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EXPLORATION DES LIENS ENTRE LES PROPRIÉTÉS OPTIQUES DE LA MATIÈRE ORGANIQUE DISSOUTE ET LA COMPLEXATION DES MÉTAUX TRACES DANS LES EAUX DOUCES NATURELLES

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Résumé

La matière organique dissoute (MOD) joue un rôle clé dans l'écotoxicologie des métaux puisqu'elle complexe les ions métalliques libres, et diminue ce faisant la concentration de l'ion libre. Une telle complexation atténue la biodisponibilité et la toxicité des métaux. Le modèle Windermere Humic Aqueous Model (WHAM) est un modèle d'équilibre chimique qui incorpore la complexation des métaux par la MOD. WHAM est partie intégrante du modèle du ligand biotique (BLM), lequel est couramment utilisé pour prédire la biodisponibilité de métaux environnementalement importants, tels que Cd, Cu, Ni et Zn, envers les organismes aquatiques dans les systèmes naturels. WHAM prend en compte la quantité mesurée de MOD, mais l'utilisateur doit définir la « qualité » de la MOD en imposant le pourcentage (%) de la MOD qui est réellement active dans la complexation des métaux. Ici, nous proposons une approche plus rigoureuse, laquelle consiste à mesurer le % de MOD active en utilisant des méthodes spectroscopiques bien établies. Nous avons mesuré les concentrations de Cd^{2+}, Cu^{2+}, Ni^{2+} et Zn^{2+} dans huit lacs et trouvé des accords raisonnables entre les mesures et les prédictions faites par WHAM pour Cd^{2+} et Zn^{2+}. Au contraire, des différences significatives ont été observées pour Cu^{2+} et Ni^{2+}. Pour les métaux Cd, Ni et Zn, le % de MOD actif requis pour ajuster les prédictions du modèle aux mesures d'ion libre est bien expliqué par les variations inter-lacs dans la qualité spectroscopique de la MOD. La proportion de MOD active dans la complexation de Cd et Zn augmente avec l'augmentation de la fluorescence caractéristique de la MOD de type allochtone; une tendance inverse a été observée pour Ni, suggérant que les sites de complexation de Cd et Zn sur la MOD sont différents que pour Ni. L'utilisation de la qualité spectroscopique de la MOD pour estimer le % de MOD actif vis-à-vis de la complexation des métaux a amélioré les capacités de WHAM à prédire les concentrations de Cu^{2+} et particulièrement Ni^{2+} libres. Nous concluons que le raffinement du modèle WHAM pour qu'il incorpore les variations dans la qualité de la MOD doit aller au-delà du simple ajustement du pourcentage de la MOD qui est actif, par exemple en tenant compte de l'affinité des différents sites de complexation des métaux, notamment Cu et Ni. Nous discutons ensuite comment les variations dans la qualité de la MOD, telles que révélées par les différences observées dans les spectres de fluorescence, peuvent être utilisées pour améliorer les prédictions du modèle WHAM vis-à-vis des concentrations des métaux environnementalement importants dans les systèmes aquatiques naturels.

Abstract

Dissolved organic matter (DOM) plays a key role in metal ecotoxicology by complexing free metal ions, an action that decreases the free metal ion concentrations and thus attenuates metal bioavailability and toxicity. The Windermere Humic Aqueous Model (WHAM) is a chemical equilibrium model that incorporates DOM-metal complexation and is an integral part of the Biotic Ligand Model, which is widely used to predict the bioavailability of environmentally significant metals, such as Cd, Cu, Ni and Zn, to aquatic organisms in natural systems. WHAM incorporates the measured quantity of DOM, but users must define the "quality" of the DOM by choosing the % of DOM that is actively involved in metal complexation. The ability to estimate this % active DOM spectroscopically would be useful. We measured Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations in eight lakes and found reasonable agreement between measured and predicted concentrations of Cd^{2+} and Zn^{2+}, but there were marked differences between measured and WHAM-modeled concentrations of Cu^{2+} and Ni^{2+}. Relationships between the model-optimized % active DOM needed to fit modeled to measured free metal concentrations and the lake-to-lake variation in the spectroscopic quality of the DOM were apparent for Cd, Ni and Zn. The proportion of DOM involved in Cd and Zn complexation was found to increase with an increase in the allochthonous DOM fluorescence signature; the opposite trend was observed for Ni, suggesting that the DOM binding sites involved in Cd and Zn binding differ from those involved in Ni complexation. Improved WHAM predictions of Cu^{2+} and, especially, Ni^{2+} concentrations were achieved when the DOM spectroscopic quality was used to estimate the % of DOM active in metal complexation. We conclude that the refinement of the WHAM model to incorporate variations in DOM quality must go beyond the simple adjustment of the "percent active DOM" and possibly include changes to DOM metal binding affinities, notably for Cu and Ni. We discuss the application of variations in DOM quality, as revealed by differences observed in the fluorescence spectra, to refine metal speciation predictions obtained with WHAM for environmentally significant trace metals in natural aquatic systems.

Avant-propos

La thèse comprend un sommaire récapitulatif (en français) ainsi qu'une première partie qui comporte une synthèse générale (en anglais) de l'ensemble des recherches, composée d'une introduction, des objectifs et hypothèses de recherche, d'une discussion des résultats majeurs et des perspectives de la recherche. La deuxième partie de la thèse consiste en trois publications scientifiques : la première est déjà publiée, la seconde est provisoirement acceptée pour publication (en révision), et la troisième est en préparation pour être soumise à une revue avec comité de lecture. Finalement, la troisième partie contient une annexe qui décrit une expérience complémentaire qui a été réalisée pour évaluer l'effet d'entreposage des échantillons sur la spéciation des métaux et la qualité de la matière organique dissoute.

La contribution des auteurs aux différents articles s'établit comme suit :

1. Mueller, K.K., C. Fortin, P.G.C. Campbell. 2012. "Spatial variation in the optical properties of dissolved organic matter (DOM) in lakes on the Canadian Precambrian Shield and links to watershed characteristics", *Aquatic Geochemistry*, **18**(1): 21-44.

   K.K. Mueller : Conception et réalisation du projet (échantillonnage, analyses, traitement et interprétation des données); rédaction initiale et finale de l'article.
   C. Fortin : Conception et réalisation du projet (contribution au traitement et l'interprétation de données); contribution à la rédaction finale de l'article.
   P.G.C. Campbell : Conception et réalisation du projet (contribution au traitement et l'interprétation de données); contribution à la rédaction finale de l'article.

2. Mueller, K.K., S. Lofts, C. Fortin, P.G.C. Campbell. 2012. "Trace metal speciation predictions in natural aquatic systems: incorporation of dissolved organic matter (DOM) spectroscopic quality", *Environmental Chemistry*, **9**: 356-368.

   K.K. Mueller : Conception et réalisation du projet (échantillonnage, analyses, traitement et interprétation des données); rédaction initiale et finale de l'article.
   S. Lofts : Modifications du model WHAM (contribution au traitement et l'interprétation de données; optimisation du modèle); contribution à la rédaction finale de l'article.
   C. Fortin : Conception et réalisation du projet (contribution au traitement et l'interprétation de données); contribution à la rédaction finale de l'article.
   P.G.C. Campbell : Conception et réalisation du projet (contribution au traitement et l'interprétation de données); contribution à la rédaction finale de l'article.

3. Mueller, K.K., C. Fortin, P.G.C. Campbell. 2012. "Nickel and copper complexation by dissolved organic matter in lake waters", en preparation.

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Forward

The thesis consists of a summary (in French) followed by a more general synthesis (in English). The synthesis comprises the following sections: 1) an introduction; 2) the research hypothesis, objectives and originality of the thesis; 3) the methodology; 4) the results and discussion; and finally, 4) the main conclusions of the thesis. The second part of the thesis presents three scientific publications, the first of which is already published, the second has been accepted with revisions, whereas the third is in preparation for submission. The third part of the thesis is an appendix that describes the results of an experiment on the effects of sample storage on the trace metal speciation and the spectroscopic quality of dissolved organic matter in natural water samples.

The contributions of each of the authors of the scientific articles included in the thesis are as follows:

1. Mueller, K.K., C. Fortin, P.G.C. Campbell. 2012. "Spatial variation in the optical properties of dissolved organic matter (DOM) in lakes on the Canadian Precambrian Shield and links to watershed characteristics", *Aquatic Geochemistry*, **18**(1): 21-44.

   K.K. Mueller: Design and realization of the project (sampling, analyses, data analysis and interpretation); initial and final writing of the article.
   C. Fortin: Design and realization of the project (contribution to the analysis and interpretation of data); contribution to the final writing of the article.
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2. Mueller, K.K., S. Lofts, C. Fortin, P.G.C. Campbell. 2012. "Trace metal speciation predictions in natural aquatic systems: incorporation of dissolved organic matter (DOM) spectroscopic quality", *Environmental Chemistry*, **9**: 356-368.

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   S. Lofts: Modifications to the model WHAM (contribution to the analysis and interpretation of data, model optimization); contribution to the final writing of the article.
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1 Introduction

1.1 Métaux et lacs d'intérêt

Plusieurs substances présentes dans l'environnent naturel sont considérées comme potentiellement problématiques envers la santé de l'écosystème et la santé humaine. Le cuivre (Cu), le cadmium (Cd), le nickel (Ni) et le zinc (Zn), émis par les raffineries et fonderies de cuivre et zinc vers l'atmosphère et l'environnement terrestre et aquatique, ont été identifiés comme « prioritaires » par la Loi canadienne sur la protection de l'environnement (CEPA, 2001)[1]. La toxicité potentielle et le risque écotoxicologique de ces métaux doivent être déterminés.

Les régions du Canada qui sont les plus affectées par la présence des raffineries et fonderies de cuivre et zinc sont les régions de Rouyn-Noranda, au Québec, et de Sudbury, en Ontario. Ces régions sont toutes deux localisées sur le Bouclier canadien précambrien (Carignan and Nriagu, 1985). Dans la région de Rouyn-Noranda, la roche mère de type granitique est le plus souvent recouverte d'une épaisse couche de dépôts superficiels de l'ère glacière, bien que parfois elle soit exposée (Veillette et al., 2005). En revanche, dans la région de Sudbury, la roche mère est parfois recouverte d'une mince couche de dépôt (<1 m), mais est le plus souvent exposée (Barnett and Bajc, 2002). Ces différences dans l'exposition de la roche-mère contribuent aux différences dans la topographie et, probablement, à celles dans la végétation, les sols et l'hydrologie des bassins versants des deux régions (Sobek et al., 2007).

Les deux régions d'intérêt possèdent un long historique d'activité minière et de raffinage de métaux, datant du début du XIXe siècle (Carignan and Nriagu, 1985; Couillard et al., 2004; Gallon et al., 2006; Nriagu et al., 1982). Les concentrations de métaux trouvées dans les aires entourant les raffineries sont tributaires de la direction des vents dominants et décroissent au fur et à mesure que l'on s'éloigne de la raffinerie. Certains lacs dans la région de Rouyn-Noranda sont aussi directement affectés par des rejets miniers et par le drainage minier acide (Goulet and Couillard, 2009).

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[1] Toutes les références se trouvent à la fin de la Quatrième partie de la thèse (page 205).

1.2 Spéciation des métaux en eau douce

La toxicité des métaux envers les organismes dans l'environnement aquatique dépend de leur biodisponibilité, laquelle dépend des formes spécifiques que revêtent les métaux (Allen and Hansen, 1996; Batley et al., 2004; Janssen et al., 2003). En particulier, la concentration de l'ion métallique libre est généralement reconnue comme représentative de la biodisponibilité des métaux envers les organismes aquatiques (Campbell, 1995). En eau douce, les ligands inorganiques simples tels que OH^-, HCO_3^- et CO_3^{2-} sont souvent présents à des concentrations permettant la formation de complexes avec les ions métalliques tels que Cu^{2+}, Cd^{2+}, Ni^{2+} et Zn^{2+} (Stumm and Morgan, 1996). Des monomères organiques comme le citrate, la glycine ou les acides polycarboxyliques ont aussi une grande affinité pour les ions métalliques, mais était donné leur grande labilité vis-à-vis de la dégradation par l'activité microbienne, ils ne sont normalement pas présents en concentration suffisante, dans les eaux douces, pour influencer la spéciation des métaux (Morel and Hering, 1993).

La complexation des métaux par la matière organique dissoute (MOD) est plus difficile à quantifier que celle par les ligands inorganiques mentionnés précédemment. En effet, l'hétérogénéité ainsi que les propriétés polycoordinantes et polyélectrolytiques de la MOD rendent impossible la détermination de sites de complexation précis. Des constantes conditionnelles sont par conséquent déterminées expérimentalement en titrant de la MOD isolée chimiquement avec des métaux dans des conditions très spécifiques (ex. : pH, force ionique, concentrations d'ions majeurs). Ces constantes conditionnelles ne peuvent toutefois être utilisées dans des conditions autres que celles dans lesquelles elles ont été déterminées. L'effet de ces différentes variables sur la complexation des métaux par la MOD a été exploré en détail par Tipping (2002). D'abord, la complexation des métaux dépend de la concentration du métal libre, $[M^{z+}]$, laquelle dépend de la concentration totale de métal. La complexation dépend aussi de l'affinité du métal étudié pour les sites de complexation de la MOD, ainsi que du pH de la solution. Lorsque le pH diminue, la compétition entre le cation métallique et les protons pour les sites de complexation augmente (ex. : Benedetti et al. (1995)). Les autres cations en solution peuvent aussi compétitionner avec le cation métallique, en fonction de leur affinité relative et de leur concentration en solution (ex. : Cao et al. (2006)). Enfin, l'effet de la force ionique sur la complexation des métaux par la MOD est expliqué par le fait que les autres ions atténuent les attractions électrostatiques entre la MOD et le cation métallique (Tipping, 2002).

