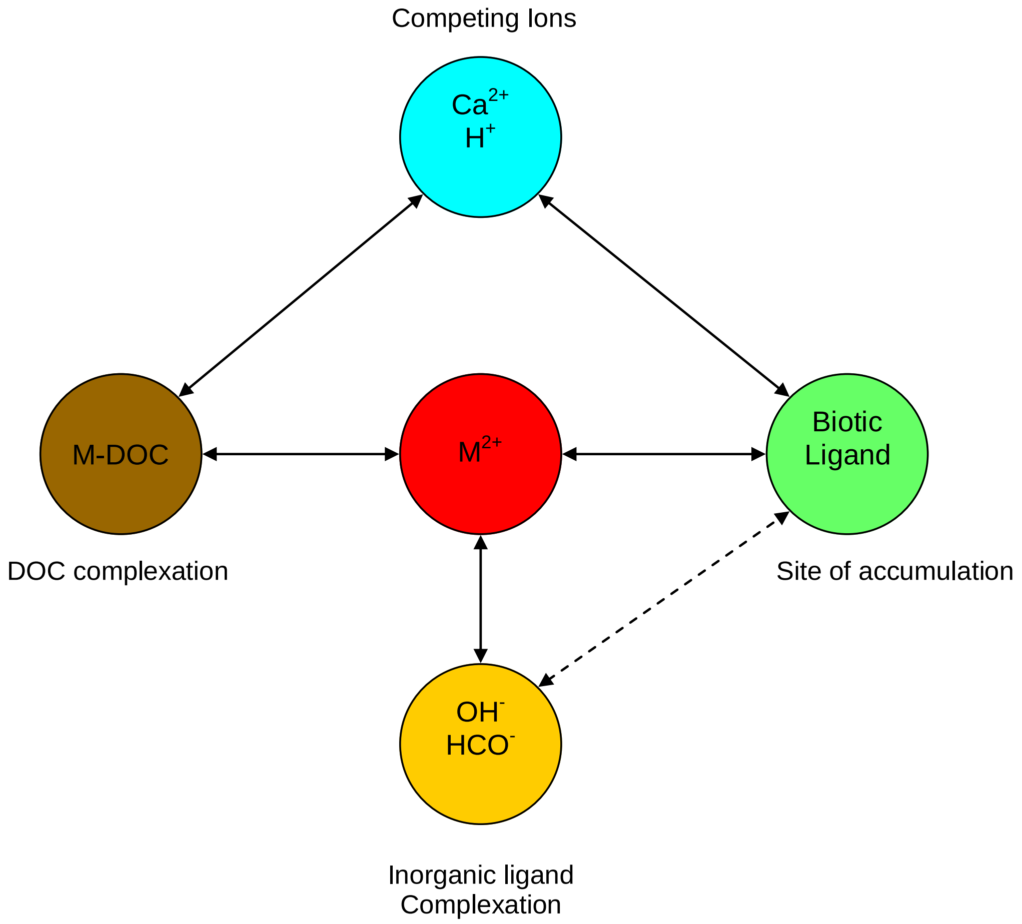

Figure 2.1 Conceptual schematic of the BLM (adapted from Di Toro et al 2001)

Although the BLM concept differed only marginally from previous approaches, its presentation in the form of a computational model (developed by the consulting firm HydroQual) that could be run on a personal computer was a significant advance, and in particular, its utilisation of a geochemical speciation model to take into account the effects of dissolved organic matter (DOM) on metal speciation. An ill-defined mixture, and ubiquitous in the aquatic environment, DOM is understood to consist largely of humic substances (humic, fulvic and hydrophilic acids) (Thurman, 1985) which contain functional groups that form complexes with metal cations. Metal ions complexed with DOM are typically not able to bind with biologically sensitive receptors, and therefore, the presence of DOM is important in influencing metal speciation and toxicity, and thus the accuracy of toxicity forecasts. Whereas researchers had previously included the effect of DOM in toxicity predictions, for example, Playle, Dixon and Burnison (1993b) estimated DOM complexation characteristics for samples obtained in their research and applied this in predictions of toxicity, the varied and complex nature of DOM suggested the application of DOM complexation characteristics determined from only a limited number of studies would be unsuitable for a model intended for a more general environmental application. Consequently, the HydroQual BLM incorporated an ion binding model from the Windermere Humic Aqueous Model (WHAM) series of humic-ion binding models (Tipping, 1994). WHAM has been calibrated to predict metal-DOM complexation from multiple data sets, and therefore its characterisation of metal-DOM complexation and was considered suitable by Di Toro *et al.* (2001) for a more general environmental application, as intended for the BLM. Whereas the WHAM model required DOM as an input value, once WHAM had been incorporated within the HydroQual model, the input parameter was modified so that dissolved organic carbon (DOC) was required as an input. The conversion between DOM and DOC is, however, relatively simple since 50% of DOM has been shown to be comprised of carbon (Thurman, 1985) and this value has been commonly applied where conversions between DOM and DOC have been required (Cheng and Allen, 2006; Bryan, Tipping and Hamilton-Taylor, 2002). Although the WHAM model applied in the original HydroQual BLM was found to provide robust predictions of complexation, Di Toro *et al.* (2001) acknowledged that further refinements would be required.

The initial HydroQual BLM calculated acute toxicity concentration values for copper and silver to *Daphnia magna* and *Pimephales promelas*, respectively, and was found to predict acute toxicity endpoint values by within a factor of two (i.e. the forecast is more than half or less than double the observed toxicity endpoint value). Indeed, this accuracy criterion has become commonly accepted as the range within which BLM forecasts are considered accurate. The initial HydroQual acute copper toxicity BLM for *D. magna* was also subsequently refined by De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere and Janssen, 2002; De Schamphelaere, Heijerick and Janssen, 2002) to take into account toxicity arising from inorganic metal species, such as $CuOH^+$ and $CuCO_3$, and to further enhance the mechanistic basis upon which toxicity forecasts were produced. Following on from this, numerous other acute toxicity BLMs for zinc (Santore *et al.*, 2002) and nickel (Deleebeeck *et al.*, 2007) as well as a number of chronic toxicity BLMs copper (De Schamphelaere and Janssen, 2004a), zinc (Heijerick *et al.*, 2005), and nickel (Deleebeeck, De Schamphelaere and Janssen, 2008) have been developed. And therefore the term 'biotic ligand model' is perhaps more appropriately considered as an approach or concept rather than in reference to any specific computational model.

2.1.2 The Biotic Ligand Model as a computational model

A BLM is a computational model that is designed to predict metal bioavailability by simulating the interactions between a metal and an aquatic organism, taking into account the influence of water chemistry characteristics, which are applied as model inputs. Typically, the output from a BLM is a dissolved metal concentration value corresponding to a specific toxicity effect (e.g. the LC50, that is, the concentration at which 50% of the organisms in a toxicity assay are killed). Whereas substantial differences might exist between various BLMs that have been developed, the manner in which they work is common to most and may be understood by assuming BLMs to comprise of two components; a chemical equilibrium sub-model and a toxicity sub-model (Di Toro *et al.*, 2001). The chemical equilibrium sub-model is typically a geochemical speciation model (e.g. WHAM) which provides the computational functionality to determine the equilibrium distribution of metal species on the basis of site-specific physico-chemical characteristics which are required as inputs. The toxicity sub-model is a database which defines the biotic ligand characteristics, including stability constant values for the various metal ions which compete for binding at the biotic ligand, and the metal-biotic ligand concentration value associated with a specific toxicity effect. When the model is ‘run’ the chemical equilibrium model includes the biotic ligand as an additional ligand in the speciation calculation and determines the dissolved metal concentration required to achieve the specified critical metal-biotic ligand concentration.

2.2 Environmental Quality Standards

2.2.1 Transition to the Water Framework Directive (WFD)

At present, water quality standards for copper, zinc, and nickel are regulated on the basis of the Dangerous Substances Directive (European Commission, 2007) (DSD) water hardness based standards (Table 2.1) which also take water body suitability for sustaining two categories of fish population into account. The hardness based standards were an initial attempt to incorporate the influence of water chemistry characteristics on metal toxicity into the regulatory framework.

Table 2.1 DSD (76/464/EEC) Hardness Related Standards for Dangerous Substances

		EQS (µg/l) for Hardness bands (mg/l $CaCO_3$)					
Substance	EQS type	**0-50**	>50-100	>100-150	>150-200	>200-250	>250
Freshwaters, suitable for all fish life							
Copper (dissolved)	Annual average	1	6	10	10	10	28
Copper (dissolved)	95th percentile	5	22	40	40	40	112
Nickel (dissolved)	Annual average	50	100	150	150	200	200
Freshwaters, suitable for Salmonid (game) fish							
Zinc (total)	Annual average	8	50	75	75	75	125
Zinc (total)	95th percentile	30	200	300	300	300	500
Freshwaters, suitable for Cyprinid (coarse) fish							
Zinc (total)	Annual average	75	175	250	250	250	500
Zinc (total)	95th percentile	300	700	1,000	1,000	1,000	2,000

In 2015, however, the DSD will be superseded by the WFD, which sets out an entirely new regulatory regime that will be used to set water quality standards for copper, zinc, and nickel. Under the WFD, water body quality will be assessed in terms of specific chemical and ecological status objectives. The chemical status classification is intended to control specific substances that have been determined to be the most polluting. The substances eligible for control in accordance with chemical status objectives are listed in Annex X of the Directive on Environmental Quality Standards (Directive 2008/105/EC, Daughter Directive of the WFD), with legally enforceable numerical concentration limits (Environmental Quality Standards) set at the European Union (EU) level. Annex X substances are divided into two categories; Priority Substances (PS), for which a progressive reduction in discharges is required, and Priority Hazardous Substances (PHS), for which complete cessation of discharges is required. Nickel has been listed in Annex X as a PS and a 20µg/L EQS has been proposed although there is anecdotal evidence that, in future, a more stringent BLM based standard may be applied. The chemical status classification has only two classes; 'good' and 'failing to achieve good' so that any water body found to contain an Annex X substance at a concentration which exceeds the EU level Environmental Quality Standard (EQS) will be classified as 'failing to achieve good'.

For the ecological status classification, waterbodies have five status classes; high, good, moderate, poor, and bad. These take into account biological, physicochemical, hydromorphological, and chemical criteria, for which specific quantifiable objectives are set. Under the ecological status classification, the chemical assessment component refers to substances which could adversely affect the ecology (not to be confused with the chemical status classification). These substances, designated 'specific pollutants', are listed in Annex VIII of the WFD, for which Member States are required to develop their own EQSs. In contrast with Annex X substances, Annex VIII lists candidate substances, from which Member States are required to select those for which relevance has been established. Whereas Member States are permitted to develop their own EQSs for Annex VIII substances, the WFD specifies that these be developed using a pre-defined methodology which has been based on that specified in an EU Technical Guidance Document (TGD) (European Chemicals Bureau, 2003), which has also formed the basis for the determination of EQS values for Annex X substances**.** Copper and zinc are listed in Annex VIII.

2.2.2 WFD EQS Criteria

The TGD described previously is aimed towards ensuring that a consistent methodology is applied in setting EQS values, and that EQSs are protective towards the most sensitive aquatic species within an ecosystem. The TGD requires EQS values be determined from actual toxicity data and that these should be based on the lowest no observable effect concentration (NOEC) values determined from chronic toxicity tests using organisms from three trophic levels; algae, invertebrates and fish, and where the lowest NOEC value is adjusted by a safety factor of 10 (i.e. lowest NOEC ÷ 10). A more precise safety factor is, however, permitted where large chronic toxicity data sets have been obtained for organisms from different taxonomic groups which are amenable to statistical extrapolation methods. For example, by fitting log transformed toxicity data values to a log normal cumulative distribution curve, representing a Species Sensitivity Distribution (SSD), for which the TGD suggests the 5th percentile may be a suitably protective value. The TGD methodology was, however, developed primarily for the assessment of organic chemicals and has generated some concern for the derivation of EQSs for metals since, as already described, metal toxicity is dependent on a bioavailable metal fraction which is not directly measureable and which varies on the basis of water body physico-chemical conditions. In addition, metals are naturally occurring so that a ‘natural’ or ‘ambient’ background concentration may occur as a consequence of underlying geological characteristics, and to which organisms may be adapted (i.e. bioavailable metal tolerance). Furthermore, it is also important to consider that trace metals, copper and zinc in particular, are micro-nutrients and essential for ecosystem health.

Whereas the consideration of bioavailability would appear to introduce substantial complexity into the formulation and implementation of EQSs in line with TGD requirements, some years prior to the introduction of the WFD, EU level risk assessments for copper, zinc and nickel had been initiated in accordance with Existing Substance Regulations (73/93/EEC) (ESR) requirements (European Commission, 2010; European Copper Institute, 2008; Denmark, 2007). These substantially improved the understanding of factors influencing metal bioavailability, and indeed, resulted in the development of so-called chronic toxicity BLMs which predict “no effect” concentration (PNEC) values for multiple aquatic organisms. The incorporation of BLMs for multiple aquatic organisms into a single modelling framework, along with the inclusion of the statistical functionality to formulate SSDs, has effectively enabled the use of BLMs as tools that are potentially useful for the determination of site-specific EQS values in a manner that is compliant with WFD (TGD) requirements.

2.2.3 The BLM as a regulatory tool

Whereas at present it is apparent that the Environment Agency (EA) for England and Wales intend to apply the BLM approach to determine environmental quality standards for copper and zinc (at least) (Peters, Merrington and Brown, 2009), in 2007, at the start of this research project, there was uncertainty as to the acceptability of the approach to regulatory authorities. Indeed, only in 2007 did the US EPA express official support for use of the HydroQual BLM for determining water quality standards for copper in the United States (United States Environmental Protection Agency, 2007a), and only in 2009 did the European Commission receive final commentary from the Scientific Committee on Health and

Environmental Risks (SCHER) on the ESR risk assessments, in which favourable views of the BLM approach were expressed (SCHER, 2009a; SCHER, 2009b; SCHER, 2007). As a consequence, views on how (and if) the BLM approach should be applied in a regulatory context have evolved rapidly in recent years. For example, it was initially anticipated that BLMs would be used to calculate a site-specific PNEC on the basis of site-specific physico-chemical characteristics which would effectively become the EQS (i.e. the approach applied in the US and in the risk assessments). More recently, however, it has become apparent that the EA intend rather to specify a permissible bioavailable metal concentration and use a BLM type model to 'convert' the bioavailability expressed value into a measureable dissolved concentration value (personal communication, Paul Whitehouse, Environment Agency). Whereas there are practical (and even philosophical) differences between these alternate approaches, the actual value against which measured metal concentration values will be compared to assess compliance will be the same under either approach (i.e. metal concentrations compared with a BLM calculated dissolved metal concentration value). Consequently, where the term 'BLM determined EQS' (or similar) is applied, the reader should interpret this as indicating the dissolved metal concentration value suggested in the BLM output regardless of the form in which the EQS value is actually expressed by the Regulator.

2.2.4 Assessing compliance with BLM determined standards

It is envisaged that compliance with BLM derived standards will be determined by comparing a measured (annual average) metal concentration with that of the BLM calculated value. Locations at which measured values exceed the BLM predicted values are deemed to 'fail', and for which some form of remediation is required, and those at which the measured values do not exceed the BLM predicted value deemed to 'pass', and for which no action is required. Typically, where the metal concentration exceeds the BLM EQS value, the EA is required to investigate, and to implement measures that result in a reduction in the metal concentration, and ultimately, compliance with the EQS. The investigation assesses the relative contribution to the exceedance from upstream sources (e.g. WwTW discharges), and where these are deemed significant, the EA will issue a legally binding numeric discharge consent, which specifies discharge criteria (e.g. a concentration value which the discharge may not exceed). The criteria by which the EA assess the requirement for a numeric discharge consent is on the basis of whether (i) the discharge is upstream of a location at which an EQS is at risk, and (ii) whether the discharge is ‘significant’, that is, it contributes to an increase in the downstream concentration by more than 10% of the EQS value (Martin and Armitage, 2005). These criteria are used not only for controlling existing discharges, but also in the assessment of quality requirements for new discharges. BLM determined EQS values will consequently influence the compliance status of WwTW discharge concentrations (after mixing with receiving waters), and therefore, the degree by which wastewaters are required to be treated.

2.2.5 A tiered approach to assessing compliance

Whereas the implementation of the BLM approach might appear relatively straightforward, the BLMs for copper and zinc developed as part of the ESR Risk Assessment process have been considered unsuitable for direct integration within the EA data management systems (Peters, Merrington and Brown, 2009). The ESR BLMs also require a reasonable degree of expertise to operate, and consequently, it has been proposed that BLMs will be applied as part of a tiered approach (Peters, Merrington and Brown, 2009). A tiered approach is characterised by lower tiers providing a simple screening mechanism so that locations that are not at risk may be unambiguously eliminated from further consideration, with the remaining locations progressing to a higher tier entailing a greater level of scrutiny. The tiered approach that has been proposed is as follows (Figure 2.2):

Tier 1: The first tier assesses compliance against a generic EQS value which is suitably conservative. This eliminates locations for which there is a high degree of confidence of compliance from further consideration.

Tier 2: At the second tier a BLM screening tool is applied. The BLM screening tool represents a conservative, purpose built, MS-Excel based version of the ESR BLMs, which are amenable to integration within the EA data management systems.

Tier 3: At the third tier, the fully functional ESR BLMs are applied.

Tier 4: At the fourth tier, a background concentration correction value is applied to the EQS value suggested by the relevant ESR BLM. The background concentration is that which is thought to represent the natural background concentration and is intended to accommodate the extent by which aquatic organisms may be adapted to local conditions.

Tier 5: Locations which have not been eliminated at earlier tiers are considered for remediation in order to reduce the concentration. Measures might include controlling upstream WwTW discharges via a discharge consent.

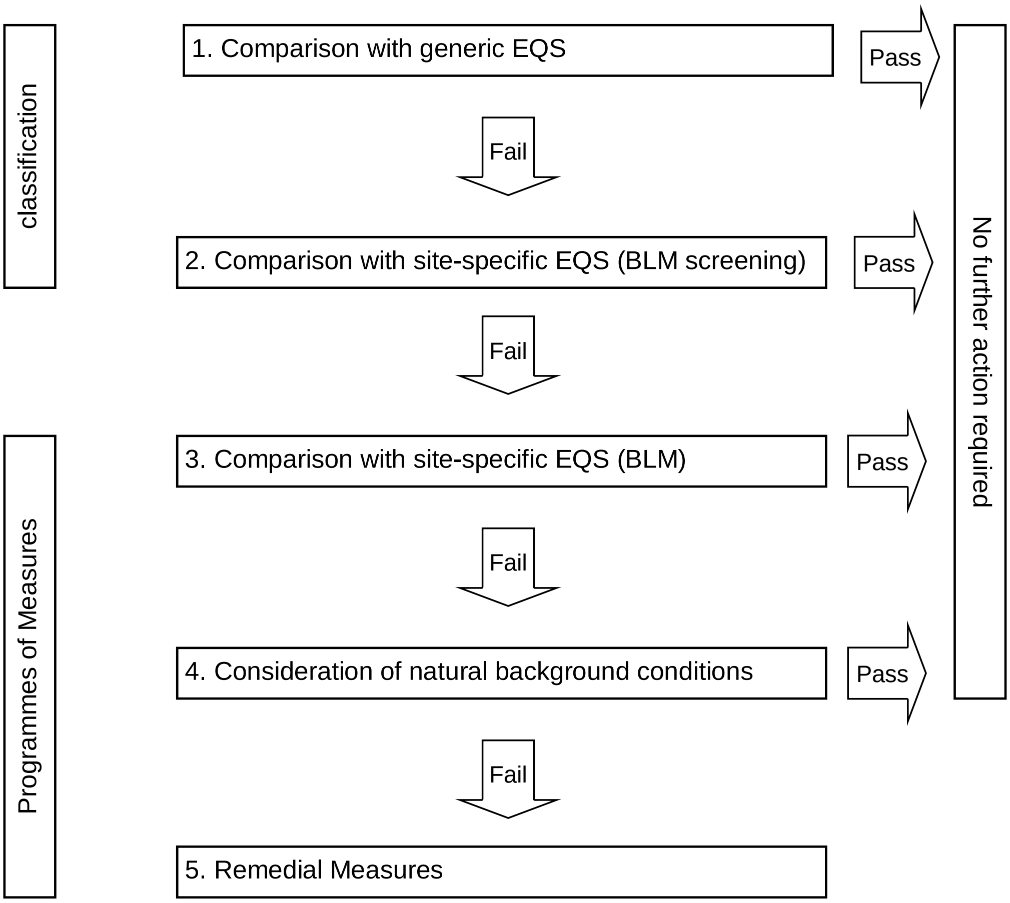

Figure 2.2 Tiered approach to compliance assessment

3. COMPUTATIONAL MODELS

3.1 The HydroQual acute copper toxicity BLM for Daphnia magna

The following description refers to the HydroQual BLM Windows Interface Version 2.2.3 (HydroQual, 2007) and draws on descriptions provided by Tipping e*t al*. (1994), Di Toro *et al.* (Di Toro *et al.*, 2001; Santore *et al.*, 2001), and the BLM version 2.2.3 User’s Guide and Reference Manual (Paquin, Santore and Mathew, 2007). The model is freely available from both the HydroQual and US EPA websites and may be run on any PC running Microsoft Windows (copy provided in chapter 3 data appendix). BLM version 2.2.3 is an update version of the original presented by Di Toro *et al.* (2001) and predicts toxicity for copper, zinc, cadmium, lead, and silver to a variety of aquatic organisms and has been approved by the US EPA for use in predicting water quality criteria for copper (United States Environmental Protection Agency, 2007b). This BLM has been applied in the study presented in Chapter 5, which evaluated the ability of this model to predict acute copper toxicity to *Daphnia magna* for assays conducted in a wastewater effluent medium. This model is expected to predict acute copper toxicity to *D. magna* by within a factor of two.

3.1.1 Model description

The HydroQual model requires temperature, pH, DOC concentration (and the % which is humic acid), inorganic carbon (or alkalinity), calcium, magnesium, sodium, potassium, sulphate, and chloride as water chemistry inputs. The model may be operated in ‘speciation mode’, whereby the equilibrium distribution of metal species is determined on the basis of the water chemistry inputs and a specified metal concentration, or in ‘toxicity mode’, whereby the model calculates the concentration value associated with an acute toxicity endpoint (e.g. LC50). Data files (.dat format) contain the biotic ligand parameters (i.e. the biotic ligand binding constants and biotic ligand critical concentration value) for the organisms and metals that are included in the model (one file per metal-organism combination) with each .dat file also referencing the thermodynamic database (.dbs format) to be applied in the calculation. A default thermodynamic database in supplied with the model and contains the organic and inorganic complexation parameters that are applied in calculations. A version of the Chemical Equilibria in Soils and Solutions (CHESS) model (Santore and Driscoll, 1995) is incorporated within the HydroQual model framework which is used in the speciation calculations. In modelling ion interactions, the biotic ligand is treated as any other solution ligand and assumed to be uniformly distributed throughout the test medium, for which ions compete for binding. The interacting model components may be visualised as in Figure 3.1. The *D. magna* biotic ligand and inorganic binding constants for the HydroQual BLM are provided in Table 3.1.

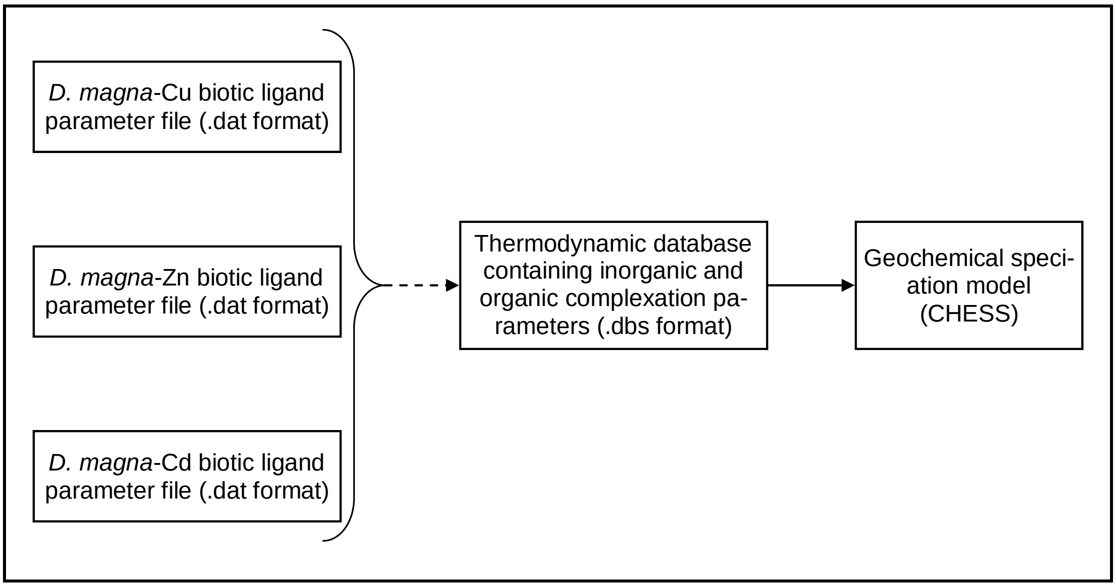

Figure 3.1 Conceptual schematic of HydroQual BLM components

3.1.2 The HydroQual BLM biotic ligand

The premise upon which BLM toxicity forecasts are founded is that a toxicity endpoint corresponds with a specific accumulation of toxic metal species at the biotic ligand. In the HydroQual BLM Cu^{2+} and $CuOH^{+}$ are considered as toxic metal species.

The total number of BL binding sites (30 nmol/gram of dry biotic ligand weight) and the biotic ligand binding constants for *D. magna* are those determined for the fathead minnow by Playle, Dixon and Burnison (1993a), but with the critical biotic ligand concentration value (0.119 nmol/gram of dry biotic ligand weight, or 0.397% of binding sites) adjusted to that which produced the most accurate predictions of acute *D. magna* copper toxicity using toxicity data reported in literature. It is useful to recognise that as a consequence of this empirically based adjustment, the basis upon which the HydroQual BLM simulates the accumulation of toxic metal species at the biotic ligand may not be mechanistically accurate.

The model uses an iterative approach to calculate the dissolved metal concentration associated with the biotic ligand critical concentration value by applying an initial trial concentration value, which is iteratively adjusted until the target critical biotic ligand concentration value (specified in the .dat file) is achieved.

3.1.3 HydroQual BLM complexation modelling

Whereas inorganic speciation may be modelled with accuracy since the formation constants well known, the simulation of DOC complexation is more complex. In order to simulate copper-DOC complexation, the BLM incorporates DOC complexation characteristics taken from the Humic Ion Binding Model V (Tipping, 1994) (WHAM V) and applied within the CHESS computational framework (i.e. CHESS simulates WHAM V). The simulation is based on the premise that metal cations and protons compete for binding at the same binding sites, and that metal binding can be determined from proton binding characteristics. In the model characterisation, DOC is assumed to comprise of two types of binding sites; type A (carboxyl) and type B (phenolic), with the site density of type B sites (n_B) half that of type A ($n_B = n_A / 2$). For each site type a distribution of pK_H (acid-dissociation) values are specified on the basis of median pK_A and pK_B values and their ranges, ΔpK_A and ΔpK_B. Individual pK_H values are determined as follows:

$$pK_{H1} = pK_A - \frac{\Delta pK_A}{2}$$

$$pK_{H2} = pK_A - \frac{\Delta pK_A}{6}$$

$$pK_{H3} = pK_A + \frac{\Delta pK_A}{2}$$

$$pK_{H4} = pK_A + \frac{\Delta pK_A}{6}$$

Comparable equations are used to calculate pK_H values for the type B sites (i.e. pK_{H5}, ..., pK_{H8}).

Since $pK_{Hi} = -\log10 K_{Hi}$ (where K_{Hi} is the proton binding stability constant) the metal stability constant values (K_{Mi}) are related to the proton binding values using an additional parameter (K_{MA}), which is specified in the model database, as follows:

$$K_{\mathrm{M}i} = \frac{K_{\mathrm{MA}}}{K_{\mathrm{H}i}} \qquad i = 1, ..., 4$$

In the model, the metal binding parameter is specified as pK_{MA} (rather than K_{MA}). Equivalent values for type B sites are similarly determined:

$$K_{\mathrm{M}j} = \frac{K_{\mathrm{MB}}}{K_{\mathrm{H}j}} \qquad j = 1, ..., 4$$

In the model database only a value for pK_{MA} is specified since the value for pK_{MB} has been related to that for pK_{MA} (Di Toro *et al.*, 2001; Tipping and Hurley, 1992) as follows:

$$\mathrm{p}K_{\mathrm{MB}} = 1.38\mathrm{p}K_{\mathrm{MA}} + 2.57$$

In addition to the binding already described, the model characterisation also allows bidentate binding. This implies that metal ions and protons bind to two sites simultaneously. Forty percent of binding sites are considered bidentate sites and the binding constants for bidentate sites are calculated as the product of the monodentate binding constants as follows:

$$K_{\mathrm{M}ij} = K_{\mathrm{M}i}K_{\mathrm{M}j}$$

The total number of stability constant values for bidentate sites may be calculated as follows:

$$P_{k,n} = \frac{n!}{k!(n-k)!}$$

Where *n* is the total number of monodentate sites (8) and *k* the number monodentate sites in each combination (2). From this, we calculate 28 stability constant values for bidentate sites, alongside the original 8 (36 in total). Since total binding site density is also given within the thermodynamic database, the distribution of binding sites (i.e. binding affinity and concentration) may be determined.

3.2 The Refined acute copper toxicity BLM for Daphnia magna

The following description refers to the Refined acute copper toxicity BLM for *D. magna* developed by De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002). This model was originally developed to enhance the mechanistic basis upon which the HydroQual acute copper toxicity forecasts for *D. magna* were based, and to improve the predictive accuracy of an earlier version that had been found to provide inaccurate forecasts above pH 8 (De Schamphelaere and Janssen, 2002). The Refined BLM utilises the HydroQual model platform but with certain modified parameter values (i.e. values within the .dat and .dbs files – modified versions provided in chapter 3 data appendix, Cu_Dmagna_acuteKACDes_2002.dat and WATER12.dbs) and, as for the HydroQual BLM, is expected to predict acute copper toxicity to *D. magna* by within a factor of two. The Refined BLM has been applied in the study presented in Chapter 5, where its predictive accuracy has been assessed in relation to that of the previously described HydroQual BLM. The critical differences between the HydroQual and Refined BLMs are described as follows and are summarised in Table 3.1.

3.2.1 Refined BLM biotic ligand

The biotic ligand parameter values applied in the Refined BLM were determined directly from toxicity data (i.e. using biological response to quantify metal speciation) and consider Cu^{2+}, $CuOH^{+}$, and $CuCO_3$ as toxic metal species. In their development of the Refined BLM, De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) derived an equilibrium equation which calculates the Cu^{2+} ion activity value required to achieve the *D. magna* biotic ligand critical concentration (i.e. $EC50Cu^{2+}$). The equilibrium equation is provided below, where square brackets indicate ion activities, and K the relevant stability constant values:

$$\mathrm{EC50}_{\mathrm{Cu}^{2+}} = \frac{f^{50\%}_{\mathrm{CuBL}} \cdot \left\{1 + K_{\mathrm{CaBL}} \cdot \left[\mathrm{Ca}^{2+}\right] + K_{\mathrm{MgBL}} \cdot \left[\mathrm{Mg}^{2+}\right] + K_{\mathrm{NaBL}} \cdot \left[\mathrm{Na}^{+}\right] + K_{\mathrm{HBL}} \cdot \left[\mathrm{H}^{+}\right]\right\}}{\left(1 - f^{50\%}_{\mathrm{CuBL}}\right) \cdot \left\{\mathrm{K}_{\mathrm{CuBL}} + \mathrm{K}_{\mathrm{CuOHBL}} \cdot \mathrm{K}_{\mathrm{CuOH}} \cdot \left[\mathrm{OH}^{-}\right] + \mathrm{K}_{\mathrm{CuCO_3BL}} \cdot \mathrm{K}_{\mathrm{CuCO_3}} \cdot \left[\mathrm{CO_3}^{2-}\right]\right\}}$$

The $f^{50\%}_{\mathrm{CuBL}}$ value represents the fraction of biotic ligand binding sites that are occupied by toxic metal species at the 50% effect level. Within the HydroQual model framework this has been reflected as an amended biotic ligand critical concentration value (i.e. 14.1nmol/gram dry BL weight, or 47% of biotic ligand binding sites).

3.2.2 Refined BLM complexation modelling

Whereas the Refined BLM applied the same humic ion binding model to simulate copper-DOC complexation (as previously described), there were differences in parameterisation. In their study, De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) found that the HydroQual BLM substantially overestimated copper complexation (i.e. measured Cu^{2+} ion activity was substantially greater than the modelled value), and consequently modified the pK_{MA} value to that which provided the best fit to their experimental data (from 1.5 to 1.9) which implied a reduction in the affinity between copper and DOC. Their model also introduced another important and well recognised concept which had not been incorporated within the HydroQual BLM. For the purposes of modelling, DOC is traditionally considered to comprise of an 'active' fraction, to which metal ions bind, and another fraction which is inert with regard to ion binding. The proportion of DOC that is considered 'active' therefore has a significant effect on modelled speciation. The 'active' fraction has, however, been found to be variable, between 40-80% as fulvic acid (Dwane and Tipping, 1998), and in their study De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) applied a default 'active' fraction value of 50% (as fulvic acid) in accordance with recommendations by Dwane and Tipping (1998) (i.e. 50% of the measured DOC concentration is applied as the model input).

3.1 *D. magna* biotic ligand and inorganic binding constants for the HydroQual and Refined biotic ligand models

	HydroQual BLM	Refined BLM
log K_{CuBL}	7.4	8.02
log K_{CuOHBL}	6.22	7.32
log K_{CuCO_3BL}		7.01
log K_{CaBL}	3.6	3.47
log K_{MgBL}	3.6	3.58
log K_{NaBL}	3.0	3.19
log K_{HBL}	5.4	5.4
$f_{CuBL}^{50\%}$ [a]	0.397%	47%
BL binding sites (nmol/g dw)	30	30
BL critical conc. (nmol Cu/g dw)	0.119	14.1
log K_{CuOH^+}	6.48	6.48
log K_{CuOH_2}	11.78	11.78
log $K_{CuHCO_3^+}$	14.62	12.13
log K_{CuCO_3}	6.75	6.77
log $K_{Cu(CO_3)_2}$	9.92	10.2
log K_{CuCL^-}	0.4	0.4
log K_{CuSO_4}	2.36	2.36

[a] The proportion of biotic ligand binding sites that are required to be occupied by copper to induce a 50% acute toxicity effect.

3.4 The chronic copper toxicity BLM

The chronic copper toxicity BLM (European Copper Institute, 2006) was developed for the EU Risk Assessment for copper (European Copper Institute, 2008) and is the BLM that will be applied at the 4th Tier stage as described in Chapter 2, and is also the version that has been applied in the risk assessment presented in Chapter 10. This BLM applies a modified version of the HydroQual model, with the inorganic and organic complexation parameters (thermodynamic database) as described for the Refined BLM (Table 3.1). Some additional functionality is, however, incorporated. This BLM contains a database with published chronic toxicity data; NOEC values as dissolved copper concentration values and the water chemistry characteristics, as required for model input, from 129 toxicity assays for 27 species from 3 taxonomic groups (alga, invertebrate and fish). The model specifies biotic ligand binding constants for each species on the basis of taxonomic group so that three sets (.i.e. three .dat files) are applied. When the model is 'run' the following calculations are performed:

- The model calculates the PNEC biotic ligand critical concentration for each of the 129 toxicity assays.
- The model then calculates the dissolved copper concentration values required to achieve the biotic ligand critical concentration, with consideration for the user input site-specific water chemistry characteristics.
- The mean NOEC values for each species are sorted, transformed into Log_{10} values, and arranged as a cumulative probability distribution.
- The final model output value is the 50% lower confidence interval value of the 5th percentile value of the cumulative probability distribution (i.e. the 5th percentile hazardous concentration, or HC5).

A copy of the chronic copper toxicity BLM is provided in the chapter 3 data appendix.

3.5 The chronic zinc toxicity BLM

The chronic zinc toxicity BLM (De Schamphelaere, Lofts and Janssen, 2005) is an MS-Excel based spreadsheet model which was developed for the EU risk assessment for zinc and its compounds (European Commission, 2010). The spreadsheet model was developed as a simplified, user-friendly version of other more complex HydroQual-type models (as described for copper) used in the risk assessment. The developers claim that it provides a good approximation (by within

2%) of the values produced by the HydroQual-type models (De Schamphelaere, Lofts and Janssen, 2005). The spreadsheet model is also the version that will be applied by the EA at the 4th Tier stage as described in Chapter 2, and is also the version that has been applied in the risk assessment presented in Chapter 10.

The spreadsheet model differs from those described previously in that it does not simulate chemical interactions. Rather, this model contains a series of lookup tables based on the outputs from HydroQual-type models, to which the model refers in predictions of toxicity. Notably, in the original HydroQual model, zinc-DOC complexation was modelled using an 'active' fraction value of 61% and pK_{MA} value of 2.3, which also reflects the lower affinity between zinc and DOC in comparison with that which exists between copper and DOC (pK_{MA} of 1.9). The spreadsheet model requires only pH, DOC and calcium/hardness as model inputs, although the output values, and the manner which they are calculated differs from that applied by the chronic copper toxicity BLM. The output is calculated as follows:

- On the basis of the user input water chemistry characteristics, the BLM determines predicted no effect concentration (PNEC) values for *Psuedokirchneriella subcapitata* (an algae)*, D. magna* (an invertebrate), and *Oncorhynchus mykiss* (a fish) (i.e. organisms from 3 trophic levels).
- The BLM calculates a bioavailability correction factor (BioF) for each of the PNEC values. The BioF is the ratio between a reference or 'realistic worst case' PNEC which represents the PNEC under conditions of high zinc bioavailability, and the calculated site specific PNEC. The 'realistic worst case' PNEC value exists as a constant within the model. The BioF is represented as follows:

$$\mathrm{BioF} = \frac{\mathrm{NOEC}_{\mathrm{reference}}}{\mathrm{NOEC}_{\mathrm{site}}}$$

- The final site specific PNEC is calculated by dividing a generic PNEC value by the highest BioF value of the considered species (i.e. the most sensitive species – usually algae). The generic PNEC value represents the HC5 value from a species sensitivity distribution determined for multiple organisms conducted under conditions of high bioavailability (Peters, Merrington and Brown, 2009) (i.e. a realistic worst case species sensitivity distribution). The generic PNEC value applied for the risk assessment described in Chapter 10 was 7.8µg/L, which was the value determined in the European Union Risk

Assessment for Zinc (European Commission, 2010). The site specific PNEC value may therefore be represented as follows:

$$\mathrm{PNEC}_{\mathrm{site}} = \frac{\mathrm{PNEC}_{\mathrm{generic}}}{\mathrm{BioF}_{\mathrm{site}}}$$

A copy of the chronic zinc toxicity BLM is provided in the chapter 3 data appendix.

3.6 The WHAM VI geochemical speciation model

The WHAM VI model, which supersedeed WHAM V, has been applied in the development of the Modified BLM described in Chapter 6 and for speciation calculations in the study described in Chapter 7.

WHAM VI has been demonstrated to be more accurate than WHAM V (Bryan, Tipping and Hamilton-Taylor, 2002), predicting Cu^{2+} ion activity by within a factor of 3.6, compared to by within a factor of 6.3 for Model V (Bryan, Tipping and Hamilton-Taylor, 2002). The crucial difference between WHAM V and WHAM VI is that WHAM VI relaxes the relationship between metal and proton binding and expresses metal-DOC affinity in terms of a central log K_{MA} value, with a distribution of binding constants determined on the basis of a primary ‘spread factor’ (ΔLK1) and a secondary ‘spread factor’ (ΔLK2). The secondary ‘spread factor’ enables the creation of high affinity binding sites for which a range of metals compete for binding, and to which the improved accuracy of Model VI has been attributed. Whereas the HydroQual and Refined BLMs required DOC as an input (in mg/L), WHAM VI required dissolved organic matter (DOM) as an input. Therefore, for WHAM VI the input values are two times the DOC concentration, where the factor of two converts DOC to DOM by assuming carbon to comprise 50% of organic matter by weight (Cheng *et al.*, 2005).

A copy of WHAM VI (license number SL0095) was purchased from the Centre for Ecology and Hydrology (CEH, UK).

4. METHODS

This chapter described the various methods that have been applied and is divided into four sections. The first section describes the general analytical and laboratory techniques that have been applied in this research project. The second section describes the Chelex column method which has been used as the metal speciation technique in this research project (Chapters 7, 8, and 9) and the rationale for its selection. The third section describes the techniques relating to the *D. magna* toxicity assays that have been applied in the study described in Chapter 5. The fourth section describes the approach adopted for the compliance risk assessment, presented in Chapter 10.

4.1 General analytical and laboratory techniques

4.1.1 Reagents and Materials

All chemicals, unless specified, were obtained from Sigma (Gillingham, UK). All water was obtained from a Millipore Milli-Q ultrapure water system (hereafter referred to as MQ). All glassware was soaked in 1% HNO_3 MQ and rinsed with copious quantities of MQ prior to use.

4.1.2 Sampling

Clean sample collection vessels were rinsed with the water of interest prior to collecting the sample. Samples were stored in 1 L or 20 L storage vessels (depending on the sample volume required) and filtered through 0.45µm cellulose nitrate membrane filters (Whatman, UK) upon return to the laboratory using a vacuum pump (20 L samples), or immediately on site using a Nalgene® hand-operated vacuum pump (Sigma, UK) (1 L samples). Samples were refrigerated at 4°C immediately after filtration. Steel-capped boots and a fluorescent jacket/vest were worn at all times, as well as protective headgear at Severn Trent Water WwTWs. Nitrile gloves were worn to prevent sample contamination and to prevent direct contact with the sampled effluents.

4.1.3 Analytical techniques

Sample pH was determined using a SENTEK P11 pH probe (Sentek, UK) for laboratory based measurements, and a Hanna Instruments HI 991003 portable pH/mV meter for field based measurements. Dissolved oxygen was measured using a Hanna Instruments HI9146 dissolved oxygen meter. Copper and nickel were quantified by graphite furnace atomic absorption spectrophotometer (GFAAS) using a Zeeman 4100ZL (PerkinElmer, Beaconsfield, UK). The limits of detection (LOD) were 0.5 µg/L and 2.0 µg/L, respectively. Zinc was quantified by flame atomic absorption spectrometry, with a limit of detection was 5 µg/L. Iron, calcium, magnesium, and potassium were quantified using flame atomic absorption spectrometry and sodium by flame atomic emission spectrometry also using an AAnalyst 100 (PerkinElmer). The LODs for iron, calcium, magnesium, potassium and sodium were 26 µg/L, 4 µg/L, 21 µg/L, 16 µg/L, and 1,239 µg/L, respectively. The LODs were calculated from the calibration as the absorbance value for a blank sample (i.e. not containing the analyte of interest) plus 3 standard deviations of the blank. Instruments were operated in accordance with the manufacturer's recommendations and calibrated within a linear range ($r \geq 0.99$) using standards prepared from 1,000 mg/L spectroscopic standards diluted in MQ with 2% Optima grade HNO3 (Fisher, UK). The characteristic concentration check values for each analysis were confirmed to be within 20% of expected values to ensure the instruments were performing up to specification. All samples were measured in triplicate with mean and 95% confidence interval range values reported to provide an indication of the accuracy of the determination.

Chloride was quantified via ultraviolet spectrometry (Lambda 2, PerkinElmer) using an Aquanal®-plus chloride test kit (Sigma-Aldrich 2009).

4.1.4 Carbon

Total organic carbon (TOC), dissolved organic carbon (DOC) and inorganic carbon were measured in triplicate using a Model 700 TOC Analyser (O.I Corporation, USA) in accordance with manufacturer's recommendations. The reagents were sodium persulphate ($Na_2S_2O_8$) (100 g/L) and a dilute (5%) phosphoric acid solution. A 5 mg/L carbon standard was prepared weekly using potassium hydrogen phthalate. The instrument was operated continuously until readings for blanks (MQ) and standards stabilised (~2 hours).

4.1.5 Synthetic chelating agents

The aminopolycarboxylate chelating agents ethylenediaminetetratacetic acid (EDTA), nitrilotriacetic acid (NTA), and diethylenepentaacetic acid (DTPA) were quantified using the method of Laine *et al.* (Laine and Matilainen, 2005). Analysis was performed on a Series 200 HPLC system (Perkin Elmer, Beaconsfield, UK) using a 20 µl loop. Analytes were separated using a 250mm x 4.6mm Kromasil C18 column. The mobile phase consisted of 0.03 mol/L sodium acetate, 0.002 mol/L tetrabutylammonium bromide as the ion-pair, and 5% methanol, adjusted to pH 3.15 using formic acid. Calibration standards were prepared from ferric chloride, EDTA, DTPA and NTA as 0.1, 0.5, 1.0 and 5.0 µM/L solutions. The limits of detection were 0.21 µM/L (63 µg/L), 0.21 µM/L (83 µg/L), and 0.77 µM/L (148 µg/L), respectively.

The analytical protocol for aminopolycarboylates required these to be reacted with Fe^{3+} since aminopolycarboylate-Fe^{3+} complexes are highly stable and display strong UV absorbance characteristics, which forms the basis for their quantification. The high stability of the Fe^{3+} complexes also suggests that, if initially present in this form, these will be unable to dissociate and complex other metal ions (i.e. is inert). Consequently, concentrations of aminopolycarboxylate chelating agent were also distinguished in terms of their labile and non-labile (inert) concentrations. The analytical protocol required 1 ml of 1 mg/L $FeCl_3$ solution to be added to 9 ml of 0.45 µm filtered sample and left to stand overnight. This converted the entire aminopolycarboxylate concentration into Fe^{3+} form, which was used as the basis for quantifying the total aminopolycarboxylate concentrations. Each sample was therefore also analysed without the addition of $FeCl_3$ (MQ water was added instead) which enabled a measure of the inert aminopolycarboxylate. The labile aminopolycarboxylate concentration was calculated as the difference between the $FeCl_3$ spiked and unspiked samples. Samples were analysed in triplicate.

4.2 Metal speciation

The Chelex column method has been applied as the metal speciation technique whereby complexation characteristics were assessed. Since a range of other techniques are also available, this section describes some of the other commonly employed techniques and the rationale for the selection of the Chelex method.

4.2.1 Method selection

The most commonly employed metal speciation techniques may be broadly categorised as potentiometric, voltammetric, and resin adsorption methods. The suitability of any particular technique depends on its intended purpose. For this study, the following two characteristics were considered desirable:

i) Reliability – wastewater effluents potentially contain an array of substances which might distort speciation measurements. The selected method should therefore be robust to the extent that sensitivity to interference is minimised, while maintaining an acceptable degree of detection sensitivity.

ii) Ease of use – the method should be amenable to routine operation, high sample throughput, use for multiple metals, and not entail excessive cost.