Pour les métaux cationiques, la mesure d'une ou plusieurs espèces chimiques dans les eaux douces naturelles n'est pas une opération de routine. Selon la composition chimique de l'échantillon, des changements réversibles dans la coordination ou dans l'état d'oxydation des éléments d'intérêt peuvent survenir. De plus, la mesure elle-même peut perturber l'équilibre initialement présent. En dépit de ces difficultés, des méthodes analytiques pour déterminer la forme spécifique des métaux dans les eaux naturelles se sont grandement améliorées au cours des dernières décennies. Ces techniques comprennent par exemple les électrodes sélectives (ISE), la voltampérométrie anodique (ASV) et cathodique (CSV) et la diffusion sur gel en couches minces (DGT) (Batley et al., 2004).

1.3 Matière organique dissoute (MOD)

La nature ubiquiste et persistante de la MOD dans l'environnement terrestre et aquatique est responsable de son rôle clé dans plusieurs processus biogéochimiques. Le cycle du carbone (Schiff et al., 1990), la pénétration de la lumière dans les eaux de surface (Scully and Lean, 1994), les réactions photochimiques (Molot and Dillon, 1997), l'acidification des eaux de surface (Schindler and Curtis, 1997), les cycles des nutriments (Dillon and Molot, 1997) et la spéciation de métaux (Buffle, 1988; Perdue and Ritchie, 2003; Wood et al., 2011) sont autant d'exemples de processus dans lesquels la MOD joue un rôle.

La MOD se définit par un mélange complexe et désordonné de molécules polyélectrolytiques provenant des plantes, microbes, et produits animaux à divers stades de décomposition (Aiken et al., 1985; Thurman, 1985; Wetzel, 2001). C'est la présence d'une variété de groupements fonctionnels qui permet à la MOD d'exercer un grand nombre de fonctions écologiques. La variabilité dans les sources de la MOD, son transport et les mécanismes de sa dégradation contribuent aussi à sa nature hétérogène. Dans la plupart des lacs, la MOD provient principalement de sources allochtones extérieures au système aquatique, c'est-à-dire depuis les sols et les plantes terrestres. La MOD allochtone consiste en un mélange de matière organique provenant du sol, à la fois la matière organique humifiée au cours du temps et la matière organique fraichement issue des plantes et du sol (Thurman, 1985). Dans un lac, les sources de matière organique autochtone sont le résultat de la sécrétion, de la décomposition et la de lyse des macrophytes, algues et cyanobactéries présents dans les eaux littorales et profondes (Thurman, 1985; Wetzel, 2001). La composition de la MOD dans un lac reflète donc la contribution relative de la matière organique provenant de sources autochtones et allochtones, et la transformation de la MOD qui survient le long de son transport dans le bassin versant vers le lac, et dans le lac lui-même (affectés par exemple par la sorption de la MOD sur les particules de sol (Schumacher et al., 2006) et par sa photo- et biodégradation (Köhler et al., 2002)). Par conséquent, les caractéristiques du bassin versant comme sa géomorphologie, son régime hydrologique et l'existence de lacs en amont, la couverture végétative, et l'utilisation du territoire influencent la nature de la MOD (Jaffé et al., 2008; Mattsson et al., 2005).

La caractérisation de la MOD requiert généralement la séparation de cette dernière en isolat en utilisant la méthode développée par Thurman et Malcolm (1981). D'abord la MOD est séparée en substances humiques et en substances non humiques. Les substances humiques sont d'origine biologique et sont composées de molécules à haut poids moléculaire, réfractaires à la biodégradation. Ces substances sont réputées représenter jusqu'à 70 ou 80 % de la MOD dans les eaux naturelles (Thurman, 1985). Les substances humiques peuvent être ensuite séparées en acides humiques et en acides fulviques, ou humine, en tirant avantage de leurs différentes solubilités en fonction du pH (Thurman and Malcolm, 1981). En effet, les acides humiques sont solubles seulement à pH plus grand que 2, alors que les acides fulviques sont solubles à tous les pH. Les acides fulviques sont plus solubles parce qu'ils contiennent une grande proportion de groupes fonctionnels carboxyliques et hydroxyles. Leurs masses moléculaires sont aussi plus petites que celle des acides humiques, qui sont plus lourds et contiennent une plus grande proportion de groupements ayant des pK_a similaires à ceux des groupements phénoliques

(Thurman, 1985). Malgré les efforts, une description précise de la structure de la matière organique dissoute n'est toujours pas disponible (Abbt-Braun et al., 2004; Sutton and Sposito, 2005).

La qualité de la MOD est un paramètre qu'il est utile d'estimer afin de mieux comprendre les rôles de la MOD dans le milieu aquatique, incluant son rôle dans la complexation et le transport des métaux. Le terme « qualité » est utilisé ici pour classifier la MOD quant à sa composition et à ces sources. Les propriétés optiques de la MOD, en particulier son absorbance (Gondar et al., 2008; Weishaar et al., 2003) et sa fluorescence (Baker, 2002; Coble, 1996; McKnight et al., 2001), sont souvent utilisées pour distinguer entre différents types de MOD provenant d'environnements contrastés. Par exemple, Al-Reasi et al. (2011) ont récemment publié un article de synthèse portant sur l'utilisation des propriétés spectroscopiques de la MOD pour distinguer entre différents types de matière organique et pour prédire leurs capacités à protéger les organismes aquatiques contre la toxicité des métaux.

Les mesures d'absorbance et de fluorescence sont particulièrement utiles puisqu'elles sont relativement simples, rapides et non destructives. En général, la MOD est caractérisée par la fluorescence de type humique ou de type protéique d'origine allochtone ou d'origine autochtone. Les mesures de fluorescence sont maintenant effectuées à l'aide de matrices d'excitation-émission (MEE), puisque cette approche permet d'obtenir une grande quantité d'information et de cartographier la fluorescence de la MOD en trois dimensions. De plus, l'analyse statistique multivariée, comme l'analyse en composantes principales (ACP), a été utilisée avec succès pour extraire de l'information des spectres MEE complexes (Andersen and Bro, 2003; Stedmon et al., 2003). Lorsque l'ACP est utilisée conjointement avec les MEE, des composantes de fluorescence individuelles peuvent être identifiées ainsi que leurs contributions relatives à la fluorescence totale. Cette approche permet ainsi de caractériser la MOD le long des gradients environnementaux (Al-Reasi et al., 2011; Baken et al., 2011; Jaffé et al., 2008; Miller and McKnight, 2010).

Étant donné que la MOD forme des complexes avec les métaux traces dans l'environnement aquatique, et puisque les caractéristiques de la MOD vis-à-vis de la complexation de métaux dépendent du type et de la source de cette dernière (Baken et al., 2011; Cao et al., 2006; Cheng et al., 2005; Xue and Sigg, 1999), les propriétés optiques, en tant que mesures de la qualité de la MOD, sont susceptibles d'être un outil puissant pour prédire l'intensité des interactions entre la MOD et les métaux cationiques. En particulier, la qualité de la MOD, telle que déterminée en utilisant les MEE de fluorescence et l'ACP, pourrait permettre de raffiner les modèles de spéciation à l'équilibre conçus pour prédire la biodisponibilité des métaux dans les eaux naturelles (Dudal and Gérard, 2004).

1.4 Modélisation de la spéciation à l'équilibre

Les modèles de spéciation à l'équilibre sont souvent utilisés pour prédire la forme spécifique des métaux dans les eaux naturelles en utilisant une approche thermodynamique avec des équations d'équilibres chimiques à stœchiométries précises. Toutefois, la nature hétérogène de la MOD rend difficile la définition de constantes thermodynamiques spécifiques qui pourraient être associées à des complexes. Néanmoins, en utilisant des hypothèses raisonnables, basées sur les connaissances actuelles sur les groupements fonctionnels de la MOD, sa propension à complexer les cations dans les eaux naturelles a pu être modélisée avec un certain succès (ex. : Kinniburgh et al. (1996), Tipping (1998)). Le logiciel de spéciation à l'équilibre Windermere Humic Aqueous Model (WHAM), combiné au modèle Humic Ion-Binding Model VI (Tipping, 1998), prend en compte l'hétérogénéité naturelle de la matière organique naturelle (MON), la compétition des ions, la stœchiométrie des réactions, et les interactions électrostatiques.

Le logiciel WHAM-VI incorpore des paramètres de complexation issus de l'analyse de centaines de jeux de données existant pour la complexation du proton et des métaux par les acides fulviques et humiques, obtenus pour une large gamme de conditions expérimentales. WHAM-VI a été abondamment testé en laboratoire pour étudier les interactions entre la MOD et les ions métalliques dissous (Tipping, 2002). La robustesse du modèle et de ces paramètres généraux a été testée fréquemment en comparant ces prédictions avec les résultats d'expériences en laboratoire. Toutefois, il y a peu d'exemples d'études où WHAM VI a été testé pour les eaux naturelles (ex. : Bryan et al. 2002; Cheng et al. 2005; Guthrie et al. 2005; Fortin et al. 2010; Doig and Liber 2007).

Il est important de spécifier que le modèle WHAM-VI est basé sur l'hypothèse que l'affinité de la MOD pour les cations dans les eaux naturelles peut être représentée par l'affinité respective des acides humiques et fulviques, lesquels peuvent être isolés des eaux naturelles et étudiés en laboratoire. Il est de plus supposé que le rapport « matière organique dissoute : carbone organique dissous » (MOD : COD) est de 2 : 1. En effet, puisqu'aucune technique permettant de mesurer directement la concentration de substances humiques dans les eaux naturelles n'a été développée, il est plus simple de mesurer le COD et d'extrapoler la quantité de MOD. Enfin, puisqu'une telle estimation de la quantité de MOD inclut à la fois les substances humiques et non humiques, l'utilisateur du modèle doit aussi estimer la proportion de la MOD qui correspond aux acides fulviques et humiques menant à la complexation des métaux. Cette proportion est souvent considérée comme un paramètre ajustable lors de l'ajustement du modèle aux données mesurées. Il en ressort que les choix, faits par l'utilisateur, quant à la proportion de la MOD jouant un rôle dans la complexation des métaux (fraction dite « active »), affectent considérablement (par 4 ordres de grandeur pour le Cu) les prédictions faites par la suite (Kalis et al. 2006).

La sélection de la fraction active de la MOD est un défi de taille, et les études dans lesquelles ce paramètre est réellement mesuré, plutôt que simplement estimé par ajustement des données, sont rares. Par conséquent, des études qui mesurent à la fois la spéciation des métaux dans les eaux naturelles et la qualité de la MOD doivent être menées à bien pour combler cette lacune (Lofts and Tipping, 2011). De telles études permettront de mieux comprendre la qualité de la MOD, et en principe permettront d'éliminer le besoin de faire des suppositions quant à la proportion « active » de la MOD lors de l'utilisation des modèles. Elles permettront aussi de diminuer les écarts entre la spéciation des métaux prédite et celle mesurée. Toutefois, il faut admettre que même si on réussit à éliminer les artefacts provoqués par l'utilisation de la MOD isolée en laboratoire, il restera néanmoins des fluctuations temporelles et spatiales à l'échelle du bassin versant qui induiront aussi une variabilité vis-à-vis de la complexation des métaux.

Ainsi, les modèles d'équilibres chimiques bénéficieraient de recherches dans les domaines suivants : 1) l'obtention de données de haute qualité sur la complexation des métaux par la matière organique sous une large gamme de conditions environnementales, 2) la mesure de la spéciation des métaux dans les eaux naturelles, 3) la mesure de la qualité de la NOM naturelle en ce qui a trait à son affinité pour les métaux et 4) la validation des modèles de complexation avec des données de terrain (Tipping, 2002).

2 Hypothèse de recherche, objectifs et originalité

2.1 Hypothèse de recherche

D'après des études récentes (Fortin et al., 2010; Guthrie et al., 2005; Unsworth et al., 2006), les concentrations d'ions métalliques libres ($[M^{Z+}]$) mesurées pour le Cu, le Cd, le Ni et le Zn dans les eaux naturelles divergent souvent de celles modélisées en utilisant le logiciel WHAM-VI. Sur la base de ces résultats, nous avons fait l'hypothèse que l'échec du modèle à prédire la spéciation est causé par des variations dans la qualité de la MOD dans les différents plans d'eau étudiés, et que par conséquent l'incorporation de la qualité de la MOD dans le modèle de spéciation à l'équilibre permettrait de mieux prédire la spéciation des métaux dans les eaux naturelles.

2.2 Objectifs de recherche

L'objectif de ce projet de recherche est d'améliorer les modèles de spéciation à l'équilibre en tenant en compte la variabilité spatiale de la qualité de la MOD. Un tel modèle pourrait ainsi mieux prédire la spéciation, et, par conséquent la biodisponibilité du Cu, du Cd, du Ni et du Zn dans l'environnement aquatique naturel. Pour atteindre cet objectif, il est important :

1- D'identifier une série de lacs représentant un large gradient de concentrations de métaux, de MOD et de conditions physicochimiques.

2- De déterminer les qualités spectroscopiques de la MOD et de relier ces variations entre les lacs aux caractéristiques de ces derniers et de leurs bassins versants.

3- De déterminer expérimentalement les concentrations de métal libre ($[M^{z+}]$) dans des lacs choisis et d'explorer les liens entre la concentration de métal libre et différents facteurs environnementaux (concentration de métal total, pH, quantité et qualité de la MOD).

4- De s'assurer que les conditions expérimentales s'approchent des conditions naturelles, par exemple en titrant des échantillons de MOD provenant de lacs contrastés (quantité et qualité de la MOD, spéciation des métaux) avec les métaux dont les concentrations en ions libres prédites et mesurées divergent le plus et de comparer les constantes de complexation conditionnelles observées avec les propriétés spectrales de la MOD.

5- D'améliorer les prédictions du modèle WHAM-VI vis-à-vis de la spéciation des métaux dans les lacs choisis en incorporant la qualité de la MOD dans le modèle. Cette dernière étape est effectuée en estimant la proportion de MOD active dans la complexation des métaux (% d'acides fulviques actifs).