The considered methods were as follows:

4.2.2 Potentiometric methods

These involve the use of an ion selective electrode (ISE); a probe type instrument which measures ion activity directly (e.g. Cu^{2+}). ISEs have been used in characterising biotic ligand characteristics for numerous organisms (De Schamphelaere *et al.*, 2005; De Schamphelaere and Janssen, 2002), as well as for characterising metal complexing properties of organic matter from a wide variety of sources (Sarathy and Allen, 2005; Bryan, Tipping and Hamilton-Taylor, 2002). Whereas ISEs offer many 'ease of use' benefits, these are also sensitive to interference from substances which might reasonably be expected to occur in wastewater effluents (e.g. Ag^{+}, Hg^{2+}, S_2, Fe^{2+}, Cd^{2+}, Cl^{-} and Br^{-}) (Sentek, UK). In addition, whereas ISEs are available for copper, there are no commercial equivalents for nickel and zinc. Consequently, potentiometric methods were not considered optimal for assessing complexation characteristics for this study.

4.2.3 Voltammetric methods

Commonly employed voltammetric methods include anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV). These involve the application of an electric potential to an electrode, upon which an electrochemically labile metal fraction is reduced, the response from which is used for quantification. The labile metal fraction is often used as a proxy for the free metal ion, and includes the free ion, inorganic metal species, and potentially, other weakly complexed metal species. The labile fraction is operationally defined and dependent upon the method by which it has been determined. Whereas voltammetric techniques no doubt represent the most commonly employed speciation techniques, these suffer from a number of drawbacks, including the adsorption of humics onto the electrode surface (Neubecker and Allen, 1983), and changes to metal complexes as a consequence of the applied potential. In addition, voltammetric methods also require specialist equipment that lacks the relative simplicity that could enable low cost, high sample throughput. Consequently, voltammetric techniques were considered sub-optimal for the purposes of this study.

4.2.4 Resin adsorption methods

These involve exposing a water sample to a resin that has some chelating functionality, where a labile metal fraction is retained by the resin. Labile metal species include the free ion, inorganic species, as well as weakly complexed organic species. The concentration of metal considered labile is dependent on a number of factors including the type of resin, the exposure method, and exposure duration. Whereas resin adsorption methods lack the degree of sensitivity offered by potentiometric and voltammetric methods, these are robust and have been successfully employed in a broad spectrum of media, including sea water, river water, and wastewater effluents (Bowles *et al.*, 2006; Buzier, Tusseau-Vuillemin and Mouchel, 2006; Florence and Batley, 1976) for copper, nickel and zinc. Two of the most commonly employed resin adsorption methods are the diffusive gradients in thin films (DGT) method (Zhang and Davison, 1995), and the Chelex column method (Bowles *et al.*, 2006).

The DGT method is commercially available and employs a Chelex ion exchange resin as a complexant, enclosed within a polyacrylamide hydrogel. When immersed into water, metal ions diffuse through the gel layer and are complexed by the Chelex resin. The method enables a selective determination of different labile species depending on the type and thickness of gel used and the exposure period. The method, however, requires the complexed metal to be eluted from the Chelex for quantification which, restricts sample throughput and therefore limits the determination of complexation characteristics.

The Chelex column method involves passing a water sample through a column packed with Chelex resin, within which labile metal species are retained. The concentration of labile metal may be determined by either eluting the metal from the resin using acid (as in the DGT method), or as the difference between the metal concentrations detected in pre-column and post-column samples. The method enables a selective determination of labile metal by controlling the flow rate through the column. A particular advantage of the column method is that if the binding capacity of the resin within each packed column is high, each column may be used multiple times in quick succession. Since the Chelex method is also amenable to routine operation and does not entail excessive cost it was considered most suitable for the purposes of this study.

4.2.5 The Chelex column method

The description of the Chelex method as applied has been adapted from Bowles *et al.* (2006).

Calcium-form Chelex columns were prepared in borosilicate glass columns (Bio-Rad, USA) 50 mm (height) by 7 mm (internal diameter) with Chelex-100 as the chelating resin (200-400 mesh, Bio-Rad, USA). 1 ml of wet sodium-form Chelex slurry (equivalent to 0.15 g dry weight of resin) was inserted into each column, and plugged with glass wool. The Chelex resin was converted to calcium form by pumping at least 10 ml of 2 M calcium chloride through each column at a flow rate of approximately 20 ml/minute. Flow through the column was controlled using a 302S/RL Watson Marlow peristaltic pump.

For each sample run flow through the column was set at 50 (±2) ml/minute. The pH of the column was stabilised between samples by passing at least 30ml of reagent grade 0.1 M 3-(N-morpholino)propanesulphonic acid (MOPS) solution, amended to the sample pH, through the column. At least 12 aliquots of 200 ml filtered sample were spiked with a metal standard prepared as 50 mg/L solutions in dilute Optima grade nitric acid (1%) (Fisher, UK) using reagent grade commercially available metal salts (Fisher, UK). Samples were spiked with either copper in increments of 50 µg/L, or with nickel or zinc in increments 10 µg/L in order of increasing concentration. The volume of resin applied has capacity to retain in excess of 10mg of the considered metals so there was good confidence that the spiked metal would not exceed the resin capacity.

The pH of each aliquot was stabilised by adding 1ml of reagent grade 1 M MOPS solution, prepared at the sample pH. The spiked aliquots were equilibrated for one hour to standardise the kinetic conditions, and passed through two separate columns (i.e. duplicate measurements for each spiked sample). At least 40 ml was collected from each aliquot before being passed through the columns and 50 ml from after each column. Pre and post column samples were preserved by adding 1 ml of Optima grade nitric acid. Columns were replaced after each titration.

Metal concentrations were quantified using either flame or graphite furnace spectrometry. The pre and post column concentrations represent dissolved and non-labile copper concentrations, respectively. The labile concentration may be determined as the difference between the pre and post column concentrations.

4.2.6 Chelex titration data processing

The results from the Chelex method titration may be represented as in Figure 4.1, where the post-column metal (non-labile) concentration (y-axis) is plotted in relation the pre-column (total) metal concentration (x-axis). As the metal concentration increases, uncomplexed ligands bind with the added metal until the available ligands become saturated. The horizontal portion of the curve therefore represents the complexation capacity, with the curve providing an indication of the binding affinity of the available ligands.

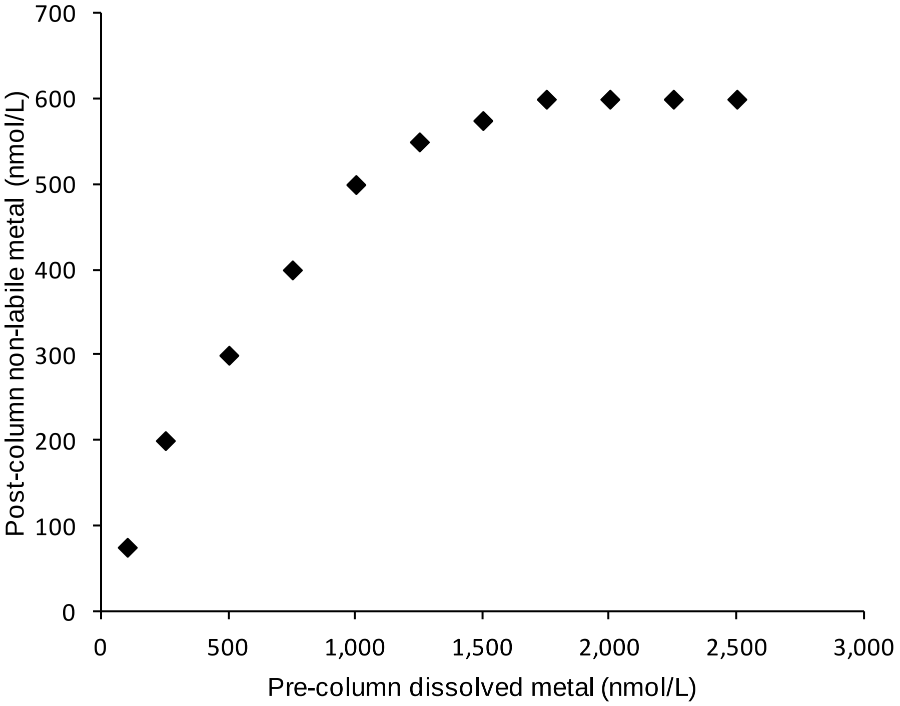

Figure 4.1 Example of the binding curve generated from Chelex method data

Complexation characteristics (complexation capacity and binding affinity) were determined from the Chelex titration data by fitting a non-linear receptor binding equation to the data (described in Motulsky and Christopoulos, 2004a). This approach was advantageous in that it avoided the more traditional approaches (e.g. Scatchard plots, Van den Berg/Ruzic transformations) which linearize the binding data for analysis, but which have been reported to distort the representation of experimental error (Gerringa, Herman and Poortvliet, 1995; Motulsky and Christopoulos, 2004a).

$$Y = \frac{B\max \cdot X}{Kd + X}$$

In the equation, Y represents the post-column or non-labile metal concentration (in molar units, normalised to the DOC concentration as nmol/mg of DOC). The value for X represents the dissolved (pre-column) metal concentration, also in molar units. B_{max} represents the complexation capacity (in molar units), and K_d the equilibrium dissociation constant (in molar units). K_d represents the equilibrium relationship between the free and complexed ligand receptors and is also the reciprocal of K (the complex formation stability constant) which is commonly used to describe binding affinity. The values for B_{max} and K_d are conditional since these are dependent on the method by which the data were obtained.

For the Chelex method data values for B_{max} and K_d were calculated using a custom-built Excel spreadsheet which uses the MS Excel Solver application to find the best fit values for B_{max} and K_d. For each titration the data from both columns were pooled which allowed the effect of differences between individual columns and differences due to random variation in the measured values to be taken into account. The best fit values for B_{max} and K_d were those that minimised the sum of squared residuals. Confidence interval values for B_{max} and K_d were determined using a Monte Carlo method, also incorporated within the custom-built spreadsheet, whereby the residuals were applied randomly (with replacement) to each measured data point and new best fit parameters determined (Motulsky and Christopoulos, 2004b). The confidence interval limits B_{max} and K_d were the 2.5%ile and 97.5%ile values for each produced from 1,000 simulations (i.e. 95% confidence interval range). It is notable that since the approach requires no assumptions about the distribution of error the upper and lower confidence interval values may not be symmetrical around the best fit value. A copy of the titration analyser is provided in the data appendix for chapter 4 (ChelexAnalyser.xlsm).

4.2.7 Comparison with WHAM

A direct comparison of complexation as measured using the Chelex column method with that predicted by WHAM VI is complicated since, as a consequence of its operational nature, the Chelex method non-labile fraction excludes weakly bound organic metal complexes that dissociate within the Chelex column, but which are included in the WHAM VI prediction. The effect of this difference is that the total complexation capacity estimated by the Chelex method will be lower than that simulated by WHAM VI, although by an indeterminate amount. The study by Bowles *et al.* (2006) (as described in section 4.2.5), however, also assessed the Chelex method selectivity for a range of synthetic ligands, and determined that 50% complex non-lability may be expected for complexes with stability constants (as log K) of 12-15 for copper, 10-12 for zinc, and 7 for nickel. This implies that the point on the Chelex binding curve at which the non-labile metal concentration is ≥50% of the input (pre-column) metal concentration represents the concentration of ligands with a binding affinity greater than or equal to the values given above (assuming 1:1 molar stoichiometry). The concentration value corresponding with 50% non-lability may, however, be calculated by simple rearrangement of the receptor binding equation as follows:

$$Y = \frac{B\max \cdot X}{Kd + X}$$

$$\frac{X}{2} = \frac{B\max \cdot X}{Kd + X}$$

$$X = 2B\max - Kd$$

The concentration corresponding with 50% non-lability was therefore used as a basis for developing a method whereby Chelex method complexation data can be compared with WHAM VI. This method involved simulating each Chelex titration using WHAM VI, but where an additional ligand, representing the complexation capacity of the ligands with a binding affinity lower than the threshold values described above (which for the purposes of distinction have been described as relatively weak ligands), was included as a solution component in speciation modelling. The complexation capacity (CC) (i.e. the concentration applied as model input in molar units) for this additional ligand was determined as follows:

$$CC = \frac{DOC \times 2}{1000} \times \left(\left(\frac{N \times 0.55}{1} \right) + \left(\frac{N \times 0.42}{2} \right) + \left(\frac{N \times 0.03}{3} \right) \right) \times AF$$

Where:

- DOC is the measured DOC concentration in mg/L. This value is multiplied by 2 in order to convert to DOM (Thurman, 1985), and divided by 1000 to convert to grams (the unit required for model input).

- *N* represents the number of binding sites for each gram of DOM. The value for *N* is multiplied by the fraction of binding sites which form monodentate, bidentate, and tridentate complexes, and divided by the number of binding sites corresponding with each (i.e. the stoichiometric binding capacity of each binding site type). Values for *N* and the fraction of binding sites for each complex type were obtained from the WHAM VI user manual (Tipping, 2001) (Table 4.1).

- AF is the fraction considered active with regard to metal binding.

This additional ligand was assigned a binding affinity equivalent to the threshold values determined in the study by Bowles *et al.* (2006) (e.g. for nickel, the additional ligand was assigned a stability constant value of log K 7, for the free ion and first hydrolysis product). The metal fraction subsequently predicted by WHAM VI to be complexed would therefore represent the ligand concentration with an affinity which exceeded that of the additional ligand (i.e. strong ligands only), and which would be comparable with the ≥50% non-labile concentration values obtained from the Chelex titration data.

The WHAM VI ion binding parameters were adjusted from their default values to those given in Table 4.2, that have been demonstrated by Bryan *et al.* (2002), Van Leer *et al.* (2006) and Cheng *et al (2005)* to provide more accurate representations of speciation for copper, nickel and zinc, respectively.

Table 4.1WHAM VI fulvic acid parameters

Parameter	Fulvic acid
Molecular weight (Da)	1500
Molecular radius (nm)	0.8
Total site density (binding sites mmol/ gram)	7.2
Type A site density (mmol/gram)	4.8
Type B site density (mmol/gram)	2.4
% of binding sites which are monodentate	55%
% of binding sites which are bidentate	42%
% of binding sites which are tridentate	3%
pK_A	3.2
pK_B	9.4
ΔpK_A	3.3
ΔpK_B	4.9

Table 4.2 WHAM VI ion binding parameters

Parameter	Copper	Nickel	Zinc
Central log K_{MA}	2.10	1.75	1.80
DLK1	2.80	2.80	2.80
DLK2	2.34	1.57	1.28
Active Fraction	68%	40%	64%
Reference	Bryan *et al.* (2002)	Van Laer *et al.* (2006)	Cheng *et al.* (2005)

4.3 *Daphnia magna* toxicity

4.3.1 Method selection

The most direct method, by which the influence of sewage effluents on BLM forecast accuracy could be assessed, was to conduct toxicity assays in an effluent medium, and compare the observed results with those predicted by the relevant BLM. Indeed, this was the rationale for the study described in Chapter 5. For this test, the cladoceran *D. magna* was deemed most suitable test organism on the basis their parthenogenetic mode of reproduction (reduces genetic variability), short life cycle, since BLM accuracy has been extensively assessed for *D. magna* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere and Janssen, 2002; De Schamphelaere, Heijerick and Janssen, 2002; Santore *et al.*, 2001), and since the organism has been proved to be tolerant of wastewater effluents (van Veen *et al.*, 2002). An acute toxicity endpoint was deemed most suitable since effluents are known to contain other compounds that might influence chronic toxicity, the effects of which may have been impossible to disentangle from those attributable to toxicity from metals.

4.3.2 *Daphnia magna* acute copper toxicity tests

D. magna acute toxicity assays were conducted in accordance with OECD guidelines (OECD, 2004) using juvenile daphnids less than 24 hours old (neonates) at the start of each test. The test organisms were IRCHA clone type 5 (clone type A as per Baird *et al.*, 1991) and originated from the culture maintained by the School of Biological Sciences at the University of Reading, UK. In each assay, five concentrations and a control group were assessed. For each concentration and control, four groups of five neonates were used, so that each assay required 120 neonates. The exposure concentrations were arranged in a geometric series with a separation factor of 1.7 so that the maximum concentration produced 100% immobilisation and the lowest concentration produced no observable effect. In the study described in Chapter 5, the test concentrations were 80 µg/L, 136 µg/L, 231 µg/L, 392 µg/L and 664 µg/L, without consideration for the concentration in the effluent.

For each toxicity assay, at least 1,200 ml of 0.45 µm filtered effluent was utilised. The filtrate was apportioned into six 200 ml measures in 250 ml borosilicate glass beakers. One 200 ml measure was allocated for use as the control group and copper was added to the other five 200 ml measures to achieve the requisite concentrations. The spiked solutions were allowed to equilibrate for 1 hour. A 40 ml sample from each test concentration, including from the control, was collected so that the initial metal concentration could be quantified. The remaining 160 ml from each test concentration was then allocated into four equal portions of 40 ml each in 50 ml borosilicate glass beakers. Five neonates were then carefully added to each 50 ml test vessel using a plastic pipette. A glass cap was placed over each beaker in order to minimise evaporation and the risk of contamination. Immobilisation was recorded after 24 and 48 hours. Immobilisation, as opposed to lethality, was the recorded endpoint since mortality cannot be confirmed in the test organisms (i.e. an EC50 as opposed to LC50). Organisms were deemed immobilised if they were not able to swim within 15 seconds after gentle agitation of the test vessel. After recording immobilisation at 48 hours, the samples from each concentration were re-combined and a 40ml post test sample collected so that the final test concentration could be quantified. Care was taken to ensure that daphnids were excluded from this post test sample. These samples were preserved by adding 1 ml of Optima grade HNO_3.

Dissolved oxygen and pH were monitored in the 200 ml measure at the beginning of the test and in the highest test concentration and control at the end of each test. Measurements were recorded from lowest to highest concentration in order to avoid entrainment effects. Ambient air temperature was monitored throughout. The EC50 values were determined by the trimmed Spearman-Karber method (Hamilton, Russo and Thurston, 1977), using a USEPA statistical program (U.S. Environmental Protection Agency, 2006).

4.3.3 *Daphnia magna* genotype

The *D. magna* clone type selected for toxicity testing (IRCHA clone type 5 / clone A) differed from the clone type that had been used to develop the Refined BLM *D. magna* biotic ligand parameters (clone K6). Since *D. magna* sensitivity to metals has been observed to vary by clone type (Barata, Baird and Markich, 1998), the compatibility of clone type K6 biotic ligand parameters (i.e. the Refined BLM) for use with clone type A (the test organism used in the present study) was examined to ascertain the extent by which differences between observed and simulated EC50 values may be attributable to differences in *D. magna* genotype, rather than due to the influence of effluent specific characteristics.

The examination was conducted by comparing the EC50 values reported in studies which had utilised clone A daphnids with BLM simulated values. The studies for comparison were selected on the basis of those for which *D. magna* genotype and water chemistry characteristics had been reported (which could be used as BLM inputs), and where the influence of other potentially confounding factors had been minimised (e.g. variability in DOC complexation capacity).

D. magna copper toxicity data from studies by Guilhermino *et al.* (1997) and Baird *et al.* (1991), conducted in ASTM hard water (American Society for Testing and

Materials, 2007) (EC50 range 21 to 41 µg/L, mean = 31 µg/L), and the data from another by Barata, Baird and Markich (1998), conducted in APHA hard water (EC50 = 32 µg/L), were selected for the comparison. Since pH had not been reported for the studies conducted in ASTM water, BLM EC50 values were simulated across a pH range of 7.4 and 8.5 and with a nominal concentration of DOC (as fulvic acid) of between 101 and 175 µg/L included as a model input (the 95% confidence interval range that De Schamphelaere and Janssen (2002) had determined was present in bioassay test media).

The simulated EC50 values ranged between 30 µg/L and 50 µg/L (mean = 40 µg/L), which was similar to the values in the other studies, and suggested that the biotic ligand characterisation developed for clone type K6 was broadly compatible with the clone type employed in the toxicity assays, and therefore that differences between observed and predicted EC50 values were unlikely to be attributable to differences in genotype (the simulation data are provided in the chapter 4 data appendix in the spreadsheet 'DMagnaGenoSim.xls').

4.3.4 *Daphnia magna* culture set up and maintenance

4.3.4.1 Culturing Daphnia magna

The *D. magna* culture was maintained in accordance with the standard operating procedure (SOP) established by the Daphnia research group at the University of Reading (UoR) (Heckmann and Connon, 2007). Two cultures with 15 individual organisms each were maintained in two 2L borosilicate glass beakers in 1.2 L of culture medium. The culture medium was prepared from stock solutions and consisted of 195.87 mg/L calcium chloride, 82.20 mg/L magnesium sulphate, 64.8 mg/L sodium bicarbonate, and 5.8 mg/L potassium chloride, and inoculated with a 3 ml marinure nutritional supplement (Glenside Organics, UK) prepared in accordance with the UoR SOP. The culture medium was renewed at least once a week. Glass caps were placed over the beakers to minimise evaporation and to reduce the risk of contamination. All cultures were maintained at 20°C (±2°C) with a controlled photo period of 16 hours light and 8 hours darkness. New cultures were established every 2-3 weeks using 3rd, 4th, or 5th generation progeny. Daphnids were fed once daily (Monday to Friday) with 0.5 – 3 ml of a *Chlorella vulgaris* feedstock, supplemented with 0.5 ml of a 100 mg/L Baker's yeast solution. A record of neonate production is provided in appendix 3.

4.3.4.2 Culturing Chlorella vulgaris

The *C. vulgaris* culture was maintained in accordance with the SOP established by the Daphnia research group at UoR (Connon, 2007). *C. vulgaris* was obtained from Blades Biological (Endenbridge, UK) and from the University of Reading and maintained in a Bold Basal Medium (BBM) solution (Sigma, UK) in two small scale fermenters. The fermenters consisted of two 1,000 ml Drechsel bottles with Nalgene tube which was fit to the gas-inlet tube through which the air supply was delivered using an aquarium air pump. A 0.45 µm syringe filter unit was fit within the Nalgene tubing to prevent contaminants from being introduced and the air flow set to ensure the algal cells remained in suspension. All glassware, tubing and fittings were autoclaved prior to use in order to reduce the risk of microbial contamination.

Between 300 ml and 500 ml of algal suspension was removed from each fermenter on a weekly basis and replaced with fresh BBM. The harvested algal suspension was centrifuged at 3,000 rpm for 30 minutes and the supernatant discarded. The remaining concentrate was re-suspended in MQ until a 1:10 dilution had an optical density of 0.800 at 400 nm (determined using a Perkin Elmer Lambda 2 spectrophotometer) in order to ensure consistency in nutritional inputs. The re-suspended concentrate was refrigerated until required. A record of *C. vulgaris* production is provided in appendix 4.

4.4 Compliance Assessment

The compliance assessment was intended to identify Severn Trent Water WwTWs for which reductions in effluent discharge concentrations may be required as a consequence of BLM determined EQSs for copper and zinc, and also as a consequence of the 20 µg/L WFD EQS for nickel. The criteria for identifying these potentially ‘at risk’ WwTWs were those applied by the EA for assessing eligibility for a numeric discharge consent, which specifies quantifiable effluent quality criteria that must be achieved. The eligibility criteria are:

- WwTWs that are upstream of a location at which the EQS is exceeded
- WwTWs discharge that contribute to an increase in the downstream concentration by more than 10% of the EQS value.

For the purposes of comparison, compliance was also assessed in relation to DSD hardness based standards to examine the relative effect on compliance risk arising from the BLM and WFD EQS values. The WwTWs at risk of becoming eligible for numeric discharge consents were identified via a three stage process:

1. Determine river water EQS values
2. Compare river water metal concentrations with EQS values
3. Determine WwTWs contribution to downstream concentration

Stage 1: Determination of river water EQS values

The copper and zinc chronic toxicity BLMs were applied to estimate site specific EQS values for 1,636 EA river water monitoring locations within the STW operating area. A database containing environmental monitoring data for the EA water monitoring locations within the STW region was obtained which covered the period 01/09/2005 to 31/08/2006 and included the data required as BLM water chemistry inputs, as well as measured concentrations of Cu, Zn, and Ni. A review of the monitoring data, however, revealed, with the exception of pH, an absence of BLM data inputs, for calcium and DOC in particular. In order to apply the BLMs in was therefore necessary to estimate values for the missing BLM inputs. Estimates for missing input values were obtained from observed relationships between water chemistry characteristics. For example, the concentration of calcium for locations that did not contain calcium data, could be estimated from the correlation between pH and calcium for the locations at which both were recorded. The regression characteristics that have been applied are given in Table 4.3.

Table 4.3 Correlations between water chemistry characteristics

Determinand	Relationship	r^2	n	p
Ca (mg/L) from pH	$\text{Log}_{10}(\text{Ca}) = 0.74\text{pH} – 3.93$	0.59	250	<0.01
Alkalinity (as CaCO3 mg/L) from pH	$\text{Log}_{10}(\text{alk}) = 0.62\text{pH} – 2.63$	0.59	199	<0.01
Mg (mg/L) from Ca (mg/L)	Mg = 0.21Ca + 2.58	0.52	256	<0.01
SO4 (mg/L) from Ca (mg/L)	SO4 = 2.0Ca – 32.68	0.69	78	<0.01
Na(mg/L) from Cl (mg/L)	Na = 0.67Cl + 3.38	0.81	72	<0.01
K (mg/L) from Cl (mg/L)	K = 0.08Cl + 3.45	0.32	102	<0.01

The concentrations of dissolved organic carbon did not correlate with any of the other water chemistry characteristics, and only a limited number of data points were available. Therefore, the mean value for each of the river catchments within the Severn Trent Water region was applied to corresponding monitoring points for where DOC data was not available (Table 4.4).

Table 4.4 DOC concentrations by river catchment

River Catchment	Mean DOC (mg/L)	No. of locations with DOC data	No. of monitoring locations assessed for compliance
Ouse M	5.98	2	23
Ouse N	5.84[1]		4
Ouse S	5.7	3	110
Severn Mid	5.17	7	78
Severn N	5.41	62	367
Severn S	6.99	9	303
St. Helens	4.12	3	27
Teifi N	3.96	17	35
Trent	6.46	43	576
Weaver	4.25	8	75
Wye	2.69	1	38

[1] The average of the concentrations in ‘Ouse M’ and ‘Ouse S’.

Stage 2: Comparison of river water metal concentrations with EQS values

To determine the compliance status of each monitoring location and treatment works, it was necessary to compare the BLM EQS value with the location metal concentration. A review of the monitoring data for metals revealed much of the data was for the total metal concentration, and thus not directly comparable with BLM values, which are given as dissolved concentration values. Consequently, dissolved phase concentrations for locations at which the total metal concentration and suspended solids concentration were known were estimated by using a partition coefficient (Kp), as indicated by the formula below:

$$\text{Dissolved Metal } (\mu\text{g/L}) = \frac{\text{Total Metal } (\mu\text{g/L})}{\text{Suspended Solids (g/L)}} \times \frac{10^6}{\text{Kp}}$$

The partition coefficient values applied in this study were selected from a review of surface water partition coefficients by Allison and Allison (2005) in which values of (as Log Kp) 4.7, 4.6, and 5.1 for copper, nickel and zinc, respectively, had been found to be most representative for surface waters. In reality, however, a single value may not always provide a good representation since binding to particulate will depend on the particulate type and size.

Dissolved metal data was available for 76%, 58%, and 26% of the monitored locations for copper, zinc and nickel, respectively. EQS status was determined by face value comparison of the BLM determined EQS and the average of the measured concentration values.

Stage 3: Determination of WwTWs contribution to downstream concentration

A database containing effluent quality data for 705 Severn Trent Water WwTW was obtained from the EA covering the same period as the monitoring data. Additional data on Severn Trent Water wastewater treatment works characteristics, including location, treatment capacity (in population equivalents, abbreviated as p.e.), treatment type, dry weather discharge flow, and receiving water flow was obtained from Severn Trent Water.

The EA methodology whereby the relative contribution to the river water concentration from a discharge is determined using a *Mass Balance* computational model (Environment Agency, 2001). The Mass Balance model applies a Monte Carlo approach which simulates combinations of upstream and effluent flows and substance concentrations to predict the downstream concentration. The *Mass Balance* model requires as inputs values for the upstream river flow and standard deviation, substance concentration and standard deviation, and equivalent information for the effluent for which the model assumes to have a lognormal distribution. The model generates 1,000 predictions of the downstream substance concentration by simulating the mixing of the effluent and river water. The simulation is conducted by applying random instances of river and effluent substance and flow combinations where the final downstream substance concentration is estimated as the average of the one thousand predictions. The *Mass Balance* model was, however, difficult to apply for the assessment of 705 treatment works since the model required that the discharges for each treatment works to be modelled individually, which would require an excessive number of individual runs and therefore an MS Excel spreadsheet approximation of the *Mass Balance* model was developed which enabled greater flexibility and a high degree of automation. The spreadsheet utilised a custom-built visual basic macro to run the simulation. A comparison of results between the spreadsheet model and EA model demonstrated that the spreadsheet model predictions of downstream substance concentration were a good approximation of those produced using the EA model ($r > 0.999$, $n = 20$, $p \leq 0.001$) (data in appendix 5; the spreadsheet simulator and the EA *Mass Balance* models are also provided in the chapter 4 data appendix).

Since the dataset containing the river and effluent flows did not, however, provide a measure of their variability, the standard deviation for both the river and effluent flows was based on EA guidance, that is, the standard deviation of the flows is assumed to be to one third of the mean flow. Whereas some indication of the variability in substance concentration could be calculated from the monitoring data, this was only possible for a limited number of works, and consequently, the standard deviation was also based on Environment Agency guidance for where this information is not available, that is, that the standard deviation is assumed to be one half of the mean concentration. In addition, where upstream substance concentration data was not available, these values were also assumed in accordance with EA guidance. The guidance, however, is explicit in that it allows a differentiation on the basis of whether upstream inputs of the modelled substance may be considered likely or unlikely. For this assessment, upstream inputs of nickel were considered unlikely, and therefore, an upstream concentration of 10% of the downstream EQS value was assumed, whereas upstream inputs of copper and zinc were considered likely, and therefore an upstream concentration of 50% of the downstream EQS was assumed.

In order to determine the effect of effluent discharges on the metals concentrations in receiving waters, dissolved metal concentrations were required. However, metals data was generally limited to large treatment works (>20 thousand p.e., $n = 71$). In addition, the monitoring data was typically as a total, rather than as the dissolved concentration. As such, dissolved metal concentrations were estimated using partition coefficients from total metal and suspended solids data, as previously described. For works without sufficient monitoring data, the mean dissolved metal concentration for the works for which data were available was applied (copper = 8.59 µg/L, $n = 84$; nickel = 5.23 µg/L, $n = 84$; and zinc = 34.34 µg/L, $n = 84$).

5. AN EVALUATION OF BLM PREDICTIVE ACCURACY

The study described in this chapter was undertaken to determine whether the Biotic Ligand Model approach is appropriate for effluent impacted waters. This was useful since there is good evidence the EA will apply BLMs to determine water quality standards for waters which receive substantial wastewater effluent inputs. To test the suitability of the approach for use in this context an evaluation of the predictive accuracy of the Refined and HydroQual acute copper toxicity BLMs was conducted using a selection of wastewater effluents with *D. magna* as the test organism. The reasons underpinning the differences between the Refined and HydroQual BLM forecasts were also examined. This was of particular interest since the speciation parameter values for the Refined BLM parameters are also those applied in the chronic copper toxicity BLM, and consequently, reflect one of the differences in the BLM approach as applied within the EU and in the US.

A contribution to knowledge from this study has been the finding that, in general, BLMs did provide accurate predictions of copper toxicity in the wastewater effluents, and that the Refined acute copper toxicity BLM for *D. magna* provided the most accurate predictions. These findings therefore support the use of BLMs in waters receiving wastewater effluent inputs. The findings have been peer reviewed and presented in the journal *Environmental Toxicology and Chemistry* (Constantino *et al.*, 2011) (appendix 1).

The findings from the parameter sensitivity analysis also suggest a requirement for regulators and researchers to understand the limitations and implications of BLM thermodynamic parameters.

5.1. Experimental approach

5.1.1 *Daphnia magna* toxicity assay

Eight acute toxicity assays, with *D. magna* as the test organism, were conducted in effluents from four wastewater treatment works. Observed EC50 values were compared with those predicted by the Refined *D. magna* BLM proposed by De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) and the HydroQual BLM (HydroQual, 2007; Paquin, Santore and Mathew, 2007). Model forecasts were considered accurate if the forecast EC50 value was less than double or more than half the observed value (i.e. within a factor of two).

5.1.2 BLM comparison by simulation

The non-linear nature of speciation modelling is such that the effect of differences in BLM parameter values (Table 3.1) on model output cannot be easily determined, although differences between model forecasts may be broadly grouped in terms of those attributable to differences in:

1. Biotic ligand parameters (critical concentration values, the metal species considered toxic, and stability constant values of competing ions)
2. Speciation parameters (that arise as a consequence of differences in the organic and inorganic species formation constants)

To assess the relative significance of each parameter group type, 5,000 EC50 values for *D. magna* were simulated using the HydroQual and Refined BLMs, with matched water chemistry characteristics (pH, DOC, Ca, Mg, Na, Cl, SO4 and CO3) as model inputs (except that the DOC concentration applied as the input for the Refined BLM was 50% of that applied as the HydroQual BLM input). The values used as the water chemistry characteristics were generated at random although to ensure these were realistic these were related to the concentrations of calcium using a ratio range suggested by the EA (Table 5.1) (Peters, Merrington and Brown, 2009). Consequently, randomly generated calcium concentrations (covering the range 4 to 100 mg/L) were multiplied by random instances of the ratio values within the given range to obtain the BLM input water chemistry characteristics for sodium, potassium, magnesium, chloride and sulphate. Values for pH (range 6.0 to 9.0, increments of 0.1 pH units) and inorganic carbon (5 mg/L to 80 mg/L, increments of 1mg/L) were also assigned randomly, but were not correlated with calcium.

Table 5.1. Ratio ranges used to estimate major ion concentrations from Ca

Ion	Na	K	Mg	Cl	SO4
Ratio	0.77 to 3.00	0.06 to 0.24	0.13 to 0.52	0.64 to 2.46	0.34 to 1.36

The output from each BLM provides the dissolved copper concentration ($Cu_{EC50diss}$) associated with the toxicity endpoint (i.e. the EC50), and the concentration complexed with DOC ($Cu_{EC50DOC}$). The difference between each BLM toxicity forecast *i* may be calculated as follows:

$$Cu_{EC50diss\_refined\textit{i}} - Cu_{EC50diss\_HydroQual\textit{i}} = \Delta Cu_{EC50diss\textit{i}} \qquad i = 1, ..., 5000$$

The concentration of dissolved copper required to achieve the critical concentration (i.e. required for the toxic effect to occur) in the absence of DOC may be calculated for each BLM as follows, where x refers to either the Refined or HydroQual BLM:

$$Cu_{EC50diss\textit{xi}} - Cu_{EC50DOC\textit{xi}} = Cu_{EC50BL\textit{xi}} \qquad i = 1, ..., 5000$$

The differences between BLM forecasts that arise as a consequence of differences in biotic ligand parameterisation may therefore be quantified as follows:

$$Cu_{EC50BL\_Refined\textit{i}} - Cu_{EC50BL\_HydroQual\textit{i}} = \Delta Cu_{EC50BL\textit{i}} \quad i = 1, ..., 5000$$

Similarly, the differences between model forecasts that arise as a consequence of differences in the parameters governing speciation reactions may be quantified as follows:

$$Cu_{EC50DOC\textit{Refinedi}} - Cu_{EC50DOCHydro\textit{Quali}} = \Delta Cu_{EC50DOC\textit{i}} \qquad i = 1, ..., 5000$$

To identify the model inputs which most substantially influenced the differences between the model outputs, the difference values described above (i.e. $\Delta Cu_{EC50BL\textit{i}}$ and $\Delta Cu_{EC50DOC\textit{i}}$) were evaluated by Multiple Linear Regression (MLR) with pH, DOC and calcium as explanatory variables. The MLR was conducted using an Excel spreadsheet. The explanatory variables were constrained to pH, DOC and calcium since these have been reported to be the most important factors influencing the outputs for both models (De Schamphelaere and Janssen, 2002; Di Toro *et al.*, 2001). The extent by which the explanatory variables influenced the difference values was determined from a *t* statistic, calculated by dividing the explanatory variable coefficient (slope), by the standard error of the slope (Moore, 2010). The variables with the highest *t* statistic value (i.e. lowest *p* value) were considered the most significant in terms of explaining the differences between the model outputs, which were also indicative of the significance of the parameter group type.

The data from this assessment have been illustrated graphically in the form of contour plots which were produced using the programming language R (R Development Core Team, 2010). The R code is given in Appendix 2 and was adapted from code provided in R-Bloggers (2011).

5.2 Description of sampled effluents

Two effluent samples were collected from each of four WwTWs (designated A, B, C and D), at least one week apart, between November 2008 and February 2009. All WwTWs utilised the activated sludge process and received mostly domestic wastewater inputs. The activated sludge process treatment type is used widely and produces a relatively high quality effluent that is low in ammonia and chemical oxygen demand, which might otherwise exert some toxic influence. Two treatment works (A and B) were known to receive inputs from dairies and were expected to contain high concentrations of EDTA as a consequence of its widespread use in cleaning detergents that are used within the dairy industry (European Chemicals Bureau, 2004). Effluents from the other works (C and D) were expected to contain EDTA concentrations resulting from domestic inputs only. At two of the WwTW (A and D) iron dosing (FeCl) was being undertaken to remove phosphate.

5.3 Results

5.3.1 Water chemistry characteristics

The pH and DOC concentration of all the sampled effluents were broadly similar (7.5 to 8.1 and 7 mg/L to 9 mg/L, respectively) and within the range expected for these effluents and wastewater treatment type. The effluent water hardness ranged from hard to very hard (155 mg/L to 283 mg/L as CaCO3), which was also within the range expected for waters within the region. The calcium and magnesium ions associated with water hardness were expected to offer significant protective influence by competing with toxic metal species for binding at the biotic ligand. High concentrations of sodium and chloride detected in effluents B1 and B2 which occurred as a consequence of large natural salt deposits that are characteristic of the local geology. The concentrations of iron were also greatest in effluents B1 and B2, which were not dosed with iron to remove phosphate, and which occurred also as a consequence of local geological characteristics.

EDTA was the only synthetic chelating agent detected, and was found in all the effluents (41 µg/L to 547 µg/L). EDTA concentrations were greatest in the treatment works which received inputs from dairies (A and B, with 164 µg/L and 547 µg/L, respectively). The EDTA in the effluents from works which were treated with iron (A and D) was found to exist predominately (>79%) in the highly stable Fe^{3+}-EDTA complex form. Whereas the concentrations of iron were greatest in effluents B1 and B2, these did, however, contain a substantial concentration of labile EDTA. This suggested that the iron in effluents B1 and B2 was not in Fe^{3+} form. The concentrations of zinc in the effluents were within the range expected for treatment works which receive predominately domestic inputs (34 µg/L to 86 µg/L), as were the concentrations of copper (2 µg/L to 5 µg/L). The water chemistry characteristics are summarised in Table 5.2.

5.3.2 BLM Forecast accuracy

In the assays no immobilisation occurred in any of the controls, indicating that the effluents were not acutely toxic to *D. magna*. However, neither the HydroQual nor the Refined BLM predicted all EC50 values by within a factor of two (i.e. observed EC50 values were less than half or more than double the predicted EC50 value) (Figure 5.1). The HydroQual predicted the EC50 values by within a factor of 2.5, with two effluents exceeding the factor of two accuracy criteria (A1 and A2), whereas the Refined BLM predicted EC50 values by within a factor of 3.1, but with only one effluent (B2) exceeding the factor of two accuracy criteria. In accordance with the accuracy assessment criteria the Refined BLM was therefore the most accurate in that fewer effluents exceeded the factor of two accuracy criteria. Notably, the values predicted for effluents B1 and B2 were substantially greater than the values predicted for the other effluents (by more than a factor of two) and were also well in excess of the observed values for those effluents. The forecast EC50 values are given in Table 5.2 alongside the water chemistry characteristics. The data for each of the toxicity assays are given in appendix 6.

Table 5.2. Water chemistry characteristics and results of acute *Daphnia magna* toxicity tests

				Concentration of major ions (mg/L)					Effluent characteristics (µg/L)				Observed EC50 (µg/L)[c]	Forecast EC50 (µg/L)	
Site	pH	DOC[a]	IC[b]	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	Cu^{2+}	Zn^{2+}	Fe	EDTA[d]		Refined	HydroQual
A1	7.6	7.5 (±0.1)	44 (±1)	52 (±1)	6 (±1)	87 (±3)	15 (±1)	90	2 (±0)	43 (±3)	87 (±19)	547(±98) [0]	401 (356-452)	249	156
A2	7.5	7.0 (±0.1)	40 (±1)	54 (±1)	6 (±1)	95 (±2)	15 (±1)	95	5 (±0)	34 (±2)	98 (±14)	393 (±76) [0]	309 (272-352)	233	131
B1	8.0	9.0 (±0.2)	60 (±1)	69 (±1)	24 (±1)	1,586 (±23)	19 (±2)	2,347	3 (±0)	68 (±1)	131 (±30)	234 (±29) [120]	483 (402-581)	961	558
B2	8.1	8.2 (±0.1)	63 (±1)	71 (±1)	26 (±1)	1,452 (±26)	19 (±1)	2,173	2 (±0)	60 (±2)	109 (±36)	164 (±10) [81]	301 (254-355)	929	574
C1	7.9	7.9 (±0.1)	41 (±1)	54 (±1)	13 (±1)	136 (±2)	20 (±1)	135	3 (±0)	46 (±6)	26 (±24)	47 (±4) [0]	288 (245-337)	307	241
C2	7.9	7.9 (±0.1)	38 (±1)	52 (±1)	11 (±1)	118 (±1)	16 (±1)	121	4 (±0)	87 (±5)	69 (±23)	41 (±3) [16]	331 (308-357)	301	237
D1	7.9	7.9 (±0.1)	42 (±1)	40 (±1)	16 (±1)	71 (±1)	12 (±1)	96	4 (±0)	57 (±2)	36 (±5)	67 (±2) [0]	347 (307-392)	275	227
D2	7.9	8.3 (±0.1)	50 (±1)	44 (±1)	20 (±1)	87 (±7)	16 (±1)	100	3 (±0)	34 (±2)	53 (±16)	124 (±8) [0]	305 (280-332)	291	237

[a] DOC = dissolved organic carbon (mg/L)
[b] IC = inorganic carbon (mg/L)
[c] Numbers between brackets indicate 95% confidence interval range values except for EC50 values which are the 95% confidence interval limits.
[d] The values in square brackets indicate the EDTA concentration that was labile (i.e. not complexed with Fe^{3+}).

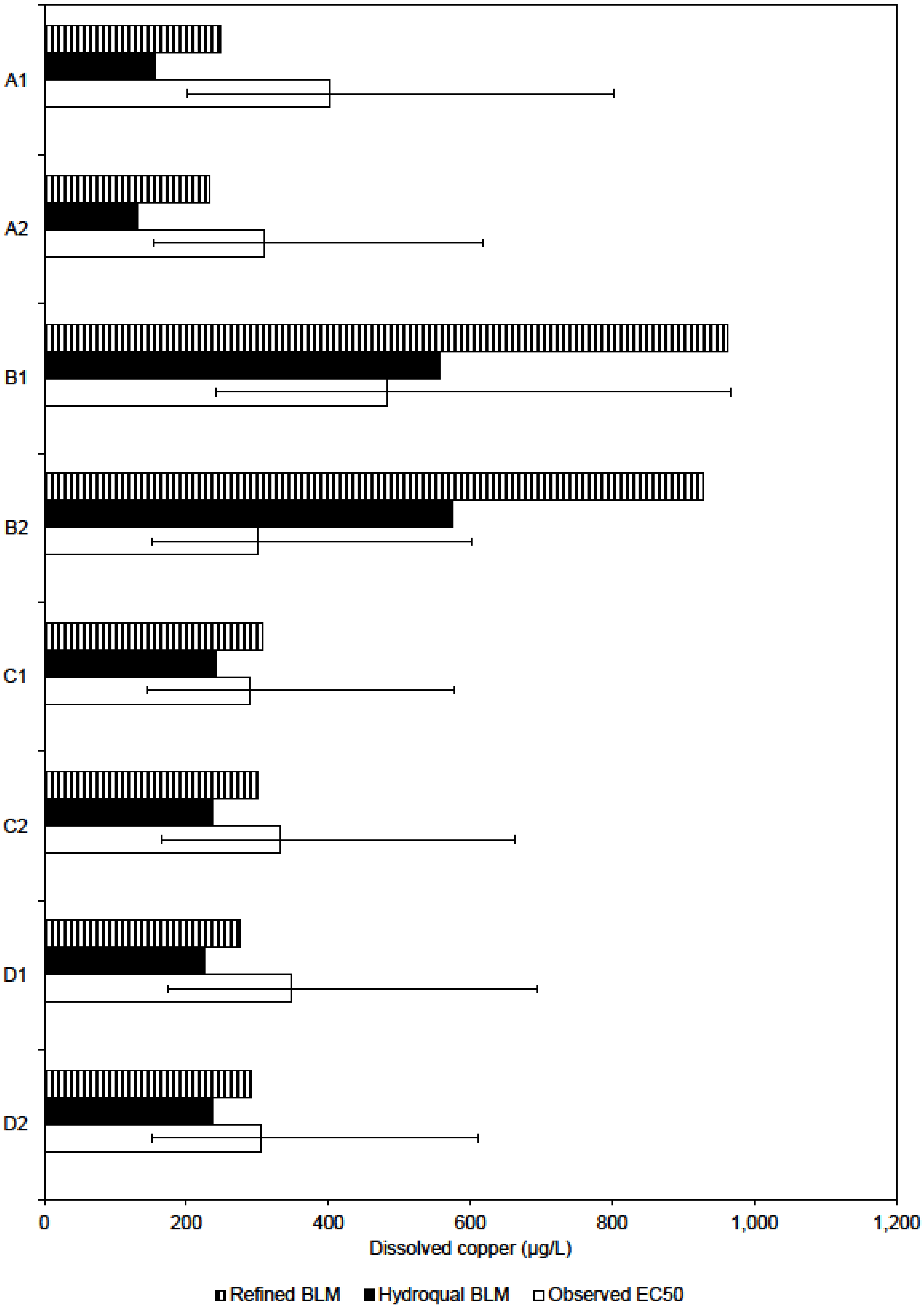

Figure 5.1 Observed EC50 in relation to BLM forecasts. Error bars represent the factor of two range so that the upper error bar value is twice the observed EC50 value, and the lower error bar value half the observed EC50 value.

5.3.3 Differences between Refined and HydroQual BLM acute copper toxicity forecasts

The EC50 values predicted by the Refined BLM were, in all instances, greater than those predicted by the HydroQual BLM (i.e. less conservative) and the effluents for which EC50 values were inaccurately predicted were not common to both models. The HydroQual BLM did not accurately predict EC50 values for effluents A1 and A2, whereas the Refined BLM did not accurately predict the EC50 value for effluent B2. The differences between the forecast values for effluents A1, A2, B1 and B2, were also large (93 µg/L, 103 µg/L, 403 µg/L, and 355 µg/L, respectively) in comparison with the differences between the forecasts for the other effluents (<67 µg/L). With regard to distinguishing characteristics, effluents A1 and A2 had the lowest concentrations of DOC, and the lowest pH values, whereas effluents B1 and B2 contained the most substantial concentrations of sodium and chloride.