2.3 Originalité

À notre connaissance, cette étude est la première à utiliser l'analyse APC de matrices de fluorescence comme une mesure de la qualité de la MOD, et à incorporer cette information dans les modèles de spéciation chimique à l'équilibre pour les eaux douces naturelles. Il s'agit de plus d'une des rares études à comparer les concentrations d'ions métalliques libres prédites avec celles mesurées expérimentalement, et ce dans variété d'échantillons provenant de 18 lacs dans deux régions contrastées, dans lesquelles la qualité de la MOD et les concentrations de Cu^{2+}, Cd^{2+}, Ni^{2+} et Zn^{2+} ont été mesurées. Le projet contribue donc à une meilleure connaissance de la biodisponibilité de métaux d'importance environnementale.

3 **Méthodologie**

Les méthodes et le matériel employés pour l'échantillonnage et l'analyse des échantillons d'eau étudiés sont décrits succinctement ci-dessous. Une description détaillée est donnée dans les articles faisant partie de cette thèse (Mueller et al., 2012a; Mueller et al., 2012b; Mueller et al., 2012c) et dans la Deuxième partie de la thèse.

3.1 Échantillonnage

Dix-huit lacs répartis dans les régions de Rouyn-Noranda et de Sudbury ont été échantillonnés à l'été 2007 avec des dialyseurs (cellules à diffusion; voir la figure 3 de la Première partie, p.48). Huit d'entre eux ont été échantillonnés à nouveau en 2008. La qualité de la MOD a été caractérisée dans chaque lac (Mueller et al., 2012b), puis la spéciation des métaux Cd, Cu, Ni et Zn, mesurée et prédite, a été comparée pour les échantillons prélevés en 2008 (Mueller et al., 2012c). Une paire de lacs témoins avec de faibles concentrations de métaux totaux (Lac Opasatica dans la région de Rouyn-Noranda et Lac Geneva dans la région de Sudbury) ont été échantillonnés en 2009 pour déterminer les constantes conditionnelles de complexation des métaux Ni et Cu par la MOD à l'aide d'un titrage. Enfin, les lacs Vaudray, dans la région de Rouyn-Noranda, et Geneva, dans la région de Sudbury, ont été échantillonnés en 2009 pour vérifier l'effet du stockage de la MOD sur sa qualité spectroscopique et la spéciation des métaux. Une description détaillée des lacs est donnée dans le tableau 1 (p.54).

3.2 Analyses

Le matériel de laboratoire et d'échantillonnage en plastique a été lavé à l'acide HNO_3 10% (v/v) pour au moins 24 h, rincé avec l'eau ultrapure (>18 Mohms·cm), et séché sous une hotte à flux laminaire de classe 100. La verrerie a été lavée à l'acide HCl 2 N, puis rincée à l'eau ultrapure. Les flacons de polystyrène pour l'analyse des anions ont été rincés à l'eau ultrapure uniquement.

Des sous-échantillons ont été prélevés pour l'analyse des cations majeurs, des anions, des concentrations totales de métaux, du carbone organique total (COT) ainsi que pour les mesures d'absorbance et de fluorescence. La mesure d'absorbance a été utilisée pour calculer l'absorbance spécifique à 254 nm ($SUVA_{254}$), tandis que les mesures de fluorescence ont été utilisées pour calculer l'indice de fluorescence (FI, le ratio entre l'intensité de l'émission de fluorescence à 470 nm sur celle de 520 nm, pour une excitation à 370 nm) et, en utilisant l'analyse de composantes principales (ACP ou PARAFAC pour *parallel factor analysis*), afin d'identifier les fluorophores individuels qui contribuent à la fluorescence totale dans chaque lac (Gondar et al., 2008; Koster and Schmuckler, 1967; McKnight et al., 2001). Les caractéristiques des bassins versants ont aussi été identifiées en utilisant des données hydrographiques et topographique géospatiales intégrées dans le logiciel ArcGIS 10 (Environmental Systems Research Institute, Inc., Redlands, CA).

Les concentrations des ions Cd^{2+}, Ni^{2+} et Zn^{2+} dans les lacs ont été mesurées par la technique d'échange d'ions (IET), et la concentration de Cu^{2+} par électrode sélective (ISE). Enfin, les concentrations d'ions métalliques libres dans chaque lac ont été calculées avec le logiciel WHAM version 6.1.

Les échantillons des lacs Opasatica et Geneva prélevés en 2009 ont été filtrés sur des membranes de polycarbonate (membrane finale 0,2 µm) et titrés avec le Cu et le Ni. Pour les titrages avec le Cu, des quantités connues d'une solution mère de Cu (0,2 mM, $Cu(NO_3)_2$) ont été ajoutées à l'échantillon, réparti en huit portions de 200 mL. Le pH des solutions a été ajusté à celui de l'eau naturelle du lac en utilisant de petits volumes de solutions concentrées de HNO_3 et de NaOH; les solutions étaient ensuite équilibrées pendant 12 h sous la hotte à flux laminaire et à l'abri de lumière. La concentration de Cu^{2+} libre a été ensuite mesurée par ISE. Une approche similaire a été utilisée pour les titrages au Ni, avec l'échantillon réparti en sept portions de 250 mL et des ajouts d'une solution de $Ni(NO_3)_2$ (1 mM), en laissant le pH s'équilibrer pendant 12 h avant d'effectuer la mesure par la méthode IET. Les mesures de Cu^{2+} et Ni^{2+} ont été prises en triple sur un total de 27 portions pour le Cu et 24 pour le Ni. Après les mesures de Cu^{2+} et Ni^{2+}, les sous-échantillons ont été analysés pour le Cu et le Ni total.

4 Résultats et Discussion

Cette section contient une brève discussion des résultats principaux de la thèse. Ces derniers sont présentés en détail dans les articles de la Deuxième partie.

4.1 Bassins versants

La recherche a été effectuée dans des lacs de la région de Rouyn-Noranda, dans le Nord-est québécois, ainsi que dans la région de Sudbury, dans le Nord ontarien. Les lacs ont été échantillonnés durant l'été au cours des trois années d'études (voir la figure 4 et le tableau 1 de la Première partie, pp. 53-54). Les caractéristiques des bassins versants des lacs diffèrent d'une région à l'autre, et d'un lac à l'autre. En effet, les lacs de la région de Rouyn-Noranda ont un temps de résidence plus court que les lacs de la région de Sudbury, et par conséquent ces derniers sont moins influencés par les apports du bassin versant. Il résulte de ces différences que la matière organique dissoute (MOD) provient davantage des lacs eux-mêmes dans la région de Sudbury et davantage des bassins versants dans la région de Rouyn-Noranda.

4.2 Chimie des eaux lacustres

Les différentes caractéristiques des bassins versants, comme la géologie, l'hydrologie et le couvert végétal, jouent un rôle important dans la variabilité régionale de la chimie des eaux de surface. Les paramètres tels que le pH et la concentration des ions majeurs et des métaux sont influencés, de même que la qualité et la quantité de la MOD (Sobek et al., 2007; Wetzel, 2001). Néanmoins, le pH et les concentrations de cations majeurs n'ont varié que relativement peu entre les deux régions d'échantillonnages (voir le tableau 2 de la Première partie, p.56). Les lacs des deux régions ont un pH près de la neutralité et de faibles concentrations de Ca et Mg, ce qui est typique des eaux douces du Bouclier canadien.

Les concentrations totales en Cd, Cu, Ni et Zn dissous varient largement entre les régions et entre les lacs (voir le tableau 2 de la Première partie, p.56), ce qui témoigne du choix judicieux des sites d'étude. Les plus grandes concentrations de Cd et de Zn se trouvent dans la région de Rouyn-Noranda, de même que les plus grands écarts de concentrations entre lacs. Dans la région de Sudbury, le Ni et le Cd montrent les plus grands gradients de concentrations, tandis que les concentrations de Cu et de Zn sont moins variables. La variabilité inter-lacs dans les concentrations totales de métaux dans une région donnée est principalement due aux gradients dans le dépôt atmosphérique des contaminants, ces dépôts étant influencés par les vents dominants qui transportent les émissions des fonderies et raffineries de métaux (Borgmann et al., 1998; Dixit et al., 2007). Certains lacs sont également influencés par des sources locales de métaux, par exemple le drainage minier acide provenant de résidus miniers.

4.3 Matière organique dissoute (MOD)

Les concentrations de MOD dans les lacs d'une région donnée sont influencées par la végétation terrestre, les sols et l'hydrologie, lesquels sont en retour influencés par le climat et la topographie. Néanmoins, à l'échelle locale, les propriétés du bassin versant et du lac lui-même influencent les concentrations et la nature de la MOD (Sobek et al., 2007). Pour les dix-huit lacs échantillonnés, la variabilité dans les concentrations de MOD est bien expliquée par les caractéristiques des bassins versants (voir le tableau 3 de la Première partie, p.59). En général, les lacs de Rouyn-Noranda affichent une plus grande concentration moyenne de MOD (7.2 ± 2.4 mg $C{\cdot}L^{-1}$) ainsi qu'une plus large gamme de concentrations (3.8 à 13.4 mg $C{\cdot}L^{-1}$) que les lacs de la région de Sudbury région (moyenne de 3.4 ± 1.1 mg $C{\cdot}L^{-1}$; concentrations de 0.8 à 6.0 mg $C{\cdot}L^{-1}$). Nous formulons l'hypothèse que les lacs de la région de Rouyn-Noranda reçoivent de grandes quantités de MOD de leurs bassins versants comparativement plus grands, alors que les lacs de la région de Sudbury reçoivent moins de MOD, cette dernière étant alors produite principalement dans le lac même (MOD autochtone).

Outre les différences dans la quantité de MOD, sa qualité, telle que mesurée par spectroscopie, varie aussi grandement entre les lacs (voir le tableau 3 de la Première partie, p.59). Les lacs de la région de Rouyn-Noranda possèdent un indice $SUVA_{254}$ (8.3 ± 1.5 $L{\cdot}m^{-1}{\cdot}mg\ C^{-1}$) plus élevé et un indice FI (1.32 ± 0.04) plus bas que ceux de la région de Sudbury ($SUVA_{254}$=5.3 ± 1.3 $L{\cdot}m^{-1}{\cdot}mg\ C^{-1}$ et FI=1.36 ± 0.08), témoignant d'apports accrus de MOD allochtone dans les lacs de la région de Rouyn-Noranda. Le rapport élevé entre l'aire des bassins versants et celle des lacs (B/L), la grande proportion de couvert végétatif et l'épaisse couche de sols dans les bassins versants de cette région font en sorte que la MOD y provienne davantage du bassin versant. Des études précédentes ont par ailleurs trouvé une relation similaire entre les sources de MOD et la fluorescence et l'absorption de cette dernière (McKnight et al., 2001; Weishaar et al., 2003). Par exemple, Jaffé et al. (2008) ont trouvé des valeurs de SUVA faibles (0.5 à 5 $L{\cdot}m^{-1}{\cdot}mg\ C^{-1}$) et des valeurs de FI élevées (1.4 to I.75) pour des eaux de surface où il y avait une haute proportion de matériel d'origine microbienne, associé à des sources autochtones. Dans d'autres eaux de surface, ils ont noté des valeurs de SUVA élevées (1 à 7.5 $L{\cdot}m^{-1}{\cdot}mg\ C^{-1}$) et des valeurs de FI faibles (1.1 à 1.5), indicatives de matière organique allochtone, provenant de sources terrestres.

Les matrices tridimensionnelles d'excitation-émission de fluorescence (MEE) contiennent des données reflétant l'intégralité du spectre de fluorescence de la MOD. Un outil d'analyse statistique multivariée, plus spécifiquement l'analyse de composantes principales (ACP), a été utilisé pour identifier les quatre fluorophores distincts contribuant à la fluorescence totale de la MOD dans les lacs, lesquels ont été attribués à différents groupements fonctionnels de la MOD en s'appuyant sur des données acceptées dans la littérature (Coble, 1996; Cory and McKnight, 2005; Koster and Schmuckler, 1967). Les composantes 1 et 2 sont attribuées aux fluorophores typiques de la quinone réduite de la matière humique présente dans une grande variété d'environnements aquatiques. La composante 3 est attribuée à un fluorophore typique de la quinone oxydée et réfractaire de la matière humique d'origine allochtone, tandis que la composante 4 est attribuée à un fluorophore typique des groupements tryptophanes des protéines d'origine autochtone.

Selon l'analyse de la fluorescence de leur MOD, les lacs de la région de Rouyn-Noranda possèdent une plus grande proportion de MOD d'origine allochtone que ceux de la région de Sudbury, c'est-à-dire que ces derniers ont une plus grande proportion de MOD d'origine autochtone. Puisque la MOD est un ligand important qui influe sur la spéciation des métaux dans les eaux naturelles, nous formulons l'hypothèse que la spéciation des métaux dissous dans ces systèmes est influencée par les différences dans les types de MOD.

4.4 Spéciation des métaux traces

Le terme « spéciation » réfère ici à la distribution des métaux entre leurs différentes formes spécifiques. Parmi ces formes possibles, l'ion libre revêt un intérêt particulier pour les écotoxicologues. La complexation de l'ion libre dans les eaux naturelles dépend des concentrations de métaux et de ligand disponibles, qu'ils soient organiques ou inorganiques, de même que de l'influence d'autres cations comme le proton et les cations majeurs qui compétitionnent pour les sites de complexation des ligands (Stumm and Morgan, 1996). Pour faciliter la comparaison entre les lacs, la proportion relative d'ions libres, plutôt que la concentration en M^{z+} seule, a été calculée en divisant cette dernière par la concentration totale du métal dissous. Ainsi, dans la région de Rouyn-Noranda, le lac Opasatica possède le plus bas pourcentage de métal libre ($\%M^{z+}$) pour tous les métaux à l'exception de Cu, alors que le lac Dasserat affiche le pourcentage le plus élevé. Dans la région de Sudbury, le lac Bethel possède le plus petit $\%M^{z+}$ pour tous les métaux, alors que le lac Raft montre les plus grands $\%M^{z+}$ pour tous les métaux, à l'exception de Cu. En général, le $\%M^{z+}$ augmente lorsque la concentration de métal total augmente et que le pH décroit, témoignant respectivement de l'importante du rapport métal/ligand et de la compétition entre le proton et M^{z+} pour des ligands.

4.5 Spéciation des métaux traces mesurée et prédite

Les modèles qui calculent la spéciation à l'équilibre des métaux, tels que le modèle WHAM, sont très utiles pour prédire la concentration d'ions métalliques libres dans les systèmes naturels qui contiennent de la MOD. Afin d'utiliser le modèle WHAM pour prédire la spéciation des métaux dans les eaux naturelles, l'utilisateur doit estimer la proportion d'acides humiques et fulviques présents dans l'eau. Cette procédure ne prend cependant pas en compte les variations dans la qualité de la MOD provenant de différentes sources. Dans cette thèse, nous avons d'abord prédit les concentrations d'ions métalliques libres pour Cd^{2+}, Cu^{2+}, Ni^{2+} et Zn^{2+} en utilisant la proportion généralement suggérée de MOD active dans la complexation des métaux, c'est-à-dire 65% (Bryan et al., 2002). Pour tous les lacs étudiés, WHAM VI prédit des concentrations de Cd^{2+} similaires à celles mesurées (voir la figure 5 de la Première partie, p.64). Les prédictions du modèle sont bonnes pour le Zn aussi, bien qu'à de faibles concentrations de

Zn total et libre, le modèle WHAM surestime ces dernières. Au contraire, dans le cas de Cu, WHAM sous-estime l'ion libre dans la moitié des lacs, plus précisément pour ceux dans lesquels les concentrations totales de Cu sont les plus faibles. De plus, WHAM prédit des concentrations de Ni libre systématiquement plus élevées que celles mesurées, et ce pour tous les lacs dans lesquels des quantités mesurables de Ni libre ont été trouvées.

Afin de prendre en compte la variabilité naturelle de la MOD entre les différents lacs, ainsi que les effets de cette variation sur la spéciation des métaux traces, nous avons testé la sensibilité du modèle WHAM vis-à-vis de la proportion de MOD active définie par l'utilisateur (%aFA). Un intervalle arbitraire a été défini en diminuant de moitié (33 %) et en doublant (130 %) la proportion de 65 % utilisée pour calculer les concentrations d'ions métalliques libres (voir la figure 5 de la Première partie, p.64). Selon cette deuxième série de simulations, le modèle WHAM prédit une proportion de métal libre similaire à celle mesurée pour la plupart des lacs pour le Cd et le Zn. Toutefois, malgré la plus large gamme de %aFA employée, l'accord entre les prédictions et les mesures fut satisfaisant seulement pour la moitié des lacs pour Cu et dans aucun cas pour Ni. Nous avons ensuite calculé, pour chaque lac et pour chaque métal, la proportion d'acide fulvique optimale (%aFAopt) qui permettait aux prédictions de WHAM et aux mesures de concentrations d'ions métalliques libres de correspondre parfaitement. Bien que l'augmentation ou la diminution de %aFA ait pour effet de modifier le nombre de sites impliqués dans la complexation des métaux, cela n'affecte pas l'affinité des métaux pour les sites. Pour le Cu, les valeurs de %aFA optimisés varient entre 7 et 90 % des concentrations de MOD totales. Pour le Cd, le Zn et le Ni, les valeurs de %aFAopt sont toutes très élevées, souvent au-dessus de la limite raisonnable de 100 % : entre 61 et 250 % pour le Cd, entre 65 et 410 % pour le Zn et entre 440 et 1900 % pour le Ni. Ces résultats suggèrent fortement que le seul ajustement de la capacité de complexation des acides fulviques (c'est-à-dire le nombre de sites) ne suffit pas, et qu'il faudra par conséquent ajuster aussi l'affinité de complexation actuellement utilisée par WHAM pour le Cd, le Zn et particulièrement le Ni.

Enfin, nous avons comparé la proportion optimisée de la MOD active dans la complexation des métaux aux variations inter-lacs de la qualité spectroscopique de la MOD. Une relation entre les valeurs de %aFAopt calculées et la proportion relative de la composante de fluorescence humique (C1) est observée pour le Cu et le Ni; de même, une relation se dessine entre les valeurs de %aFAopt et la composante de fluorescence allochtone (C3) pour le Cd et le Zn (voir la figure 6 de la Première partie, p.65). De plus, la direction des relations entre les rapports $C1/C_T$ ou $C3/C_T$ et les %aFAopt pour le Cd, le Cu et le Zn diffère nettement de la direction de ces relations pour le Ni. Ces tendances distinctes suggèrent que les sites de complexation de la MOD pour le Cd, le Cu et le Zn sont différents de ceux pour le Ni.

Les relations entre les valeurs de %FAopt calculées et la proportion relative des composantes de fluorescence C1 et C3 ont été utilisées pour calculer à nouveau la concentration des ions métalliques libres pour le Cd, le Cu, le Ni et le Zn dans les lacs d'étude en utilisant WHAM VI. Cette approche a permis d'améliorer les performances du modèle WHAM VI, en particulier quant aux prédictions des concentrations de Ni libre (une amélioration jusqu'à un facteur de 6 pour le Ni; voir la figure 7 de la Première partie, p.67). Ces résultats appuient notre hypothèse selon laquelle la qualité spectroscopique de la MOD pourra être utilisée pour prédire la proportion de MOD active dans la complexation des métaux.

4.6 Paramètres de complexation mesurés

Les raisons suivantes peuvent expliquer les différences entre les concentrations d'ions métalliques libres mesurées et celles prédites par le modèle WHAM : 1) la sous-estimation de la concentration des sites de complexation de métaux par unité de carbone organique dissous, et 2) la sous-estimation de l'affinité de ces sites pour les métaux d'intérêt. La première explication a été explorée ci-haut en calculant les valeurs de %FAopt pour chaque métal et pour chaque lac. Toutefois pour le Cd, le Cu, le Zn et le Ni, les valeurs de %FAopt étaient supérieures à la valeur maximale raisonnable de 100 %. Cela nous amène à supposer que c'est la sous-estimation de l'affinité des sites complexation qu'il nous fait maintenant explorer. En effet, les constantes de complexation prises en compte par WHAM sont obtenues depuis des titrages en laboratoire en utilisant des isolats d'acides humiques et fulviques. De plus, de telles expériences sont souvent réalisées en utilisant des rapports métaux : MOD beaucoup plus élevés que ceux normalement observés dans les eaux naturelles.

Nous avons mesuré la complexation de Ni et Cu par la MOD par l'entremise de titrages pour deux lacs où les concentrations naturelles de métaux totaux sont faibles et pour lesquels la qualité spectroscopique de la MOD est différente. Ensuite, nous avons comparé les courbes de titrage obtenues à celles prédites par WHAM VI en utilisant les paramètres suggérés par le fabricant du logiciel, en fixant à 65 % la proportion de la MOD active dans la complexation des métaux, et en supposant que toute cette fraction active pourrait être représentée par les acides fulviques (voir la figure 8 de la Première partie, p.68). Les résultats montrent que, d'une part, la MOD présente dans le lac Geneva complexe plus de Ni par unité de carbone que celle du lac Opasatica, et que, d'autre part, elle complexe moins de Cu par unité de carbone. Nous nous sommes ensuite appuyés sur ces résultats pour calculer des constantes conditionnelles pour la complexation de Ni et de Cu par la MOD des lacs Opasatica et Geneva.

Considérons d'abord la complexation du Ni. Son affinité pour les sites faibles de la MOD du lac Opasatica (Log $K_{1,Ni}$=6.98) est significativement plus élevée (*P*=0.009) que pour la MOD du lac Geneva (Log $K_{1,Ni}$=6.30), malgré le fait que les sites faibles dans le lac Opasatica ($L_{1,Ni}$=34.2 nmole·mg C^{-1}) soient présents à des concentrations quatre fois plus basses (*P*=0.01) que dans le lac Geneva ($L_{1,Ni}$=137 nmole·mg C^{-1}). L'affinité du Ni pour les sites forts dans la MOD n'est pas significativement différente (Log $K_{2,Ni}$=11.6 et 11.07 pour le lac Opasatica et Geneva, respectivement), encore une fois malgré le fait qu'il y ait environ six fois moins (*P*=0.025) de sites forts par unité de carbone dans le lac Opasatica ($L_{2,Ni}$=0.70 nmole·mg C^{-1}) que dans le lac Geneva ($L_{2,Ni}$=3.98 nmole·mg C^{-1}).

Malgré que l'affinité de complexation de Cu pour les sites faibles de la MOD ne soit pas significativement différente entre les deux lacs (Log $K_{1,Cu}$=7.59 pour le lac Opasatica et Log $K_{1,Cu}$=7.63 pour le lac Geneva), le nombre de sites faibles par unité de carbone est environ trois fois plus élevé ($P<0.001$) dans le lac Opasatica ($L_{1,Cu}$=244 nmole·mg C^{-1}) que dans le lac Geneva ($L_{1,Cu}$=75.6 nmole·mg C^{-1}). Les sites forts pour la complexation du Cu, quant à eux, ont une affinité (Log $K_{2,Cu}$=9.46) significativement plus forte (P=0.014) et sont deux fois plus nombreux (P=0.003) dans la MOD du lac Opasatica que dans la MOD du lac Geneva (Log $K_{2,Cu}$=8.90 et $L_{2,Cu}$=27.0 nmole·mg C^{-1}). Ces résultats suggèrent fortement que la MOD diffère entre les lacs Opasatica et Geneva en ce qui a trait à son affinité pour le Ni et le Cu.

Les mesures d'absorbance et de fluorescence suggèrent que la MOD du lac Opasatica possède les caractéristiques d'une MOD provenant de sources allochtones, alors que celle du lac Geneva affiche une signature d'origine autochtone. Il est par conséquent raisonnable de conjecturer que les différentes affinités de complexation pour Cu et Ni trouvées pour la MOD de ces deux lacs sont reliées aux différentes proportions de MOD allochtones et autochtones. La MOD de type aromatique provenant de sources allochtones possède une affinité de complexation significativement plus grande pour Cu – aux sites forts, K_2 – que la MOD de type protéique provenant de sources autochtones. Au contraire, une tendance opposée est observée pour Ni. En effet, l'affinité de Ni pour les sites faibles K_1 est significativement moindre pour le lac possédant la MOD plus aromatique de type humique provenant de source allochtone. La capacité de complexation des deux types de sites pour le Ni était significativement plus faible pour le lac possédant la MOD aromatique de type humique provenant de source allochtone. De nouveau, ces résultats suggèrent que le Ni et le Cu se lient à des types de sites de complexation différents.

À notre connaissance, ces résultats sont les premiers à indiquer la présence de sites spécifiques pour certains métaux dans la structure complexe de la MOD. Ces résultats indiquent l'importance de spécifier la qualité de la MOD afin de mieux prédire la complexation des métaux par la MOD dans les systèmes naturels.

5 Conclusions

L'objectif principal de cette thèse était de démontrer que les calculs de la spéciation des métaux dans les eaux naturelles puissent être améliorés en incorporant des indices de la qualité de la MOD dans les modèles de spéciation à l'équilibre existants : nous avons formulé l'hypothèse que la qualité spectroscopique de la MOD peut être utilisée à cette fin.

Fort des résultats d'études précédentes montrant les différences entre les concentrations d'ions libres mesurées et celles prédites par le code WHAM dans les lacs (Fortin et al., 2010; Unsworth et al., 2006), nous avons caractérisé la spéciation des métaux dans une série des lacs situés dans deux régions différentes du Bouclier canadien.

Malgré un accord raisonnable entre les concentrations de métaux libres prédites par WHAM quant aux concentrations libres de Cd^{2+} et Zn^{2+} pour la majorité des lacs, des différences notables ont été observées pour le Cu^{2+} et le Ni^{2+}. En particulier, des différences marquées ont été observées pour le Cu^{2+} lors que les concentrations de Cu^{2+} libre étaient sous 0.4 nM (c'est-à-dire pour des concentrations totales de Cu sous 40 nM). Or, de telles concentrations sont fréquentes dans les eaux naturelles. Dans le cas de Ni, WHAM surestime systématiquement les concentrations de Ni^{2+} dans chaque lac.

Les raisons possibles pour les différences entre la spéciation mesurée et celle prédite par WHAM ont récemment été résumées par Lofts et Tipping (2011). Ces derniers ont noté que les facteurs suivants peuvent influencer les prédictions de WHAM : 1) la précision des intrants du modèle ainsi que celle de ces paramètres, 2) l'incertitude sur les mesures de concentrations d'ions métalliques libres et 3) les différences entre les propriétés complexantes des substances humiques présentes dans les eaux naturelles et celles des isolats humiques et fulviques utilisés pour calibrer le modèle. C'est précisément cette dernière explication qui a été explorée durant cette thèse, en caractérisant la MOD et la spéciation des métaux dans les lacs d'étude.

Nous nous sommes intéressés à une méthode de caractérisation de la MOD qui, en principe, pourrait être utilisée par des non-spécialistes. Nous avons donc choisi des techniques spectroscopiques simples, lesquelles sont disponibles dans la plupart des laboratoires de chimie de l'eau, par opposition aux techniques fastidieuses utilisées pour isoler les substances humiques. Premièrement, nous avons mis en évidence des différences dans la composition de la MOD dans un ensemble de petits lacs du Bouclier canadien en utilisant différents paramètres optiques tels que l'absorbance spécifique UV ($SUVA_{254}$), les indices de fluorescence (FI) et les matrices d'excitation-émission de fluorescence (MEE) interprétées par l'analyse de composantes principales (ACP). Nous avons ensuite exploré comment les propriétés optiques de la MOD peuvent être utilisées pour améliorer la prédiction, par les modèles d'équilibres chimiques, de la spéciation des métaux dans les eaux naturelles.