5.3.4 BLM simulation

5.3.4.1 The effect of differences in biotic ligand parameterisation

The simulation results revealed that a greater concentration of copper was required to achieve the Refined BLM biotic ligand critical concentration value than for the HydroQual BLM biotic ligand critical concentration value, with the difference ranging between 1 µg/L and 178 µg/L (i.e. the values for $\Delta Cu_{EC50BLi}$). This suggests that in the absence of DOC, the EC50 values predicted by the Refined BLM will be greater than those predicted by the HydroQual BLM and consequently, that HydroQual BLM description of the *D. magna* biotic ligand is more sensitive. Whereas the $\Delta Cu_{EC50BLi}$ values increased in line with both pH and Ca, pH was of marginally greater significance to the differences between the forecasts (as determined by the t-ratio produced from the MLR, $\Delta Cu_{EC50BL} = 21.26pH + 0.62Ca - 154$, $r^2 = 0.76$, $p < 0.001$, pH t-ratio = 91.07, Ca t-ratio = 85.89). The results from this comparison are presented in the form of a surface contour plot (Figure 5.2) which demonstrates, in increments of 10µg/L, the difference between the Refined and HydroQual BLM forecasts attributable to differences in the biotic ligand parameterisation in relation to pH and the concentration of calcium. The plot shows that as pH and the concentration of calcium increase (from the blue region towards the orange in Figure 5.2) so does the difference in the concentration of dissolved copper required to achieve the Refined BLM biotic ligand critical concentration value relative to that required to achieve the HydroQual BLM biotic ligand critical concentration value.

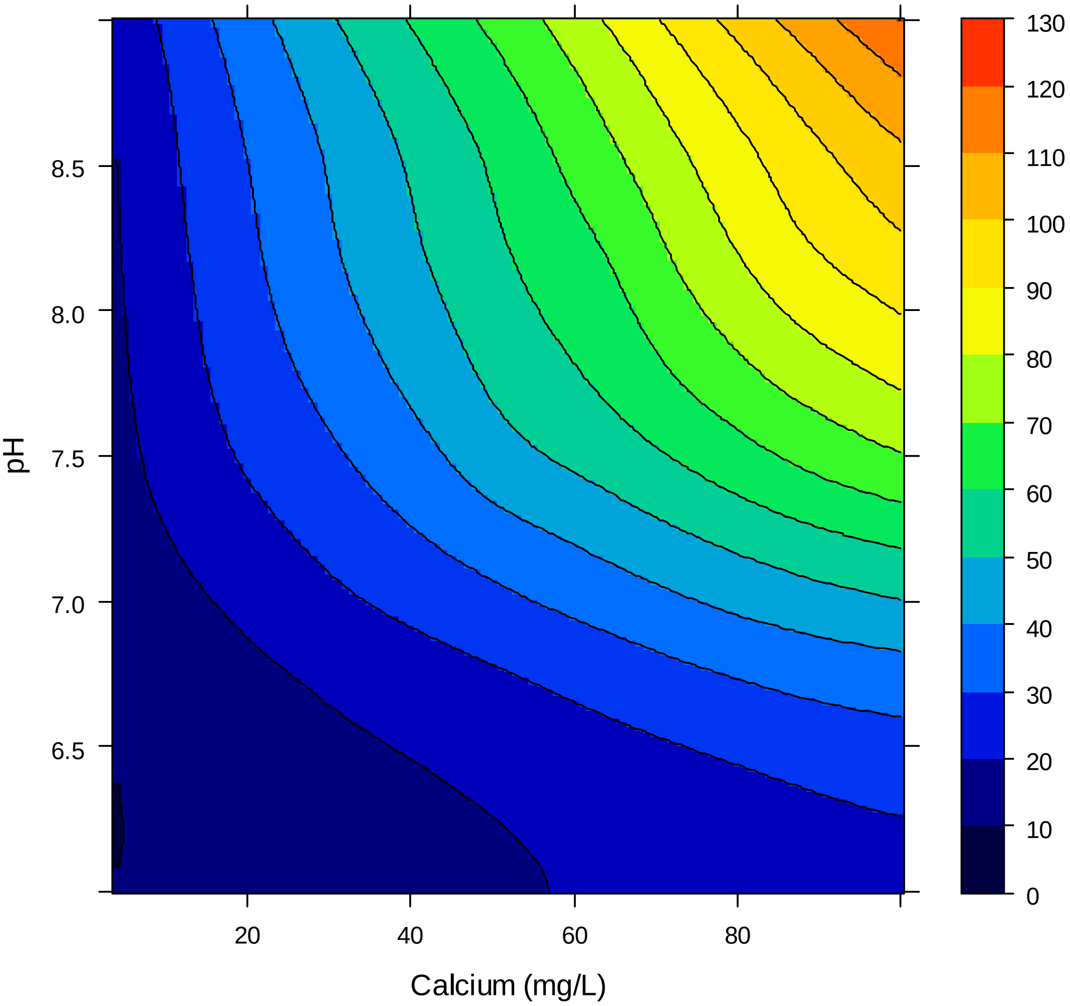

Figure 5.2 Contour plot demonstrating the differences between Refined and HydroQual *D. magna* biotic ligand in relation to the effects of calcium and pH. The colour key indicates in bands of 10µg/L increments, the difference between the Refined and HydroQual BLM forecasts attributable to differences in the *D. magna* biotic ligand parameterisation.

5.3.4.1 The effect of differences in speciation parameterisation

The simulation results revealed that the difference between the Refined and HydroQual BLM forecasts attributable to differences in the parameters governing speciation reactions ($\Delta Cu_{EC50DOCi}$) represented a broad difference range (>1,000 µg/L) that was substantially greater than differences which might occur as a consequence of differences in the biotic ligand parameterisation (1 µg/L to 178 µg/L) indicating that differences in the parameters governing speciation would be most significant in terms of explaining differences in model forecasts. The MLR also indicated pH to be the most significant in explaining these differences, followed by

DOC and calcium ($\Delta Cu_{EC50DOC\mathit{i}}$ = -165.52pH + 3.71DOC – 0.41Ca + 1265, $r^2 = 0.63$, $p < 0.001$, pH t-ratio = 89.3, DOC t-ratio = 16.3, Ca t-ratio = 7.2). The results of this comparison are also presented in the form of a contour plot (Figure 5.3). The figure shows that the differences between the Refined and HydroQual BLM forecasts attributable to differences in the parameters governing speciation reactions (i.e. $\Delta Cu_{EC50DOC\mathit{i}}$) in 100µg/L increments.

Figure 5.3 shows that, in general, below pH 8.0, the Refined BLM values were greater (i.e. $\Delta Cu_{EC50DOC\mathit{i}}$ is positive) than the equivalent HydroQual BLM values, with the difference increasing in accordance with the DOC concentration. This suggested that below pH 8 the Refined BLM toxicity forecasts were likely to be higher (i.e. less conservative) than those produced by the HydroQual BLM, by between 100 µg/L and 300 µg/L within pH 7-8 and a DOC concentration of 0-10 mg/L (notwithstanding the effect of the difference attributable to differences in the biotic ligand characterisation). Indeed, this is supported by the toxicity forecasts in the present study, for which effluent water chemistry characteristics were broadly within the range described, since the Refined BLM EC50 forecasts exceeded all the HydroQual BLM predicted values. The comparison, however, suggests that above pH 8.2, the EC50 values predicted by the HydroQual BLM would tend to be greater than values predicted by the Refined BLM (also notwithstanding the effect of the difference attributable to differences in the biotic ligand characterisation).

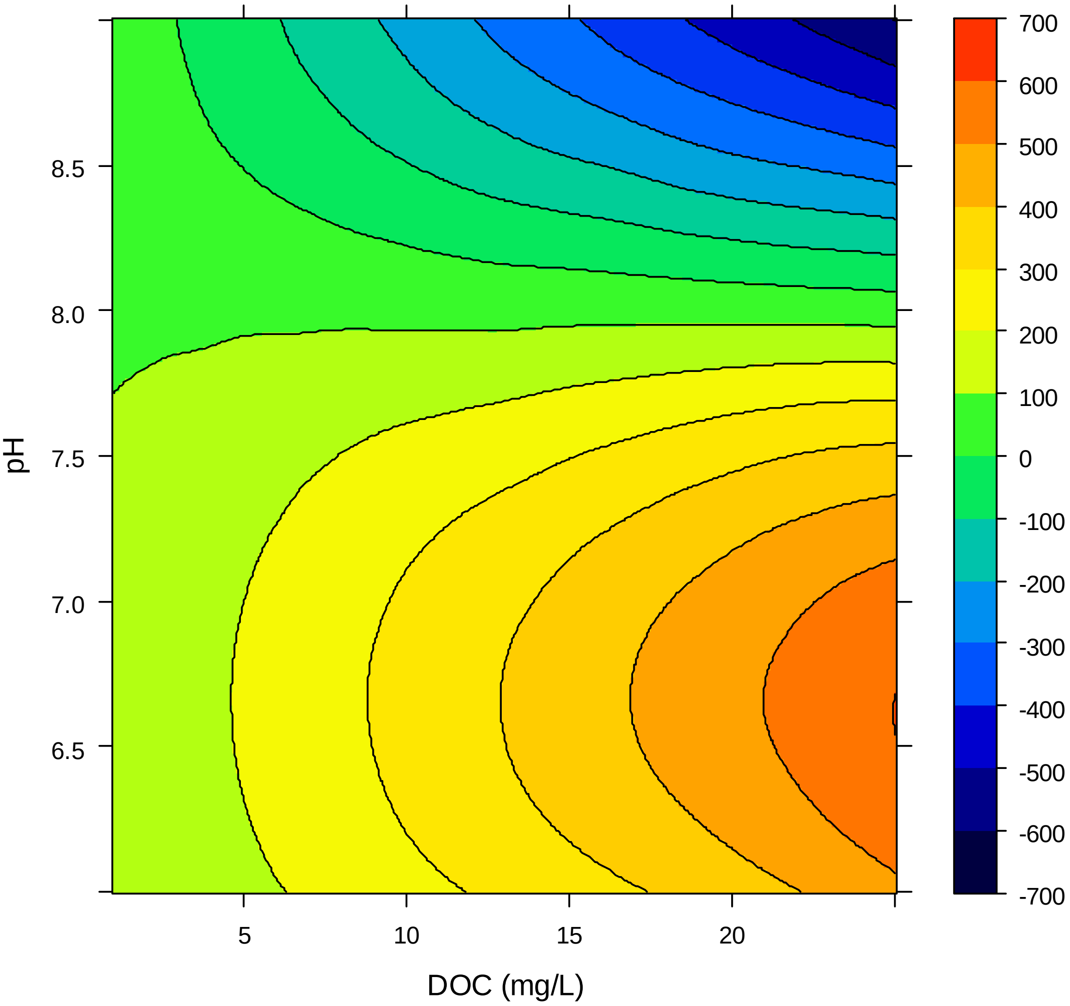

Figure 5.3 Contour plot demonstrating the effect differences between Refined and HydroQual *D. magna* BLM speciation parameters. The colour key indicates, in bands of 100 µg/L increments, the difference between the Refined and HydroQual BLM forecasts attributable to differences in the BLM speciation parameterisation.

5.4 Discussion

5.4.1 The significance of EDTA in influencing toxicity forecasts

The EDTA concentration was in all cases, below the provisional UK EDTA EQS (Murgatroyd *et al.*, 1997), and below the predicted no effect concentration (2.2 mg/L) determined in the EU risk assessment for EDTA (European Chemicals Bureau, 2004) and was therefore not expected to have contributed toxicity in any of the assays. Conversely, for effluents A1 and A2, which contained the highest concentrations of EDTA, the EDTA was found to be entirely in non-labile form and could therefore not have been expected to have exerted any protective influence by complexing copper ions. Indeed, the highest concentration of labile EDTA (effluent B1, 120 µg/L) was also potentially sufficient to complex only 26 µg/L of copper (assuming 1:1 molar stoichiometry) suggesting that EDTA was likely to have been of only minor significance in influencing copper toxicity. The high stability of the Fe^{3+}-EDTA complex (Log *K* 20.5, Stumm and Morgan (1996)) also suggests that, if initially present in this form, it would be unlikely to dissociate from the iron and complex the added copper, at least within the time frame of the toxicity assay. The slow rate of dissociation of Fe^{3+}-EDTA has also been noted in other studies which have demonstrated that the dissociation of this complex occurs over a time scale of many days (Xue, Sigg and Kari, 1995). This finding therefore contributes further evidence that consideration for the initial EDTA speciation is important for assessing the impact of EDTA on metal speciation and toxicity; a factor already well recognised in a number of studies which have investigated the extent by which EDTA mobilises metal through the wastewater treatment process (Christianne Ridge and Sedlak, 2004; Kari and Giger, 1996).

5.4.2 The significance of sodium in influencing toxicity forecasts

Whereas the EC50 forecasts for both the Refined and HydroQual BLMs suggested effluents B1 and B2 to be more toxic than might have been expected (i.e. the predicted EC50 values were substantially greater than the observed value), it was notable that the observed EC50 values were within a similar range to the values for the other effluents. It was also notable that the high concentrations of sodium and chloride in effluents B1 and B2 far exceeded the upper range concentration values used in the development of the Refined *D. magna* biotic ligand characterisation (347 mg/L and 553 mg/L, respectively) and those specified for use with the HydroQual BLM (236.9 mg/L and 279.7 mg/L, respectively). Whereas chloride is included as a model input only for its contribution to ionic strength, and is not expected to directly influence copper toxicity (Paquin, Santore and Mathew, 2007), sodium has a significant influence since it is assumed to compete with toxic metal species for binding at the biotic ligand. Waters with higher sodium concentrations would therefore be expected to be more protective which would, indeed, explain the high EC50 values predicted by both the Refined and HydroQual BLMs for B1 and B2.

In the development of the Refined BLM for *D. magna*, De Schamphelaere and Janssen (2002), however, commented that the biotic ligand stability constant value for sodium (log K 3.19) was high in comparison with values for calcium (log K 3.47) and magnesium (log K 3.58) considering their relative affinities for other synthetic chelating agents, and suggested that the actual protective influence offered by sodium may be attributable to some other direct physiological effect, rather than competition with the Cu^{2+} ion alone. This explanation is plausible since studies have shown environmental sodium to ‘assist’ sodium uptake (osmoregulation) in *D. magna*, and juvenile *D. magna* in particular (Bianchini and Wood, 2008). Consequently, the biotic ligand stability constant for sodium potentially incorporates the effect of both competitive binding and some other benefit, potentially as a consequence of the reduction in the energy burden associated with maintaining osmoregulation.

Following on from this, Bianchini and Wood (2008) also identified a Michaelis-Menton type relationship between environmental sodium and sodium uptake in juvenile *D. magna*, and found that the sodium uptake ‘assistance’ is saturable. Consequently, where environmental sodium concentrations exceed the organism’s uptake ability, only the true competitive binding influence is likely to exist, and for which a smaller stability constant value is likely to be representative. Therefore, in effluents B1 and B2, the concentrations of sodium may have exceeded the organism’s uptake ability, but with the effect that the biotic ligand stability constant for sodium overstated its protective influence, causing the BLMs to predict EC50 values that were much greater than was observed. This demonstrates that the existing stability constant for sodium may therefore result in inaccurate forecasts where sodium concentrations exceed the range within which the biotic ligand stability constant value had been determined (for example, in estuaries).

Relative inaccuracy of the Refined BLM forecasts compared to the HydroQual BLM forecasts for effluents B1 and B2 was plausibly as a consequence that the *D. magna* biotic ligand as characterised by the Refined BLM had been found in the assessment of parameter differences to be more sensitive to the water chemistry inputs, and potentially also, although to a lesser extent, since the Refined BLM biotic ligand stability constant value for sodium exceeds that of the HydroQual BLM (log K 3.19 vs. log K 3.0). These findings, however, further reinforce the need for consideration for the environmental context within which BLMs are applied and the limitations of their thermodynamic parameters.

5.4.3 The significance of BLM parameter differences

The substantial difference between the Refined and HydroQual BLM forecasts above and below pH 8, and that the difference was substantially more sensitive to pH than DOC (as per the MLR), suggested that the difference between refined and HydroQual BLM forecasts was unlikely to be attributable to the difference in pKH_{MA} alone (i.e. the model parameter which relates metal binding to proton binding values, 1.5 for the HydroQual BLM and 1.9 for the Refined BLM). Indeed, the significance of pH as an explanatory variable should also be viewed in the context of other differences between the models for which pH is significant, and in particular, the higher formation constant value for $CuHCO_3^+$ applied by the HydroQual BLM (log K 14.62 vs. 12.12 for the HydroQual and Refined BLMs, respectively). The higher formation constant value used in the HydroQual BLM implies that in the typical pH range (pH 6 to 9) the $CuHCO_3^+$ species will dominate copper speciation, but which subsequently reduces the significance of DOC in the HydroQual model forecasts. Therefore, whereas the Refined BLM pKH_{MA} value implies a lower affinity between Cu^{2+} and DOC, the lower formation constant value for $CuHCO_3^+$ in the Refined BLM also implies that more Cu^{2+} is available to bind with DOC. Indeed, this could also explain why the Refined BLM forecasts become more protective in comparison with HydroQual forecasts as DOC increases (Figure 5.3). The threshold effect at pH 8 appears to relate to an aspect of carbonate speciation. Above pH 8.0, as carbonate becomes important in influencing copper speciation (in both models), the Refined BLM EC50 forecasts become progressively more conservative (i.e. predicts lower EC50 values) since, unlike the HydroQual model, the Refined BLM considers $CuCO_3$ as a toxic metal species.

Given the other uncertainties in the BLM approach (DOC complexation in particular) it was interesting that differences between the model forecasts might be attributable to differences in carbonate speciation parameters. It is notable, however, that the potential significance of the $CuHCO3^+$ formation constant applied in the HydroQual model was also clearly identified almost a decade ago in a study by Bryan, Tipping and Hamilton-Taylor (2002), who pointed out that the value was originally based on a much earlier estimate by Mattigod and Sposito (1979) and commented that the value was probably too high. As a consequence it is possible that, the HydroQual BLM would, in a regulatory context, provide a precautionary assessment of risk where applied in water with low pH and high DOC. This, however, further demonstrates the significance of BLM parameterisation on model forecasts.

5.5 Conclusions

This study has demonstrated that the Refined BLM predicted copper toxicity to *D. magna* in the wastewater effluents by within a factor of two, for seven out of eight effluents, compared to six out of eight for the HydroQual model and that differences between the forecasts related not only to differences in biotic ligand and DOC parameterisation, but also to differences in the treatment of carbonate speciation. This study has also demonstrated that the existing biotic ligand stability constant for sodium in both the Refined and HydroQual BLMs may be an inaccurate approximation of the mechanisms that govern the influence of sodium, in particular, where sodium concentrations exceed the concentration range within which the biotic ligand stability constant value had been determined. In addition, whereas EDTA was prevalent in the assessed wastewater effluents, its speciation suggested that it occurred predominantly in an inert form that was not relevant at acutely toxic concentrations. These findings contribute knowledge supporting the use of BLMs in waters impacted by wastewater effluents, but reinforce the need for regulators and researcher to understand the limitations and implications of their thermodynamic parameters.

6. A MODIFIED ACUTE COPPER TOXICITY BLM FOR *DAPHNIA MAGNA*

The study described in this chapter was undertaken to determine whether a Modified BLM, developed in the course of this study, which used WHAM VI for speciation calculations and included effluent water chemistry characteristics as model inputs, could produce *D. magna* toxicity forecasts that were more accurate than the forecasts produced by either the HydroQual or Refined BLMs. This was intended to investigate the extent by which the environmental relevance of compliance criteria determined using the BLM approach could be improved.

The findings from this study were that, whereas the Modified BLM did produce toxicity forecasts which were more accurate than either the HydroQual or Refined BLMs, this was dependent on optimising the DOC fraction considered active with regard to ion binding, rather than the inclusion of effluent water chemistry characteristics in the speciation modelling. A contribution to knowledge from this study has been a set of active fraction values for sewage effluent derived DOC that may be used with the HydroQual, Refined and Modified BLMs for predicting copper toxicity in sewage effluents. These findings have been peer reviewed and were presented alongside those described in Chapter 5 in the journal *Environmental Toxicology and Chemistry* (Constantino *et al.*, 2011).

6.1 Experimental approach

The Modified BLM was created as part of this research using the WHAM VI model for speciation calculations, and which included effluent specific water chemistry characteristics as model inputs. The accuracy of the Modified BLM forecasts was assessed by using the model to produce forecasts for the assays described in Chapter 5. These forecasts were compared with the observed toxicity values and the equivalent Refined and HydroQual BLM forecasts. The Modified BLM was used to produce two forecasts in order to determine the significance of the effluent characteristics to forecast accuracy; one which used the same inputs as the Refined and HydroQual BLMs, and another which included the additional effluent characteristics as inputs which the modifications allowed for.

Since the actual DOC active fraction for these samples was unknown, but known to be variable (Bryan, Tipping and Hamilton-Taylor, 2002), and because Sarathy and Allen (2005) had demonstrated that DOC derived from sewage effluent contained a greater concentration of complexing ligands compared with DOC derived from natural sources, an additional assessment was conducted whereby the DOC active fraction was treated as an adjustable parameter and optimised to the value which resulted in inevitable perfect agreement between observed and predicted toxicity. This optimisation was similar to that conducted by Bryan, Tipping and Hamilton-Taylor (2002) and Cheng *et al.* (2005) in their respective assessments of copper and zinc binding to natural organic matter using WHAM VI, except that in the present study, *D. magna* response was used as a type of speciation probe (i.e. to measure of free ion activity).

6.2 Description of the Modified acute copper toxicity BLM

The Modified BLM was constructed by utilising the Refined BLM *D. magna* biotic ligand characterisation (Table 3.1) within the WHAM VI geochemical speciation model. Whereas the *D. magna* biotic ligand may easily be included as an additional ligand within WHAM VI, the model lacks the computational functionality to iteratively apply dissolved phase copper concentration values to determine the concentration that results in the critical accumulation of copper at the biotic ligand (as the HydroQual BLM). WHAM VI is, however, able to accept free ion activity as an input, and the functionality to calculate, with consideration for the influence of the other specified water chemistry characteristics, the dissolved phase concentration required for the specified ion activity value to occur. Consequently, the equilibrium equation developed by De Schamphelaere *et al*. (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) (Chapter 3.2.1**)** was used as the basis for calculating the free ion activity input value required to immobilise 50% of the test organisms (EC50Cu^{2+}), which was subsequently used as the WHAM VI input to calculate the corresponding dissolved phase copper concentration (i.e. the predicted EC50 value). The inorganic and biotic ligand complex formation constants were as provided for the Refined BLM in Table 3.1.

6.3 Modified BLM inputs

The standard BLM water chemistry characteristics were applied as model inputs (ph, DOC, calcium, magnesium, sodium, potassium, sulphate, and chloride), with the following additions and amendments:

6.3.1 Copper

As already described, copper ion activity required to immobilise 50% of the daphnids (EC50Cu^{2+}) was applied as the Modified BLM (WHAM VI) input value, from which the corresponding dissolved phase EC50 copper concentration was calculated. The Cu^{2+} activity value applied as the model input was calculated using the equilibrium equation described in Chapter 3.2.1, using an Excel spreadsheet, where the ion activity values for the equation inputs were calculated with WHAM VI. Since the addition of the EC50Cu^{2+} value as a model input also influenced the solution ionic strength, and thus the ion activities of other solution components, this process was repeated until the target EC50Cu^{2+} stabilised. However, since the copper concentration had only a minor influence on ionic strength, the target EC50Cu^{2+} value stabilised within two or three repetitions of these steps.

6.3.2 Sodium

As a consequence that the biotic ligand stability constant for sodium was suspected of inaccurately representing the influence of sodium in two of the effluents (B1 and B2, Chapter 5.5.2), the model input concentrations for sodium (and chloride) for these effluents were limited to the upper threshold values specified for the HydroQual model (236.9 mg/L and 279.7 mg/L, respectively). Whereas the HydroQual model upper threshold values were lower than the highest concentrations that were used in the development of the Refined *D. magna* BLM (347 mg/L and 553 mg/L, respectively), it was preferable to apply the HydroQual model upper threshold values in order to ensure that the input values would be appropriate for the purposes of model comparison. More elaborate schemes could have been tried to more accurately take account of the influence of sodium, for example, by assuming a multiple-site binding model for sodium, with differing binding affinities and saturation thresholds, however, in the absence of experimental evidence upon which to base such a characterisation, the simplest approach was considered to be the most appropriate, that is, to apply an upper threshold input value.

6.3.3 Dissolved organic carbon

Although De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) assumed 50% of DOC to be active for the Refined BLM, the evaluation of WHAM VI by Bryan, Tipping and Hamilton-Taylor (2002) found that, on average, copper speciation was most accurately described by WHAM VI with 68% of DOC considered to behave as active fulvic acid. Whereas WHAM VI allows DOC to be specified as either humic or fulvic acid, the active fraction values provided in the scientific literature have been determined where DOC is considered to behave as fulvic acid only. The extent by which the 50% value applied by De Schamphelaere *et al.* (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002) is different from the 68% value suggested by Bryan, Tipping and Hamilton-Taylor (2002) was not evident, since the effect of this difference is also dependent on other model differences (for example, as described in Chapter 5.3.3). However, since the Modified BLM applied WHAM VI, the 68% active fraction value suggested by Bryan, Tipping and Hamilton-Taylor (2002) was applied as the default value for this study, and DOC was considered to be fulvic acid only.

In addition, whereas the HydroQual and Refined BLMs required DOC as an input (in mg/L) WHAM VI requires dissolved organic matter (DOM) as an input (rather than DOC), therefore, the input values were two times the DOC concentration, multiplied by the active fraction value. The factor of two converts DOC to DOM by assuming carbon to comprise 50% of organic matter by weight (Thurman, 1985; Cheng *et al.*, 2005).

6.3.4 Effluent water chemistry characteristics

6.3.4.1 EDTA

The total measured concentration of EDTA was used as the model input value, regardless of the form in which it was detected. This was useful since it allowed consideration of the maximum potential influence of EDTA. Stability constants for EDTA and relevant metal cations (and their hydroxide products) which were used to regulate the influence of EDTA were obtained from Stumm and Morgan (1996) and are presented in Table 6.1.

Table 6.1 Thermodynamic parameters governing EDTA speciation

Reaction components					Product	Log *K*
$EDTA^{4-}$+	H^{+}			↔	$H(EDTA)^{3-}$	11.12
$EDTA^{4-}$+	$2H^{+}$			↔	$H2(EDTA)^{2-}$	17.8
$EDTA^{4-}$+	$3H^{+}$			↔	$H3(EDTA)^{-}$	21.04
$EDTA^{4-}$+	$4H^{+}$			↔	$H4(EDTA)^{0}$	23.76
$EDTA^{4-}$+	$5H^{+}$			↔	$H5(EDTA)^{+}$	28.34
$EDTA^{4-}$+	Na^{+}			↔	$Na(EDTA)^{3-}$	2.5
$EDTA^{4-}$+	K^{+}			↔	$K(EDTA)3^{-}$	1.7
$EDTA^{4-}$+	Ca^{2+}			↔	$Ca(EDTA)^{2-}$	12.4
$EDTA^{4-}$+	Ca^{2+}	+	H^{+}	↔	$HCa(EDTA)^{-}$	16.0
$EDTA^{4-}$+	Mg^{2+}			↔	$Mg(EDTA)^{2-}$	10.6
$EDTA^{4-}$+	Mg^{2+}	+	H^{+}	↔	$HMg(EDTA)^{-}$	15.1
$EDTA^{4-}$+	Fe^{3+}			↔	$Fe(EDTA)^{-}$	27.7
$EDTA^{4-}$+	Fe^{3+}	+	H^{+}	↔	$HFe(EDTA)^{0}$	29.2
$EDTA^{4-}$+	Fe^{3+}	+	OH^{-}	↔	$FeOH(EDTA)^{2-}$	33.8
$EDTA^{4-}$+	Fe^{3+}	+	$2OH^{-}$	↔	$Fe(OH)2(EDTA)^{3-}$	37.7
$EDTA^{4-}$+	Cu^{2+}			↔	$Cu(EDTA)^{2-}$	20.5
$EDTA^{4-}$+	Cu^{2+}	+	H^{+}	↔	$HCu(EDTA)^{-}$	23.9
$EDTA^{4-}$+	Cu^{2+}	+	OH^{-}	↔	$CuOH(EDTA)^{3-}$	22.6
$EDTA^{4-}$+	Zn^{2+}			↔	$Zn(EDTA)^{2-}$	18.3
$EDTA^{4-}$+	Zn^{2+}	+	H^{+}	↔	$HZn(EDTA)^{-}$	21.7
$EDTA^{4-}$+	Zn^{2+}	+	OH^{-}	↔	$ZnOH(EDTA)^{3-}$	19.9

6.3.4.2 Iron

It was desirable to include iron as a model input to reflect its ability to occupy high affinity binding sites on DOC (Tipping *et al.*, 2002), as well as its influence on EDTA speciation. Iron speciation is, however, complex, since the proportion of dissolved phase iron existing in Fe^{3+} form would, ordinarily, be extremely low due to hydrolysis and precipitation as colloidal hydroxides which occurs in the typical pH range (6 to 9). The presence of EDTA might, however, alter iron speciation significantly in that a larger proportion may exist in Fe^{3+} form as part of an Fe^{3+}-EDTA complex. Iron speciation was therefore simulated by specifying the WHAM VI input as the Fe^{3+} ion activity (i.e. that remains after the influence of other water chemistry characteristics has been taken into account - similar to what has been described for copper). The Fe^{3+} ion activity used as the model input was determined using the method suggested by Lofts, Tipping and Hamilton-Taylor (2008) which takes account of temperature and pH solubility and is represented by the equation below:

$$\mathrm{Log}\alpha_{\mathrm{Fe}^{3+}} = 2.93 - 2.70\mathrm{pH} + \frac{\Delta H^{\circ}}{2.30R}\left(\frac{1}{283} - \frac{1}{T}\right)$$

Where T represents temperature, $\Delta H°$ the standard enthalpy change for solubility equilibrium with $Fe(OH)_3$ (-24.37kcal/mol), and R the log solubility product of $Fe(OH)_3$ (for these calculations a value of 2 was applied as R in accordance with the recommendation by Lofts, Tipping and Hamilton-Taylor,(2008)). The default WHAM VI thermodynamic parameters for Fe^{3+} were applied in speciation modelling, except those controlling reactions with EDTA, which were as described previously.

6.3.4.3 Zinc

The measured dissolved zinc concentration was included as a model input due to its relative abundance in wastewater effluents, and strong affinity for EDTA. Zinc was not included in the calculation for binding at the biotic ligand, or assumed to contribute towards toxicity since the concentrations of zinc in the effluents were well below the range at which concentration of zinc were likely to be acutely toxic to *D. magna* (>1,000 µg/L) (De Schamphelaere, Lofts and Janssen, 2005).

6.4 Dissolved organic carbon active fraction optimisation

The optimum active fraction value for each BLM-effluent assay combination was determined by simulating EC50 forecasts for each assay with each BLM over a range of DOC active fraction values. Since all other model inputs are held constant the relationship between the simulated EC50 values and the active fraction is expected to be linear. The active fraction which would result in perfect agreement between simulated and observed EC50 value may therefore be obtained from the regression characteristics which represent the relationship between the EC50 and the active fraction. These calculations were performed using an Excel spreadsheet (included in the data appendix for chapter 6). The optimised active fraction values were subsequently confirmed by applying the value estimated from the regression as the model input and comparing the value with the observed EC50 value.

6.5 Results

6.5.1 Modified BLM forecast accuracy

The Modified BLM which included the effluent characteristics as inputs (denoted with the '‡' symbol in Figure 6.1) and applied the default DOC active fraction value (68%) predicted all EC50 values by within a factor of 1.7 of the observed value. The predicted EC50 values were lower than the observed values in 7 out of the eight assays (i.e. excepting B2), suggesting the effluents were generally less toxic than predicted. The Modified BLM which applied standard inputs (i.e. did not include effluent characteristics as inputs) and default DOC active fraction value predicted the EC50 values by within a factor two for only 7 out of the 8 toxicity assays (Figure 6.1) and all values by within a factor of 2.1 of the observed value. This suggested that the inclusion of effluent characteristics in speciation modelling could indeed improve the accuracy of the toxicity forecasts. It was also notable that the differences between the two Modified BLM forecasts were greatest for effluents A1 and A2 which also contained the highest concentrations of EDTA (Table 5.2). Whereas the Modified BLM which included effluent characteristics as inputs predicted all EC50 values by within a factor of 1.7, which was an improvement on the values reported for the study in Chapter 5, this was as a consequence of adjusting the sodium concentration input values for effluents B1 and B2. As a consequence of this adjustment, the forecast accuracy for the Refined BLM also improved substantially so that all EC50 values were predicted by within a factor of 1.6 of the observed value. The HydroQual model predicted only 6 forecasts within a factor of two of the observed EC50 value and therefore provided the least accurate forecasts. In accordance with the accuracy assessment criteria, the Refined BLM was therefore considered to provide the most accurate toxicity forecasts.

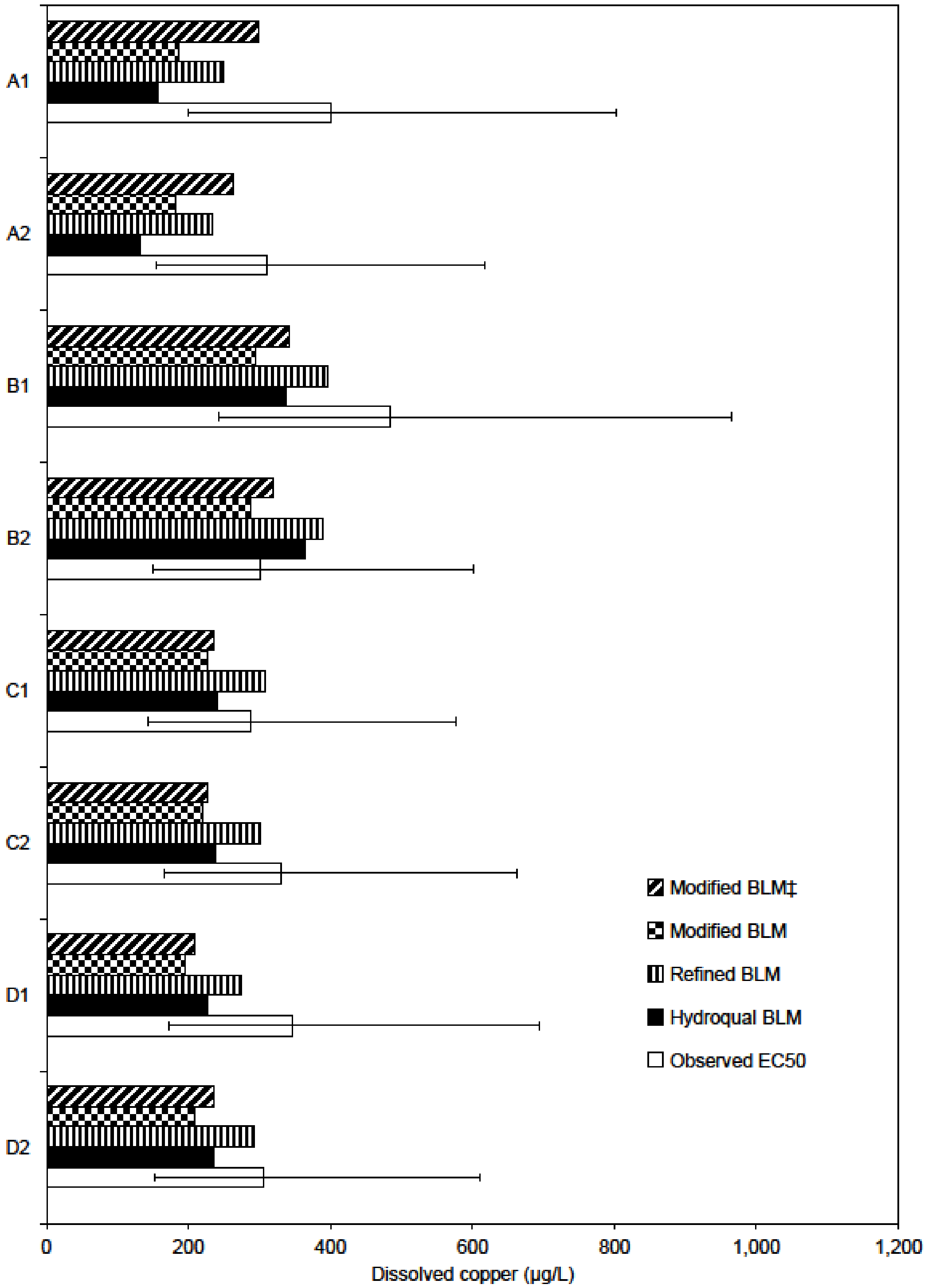

Figure 6.1 Observed EC50 in relation to BLM forecasts. Error bars represent the factor of two range so that the upper bar is twice the observed EC50 value, and the lower bar half the observed EC50 value. The '‡' symbol indicates the modified BLM in which effluent water chemistry characteristics were applied as model inputs.

6.5.2 Optimisation of dissolved organic carbon active fraction

The regression characteristics determined from simulating EC50 values for a range of active fraction values are given in Table 6.3, where y represents the EC50 (in µg/L) and x the active fraction value. The high coefficient of determination (r^2) confirmed that where all other water chemistry characteristics are held constant, the BLM predicted EC50 value increases directly in proportion with the DOC fraction considered active. Since for these effluents the actual EC50 values were known, the optimised active fraction values for these effluents could be determined by rearranging the regression characteristics given in Table 6.3 as follows:

$$AF\% = \frac{y - b}{m}$$

Where AF% represents the active fraction value for which perfect agreement between the simulated and observed EC50 is expected to occur, b the intercept and m the slope of the regression. The calculated optimised active fraction values are given in Table 6.4. Where these were applied to determine the DOC concentration used as the BLM input, the EC50 values were predicted by within 1% of the observed EC50 values, thereby confirming the suitability of this approach for estimating optimised active fraction values (the raw data are available in the data appendix for chapter 6, DOC_optimisation.xlsx).

With the exception of effluent B2, the optimised active fraction values exceeded the literature values, suggesting the effluent DOC incorporated a ligand intensity greater than was assumed in the standardised description of DOC complexation characteristics. Similarly, the mean active fraction values also exceeded the literature values. An active fraction value of greater than 100% (for the HydroQual and Modified BLMs) did not present any conceptual difficulty since this was indicative that the sample DOC contained a ligand intensity greater than was assumed in the model description of DOC ion binding capacity.

Where the mean optimised active fraction values were applied in predictions of toxicity (Figure 6.2), the Modified BLM which included effluent characteristics as inputs provided the most accurate forecasts, and predicted all EC50 values by within a factor of 1.35. Where the effluent characteristics were not included as inputs for the Modified BLM, the predictive accuracy was, however, only slightly worse in that all EC50 values were predicted by within a factor of 1.39. For the Refined and HydroQual BLMs, the optimised active fraction values resulted in predictions that were within a factor of 1.5 and 1.9, respectively, of the observed EC50 values. This demonstrated that the active fraction value was relatively more important to model accuracy than the inclusion of effluent water chemistry characteristics (e.g. EDTA, Fe^{3+}, and Zn^{2+}). This also demonstrated that the default DOC active fraction value may substantially underestimate the concentration of complexing ligands in sewage effluents, with the result that BLM forecasts may be over-precautionary.

Table 6.2 Regression parameters used to estimate optimum active fraction values

	HydroQual BLM			Refined BLM			Modified BLM			Modified BLM‡		
Site	Regression	r^2	*n*	Regression	r^2	*n*	Regression	r^2	*n*	Regression	r^2	*n*
A1	y = 152.5x + 4.3	1.0	3	y = 413.9x + 43.5	1.0	3	y = 212.2x +41	1.0	3	y = 211.9x +154.1	1.0	3
A2	y = 127.8x + 3.8	1.0	3	y = 385.1x + 42.2	1.0	3	y = 207x +39.9	1.0	3	y = 206.7x +122.2	1.0	3
B1	y = 317.5x + 20.5	1.0	3	y = 567.7x + 114.2	1.0	3	y = 280.2x +103.7	1.0	3	y = 281x +150.8	1.0	3
B2	y = 338.1x + 28.2	1.0	3	y = 532.3x + 124.4	1.0	3	y = 255.3x +112.8	1.0	3	y = 254.9x +145.7	1.0	3
C1	y = 235.9x + 7.0	1.0	3	y = 485.6x + 65.9	1.0	3	y = 243.2x +61.4	1.0	3	y = 242.7x +71	1.0	3
C2	y = 232.6x + 5.5	1.0	3	y = 486.2x + 59.4	1.0	3	y = 242x +55.7	1.0	3	y = 240.9x +63.4	1.0	3
D1	y = 222.1x + 5.7	1.0	3	y = 458.3x + 47.5	1.0	3	y = 221.9x +44.6	1.0	3	y = 221.1x +57.5	1.0	3
D2	y = 229.7 + 7.8	1.0	3	y = 475.5x + 55.1	1.0	3	y = 231.1x +51.6	1.0	3	y = 230.9x +77.2	1.0	3

Modified BLM‡ is the Modified BLM which included effluent chemical characteristics in speciation modelling
Where x is the active fraction value and y the EC50 value

Table 6.3 The optimised DOC active fraction values for the HydroQual, Refined and Modified BLMs

Site	HydroQual BLM	Refined BLM	Modified BLM	Modified BLM‡
A1	260%	86%	170%	117%
A2	239%	69%	130%	90%
B1	146%	65%	135%	118%
B2	81%	33%	74%	61%
C1	120%	46%	93%	89%
C2	140%	56%	114%	111%
D1	154%	65%	136%	131%
D2	129%	53%	110%	99%
Mean	158%	59%	120%	102%
S.D	61%	16%	29%	22%
Literature	100%	50%	68%	68%

‡ Modified BLM including effluent water chemistry characteristics as inputs

[a] Indicates the best fit active DOC fraction determined by minimising the sum of squared forecast residuals.

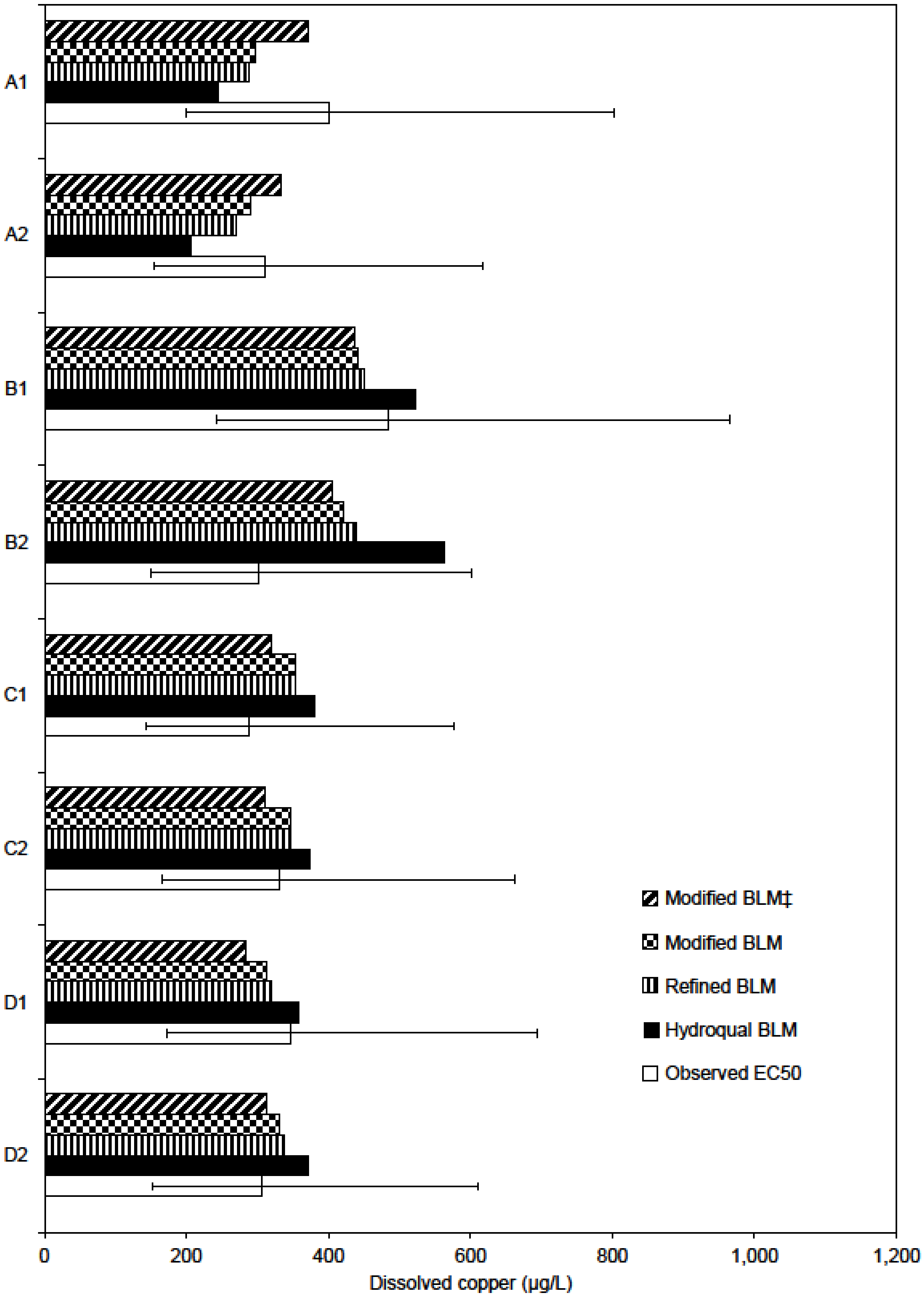

Figure 6.2 Observed EC50 in relation to BLM forecasts using the mean optimised active fraction value. Error bars represent the factor of two range so that the upper bar is twice the observed EC50 value, and the lower bar half the observed EC50 value.

6.6 Discussion

The active fraction values determined for the effluents in the present study suggested that these effluents contained a greater concentration of copper complexing ligands per milligram of DOC compared with organic matter derived from natural sources. This was in general agreement with the findings from the study by Sarathy and Allen (2005) who also proposed that effluent derived organic matter should be considered as an additional ligand category in speciation modelling. However, the substantial improvement in forecast accuracy that arose from adjusting the active fraction value, in relation to that which occurred by including additional components in speciation modelling (e.g. EDTA), also suggested that adjusting the DOC active fraction could be more useful to reflect differences in complexation characteristics than an approach which aims to improve forecast accuracy by enhancing the mechanistic basis upon which BLM forecasts are based (for example, by including additional substances as model inputs).