Nous avons fait l'hypothèse que la mesure spectroscopique de la qualité de la MOD peut être utilisée pour estimer la proportion de MOD active dans la complexation des métaux. Comme mentionné précédemment, l'utilisateur de WHAM doit d'abord estimer la proportion du carbone organique dissous présent dans l'échantillon naturel considéré (mesuré en mg $C{\cdot}L^{-1}$) qui est composé de substances humiques, ainsi que la répartition de ces substances humiques entre acides fulviques et acides humiques. La pratique courante consiste à présumer un rapport MOD : COD de 2 (Thurman, 1985) et à *supposer que* 65 % de cette matière organique dissoute possède des propriétés de complexation des cations similaires à celles des isolats d'acides fulviques (Bryan et al., 2002). En utilisant ces principes suggérés par les créateurs de WHAM, nous avons trouvé de grandes différences entre les concentrations de Cu^{2+} et de Ni^{2+} mesurées et celles prédites par le modèle.

Nous avons travaillé à améliorer les prédictions du modèle WHAM en calculant le pourcentage optimal d'acide fulvique impliqué dans la complexation des métaux (%FAopt) qui permet de reproduire précisément les mesures d'ion libre pour chaque métal, et dans chaque lac. Les relations entre la valeur de %FAopt et les propriétés spectroscopiques de la MOD ont été explorées. Des relations significatives ont été trouvées entre la composante de fluorescence de type humique (C1) et le %FAopt pour le Cu et le Ni, et entre la composante d'origine allochtone (C3) et le %FAopt pour le Cd et le Zn (voir la figure 6 de la Première partie, p.65).

Ainsi, nous avons amélioré les capacités de WHAM à prédire la spéciation de Cu^{2+} et Ni^{2+} libres dans les lacs en utilisant les mesures de fluorescence pour estimer la proportion de MOD active dans la complexation des métaux. La plus grande amélioration a été observée quant aux prédictions de Ni^{2+} libre (jusqu'à un facteur de 6), suggérant que la signature de fluorescence de la MOD peut être utilisée comme indice de son activité de complexation dans les modèles d'équilibres chimiques, tels que WHAM.

En fait, il serait utile de systématiquement déterminer : i) la matrice d'excitation-émission pour une eau lacustre donnée, ii) de calculer la proportion relative de la composante de fluorescence $C1/C_T$ ou $C3/C_T$, et iii) d'utiliser cette proportion pour estimer %FA dans le lac en se basent sur une relation telle que celle illustrée dans la figure 6 de la Première partie (p.65). Bien que cette approche soit prometteuse, elle devrait idéalement être vérifiée sur une plus large gamme d'échantillons de MOD dans différents types d'eau naturelle.

Afin de permettre au modèle WHAM d'estimer encore plus précisément la spéciation de Cu et de Ni, des ajustements non pas seulement à sa capacité de complexation mais aussi à son affinité pour ces métaux seront éventuellement nécessaires. En effet, nous avons trouvé que les différences dans la capacité de complexation de la MOD, c'est-à-dire dans le nombre de sites, pour Cu et Ni pouvaient être expliquées par les qualités spectroscopiques de la MOD. Dans un petit nombre de lacs (N=2) nous avons aussi trouvé que les variations entre lacs et entre métaux de l'affinité de complexation de la MOD pour Ni et Cu pouvant également être expliquée par la qualité spectroscopique de la MOD. Puisque le nombre de lacs utilisé pour cette dernière observation est petit, la relation tirée est conjecturale et devrait être étudiée dans une plus grande variété d'eaux de surface. Néanmoins, ces résultats confortent notre hypothèse à l'effet que la qualité de la MOD, telle qu'exprimée par la signature fluorescente de cette dernière, soit un paramètre clé qui permettra d'estimer le rôle de la MOD dans la spéciation des métaux traces dans les eaux naturelles.

L'amélioration des modèles d'équilibres chimiques permet de mieux prédire la spéciation des métaux traces dans les écosystèmes aquatiques. La justesse de ces précisions est essentielle pour pouvoir estimer la biodisponibilité des métaux et, par conséquent, leur toxicité envers les organismes aquatiques. Le modèle du ligand biotique (BLM) est couramment utilisé par les scientifiques et les législateurs pour prédire la toxicité des métaux envers les organismes aquatiques. Ce modèle utilise WHAM pour calculer la concentration d'ion libre aqueuse à proximité du ligand biotique considéré. Bien que le BLM fonctionne raisonnablement bien lorsqu'il considère des concentrations de métaux élevées et leur effets toxiques aigues, il doit être amélioré pour mieux prédire la toxicité des métaux à faible concentrations de ces derniers, c'est-à-dire lorsqu'ils sont susceptibles d'engendrer des effets toxique chroniques (Campbell et al., 2006). Augmenter la justesse des prédictions du modèle WHAM lorsqu'il considère de faibles concentrations de métaux permettrait directement de mieux prédire la toxicité chronique des métaux. La portée de cette recherche est donc basée sur le fait qu'une amélioration des prédictions de la spéciation de métaux traces dans l'environnement aquatique est nécessaire afin de mieux estimer et gérer le risque écologique posé par les métaux traces (Luoma and Rainbow, 2008).

Première partie : Synthèse

1 **Introduction**

1.1 Metals of interest

Many substances present in our natural environment may be considered as problematic with respect to human or ecosystem health. The Canadian Environmental Protection Act defines a toxic substance as one that "entering or may enter the environment in a quantity or a concentration or under conditions that: a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity, or b) constitute or may constitute a danger to the environment on which life depends, or c) constitute or may constitute a danger in Canada to human life or health". Environment Canada and Health Canada published a Priority Substances List Assessment Report in 2001 (CEPA, 2001) within which Cu, Cd, Ni and Zn released from copper and zinc smelters and refineries to the atmospheric, terrestrial and aquatic environments were identified as priority substances, as defined by CEPA, the potential toxicity of which should be determined. What was not clear from this assessment was the chemical speciation of the metals released and their fate in the eventual receiving water, therefore limiting the regulators' ability to estimate their bioavailability and toxicity (Hornbrook et al., 1986).

1.2 Sites of interest

Areas in Canada that are greatly affected by the presence of copper smelting and refining include the Rouyn-Noranda region of Québec and the Sudbury region of Ontario. Rouyn-Noranda, Québec, is home to the Xstrata Copper-Horne smelter, which has been in operation since 1927, and lakes in the area have been contaminated by atmospheric deposition of smelter emissions (Couillard et al., 2004; Gallon et al., 2006). The Xstrata Copper-Horne smelter is the highest producing copper smelter in Canada (190 000 tonnes in 2001) and releases large amounts of Cu, Zn and Cd into the atmosphere (42, 18 and 2.5 tonnes in 2001) (Bonham-Carter, 2005). Due to the changes in smelter operations and legislated emission controls, the atmospheric emissions of Cd and Zn from the Horne smelter have decreased significantly (75% and 90%, respectively) in recent history (1989-2001), although the emission of Cu has varied considerably (Bonham-Carter, 2005). Furthermore, the concentrations of metals found in the surrounding area depend on the direction of the prevailing winds and decrease with increasing distance from the smelter (Bonham-Carter et al., 2006; Telmer et al., 2006). There have been many studies of the total dissolved concentrations of metals in the lakes in the surrounding area of Rouyn-Noranda (e.g., Borgmann et al. (2004), Croteau et al. (2002), Guthrie et al. (2005)). In recent years, the concentration of total dissolved Cu in area lakes has been shown to vary from 33 nM (Giguère et al., 2006) to 608 nM (Fortin et al., 2010). The concentration Cd varies from 0.06 nM to 6.8 nM (Fortin et al., 2010). The widest concentration range is that of Zn from 7 nM (Bonneris et al., 2005) to 3700 nM (Fortin et al., 2010). Conversely, Ni varied the least in the region with concentrations ranging from 8 to 12 nM (Giguère et al., 2006). It should also be noted that certain lakes in the Rouyn-Noranda area are affected by direct run-off from past or present mining activities in addition to atmospheric deposition (Goulet and Couillard, 2009).

Sudbury, Ontario, also has a long history of mining, smelting and refinery activities dating back to 1929. There are currently two nickel/copper smelters (Vale-Copper Cliff and Xstrata Nickel-Sudbury) and one copper refinery (Vale-Copper Cliff) operating in the area (CEPA 2001). The Vale-Copper Cliff smelter was reported to release 132, 9.5, 87, and 2.4 tonnes/year of Cu, Zn, Ni and Cd, respectively to the atmosphere in 1995 (CEPA 2001). The Xstrata Nickel-Sudbury smelter was reported to release 9.0, 1.9, 10.2, and 4.5 tonnes/year of Cu, Zn, Ni and Cd, respectively to the atmosphere in 1995 (CEPA 2001). Similar to the Rouyn-Noranda lakes, the Sudbury lakes are greatly affected by the mining and smelting activities in the area (Carignan and Nriagu, 1985; Nriagu et al., 1982). Moreover, the metal concentrations in lakes surrounding the smelters decrease with increasing distance from the smelters (Keller et al., 1999; Pyle et al., 2005; Shuhaimi-Othman et al., 2006). Similar to the Rouyn-Noranda region mentioned above, a substantial decrease in lake water metal concentrations, especially Cu and Ni, over the past 30 years has been observed following reductions in smelter emissions (Keller et al., 1999; Tropea et al., 2010). Nriagu et al. (1998) found that the concentrations of total dissolved metal in lakes in the region varied from 27-4600 nM for Cu, 0.03-11 nM for Cd, 87-1100 nM for Ni and 3-1300 nM for Zn.

Both the Rouyn-Noranda and Sudbury regions are found on the Canadian Precambrian Shield characterized by bedrock made up of quartzite, gabbro and felsic gneisses (Carignan and Nriagu, 1985). However, the superficial deposits covering this underlying geology vary among regions. In the Rouyn-Noranda region, located in the Abitibi sub-province and containing the Abitibi Greenstone Belt, the bedrock is covered with a thick layer of glacial deposits, although clusters of bedrock do break through to the surface occasionally (Veillette et al., 2005). In contrast, the Sudbury region, located on three structural provinces (Grenville, Southern and Superior) of the Shield (Rousell et al., 2002), is covered by a thin (<1 m) and discontinuous layer of glacial deposits (Barnett and Bajc, 2002). These differences in surficial geology contribute to differences in the sensitivity of the two regions to acid precipitation (Jeffries et al. 2003), and they also affect the movement of organic matter from the terrestrial to the aquatic environment (see section 1.4). The differences in surficial geology also contribute to differences in the topography and thus, presumably, to the differences in vegetation, soils and hydrology of the watersheds within these two regions (Sobek et al., 2007).

1.3 Freshwater trace metal speciation

The toxicity of the metals to organisms in the aquatic environment depends on their bioavailability and in turn, the bioavailability of metals in aquatic systems depends on their chemical speciation (Allen and Hansen, 1996; Batley et al., 2004; Janssen et al., 2003). In particular, it is the free metal ion that best predicts the bioavailability of a metal to aquatic organisms (Campbell, 1995). The free metal ion may form complexes with many ligands present in natural freshwater system. For a given complexation reaction between a metal (*M*) and a ligand (*L*) at equilibrium,

$$M + L \leftrightarrow ML \qquad (1)$$

the equilibrium constant *K*,

$$K = \frac{[ML]}{[M][L]} \qquad (2)$$

is a measure of the stability of the formed complex (charges are omitted for brevity). In freshwater systems, simple inorganic ligands, such as OH^-, HCO_3^-, CO_3^{2-} are present at significant concentrations and can form complexes with metals ions such as Cu^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (Stumm and Morgan, 1996). Simple monomeric organic ligands such as citrate, glycine or polycarboxylates have high affinities for many metal ions, but due to their microbial lability, these ligands are not normally present in natural freshwater systems at high enough concentrations to influence the speciation of most metal ions (Morel and Hering, 1993). The speciation of metal ions in simple aquatic systems (i.e. inorganic and simple organic ligands) can be successfully modeled using computer speciation codes (Turner, 1995).

The speciation of metals in natural waters depends on their affinity for the available ligands, and on the concentrations of these ligands. Equilibrium calculations for the four metals of interest in this thesis (Cd, Cu, Ni and Zn) suggest that the most abundant inorganic metal species in natural freshwater are: $CuCO_3^0$ and $Cu(OH)_2^0$; Cd^{2+} and $CdCO_3^0$; Ni^{2+} and $NiCO_3^0$; Zn^{2+} and $ZnCO_3^0$ (Stumm and Morgan, 1996). The carbonate ligand is very important in natural freshwater due to its presence at relatively high concentrations and its affinity for many divalent metals. However, the availability of the carbonate ligand will depend on the pH of the system (Stumm and Morgan, 1996). Alkalinity and pH, therefore, play significant roles in the speciation of many metals in natural aquatic systems.

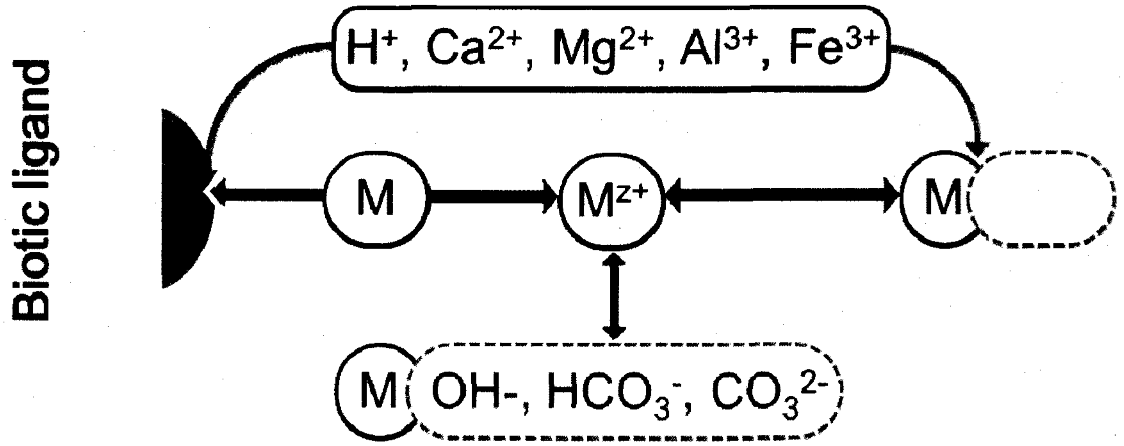

Figure 1. Schematic representation of the interconnectedness between water chemistry and bioavailability / Représentation schématique de la relation entre la chimie des eaux naturelles et la biodisponibilité des métaux.