Indeed, the approach proposed by Sarathy and Allen (2005) presents a number of disadvantages within the context of biotic ligand modelling. For example, their approach implies that the complexation characteristics of naturally derived organic matter are standardised, which may not be realistic since Dwane and Tipping (1998) found active fraction values for naturally derived organic matter to range between 40% and 80%. Similarly, the approach proposed by Sarathy and Allen (2005) also suggests that the complexing characteristics of effluent derived organic matter to be similar, which was also not supported by the findings from the present study. Furthermore, an approach aimed at improving the mechanistic basis upon which forecasts are produced would also potentially require some account of the contribution to complexation capacity from other substances found in sewage effluents (e.g. organophosphonates, iminodisuccinic and gluconic acids (Knepper, 2003)) but which may also risk erroneous conclusions as to their significance. For example, whereas including EDTA in the speciation modelling improved forecast accuracy for effluents A1 and A2 this may rather have been incidental since the chemical analysis had revealed the EDTA to be in an inert form (Chapter 5.5.1).

The findings from this study therefore suggest that the variability in metal complexation characteristics should rather be taken into account in the most straightforward manner and which requires the fewest assumptions. Indeed, this also presents a practical opportunity to take into account variability in complexation characteristics and therefore, to improve the environmental relevance of water quality standards derived using the BLM approach.

6.7 Conclusions

The Modified BLM predicted copper toxicity to *D. magna* with greater accuracy than the HydroQual and Refined BLMs although this was dependant on optimising the DOC active fraction considered active. Whereas including effluent specific characteristics as inputs into the Modified BLM also improved forecast accuracy, the accuracy improvement attributable to optimising the DOC fraction considered active was substantially more significant. This suggests that optimising the DOC fraction considered active may be a more useful approach for reflecting differences in complexation characteristics, and therefore to ensure the environmental relevance of water quality standards derived using the BLM approach. Since the optimised active fraction values also exceeded the default values, these findings suggest that sewage effluents contain a greater concentration of complexing ligands per milligram of DOC in comparison with organic matter derived from natural source, these findings also suggests that effluent derived organic matter may offer greater protection against potentially adverse effects of copper than is offered by naturally derived organic matter.

7. INCORPORATING THE EFFECT OF EFFLUENT DERIVED COMPLEXING LIGANDS WITHIN THE BIOTIC LIGAND MODEL

The results from the study in Chapter 6 demonstrated that increasing the DOC fraction considered active was more significant to the accuracy of *D. magna* toxicity forecasts than the inclusion of effluent specific characteristics in the speciation modelling. Since the increased active fraction values imply a greater concentration of copper complexing ligands per milligram of DOC (i.e. complexation capacity) than would ordinarily be expected to occur, the studies described in this chapter compared the concentrations of complexing ligands per milligram of DOC in those effluents with values for organic matter derived from a natural source. This study has identified a method that enables a relatively straightforward estimation of the active fraction that therefore allows for some consideration of the variability in surface water complexation characteristics in biotic ligand modelling, and therefore the prospect of improving the ecological relevance to EQSs determined using the BLM approach. The findings from this study were that the effluents had, on average, 6 times the number of copper complexing ligands per milligram of DOC compared with DOC derived from natural sources.

A set of empirical regression characteristics have also been derived with which Chelex method titration data may be applied to estimate DOC active fraction values for both the Modified and Refined BLMs described in Chapter 6. These regression characteristics substantially improved the accuracy of toxicity forecasts compared with those produced using the default active fraction values, and indicates that the Chelex method may be used to improve the ecological relevance of BLM determined EQSs.

7.1 Comparison of complexation capacities of effluent and river water

7.1.1 Background

Many waterbodies receive substantial inputs of effluent derived organic matter (OM), which other studies (Guo *et al.*, 2011; Pernet-Coudrier *et al.*, 2008; Sarathy and Allen, 2005) have shown to have a substantially different effect on metal speciation than OM derived from natural sources. Indeed, Sarathy *et al* (2005) also proposed that effluent derived OM should be included as an additional ligand type in biotic ligand modelling, which has been supported by findings from other recent studies (Pernet-Coudrier *et al.* ,2008; Baken *et al.*, 2011). Whereas improving the accuracy of biotic ligand models is, from a scientific perspective, valuable in itself, the prospect that BLMs will be applied within a compliance-based regulatory framework, however, provides an additional imperative since failure to take into account real-world effects may result in statutory standards which are either insufficiently protective so that harm may occur, or over-precautionary so that measures to reduce metal concentrations may be required, but which deliver no environmental benefit. The significance of taking into account the effect of effluent derived OM for the purposes of biotic ligand modelling was also of particular interest to Severn Trent Water since much of its operating region was considered to be characterised by low effluent dilution capacity. The following study was therefore conducted to examine some of the differences in the metal complexing characteristics of OM derived from river water and that discharged from Severn Trent Water WwTWs.

7.1.2 Experimental Approach

Chelex column titrations, as described in Chapter 4.2.5, were conducted for the effluents described in Chapters 5 and 6, and complexation characteristics determined as described in Chapter 4.2.6 (DOC normalised). The concentrations of complexing ligands in sewage effluent per milligram of DOC were compared with equivalent values for river water samples obtained from the River Frome in the Gloucestershire Cotswolds. The River Frome was selected since significant anthropogenic inputs were not expected to occur upstream of the sample location and, therefore, these were expected to be representative of DOC as described within the BLM, but also because the River Frome was expected to be broadly representative of river waters found within the Severn Trent Water region (i.e. hard and highly alkaline). River water samples were collected on four separate occasions within a two week period in summer 2008.

7.1.3 Results

7.1.3.1 River water chemistry characteristics

The sampled river waters were hard (210 to 218 mg/L as $CaCO_3$), highly alkaline (152 to 179 mg/L as $CaCO_3$), and contained high concentrations of iron (0.2 to 0.6 mg/L), which were all attributable to the underlying geology (Table 7.1). The DOC concentrations were low, and ranged from 2.2 to 3.3 mg/L. The background concentrations of trace metals were also low and EDTA was not detected in any of the samples.

Table 7.1 Water chemistry characteristics of samples collected from the River Frome

Sample	pH	DOC (mg/L)	Hardness[a]	Alkalinity[a]	Cu (µg/L)	Fe (µg/L)
F1	7.6	2.6 (±0.1)	216	166	1	229 (±47)
F2	7.5	3.3 (±0.1)	201	152	1	355 (±37)
F3	7.4	2.2 (±0.1)	218	179	1	461 (±45)
F4	7.2	2.4 (±0.2)	213	150	2	557 (±10)

[a] Hardness and alkalinity as mg/L $CaCO_3$
Values in parentheses indicate the 95% confidence interval range

7.1.3.2 Complexation characteristics

Complexation characteristics were determined for all samples (Table 7.2). The conditional stability constant values (Log *K'*) indicated that the average binding affinity of the effluent derived ligands was lower than those which occurred in the river water samples. This suggested that these effluents contained substantial concentrations of a category of moderately strong ligand (i.e. distinguishable by the Chelex method) but which were, nonetheless, weaker on average than the ligands detected in river water sample. The average concentration of copper complexing ligands per milligram of DOC in the effluent samples was, however, 6 times greater than the average for the DOC derived from the River Frome, indicating that the effluent derived DOC contained a substantially greater concentration of complexing ligands than found in unimpacted river water samples.

The effluent complexation capacities per milligram of DOC varied within a 4-fold range of one another, and the river water samples within a 6-fold range suggesting the complexation characteristics of the river water samples to be more variable than those for the effluents. However, the large variance in the complexation capacity values for the river water samples was primarily attributable to sample F4 which, in relation to the other river water samples, displayed unusual complexation characteristics, more typical of those of the effluents. Sample F4 also had the lowest pH and contained the highest concentration of iron although the extent by which these might have influenced complexation characteristics was uncertain.

Table 7.2 Chelex measured conditional stability constants for copper-ligand complexes and the ligand concentrations [LT] per mg of DOC

Sample	Log *K'*	[LT] as µM/mg of DOC	[LT] as µg/mg of DOC
A1	5.16 (4.95 – 5.32)	0.54 (0.44 – 0.74)	34 (28 – 47)
A2	5.06 (4.79 – 5.26)	0.63 (0.49 – 0.97)	40 (31 – 62)
B1	4.97 (4.86 – 5.08)	0.82 (0.70 – 0.96)	52 (45 – 61)
B2	4.67 (4.59 – 4.74)	1.76 (1.55 – 2.01)	112 (99 – 128)
C1	4.94 (4.86 – 5.00)	0.56 (0.52 – 0.63)	36 (33 – 40)
C2	4.42 (4.09 – 4.57)	1.47 (1.09 – 2.84)	94 (70 – 181)
D1	4.60 (4.49 – 4.69)	1.27 (1.10 – 1.54)	81 (70 – 98)
D2	4.47 (4.31 – 4.59)	1.85 (1.49 – 2.50)	118 (95 – 159)
F1	7.01 (6.67 – 7.37)	0.05 (0.01 – 0.05)	3 (3 – 3)
F2	6.03 (5.91 – 6.12)	0.06 (0.06 – 0.07)	4 (4 – 5)
F3	6.04 (5.85 – 6.22)	0.10 (0.09 – 0.12)	7 (6 – 8)
F4	4.90 (1.64 – 5.22)	0.56 (0.31 - 869)	36 (20 – 55,213)

Samples A-D represent the effluents as described in Chapter 5.3.1 and F1-F4 the samples obtained from the River Frome.
Values in parenthesis indicate the 95% confidence interval limits except for sample F4 where these represent the 80% confidence interval range.

The Chelex titration data are provided in the data appendix for chapter 7.1.

7.1.4 Discussion

7.1.4.1 Sewage effluent complexation capacity

The complexation characteristics presented in Table 7.2 clearly demonstrate that for these samples effluent derived OM offers a substantially greater (DOC normalised) capacity to complex copper than OM derived from natural sources, which was also consistent with findings from other similar studies (van Veen *et al.*, 2002; Sarathy and Allen, 2005. In their study, however, Sarathy and Allen (2005) proposed that up to 90% of the difference between river water and effluent complexation might be attributable to the influence of sulphide for which unprotonated sulphide clusters have been observed by others to form highly stable complexes with copper (Rozan *et al.*, 2000) (>Log K 54), however, since the average binding affinity values for the effluents in the present study were lower than the values for the river water samples, the additional complexation capacity in the sewage effluents was unlikely to have been attributable substantially to such a strongly binding ligand.

In addition to sulphide Sarathy and Allen (2005), also recognised that other copper complexing ligands were likely to occur in sewage effluent, and that these were significant to copper speciation although they did not speculate on the identity of these ligands other than to suggest that these might be non-humic biological macromolecules. This suggestion was, however, supported by the findings from other studies which have investigated the chemical composition of sewage effluents (Ma, Allen and Yin, 2001; Pernet-Coudrier *et al*., 2008) which that found approximately 50% of the carbon content in sewage effluent derived OM was comprised of an acid soluble hydrophilic fraction made up of high molecular weight (>3500 Da) polysaccharides, in excess of the proportion which occurs in naturally derived organic matter (between 9% and 30%). The study by Ma, Allen and Yin (2001) also investigated the copper binding characteristics of the hydrophilic fraction and found this to have an affinity for copper which was lower than that between copper ions and humic or fulvic acids. This provides a plausible explanation for the finding in the present study that the binding affinity of the effluent derived ligands was, on average, lower than the binding affinity of the ligands in the river samples, which consequently, implies that a substantial proportion of the additional complexation capacity observed in the present study may indeed have been attributable to high molecular weight non-humic substances.

It was, however, surprising that the studies by Ma, Allen and Yin (2001) and Pernet-Coudrier *et al.* (2008) did not consider the potential significance of anthropogenic ligands, such as EDTA, which is known to have a high affinity for copper and to occur in sewage effluents (Knepper, Werner and Bogenschütz, 2005; Knepper, 2003). Indeed, a recent study by Baken *et al.* (2011)**,** which investigated the metal complexing properties of organic matter in anthropogenically impacted surface waters also identified the significance of synthetic chelating agents such as EDTA to trace metal speciation. However, whereas EDTA is perhaps the most well known synthetic chelating agent, numerous other anthropogenic substances with chelating properties, such as organophosphonates, are also known to, or may reasonably be expected to occur in sewage effluents (HERA, 2004; Knepper, 2003). In addition to occurring in their primary form, chelating agents might also occur as breakdown products; for example, ethylenediaminetriacetic acid (ED3A) has been identified as a common EDTA breakdown product (Nowack and VanBriesen, 2005a), which is also known to have a strong affinity for copper (log K 10.5) (Stumm and Morgan, 1996).

In the context of the present study, whereas it is plausible that some proportion of the difference between the complexation capacity values for the effluent and river water samples may have been attributable to sulphide, it is perhaps more plausible that this was attributable to the presence of biological macromolecules, although potentially also to synthetic chelating agents and/or their breakdown products. The findings from the present study therefore contribute support to the view that the influence of effluent derived organic matter should be taken into account within the context of biotic ligand modelling.

7.2 Using the Chelex method data to estimate the DOC active fraction

7.2.1 Background

The previous section provided evidence that effluent derived organic matter offers a greater concentration of complexing ligands per milligram of DOC than occurs in naturally derived organic matter. The ability to distinguish such differences is, however, only of limited practical value unless these differences may be taken into account, and applied for the purposes of biotic ligand modelling.

The DOC fraction considered active (i.e. the concentration value used as a model input) reflects the density of ion binding sites relative to that assumed within the BLM (e.g. models in the WHAM series of humic ion binding models) so that adjusting this value offers the ability to take account of differences in complexing characteristics. Default active fraction values are, however, typically used since the techniques for determining active fraction values are complex and not amenable to application as part of routine water quality monitoring. For example, active fraction values are typically determined by comparing BLM modelled and measured free ion activity values, with the optimum active fraction value that which minimises the error between the BLM modelled and measured values (Bryan, Tipping and Hamilton-Taylor, 2002; Dwane and Tipping, 1998) and where free ion activity is measured using an ion selective electrode or a voltammetric technique, or on the basis of toxicity data as described in Chapter 6.

Whereas the default active fraction values have generally demonstrated good accuracy in predicting toxicity, the accuracy of model forecasts, and hence, the ecological relevance of EQSs determined using this approach, might further be improved by taking account of the variability in OM binding characteristics. Indeed, this was the motivation behind a study by De Schamphelaere *et al.* (2004) who assessed the usefulness of UV absorbance characteristics for estimating active fraction values. De Schamphelaere *et al.* (2004) found that using UV absorbance to estimate the active fraction improved the accuracy of BLM toxicity forecasts from within a factor of 2, to within a factor of 1.3. However, the samples used in their study were derived from sources with predominately natural inputs and for which the functional groups primarily responsible for complexing metal ions (e.g. carboxylates) display strong UV absorbance characteristics so that a strong correlation between UV absorbance and the DOC fraction considered active might have been reasonably expected to exist. In the context of the present study, however, other studies involving effluents (Pernet-Coudrier *et al.*, 2008; Ma, Allen and Yin, 2001) have demonstrated effluent derived OM to be comprised substantially of a hydrophilic fraction which complexes metal ions but displays low UV absorbance, which suggests that UV absorbance is unlikely to be useful for predicting complexation characteristics for effluents and effluent influenced waters. Indeed, a recent study by Baken *et al.* (2011) found UV absorbance was only useful for explaining metal binding affinity for waters which did not receive anthropogenic inputs and recommended that some other method was required for estimating binding site density in effluent influenced waters.

This study was therefore aimed at developing a simple method whereby active fraction values could be estimated for anthropogenically impacted waters for application in biotic ligand modelling, and thereby improving the ecological relevance of BLM determined EQSs.

7.2.2. Experimental approach

The Chelex method (Chapter 4.2.5) was assessed for suitability for estimating the DOC active fraction values. The objective was to identify a set of regression parameters that could allow the DOC-normalised non-labile copper concentration that occurs from a fixed (spiked) dissolved copper concentration to be used for estimating DOC active fraction values, which would allow the DOC active fraction value to be determined from the DOC concentration and the Chelex titration data alone.

In order to relate Chelex data and the DOC active fraction, the Chelex titration characteristics for the effluents described previously (Table 7.2) were assumed to approximate the binding characteristics implied by the optimised active DOC fraction values for the Refined (WHAM V) and Modified (WHAM VI) BLMs (determined from the *D. magna* toxicity assay results described in Chapter 6, Table 6.3). This suggests that, in the first instance, the concentration of complexing ligands which are not distinguishable by the Chelex method (i.e. weak ligands) may be extrapolated from the ligands that are distinguishable, and in the second instance, that the difference between BLM predicted and observed toxicity is attributable to differences in the active fraction alone. These assumptions are plausible since, in the case of the former, the study by Sarathy and Allen (2005) demonstrated that for both effluent and naturally derived DOC, the weakest ligand category (in their study with an average binding affinity approximately log *K* 6.5) represented a relatively constant proportion (approximately 75%) of the total concentration of complexing ligands, and for the latter, since using an organism as a type of biological speciation probe to estimate site-specific complexation characteristics has already been demonstrated to provide reasonably accurate estimations of active fraction values (Heijerick *et al.*, 2005; De Schamphelaere and Janssen, 2004b; De Schamphelaere *et al.*, 2004; De Schamphelaere, Heijerick and Janssen, 2002)**.**

The correlation between the DOC-normalised non-labile copper and the optimised active fraction values was evaluated by simulating the non-labile concentrations (i.e. post Chelex column) that occurred for each effluent across a range of dissolved (i.e. pre Chelex column) copper concentrations (between 1 µg/L to 1000 µg/L, in 1µg/L increments) and plotting these in relation to their corresponding optimised active fraction. Since each unique dissolved copper concentration value generates a unique non-labile concentration for each effluent, 1,000 combinations of non-labile copper and active fraction were produced, which served as the dataset from which the optimum regression was selected. Effluent samples B1 and B2 were excluded from this assessment in order to eliminate the potential for confounding influences which might occur since the optimised active fraction values for these had been determined on the basis of an adjusted sodium concentration value (Chapter 6.3.2). This simulation was performed using an Excel spreadsheet macro which iteratively adjusted the copper concentration across the range (the spreadsheet model is included in the data appendix for chapter 7.2 in the spreadsheet ‘Simulation_Complexation_ActiveFraction.xlsm’).

7.2.3 Results

The correlation between the optimised active DOC fraction of each effluent and the DOC-normalised non-labile copper concentration that occurred for the dissolved copper concentration at the observed EC50 value was statistically significant (*r* = 0.81, *p* = <0.05, *n*= 6; and *r* = 0.83, *p* < 0.05, *n*= 6, for the Refined and Modified BLMs, respectively) (Figures 7.1 and 7.2), thus demonstrating that the Chelex method data could be used to support predictions of the DOC active fraction. This positive relationship was unsurprising since the active fraction and the complexation capacity per mg of DOC are, theoretically, analogous; however, confirmation of this relationship was necessary as a first step in ensuring that these results could support active fraction predictions.

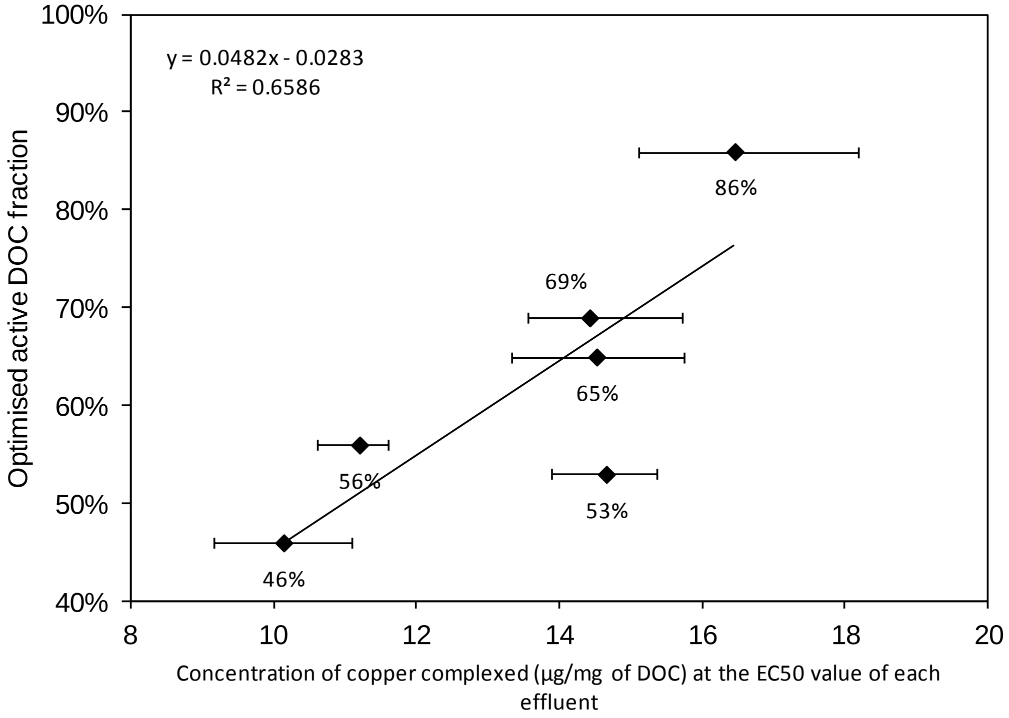

Figure 7.1 Concentration of DOC-normalised non-labile copper at the EC50 value in relation to the Refined BLM optimised active fraction values (Chapter 6). Error bars represent the complexation capacity 95% confidence interval range.

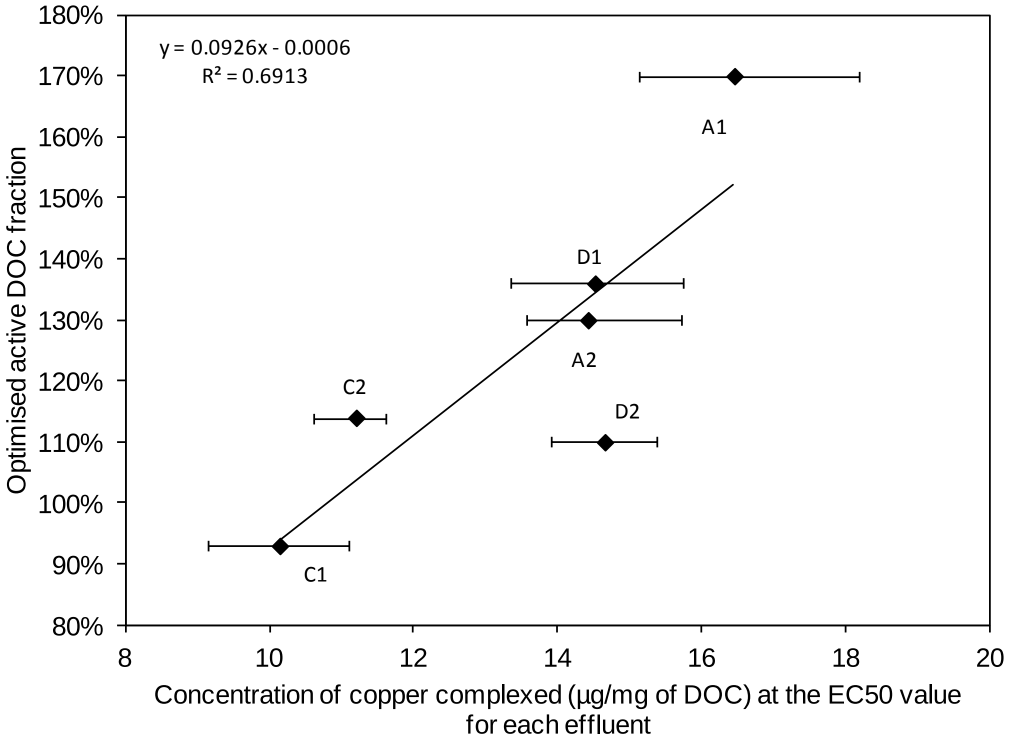

Figure 7.2 Concentration of DOC-normalised non-labile copper at the EC50 value in relation to the Modified BLM optimised active fraction values (Chapter 6). Error bars represent the complexation capacity 95% confidence interval range.

The correlation between the optimised active fraction and copper complexation at the EC50 value is given in Figure 7.3, where the y-axis indicates the coefficient of determination for the relationship between the non-labile copper and the optimised DOC active fraction (for the Refined and Modified BLMs), and the x-axis the simulated copper concentration. The figure shows that the correlation between the DOC-normalised non-labile copper concentration (i.e. the post column copper concentration) and the DOC active fraction decreased as the dissolved copper concentrations increased, indicating that the strongest correlation between the DOC-normalised non-labile copper concentration and the DOC active fraction would occur at a low dissolved copper concentration. However, at very low copper and DOC concentrations the resultant non-labile concentrations might not be easily distinguishable analytically (e.g. post column copper concentrations <1 µg/L), and small differences in DOC-normalised non-labile copper might also result in large differences in the estimated active fraction value. It is therefore desirable to ensure that where the observed relationships are used for the purposes of prediction, that the regression characteristics are not overly sensitive to small differences in the non-labile copper concentration. Indeed, Bryan, Tipping and Hamilton-Taylor (2002) also reported a mean variability of 10% in the ‘best-fit’ active fraction value for individual samples, indicating that even sophisticated techniques are limited in their ability to provide precise estimations of the active fraction value. This sensitivity requirement may therefore be accommodated by limiting the data set from which the optimal regression is selected to those for which a 1 µg/L difference in non-labile copper (i.e. a difference plausibly attributable to the analytical method detection sensitivity) results in a difference in active fraction of no more than 10% (i.e. the range within which active fraction values might otherwise have been determined using some other technique). This sensitivity is, however, reflected in the slope of each of the simulated regressions (i.e. the change in active fraction for each µg of non-labile copper per milligram of DOC) and consequently this sensitivity constraint may be accommodated by limiting the set from which the optimal regression is selected to those for which the slope is less than or equal to 0.1; that is, where a 10% difference in active fraction occurs for a 1 µg/L difference in non-labile copper.

The ‘goodness-of-fit’ of the regression characteristics was, however, not significantly affected by constraining the regression sensitivity (slope) (Figure 7.4), although in general, the ‘goodness-of-fit’ was better for the Refined BLM (WHAM V) compared with the Modified BLM (WHAM VI). The regression characteristics that most accurately reflected the agreement between the active DOC fraction and DOC-normalised non-labile copper, while minimising the sensitivity to variances in non-labile copper, are given in Table 7.3. These may be applied for estimating active fraction values from Chelex titration data using the following equation:

$$\mathrm{AF\%}_{ij} = (\mathrm{slope}_j \cdot \mathrm{NL}_j) + \mathrm{intercept}_j$$

Where:

- AF%*ij* is the active fraction for sample *i* for BLM *j* (i.e. Refined or Modified)
- slope_j is slope parameter for BLM *j*
- NL_{ij} is the DOC-normalised non-labile copper concentration occurring from the specified dissolved copper concentration for BLM *j*
- $\mathrm{intercept}_j$ is the intercept parameter for BLM *j*

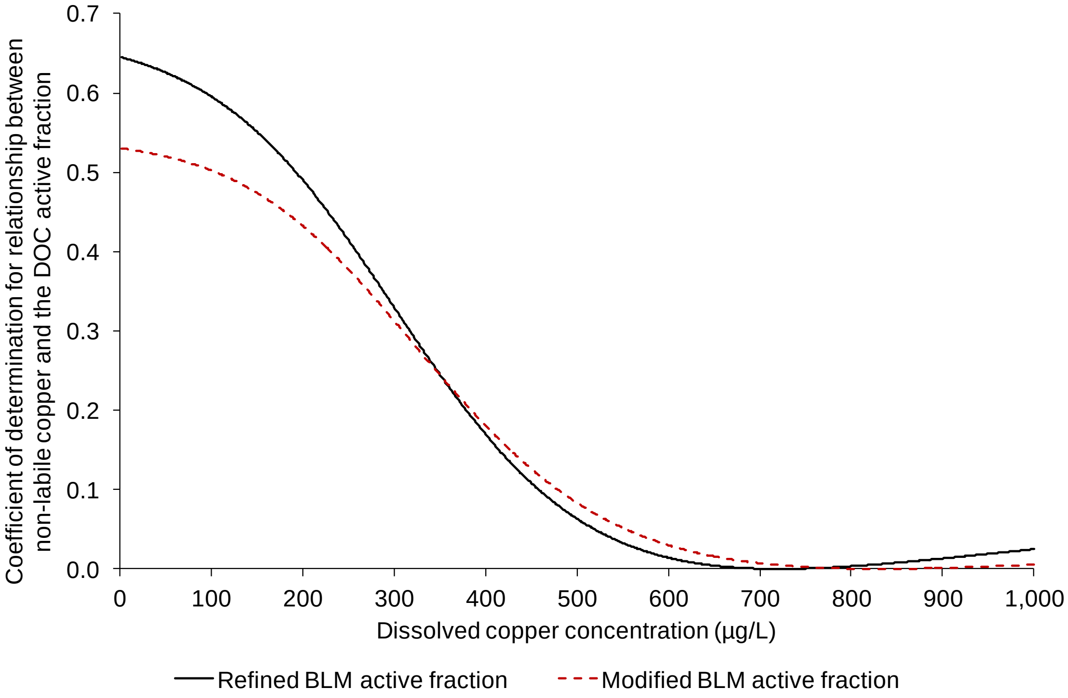

Figure 7.3 Coefficient of determination (r^2) for relationship between the DOC-normalised non-labile (post column) copper concentration and the DOC fraction considered with regard to metal ion bindings in relation to the dissolved (pre-column) copper concentration

Table 7.3 Best fit linear regression characteristics for use in estimating DOC active fraction values from Chelex titration data

Model	*n*	Dissolved copper (µg/L)	Slope	Intercept	r^2
Refined BLM	6	105	0.1003	0.0909	0.59
Modified BLM	6	225	0.1030	0.2100	0.41

The DOC active fraction values calculated using the best-fit linear regression characteristics for the Refined and Modified BLMs are given in Table 7.4. The *D. magna* EC50 values determined using the calculated active fraction values is given in Figure 7.5. These were accurate for both BLMs by within a factor of 1.2 of the observed values, which was more accurate than the factor range obtained using the default active fraction values (1.6 for the Refined BLM and 2.1 for the Modified BLM).

Table 7.4 DOC active fraction value calculated by linear regression using 'best-fit' linear regression characteristics for the Refined and Modified BLMs

	Refined BLM		Modified BLM	
Effluent	Toxicity assay optimised active fraction	Calculated active fraction value	Toxicity assay optimised active fraction	Calculated active fraction value
A1	86%	79%	170%	145%
A2	69%	69%	130%	145%
C1	46%	59%	93%	103%
C2	56%	49%	114%	103%
D1	65%	59%	136%	124%
D2	53%	59%	110%	134%

Default active fraction values are 50% and 68% for the Refined and Modified BLMs, respectively

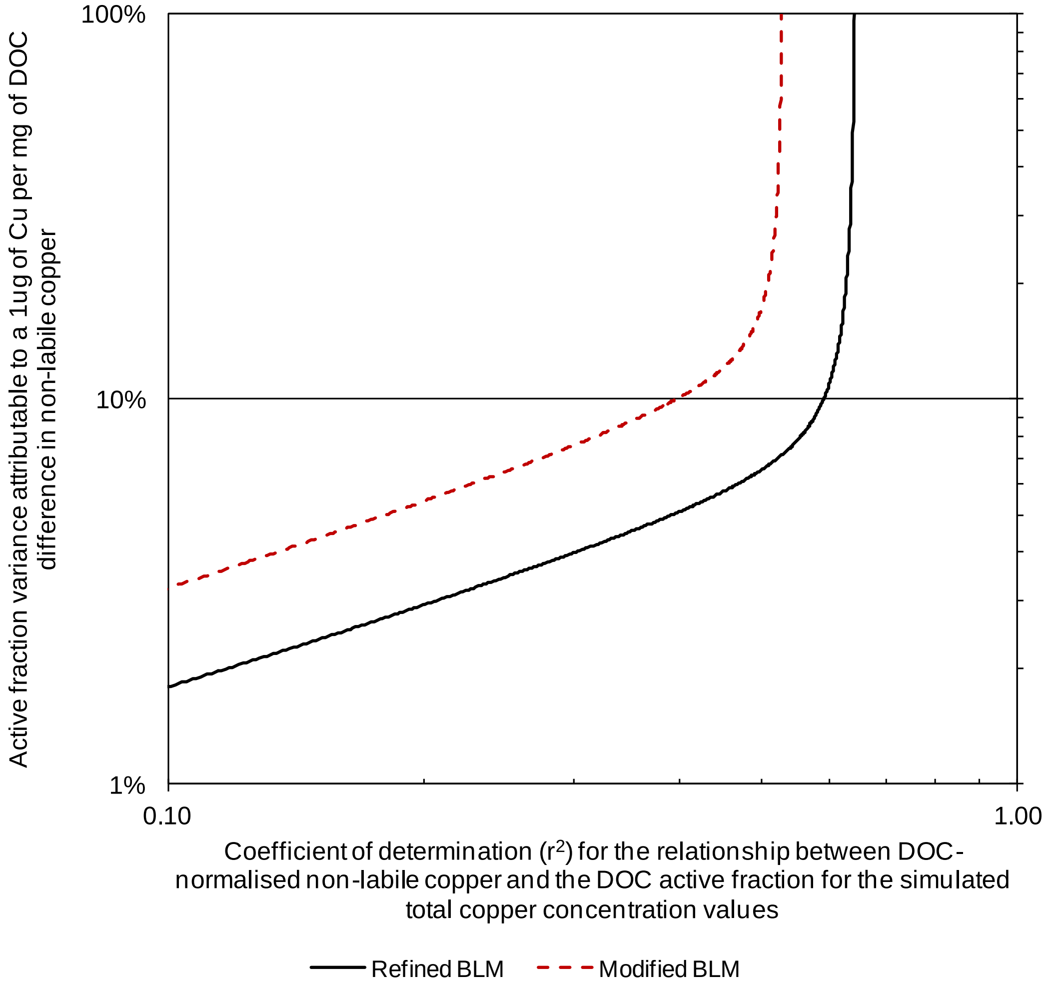

Figure 7.4 Relationship between DOC-normalised non-labile copper, the DOC active fraction (the 'goodness-of-fit' of the relationship represented collectively by the coefficient of determination (r^2) value on the x-axis) and the variance in active fraction attributable to a 1 µg/L difference in DOC-normalised non-labile copper (on the y-axis)

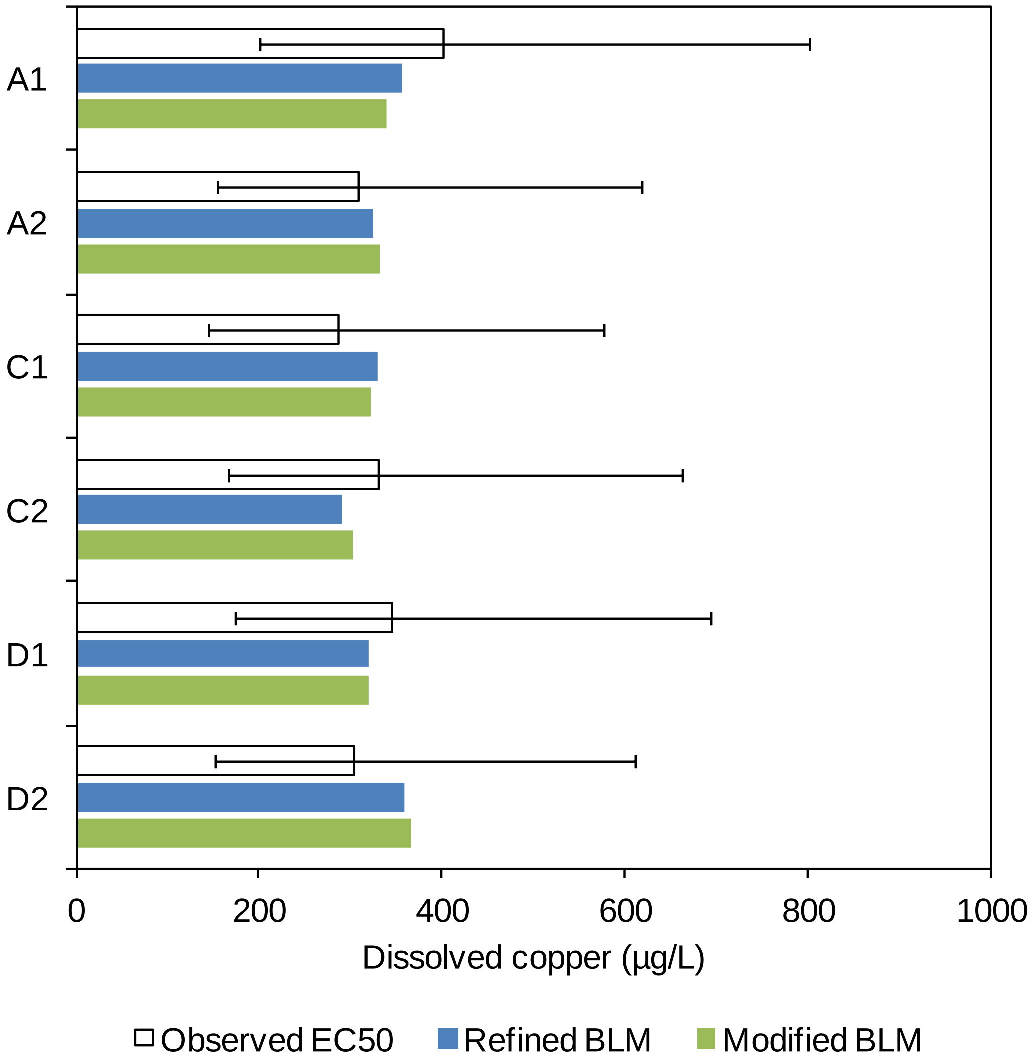

Figure 7.5 Observed EC50 in relation to BLM forecasts produced using DOC active fraction values estimated using the Chelex method. Error bars represent a factor of two range (the range within which BLM forecasts are considered accurate) so that the upper error bar is twice the observed EC50 value, and the lower error bar half the observed EC50 value.

7.2.4 Discussion

7.2.4.1 The Chelex method as a tool for predicting the active fraction values

Operationally defined speciation methods such as the Chelex method have traditionally been of interest to researchers since these are robust, relatively straightforward to apply, and the labile metal fraction (i.e. the fraction retained within the column) provides a reasonable, albeit conservative, approximation of the bioavailable metal fraction. Indeed, in their study, Bowles *et al.* (2006) found the Chelex labile copper fraction over-estimated the concentration of bioavailable copper by a factor of 5. Whereas copper toxicity predictions that are within a factor of 5 of the observed toxic effect concentration may have, historically, been regarded as accurate, the advent of biotic ligand models has allowed for more accurate estimations of bioavailability (within a factor of two) so that the usefulness of techniques such as the Chelex method for predicting bioavailability has been reduced. However, the relative complexity of the BLM is such that taking account of the variability in the capacity of OM to bind metal ions is not simple, in particular in effluent impacted waters, for which simple chemical characterisations (e.g. UV absorbance and acid-base properties) are unlikely to adequately reflect biologically relevant differences. The present study has, however, demonstrated the Chelex method provides a relatively straightforward method for estimating the DOC active fraction, and may therefore assist in improving the ecological relevance of EQSs determined by the BLM approach. Indeed, this also serves to satisfy the original objective set by Bowles *et al.* (2006) for developing the Chelex method, that is, to serve as a simple and rapid technique that may be useful as a regulatory tool.

7.2.4.2 Regression and 'goodness-of-fit' characteristics

The coefficient of determination (r^2) for the relationship between the DOC normalised non-labile copper and the toxicity assay optimised active fraction, which provides an indication of the 'goodness-of-fit' of the regression characteristics was, for the Refined BLM, in a similar range to the value for natural waters determined in the UV absorbance study by De Schamphelaere *et al.* (2004) (r^2 = 0.61, n = 7) suggesting the precision of these two methods to be similar. However, De Schamphelaere *et al.* (2004) improved the overall 'goodness-of-fit' (to r^2 = 0.85, n= 17) by including additional data obtained for samples prepared from purified pre-concentrated organic matter, which demonstrated a much stronger correlation between UV absorbance and the active fraction. Further statistical analysis by De Schamphelaere *et al.* (2004), however, demonstrated that the lines of regression for the natural and isolated samples were not statistically different, and consequently, that the line of regression for the natural water samples alone could be used to represent the relationship between the active fraction and UV absorbance. This suggests that the relationship identified in the present study may plausibly be applied to estimate active fraction value since, as in the study by De Schamphelaere *et al.* (2004), the 'goodness-of-fit' may have been constrained by the relatively small number of samples upon which the regression characteristics were determined.

A similar comparison for the values determined for the Modified BLM was not possible since equivalent toxicity data were not available for comparison. The lower r^2 value for the Modified BLM, however, also indicated that the active fraction values were less well predicted from the Chelex data. A plausible explanation for this is that the Refined BLM provided a better fit since the *D. magna* biotic ligand characteristics (e.g. the biotic ligand critical concentration value) that had been applied in the Refined and Modified BLMs were originally calibrated using the Refined BLM (De Schamphelaere, Heijerick and Janssen, 2003; De Schamphelaere, Heijerick and Janssen, 2002). Consequently, where the *D. magna* biotic ligand parameter values are applied in other models (i.e. the Modified BLM) the effect of other differences between the models (e.g. the binding affinity between calcium and DOC) is likely to have some effect on toxicity predictions, and hence the optimised active fraction value.

7.2.4.3. Applying the Chelex method approach to river water and other effluent samples

The regression characteristics determined in this study (Table 7.3) were applied to the Chelex titration data for the river water samples given in Table 7.2. The results suggested active fraction values of 37%, 35%, 52% and 51% (44% average) for the Refined BLM and 50%, 55%, 75%, and 102% (71% average) for the Modified BLM, for samples F1, F2 F3, and F4 respectively, which were also close to the default active fraction values (50% for the Refined BLM and 68% for the Modified BLM), which also confirmed the suitability of the default active fraction values for waters which do not receive sewage effluent inputs. The Chelex method and regression characteristics as described may therefore also be useful for identifying locations at which the DOC may be less protective than implied by the default active fraction values, or at locations which may be considered to be ecologically sensitive for which confirmation of the ‘true’ active fraction may be desirable. The variability in the active fraction for these river water samples, which were obtained from the same location, also suggests that further research is required in order to better understand the extent by which site-specific active fraction values may vary.

The data presented in this section have demonstrated that the Chelex column method offers a simple yet effective technique that does not require complex chemical characterisations for estimating DOC active fraction values that may be applied in biotic ligand modelling. The Chelex method estimated active fraction values improved the predictions of *D. magna* acute copper toxicity from by within a factor of 1.6 and 2.1 for the Refined and Modified BLMs, respectively, to by within a factor of 1.2 for both the Refined and Modified BLMs. The Chelex method therefore offers the opportunity to improve the ecological relevance of environmental quality standards determined using the BLM approach.

7.3 Conclusions

Sewage effluents were found to contain an average of 6 times the number of copper complexing ligands per milligram of DOC than that of control river water samples with no significant influence from sewage effluents, providing support for the view that effluent derived DOC may offer greater protection from the potentially adverse effects from metals in relation to DOC derived from natural sources. These findings support the case that the contribution to complexation capacity from effluent derived ligands should be taken into account for the purposes of biotic ligand modelling. This study has also demonstrated that the Chelex column method offers a simple yet effective technique, that does not require complex chemical characterisations, for estimating active fraction values that may be applied in biotic ligand modelling, and therefore offers the opportunity to improve the ecological relevance of environmental quality standards determined using the BLM approach.

8. COMPLEXING LIGANDS IN SEWAGE EFFLUENTS

This chapter describes two studies that were aimed at evaluating metal speciation in sewage effluents. The first study compared the concentrations of high affinity ligands in sewage effluents and surface waters in relation to that predicted by the WHAM VI speciation model. This was useful since high affinity ligands are most significant for determining the proportion of metal that is bioavailable at low, environmentally relevant metal concentrations. This study found that for copper and nickel, only certain effluents contained concentrations of high affinity ligands that exceeded the WHAM VI prediction, but varied over a 6-fold and 8-fold range, respectively. For zinc, all the effluents were found to contain substantially greater concentrations of high affinity ligands than suggested by the WHAM VI characterisation, which suggested that WHAM VI might substantially underestimate the concentration of high affinity ligands in effluent derived organic matter, although of equivalent significance, the measured concentrations of high affinity ligands were highly variable, and varied over a 16-fold range. These findings support that consideration for the influence of site-specific complexation characteristics should included as part of the regulatory framework within which BLMs will be applied.

The second study was aimed at evaluating the effect of dilution by river water on sewage effluent derived complexing ligands. This was useful since this would indicate the extent by which sewage effluent derived complexing ligands were likely to continue to exert influence once discharged into the aquatic environment, and the extent by which their effect may be predictable on the basis of dilution. The findings from this study indicated that the complexation capacity of combined effluent and river water samples were well predicted on the basis of their component complexation characteristics and the dilution ratio and consequently, that effluent derived ligands retain their influence on complexation once discharged into surface waters. These findings provide further evidence supporting the consideration of site-specific complexation characteristics as part of the regulatory framework within which BLMs will be applied.

8.1 High affinity ligands in sewage effluents: a comparison with WHAM VI

8.1.1 Background

Speciation models such as WHAM VI are parameterised such that DOC (or DOM) is assumed to be comprised of metal complexing ligands with a fixed distribution of binding affinities so that increasing the DOC fraction considered active (or the DOC concentration) proportionately increases the concentration of complexing ligands across the entire binding affinity spectrum. Whereas adjusting the DOC fraction considered active represents a plausible and straightforward method that allows variability complexing characteristics to be taken into account, differences between the WHAM VI characterisation and the DOC that occurs in the environment may, however, relate to ligands within a specific binding affinity range rather than across the entire affinity distribution so that altering the DOC fraction considered active might provide a sub-optimal approximation of the actual distribution of complexing ligands. The effect of differences between the actual ligand affinity distribution and that represented in the WHAM VI characterisation are also likely to be most significant where these differences relate to high affinity ligands since binding affinity determines the extent by which a reaction between a metal and a complexing ligand goes to completion, and therefore the proportion of metal which remains in the free ion (bioavailable) form. Errors in the estimation of the concentration of high affinity ligands are therefore likely to be of significance, in particular at low metal concentrations at which the ligands with the greatest binding affinity will most substantially influence the proportion of metal remaining in free ion (bioavailable) form. This study was therefore aimed at evaluating the concentrations of high affinity ligands present in sewage effluent in relation to that predicted by WHAM VI, as well as their variability.

8.1.2 Experimental Approach

Nickel and zinc Chelex column titrations were conducted on the effluents described in Chapters 5, 6, and 7, in addition to those described previously for copper. The comparison of the measured concentration of high affinity ligands in relation to that predicted by WHAM VI was as described in Chapter 4.2.7. The concentration of high affinity ligands detected was quantified in terms of complexation capacity in µg/L of metal (i.e. not DOC normalised).

8.1.3 Results

The Chelex titration data for this section are provided in the data appendix for chapter 8.1 in addition to the outputs from the WHAM VI speciation simulation.