The complexation of dissolved metals with natural dissolved organic matter (DOM) is less easily quantified than is their binding to the inorganic ligands mentioned above. The heterogeneous, multi-dentate and poly-electrolyte properties of DOM make it impossible to determine specific metal binding constants for DOM (Tipping, 2002). Binding characteristics of metal ions to DOM have, however, been estimated in the past by titrating chemically isolated organic matter fractions with metals under specific conditions of pH, ionic strength and concentration of competing ions (see, for example, Cabaniss and Shuman (1988), Ephraim et al. (1989), Gondar et al. (2006), Higgo et al. (1993), Saar and Weber (1980)). However, these conditional binding constants define the complexation under very specific conditions and theoretically cannot be applied to other scenarios. Tipping (2002) summarised the effect of many variables on the binding of metal ions to DOM. First, the binding of metal ions to DOM depends on the concentration of the free metal ion, [M^{z+}] (which depends on the total metal concentration) and the affinity of the DOM binding sites for the metal in question. The binding of metal cations to DOM will also depend on the pH of the solution. As the pH increases, the competition between the metal cations and protons for binding sites decreases (e.g. Benedetti et al. (1995)). Depending on their relative concentrations and binding strengths, other cations in the solution can also compete with metal cations for DOM binding sites (e.g. Cao et al. (2006)). Finally, the effect of ionic strength on the binding of cations to DOM is explained by the shielding of electrostatic attractions by other ions in solution (Tipping, 2002). Chemical equilibrium models that take into account the binding of metal cations to DOM have been developed and will be discussed in Section 1.5.

1.3.1 Measuring freshwater trace metal speciation

According to the International Union of Pure and Applied Chemistry (IUPAC), a chemical species is defined as "the specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure" and therefore, the distribution of such chemical species in a system is referred to as "speciation" (Templeton et al., 2000). The measurement of one or more chemical species in a natural freshwater sample is, however, not routine. Depending on the chemistry of the sample, reversible changes in the coordinate bonding or oxidation state of the element of interest may occur. Furthermore, the very act of measuring the chemical speciation in a sample may perturb the equilibrium state initially present. Despite these difficulties, analytical methods to measure the speciation in natural waters have largely improved in recent decades. The review by Batley et al. (2004) gives a detailed description of the recent improvements in the detection limits, sensitivities and selectivities of methods, such as those using ion-selective electrodes (ISE), anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), diffusive gradients in thin films (DGT) and permeation liquid membranes (PLM).

1.4 Dissolved organic matter (DOM)

The ubiquitous and persistent nature of organic matter in both the terrestrial and aquatic environments is responsible for its key role in many biogeochemical processes. In aquatic systems, an arbitrary separation of dissolved organic matter (DOM) from particulate organic matter (POM) is achieved by filtering natural water samples through 0.45 µm filters (Wetzel, 2001). Important aquatic biogeochemical processes affected by the presence of DOM include, carbon cycling (Schiff et al., 1990), light penetration into surface waters (Scully and Lean, 1994), photochemical processes (Molot and Dillon, 1997), the acidification of surface waters (Schindler and Curtis, 1997), nutrient cycling (Dillon and Molot, 1997) and metal speciation and transport (Buffle, 1988; Perdue and Ritchie, 2003; Wood et al., 2011).

The foremost characteristic of DOM leading to its many important roles in the environment is its complex heterogeneity. In order for DOM to be involved in such a vast range of ecologically important biogeochemical processes, a heterogeneous nature is necessary (MacCarthy and Rice, 1991). To this end, DOM is defined as a complex mixture of disordered, poly-electrolytic molecules, generated from plant, microbial, and animal products at various stages of decomposition (Aiken et al., 1985; Thurman, 1985; Wetzel, 2001). The presence of a wide variety of functional groups that, together, express average characteristics, permits the ecological functions which rely on the presence of DOM. The importance of the nature of organic matter is well described by MacCarthy and Rice (1991): "An ecologically based rationale for the chemically heterogeneous nature of humus: in the case of humus the mixture is the message". In order to persist in aquatic systems, DOM must also be resistant to rapid degradation (MacCarthy and Rice, 1991; Wetzel, 2001). The complex heterogeneous nature of DOM is in part due to disorderly decomposition processes as opposed to the ordered synthesis of specific biological compounds (MacCarthy and Rice, 1991; Thurman, 1985).

Variability in sources, transport and degradation pathways also contribute to the heterogeneous nature of DOM. In most lakes, DOM originates predominantly from allochthonous sources outside the aquatic system, i.e. from soil or terrestrial plant sources (Thurman, 1985; Wetzel, 2001). Allochthonous DOM may consist of a mixture of old soil organic matter that has been degraded and humified over a long period of time and relatively young organic matter that has recently decomposed from plant or soil organic matter (Thurman, 1985). Autochthonous sources of DOM from within the lake have been described as resulting from the active secretion, decomposition and lysis of macrophytes, algae and cyanobacteria in littoral and open water (Thurman, 1985; Wetzel, 2001). The composition of DOM in a given lake will reflect the relative contributions of its allochthonous and autochthonous sources, as well as the processing of the DOM occurring along its path from the watershed to the lake and within the lake itself (such as adsorption to soil particles (Schumacher et al., 2006) and photo- and bio-degradation (Köhler et al., 2002)). Watershed characteristics such as geomorphology, hydrologic regime, land cover, upstream waterbodies, and land use are likely to influence the composition or nature of DOM (Jaffé et al., 2008; Mattsson et al., 2005). Seasonal variability will also play a role in the relative importance of each DOM source. For example, stream and runoff flow as well as growth and decay cycles of terrestrial and aquatic vegetation vary greatly with seasonal temperature changes (Wetzel, 2001).

Attempts to characterize DOM often involve its separation into isolated fractions using a method developed by Thurman and Malcolm (1981). First, DOM is separated into humic and non-humic substances. Non-humic substances include, among other low molecular weight organic substances, carbohydrates, proteins, peptides and amino acids, which are relatively easily utilised or degraded by microorganisms and, therefore, are present at relatively low concentrations in aquatic systems (Aiken and Wershaw, 1985; Thurman, 1985). Humic substances, on the other hand, are coloured, high molecular weight organic substances of biogenic origin that are resistant to biological degradation. Humic substances are thought to make up 70 – 80% of the DOM in natural waters (Thurman, 1985). Humic substances can be further separated into humic acids, fulvic acids or humin based on their acid-base solubility (Thurman and Malcolm, 1981). Humic acids are soluble only at a pH greater than 2, whereas fulvic acids are soluble at any pH. Humin is insoluble in aqueous solutions at any pH. There has been some debate over the wide pH range used in the isolation of fulvic and humic acids from DOM and there is still no consensus as to whether or not isolation procedures lead to any major chemical decomposition of the DOM (Abbt-Braun et al., 2004). Fulvic acids are thought to be more water soluble because of their higher proportion of carboxylic and hydroxyl functional groups and their smaller molecular size, relative to humic acids, which are generally larger and have a higher phenolic content (Thurman, 1985). The greater aqueous solubility of fulvic acids leads to their presence in greater proportions than humic acid in natural waters. Despite many efforts, a precise description of the structure of DOM has not been achieved (Abbt-Braun et al., 2004; Sutton and Sposito, 2005).

The model of DOM structure outlined by Schulten and Schnitzer (1997) illustrates its heterogeneous nature (Figure 2). These authors point out the usefulness of such models for the investigation of the structure and function of aqueous humic material under environmental conditions. Sutton and Sposito (2005), however, describe a supramolecular view of the humic substances. They describe humic substances as mixture of diverse, low molecular mass components held together by hydrogen bonds and hydrophobic interactions, ultimately forming micellar structures. A similar description of humic substances was first advanced by Khan and Schnitzer (1971). The importance of describing the character of humic substances by molecular interactions is emphasised over molecular components. Sutton and Sposito, therefore, raise the need to re-evaluate the biogeochemical formation and pathways of humic substances in the natural environment.

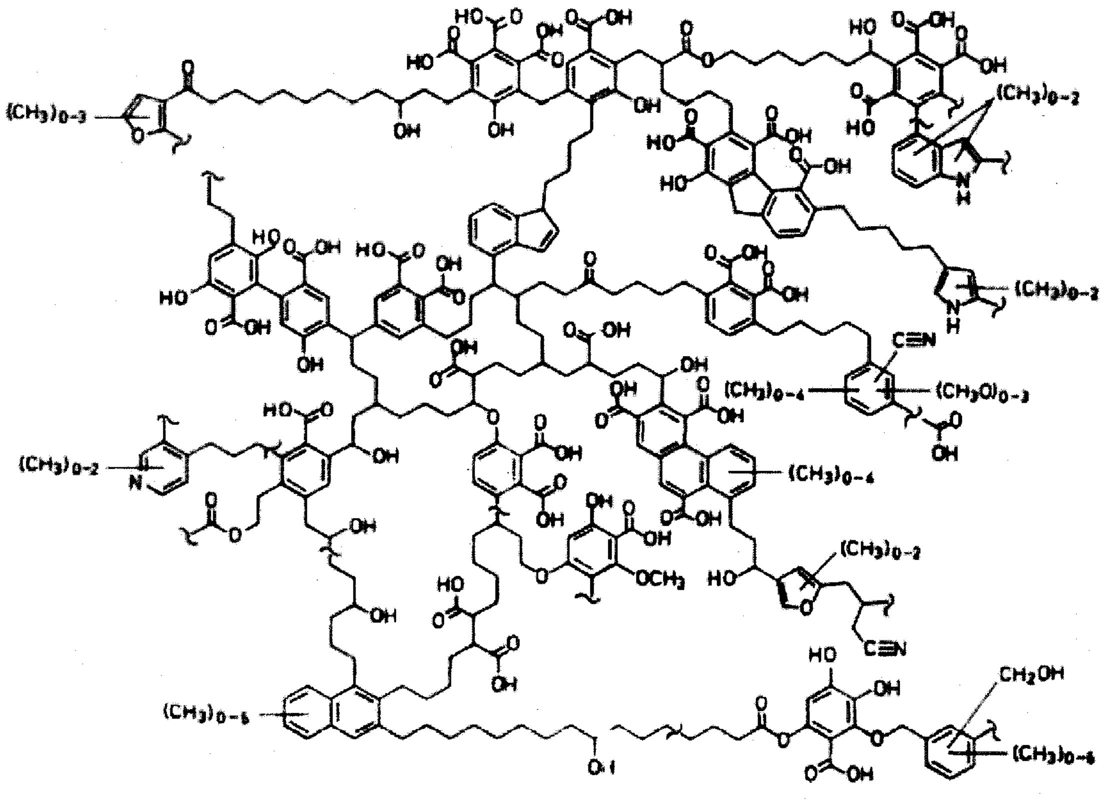

Figure 2. Representation of a proposed molecular structure for humic substances (from Schulten and Schnitzer 1997)[2] / Structure moléculaire probable de la substance humique (tiré de Schulten and Schnitzer, 1997).

In order to better understand the role of DOM in the aquatic environment, including its role in trace metal speciation and transport, the quality of DOM is often estimated. The term quality, here, is used to describe the type of DOM based on its composition and source. Abbt-Braun et al. (2004) present a useful review of quantitative and qualitative methods used to characterize DOM. Spectroscopic methods of characterisation include UV-visible, Infrared (IR), nuclear magnetic resonance (NMR) as well as absorbance and fluorescence spectroscopy. Optical properties of DOM have been used to distinguish among different types of DOM from contrasting environments, including absorbance (Gondar et al., 2008; Weishaar et al., 2003) and fluorescence (Baker, 2002; Coble, 1996; McKnight et al., 2001). For example, a recent

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[2] Note that this structure does not account for the trace amounts of sulfur that normally are found in isolated humic substances.

review by Al-Reasi et al. (2011) discusses the use of various spectroscopic properties of natural organic matter (NOM) to distinguish between different sources of NOM with different protective effects against metal toxicity towards aquatic organisms. Absorbance and fluorescence measurements are useful tools in that they are relatively simple, rapid, sensitive and non-destructive. In general, DOM fluorescence has been characterized as either humic-like or protein-like and has been attributed to either allochthonous or autochthonous sources. More recently, Cory and McKnight (2005) have used fluorescence spectroscopy to identify oxidised and reduced quinone-like fluorophores in DOM from a variety of aquatic environments. Fluorescence spectroscopy may therefore be used to further characterize DOM based on its redox state.

Fluorescence excitation-emission matrices (EEMs) have become a popular method of DOM fluorescence measurement in recent years due to the great amount of information obtained from a three-dimensional picture of DOM fluorescence. EEMs have been used to discriminate among different types of DOM from a wide range of environments such as river water (Baker, 2002), marine and estuarine environments (Coble, 1996) and Antarctic lakes and ponds (McKnight et al., 2001). Although the three-dimensional scans offer more information than traditional single wavelength scans, these methods usually involve the visual inspection of the spectra and identification of zone of fluorescence representative of different types of DOM. More recently, multivariate data analyses, such as parallel factor analysis (PARAFAC) methods, have been applied to EEM spectra to facilitate the extraction of useful data from the complex spectra (Andersen and Bro, 2003; Stedmon et al., 2003). When PARAFAC analysis is used in conjunction with fluorescence EEMs, individual fluorescence components may be identified along with their relative contribution to the overall fluorescence, allowing for a more refined characterisation of DOM along a variety of environmental gradients (Al-Reasi et al., 2011; Baken et al., 2011; Jaffé et al., 2008; Miller and McKnight, 2010).