8.1.3.1 Copper

Whereas the DOC concentration for the samples were similar (8 mg/L ±1) (Table 5.2) the titration data indicated substantial differences complexation capacity. Total complexation capacity varied across an 8-fold range and complexation capacity attributable to high affinity ligands over a 6-fold range (for the effluents in which these were detected), indicating a substantial degree of variability in the concentration of complexing ligands (Table 8.1). The Chelex titrations for copper indicated complexation capacity attributable to high affinity ligands ($\geq \log K$ 12) in effluents A1, B1, and B2 only, which also far exceeded the equivalent WHAM VI values. The high affinity ligand complexation capacity values also vastly exceeded the concentrations of copper in these effluents indicating the copper present in these effluents to be effectively inert but also that once discharged into the environment these effluents might further reduce the bioavailability of copper (or other trace metals) in receiving waters. In addition, no significant correlation between the high affinity ligand complexation capacity and the concentrations of labile EDTA was identified (the EDTA in sample A1 was entirely non-labile) ($r = 0.60$, $n = 3$, $p = 0.59$) which suggested that the high affinity ligands were unlikely to be EDTA alone although since effluents A1, B1 and B2 were all obtained from treatment works which receive inputs from dairies the unidentified high affinity ligand(s) might plausibly be attributable to some other substance(s) used within the dairy industry. These results indicate that effluents contain highly variable concentrations of ligands with an affinity for copper, in excess of that predicted by WHAM VI, but that these are not ubiquitous or attributable entirely to EDTA.

Table 8.1 Chelex method total and high affinity (≥ log *K* 12) copper complexation capacity in relation to values predicted by WHAM VI

Site	Chelex Total CC (µg/L)	Chelex High Affinity CC (µg/L)	WHAM VI High Affinity CC (µg/L)	Cu (µg/L)
A1	257 (207 – 343)	39 (0 – 58)	2	2 (±0)
A2	279 (211 – 415)	n.d	2	5 (±0)
B1	466 (396 – 560)	123 (103 – 136)	3	3 (±0)
B2	921 (808 – 1,053)	234 (234 – 236)	4	2 (±0)
C1	282 (259 – 313)	n.d	3	3 (±0)
C2	742 (543 – 1,482)	n.d	3	4 (±0)
D1	637 (539 – 781)	n.d	3	4 (±0)
D2	118 (93 – 160)	n.d	3	3 (±0)

Values in parenthesis indicate 95% confidence interval range
CC = complexation capacity
n.d = not detected

8.1.3.2 Nickel

Total complexation capacity varied across a 2-fold range and complexation capacity attributable to high affinity ligands over an 8-fold range in the effluents in which these were detected, indicating a substantial degree of variability in the concentration of complexing ligands (Table 8.1). The Chelex titrations for nickel identified complexation capacity attributable to high affinity ligands (≥ log *K* 7) in effluents A1, A2, B1 and C2; although only in effluents A1 and A2 did these exceed the corresponding WHAM VI values (Table 8.2). As with copper, where detected, the high affinity ligand complexation capacity also exceeded the concentrations of nickel in these effluents indicating the nickel present in these effluents to be effectively inert and that these might further reduce the bioavailability of nickel (or other metals) in receiving waters. Whereas effluents A1 and A2 had also been found to contain substantial concentrations of EDTA (547µg/L and 393µg/L, respectively), the EDTA was entirely non-labile and was unlikely to have impacted complexation in these effluents although, as described for copper, since effluents A1 and A2 received inputs from a dairy the unidentified high affinity ligand(s) might relate to some other substance(s) used within the dairy industry. Whereas these results have also demonstrated that, with the exception of effluents A1 and A2, the WHAM VI estimates of high affinity ligands were greater than the measured values, the relatively short equilibration period applied in this study (1hr), and the known slow rate of nickel complex formation (Guthrie *et al*, 2003) may have been insufficient for equilibration to have occurred so that the measured concentration of high affinity ligands determined in this study may have been an underestimation.

Table 8.2 Chelex method total and high affinity (≥ log *K* 7) nickel complexation capacity in relation to values predicted by WHAM VI

Sample	Chelex Total CC (µg/L)	Chelex High Affinity CC (µg/L)	WHAM VI High Affinity CC (µg/L)	Ni (µg/L)
A1	50 (46 – 54)	24 (22 – 24)	5	5 (±1)
A2	43 (38 – 49)	22 (20 – 23)	5	10 (±1)
B1	63 (55 – 72)	4 (0 – 10)	7	<2
B2	59 (52 – 68)	n.d	11	<2
C1	43 (39 – 46)	n.d	7	6 (±2)
C2	44 (40 – 47)	3 (0 – 6)	8	8 (±1)
D1	66 (61 – 74)	n.d	9	<2
D2	73 (67 – 79)	n.d	9	<2

Values in parentheses indicate 95% confidence interval range
CC = complexation capacity
n.d. = not detected

8.1.3.3 Zinc

Total complexation capacity varied across a 6-fold range and complexation capacity attributable to high affinity ligands over a 16-fold range, indicating a substantial degree of variability in the concentration of complexing ligands (Table 8.3). The Chelex titration for zinc identified complexation capacity attributable to high affinity ligands (≥ log *K* 10) in all the effluents which also far exceeded the WHAM VI values indicting these to have substantial capacity to reduce zinc bioavailability. The high affinity ligand complexation capacity values also exceeded the concentrations of zinc in 4 out of 8 effluents (A2, B1, B2, and D2) indicating the zinc in these effluents to be effectively inert but also that these effluents have the capacity to further reduce the bioavailability of zinc in receiving waters. Furthermore, the complexation capacity attributable to high affinity ligands represented 68% (on average) of total complexation capacity indicating that, for these effluents, the available complexation capacity was primarily attributable to high affinity ligands. Whereas concentrations of high affinity ligands were strongly correlated with the concentration of labile EDTA (r=0.92, n=8, p=<0.01), the concentrations of labile-EDTA were, however, insufficient to provide a causal explanation for this correlation (i.e. the labile-EDTA complexation capacity was less than the high affinity complexation capacity) suggesting that the high affinity ligands could not be attributable to EDTA alone. The concentrations of high affinity ligands were also correlated with the concentration of DOC, although the correlation not statistically significant (r=0.64, n = 8, p = 0.09) and influenced substantially by effluents B1 and B2. This suggested that the high affinity ligands were unlikely to be attributable to an underestimation of complexation capacity attributable to DOC. In summary, these results indicate that WHAM VI substantially underestimates the concentrations of ligands with a high affinity for zinc in sewage effluents and also that these are highly variable.

Table 8.3 Chelex method total and high affinity (≥ log *K* 10) zinc complexation capacity in relation to values predicted by WHAM VI

Sample	Chelex Total CC (µg/L)	Chelex High Affinity CC (µg/L)	WHAM VI High Affinity CC (µg/L)	Zn (µg/L)
A1	54 (47 – 62)	41 (41 – 86)	<1	43 (±3)
A2	52 (48 – 56)	41 (41 – 83)	<1	34 (±2)
B1	197 (184 – 213)	146 (146 – 305)	<1	68 (±1)
B2	171 (167 – 175)	126 (126– 260)	<1	60 (±2)
C1	26 (24 – 28)	18 (18 – 39)	<1	46 (±6)
C2	35 (31 – 38)	9 (8 – 24)	<1	87 (±5)
D1	43 (42 – 45)	31 (31 – 64)	<1	57 (±2)
D2	57 (55 – 59)	43 (43 – 87)	<1	34 (±2)

Values in parentheses indicate 95% confidence interval range
CC = complexation capacity

8.1.3.4 Summary

The Venn diagram below illustrates the overlap in the effluents for which high affinity ligands for each of the considered metals were detected. Notably, effluents A1 and B1 were found to contain ligands with a high affinity for each of the considered metals and effluents A2, B2 and C2 found to contain ligands with a high affinity for two of the considered metals. All the effluents were found to contain concentrations of ligands with a high affinity for zinc in excess of that estimated by WHAM VI.

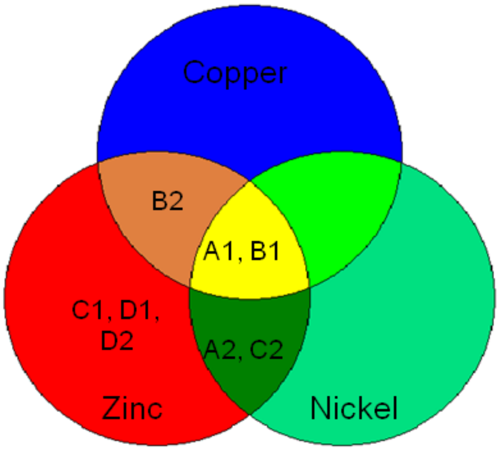

Figure 8.1 Venn diagram showing the overlap in the effluents for which high affinity ligands for the considered metals were detected

8.1.4 Discussion

Whereas the complexation capacity values were highly variable, the complexation capacity attributable to high affinity ligands in particular, the effluents which received inputs from dairies (WwTWs A and B) were notable in these tended to demonstrate the highest total complexation capacity and that attributable to high affinity ligands. Whereas dairies are known to use synthetic chelating agents (EDTA in particular) to prevent the formation of milk-stone linings (Knepper, 2003), the EDTA detected in the effluents was either entirely (A1 and A2), or predominately (B1 and B2) in a non-labile form, and therefore EDTA could not alone explain the presence of these high affinity ligands. Whereas it is plausible that the high affinity complexation capacity may have been attributable to an EDTA substitute, for example, methyl glycine diacetic acid (MGDA) and LinearAlkyl-ED3A have both been suggested as possible

EDTA substitutes and have also been reported to have a strong affinity for trace metals (Nowack and VanBriesen, 2005b; Knepper, 2003), the extent by which these substitutions may have been implemented is uncertain. These would also be expected to be more degradable than EDTA and therefore less likely to survive the wastewater treatment process.

An alternate explanation is that the high affinity ligands may be attributable to some EDTA breakdown product. Although EDTA is generally resistant to degradation this does, to a significant extent, depend on environmental conditions (EDTA is more prone to degradation under alkaline conditions and in summer), its speciation (Fe-EDTA species are known to be susceptible to photochemical degradation, with other forms, such as Ca-EDTA, susceptible to microbial degradation) and the acclimatisation of microbial organisms (Schmidt and Heinz-jurgen, 2006; European Chemicals Bureau, 2004). Indeed, EDTA removal rates of between 30% and 90% have been reported for WwTWs which receive dairy inputs (European Chemicals Bureau, 2004) and consequently, it is highly probable for EDTA breakdown products to have been present, which include (in order of degradation) N-carboxymethyl-N,N'-ethylenediglycine (ED3A), N-carboxymethyl-N-aminoethyleneglycine (N,N-EDDA), iminodiacetic acid (IDA), N-aminoethyleneglycine (EDMA), and glycine (European Chemicals Bureau, 2004). Although only a later stage breakdown product, even IDA is known to have a strong affinity for trace metals, with stability constants (as Log K) of 11.5, 9.1 and 8.2 for copper, nickel and zinc respectively (Stumm and Morgan, 1996). It is therefore plausible that the high affinity ligands detected in effluents from WwTWs A and B might relate to some EDTA breakdown product, a possibility not usually considered due to the general (mistaken) impression that EDTA is not degraded within the wastewater treatment process (European Chemicals Bureau, 2004).

The plausibility that the high affinity complexation capacity may be attributable to EDTA breakdown products, however, also illustrates some of the challenges that are likely to exist in attempting to identify specific ligands in sewage effluent and unambiguously attributing to these a quantifiable complexation capacity. The approach applied in the present study, however, suggests that it may be unnecessary for these ligands to be specifically identified for their influence to be accommodated. For example, the complexation capacity attributable to high affinity ligands that is in excess of that implied within the WHAM VI parameterisation (or some other model) might, as an alternative to increasing the DOC fraction considered active (as described in Chapter 7) be included as an additional generic ligand in speciation and biotic ligand modelling to take account of the variability in the concentrations of high affinity complexing ligands. Indeed, the Chelex method has been demonstrated to provide a relatively straightforward method by which excess complexation capacity within a specific affinity range may be quantified and applied as suggested.

8.2 The effect of dilution on effluent derived complexing ligands

The objective of the study described in this section was to evaluate the effect of dilution by river water on the complexing ligands present in effluent. This was useful in order to assess the extent by which complexing ligands present in effluents may continue to exert influence once discharged into the aquatic environment, and the extent by which their influence may be predicted on the basis of dilution. This study focused on nickel and zinc since other studies have already demonstrated the suitability of a dilution based approach for copper.

8.2.1 Background

The results from the previous study demonstrated the presence of a category of strong ligand in sewage effluents, however, the extent by which these might continue to exert some influence once discharged into receiving waters is uncertain due to physico-chemical changes that might occur as a consequence of combining effluent and river water samples. For example, mixing may result in the precipitation of humic substances and partitioning of metals between the particulate and dissolved phases. Similarly, changes in the concentrations of H^+, Ca^{2+}, and Mg^{2+} may also influence competition at binding sites. Previously, however, a study by van Veen *et al.* (2002) demonstrated that effluent copper complexation capacity could be predicted reasonably well over a 10-fold dilution range, and another study by Chakraborty and Chakrabarti (2006), which investigated metal speciation in mine waters, obtained similar findings on the effects of dilution for copper. Chakraborty and Chakrabarti (2006), however, also found that dilution tended to increase the strength of complexes for nickel and zinc and attributed this effect to changes in the polymeric structure of organic matter that, as a consequence of dilution, increased the number of available binding sites that were of greater significance to nickel and zinc. The study described in this section therefore focuses on the effect of dilution on nickel and zinc complexation. The objective of the study was to determine the extent by which the complexation characteristics of a combined river water and effluent sample could be predicted on the basis of their individual complexation characteristics and the dilution ratio, and thus the extent by which effluent derived ligands may exert influence once discharged into receiving waters.

8.2.2 Experimental Approach

Four effluent samples were obtained from WwTW A (described in the studies in Chapter 5 and Chapter 6) over a two week period in June 2008. For each effluent sample a river water sample was obtained from the River Frome (as described in Chapter 7.1.2) at a location upstream of the point at which the effluent from WwTW A is discharged. Combined samples were produced by mixing equal portions of unfiltered river water and effluent and allowing these to equilibrate for two hours prior to filtration. Complexation capacities were determined for the individual and combined samples using the Chelex column method. The effect of dilution was assessed on total complexation capacity and on the complexation capacity attributable to high affinity ligands.

8.2.3 Results

8.2.3.1 Water chemistry characteristics

The river water characteristics were as described in Table 8.4 (sample label ‘F’). The effluent (sample label ‘SD’) and mixed water samples (sample label ‘SDF’) were hard (192 to 231 mg/L as $CaCO_3$), highly alkaline (136 to 182 mg/L as $CaCO_3$) and contained high concentrations of iron as a consequence of the underlying geology, but also as a consequence of effluent iron dosing. The pH of the effluent and river water samples was reasonably similar and ranged from 7.1 to 7.6. The DOC concentrations for the effluent and the combined samples ranged from 7.3 to 11.8 mg/L, and 4.8 to 7.2 mg/L, respectively. The combined sample DOC concentrations closely matched the mean concentration of the constituent river water and effluent samples (±5%) suggesting that the process of combining the unfiltered aliquots did not substantially influence the dissolved phase concentrations of carbon. EDTA was not detected in any of the river water samples, but was detected in the effluent and combined samples at between 583 and 932 µg/L and 171 and 354 µg/L, respectively. The EDTA was predominately (>63%) in Fe^{3+}-form (non-labile). In the mixed samples, the proportion of EDTA in non-labile form was also lower than in the effluent sample (by between 11-22%), potentially suggesting some partial dissociation of the Fe^{3+}-EDTA complex as a consequence of mixing. The combined sample EDTA was 50% (±12%) of the effluent EDTA concentration, indicating that the process of combining the unfiltered river water and effluent aliquots only marginally influenced the dissolved phase concentrations.

8.2.3.1 Nickel

The measured complexation capacities of the mixed samples were lower than the values predicted on the basis of simple dilution (i.e. the mean of the river and effluent complexation capacity values) in all samples, by 49% on average, suggesting some incremental reduction in complexation capacity (Figure 8.2). The relatively small difference between the estimated and measured DOC concentrations (<5%) in the combined samples also suggested this was unlikely to have been attributable to differences in the concentration of DOC. Three of the 4 predicted values were, however, within a factor of 1.3 of the measured values (i.e. the range within which BLM toxicity forecasts are considered accurate) and all the predicted values were within a factor of 2.4 of the measured values, and were substantially less variable than the complexation capacity values described in the previous study (section 8.1).

High affinity ligands were detected in only the effluent and mixed samples and not in the river water samples (Figure 8.3). The measured complexation capacity for the high affinity ligands was also lower than that predicted on the basis of dilution, by 57% on average, although 3 of the 4 values were predicted by within a factor of 1.8 of the measured values and all values were predicted by within a factor of 2.3, which was less variable that the complexation capacities for the samples described previously. The Chelex titration data are provided in the data appendix for chapter 8.1.

Table 8.4Water chemistry characteristics of river water, effluent, and combined samples

Sample	Type	pH	DOC[a]	Hardness[b]	Alkalinity[b]	Ni (µg/L)	Zn (µg/L)	Fe (µg/L)	EDTA (µg/L)[c]
F1	River	7.6	2.6 (±0.2)	216 (±7)	166 (±4)	<2	<5	229 (±47)	n.d.
SD1	Effluent	7.4	9.9 (±0.1)	231 (±7)	156 (±4)	4 (±0)	47 (±5)	384 (±92)	584 [148]
SDF1	Mixed	7.5	6.2 (±0.1)	220 (±7)	165 (±4)	2 (±1)	30 (±5)	321 (±20)	207 [74]
F2	River	7.5	3.3 (±0.1)	201 (±7)	152 (±4)	<2	<5	355(±37)	n.d.
SD2	Effluent	7.1	11.8 (±0.1)	192 (±7)	136 (±4)	4 (±1)	38 (±5)	388 (±74)	479 [58]
SDF2	Mixed	7.2	7.2 (±0.1)	198 (±7)	143 (±4)	<2	29 (±5)	321 (±127)	172 [57]
F3	River	7.4	2.2 (±0.1)	218 (±7)	179 (±4)	<2	<5	461(±45)	n.d.
SD3	Effluent	7.4	7.3 (±0.1)	219 (±7)	182 (±4)	2 (±0)	52 (±7)	601(±47)	666 [0]
SDF3	Mixed	7.4	4.8 (±0.1)	221 (±7)	177 (±4)	<2	31 (±2)	528 (±77)	254 [47]
F4	River	7.2	2.4 (±0.1)	213 (±7)	150 (±4)	<2	<5	557 (±10)	n.d.
SD4	Effluent	7.2	8.1 (±0.1)	227 (±7)	148 (±4)	3 (±1)	56 (±5)	667 (±75)	931 [0]
SDF4	Mixed	7.3	5.0 (±0.1)	222 (±7)	147 (±4)	4 (±0)	35 (±5)	653 (±30)	353 [65]

[a] DOC in mg/l

[b] Hardness and Alkalinity as mg/L as $CaCO_3$

[c] Figures in square brackets indicate the EDTA concentration that was non-labile (single determination)

Figures in parentheses indicate the 95% confidence interval range

n.d. = not detected

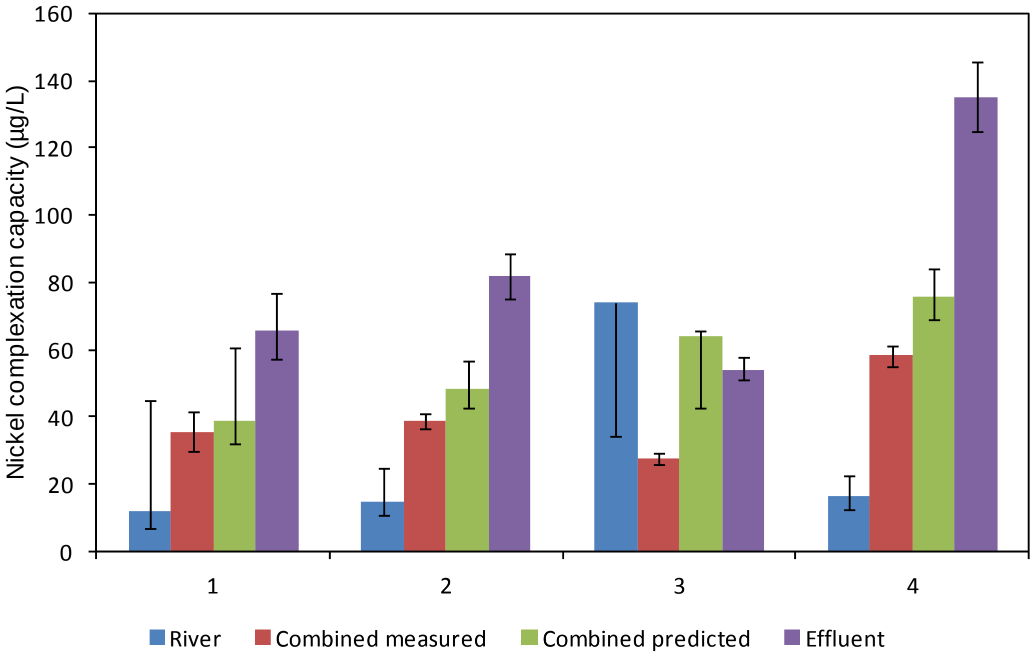

Figure 8.2 Nickel complexation capacities for the river water, combined (measured and predicted) and effluent samples. Error bars indicate complexation capacity 95% confidence interval range. The confidence interval values for the predicted samples were the average of the confidence interval values for the river and effluent samples. The upper error bar for river water sample F3 and that for the combined predicted value have not been rendered as a consequence that these were large.

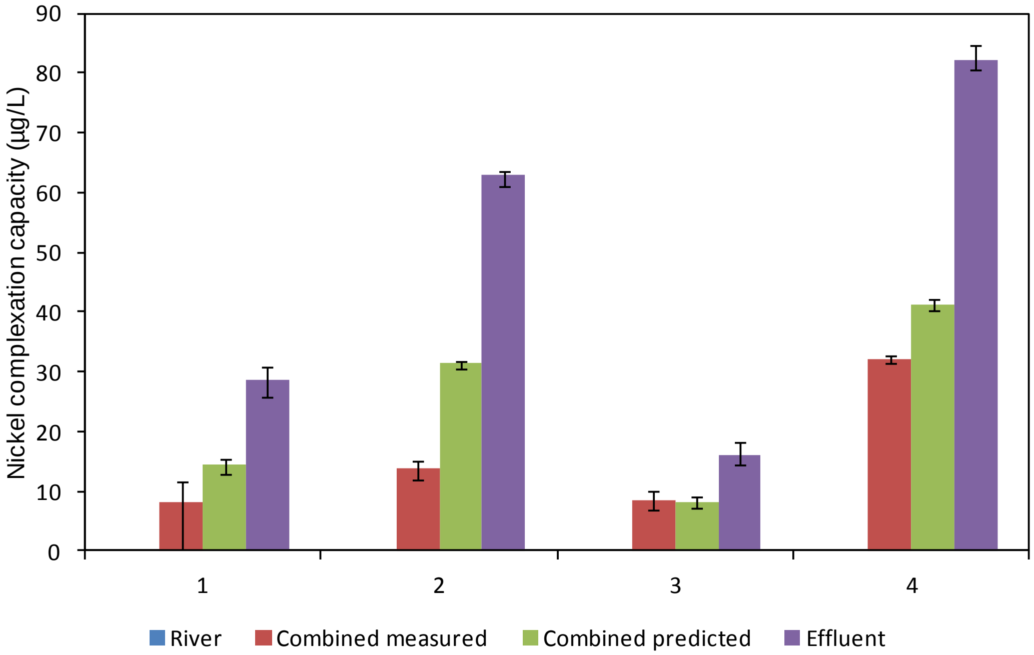

Figure 8.3 Nickel high affinity ligand complexation capacities for river water, combined (measured and predicted) and effluent samples. Error bars indicate complexation capacity 95% confidence interval range.

8.2.3.2 Zinc

The dilution predicted complexation capacities were all within a factor of 1.6 of the observed values and, in contrast to the result for nickel, the measured complexation capacity was greater than the predicted value in 3 out of 4 of the dilutions. These were also substantially less variable than the complexation capacity values described in the previous study (section 8.1).

High affinity ligands were detected in all effluent and combined samples, but in only one river water sample (Figure 8.5). The measured values were also greater than the predicted values (by 10% on average) and all were within a factor of 1.5 of the predicted values and suggested that, in general, the high affinity ligands present in the effluent samples were not substantially altered by mixing with river water and that these may continue to exert influence once introduced into the aquatic environment. The Chelex titration data are provided in the data appendix for chapter 8.1.

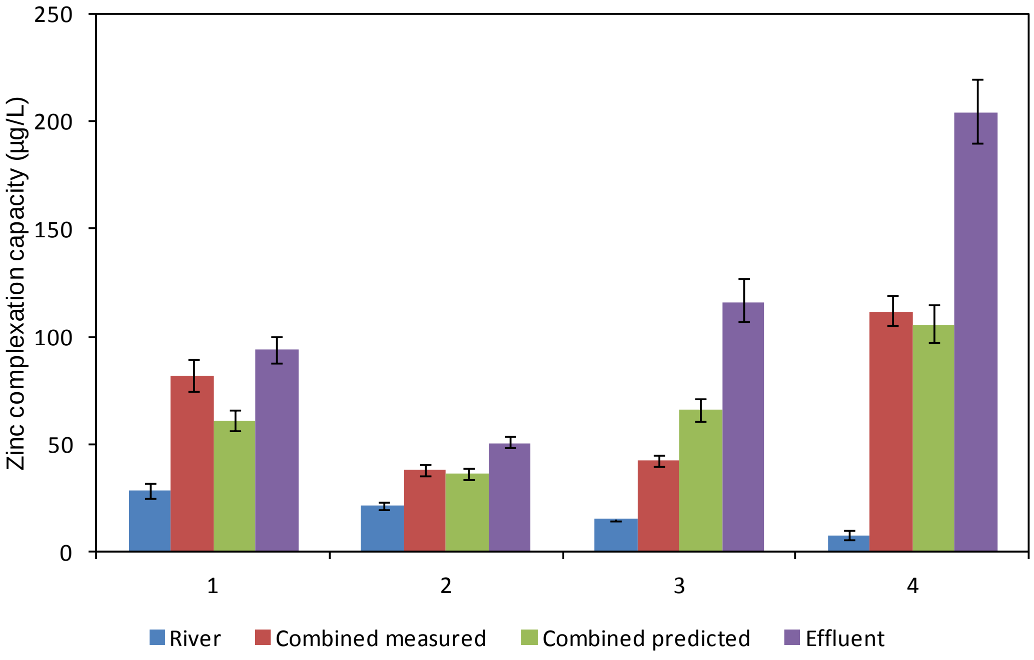

Figure 8.4 Zinc complexation capacities for the river water, combined (measured and predicted) and effluent samples. Error bars indicate complexation capacity 95% confidence interval range.

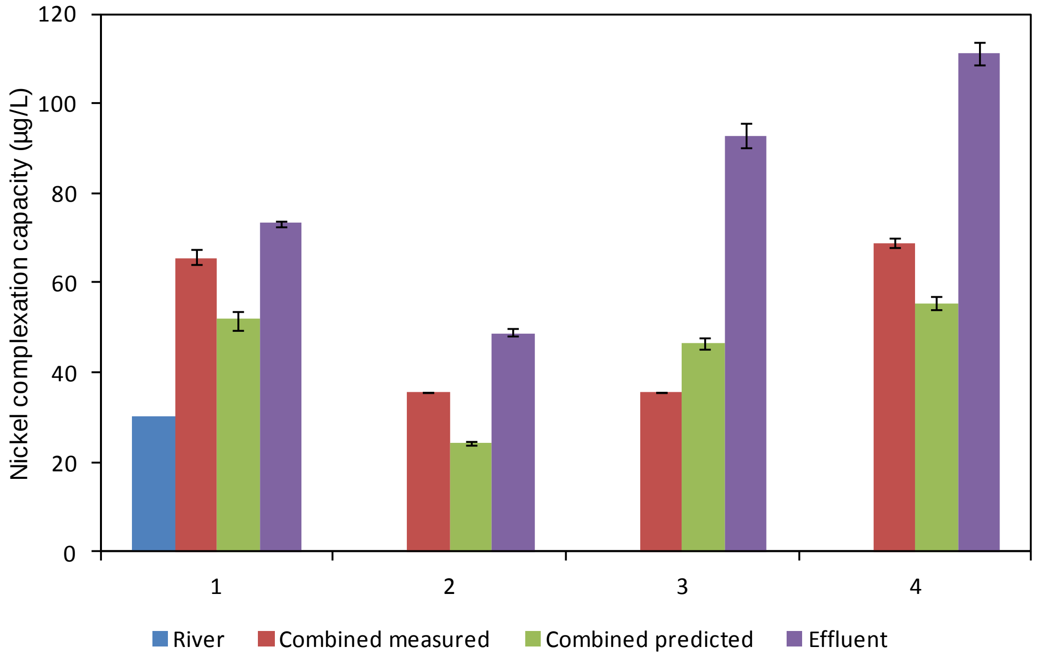

Figure 8.5 Zinc high affinity ligand complexation capacities for river water, combined (measured and predicted) and effluent samples. Error bars indicate complexation capacity 95% confidence interval range.

8.2.4 Discussion

The range within which the dilution predicted complexation capacity values varied from the observed values was substantially smaller than the range within which samples obtained from different locations or effluent might vary, and therefore suggests that complexation characteristics can be predicted on the basis of the characteristics of the component parts, and therefore demonstrates that effluent derived ligands are likely to continue to exert influence once discharged into the environment.

Whereas the complexation capacity values for zinc in the mixed samples were, on average, greater than the measured values, suggesting that dilution based estimates of complexation may be conservative, and which was in also agreement with findings from the study by Chakraborty and Chakrabarti (2006), the values for nickel were, on average, lower. A plausible explanation for the difference in the direction of the effect on complexation capacity between zinc and nickel may relate to the slow ligand exchange kinetics of nickel. A study by Guthrie *et al.* (2003) demonstrated an inverse relationship between the Ni^{2+}:DOC mole ratio and the rate of Ni^{2+}complex dissociation (which would also be expected to apply to the rate of complex formation) and therefore, since the concentration of complexing ligands in the combined sample was lower than in the effluents, the rate of complexation would therefore also be expected to slower. Since the equilibration period had been standardised (1hr) it is therefore plausible that the difference between the observed and predicted complexation capacity might rather reflect a difference attributable to the rate at which nickel ions complexed with the available binding sites rather than due to an absolute difference in complexation capacity.

Whereas these results indicate that the effect of effluent derived ligands on the complexation characteristics of surface waters may be determined on the basis of dilution, these also suggest additional consideration of the significance of the rates of complexation formation (with DOC and the biotic ligand) may be required. This is, perhaps, of particular significance for metals such as nickel for which the rate of complex formation is known to be slow, since the speciation calculations determined by BLMs assume instantaneous equilibrium.

8.3 Conclusions

The results from these studies have demonstrated total complexation capacity, and that attributable to high affinity ligands to be highly variable and not well predicted on the basis of the DOC concentration. The results have also demonstrated that for copper and nickel, only certain effluents contained concentrations of high affinity ligands that exceeded the WHAM VI characterisation, for zinc all the effluents contained concentrations in excess of that suggested by WHAM VI and also consequently, that effluents might offer an enhanced level of protection.

The dilution experiment demonstrated that range within which the dilution predicted complexation capacity values varied from the observed values was substantially smaller than the range within which samples obtained from different locations or effluent might vary. This suggests that complexation can be predicted on the basis of the complexation characteristics of solution component, and therefore demonstrated that effluent derived ligands are likely to continue to exert influence once discharged into the environment. In summary, these findings indicate that consideration of site-specific complexation characteristics within the regulatory framework will enhance the environmental relevance of the compliance criteria derived from the approach.

9. COMPLEXING LIGANDS IN SURFACE WATERS

The purpose of the study described in this chapter was to assess the variability in the concentrations of metal complexing ligands in surface waters, with a focus on zinc and nickel. This study was useful in that it enabled an assessment of the extent by which a standardised description of metal complexation, such as that inherent in BLMs, may be appropriate for determining water quality criteria for nickel and zinc. The findings from this study indicated that nickel complexation capacity could be predicted from the DOC concentration by within a factor of 1.8, which was within a similar to range within which complexation capacity could be predicted from dilution (2.3). Zinc complexation capacity could be predicted by within a factor range of 2.6, which was greater than the range within which complexation capacity could be predicted from dilution (1.6). In addition, zinc complexation capacity could not be quantified for all the sample locations and was therefore indicative of substantial variability in the complexation characteristics. High affinity ligands were detected at locations immediately downstream of WwTWs, indicating these to be significant sources of high affinity ligands, however, high affinity ligands were also detected in surface water samples obtained from rural locations which did not receive effluent inputs from WwTWs, suggesting there may be other sources of high affinity ligands. These findings contribute further support to the view that site-specific complexation capacity should be incorporated within the regulatory framework that has been proposed by the Regulator.

9.1 Experimental Approach

Complexation capacities were determined using the Chelex column method, as described in Chapter 4.2.7 (i.e. not DOC normalised), except that pH was buffered to pH 7.0, (±0.02) using MOPS (5mM), and 3 aliquots of 200ml of filtered sample were spiked in order to determine complexation characteristics (in addition to an unspiked sample). DOC concentrations were quantified by an external laboratory (AlControl Laboratories, Hawarden, UK).

9.2 Sample locations

River water samples were collected in summer 2010 within the Tame-Anker-Mease river catchment along the River Tame and its tributaries (sample label ‘T’), and within the Mid-Severn river catchment at points along the River Stour and Smestow Brook – a tributary of the Stour (sample label ‘S’) (Figure 9.1 – sample numbers assigned in the order starting from the furthest upstream location and ending at the furthest downstream location). The River Tame follows a course through the Birmingham-Wolverhampton metropolitan area and receives a diverse range of inputs including landfill leachate, surface water run-off and, in particular, from numerous large WwTWs. With the exception of the sample obtained from the furthest upstream point, all samples were expected to contain some proportion of sewage effluent. The furthest upstream location (T1) has, however, been reported to receive leachate inputs from a disused landfill site (Slacky Lane, closed 31/01/1972) (Environment Agency, 2011) known to contain significant concentrations of nickel (personal communication, John Churchley, formerly Severn Trent Water Limited). The River Tame is broadly typical of a heavily modified river.

The Smestow Brook flows to the west of Dudley and converges with the River Stour to the southwest of Dudley prior to its confluence with the River Severn. The Smestow Brook receives inputs from only one large WwTW, but is also expected to receive diffuse inputs from surface water runoff. The River Stour bisects Dudley and Stourbridge (prior to its confluence with the Smestow Brook) and does not receive any inputs from WwTW although is expected to receive surface water runoff from adjacent impermeable surface areas and potentially also and from misconnected pipes (i.e. where domestic foul water is incorrectly routed to the surface water drainage system) and from combined sewer (i.e. which receive foul and surface water inputs) overflows in periods of heavy rainfall. Detailed descriptions of the sample locations are provided in Table 9.1 and Table 9.2.

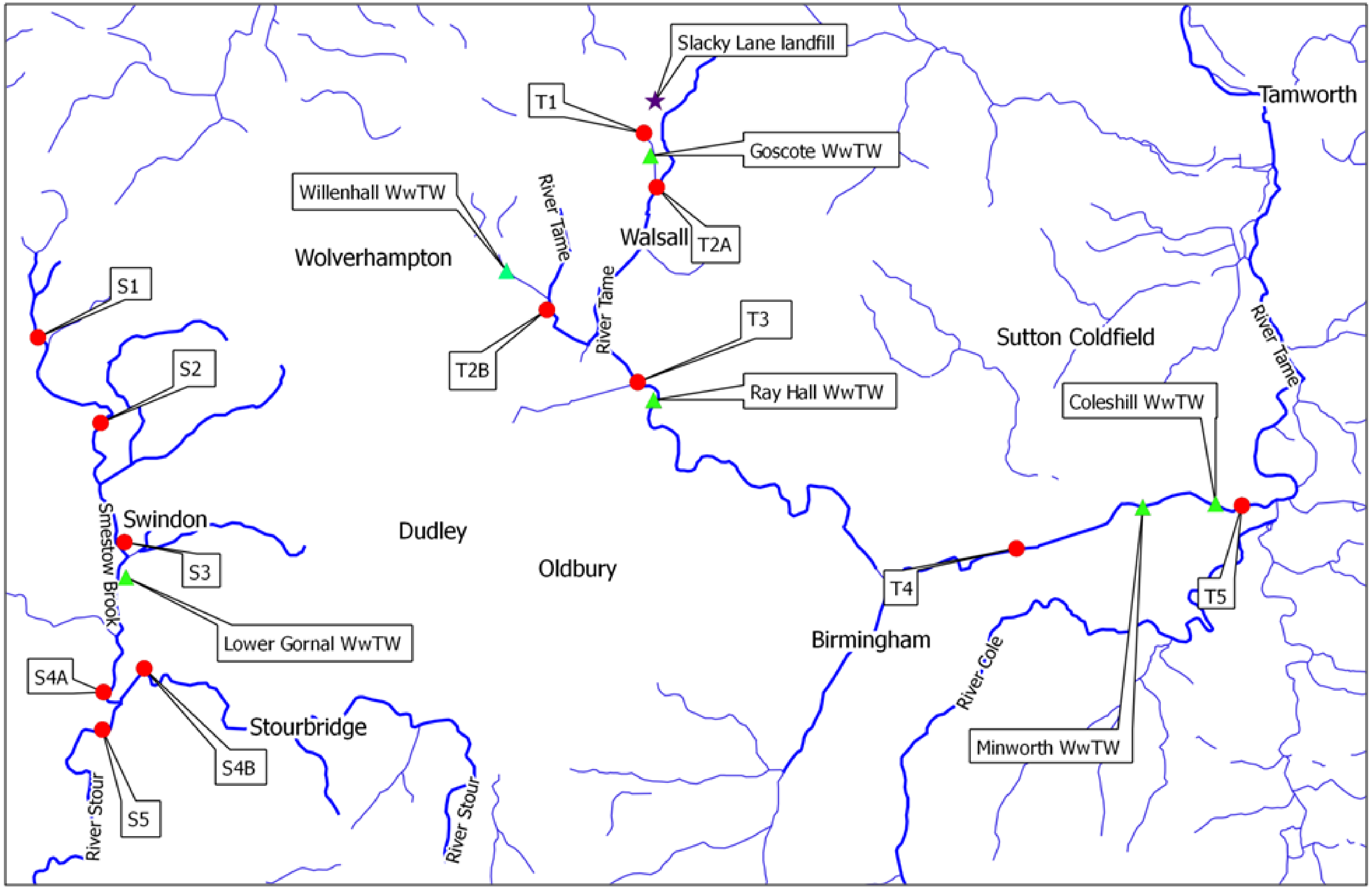

Figure 9.1 River Tame, River Stour and Smestow Brook sampling locations in relation to WwTWs and the Slacky Lane landfill

Table 9.1 Description of River Tame sample locations

Sample	Location (x,y coordinates)	Nearest post code	Description	Location description
T1	401897, 302263	WS3 4NW	Rough Brook (tributary of River Tame), immediately upstream of Goscote WwTW	Sub-urban/Roadside setting; in proximity to dwellings, visible inputs from disused landfill (Slackey Lane)
T2A	402205, 301133	WS3 1RU	Rough Brook(tributary of River Tame), immediately downstream of Goscote WwTW	Sub-urban/Roadside setting; in proximity to dwellings, anecdotal evidence suggests receives some inputs from disused landfill (Slackey Lane)
T2B	398957, 297503	WS2 9SF	River Tame, downstream of Willenhall WwTW	Urban/Roadside/Rail setting; along A4031 alongside railway line, in proximity to urban dwellings and light industrial units
T3	401767, 295401	WS5 4AN	River Tame, immediately upstream of Rayhall WwTW	Urban/Roadside/Rail setting; along A4031 under M5/M6 motorway alongside railway line, in proximity to urban dwellings
T4	413635, 290453	B35 7RD	River Tame, immediately upstream of Minworth (one of the largest WwTW in the UK) and Coleshill WwTW	Light industrial/Urban/Roadside setting; along A452 directly under M6 motorway, in proximity to business office parks and light industrial units
T5	420193, 291551	B46 1AB	River Tame, immediately downstream of Minworth and Coleshill WwTWs	Light industrial setting; in proximity to business office parks and light industrial units

Table 9.2 Description of River Stour and Smestow Brook sample locations

Sample	Location (x,y coordinates)	Nearest post code	Description	Location description
S1	383720, 296363	WV5 7HR	Sample location in headwaters of Smestow Brook	Rural/Agricultural setting
S2	385651, 293793	WV5 7HP	Smestow Brook	Rural/Agricultural setting
S3	386089, 290553	DY3 4NT	Smestow Brook, immediately upstream of Lower Gornal WwTW	Urban/Roadside setting; Swindon town centre along High Street
S4A	385982, 286375	DY7 5BA	Smestow Brook, downstream of Lower Gornal WwTW, immediately before to confluence with River Stour	Semi-rural setting
S4B	386926, 286293	DY7 5AJ	River Stour, prior to confluence with Smestow Brook, downstream of Dudley urban area	Roadside, semi-rural setting
S5	385708, 285603	DY7 5BD	Smestow Brook, downstream of convergence of River Stour and Smestow Brook	Semi-rural setting

9.3 Results

9.3.1 Water chemistry characteristics

The concentrations of nickel, and zinc in the River Tame samples were high (Table 9.3) in comparison with the River Stour and Smestow Brook (Table 9.4), reflecting the greater industrial nature (and legacy) of the River Tame catchment. The sample collected from location T1 (i.e. the furthest upstream sample location) contained the highest concentration of nickel which was attributable to leachate inputs from the Slacky Lane landfill. The concentrations of DOC were typically higher at locations immediately downstream of WwTWs (e.g. T2A, T5 and S4A) than elsewhere suggesting these effluents may represent a significant source of DOC. EDTA was not detected in any of the samples.

Table 9.3 Water chemistry characteristics of samples obtained from the River Tame

Sample	DOC (mg/L)	Ni (µg/L)	Zn (µg/L)
T1	4.4	335 (±29)	<5
T2A	9.2	56 (±3)	23 (±15)
T2B	3.4	137 (±13)	124 (±27)
T3	6.3	36 (±3)	31 (±12)
T4	6.8	16 (±9)	22 (±4)
T5	8.6	22 (±7)	54 (±4)

Table 9.4 Water chemistry characteristics for samples obtained from the River Stour and Smestow Brook

Sample	DOC (mg/L)	Ni (µg/L)	Zn (µg/L)
S1	3.3	<2	<5
S2	8.6	<2	<5
S3	8.2	<2	21 (±14)
S4A	9.3	<2	28 (±6)
S4B	5.0	<2	16 (±13)
S5	6.6	<2	<5

9.3.2 Nickel complexation characteristics

Nickel complexation characteristics were determined for the samples obtained from the River Tame (Table 9.3). With the exception of the samples obtained from locations T4 and T5 the concentrations of nickel exceeded the complexation capacity, suggesting much of the nickel in these samples to be bioavailable.

Total complexation capacity could be predicted from the DOC concentration by within a factor of 1.8 (Figure 9.2). Complexation capacity attributable to high affinity ligands (≥ log K 7) was detected in only two samples (T2A and T5), which were also directly downstream of sewage treatment works (Goscote and Minworth/Coleshill, respectively). These samples also contained the highest concentrations of DOC for the River Tame samples. The concentrations of high affinity ligands were also within a similar range as those determined previously for sewage effluents (Table 8.2, Chapter 8.1.3.2), further suggesting these to be comprised substantially of sewage effluent.

The reduction in the concentration of nickel between locations T1 (furthest upstream sample point, with inputs from Slacky Lane landfill) and T2A (immediately downstream of Goscote WwTW) was as a consequence dilution by the effluent discharged from Goscote WwTW. The increased in complexation capacity, along with the presence of high affinity ligands, suggests that the Goscote WwTW effluent further reduces the bioavailability of the nickel discharged from Slacky Lane. Whereas the sample obtained at location T2B, which was also downstream of a WwTW (Willenhall), did not demonstrate complexation capacity attributable to high affinity ligands, the relatively low DOC concentration (3.4 mg/L) (in comparison with that detected at the other locations directly downstream of effluent discharges) and since sample location was downstream of a point at which the flow from an alternate arm of the River Tame had converged (Figure 9.1), suggested that it was plausible that the high affinity ligands in the effluent had been diluted to the extent that their influence was not detectable. The relatively high concentration of nickel (and zinc) detected at location T2B, however, also indicates the existence of some other significant source of metal inputs into the River Tame upstream of the sample location. In summary, these findings indicate that high affinity ligands do occur in surface waters at a level at which they are detectable, and that sewage effluents may be a significant source. This was also in agreement with the findings from the dilution study described in Chapter 8.2. The Chelex titration data are provided in the data appendix for Chapter 9.

Table 9.5 Chelex method nickel complexation capacity and complexation capacity attributable to high affinity ligands (≥ log *K* 7)

Sample	Log K'	Chelex Total CC (µg/L)	Chelex High Affinity CC (µg/L)	Ni (µg/L)
T1	4.6 (3.7 – 5.0)	39 (21 – 118)	0	335 (±29)
T2A	5.9 (5.8 – 6.0)	54 (50 – 57)	18 (15 – 21)	56 (±3)
T2B	5.4 (5.0 – 5.7)	25 (18 – 38)	0	137 (±13)
T3	6.1 (5.9 – 6.3)	19 (17 – 22)	0	36 (±3)
T4	5.7 (5.5 – 6.0)	28 (23 – 36)	0	16 (±9)
T5	6.0 (5.9 – 6.2)	35 (31 – 39)	8 (0 – 12)	22 (±7)

Values in parentheses indicate 95% confidence interval range
CC = complexation capacity

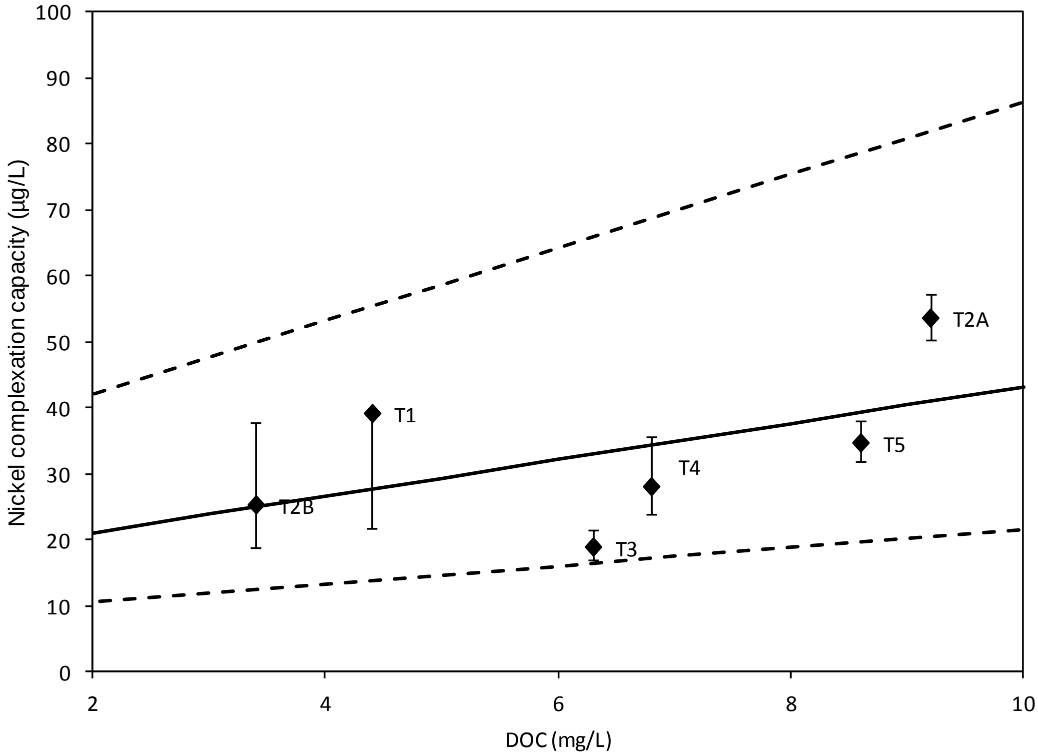

Figure 9.2 Nickel complexation capacity in relation to DOC for samples collected along the River Tame. Error bars represent the complexation capacity 95% confidence interval range. The upper confidence interval range for sample T1 has not been rendered due to its large size. The solid line represents the regression for the relationship between DOC and complexation capacity. The dashed lines represent the represent a factor of two range of the line of the regression.