As DOM is known to form complexes with trace metals in aquatic environments, and since DOM metal-complexing characteristics depend on the character and source of DOM (Baken et al., 2011; Cao et al., 2006; Cheng et al., 2005; Xue and Sigg, 1999), optical properties, as a measure of DOM quality, may be an important tool for predicting the role of DOM in metal speciation and transport. However, the percent of DOM that is actually fluorescent has been described to be as low as 1% (Green and Blough, 1994; Senesi, 1990). Although we are unable to directly determine the proportion of DOM that is represented by the fluorescence spectrum, this approach has been successfully used to estimate metal-DOM complexation in titrations of isolated organic matter samples (Saar and Weber, 1980) and in natural untreated water samples (Yamashita and Jaffé, 2008). The incorporation of DOM quality, determined from fluorescence EEMs and PARAFAC analysis, would be a valuable addition to existing chemical equilibrium models used for the prediction of metal bioavailability in natural freshwater (Dudal and Gérard, 2004).

1.5 Chemical equilibrium speciation modelling

Chemical equilibrium modelling is often employed in natural aqueous systems to predict the chemical speciation of various aqueous solutes based on established thermodynamic equilibrium reactions with defined stoichiometries. Simple chemical equilibrium modelling systems, such as MINEQL+, calculate the speciation, precipitation-dissolution and adsorption of aqueous inorganic chemical species using an extensive thermodynamic database included in the model (Schecher and McAvoy, 1992). The heterogeneous nature of DOM, however, makes it difficult to define specific thermodynamic data associated with its complexes. Nevertheless, with the use of some reasonable assumptions based on the current knowledge of the character of DOM, the complexation of DOM with cations in natural aqueous systems has been modeled with success (e.g. Kinniburgh et al. (1996), Tipping (1998)).

The calculation of complexation reactions in natural aquatic systems is possible using the Windermere Humic Aqueous Model (WHAM) speciation code combined with the Humic Ion-Binding Model VI[3] (Tipping, 1998). The model takes into account the heterogeneity of natural

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[3] WHAM has very recently been updated to include the Humic Ion-binding model VII (Tipping et al. 2011). The present thesis work was, however, based on model VI which was available throughout the majority of the research project.

organic matter (NOM), ion competition, reaction stoichiometry variability and electrostatic interactions, but it is not yet well tested in natural aquatic systems (Tipping, 2002). Tipping (1998) describes the Model VI as a "discrete site-electrostatic model of the interactions of protons and metals with fulvic and humic acids". The dissociation of protons on the surface of humic and fulvic acids, assumed to be rigid spheres of uniform size, is represented by eight groups having different acidities characterized by intrinsic equilibrium constants (Tipping, 1998).

$$R^Z H \leftrightarrow R^{Z-1} + H^+ \tag{3}$$

The eight groups are further divided into four strongly acidic type A groups representing carboxylic acids and four weakly acidic type B groups representing phenolic acids. The pK_i values associated with these groups are calculated as follows:

$$\text{for i = 1-4} \quad pK_i = pK_A + \frac{(2i-5)}{6}\Delta pK_A \tag{4}$$

$$\text{for i = 4-8} \quad pK_i = pK_B + \frac{(2i-13)}{6}\Delta pK_B \tag{5}$$

where pK_A and pK_B are average pK values of each type of group and ΔpK_A and ΔpK_B signify the spread of the pK values around means. Furthermore, it is written into the model that the type A groups are twice as abundant as the type B groups. With respect to metal binding to type A and B groups, metal ions will compete with each other and with protons. Monodentate binding is described by the following general equation and equilibrium constants:

$$R^Z + M^z \leftrightarrow RM^{Z+z} \tag{6}$$

$$\text{Type A groups; } K(i) = \log K_{MA} + \frac{(2i-5)}{6}\Delta LK_1 \tag{7}$$

$$\text{Type B groups; } K(i) = \log K_{MB} + \frac{(2i-13)}{6}\Delta LK_1 \tag{8}$$

where ΔLK_1 is a constant estimated from the fitting of experimental data. Bidentate and tridentate binding is also incorporated into the model.

$$\log K(i,j) = \log K(i) + \log K(j) + x \cdot \Delta LK_2 \tag{9}$$

$$\log K(i,j,k) = \log K(i) + \log K(j) + \log K(k) + y \cdot \Delta LK_2 \tag{10}$$

Tridentate binding sites and adjustable parameters ΔLK_1[4] and ΔLK_2 were added to take into account the heterogeneity of binding sites, resulting in 80 different sites in all. In general, WHAM assumes fulvic acids to have a higher density of binding sites, as well as stronger strong acid sites and weaker weak acid sites than humic acids (Tipping, 2002).

The non-specific binding caused by the electrostatic attraction between the generally negative humic substances and the cations in their immediate environment must also be taken into account. The accumulation of counterions in the electrical double layer formed at the surface of humic substances is approximated by a simple Donnan sub-model. The non-specific binding of counterions in the double layer adjacent to the binding surface is most pronounced at low ionic strengths, such as is the case for most freshwaters on the Canadian Precambrian Shield.

A set of generic default parameters for the WHAM/Model VI has been determined by Tipping (1998). Hundreds of existing data sets for proton and metal binding to humic and fulvic acids, obtained over a wide range of experimental conditions, were analysed to generate average binding parameters. Generic default parameters offer a good starting point when applying the models to experimental data. Once the models are applied to experimental data, and based on the overall goodness of fit, several model parameters can be adjusted. In addition, in situations where experimental data are limited, the number of parameters needing to be optimised may be reduced; in the case of WHAM/Model VI, the number of adjustable parameters can be reduced to a single parameter, log K_{MA}, where K_{MA} is the intrinsic equilibrium constant for the binding of metal M to the type "A" binding sites (Tipping, 1998).

1.5.1 WHAM field validations

WHAM/Model VI has been extensively applied and tested in laboratory experiments to investigate the interaction between DOM and dissolved metal ions (Tipping, 2002). The robustness of this model and its default parameters have been tested by comparing simulated

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[4] During the development of WHAM Model VII, Tipping et al. (2011) found a coding error in Model VI, which prompted the removal of LK1 parameters from Model VII. All calculations using the Model VI are still correct, but do not refer to the model described by Tipping (1998).

results to those obtained from laboratory experiments. The reviews by Tipping (2002) and Dudal and Gérard (2004) describe at length the laboratory application and optimisation of the most recent versions of the WHAM/Model VI.

Applications of WHAM to predict the speciation of trace metals in natural waters, as opposed to laboratory solutions, should be made while keeping in mind that it is a semi-empirical model based on some major assumptions, such as the size and shape of the natural organic matter as well as density, distribution and heterogeneity of the metal-binding sites (Tipping, 2002). Another major assumption of WHAM worth mentioning is that the cation-binding properties of organic matter in natural waters can be represented by those of humic and fulvic acids that have been isolated from natural waters and studied in the laboratory. Note too that no technique has been developed that directly measures the concentration of humic substances in natural waters. To circumvent this difficulty, it is normally assumed that the ratio of DOM to DOC is about 2:1, the concentration of DOC being readily available analytically. However, since this estimate of DOM includes both humic and non-humic components, the model user must also estimate the proportion of DOM that corresponds to fulvic and humic acids and is active in metal complexation. This proportion is often used as an optimisation parameter to empirically fit model predictions to experimental data. For example, Bryan et al. (2002) optimised the proportion of DOM as "active" in metal complexation to be used in WHAM VI as 65% by comparing predicted and measured Cu speciation in 15 natural water samples. Cheng et al. (2005) also found that a similar 64% of DOM was "active" in Zn complexation, but only after the binding constant for Zn to fulvic acid was optimised to fit predicted to experimental speciation data. Similarly, Van Laer et al. (2006) found that the best agreement between predicted and experimental Ni speciation data for a series of natural surface water samples was found when the fraction of FA "active" in complexing Ni was optimised to 65%, along with the K_{MA} constant for Ni, and the binding parameter determining the range of binding strengths of multidentate sites, ΔLK_2. It is clear that the model user's choice of the proportion of DOM that is active in metal complexation greatly affects the predicted metal speciation in natural waters (Kalis et al., 2006). However, choosing the "correct" active fraction of DOM is a major challenge and studies where this parameter is actually measured in the natural water samples, as opposed to being estimated after-the-fact by optimisation, are lacking. Studies that simultaneously measure the speciation of metals in natural waters and the character of DOM are needed (Lofts and Tipping, 2011).

One of the few studies to test the ability of chemical equilibrium speciation models (such as WHAM) to predict measured metal speciation in natural fresh waters is described in the paper by Unsworth et al. (2006). The free metal concentrations were measured by hollow fiber permeable liquid membrane and Donnan membrane techniques and 50% of the DOC was assumed to be "active" in metal complexation and to consist of 90% fulvic acids and 10% humic acids. The authors of this study found that the model predictions of free-ion activities often diverged from the observed values. The authors found that although there was reasonable agreement between the predicted and measured free metal ion concentrations for Cd^{2+}, there was a poor agreement between the predicted and measured free ion concentrations of Cu^{2+} and Pb^{2+}. The authors suggested that the poor prediction of free Cu^{2+} and Pb^{2+} concentrations might be due to the use of default model parameters determined in laboratory experiments with isolated humic substances. Kalis et al. (2007) found modeled free Ni^{2+} to be consistently higher than that measured using the Donnan membrane technique (DMT) and cited the binding of Ni by a ligand unaccounted for by the model as an explanation. A better understanding of the quality of DOM would in principle alleviate the need to make assumptions about the activity of DOM and minimise the discrepancies between model predicted and measured free metal concentrations.

The ideal application of chemical speciation models to the binding of metal ions to organic matter in a complex, natural aquatic system draws upon an appropriate set of known parameters that describe the binding characteristics of individual metal ions under conditions where the pH, ionic strength, metal-ion concentration and speciation, and presence of competing ions are known (Milne et al., 2003). Unfortunately, this is rarely the case. One of the key assumptions outlined by Lofts and Tipping (2011) is that the cation-binding data generated from laboratory experiments on samples of isolated humic substances used to parameterise chemical equilibrium models, such as WHAM, represent the cation-binding properties of DOM in natural waters. First, there is a large variability in the data sets used to generate default model parameters (Tipping, 2002). The variability may result from the use of various experimental conditions and analytical methods, and also from different sample origins. Furthermore, there is no consensus on whether or not isolation procedures used to isolate humic and fulvic acids from DOM affect the proportion of reactive sites and binding site affinities. In addition to the variability in DOM that may result from laboratory procedures, there is natural spatial and temporal variability in DOM with respect to its cation binding character. Chemical equilibrium models would, therefore, benefit from future research in the following areas: 1) the generation of high quality data sets of metal binding to organic matter under a wide range of environmentally significant conditions; 2) the simultaneous prediction and measurement of metal speciation in natural samples; 3) a measure of the natural quality of NOM with respect to metal binding activity; and 4) the validation of modeled metal binding characteristics with reliable field data (Tipping, 2002).

2 Research hypothesis, objectives and originality

2.1 Research hypothesis

Based on recently published results (Fortin et al., 2010; Guthrie et al., 2005; Unsworth et al., 2006), it was anticipated that the measured [M^{Z+}] values for Cu, Cd, Ni and Zn would not agree well with those predicted by the "off the shelf" or default versions of WHAM VI. It was thus hypothesised that this failure of the models to predict the free metal ion concentrations accurately is primarily due to variations in the quality of the dissolved organic matter (DOM) in the different water bodies, and that the incorporation of DOM quality into the chemical equilibrium models would result in predicted values in closer agreement with the measured values in natural freshwater samples.

2.2 Research objectives

The overall objective for this research was to enhance existing chemical equilibrium models by taking into account spatial variability in the quality of DOM. The resulting model should better predict the speciation, and therefore, bioavailability of Cu, Cd, Ni and Zn in the natural aquatic environment. In order to attain this proposed objective, five specific sub-objectives were identified.

Sub-objective 1

Identify a series of lakes representing a wide gradient of dissolved metal concentrations, DOM concentrations and physico-chemical conditions.

Sub-objective 2

Determine the quality of DOM using UV-visible absorbance, 3D-EEM fluorescence and PARAFAC analysis, and link variations among lakes to watershed and lake characteristics.

Sub-objective 3

Experimentally determine the concentrations of the free metal ion ($[M^{z+}]$) for Cd, Cu, Ni and Zn in the chosen lakes and explore the link between the concentration of the free metal ion and its proportion (i.e., its contribution to the total dissolved metal concentration) and various environmental factors (total metal concentration, pH, quantity and quality of DOM).

Sub-objective 4

Based on the results of sub-objectives 2 and 3, identify a subset of lakes that exhibit marked differences in DOM (quantity and quality) and in metal speciation, and titrate lake water samples from these lakes with Cu and Ni under environmentally realistic conditions (i.e., natural DOM lake samples and low metal concentrations). Generate conditional binding constants for each sample, and investigate potential links between the observed binding constants and the spectral properties of the lake water DOM.

Sub-objective 5

Improve WHAM VI metal speciation predictions in the chosen lakes through the incorporation of DOM quality by adjusting the estimated proportion of DOM active in metal complexation (% active FA).

2.3 Originality

Several factors contribute to the originality and significance of this doctoral thesis. First, to the best of our knowledge, this is the first study to apply fluorescence PARAFAC analysis as a measure of DOM quality to chemical equilibrium modelling for the prediction of metal speciation in natural freshwater systems. Second, the study measured DOM quality and the free Cu^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} concentrations in several lakes (N=18) in two different regions of Canada with wide gradients in dissolved organic carbon and metal concentrations and water chemistry. This is therefore one of the rare studies that compare predicted free metal concentrations with experimentally measured concentrations in a wide variety of natural samples. Finally, the project was designed within the research objectives of the Metals in the Human Environment Research Network (MITHE-RN). The project yields a better understanding of the bioavailability of environmentally significant metals.