9.3.3 Zinc complexation characteristics

Zinc complexation capacity titrations were conducted for samples from both river catchments, although values could only be determined for 9 out of the 12 samples (Table 9.6) since the post column concentrations of zinc for samples S1, S4A and S5 were below the limit of detection. This did, however, suggest that the complexing ligands in these samples may be weak in relation to the other samples.

Total complexation capacity was not well correlated with the concentration of DOC (Figure 9.4) and could only be predicted by within a factor of 2.6 (i.e. greater than the range within which BLM toxicity forecasts are considered accurate) using the relationship between DOC and complexation capacity as a basis for prediction, however, since this included only the samples for which complexation capacity could be determined the true factor range will be larger.

For the River Tame samples no statistically significant relationship between zinc and nickel complexation capacity values was found (Spearman rank correlation, $rs = 0.54$, $n = 6$, $P > 0.05$) suggesting that different ligands may be responsible for complexing either metal. Complexation capacity attributable to high affinity ligands for both metals was detected in samples T2A (immediately downstream of Goscote WwTW) and T5 (immediately downstream of Minworth and Coleshill WwTWs) and suggested that the high affinity ligand complexation capacity may be attributable to the same ligand(s). The samples obtained from T2A and T5 were also both immediately downstream of WwTWs, and further suggests that sewage effluents may be a significant source of high affinity ligands. However, complexation capacity attributable to high affinity ligands was detected in 2 samples which were not immediately downstream of any WwTWs (S3 – Swindon town centre, S4B – road side semi-rural setting), and which also exceeded the complexation capacity values for T2A and T5. This suggested that there may also be sources of high affinity ligands which are not derived from sewage effluents. The Chelex titration data are provided in the data appendix for Chapter 9.

Table 9.6Chelex method zinc complexation capacity and complexation capacity attributable to high affinity ligands (≥Log K 10)

Sample	Log K’	Chelex Total CC (µg/L)	Chelex High Affinity CC (µg/L)	Zn (µg/L)
T1	5.6 (4.7 – 6.3)	7 (3 – 217)	n.d	<5
T2A	6.9 (6.6 – 7.5)	18 (15 – 21)	14 (14 – 15)	23 (±15)
T2B	7.8 (5.5 – n.d)	7 (4 – 17)	6 (n.d.)	124 (±27)
T3	5.2 (3.8 – 5.6)	6 (2 – n.d.)	n.d	31 (±12)
T4	6.5 (6.1 – 7.4)	12 (10 – 16)	1 (0 – 11)	22 (±4)
T5	6.6 (6.3 – 7.2)	23 (20 – 27)	15 (9 – 20)	54 (±4)
S1	n.d	<5	n.d	<5
S2	6.9 (6.4 – n.d)	24 (19 – 32)	n.d	<5
S3	7.2 (6.8 – n.d)	21 (17 – 24)	19 (0 – 19)	21 (±14)
S4A	n.d	<5	n.d	28 (±6)
S4B	7.2 (6.9 – 7.8)	26 (24 – 29)	24 (0 – 24)	16 (±13)
S5	n.d	<5	n.d	<5

Values in parentheses indicate 95% confidence interval range
CC = complexation capacity
n.d. = could not be determined

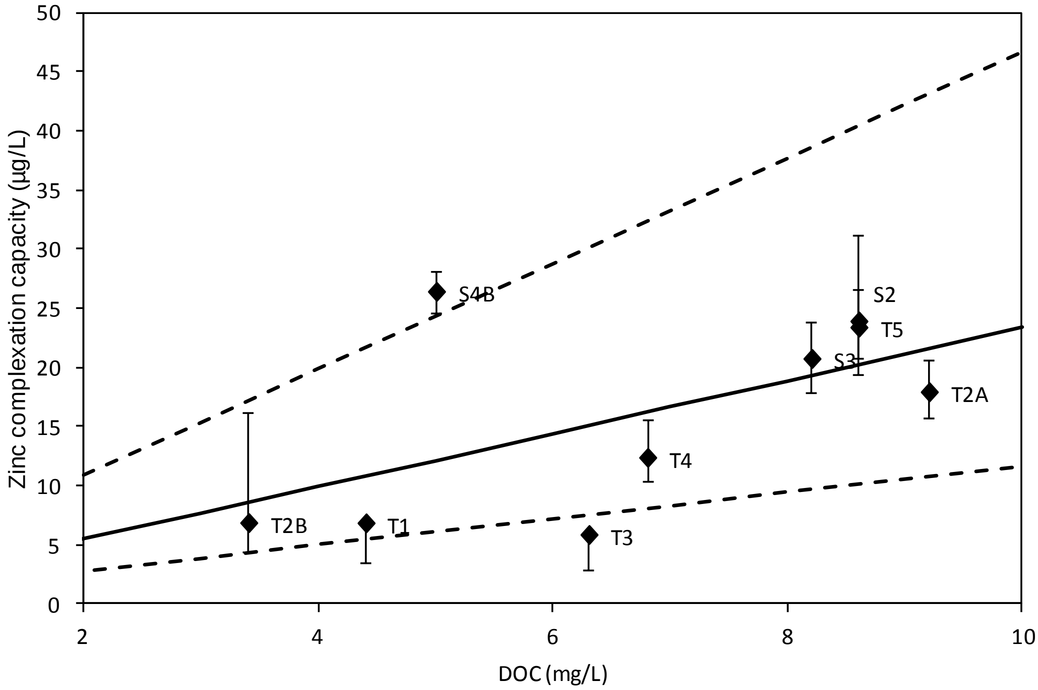

Figure 9.3 Zinc complexation capacity in relation to DOC concentration. Error bars represent the complexation capacity 95% confidence interval range. The upper confidence interval range for samples T1 and T3 have not been rendered due to its large size. The solid line represents the regression for relationship between DOC and complexation capacity. The dashed lines represent the represent a factor of two range of the line of the regression.

9.4 Discussion

The occurrence of high affinity ligands in anthropogenically impacted waters has been observed recently by Baken *et al.* (2011), who investigated the metal binding characteristics of DOM. Baken *et al.* (2011) identified an excess binding capacity (i.e. the difference between that predicted by WHAM VI and the observed complexation capacity) which they attributed to the presence of aminopolycarboxylate chelating agents (EDTA in particular) although they also found that these could only explain between 10-43% of the excess capacity and hypothesised that the difference may be attributable to some other synthetic ligand. However, whereas the occurrence of ligands with a high affinity for trace metals has typically been associated with sewage effluents (Cheng and Allen, 2006; Cheng, 2005; Sarathy and Allen, 2005), which the findings from the present study support, the results have also, however, indicated that high affinity ligands may potentially also be derived from sources other than WwTWs (e.g. the high affinity ligands detected in samples S3 (Swindon town centre) and S4B (semi-rural)). Although the identity of these ligands was not determined it is plausible that these might also be attributable to anthropogenic inputs; for example, from sewerage network misconnections where domestic wastewater is incorrectly routed into the surface water drainage network, and from wastewater discharges from septic tanks which serve individual houses and that are common in rural areas not served by a public sewerage connection. Indeed, substances such as EDTA are also used as a component in fertiliser formulations (to enhance crop uptake of trace metals) and as a stabiliser in plant protection products (European Chemicals Bureau, 2004). Consequently EDTA, and other similar substances, might also occur in rural areas as a result of surface water run-off. The occurrence of anthropogenic ligands in surface waters in rural or agricultural areas may therefore not be uncommon.

The variability in complexation characteristics observed in the studies throughout this research argues in favour that some real-world validation of complexation characteristics should be incorporated within the approach for assessing compliance with environmental quality standards proposed by the Regulator (as described in Chapter 2.2.4). A recent discussion with the Environment Agency (meeting with Paul Whitehouse, 01/11/2011) indicated some support for an approach whereby real-world complexation characteristics are taken into account in assessing metals risk, and for determining compliance with environmental quality standards. However, the manner in which such approach might be implemented requires further investigation although the approaches described in Chapter 7 (site-specific active fraction) and in Chapter 8 (additional high affinity ligand complexation capacity) may be suitable.

9.5 Conclusions

The findings from this study indicated substantial variability in the zinc complexation characteristics of water samples obtained even within the same river. High affinity ligands were detected at locations immediately downstream of WwTWs, indicating these to be significant sources of high affinity ligands, although were also detected in surface water samples obtained from rural locations, suggesting there may be sources of high affinity ligands other than WwTW. These findings contribute support to the view that some consideration for the influence of site-specific complexation characteristics should be incorporated within the tiered approach to compliance that has been proposed by the Environment Agency.

10. COMPLIANCE IMPLICATIONS OF THE BLM APPROACH

The study described in the following chapter was aimed at assessing the implications for Severn Trent Water Limited of the BLM approach being applied to determine EQSs for copper and zinc, and of the WFD 20 µg/L EQS for nickel. The objective of this study was to identify the wastewater treatment works that may become eligible for a discharge consent as a consequence of the new standards. This was useful for Severn Trent Water in order to better understand the implications of the new standards, and in particular, to ascertain the extent by which additional measures (treatment capacity or infrastructure investment) may be required as a consequence of these new standards.

The findings from this study indicated different impacts for the considered metals. For copper, reductions in discharge concentrations may be required for only a small number of WwTWs. For nickel, whereas the WFD EQS is substantially more stringent than under the DSD, WwTWs discharge into River Tame, or tributaries thereof, are particularly impacted. The findings for zinc indicate the likelihood of widespread EQS exceedances throughout the Severn Trent Water region, with numerous WwTWs likely to contribute to these EQS exceedances so that substantial reductions in the concentrations of zinc in sewage effluent discharges may be required.

10.1 Experimental Approach

Biotic ligand models for copper and zinc were applied to estimate site specific water quality standards for Environment Agency (river) water quality monitoring locations within the Severn Trent Water operating area. The compliance status of each location, and metal, was determined by comparison of the calculated water quality standard with the average of the measured concentrations. The locations at risk of non-compliance were those where the average of the measured concentrations exceeded the calculated standard. For nickel, compliance status was determined by comparison with the 20µg/L standard specified by the WFD. Compliance was also assessed relative to the standards specified by the Dangerous Substances Directive in order to evaluate the impact of new standards. The WwTWs at which reductions in discharge concentrations may be required were identified using the Environment Agency criteria that are applied to determine whether treatment works require numeric discharge consents (Martin and Armitage, 2005). Further details on the approach applied in this assessment are provided in Chapter 4.4.

10.2 Results

10.2.1 Compliance at water quality monitoring locations

Copper. The data permitted an assessment of compliance for 1,245 water quality monitoring locations (76% of the total number of water quality monitoring locations). The copper concentration exceeded the BLM EQS value at 20 locations (1.6% of the total) (Figure 10.1) suggesting relatively few locations to be at risk of exceeding the BLM based value. The copper concentration exceeded the DSD EQS value at 40 locations (Figure 10.2), of which 10 were in common with exceedances of the BLM EQS. This suggested that the BLM approach is unlikely to substantially alter the overall compliance status of the monitoring locations within the Severn Trent Water region but also that EQS exceedances are likely to occur at locations at which the concentrations of copper are already of concern. Whereas the overall effect of the BLM approach on compliance appears limited, a comparison of the EQS values (Table 10.1) revealed substantial changes in site-specific EQS values. For example, 1,026 locations which (currently) qualify for an EQS of 28 µg/L under the DSD will, under the BLM approach, become eligible for a standard that is more stringent (i.e. <28 µg/L), and 468 locations which are eligible for an EQS of 10µg/L under the DSD will become eligible for an EQS which is less stringent (>10 µg/L). The effect of the BLM approach on compliance risk therefore appears limited since the concentrations of copper at the locations which may become eligible for more stringent standards were generally sufficiently low so that no additional risk to compliance was expected to occur, albeit that the ‘headroom’ between the observed concentration and the compliance statistic would be eroded.

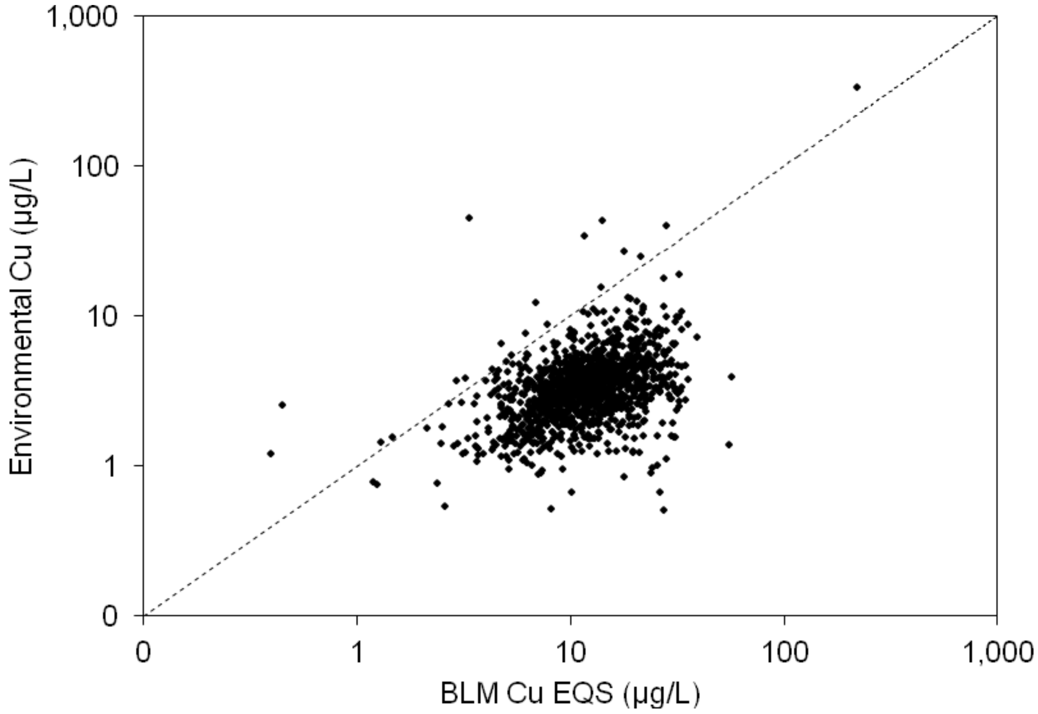

Figure 10.1 Concentrations of dissolved copper relative to the EQS value suggested by the copper BLM. The dashed line divides compliant (below line) and non-compliant (above line) locations.

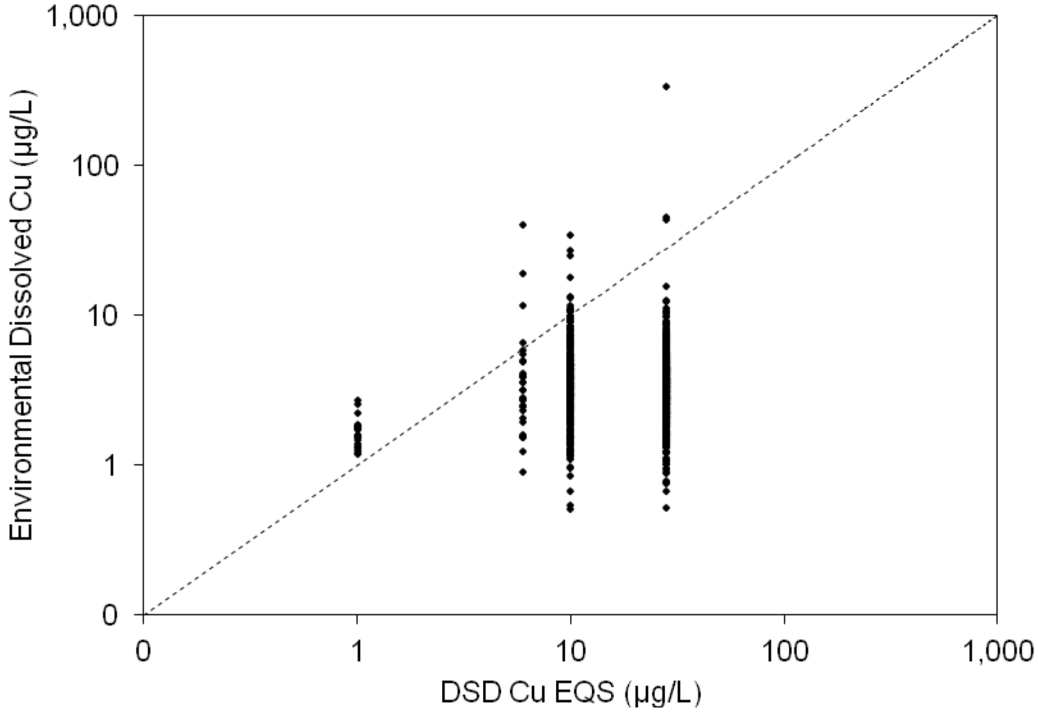

Figure 10.2 Concentrations of dissolved copper relative to the EQS value as specified by the DSD. The dashed line divides compliant (below line) and non-compliant (above line) locations.

Table 10.1 Comparison of BLM and DSD EQS values for copper at monitored locations

No. of water quality monitoring locations		DSD EQS Band (µg/L)			
		1	6	10	28
BLM EQS (Banded) (µg/L)	≤1	2	0	0	0
	≤6	9	0	19	119
	≤10	6	4	43	411
	≤28	8	21	433	496
	>28	13	13	35	4

Nickel. Data were available to assess the compliance status for nickel at 421 (26%) of the monitored locations in relation to the 20µg/L WFD EQS. Nickel concentrations exceeded the WFD EQS value at 28 locations (Figure 10.3) compared with only 3 locations under the DSD EQS (Figure 10.4), indicating a substantial increase in compliance risk. A review of the locations at risk of exceeding the 20 µg/L standard indicated that most (15) occurred along the River Tame (or its tributaries) on its course through the Birmingham-Wolverhampton metropolitan area (described in the study in Chapter 9) for which nickel inputs are expected to occur as a consequence of both current and historic anthropogenic inputs.

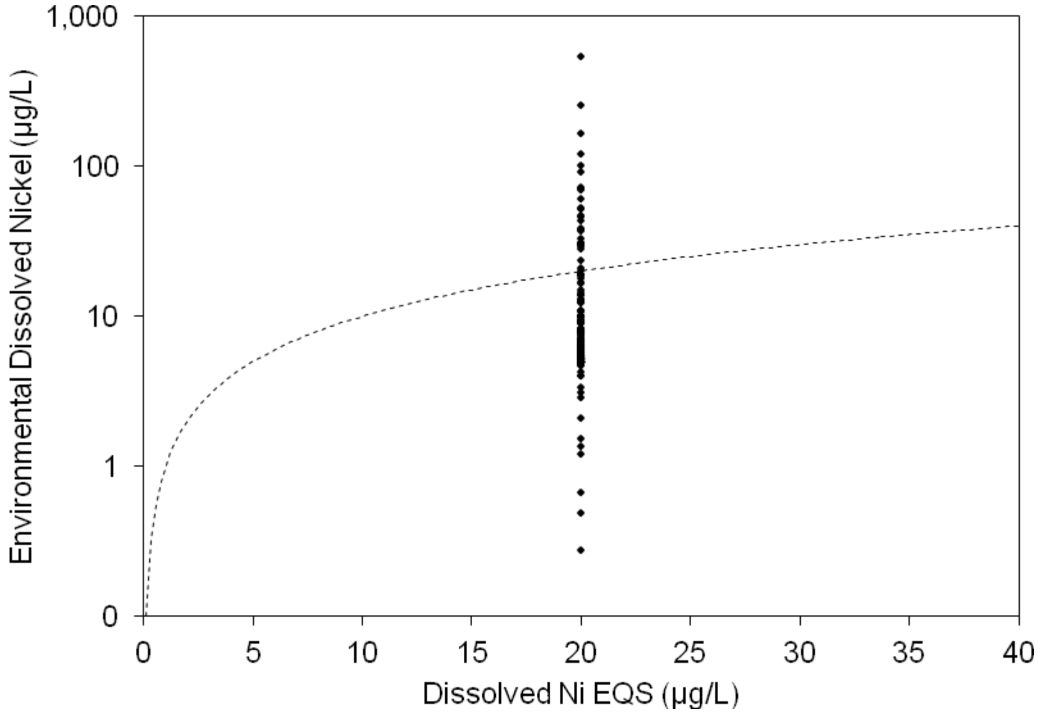

Figure 10.3 Concentrations of dissolved nickel relative to the WFD 20µg/L EQS value. The dashed line divides compliant (below line) and non-compliant (above line) locations.

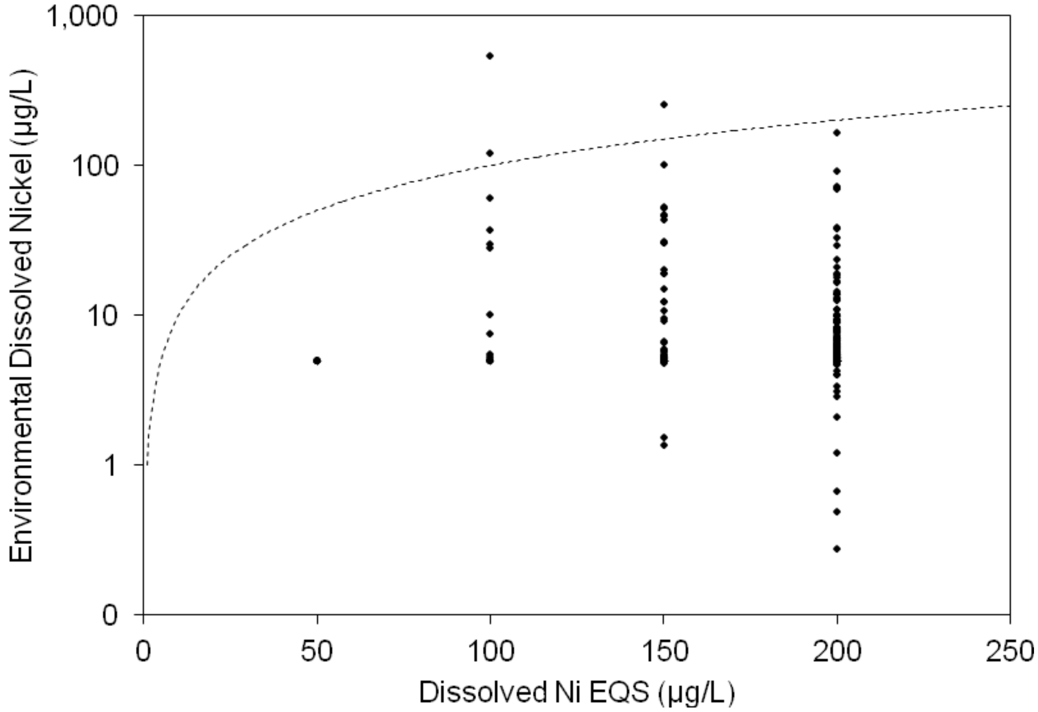

Figure 10.4 Concentrations of dissolved nickel relative to the EQS as specified by the DSD. The dashed line divides compliant (below line) and non-compliant (above line) locations.

Zinc. Data were available to assess EQS compliance for zinc at 934 of the monitoring locations (57% of the total), of which 246 locations (26%) were found to be at risk of exceeding the BLM EQS value (Figure 10.5), of which 131 sites (53%) exceeded the BLM EQS by more than 10µg/L. This suggested that the EQSs were typically exceeded by a substantial margin. Only 38 locations were determined to be at risk of exceeding the relevant DSD value (Figure 10.6), of which only 7 were in common with the locations deemed to be at risk of exceeding the BLM based EQS. This indicates a potentially substantial increase in the compliance risk for zinc within the Severn Trent Water region. A review of the geographical distribution of the locations at risk (Figure 10.7) indicated these to be spread throughout the Severn Trent Water region, with a number of risk clusters occurring in and around urban areas such as Birmingham, Wolverhampton, Stoke-on-Trent, Derby, Doncaster and Chesterfield, but also in a number of non-urban areas, such as along the River Derwent in the Pennines, and in Mid Wales, both regions associated with historical or ancient mining activities rather than sewage inputs.

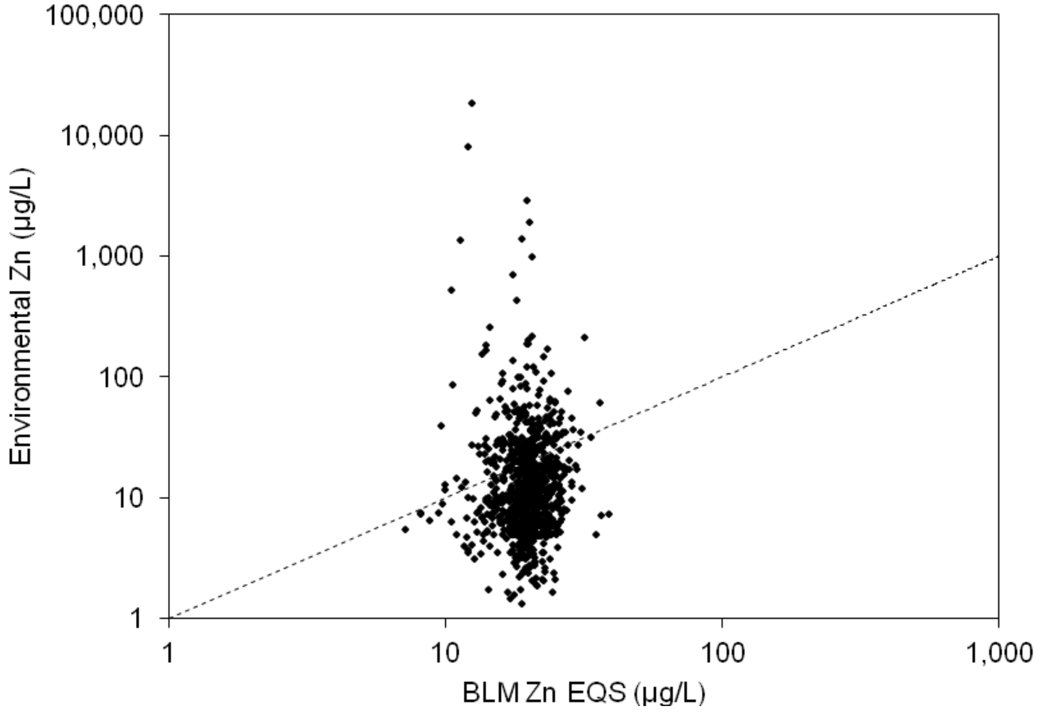

Figure 10.5 Concentrations of dissolved zinc relative to the EQS value suggested by the zinc BLM. The dashed line divides compliant (below line) and non-compliant (above line) locations.

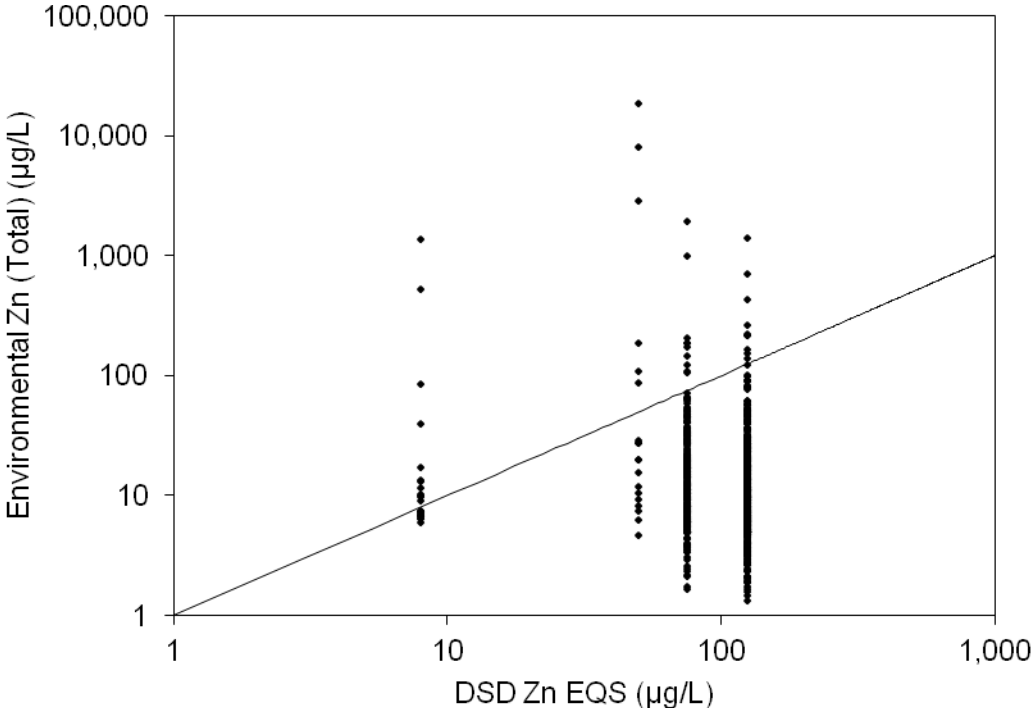

Figure 10.6 Concentrations of dissolved zinc relative to DSD EQS values. The dashed line divides compliant (below line) and non-compliant (above line) locations.

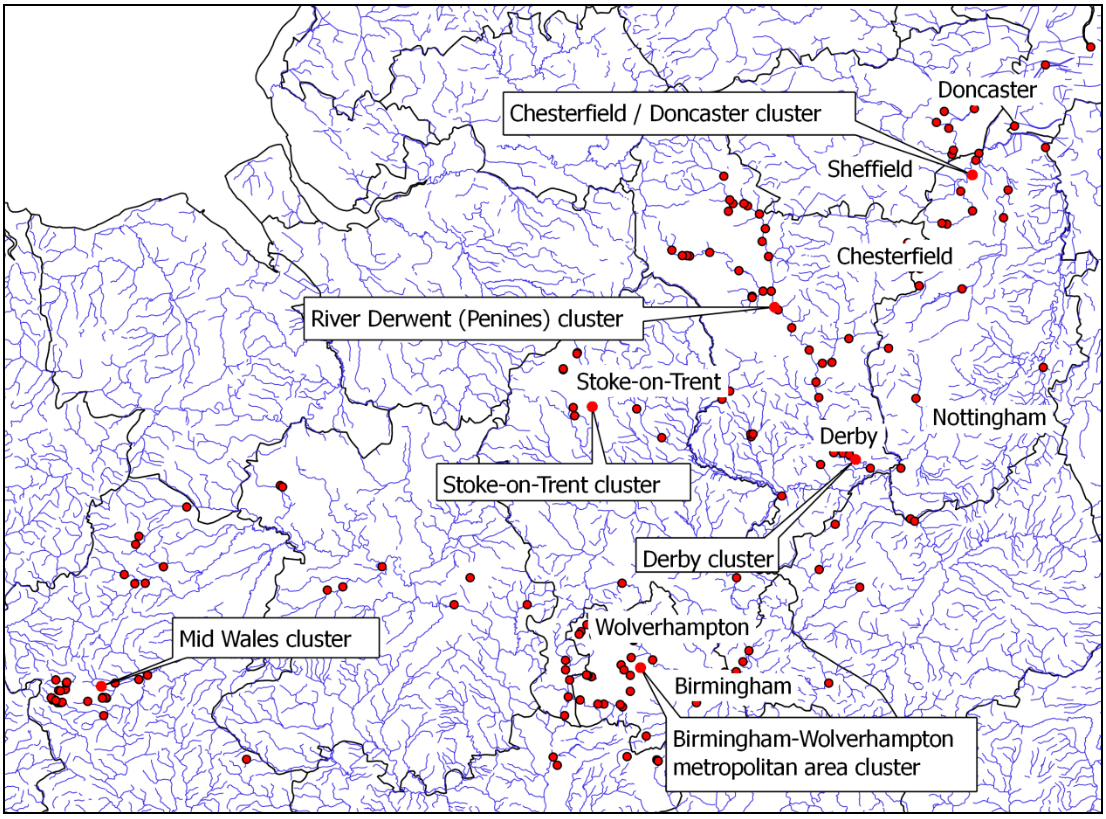

Figure 10.7 Water quality monitoring locations where the concentrations of zinc exceed EQS values determined using the BLM approach.

10.2.2 Implications of EQS exceedances for WwTW discharges

Copper. The compliance simulation for copper indicated only 9 WwTWs to be at risk of contributing to an exceedance of the downstream EQS value as determined using the copper BLM (Table 10.2), and a further 43 for which the discharge concentrations were considered ‘significant’ (i.e. increase in downstream concentration by >10% of EQS value). Only 3 of the WwTWs which were at risk were, however, considered large (>20k population equivalent treatment capacity), namely, Tamworth (91k p.e.), Malvern (39k p.e.) and Tewksbury (21k p.e.). The discharge concentrations from these WwTWs were also relatively high, representing the 98%ile (55µg/L), 78%ile (16µg/L) and 100%ile (100µg/L) of the WwTW discharge concentration values, respectively, suggesting these works may receive trade inputs with high concentrations of copper which might therefore contribute substantially to the downstream EQS exceedance.

For the purposes of comparison, the WwTWs at risk of contributing to an exceedance of DSD EQS values (i.e. those which might currently be expected to have discharge consents for copper) were also identified. In this comparison 23 WwTWs were found to be at risk, 8 of which were large, namely Coleshill, Goscote, Tamworth, Yaddlethorpe, Bromsgrove, Lower Gornal, Burntwood and Walsall Wood, with only one of these in common with the ‘at risk’ treatment works identified using BLM EQS values (Tamworth) (Table 10.2). Similarly, the discharge concentrations from the 43 treatment works which had been identified as ‘significant’ in accordance with BLM EQS values were also ‘significant’ in relation to the DSD EQS values. In summary, this assessment suggests only a minimal impact on compliance risk as a consequence of EQSs for copper determined via the BLM approach.

Table 10.2 Comparison of the number of WwTWs contributing to downstream copper EQS exceedances under the BLM and DSD regulatory regimes

	BLM EQS AT RISK	BLM EQS NOT AT RISK	Total
DSD EQS AT RISK	1	22	23
DSD EQS NOT AT RISK	8	674	682
Total	9	696	705

Nickel. The assessment for nickel found 13 treatment works to be at risk of contributing to an exceedance of the downstream WFD EQS value (20 µg/L), and a further 15 for which the discharge concentrations were considered ‘significant’. Of the works at risk, 5 were large (Goscote, Minworth, Walsall Wood, Rayhall and Willenhall), and all discharged directly into the River Tame, or tributaries thereof. The concentrations of nickel discharged from these works were, however, relatively high (98%ile, 71%ile, 94%ile, 93%ile, and 100%ile of the WwTWs discharge concentration values, respectively) although none were in common with the works at risk for copper.

For the purposes of comparison, the WwTW at risk of contributing to an exceedance of DSD EQS values (i.e. those which might currently be expected to have discharge consents for nickel) were also identified, of which only one treatment works (Goscote) was found to be at risk of contributing to the downstream exceedance of the DSD EQS value, which was also in common with the WFD ‘at risk’ WwTWs (Table 10.3). The relatively small increase in the number of works at risk, in spite of the substantially more stringent EQS value was as a consequence that the concentrations of nickel in effluent discharges were generally low (<5 µg/L) throughout the region.

Table 10.3 Comparison of the number of WwTWs contributing to downstream nickel EQS exceedances under the WFD and DSD regulatory regimes

	WFD EQS AT RISK	WFD EQS NOT AT RISK	Total
DSD EQS AT RISK	1	0	1
DSD EQS NOT AT RISK	12	692	704
Total	13	692	705

Zinc. The assessment for zinc found 126 WwTWs to be at risk of contributing to downstream exceedances of the zinc BLM EQS values, of which 36 of the WwTWs were considered large. The discharge concentrations from a further 112 WwTWs were also considered ‘significant’.

For the purposes of comparison, the WwTWs at risk of contributing to an exceedance of DSD EQS values (i.e. those which might currently be expected to have discharge consents for zinc), only twelve works were found to be at risk, with only one of these works considered to be large (Willenhall), which was also in common with the works at risk under the BLM approach (Table 10.4). The discharge concentrations from a further 21 works were considered ‘significant’. In comparison with the DSD, the BLM approach appears likely to generate a substantial increase in compliance risk.

Table 10.4 Comparison of the number of WwTWs contributing to downstream zinc EQS exceedances under the DSD and BLM regulatory regimes

	BLM EQS AT RISK	BLM EQS NOT AT RISK	Total
DSD EQS AT RISK	11	1	12
DSD EQS NOT AT RISK	115	578	693
Total	126	579	705

10.3 Discussion

10.3.1 Consideration of the ambient background concentration

The compliance assessment was conducted on the basis of a face value comparison of the metal concentrations and the simulated dissolved metal EQS, however, it has been envisaged that some allowance for an ambient or natural background concentration will be incorporated within the final EQS value (Peters, Merrington and Brown, 2009), which is intended to reflect the acclimation and adaptation by organisms to geological influences that may have occurred over time. Since the BLM calculated EQS values have not incorporated such an allowance, the assessment may, to some extent, provide a relatively conservative perspective on risk; however, the current view on the allowance for natural background concentrations indicates that these are unlikely to be substantial. For copper, a value of 0.5 µg/L has been proposed, and for zinc, values of 3.0 µg/L, 3.7 µg/L, and 5.0 µg/L have been tentatively proposed (Peters, Merrington and Brown, 2009). Since more than 50% of the monitoring locations exceeded the predicted zinc EQS by more than 10µg/L, the findings from this assessment are unlikely to be sensitive to this allowance and consequently EQS exceedances throughout the Severn Trent Water region appear plausible.

Whereas some consideration for the natural background is sensible since zinc is also a micro-nutrient and essential for ecosystem health, it was, notable that Peters, Merrington and Brown (2009) considered only inputs from the underlying geology (e.g. soil) to represent the natural background. The implication is that the proposed values will not take into account the effect of acclimation and adaptation that might already have occurred in response to anthropogenic inputs. Indeed, the broad scale at which environmental concentrations of zinc have been predicted to exceed the BLM derived EQS values would suggest there should be evidence of widespread environmental harm attributable to zinc. Whereas the extent by which this is supported by evidence is uncertain, a recent assessment of the relationship between concentrations of zinc and ecological quality (Comber and Georges, 2008) found that some catchments supported ecology of good or high status even where concentrations of zinc are elevated (>100 µg/L). Furthermore, a study by Muyssen, Janssen and Bossuyt (2002) also found that wild populations of *D. magna* were substantially more tolerant of elevated concentrations of zinc in comparison with laboratory cultured populations (wild population EC50 was greater than the laboratory population EC50 by a factor of 4) and another similar study (Muyssen and Janssen, 2001) found that two algal species (*Raphidocelis subcapitata* and *Chlorella vulgaris*) commonly used in toxicity testing were able to increase their tolerance for zinc by a factor of 3 within a relatively short period of time (over a period of 100 days). Indeed, numerous other studies (De Schamphelaere *et al.*, 2004; Muyssen and Janssen, 2004; Muyssen and Janssen, 2002; Muyssen and Janssen, 2000) have also demonstrated the significance of acclimation and shifts in tolerance as a consequence of exposure to zinc. Consequently, the extent by which zinc EQS compliance may be indicative of environmental harm, without consideration for acclimation to anthropogenic inputs, is uncertain. Further research into how acclimation may be accommodated within the proposed regulatory framework is clearly required, although perhaps in particular, to consider the extent by which acclimation to anthropogenic influences should be taken into account.

10.3.2 Elements of uncertainty in the underlying basis for BLM derived environmental quality standards for zinc

In calculating site-specific EQS values for zinc as part of this risk assessment, it was notable that the bioavailability correction factor (BioF) for *P. Subcapitata* (algae) most substantially influenced the calculated EQS values (the BioF is used to calculate the site specific EQS, as described in Chapter 3.5, and is indicative of the organism's sensitivity to the effects of exposure to zinc). Further examination of the original study in which the biotic ligand characteristics for *P. Subcapitata* were determined (De Schamphelaere, Lofts and Janssen, 2005), found that chronic zinc toxicity could not be adequately explained using the traditional BLM approach (i.e. a single binding site for which ions compete) and consequently, the model developed to predict zinc toxicity to *P. Subcapitata* was based on an empirical relationship between toxicity, Zn^{2+} activity and pH. Although the empirical model predicted NOEC values by within a factor of two, the authors of the study (De Schamphelaere, Lofts and Janssen, 2005) recommended further testing (with *P. Subcapitata)* to improve the basis upon which the toxicity forecasts had been produced. Whereas the recommendation for additional data is not necessarily an unusual outcome of toxicity testing, this should, however, also be seen within the context that the generic PNEC value (7.8 µg/L), which was applied in calculating site-specific EQS values, had been derived by dividing the original experimentally determined generic PNEC (i.e. the HC5 value from a species sensitivity distribution determined under conditions of high zinc bioavailability) of 15.6 µg/L by a factor of two on the basis of concern that this may not have been sufficiently protective of sensitive organisms (algae in particular) (European Commission, 2010).

Consequently, the EQS values for zinc may be precautionary due to uncertainty in the sensitivity of *P. Subcapitata* to zinc; however, this also suggests that reducing uncertainty in the prediction of toxicity to *P. Subcapitata* might also eliminate the need for an assessment factor, and therefore enable the determination of EQS values that are, arguably, less precautionary.

Indeed, with further consideration for the conservatism in the EQS values that may exist as a consequence of failing to take into account the influence of acclimation to anthropogenic inputs, the potentially substantial implications of numerous and widespread EQS exceedances may be avoided by reducing uncertainty and conservatism in the prediction of toxicity to *P. subcapitata*.

10.4 Conclusions

The results from the assessment have indicated substantially different impacts on compliance risk for the considered metals. For copper, the results suggest the BLM approach might reduce the number of WwTWs that contribute to downstream EQSs exceedances, while for nickel, whereas the WFD EQS is substantially more stringent than under the DSD, the compliance risk impact appears likely to occur in only a limited number of locations, primarily along the River Tame within the Birmingham-Wolverhampton metropolitan area, and largely as a consequence of the region's industrial nature (and legacy). The results for zinc, however, suggest the likelihood of widespread EQS exceedances in both rural and urban areas, and also that numerous WwTWs are likely to contribute to these EQS exceedances. However, there also appears also be a substantial degree of conservatism inherent in the BLM predicted EQS values arising from, in the first instance, uncertainty in the underlying toxicity data for P. *subcapitata*, and in the second instance, as a consequence of failing take into account the extent by which aquatic organisms may be adapted to the current conditions. More research is required reduce uncertainty in the zinc BLM parameterisation.

11. FINAL DISCUSSION

11.1 Accommodating environmental variability within the tiered approach

The BLM approach has been demonstrated to provide a useful framework for assessing risk in that it takes account of the influence of organism sensitivity and chemical speciation, and consequently, the factors that influence metal bioavailability. Indeed, the results from the studies in Chapter 5 and 6 demonstrated that BLMs are able to accurately predict metal bioavailability, even in complex chemical mixtures such as sewage effluents. The results have, however, also demonstrated that effluent derived organic matter may offer greater protection against potentially adverse effects from metals as a consequence of its greater intensity of complexing ligands compared with organic matter derived from natural sources. Consequently, BLM forecasts may be precautionary when applied in effluent impacted waters, however, the findings also demonstrated that forecast accuracy could be improved by taking account of the differences between effluent and naturally derived organic matter, which further suggests an opportunity to improve the environmental relevance of compliance criteria determined using this approach.

Whereas from the perspective of assessing risk it may be desirable to adopt a conservative approach and the use the BLM in ‘default’ mode, for the purposes of specifying statutory water quality standards, which must be achieved, the corresponding environmental and socioeconomic consequences of such a precautionary approach might themselves be undesirable (e.g. the financial cost associated with additional wastewater treatment). Indeed, as a consequence that BLMs will be used in a regulatory context (i.e. for setting standards and discharge consents) it should further be considered that environmental quality standards must also ensure that the level of protection afforded is commensurate with the cost of achieving the desired degree of protection (Farmer *et al.*, 2010; Royal Commission on Environmental Pollution, 1998), which is also in accordance with WFD recommendations. These factors argue in favour of taking account of the influence of effluent derived organic matter, but also the natural variability in complexation characteristics which may occur.

The manner in which the Regulator has formulated the policy within which the BLM approach will be applied (i.e. a tiered approach, Chapter 2) can, however, better accommodate environmental variability, and thereby reduce the risk that BLM derived standards may be over-precautionary, with only minor modification. For example, Figure 11.1 shows a modified version of the tiered approach proposed by the Regulator, within which it is suggested that site specific complexation characteristics are taken into account at the 4th tier stage (4b), alongside that of the natural background conditions. Such an approach would also have the effect of limiting the number of locations at which it may be necessary to evaluate site specific complexation capacity so as to be practicable. The method suggested in Chapter 7 (i.e. using the Chelex method to estimate active fraction values) offers a plausible method whereby this may be achieved although research into other methods would also be beneficial.

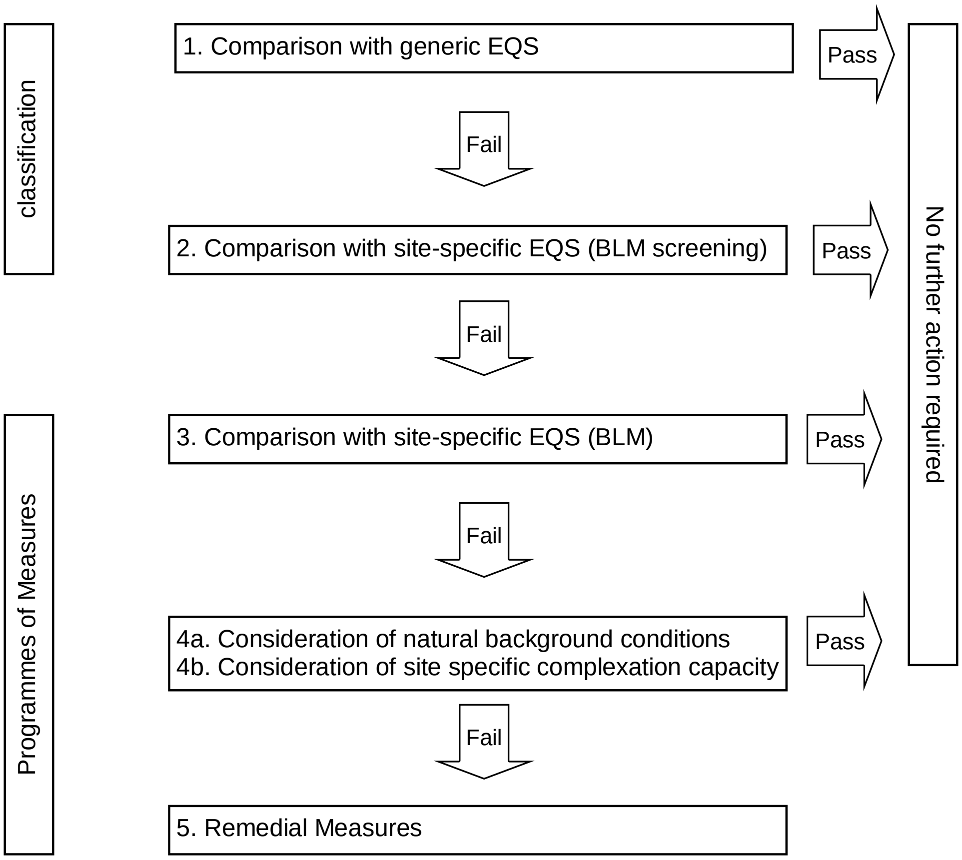

Figure 11.1 Modified tiered approach to compliance which includes the consideration of site specific complexation capacity at the 4th tier stage

11.2 Exceptions to the BLM approach

Although numerous studies have demonstrated that metal toxicity is reduced by complexation with organic matter (De Schamphelaere and Janssen, 2004b; De Schamphelaere *et al.*, 2004; Paquin *et al.*, 2000; Allen and Hansen, 1996) or synthetic complexants such as EDTA (European Chemicals Bureau, 2004), there is also some evidence to the contrary which should be objectively considered within the context of biotic ligand modelling. For example, in their study Phinney and Bruland (1997) demonstrated that certain organic compounds form neutral lipophilic copper complexes that are transported rapidly across biological membranes so that the complex exhibits a relatively higher degree of toxicity than the free copper ion. In their study, Phinney and Bruland (1997) identified a number of ingredients used in dithiocarbamate fungicides that exhibit these characteristics and suggested this to be an important consideration where these are applied to agricultural fields adjacent to water. Furthermore, other organic compounds, which might plausibly be expected to occur in sewage effluent, have also been demonstrated to increase metal bioavailability. For example, there are reports of toxicity to *D. Magna* arising from the Cu^{2+}-glycine complex (Campbell, 1995; Borgmann and Ralph, 1983), and of enhanced zinc bioavailability in the presence of citrate and ethylenediamine (Campbell, 1995; Guy and Ross Kean, 1980). Indeed, a recent study by Tusseau-Vuillemin *et al.* (2004) found 10µM/L of glycine (~12µg/L) reduced the concentration of Cu^{2+} required to induce toxicity at the 50% effect level by three quarters. Although these substances would not ordinarily be expected to occur in significant concentrations in natural waters (if at all), their occurrence in wastewater effluents is certainly plausible; for example, ethylenediamine may be derived as a breakdown product of EDTA which was detected in all effluents described in the studies in Chapters 5 to 8. Whereas there is no evidence of environmental harm attributable to substances which enhance metal bioavailability, the evidence from the laboratory studies described suggests that the environmental relevance of substances which might enhance metal bioavailability is certainty worthy of additional research.

Another, traditionally unconsidered exception to the current BLM approach for copper, relates to toxicity from monovalent metal species. Current BLMs do not take into account the effect monovalent copper since, ordinarily, the proportion of total copper present in monovalent form would be expected to be extremely low. Monovalent copper is, however, used in antifouling paints applied to the hulls of boats and other underwater structures to prevent colonisation by a variety of marine organisms. Indeed, a recent study by Turner, Pollock and Brown (2009) suggested that sediments in harbour areas may be comprised of antifouling paint particles by up to about 1% by weight. Consequently, there may be a need to further expand the current BLM approach to take into account toxicity from monovalent species. This may, however, perhaps be of greater significance for the development of a marine or estuarine BLM, where inputs from antifouling paints are likely to be of greater significance than in the freshwater environment.

11.3 Extending the BLM approach

11.3.1 Non-metal toxicants

The results presented in Chapter 5 and 6 have demonstrated the ability of a number of BLMs to predict acute copper toxicity to *D. magna* in a sewage effluent medium, and as already described contribute further support for the robustness of the BLM approach. However, in addition to assessing the impact of metals in the aquatic environment, the BLM approach has also recently been applied to predict ecotoxicological impacts in the terrestrial environment, and a number of models have been developed which predict metal bioavailability to plants and soil based organisms such as earthworms (Wang *et al.*, 2010; Li *et al.*, 2009; Luo, Li and Zhou, 2008; Lock *et al.*, 2007; Koster *et al.*, 2006), further underlining the significance of environmental factors on the impact of metals, but also the usefulness of the BLM as a platform for quantifying ecotoxicological impacts.

Whereas at present the BLM approach has been applied only to metals, it is plausible that the BLM approach may be applied to other substances, perhaps in particular where the chemical form of a substance (i.e. its speciation) influences its bioavailability. At present there do not appear to have not been any reports of attempts to do so, although the academic literature suggests a number of opportunities. For example, a study by Akkanen and Kukkonen (2003) which evaluated the toxicity of two common organic toxicants, benzo(a)pyrene (a Priority Hazardous Substance under the WFD) and 3,3’,4,4’-tetrachlorobiphenyl, using toxicity assays with *D. magna* as the test organism, found that toxicity was reduced in the presence of DOM and consequently, that toxicity was correlated with what the authors termed the ‘freely dissolved fraction’. The evidence that organic matter influences the bioavailability of substances other than metals therefore suggests an opportunity to improve the environmental relevance of EQSs for organic substances, for example, the fixed value EQS for benzo(a)pyrene (50 ng/L) as specified under the WFD.

Another study by Zhang *et al.* (2011), which assessed the environmental fate and toxicity of two ionic liquids, 1-butyl-3-methylimidazolium and 1-octyl-3-methylimidazolium chloride, which are used as organic solvents, also found that their toxicity was correlated with their freely dissolved concentration, and that their toxicity to medaka fish was reduced in the presence of DOM. Whereas these studies also identified subtle differences in the chemical behaviour of the individual substances, critically, both the study by Zhang *et al.* (2011) and Akkanen and Kukkonen (2003) demonstrated the significance of DOM in influencing the bioavailability of non-metals, although neither of the studies considered the significance of other water chemistry characteristics, such pH or water hardness, on influencing toxicity or adsorption to DOM which would be required to develop BLMs for the aforementioned substances.

Whereas the two studies described suggest additional research would be required in order to fully develop a functioning BLM, there are also other studies which have more extensively considered the influence of pH on substance adsorption to DOM, and for which perhaps a more imminent opportunity to develop a non-metal BLM exists. For example, a recent study by Neale, Escher and Schäfer (2009), evaluated the influence of pH on the adsorption of steroid hormones to DOM and identified partition coefficients for 4 steroid hormones and 3 different kinds of DOM, alongside the partition coefficient sensitivity to pH. Indeed, these data are already useable for developing model parameters where freely dissolved steroid hormone concentrations may be predicted and are also similar to the kind of data applied for parameterising speciation models such as WHAM VI. Consequently, in combination with exposure-response data it may already be possible to develop a BLM type model for the steroid hormones on the basis of the data presented by Neale, Escher and Schäfer (2009), however, as also acknowledged by Neale, Escher and Schäfer (2009), further information on the adsorption characteristics of other kinds of DOM should also be investigated, in particular since the DOM assessed in the study by Neale, Escher and Schäfer (2009) was of synthetic origin and is likely to differ that which occurs naturally or in sewage effluents. Indeed, a study by De Schamphelaere, Lofts and Janssen (2005), which investigated the binding properties of zinc to DOC from a range of surface waters found that synthetic DOC contained a substantially smaller active fraction than DOC from other sources (2-3% vs. ~40%) suggesting that the partition coefficient values in the study by Neale, Escher and Schäfer (2009) might understate the true affinity between organic compounds and DOM. Furthermore, a BLM type model may also be of particular interest to regulators given that a number of steroid hormones (e.g. 17α-Ethinylestradiol and Estradiol) have been demonstrated to produce developmental abnormalities in fish (Jobling *et al.*, 1998), and are also being considered for classification as Priority or Priority Hazardous Substances under the WFD. In summary, the studies described above, to some extent, resemble the early studies within which the significance of the chemical form of a metal (i.e. the FIAM) (Campbell, 1995; Morel, 1983), and the significance of metal complexation (Di Toro *et al.*, 2001) had been proposed, and consequently, demonstrates that the BLM approach could be useful for developing models to predict ecotoxicological impacts for substances other than metals.

11.3.2 Chemical mixtures

Whereas BLMs have been demonstrated to accurately predict both acute and chronic toxicity effects from exposure to a single metal, in the aquatic environment organisms are exposed to chemical mixtures which comprise of multiple metals and other compounds with toxic characteristics. To further improve the toxicological basis upon which the risk from metals (and other substances) is assessed, it is prudent to consider how these may impact aquatic organisms in combination, and also to identify chemical combinations which might either enhance or reduce bioavailability so as to present options that might allow risk to be managed. From a toxicological perspective, however, the assessment of the toxicity of chemical mixtures is complex since individual substances may either reduce or enhance the effect of other toxicants so that the toxic effect of a mixture may not be the sum of the individual components. Indeed, this phenomenon has been demonstrated in a number of studies; for example, Altenburger, Walter and Grote (2004) investigated the extent by which the toxicity of mixtures comprising of a series of up to 10 individual organic toxicants (benzo[ghi]fluoranthene, benz[a]anthracene, fluoranthene, pyrene, 2-phenylnaphthalene, anthracene, phenanthrene, prometryn, N-phenyl-2-naphthylamine, and parathion-methyl) and found that the toxicity of combined samples could not be predicted on the basis of their individual toxicity. Furthermore, they also found that sub-set combinations of the 10 toxicants produced an effect equivalent to that of the mixture comprising of all 10 of the individual toxicants, which demonstrated a need for the consideration of the mode of action of the individual toxicants. More recently, Vandenbrouck *et al.* (2009)

investigated the effect of equitoxic concentrations of nickel, cadmium and lead individually, and in binary combinations, to *D. magna* using a gene expression microarray. Their findings identified that the gene transcription profiles for the metal mixtures were not the sum of the individual profiles (i.e. evidence of reduction or enhancement effects, also in agreement with the findings of Altenburger *et al.* (2009)) but also that mixtures elicited gene responses that did not occur in the individual substance exposures. Further research is clearly required in order to better understand the biological mechanisms responsible for the toxicity of mixtures, however, the BLM approach may still provide a useful platform upon which to develop a model for predicting the effects of chemical mixtures, and the approach adopted in study by Vandenbrouck *et al.* (2009), suggests an interesting opportunity.

In their study, Vandenbrouck *et al.* (2009), identified discrete sets of genes that were activated as a consequence of exposure to individual metals, and those activated in response to the metal combinations. This indicates that toxicity might reasonably be predicted on the basis of the gene expression pattern, but also that different gene expression patterns (i.e. responses to different substance combinations) might be equated with a specific degree of toxicity (e.g. EC50, NOEC). Therefore once the combination of gene expression patterns corresponding with a specific degree of toxicity for a set of toxicant combinations may be reasonably predicted, the toxicity of various substance combinations may also be forecast.

Within the context of the BLM approach, this suggests that gene expression patterns could replace direct toxicity data as the basis for parameterising the biotic ligand-toxicant critical concentration values. Indeed, this would also enhance the mechanistic basis upon which toxicity forecasts are produced. Furthermore, from a practical perspective, the gene expression patterns would also be useful for identifying substance combinations which are likely to be most significant in terms of reducing or enhancing the effect of individual toxicants, thereby limiting (or prioritising) the substances for which mixture exposure effects are assessed.Following on from this, only a moderate extension of the BLM approach would be required in order to reflect to toxicity of mixtures. For example, by including the toxicants of which the chemical mixture is comprised in the speciation modelling. This then allows them to compete for binding at the biotic ligand (where the BL binding affinity of each toxicant is that determined from single substance exposure assays) and inclusion of the combination of toxicant-BL critical concentration values (as determined from gene expression data) that correspond with the toxic effect. It would subsequently be relatively straightforward, from a computational point of view, to determine the combination of concentration values for each toxicant that would be required to elicit the specified level of toxicity and, consequently, to determine the lowest concentration values for each toxicant that correspond with the level of toxicity specified by the model parameterisation. Such an approach would be useful for the purposes of assessing environmental risk and would effectively enable the current metal-specific BLMs to be combined within a single modelling framework, potentially alongside other toxicants. An additional advantage would be that including the toxicants in speciation modelling would also enable consideration for the influence of environmental factors, such as DOC, on toxicant bioavailability, as already demonstrated for metals, and also as described for non-metals in the previous section. A conceptual model of the BLM approach for mixtures is given in Figure 11.2, which has been adapted from the schematic model of the binding of lead to the gill of a rainbow trout given in Macdonald *et al.* (2002).

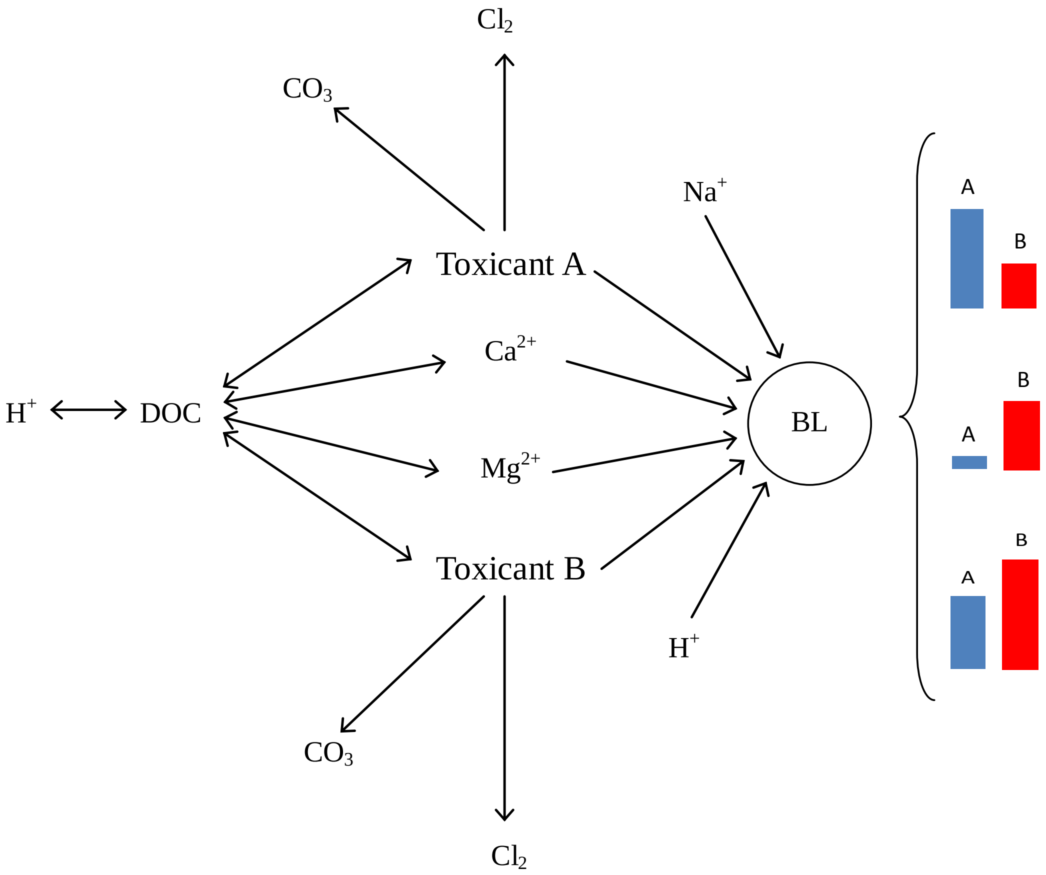

Figure 11.2 Conceptual model of BLM for toxicant mixtures (adapted from Macdonald et al 2002). The bar charts represent the equitoxic combinations of biotic ligand critical concentration values for toxicant A and B, determined from gene expression data.

11.3.3 Human health impacts assessment

It is interesting to note some of the similarities between the BLM approach proposed for mixtures and an approach that has been suggested for assessing risk to human health. Within the field of human health the concept of the ‘exposome’ has recently been proposed (Wild, 2005), which represents an inventory of the exposure pathways through which contact with a toxicant or toxicants might occur. The context within which the exposome concept had been developed was that, whereas the sequencing and mapping of the human genome had enabled the identification of biochemical signals that are useful for identifying an adverse response to toxicant exposure, knowledge of the exposure pathways was less advanced, but necessary for predicting, preventing or reducing exposure. The BLM approach proposed for mixtures takes into account both exposure and biochemical response factors and therefore, to some extent, aligns the approaches that have been proposed for assessing risk to human health with that of assessing risk to the environment. It might also be feasible to develop a BLM type model with a human as the biologically sensitive receptor, although would require adaptation in order to simulate human exposure. Nonetheless, the BLM approach has been demonstrated to provide a useful and adaptable platform for assessing the impact of chemicals in the environment and may consequently also be useful assessing human health impacts arising from exposure to chemicals.

12. CONCLUSIONS

The aims of this research were to investigate the extent by which the BLM approach may be appropriate for assessing risk in waters which receive sewage effluent inputs, and the regulatory implications of the approach for Severn Trent Water. These were investigated via a series of specific research problems which have been listed below along with a description of the key findings from the studies relating to each research problem, and how these relate to the project aims.

1. **Can BLM predict metal bioavailability with sufficient accuracy in effluent impacted waters?**

   The study described in chapter 5 investigated the extent by which sewage effluent might impact BLM forecast accuracy by comparing the results from a series of acute copper toxicity assays conducted in undiluted sewage effluent, using *Daphnia magna* as the test organism, with toxicity forecasts produced using the HydroQual BLM and the Refined BLM for *D. magna*. In general, both BLMs predicted the acute copper toxicity endpoint by within a factor of two (the range within which BLM forecasts are considered accurate), thus contributing support for using BLMs even in waters which receive sewage effluent inputs. The findings from this study have also provided further evidence that the *D. magna* biotic ligand stability constant for sodium may, at elevated sodium concentrations, provide an inaccurate approximation of the mechanisms that govern the influence of sodium. This suggests the need for regulators (and researchers) to understand the limitations and implications of the BLM thermodynamic parameters.

2. **Can the accuracy of BLM forecasting be improved through model optimisation?**

   The findings from the studies in chapter 6 and chapter 7 demonstrated that the accuracy of BLM toxicity forecasts could be further improved, and therefore, the environmental relevance of water quality standards derived using the BLM approach enhanced. The findings also indicated that the DOC fraction considered active with regard to ion binding, which determines the DOC concentration applied as the model input, was of much greater significance to forecast accuracy than using either a more advanced speciation model, such as

WHAM VI, to simulate ion interactions, or including additional effluent specific chemical characteristics, such as the concentration of EDTA, as model inputs.

The findings from these studies have also demonstrated the active fraction of sewage effluent derived DOC to be greater than the recommended default values and consequently, that the default values under-represent the actual concentration of the metal complexing ligands present in sewage effluents. This suggests that BLM predictions of toxicity may be conservative in effluent impacted waters. A set of DOC active fraction values suitable for use in sewage effluent and a method whereby more precise determinations of the DOC active fraction may be determined have been derived.

3. **To what extent do model simulations describe the influence of effluent derived organic matter on metal speciation?**

The study described in chapter 8 investigated differences between the complexation capacity in sewage effluent that was attributable to ligands with a high affinity for copper, nickel and zinc (≥ log *K* 12, 7, and 10, respectively) in relation to that predicted by the WHAM VI speciation model. The study found that the concentrations of high affinity ligands were not well predicted by WHAM VI (only 2 out of 24 simulated values were within the 95% confidence interval range of the measured values). The study also found that whereas certain effluents contained concentrations of ligands with a high affinity for copper and nickel in excess of that predicted by WHAM VI, all the effluents contained concentrations of ligands with a high affinity for zinc that were well in excess of that predicted by WHAM VI. The capacity of effluent derived organic matter to complex zinc ions may therefore differ substantially from that of naturally derived organic matter.

The study also found that for half of the effluents, the concentration of ligands with a high affinity for zinc exceeded the concentration of zinc detected in the effluent, suggesting the zinc discharged into receiving waters to be effectively inert, but also that these ligands might further reduce zinc bioavailability in the aquatic environment. An additional study involving a series of dilution experiments conducted for nickel and zinc also found that effluent derived ligands were also likely to retain their influence upon mixing with river water.

In summary, these findings contributes evidence in support of taking the effect of effluent derived organic matter into account for the purposes of assessing risk, and for assessing compliance to water quality standards derived using the BLM approach.

4. **To what extent do the metal complexing characteristics of surface waters vary?**

   The findings from the study described in Chapter 9 demonstrated the concentrations of DOC in river water samples to be highly variable, even in samples collected from the same river on the same day (between 3mg/L and 10mg/L in the Tame and Stour/Smestow Brook catchments).

   Complexation capacity attributable to high affinity ligands was also detected at locations downstream of WwTWs, suggesting these to be a significant source of high affinity ligands, however, high affinity ligands were also detected in samples obtained from rural locations which do not receive effluent inputs, suggesting there may be sources high affinity ligands other than WwTWs. The variability in complexation capacity, for zinc in particular, suggests that in order to ensure the environmental relevance of water quality standards derived via the BLM approach, the consideration of site-specific complexation characteristics should be incorporated within the tiered approach that has been proposed by the Regulator.

   An additional finding from this study has been that landfill leachate, such as that derived from the Slacky Lane landfill, may also represent a significant source of metal inputs into the River Tame.

5. **Could the use of the BLM approach within a compliance based regulatory framework require Severn Trent Water to reduce the concentrations of trace metals in sewage effluent discharges?**

   The results of the compliance assessment described in chapter 10 found that, whereas water quality standards derived using the BLM approach are likely to be more stringent that the current standards, the extent by which it may require a reduction in effluent discharge concentrations varied by metal. For copper, the concentrations in surface waters were generally already sufficiently low so that reductions in effluent concentrations may be required for only a small number of WwTWs. For zinc, however, the concentrations in surface waters generally exceeded BLM derived water quality standards, which suggested that a reduction in the concentration of zinc may be required for the discharges from numerous WwTWs. There does, however, also appear to be significant elements of conservatism incorporated within the zinc BLM which suggests that water quality standards derived using the current zinc BLM may be over-precautionary for reasons other than inaccuracies in the manner in which speciation is simulated.

An additional assessment which examined the potential impact of the 20µg/L WFD standard for nickel found that a reduction in the discharge concentration may be required for only a small number of WwTWs, but that these included some of the largest WwTWs within the Severn Trent Water network, and in particular, for WwTWs discharging into the River Tame or tributaries thereof. The elevated concentrations of nickel in the River Tame, however, appear to be substantially as a consequence of the region's greater industrial nature and legacy and may not be entirely attributable to inputs from WwTWs.

LIST OF ABBREVIATIONS

AF	Active fraction; the fraction of organic carbon considered to participate in complexation reactions
ASV	Anodic stripping voltametry; voltammetric technique used to quantify an electrochemically labile metal concentration
BBM	Bolds Basal Medium
BioF	Bioavailability Correction Factor; the ratio between a reference or ‘realistic worst case’ PNEC which represents the PNEC under conditions of high zinc bioavailability, and the calculated site specific PNEC
BL	Biotic ligand
BLM	Biotic ligand model; a computational model designed to predict metal bioavailability by simulating the interactions between a metal and an aquatic organism with consideration for the influence of water chemistry characteristics
BMAX	The parameter representing complexation capacity in the mass action binding model
CC	Complexation capacity
CHESS	Chemical Equilibria in Soils and Solutions model
CSV	Cathodic stripping voltametry; voltammetric technique used to quantify an electrochemically labile metal concentration
DGT	Diffusive gradients in thin films; a Chelex ion exchange resin enclosed within a polyacrylamide hydrogel that, when immersed into water, metal ions diffuse through the gel layer and are complexed by the Chelex resin.

DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DSD	Dangerous Substances Directive (76/464/EEC)
DTPA	Diethylenepentaacetic acid
EA	Environment Agency
EC50	Median effects concentration; the concentration of a chemical that causes a nonlethal adverse effect in 50% of test organisms
ED3A	Ethylenediaminetriacetic acid
EDDA	N-carboxymethyl-N-aminoethyleneglycine
EDMA	N-aminoethyleneglycine
EDTA	Ethylenediaminetetraacetic acid
EQS	Environmental quality standard
ESR	Existing Substance Regulations (73/93/EEC)

EU	European Union
FIAM	Free Ion Activity Model
GFAAS	Graphite furnace atomic absorption spectrometry
GSIM	Gill Surface Interaction Model
HC5	The 5th percentile hazardous concentration
HPLC	High performance liquid chromatography
IDA	Iminodiacetic acid
IRCHA	Institut National de Recherche Chimique Appliquée
ISE	Ion selective electrode
Kd	The parameter representing the equilibrium dissociation constant in the mass action binding model
LC50	Median lethal concentration; the concentration that causes 50% mortality in bioassay test organisms
LOD	Limit of detection

MGDA	Methyl glycine diacetic acid
MLR	Multiple linear regression
MOPS	3-(N-morpholino)propanesulphonic acid
MQ	Milli-Q water; ultrapure water
NOEC	No observed effects concentration; the highest concentration that did not cause a statistically significant effect on test organisms
NTA	Nitrilotriacetic acid
OECD	Organisation for Economic Co-operation and Development
OM	Organic matter
PHS	Priority hazardous substance
PNEC	Predicted no effect concentration; the highest chemical concentration that is predicted will not cause a statistically significant effect on test organisms
PS	Priority substance
SCHER	Scientific Committee on Health and Environmental Risks

SETAC	Society for Environmental Toxicology and Chemistry
SSD	Species sensitivity distribution; a probability distribution of the toxicity of a chemical to a population of animal species
TGD	EU Technical Guidance Document on Risk Assessment
TOC	Total organic carbon
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive
WHAM	Windermere Humic Aqueous Model
WwTW	Wastewater treatment works

REFERENCES

Akkanen, J. and Kukkonen, J.V.K. (2003) "Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter", *Environmental Toxicology and Chemistry,* vol. 22, no. 3, pp. 518-524.

Allen, H.E. and Hansen, D.J. (1996) "The importance of trace metal speciation to water quality criteria", *Water Environment Research,* vol. 68, no. 1, pp. 42-54.

Allison, J. and Allison, T. (2005) *Partition Coefficients for Metals in Surface Water, Soil, and Waste*, EPA, Washington, DC.

Altenburger, R., Walter, H. and Grote, M. (2004) *What Contributes to the Combined Effect of a Complex Mixture?*, American Chemical Society.

American Society for Testing and Materials (2007) "Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates and amphibians" in *Annual Books of ASTM Standards* American Society for Testing and Materials, West Conshohocken, PA, pp. 1-22.

Baird, D.J., Barber, I., Bradley, M., Soares, A.M.V.M. and Calow, P. (1991) "A comparative study of genotype sensitivity to acute toxic stress using clones of Daphnia magna Straus", *Ecotoxicology and Environmental Safety,* vol. 21, no. 3, pp. 257-265.

Baken, S., Degryse, F., Verheyen, L., Merckx, R. and Smolders, E. (2011) "Metal Complexation Properties of Freshwater Dissolved Organic Matter Are Explained by Its Aromaticity and by Anthropogenic Ligands", *Environmental Science and Technology,* vol. 45, no. 7, pp. 2584-2590.

Barata, C., Baird, D.J. and Markich, S.J. (1998) "Influence of genetic and environmental factors on the tolerance of Daphnia magna Straus to essential and non-essential metals", *Aquatic Toxicology,* vol. 42, no. 2, pp. 115-137.

Bianchini, A. and Wood, C.M. (2008) "Sodium uptake in different life stages of crustaceans: the water flea Daphnia magna Strauss", *Journal of Experimental Biology,* vol. 211, no. 4, pp. 539-547.

Borgmann, U. and Ralph, K.M. (1983) "Complexation and toxicity of copper and the free metal bioassay technique", *Water Research,* vol. 17, no. 11, pp. 1697-1703.

Bowles, K.C., Apte, S.C., Batley, G.E., Hales, L.T. and Rogers, N.J. (2006) "A rapid Chelex column method for the determination of metal speciation in natural waters", *Analytica Chimica Acta,* vol. 558, no. 1-2, pp. 237-245.

Bryan, S.E., Tipping, E. and Hamilton-Taylor, J. (2002) "Comparison of measured and modelled copper binding by natural organic matter in freshwaters", *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* vol. 133, no. 1-2, pp. 37-49.

Buzier, R., Tusseau-Vuillemin, M. and Mouchel, J. (2006) "Evaluation of DGT as a metal speciation tool in wastewater", *Science of The Total Environment,* vol. 358, no. 1-3, pp. 277-285.

Campbell, P.G.C. (1995) "Interactions between Trace Metals and Aquatic Organisms: A Critique of the Free-ion Activity Model" in *Metal Speciation and Bioavailability in Aquatic Systems* John Wiley and Sons, Chichester, England, pp. 45-102.

Chakraborty, P. and Chakrabarti, C.L. (2006) "Chemical speciation of Co, Ni, Cu, and Zn in mine effluents and effects of dilution of the effluent on release of the above metals from their metal–dissolved organic carbon (DOC) complexes", *Analytica Chimica Acta,* vol. 571, no. 2, pp. 260-269.

Cheng, T. (2005) "Comparison of zinc complexation properties of dissolved organic matter from surface waters and wastewater treatment plant effluents", *Journal of Environmental Sciences,* vol. 17, no. 4, pp. 535-539.

Cheng, T. and Allen, H.E. (2006) "Comparison of zinc complexation properties of dissolved natural organic matter from different surface waters", *Journal of Environmental Management,* vol. 80, no. 3, pp. 222-229.

Cheng, T., Schamphelaere, K.D., Lofts, S., Janssen, C. and Allen, H.E. (2005) "Measurement and computation of zinc binding to natural dissolved organic matter in European surface waters", *Analytica Chimica Acta,* vol. 542, no. 2, pp. 230-239.

Christianne Ridge, A. and Sedlak, D.L. (2004) "Effect of ferric chloride addition on the removal of Cu and Zn complexes with EDTA during municipal wastewater treatment", *Water Research,* vol. 38, no. 4, pp. 921-932.

Comber, S. and Georges, K. (2008) *Tiered approach to the assessment of metal compliance in surface waters*, Environment Agency, Bristol, UK.

Connon, R. (2007) *Culturing of Chlorella vulgaris - Standard Operating Procedure*, University of Reading, Reading, UK.

Constantino, C., Scrimshaw, M., Comber, S. and Churchley, J. (2011) "An evaluation of biotic ligand models predicting acute copper toxicity to *Daphnia magna* in wastewater effluent", *Environmental Toxicology and Chemistry,* vol. 30, no. 4, pp. 852-860.

De Schamphelaere, K.A.C. and Janssen, C.R. (2002) "A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: The effects of calcium, magnesium, sodium, potassium, and pH", *Environmental Science and Technology,* vol. 36, no. 1, pp. 48-54.

De Schamphelaere, K.A.C., Lofts, S. and 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,* vol. 24, no. 5, pp. 1190-1197.

De Schamphelaere, K.A.C., Heijerick, D.G. and Janssen, C.R. (2003) "Erratum to “Refinement and field validation of a biotic ligand model predicting acute copper toxicity to *Daphnia magna*” [Comp. Biochem. Physiol. C 133 (2002) 243–258]", *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* vol. 134, no. 4, pp. 529.

De Schamphelaere, K.A.C., Heijerick, D.G. and Janssen, C.R. (2002) "Refinement and field validation of a biotic ligand model predicting acute copper toxicity to *Daphnia magna*", *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* vol. 133, no. 1-2, pp. 243-258.

De Schamphelaere, K.A.C. and Janssen, C.R. (2004a) "Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*", *Environmental Toxicology and Chemistry,* vol. 23, no. 6, pp. 1365-1375.

De Schamphelaere, K.A.C. and Janssen, C.R. (2004b) "Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to *Daphnia magna*", *Environmental Toxicology and Chemistry,* vol. 23, no. 5, pp. 1115-1122.

De Schamphelaere, K.A.C., Stauber, J.L., Wilde, K.L., Markich, S.J., Brown, P.L., Franklin, N.M., Creighton, N.M. and 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,* vol. 39, no. 7, pp. 2067-2072.

De Schamphelaere, K.A.C., Vasconcelos, F.M., Tack, F.M.G., Allen, H.E. and Janssen, C.R. (2004) "Effect of dissolved organic matter source on acute copper toxicity to *Daphnia magna*", *Environmental Toxicology and Chemistry,* vol. 23, no. 5, pp. 1248-1255.

De Schamphelaere, K.A.C., Canli, M., Van Lierde, V., Forrez, I., Vanhaecke, F. and Janssen, C.R. (2004) "Reproductive toxicity of dietary zinc to *Daphnia magna*", *Aquatic Toxicology,* vol. 70, no. 3, pp. 233-244.

De Schamphelaere, K.A.C., Unamuno, V.I.R., Tack, F.M.G., Vanderdeelen, J. and Janssen, C.R. (2005) "Reverse osmosis sampling does not affect the protective effect of dissolved organic matter on copper and zinc toxicity to freshwater organisms", *Chemosphere,* vol. 58, no. 5, pp. 653-658.

Deleebeeck, N.M.E., De Schamphelaere, K.A.C. and 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,* vol. 27, no. 10, pp. 2097-2107.

Deleebeeck, N.M.E., De Schamphelaere, K.A.C., Heijerick, D.G., Bossuyt, B.T.A. and Janssen, C.R. (2007) "The acute toxicity of nickel to *Daphnia magna*: Predictive capacity of bioavailability models in artificial and natural waters", *Ecotoxicology and Environmental Safety,* vol. In Press, Corrected Proof.

Denmark, 2. (2007) *European Union Risk Assessment Report on nickel, nickel sulphate,nickel carbonate, nickel chloride, nickel dinitrate, Denmark, Draft report October 2007. Prepared by Denmark, Danish Environmental Protection Agency on behalf of the European Union.*

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R. and Santore, R.C. (2001) "Biotic ligand model of the acute toxicity of metals. 1. Technical basis", *Environmental Toxicology and Chemistry,* vol. 20, no. 10, pp. 2383-2396.

Dwane, G.C. and Tipping, E. (1998) "Testing a humic speciation model by titration of copper-amended natural waters", *Environment International,* vol. 24, no. 5-6, pp. 609-616.

Environment Agency (2011) 17/11/2011-last update, *Historic landfill*. Available: http://maps.environment-agency.gov.uk/wiyby/wiybyController?latest=trueandtopic=wasteandep=queryandlang=_eandx=402052.4166666667andy=302411.4166666667andscale=9andlayerGroups=2andqueryWindowWidth=25andqueryWindowHeight=25 [2011, 30/12/2011].

Environment Agency (2001), *Mass Balance Calculatio*n, Bristol, UK.

European Chemicals Bureau (2004) *European Union Risk Assessment Report: Edetic Acid (EDTA*), European Commission, Luxembourg.

European Chemicals Bureau (2003) *Technical Guidance Document on Risk Assessment (in support of Commission Directive 93/67/EEC, Commission Regulation (EC) No 1488/94, Directive 98/8/EC*), European Chemicals Bureau, Ispra, Italy.

European Commission (2010) *European Union risk assessment report on zinc meta*l, European Commission, Luxembourg, Luxembourg.

European Commission (2007) 02/04/2007-last update*, Directive 76/464/EE*C [Homepage of European Commission], [Online]. Available: http://ec.europa.eu/environment/water/water-dangersub/76_464.htm [2007, 07/16].

European Copper Institute (2008) *European Union risk assessment report on copper, copper(II) sulphate pentahydrate, copper(I) oxide, copper(II) oxide, dicopper chloride trihydroxide. Voluntary risk assessment.*

European Copper Institute (2006) *EU Copper Risk Assessment Software Version 0.0.0.17*.

European Parliament (2000) *DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCI*L, Directive edn, European Union, Brussels.

Farmer, A., Lee, R., Loutseti, S., Stanley, K., Warinton, J. and Whitehouse, P. (2010) "Setting Environmental Standards within a Socioeconomic Context" in *Derivation and Use of Environmental Quality and Human Health Standards for Chemical Substances in Water and Soil*, eds. M. Crane, P. Mattiessen, D. Stretton-Maycock, G. Merrington and P. Whitehouse, CRC Press, Pensacola, USA, pp. 5-29.

Florence, T.M. and Batley, G.E. (1976) "Trace metals species in sea-water—I : Removal of trace metals from sea-water by a chelating resin", *Talanta,* vol. 23, no. 3, pp. 179-186.

Gerringa, L.J.A., Herman, P.M.J. and Poortvliet, T.C.W. (1995) "Comparison of the linear Van den Berg/Ružić transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation data in the estuarine environment", *Marine Chemistry,* vol. 48, no. 2, pp. 131-142.

Guilhermino, L., Diamantino, T.C., Ribeiro, R., Gonçalves, F. and Soares, A.M.V.M. (1997) "Suitability of test media containing EDTA for the evaluation of acute metal toxicity to Daphnia magna Straus", *Ecotoxicology and Environmental Safety,* vol. 38, no. 3, pp. 292-295.

Guo, J., Peng, Y., Guo, J., Ma, J., Wang, W. and Wang, B. (2011) "Dissolved organic matter in biologically treated sewage effluent (BTSE): Characteristics and comparison", *Desalination,* vol. 278, no. 1-3, pp. 365-372.

Guthrie, J.W., Mandal, R., Salam, M.S.A., Hassan, N.M., Murimboh, J., Chakrabarti, C.L., Back, M.H. and Grégoire, D.C. (2003) "Kinetic studies of nickel speciation in model solutions of a well-characterized humic acid using the competing ligand exchange method", *Analytica Chimica Acta,* vol. 480, no. 1, pp. 157-169.

Guy, R.D. and Ross Kean, A. (1980) "Algae as a chemical speciation monitor—I A comparison of algal growth and computer calculated speciation", *Water Research,* vol. 14, no. 7, pp. 891-899.

Hamilton, M.A., Russo, R.C. and Thurston, R.V. (1977) "Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays", *Environmental Science and Technology,* vol. 11, pp. 714-719.

Heckmann, L. and Connon, R. (2007) *Culturing of Daphnia magna - Standard Operating Procedure*, University of Reading.

Heijerick, D.G., De Schamphelaere, K.A.C., Van Sprang, P.A. and Janssen, C.R. (2005) "Development of a chronic zinc biotic ligand model for *Daphnia magna*", *Ecotoxicology and Environmental Safety,* vol. 62, no. 1, pp. 1-10.

HERA (2004) *Human and Environmental Risk Assessment on ingredients of European household cleaning products: Phosphonates*.

HydroQual (2007) *The Biotic Ligand Model - Windows Interface Version*, Mahwah, NJ, USA.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G. and Sumpter, J.P. (1998) "Widespread Sexual Disruption in Wild Fish", *Environmental Science and Technology,* vol. 32, no. 17, pp. 2498-2506.

Kari, F.G. and Giger, W. (1996) "Speciation and fate of ethylenediaminetetraacetate (EDTA) in municipal wastewater treatment", *Water Research,* vol. 30, no. 1, pp. 122-134.

Keller, V.D.J., Whelan, M.J. and Rees, H.G. (2006) "A global assessment of chemical effluent dilution capacities from a macro-scale hydrological model" in *Climate Variability and Change - Hydrological Impacts*, eds. S. Demuth, A. Gustard, E. Planos, F. Scatena and E. Servat, International Association of Hydrological Sciences, Wallingford, UK, pp. 586-590.

Knepper, T.P. (2003) "Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment", *TrAC Trends in Analytical Chemistry,* vol. 22, no. 10, pp. 708-724.

Knepper, T.P., Werner, A. and Bogenschütz, G. (2005) "Determination of synthetic chelating agents in surface and waste water by ion chromatography–mass spectrometry", *Journal of Chromatography A,* vol. 1085, no. 2, pp. 240-246.

Koster, M., de Groot, A., Vijver, M. and Peijnenburg, W. (2006) "Copper in the terrestrial environment: Verification of a laboratory-derived terrestrial biotic ligand model to predict earthworm mortality with toxicity observed in field soils", *Soil Biology and Biochemistry,* vol. 38, no. 7, pp. 1788-1796.

Kozlova, T., Wood, C.M. and McGeer, J.C. (2009) "The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a biotic ligand model", *Aquatic Toxicology,* vol. 91, no. 3, pp. 221-228.

Laine, P. and Matilainen, R. (2005) "Simultaneous determination of DTPA, EDTA, and NTA by UV-visible spectrometry and HPLC", *Analytical and Bioanalytical Chemistry,* vol. 382, no. 7, pp. 1601-1609.

Lester, J.N. (1987) *Heavy metals in wastewater and sludge treatment processes,* CRC Press, Boca Raton, USA.

Li, B., Zhang, X., Wang, X. and Ma, Y. (2009) "Refining a biotic ligand model for nickel toxicity to barley root elongation in solution culture", *Ecotoxicology and Environmental Safety,* vol. 72, no. 6, pp. 1760-1766.

Lock, K., De Schamphelaere, K.A.C., Becaus, S., Criel, P., Van Eeckhout, H. and Janssen, C.R. (2007) "Development and validation of a terrestrial biotic ligand model predicting the effect of cobalt on root growth of barley (*Hordeum vulgare*)", *Environmental Pollution,* vol. 147, no. 3, pp. 626-633.

Lofts, S., Tipping, E. and Hamilton-Taylor, J. (2008) *The Chemical Speciation of Fe(III) in Freshwaters*, Springer Netherlands.

Luo, X., Li, L. and Zhou, D. (2008) "Effect of cations on copper toxicity to wheat root: Implications for the biotic ligand model", *Chemosphere,* vol. 73, no. 3, pp. 401-406.

Ma, H., Allen, H.E. and Yin, Y. (2001) "Characterization of isolated fractions of dissolved organic matter from natural waters and a wastewater effluent", *Water Research,* vol. 35, no. 4, pp. 985-996.

Macdonald, A., Silk, L., Schwartz, M. and Playle, R.C. (2002) "A lead–gill binding model to predict acute lead toxicity to rainbow trout (Oncorhynchus mykiss)", *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* vol. 133, no. 1-2, pp. 227-242.

Martin, D. and Armitage, R. (2005) *Water Quality Consenting Guidance Dangerous Substances in Discharges to Surface Waters*, Environment Agency.

Mattigod, S.V. and Sposito, G. (1979) "Chemical Modeling of Trace Metal Equilibria in Contaminated Soil Solutions Using the Computer Program GEOCHEM" in *Chemical Modeling in Aqueous Systems* AMERICAN CHEMICAL SOCIETY, pp. 837-856.

Meyer, J.S., Clearwater, S.J., Doser, T.A., Rogaczewski, M.J. and Hansen, J.A. (2007) *Effects of Water Chemistry on Bioavailability and Toxicity of Waterborne Cadmium, Copper, Nickel, Lead, and Zinc to Freshwater Organisms,* SETAC Press, USA.

Moore, D.S. (2010) "Inference for Regression" in *The Basic Practise of Statistics*, 5th edn, W.H. Freeman and Company, Basingstoke, England, pp. 595-632.

Morel, F.M.M. (1983) *Principles of Aquatic Chemistry,* John Wiley, New York, NY, USA.

Motulsky, H. and Christopoulos, A. (2004a) "Analyzing saturation radioligand binding data" in *Fitting Models to Biological Data Using Linear and Nonlinear Regression* Oxford University Press, New York, USA, pp. 199-210.

Motulsky, H. and Christopoulos, A. (2004b) "Generating confidence intervals by Monte Carlo simulations" in *Fitting Models to Biological Data Using Linear and Nonlinear Regression* Oxford University Press, New York, USA, pp. 104-108.

Murgatroyd, C., Wroath, A., Mascarenhas, R., Sutton, A. and Comber, S. (1997) *Proposed Environmental Quality Standards for Ethylene diamine tetra acetic acid (EDTA) in Water (1997.* DETR.

Muyssen, B.T.A., Janssen, C.R. and Bossuyt, B.T.A. (2002) "Tolerance and acclimation to zinc of field-collected Daphnia magna populations", *Aquatic Toxicology,* vol. 56, no. 2, pp. 69-79.

Muyssen, B.T.A. and Janssen, C.R. (2001) "Zinc acclimation and its effect on the zinc tolerance of Raphidocelis subcapitata and Chlorella vulgaris in laboratory experiments", *Chemosphere,* vol. 45, no. 4–5, pp. 507-514.

Muyssen, B. and Janssen, C.R. (2000) "Zinc deficiency and toxicity in multi-generation acclimation experiments with Daphnia magna : shifts in zinc tolerance", *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology,* vol. 126, Supplement 1, no. 0, pp. 108.

Muyssen, B.T.A. and Janssen, C.R. (2004) "Multi-generation cadmium acclimation and tolerance in Daphnia magna Straus", *Environmental Pollution,* vol. 130, no. 3, pp. 309-316.

Muyssen, B.T.A. and Janssen, C.R. (2002) "Tolerance and acclimation to zinc of Ceriodaphnia dubia", *Environmental Pollution,* vol. 117, no. 2, pp. 301-306.

Neale, P.A., Escher, B.I. and Schäfer, A.I. (2009) "pH dependence of steroid hormone—organic matter interactions at environmental concentrations", *Science of The Total Environment,* vol. 407, no. 3, pp. 1164-1173.

Neubecker, T.A. and Allen, H.E. (1983) "The measurement of complexation capacity and conditional stability constants for ligands in natural waters", *Water Research,* vol. 17, no. 1, pp. 1-14.

Nowack, B. and VanBriesen, J.M. (2005a) "Chelating Agents in the Environment" in *Biogeochemistry of Chelating Agents*, eds. B. Nowack and J.M. Van Briesen, American Chemical Society, pp. 1-18.

Nowack, B. and VanBriesen, J.M. (2005b) "Chelating agents in the Environment" in *Biogeochemistry of Chelating Agents*, eds. B. Nowack and J.M. Van Briesen, American Chemical Society, Washington DC, USA, pp. 1-18.

OECD (2004) *OECD guideline for testing of chemicals: Daphnia sp., Acute Immobilisation Test*, Organisation for Economic Co-operation and Development, Paris, France.

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,* vol. 17, no. 6, pp. 342-347.

Paquin, P., Santore, R. and Mathew, R. (2007) *Biotic ligand Model Windows interface version 2.2.3 users guide and reference manual*, HydroQual, Mahwah, NJ, USA.

Paquin, P.R., Santore, R.C., Wu, K.B., Kavvadas, C.D. and Di Toro, D.M. (2000) "The biotic ligand model: a model of the acute toxicity of metals to aquatic life", *Environmental Science and Policy,* vol. 3, no. Supplement 1, pp. 175-182.

Pernet-coudrier, B., Clouzot, L., Varrault, G., Tusseau-vuillemin, M., Verger, A. and Mouchel, J. (2008) "Dissolved organic matter from treated effluent of a major wastewater treatment plant: Characterization and influence on copper toxicity", *Chemosphere,* vol. 73, no. 4, pp. 593-599.

Peters, A., Merrington, G. and Brown, B. (2009) *Using biotic ligand models to help implement environmental quality standards for metals under the Water Framework Directive*, Environment Agency, Bristol, UK.

Phinney, J.T., and Bruland, K.W. (1997) “Trace metal exchange in solution by the fungacides Ziram and Maneb (Dithiocarbamates) and subsequent uptake of lipophilic organic zinc, copper and lead complexes into phytoplankton cells”, *Environmental Toxicology and Chemistry*, vol. 16, no. 10, pp. 2046-2053.

Playle, R.C., Dixon, D.G. and Burnison, K. (1993a) "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,* vol. 50, no. 12, pp. 2678-2687.

Playle, R.C., Dixon, D.G. and Burnison, K. (1993b) "Copper and cadmium binding to fish gills: Modification by dissolved organic carbon and synthetic ligands", *Canadian Journal of Fisheries and Aquatic Sciences,* vol. 50, no. 12, pp. 2667-2677.

R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

R-Bloggers (2010) 03/05/2010-last update, *Displaying data using level plots*. Available: http://www.r-bloggers.com/displaying-data-using-level-plots/ [2011, 06/08/2011].

Royal Commission on Environmental Pollution (1998) *Setting Environmental Standards*, The Stationary Office, London, UK.

Rozan, T.F., Lassman, M.E., Ridge, D.P. and Luther, G.W., (2000) "Evidence for iron, copper and zinc complexation as multinuclear sulphide clusters in oxic rivers", *Nature,* vol. 406, no. 6798, pp. 879-882.

Santore, R. and Driscoll, C.T. (1995) "The CHESS model for calculating chemical equilibria in soils and solutions" in *Chemical Equilibrium and Reaction Models*, eds. R. Loeppert, A.P. Schwab and S. Goldberg, American Society of Agronomy, Madison, WI, pp. 357-375.

Santore, R.C., Mathew, R., Paquin, P.R. and DiToro, D. (2002) "Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and Daphnia magna", *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* vol. 133, no. 1-2, pp. 271-285.

Santore, R.C., Toro, D.M.D., Paquin, P.R., Allen, H.E. and Meyer, J.S. (2001) "Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*", *Environmental Toxicology and Chemistry,* vol. 20, no. 10, pp. 2397-2402.

Sarathy, V. and Allen, H.E. (2005) "Copper complexation by dissolved organic matter from surface water and wastewater effluent", *Ecotoxicology and Environmental Safety,* vol. 61, no. 3, pp. 337-344.

SCHER (2009a) *Scientific opinion on the risk assessment report on nickel and its compounds, environmental part*. European Commission, Brussels, Belgium.

SCHER (2009b) *Scientific opinion on the voluntary risk assessment report on copper and its compounds, environmental part*, European Commission, Brussels, Belgium.

SCHER (2007) *Scientific opinion on the risk assessment report on zinc, environmental part*. European Commission, Brussels, Belgium.

Schmidt, C.K. and Heinz-jurgen, B. (2006) "Aminopolycarboxylate Complexing Agents" in *Organic Pollutants in the Water Cycle*, eds. T. Reemtsma and M. Jekel, Wiley-VCH, Weinheim, Germany, pp. 155-180.

SENTEK*, Ion Selective Electrode Operating Instructions*, SENTEK, United Kingdom.

Stumm, W. and Morgan, J.J. (1996) "Stability Constants" in *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd edn, John Wiley and Sons, New York, NY, USA, pp. 325-334.

Thurman, E.M. (1985) *Organic Geochemistry of Natural Waters,* Kluwer Academic Publishers.

Tipping, E. (1994) "WHAMC—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,* vol. 20, no. 6, pp. 973-1023.

Tipping, E. and Hurley, M.A. (1992) "A unifying model of cation binding by humic substances", *Geochimica et Cosmochimica Acta,* vol. 56, no. 10, pp. 3627-3641.

Tipping, E., Rey-Castro, C., Bryan, S.E. and Hamilton-Taylor, J. (2002) "Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation", *Geochimica et Cosmochimica Acta,* vol. 66, no. 18, pp. 3211-3224.

Tipping, E. (2001) *WHAM VI - equilibrium chemical speciation for natural waters*, Centre for Ecology and Hydrology, Lancaster, UK.

Turner, A., Pollock, H., and Brown. (2009) “Accumulation of Cu and Zn from antifouling paint particles by the macroalga, *Ulva lactuca*”, *Environmental Pollution*, vol. 157, pp. 2314-2319.

Tusseau-Vuillemin, M., Gilbin, R., Bakkaus, E. and Garric, J. (2004) "Performance of Diffusion Gradient in Thin Films to evaluate the toxic fraction of copper to Daphnia Magna", *Environmental Toxicology and Chemistry,* vol. 23, no. 9, pp. 2154-2161.

U.S. Environmental Protection Agency (2006) *TRIMMED SPEARMAN-KARBER (TSK) PROGRAM VERSION 1.5*, Ohio, USA.

U.S. Environmental Protection Agency (2003) *2003 Draft Update of Ambient Water Quality Criteria for Copper*, U.S. Environmental Protection Agency, Washington, DC, USA.

United States Environmental Protection Agency (2007a) 04/11/2007-last update, *2007 Updated Aquatic Life Copper Criteria* [2007, 08/23].

United States Environmental Protection Agency (2007b) *Aquatic Life Ambient Freshwater Quality Criteria - Copper*.

van Veen, E., Burton, N., Comber, S. and Gardner, M. (2002) "Speciation of Copper in Sewage Effluents and its Toxicity to Daphnia Magna", *Environmental Toxicology and Chemistry,* vol. 21, no. 2, pp. 275-280.

Van Laer, L., Smolders, E., Degryse, F., Janssen, C. and De Schamphelaere, K.C. (2006) "Speciation of nickel in surface waters measured with the Donnan membrane technique", *Analytica Chimica Acta,* vol. 578, no. 2, pp. 195-202.

Vandenbrouck, T., Soetaert, A., van der Ven, K., Blust, R. and De Coen, W. (2009) "Nickel and binary metal mixture responses in Daphnia magna: Molecular fingerprints and (sub)organismal effects", *Aquatic Toxicology,* vol. 92, no. 1, pp. 18-29.

Wang, X., Li, B., Ma, Y. and Hua, L. (2010) "Development of a biotic ligand model for acute zinc toxicity to barley root elongation", *Ecotoxicology and environmental safety,* vol. 73, no. 6, pp. 1272-1278.

Wild, C.P. (2005) "Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology", *Cancer Epidemiology Biomarkers and Prevention,* vol. 14, no. 8, pp. 1847-1850.

Xue, H., Sigg, L. and Kari, F.G. (1995) "Speciation of EDTA in Natural Waters: Exchange Kinetics of Fe-EDTA in River Water", *Environmental Science and Technology,* vol. 29, no. 1, pp. 59-68.

Zhang, H. and Davison, W. (1995) "Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution", *Analytical Chemistry,* vol. 67, no. 19, pp. 3391-3400.

Zhang, Z., Liu, J., Cai, X., Jiang, W., Luo, W. and Jiang, G. (2011) "Sorption to Dissolved Humic Acid and Its Impacts on the Toxicity of Imidazolium Based Ionic Liquids", *Environmental Science and Technology,* vol. 45, no. 4, pp. 1688-1694.

Appendix 1 R code for contour plots

R code to produce Figure 5.2

Where CuBLdiff is a file containing the values for $\Delta \mathrm{Cu}_{\mathrm{EC50BL}i}$, Ca is a file containing the values for calcium, and pH is a file containing the values for pH

1. CuOrgdiff.loess = loess(CuBLdiff~Ca*pH, data = CuOrgdiff, degree = 2, span = 0.25) (creates a quadratic function from which to estimate CuORGdiff across an expanded region)

2. CuBLdiff.fit = expand.grid(list(Ca = seq(4,100,1),pH = seq(6,9,0.01))) (creates a new file with an expanded grid region which contains x,y coordinate grid increments which are more refined than contained within the actual data)

3. z = predict(CuBLdiff.loess, newdata = CuBLdiff.fit) (calculates new values of CuORGdiff using the quadratic function for the ‘new’ locations contained within the expanded grid and places these in a file named z)

4. CuBLdiff.fit$CuBLdiff = as.numeric(z) (appends the newly calculated values for CuORGdiff, contained within the file ‘z’, to the relevant Ca-pH combination to which it applies)

5. levelplot(CuBLdiff~Ca*pH, data = CuBLdiff.fit, col.regions = terrain.colors(100)) (plots the data as a levelplot using the expanded grid and the ‘terrain.colors’ pallete)

R code to produce Figure 5.3

Where CuORGdiff is a file containing the values for $\Delta \mathrm{Cu}_{\mathrm{EC50DOC}i}$, DOC is a file containing the values for DOC, and pH is a file containing the values for pH

1. CuOrgdiff.loess = loess(CuORGdiff~DOC*pH, data = CuOrgdiff, degree = 2, span = 0.25) (creates a quadratic function from which to estimate CuORGdiff across an expanded region)

2. CuOrgdiff.fit = expand.grid(list(DOC = seq(5,25,1),pH = seq(6,9,0.01))) (creates a new file with an expanded grid region which contains x,y coordinate grid increments which are more refined than contained within the actual data)

3. z = predict(CuOrgdiff.loess, newdata = CuOrgdiff.fit) (calculates new values of CuORGdiff using the quadratic function for the ‘new’ locations contained within the expanded grid and places these in a file named z)

4. CuOrgdiff.fit$CuORGdiff = as.numeric(z) (appends the newly calculated values for CuORGdiff, contained within the file ‘z’, to the relevant DOC-pH combination to which it applies)

5. levelplot(CuORGdiff~DOC*pH, data = CuOrgdiff.fit, col.regions = terrain.colors(100)) (plots the data as a levelplot using the expanded grid and the ‘terrain.colors’ pallete)

Appendix 2 *Daphnia magna* culture data sheets

Daphnia magna - Culture Data Sheet

Initiation Date: 06-Nov-2008 **Operator: Carlos Constantino** **Culture reference #: 1**

Date	Day	Day #	Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		Remarks
			1	2	1	2	Algae (ml)	Yeast (ml)	Batch #	Marinure (ml)	Min.	Max	
06/11/08	Thu	1	15	15			1	0.5	1	3.0	22	22	pH 7.8
07/11/08	Fri	2					1.5	0.5			22	22	50% more for weekend
08/11/08	Sat	3									22	22	
09/11/08	Sun	4									22	22	
10/11/08	Mon	5					1.0	0.5			22	22	
11/11/08	Tue	6					1.5	0.5			22	22	
12/11/08	Wed	7					1.5	0.5			22	22	
13/11/08	Thu	8	13	14	~50	~40	2.0	0.5	2	4.0	23	23	1st brood, renew water
14/11/08	Fri	9			~100	~90	3.0	0.5			23	24	1st brood, 50% more for weekend
15/11/08	Sat	10									22	24	
16/11/08	Sun	11									22	24	
17/11/08	Mon	12			~200	~200	2.0	0.5			22	24	2nd brood
18/11/08	Tue	13			~40	~40	2.0	0.5			21	21	No juveniles as at 17:00
19/11/08	Wed	14			~100	~100	2.0	0.5	3	4.0	20	22	3rd brood, renew water, start culture ref #2
20/11/08	Thu	15			~10	~40	2.0	0.5			21	22	
21/11/08	Fri	16					3.0	0.5			19	22	50% extra for weekend
22/11/08	Sat	17									19	21	
23/11/08	Sun	18									19	21	
24/11/08	Mon	19	12	14	~200	~200	2.0	0.5			19	21	4th brood, 1 adult dead
25/11/08	Tue	20			~60	~40	2.0	0.5			19	21	
26/11/08	Wed	21			~100	~40							5th brood, used in tox test A1, culture ended

Daphnia magna - Culture Data Sheet

Initiation Date: 19-Nov-2008 **Operator: Carlos Constantino** **Culture reference #: 2**

			Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		
Date	Day	Day #	1	2	1	2	Algae (ml)	Yeast (ml)	Batch #	Marinure (ml)	Min.	Max	Remarks
19/11/08	Wed	1	15	15			1.0	0.5	1	3.0	20	22	New water, pH 7.4
20/11/08	Thu	2					1.0	0.5			21	22	
21/11/08	Fri	3					2.0	0.5			20	22	50% extra algae for weekend
22/11/08	Sat	4									19	21	
23/11/08	Sun	5									19	21	
24/11/08	Mon	6					1.5	0.5			19	21	
25/11/08	Tue	7					2.0	0.5			19	21	
26/11/08	Wed	8					2.0	0.5	2	4.0	20	22	New water, pH 7.4
27/11/08	Thu	9					2.0	0.5			20	22	
28/11/08	Fri	10	14	15	~40	~50	3.0	0.5			22	22	1st brood, 50% extra algae for weekend
29/11/08	Sat	11									18	22	
30/11/08	Sun	12									18	22	
01/12/08	Mon	13			~100	~100	2.0	0.5			18	22	2nd brood
02/12/08	Tue	14			~220	~150	2.0	0.5	3	4.0	20	22	3rd brood, used in tox test A2, new water, pH 7.4, start culture #3
03/12/08	Wed	15				~60	2.0	0.5			21	21	
04/12/08	Thu	16			~80	~60	2.0	0.5			22	22	4th brood
05/12/08	Fri	17			~120	~120	3.0	0.5			20	22	4th brood, 50% extra algae for weekend
06/12/08	Sat	18									21	22	
07/12/08	Sun	19									21	22	
08/12/08	Mon	20			~120	~100	2.0		4	4.0	21	22	New water, pH 7.4
09/12/08	Tue	21			~40	~60	2.0				21	22	Used in tox test B1, culture ended

Daphnia magna - Culture Data Sheet

Initiation Date: 02-Dec-2008 **Operator: Carlos Constantino** **Culture reference #: 3**

Date	Day	Day #	Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		Remarks
			1	2	1	2	Algae (ml)	Yeast (ml)	Batch #	Marinure (ml)	Min.	Max	
02/12/08	Tue	1	15	15			1.0	0.5	1	2.0	20	22	New water, pH 7.4
03/12/08	Wed	2					1.0	0.5			21	21	
04/12/08	Thu	3					1.0	0.5			22	22	
05/12/08	Fri	4					2.0	0.5			20	22	50% extra algae for weekend
06/12/08	Sat	5									21	22	
07/12/08	Sun	6									21	22	
08/12/08	Mon	7	15	15			2.0	0.5	2	4.0	21	22	
09/12/08	Tue	8					2.0	0.5			21	22	
10/12/08	Wed	9					2.0	0.5			21	22	
11/12/08	Thu	10			~80	~60	2.0	0.5			21	22	1st brood
12/12/08	Fri	11			~20	~40	3.0	0.5			21	23	1st brood, 50% extra algae for weekend
13/12/08	Sat	12									21	23	
14/12/08	Sun	13									21	23	
15/12/08	Mon	14	15	15	~250	~250	2.0	0.5	3	4.0	21	23	2nd brood
16/12/08	Tue	15			~50	~50	2.0	0.5			21	23	2nd brood
17/12/08	Wed	16			~250	~250	2.0	0.5			21	23	3rd brood
18/12/08	Thu	17			~50	~80	2.0	0.5			20	22	3rd brood, used in tox test B2
19/12/08	Fri	18			~30	~10	3.0	0.5			21	22	50% extra algae for weekend, start culture #4
20/12/08	Sat	19									21	22	
21/12/08	Sun	20									22	22	
22/12/08	Mon	21			~50	~50	2.0	0.5			22	22	Culture ended

Daphnia magna - Culture Data Sheet

Initiation Date: 20-Dec-2008 **Operator: Carlos Constantino** **Culture reference #: 4**

Date	Day	Day #	Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		Remarks
			1	2	1	2	Algae (ml)	Yeast (ml)	Batch #	Marinure (ml)	Min.	Max	
20/12/08	Sat	1	15	15			2.0	0.5	1	3.0	21	22	New water, pH 7.4
21/12/08	Sun	2									22	22	
22/12/08	Mon	3					1.0	0.5			22	22	
23/12/08	Tue	4					1.0	0.5			22	22	
24/12/08	Wed	5					2.0	0.5			19	21	
25/12/08	Thu	6					2.0	0.5			18	21	
26/12/08	Fri	7					2.0	0.5			18	21	
27/12/08	Sat	8	14	15			2.0	0.5	2	4.0	17	21	1 discoloured adult discarded, new water, pH 7.4
28/12/08	Sun	9					2.0	0.5			16	21	
29/12/08	Mon	10			~25	~35	2.0	0.5			17	21	1st brood
30/12/08	Tue	11					2.0				17	21	Both cultures unusually sedate (at flask bottom)
31/12/08	Wed	12			~200	~100	2.0	0.5			17	21	2nd brood
01/01/09	Thu	13			~100	~150	2.0	0.5	3	4.0	17	21	2nd brood, new water, pH 7.4
02/01/09	Fri	14					2.0	0.5			17	21	
03/01/09	Sat	15			~200	~200	2.0	0.5			16	22	3rd brood, start culture #5
04/01/09	Sun	16			~25	~15	2.0	0.5			16	22	3rd brood
05/01/09	Mon	17			~30	~80	2.0	0.5			16	20	3rd brood
06/01/09	Tue	18					2.0	0.5			17	18	
07/01/09	Wed	19	12	14	~80	~80	2.0	0.5			17	20	4th brood, used in tox test C1
08/01/09	Thu	20			~100	~100	2.0	0.5			19	20	
09/01/09	Fri	21			~100	~100					20	21	5th brood, start culture #6, culture ended

Daphnia magna - Culture Data Sheet

Initiation Date: 03-Jan-2009 **Operator: Carlos Constantino** **Culture reference #: 5**

Date	Day	Day #	Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		Remarks
			1	2	1	2	Algae (ml)	Yeast (ml)	Batch #	Marinure (ml)	Min.	Max	
03/01/09	Sat	1	15	15			1.0	0.5	1	3.0	16	22	New water, pH 7.8
04/01/09	Sun	2					1.0	0.5			16	22	
05/01/09	Mon	3					1.0	0.5			16	20	
06/01/09	Tue	4					1.0	0.5			17	18	
07/01/09	Wed	5					2.0	0.5			17	20	
08/01/09	Thu	6					2.0	0.5			19	20	
09/01/09	Fri	7	15	15			3.0	0.5		4.0	20	21	50% extra alage for weekend, new water, pH 7.8
10/01/09	Sat	8									20	22	
11/01/09	Sun	9									20	22	
12/01/09	Mon	10					2.0	0.5			20	22	
13/01/09	Tue	11			~40	~40	2.0	0.5			21	22	1st brood
14/01/09	Wed	12			~40	~30	2.0	0.5		4.0	20	21	
15/01/09	Thu	13					2.0	0.5			20	21	New water, pH 7.8
16/01/09	Fri	14					3.0	0.5			20	21	50% extra algae for weekend
17/01/09	Sat	15									21	22	
18/01/09	Sun	16									21	22	
19/01/09	Mon	17	15	14	~100	~100	2.0	0.5			21	22	2nd brood
20/01/09	Tue	18			~150	~150	2.0	0.5			21	22	3rd brood, used in tox test C2 & D1,culture ended
21/01/09	Wed	19											
22/01/09	Thu	20											
23/01/09	Fri	21											

Daphnia magna - Culture Data Sheet

Initiation Date: 09-Jan-2009 **Operator: Carlos Constantino** **Culture reference #: 6**

			Culture (# founding ind.)		Offspring		Food		Water renewal		Temperature (°C)		
Date	**Day**	**Day #**	**1**	**2**	**1**	**2**	**Algae (ml)**	**Yeast (ml)**	**Batch #**	**Marinure (ml)**	**Min.**	**Max**	**Remarks**
09/01/09	Fri	1	15	15			2.0	0.5	1	3.0	20	21	New water, pH 7.8
10/01/09	Sat	2									20	22	
11/01/09	Sun	3									20	22	
12/01/09	Mon	4					1.0	0.5			20	22	
13/01/09	Tue	5					2.0	0.5			21	22	
14/01/09	Wed	6					2.0	0.5			20	21	
15/01/09	Thu	7					2.0	0.5			20	21	
16/01/09	Fri	8	10	10			3.0	0.5		4.0	20	21	50% extra alage for weekend, new water, pH 7.8, 5 undersized daphnids abandoned from each culture
17/01/09	Sat	9									21	22	
18/01/09	Sun	10									21	22	
19/01/09	Mon	11					2.0	0.5			21	22	
20/01/09	Tue	12			~10	~45	2.0	0.5			21	22	1st brood
21/01/09	Wed	13			~15	~10	2.0	0.5			21	22	
22/01/09	Thu	14			~25	~20	2.0	0.5			21	23	
23/01/09	Fri	15			~50	~50	3.0	0.5			21	23	2nd brood, 50% extra alage for weekend, new water, pH 7.8
24/01/09	Sat	16									21	23	
25/01/09	Sun	17									21	23	
26/01/09	Mon	18			~135	~135	2.0	0.5			21	23	3rd brood, used in tox test D2, culture ended
27/01/09	Tue	19			~30	~40	2.0	0.5			20	23	

Appendix 3 *Chlorella vulgaris* culture data sheet

			Culture 1			Culture 2			
Date	Day	Day #	BBM added (ml)	Volume harvested (ml)	Feedstock volume recovery (ml)	BBM added (ml)	Volume harvested (ml)	Feedstock volume recovery (ml)	Notes
06-Nov	Thu	1	1,000			1,000			Culture 1 initiated with 65ml Blades solution. Culture 2 initiated with 1ml UoR concentrate.
12-Nov	Wed	7	300	300		700			Culture 2 crash. Restarted using 300ml from culture 1 and 700ml BBM.
18-Nov	Tue	13	500	500	140				
24-Nov	Mon	19	500	500	180				
01-Dec	Mon	26	500	500	160				
08-Dec	Mon	33	250			250			250ml added to each to compensate for evaporation losses.
15-Dec	Mon	40		500	50		500	50	
05-Jan	Mon	61							Cultures ended.

Appendix 4 Comparison of downstream substance concentrations simulated using the *Mass Balance* model and the spreadsheet simulator

	Upstream characteristics				Discharge characteristics				Comparison	
Scenario	Mean flow (m3/d)	Flow stdev (m3/d)	Conc. (ug/L)	Conc. stdev (ug/L)	Mean flow (m3/d)	Flow stdev (m3/d)	Mean conc. (ug/L)	Conc. stdev (ug/L)	Spreadsheet simulated mean downstream conc. (ug/L)	Mass Balance simulated mean downstream conc. (ug/L)
Test 1	27,440	9,147	8.01	4.00	341,600	113,867	7.62	3.81	7.77	7.8
Test 2	9,640	3,213	17.30	8.65	100,000	33,333	2.51	1.255	3.94	3.89
Test 3	663,140	221,047	4.19	2.10	118,370	39,457	4.47	2.235	4.12	4.27
Test 4	40,140	13,380	4.05	2.02	86,010	28,670	5.76	2.88	5.21	5.31
Test 5	13,110	4,370	4.94	2.47	73,560	24,520	7.18	3.59	6.83	6.97
Test 6	9,740	3,247	1.87	0.94	80,590	26,863	2.61	1.305	2.45	2.58
Test 7	91,820	30,607	6.83	3.41	50,330	16,777	6.39	3.195	6.72	6.75
Test 8	954,250	318,083	6.41	3.21	43,460	14,487	6.11	3.055	6.5	6.44
Test 9	13,180	4,393	3.26	1.63	32,670	10,890	3.31	1.655	3.33	3.35
Test 10	64,460	21,487	6.33	3.17	27,570	9,190	4.79	2.395	5.94	5.92
Test 11	44,610	14,870	2.84	1.42	25,800	8,600	2.69	1.345	2.83	2.82
Test 12	5,170	1,723	4.55	2.28	26,110	8,703	5.07	2.535	4.94	5.08
Test 13	10,470	3,490	40.48	20.24	23,700	7,900	7.69	3.845	17.66	17.95
Test 14	1,661,540	553,847	2.94	1.47	28,830	9,610	3.45	1.725	2.92	2.97
Test 15	12,660	4,220	6.69	3.35	23,220	7,740	10.01	5.005	8.77	8.99
Test 16	1,061,360	353,787	5.61	2.81	39,620	13,207	8.59	4.295	5.77	5.76
Test 17	126,800	42,267	5.51	2.76	19,630	6,543	8.59	4.295	5.89	5.99
Test 18	29,940	9,980	2.52	1.26	18,680	6,227	9.99	4.995	5.52	5.52
Test 19	40,340	13,447	1.11	0.55	21,540	7,180	5.07	2.535	2.5	2.55
Test 20	48,130	16,043	5.48	2.74	17,380	5,793	28.31	14.155	11.59	11.86

Goodness-of-fit characteristics: $r^2 = 0.99$, $p \leq 0.001$

Appendix 5 *Daphnia magna* toxicity data

Effluent	:	Stroud wastewater treatment works (A1) 25 November 2008
Effluent sample date	:	25 November 2008
Effluent assay date	:	25 November 2008

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	17:20	7.6	6.1-7.2	20-21	5	0	0		<5	2	-	2
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1												
	2												
	3												
	4												
Tox 2	1	18:35				5	0	0		136	124 (±2)	113 (±2)	118 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 3	1	18:45				5	0	0		231	222 (±1)	169 (±3)	196 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 4	1	19:00				5	0	0	All sluggish (48hrs)	392	358 (±3)	349 (±2)	354 (1%)
	2					5	0	2	All sluggish (48hrs)				
	3					5	0	2	All sluggish (48hrs)				
	4					5	0	1	All sluggish (48hrs)				
Tox 5	1	19:20				5	0	5		664	592 (±10)	564 (±1)	578 (2%)
	2					5	0	5					
	3					5	0	5					
	4					5	0	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Effluent : Stroud wastewater treatment works (A2)
Effluent sample date : 02 December 2008
Effluent assay date : 02 December 2008

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	15:35	7.5	7.1-7.3	20-22	5	0	0		<5	5	-	5
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1	16:00				5	0	0		80	63 (±2)	62 (±1)	62 (3%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 2	1	16:10				5	0	0		136	124 (±2)	76 (±1)	100 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0	1 sluggish (48hrs)				
Tox 3	1	16:15				5	0	0		231	210 (±4)	174 (±1)	192 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	1					
Tox 4	1	16:20				5	2	4		392	359 (±5)	325 (±1)	342 (2%)
	2					5	3	4					
	3					5	0	0	1 sluggish (24hrs)				
	4					5	2	4					
Tox 5	1	16:25				5	4	5		664	555 (±13)	517 (±3)	536 (2%)
	2					5	4	5					
	3					5	5	5					
	4					5	5	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements

[b] The value in parenthesis is the relative standard deviation.

Effluent : Droitwich wastewater treatment works (B1)
Effluent sample date : 09 December 2008
Effluent assay date : 10 December 2008

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	20:15	8.0	7.0	20-22	5	0	0		<5	3	-	3
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1	20:25				5	0	0		80	90 (±4)	69 (±1)	80 (5%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 2	1	20:30				5	0	0		136	144 (±1)	119 (±4)	132 (4%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	1					
Tox 3	1	20:35				5	0	1		231	218 (±3)	198 (±7)	208 (4%)
	2					5	0	0	1 discoloured (48hrs)				
	3					5	0	0					
	4					5	0	0					
Tox 4	1	20:40				5	0	1	1 sluggish (48hrs)	392	451 (±9)	376 (±4)	414 (2%)
	2					5	0	2	1 sluggish (48hrs)				
	3					5	0	1	1 sluggish (48hrs)				
	4					5	0	1	1 trapped at surface(48hrs)				
Tox 5	1	20:45				5	1	4	1 sluggish (24hrs)	664	620 (±5)	639 (±33)	629 (5%)
	2					5	1	4	2 sluggish (24hrs)				
	3					5	2	5					
	4					5	0	4					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Effluent : Droitwich wastewater treatment works (B2)
Effluent sample date : 18 December 2008
Effluent assay date : 18 December 2008

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	14:20	8.1	7.0	21-22	5	0	0		<5	2	-	2
	2					5	0	0					
	3					5	0	0					
	4					5	0	0	1 sluggish (24hrs)				
Tox 1	1	14:45				5	0	0		80	97 (±1)	96(±1)	96 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 2	1	14:55				5	0	0		136	156 (±2)	142 (±1)	149 (1%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 3	1	15:00				5	0	2	1 trapped at surface(48hrs)	231	213 (±1)	195 (±1)	213 (1%)
	2					5	0	0	1 discoloured (48hrs)				
	3					5	0	2					
	4					5	0	3	1 trapped at surface(48hrs)				
Tox 4	1	15:05				5	0	2	1 sluggish (24hrs)	392	411 (±1)	365 (±1)	388 (1%)
	2					5	0	4	1 sluggish (24hrs)				
	3					5	0	3	1 sluggish (24hrs)				
	4					5	0	3					
Tox 5	1	15:10				5	2	5		664	626 (±2)	538 (±2)	582 (1%)
	2					5	0	5	1 discoloured (24hrs)				
	3					5	1	4					
	4					5	3	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements

[b] The value in parenthesis is the relative standard deviation.

Effluent : Pershore wastewater treatment works (C1)
Effluent sample date : 06 January 2009
Effluent assay date : 07 January 2009

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	15:00	7.9	6.4-7.1	20-21	5	0	0		<5	3	-	3
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1	15:10				5	0	0		80	81 (±1)	75 (±1)	78 (1%)
	2					5	0	0					
	3					5	0	3					
	4					5	0	0					
Tox 2	1	15:20				5	0	0		136	160 (±2)	117 (±2)	138 (2%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 3	1	15:30				5	0	1		231	270 (±4)	192 (±1)	231 (2%)
	2					5	0	1					
	3					5	0	1	1 trapped at surface(48hrs)				
	4					5	0	2					
Tox 4	1	15:40				5	0	5		392	404 (±2)	344 (±1)	370 (1%)
	2					5	1	3					
	3					5	0	3					
	4					5	0	4	1 sluggish (48hrs)				
Tox 5	1	15:50				5	3	5		664	666 (±1)	577 (±1)	619 (1%)
	2					5	1	5					
	3					5	1	5					
	4					5	4	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Effluent : Pershore wastewater treatment works (C2)
Effluent sample date : 13 January 2009
Effluent assay date : 20 January 2009

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	17:20	7.9	7.3-8.4	20-21	5	0	0		<5	4	-	4
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1	17:55				5	0	0		80	102 (±1)	98 (±3)	100 (3%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 2	1	17:45				5	0	0		136	155 (±1)	150 (±1)	153 (1%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 3	1	17:40				5	0	0		231	249 (±2)	237 (±1)	243 (1%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 4	1	17:35				5	1	4		392	413 (±3)	373 (±3)	393 (1%)
	2					5	1	5					
	3					5	0	3					
	4					5	1	5					
Tox 5	1	17:30				5	2	5		664	669 (±2)	558 (±1)	614 (1%)
	2					5	5	5					
	3					5	3	5					
	4					5	1	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Effluent	:	Worcester wastewater treatment works (D1)
Effluent sample date	:	20 January 2009
Effluent assay date	:	20 January 2009

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu[a] (µg/L)	Final Cu[a] (µg/L)	Average[b] Cu (µg/L)
Control	1	16:00	7.9	5.3-6.5	20-22	5	0	0		<5	4	-	4
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1	16:30				5	0	0		80	81 (±1)	76 (±3)	79 (5%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 2	1	16:25				5	0	0		136	131 (±2)	129 (±1)	130 (1%)
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 3	1	16:20				5	0	0		231	228 (±1)	222 (±1)	225 (1%)
	2					5	0	1					
	3					5	0	0					
	4					5	0	0					
Tox 4	1	16:15				5	0	5		392	393 (±2)	364 (±3)	378 (1%)
	2					5	0	5					
	3					5	0	1					
	4					5	0	1					
Tox 5	1	16:10				5	4	5		664	642 (±2)	591 (±2)	616 (1%)
	2					5	2	5					
	3					5	3	5					
	4					5	4	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Effluent : Worcester wastewater treatment works (D2)
Effluent sample date : 27 January 2009
Effluent assay date : 27 January 2009

Type	No.	Start time	pH	DO (mg/L)	Temp. (°C)	Daphnids added	Immob. 24hrs	Immob 48hrs	Notes	Estimated Cu (µg/L)	Initial Cu (µg/L)	Final Cu (µg/L)	Average Cu (µg/L)
Control	1	15:40	7.9	3.6-4.0	21-22	5	0	0		<5	3	-	3
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 1	1												
	2												
	3												
	4												
Tox 2	1												
	2												
	3												
	4												
Tox 3	1	16:00				5	0	0		231	219	209	214
	2					5	0	0					
	3					5	0	0					
	4					5	0	0					
Tox 4	1	15:55				5	0	3		392	376	363	370
	2					5	1	5					
	3					5	0	5					
	4					5	0	4					
Tox 5	1	15:50				5	4	5		664	644	600	622
	2					5	5	5					
	3					5	3	5					
	4					5	4	5					

[a] The values in parenthesis for the initial and final concentrations are the standard deviations from triplicate measurements.

[b] The value in parenthesis is the relative standard deviation.

Appendix 6 Abstract for presentation at SETAC UK Annual Meeting 9-10 September 2008 (accepted)

Metals and Complexants: A Speciation Approach to Consenting and Compliance

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The toxicity of heavy metals such as copper, nickel and zinc in terrestrial aquatic environments does not necessarily relate directly to the concentration of the metal, but rather to a bioavailable fraction, usually only a small percentage of the total metal concentration. Bioavailability is effectively determined by the form (or species) of the metal present in the water, and is influenced by water characteristics such as hardness, alkalinity, pH and dissolved organic carbon. Recently, computer programs such as the Biotic Ligand Model (BLM) have demonstrated success in predicting metal toxicity for copper and zinc on the basis of these characteristics. However the complex and varied nature of wastewater effluents present a challenge due to the presence of synthetic ligands which are not accounted for in present models. Synthetic complexants, such as EDTA and NTA, are commonly found in many wastewater effluents and have a strong affinity for heavy metal ions, forming complexes from which bound metals are potentially unavailable for uptake by aquatic organisms. Initial results from a series of *Daphnia magna* toxicity tests, conducted to determine whether synthetic complexants identified in certain wastewater effluents mitigate the toxicity of heavy metals or distort the accuracy of BLM determinations, are presented. The aim of this study is to better understand the science regarding the speciation of metals in effluents to increase the confidence of the Regulator in using BLM-style approaches to the consenting of metals discharged from wastewater treatment works.

Appendix 7 Abstract for poster at EngD Conference 4 June 2008 (accepted)

Metals and Complexants: A Speciation Approach to Consenting and Compliance

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ABSTRACT

Environmental Quality Standards and Wastewater Treatment Works discharge consents for metals are generally set for dissolved or total concentrations respectively. However, metal toxicity does not necessarily relate directly to the concentration of the metal, but rather to the bioavailable fraction, usually only a small percentage of the total metal concentration.

Bioavailability is effectively determined by the form (or species) of the metal present in the water and consequently, environmental standards which do not have consideration for the speciation of the metal risk being over precautionary and potentially contributing to a need for disproportionate investment in unnecessary treatment.

The determination of metal species and bioavailability is complex. There are, however, computer models, such as the Biotic Ligand Model (BLM), which have demonstrated success in predicting bioavailable metal concentrations in natural waters on the basis of water characteristics, such as total dissolved organic carbon, pH and alkalinity that could in future be applied for consenting purposes. Bioavailability determinations for effluents have, however, been less certain. Metal speciation may be influenced by other substances likely to be present in effluents. Complexants, natural as well as synthetic (e.g. EDTA and NTA in particular) which bind strongly with metal ions, will significantly reduce metal toxicity and therefore could potentially distort the accuracy of any BLM predictions.

The objective of this study is to contribute to the science regarding the speciation of metals in effluents and receiving waters, therefore increasing the confidence of the Regulator in using BLM-style approaches to the consenting of metals (copper, zinc and nickel) discharged from wastewater treatment works.

Keywords: Metals, Copper, Nickel, Zinc, Complexants, Biotic Ligand Model, EDTA

Appendix 8 EngD Conference Poster

A Speciation Approach to Regulatory Compliance for Metals

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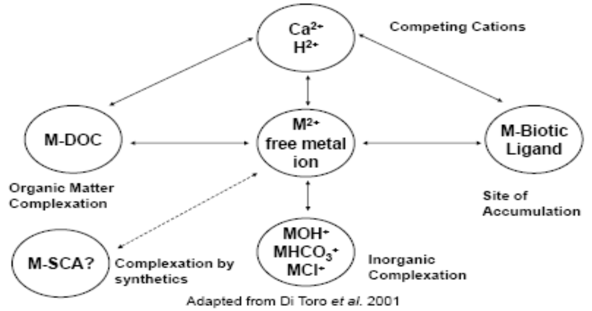
INTRODUCTION

Environmental Quality Standards (EQSs) for metals are generally set for total or dissolved (i.e. passes through a 0.45µm filter) concentrations. However, metal toxicity does not necessarily relate directly to the concentration of metal in the aquatic environment, but rather to the fraction that is bioavailable. This bioavailable fraction is usually only a small proportion of the total concentration, dependant on the form or species of the metal, and variously influenced by water characteristics such as pH, hardness, alkalinity and dissolved organic carbon (DOC).

Currently, EQSs for copper, nickel and zinc consider only the effects of water hardness. EQSs which do not take account of the full range of relevant water characteristics risk being unsupported by science and over precautionary, potentially requiring a level of wastewater treatment, with commensurate CO_2 emissions, which deliver no environmental benefit, perhaps of particular relevance with EU legislation driving more stringent water quality standards for metals.

Recently, Biotic Ligand Models (BLMs), computer programs which determine bioavailability on the basis of water characteristics, have been developed to predict metal toxicity for a variety of organisms on the basis of water characteristics, and present an opportunity to determine environmentally relevant EQSs. The accuracy of these programs in media such as wastewater effluents is however unknown. Effluents contain synthetic compounds, commonly used in many household and industrial applications, which form strong complexes with metal ions, effectively reducing their bioavailability. The purpose of this research project is to evaluate the performance of BLMs for copper, nickel and zinc in wastewater effluents and to identify effluent characteristics which may influence BLM forecast accuracy.

THE BIOTIC LIGAND MODEL



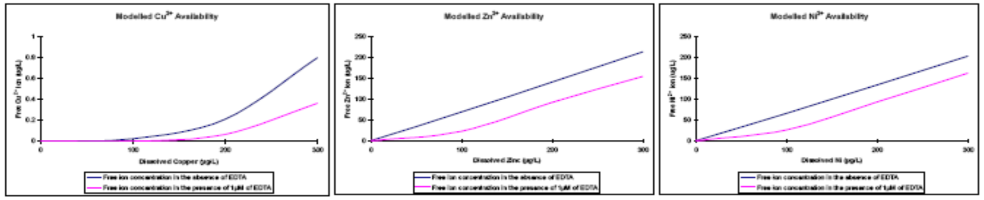
Adapted from Di Toro *et al.* 2001

'Biotic Ligand' is a generic term which refers to the biologically relevant receptor of an organism (e.g. fish gill). BLMs operate a chemical equilibrium sub-model, which determines metal speciation and accumulation at the biotic ligand, and a toxicity sub-model, which uses the accumulation to predict toxicity (Paquin *et al.* 2000).

THE INFLUENCE OF SYNTHETIC CHELATING AGENTS

Synthetic chelating agents (SCAs) are used because of their capability of binding or masking metal cations, typically Ca^{2+} and Mg^{2+}, with which they form complexes with 1:1 molar stoichiometry. SCAs have significant inputs into wastewater treatment works and consequently, are commonly found in effluents and terrestrial aquatic environments.

Formation constants for certain types of chelating agent, aminopolycarboxylates (EDTA, NTA, DTPA) and organophosphonates (HEDP, ATMP, DTPMP, EDTMP) in particular, are greater for Cu^{2+}, Ni^{2+} and Zn^{2+} ions than for Ca^{2+} and Mg^{2+} ions. These chelating agents preferentially bind with Cu^{2+}, Ni^{2+} or Zn^{2+} ions, forming complexes from which these metals are effectively unavailable to aquatic organisms. The presence of SCAs is therefore likely to influence metal bioavailability and therefore BLM accuracy.



Modelled using Visual MINTEQ geochemical speciation program for waters with pH 7.6, DOC 11mg/L, alkalinity 131mg/L as $CaCO_3$, Ca^{2+} 60mg/L, Mg^{2+} 7mg/L, $SO4^{-}$ 120mg/L, Cl^{-} 100mg/L, Na^{+} 70mg/L, K^{+} 10mg/L with (and without) 1µm EDTA (ethylenediaminetetraacetic acid).

BLM ASSESSMENT METHODOLOGY OVERVIEW

Conduct *D. magna* toxicity tests in metal spiked wastewater effluents → Compare the BLM predicted toxicity to the actual toxicity → Evaluate toxic concentration variances in relation to SCA concentrations

CONCLUSION

The BLM approach holds significant promise for use in the determination of environmentally relevant EQSs, or as a mechanism in a tiered assessment approach. An evaluation of the accuracy of BLM toxicity forecasts as well as the factors which influence the accuracy of these forecasts, such as the presence of synthetic chelating agents, will reduce uncertainty and increase the confidence of regulatory authorities in such an approach.

REFERENCES

Paquin, P., Santore, R.C., and, Wu, K.B., Kavvadas, C.D. and Di Toro, D.M., 2000. The Biotic Ligand Model: a model of the acute toxicity of metals to aquatic life. *Environmental Science & Policy*, 3(supplement 1), pp. 175-182.
Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., and Santore, R.C., Biotic Ligand Model of the Acute Toxicity of Metals. 1. Technical Basis. *Environmental Toxicology and Chemistry*, 20(10), pp. 2383-2396, 2001
Gustafsson, J.P., Visual MINTEQ ver. 2.53 (Windows version of MINTEQA2 ver 4.0), 2007

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SEVERN TRENT

Appendix 9 Extended abstract for presentation at SETAC Seville 2010 conference (accepted)

The influence of sewage effluents on water quality standards for copper and zinc

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1. Introduction

The Water Framework Directive (WFD) requires Member States to review existing environmental quality standards (EQSs) for substances of concern, such as copper and zinc. Recently, computer modelling approaches, such as Biotic Ligand Models (BLMs), have demonstrated good accuracy in predicting copper and zinc toxicity for a number of keystone aquatic organisms, and appear likely to be applied for determining new and site specific EQSs for these metals.

The accuracy of BLMs for waters that comprise significantly of treated wastewater effluent is, however, uncertain, and of particular relevance in the UK which has some of the lowest effluent dilution capacity in Europe. Effluents are known to contain synthetic chelating agents which are used in many domestic and industrial applications because of their capability to sequester metal ions, and which may influence metal speciation in a manner that may not be accommodated by BLMs. In addition, synthetic chelating agents may impact on the removal of metals during the wastewater treatment process [1] and jeopardise the ability of treatment works operators to improve the removal of metals, potentially affecting the compliance status of receiving waters. There is, however, relatively little data on the concentrations of synthetic chelating agents in UK wastewater effluents since these do not form part of routine water quality monitoring.

This research is aimed at assessing the influence of environmentally relevant concentrations of synthetic chelating agent on:

- The accuracy of BLM forecasts
- The concentrations of metals discharged via wastewater effluent into the environment
- The impact of the metal concentration contribution on the compliance status of receiving waters

2. Materials and methods

2.1 Synthetic Chelating Agents

A mass balance approach was applied to estimate the potential concentrations of the relevant compounds identified from a review of literature. Two classes of chelating agent were identified as potentially relevant, amino polycarboxylates and organophosphonates. Both have strong affinities for copper and zinc. These were quantified in effluents from four wastewater treatment work using HPLC-UV methods.

2.2 Toxicity Testing

The effect on BLM accuracy was assessed for copper using acute toxicity tests conducted in effluents from four wastewater treatment works with *D. magna* as the test species. Tests were conducted in accordance with OECD guidelines [2]. The BLM model assessed was the Hydroqual BLM version 2.2.3 [3].

2.3 Compliance Assessment

For the purposes of compliance, the metal contribution from synthetic chelating agents was only potentially relevant for locations at which the BLM EQS may be exceeded. Chronic toxicity BLMs for copper [4] and zinc [5] were used to estimate EQSs for water quality monitoring points in the UK Midlands region, and compared with the location copper and zinc concentrations. No allowances for natural background concentrations were made.

3. Results

3.1 Synthetic chelating agents

Of the two classes of chelating agent monitored (6 agents in total), only ethylenediamine tetra acetic acid (EDTA) was detected in the sampled effluents (0.1-1.9µM/L). EDTA speciation analysis revealed the EDTA to be complexed predominately with Fe^{3+}, a form typically unavailable to other metals.

3.2 Acute toxicity

The toxicity assays revealed that the BLM was able to predict the *D. magna* EC50 concentration within the accepted limits of accuracy, regardless of EDTA concentration. However, as a consequence of the predominance of the Fe^{3+} EDTA species, the EDTA fraction available to complex copper, and thus influence BLM accuracy, was found to be less than the total EDTA concentration, but also less than the error margin that the model’s accuracy limits allow. Environmentally relevant concentrations of EDTA are unlikely to produce an identifiable influence on BLM accuracy.

3.3 Compliance assessment

The compliance assessment indicated less than 2% of the considered locations to be at risk of exceeding the BLM EQS for copper, suggesting a relatively limited opportunity to mitigate compliance risk by reducing synthetic chelating agent concentrations. However, the assessment revealed 23% of the considered locations to be at risk of exceeding the BLM EQS for zinc, suggesting potentially some opportunity to mitigate compliance risk by eliminating the contribution from synthetic chelating agents.

4. Conclusions

The copper BLM predicted acute toxicity in the effluents within the accepted limits of accuracy. As a consequence of relatively low concentrations of EDTA, predominance of EDTA in Fe^{3+} form, and the model accuracy limits, environmentally relevant concentrations of EDTA appear unlikely to produce an identifiable influence on BLM accuracy.

From a compliance point of view, however, EDTA, and the non-Fe^{3+} EDTA fraction in particular, may contribute to an increase in the metal concentrations of effluents, and present risk to the compliance status of receiving waters, especially at locations where compliance is marginal. The compliance assessment suggests this effect may be especially relevant for zinc. More research regarding the speciation of synthetic chelating agents in effluents is, however, required.

5. Significance of findings

Regulatory compliance for metals may be influenced by other compounds with anthropogenic origin. Minimising the metal contribution from synthetic chelating agents may, at certain locations, present an opportunity to mitigate compliance risk for metals that might otherwise be attempted via additional wastewater treatment with commensurate cost and energy consumption implications.

6. References

[1] KNEPPER, T.P., 2003. Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment. TrAC Trends in Analytical Chemistry, 22(10), 708-724.

[2] OECD, 2004. OECD guideline for testing of chemicals: Daphnia sp., Acute Immobilisation Test. 202. OECD.

[3] The Biotic Ligand Model Windows Interface, Version 2.2.3, HydroQual, Inc, Mahwah, NJ, April 2005.

[4] European Copper Institute, EU Copper Risk Assessment Software Version 0.0.0.17 (2006), B-1150 Brussels, Belgium

[5] DE SCHAMPHELAERE, K.A.C., LOFTS, S. and 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(5), 1190-1197.

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