3 **Methodology**

The following section briefly outlines the materials and methods used for sampling and analysing the lake water samples collected throughout the study. For a more detailed description, the reader is referred to Mueller et al. (2012a; 2012b; 2012c) found in Part II of the thesis.

3.1 Sampling

As described in the Introduction section (Section 1.2), the areas of Rouyn-Noranda, Québec and Sudbury, Ontario have been impacted by past and present copper/nickel mining, smelting and refining activities. A detailed description of the study areas can be found in Mueller et al.

(2012b). Lakes where chosen to represent geochemical gradients in trace metal concentrations, pH and DOM quantity and quality.

Eighteen lakes from both of the Rouyn-Noranda and Sudbury regions were sampled in the summer of 2007 and eight of these lakes were re-sampled in the summer of 2008. The 2007 and 2008 samples were used to characterize the DOM in the chosen lakes (Mueller et al., 2012b), while the eight samples taken in 2008 were subsequently used to compare measured and predicted trace metal speciation of Cd, Cu, Ni and Zn (Mueller et al., 2012c). A further two reference lakes with low total metal concentrations (Lake Opasatica from the Rouyn-Noranda region and Lake Geneva from the Sudbury region) were sampled again in 2009 to determine conditional binding constants for Ni and Cu to DOM by titration (Mueller et al., 2012a). Lake Vaudray from the Rouyn-Noranda region and Lake Geneva from the Sudbury region were also sampled in 2009 to measure the effects of long-term sample storage on DOM spectroscopic quality and trace metal speciation. See Table 1 for a description of the lakes sampled.

Lake water was collected from the littoral zones of the chosen lakes using equilibrium diffusion samplers in the summers of 2007 and 2008 (details can be found in Mueller et al. (2012b)). This sampling technique is based on the equilibrium of dissolved species in the surface waters and the ultrapure water initially held within the sampler; the diffusion occurs across a membrane of known porosity (0.2 µm). The sample containers consisted of 250 mL polypropylene (Nalgene) jars with custom-made plastic lids fitted with a 0.2 µm polysulfone filter membrane (HT Tuffryn, Pall). Before being transported into the field, the assembled jars were filled with ultrapure water (>18 Mohms) and bagged to avoid contamination. Two sample jars were horizontally fixed to plastic rods and anchored to the lake bottom (Figure 3). Three rods (i.e., six sample jars) were installed in each lake at a depth of 1 m from the lake surface. The samplers were left to equilibrate in the lakes for 13 to 19 days before being removed.

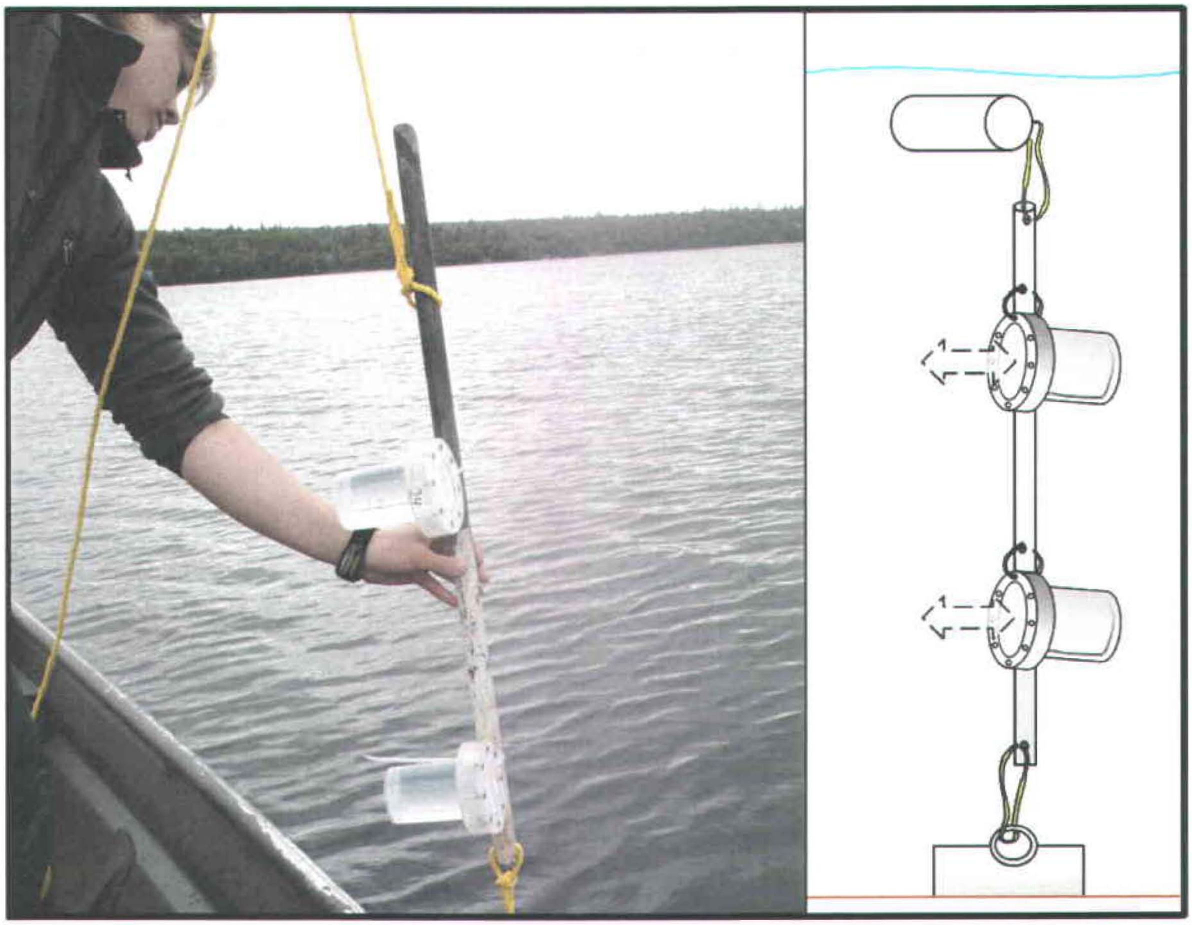

Figure 3. Example of the sampling apparatus / Montage utilisé pour l'échantillonnage.

The membrane of each sample jar was rinsed with ultrapure water and then gently pierced with a clean plastic pipette tip before replacing the membrane cover with a clean polypropylene screw lid. The samplers were bagged and stored in the dark at 4°C until transported back to the lab. In the field, lake water temperature, conductivity and pH were measured.

In the summer of 2009, large volume grab samples were collected from the surface of the lakes for the titration and sample storage experiments. For the water needed for the titration experiments, two 4-L amber glass bottles (I-CHEM Brand) were used for each of the two lakes sampled. For the sample storage experiments, one 10-L polypropylene carboy (Nalgene) was used for each of the two lakes sampled. All grab samples were taken from approximately the same location as the equilibrium diffusion samplers and transported back the laboratory where they were stored in the dark and at 4°C.

3.2 Sample analysis

All plastic laboratory and sampling equipment was soaked in a 10% (v/v) HNO_3 solution for at least 24 h and then rinsed a minimum of six times with ultrapure (>18 Mohms·cm) water and dried under a Class 100 laminar flow hood. All glassware was soaked in a 2 N HCL solution before being rinsed with ultrapure water. The polysulfone membrane for the diffusion samplers was soaked in ultrapure water, changed daily, for 1 week. The polycarbonate filters for vacuum filtration were soaked in 10% HNO_3 for at least 24 h and then ultrapure water for at least 24 h. Polystyrene vials for anion analyses were rinsed three times with ultrapure water only.

3.2.1 Lake DOM quality

Details of the sample preparation and analyses of the samples taken in 2007 and 2008 to characterize the lake DOM can be found in Mueller et al. (2012b). Briefly, subsamples were taken for analyses of major cation concentrations, total organic carbon (TOC) as well as absorbance and fluorescence measurements. Absorbance measurements were used to calculate the specific absorbance at 254 nm ($SUVA_{254}$), while the fluorescence measurements were used to calculate the fluorescence index (FI; the ratio between the fluorescence emission intensity at 470 nm over that at 520 nm, for an excitation at 370 nm) and, using parallel factor analysis (PARAFAC), to identify the individual fluorescent components contributing to the overall DOM fluorescence for each lake (Cory et al., 2010; Gondar et al., 2008; Koster and Schmuckler, 1967). Lake watershed characteristics were also identified using hydrographic and topographic geospatial data integrated using ArcGIS 10 (Environmental Systems Research Institute, Inc., Redlands, CA).

3.2.2 Cd, Cu, Ni and Zn metal speciation

Details of the sample preparation and analyses of the samples taken in 2008 to compare measured and predicted trace metal speciation can be found in Mueller et al. (2012c). Briefly, subsamples were taken for determination of major cation, anion and total metal concentrations. Total organic carbon (TOC), total inorganic carbon (TOC), as well as absorbance ($SUVA_{254}$) and fluorescence (FI and PARAFAC components) measurements were also made. The concentrations of Cd^{2+}, Ni^{2+} and Zn^{2+} in the lake samples were measured using an ion exchange technique (IET), whereas the concentration of Cu^{2+} was measured using a cupric ion selective electrode (ISE). The concentrations of free metal ions for each lake were also calculated using the Windermere Humic Aqueous Model (WHAM model 6.1).

3.2.3 DOM titrations with Cu and Ni

The grab samples taken from Lake Opasatica (8 L) and Lake Geneva (8 L) in 2009 were transported back to the laboratory and vacuum filtered under a clean laminar flow hood using polycarbonate filter holders (Advantec MFS, Inc.) and a series of 47-mm polycarbonate filters (5, 2, 0.4 and 0.2 µm; Millipore and AMD Manufacturing Inc.). Before filtering the lake water samples, 1 L of ultrapure water and a small volume of sample were filtered through the filtration apparatus and discarded. Following filtration, the samples were stored in the dark and at 4°C until analysis.

Samples from both Lakes Opasatica and Geneva were individually titrated with Cu and Ni. For the Cu titrations, known amounts of a stock solution of 0.2 mM copper nitrate ($Cu(NO_3)_2$, PlasmaCal) were added to eight 200-mL aliquots of the sample, which had been amended with KNO_3 (99.995%, Fluka; a known amount of a 1.23 M stock solution was added to achieve a final concentration of 10 mM KNO_3 for all titration aliquots). The pH of the titration aliquots was adjusted to the natural lake pH using small volumes of either HNO_3 or NaOH, and left to equilibrate overnight under a laminar flow hood and in the dark before measuring the free Cu^{2+} concentration using a cupric ISE. An initial sample aliquot with no added Cu was also analysed. For details on the calibration of the electrode and measurement of the free Cu^{2+} ion in natural lake water samples, the reader is referred to Mueller et al. (2012c). Triplicate Cu titrations were carried out for a total of 27 Cu titration aliquots analysed. After the measurement of the free Cu^{2+} concentration, subsamples of each titration aliquot were taken for measurement of the total dissolved Cu concentration.

For the Ni titrations, known amounts of a stock solution of 1 mM nickel nitrate ($Ni(NO_3)_2$, PlasmaCal) were added to seven aliquots of 250 mL of sample, which had been amended with $Ca(NO_3)_2$ (99.0%, Sigma-Aldrich; a known amount of a 0.025 M stock solution was added to achieve a final concentration of 0.46 mM $Ca(NO_3)_2$ for all titration aliquots). Again, the pH of the titration aliquots was adjusted to the natural lake pH and left to equilibrate overnight before measuring the free Ni^{2+} concentration using the IET. An initial sample aliquot with no added Ni was also analysed. For details on the calibration and measurement of the free Ni^{2+} ion in natural lake water samples, the reader is referred to Mueller et al. (2012c). Triplicate Ni titrations were carried out for a total of 24 Ni titration aliquots analysed. In the same manner as the Cu titrations, subsamples of each titration aliquot were taken for measurement of the total dissolved Ni concentration.

3.2.4 Effect of sample storage on metal speciation and DOM quality

Following the same protocol as described above (Section 3.2.3), the 10-L samples collected from Lake Vaudray and Lake Geneva in July 2009 were filtered upon return to the laboratory. Subsamples were taken for analyses of pH, major ion concentrations, DOM optical properties (absorbance and fluorescence) as well as total and free Cd, Cu, Ni and Zn concentrations immediately after filtration and at regular intervals (approximately 3 months) over an 8-month period. Between subsampling events, the large volume samples were stored in the dark at 4°C, as was the case for the lake water samples collected with the *in situ* diffusion samplers. See Appendix A for further details.

4 Results and Discussion

The following section includes a discussion of the major results found during the completion of this thesis. More detail can be found in the papers found in Part II of the thesis. The overall objective of this thesis was to enhance existing chemical equilibrium models by incorporating the quality of DOM, resulting in improved predictions of Cd, Cu, Ni and Zn speciation, and therefore, bioavailability in natural aquatic environments. To this end, the effects of watershed characteristics and in-lake processes on lake water chemistry are first presented, with an emphasis on the DOM present in the various lakes. For a subset of these lakes, chosen to represent differences in DOM and in water chemistry (including total dissolved metal concentrations), we then examine the free-metal ion concentrations for four metals (Cd, Cu, Ni and Zn) and consider how the trace metal speciation varies among the metals and for a given metal among the various lakes. The measured trace metal speciation is then compared to that predicted by the commonly used chemical equilibrium speciation model, the Windermere Humic Aqueous Model (WHAM) and possible reasons for differences between the measured and WHAM modeled trace metal speciation are discussed. Finally, the conditional binding parameters for the complexation of Cu and Ni by DOM are calculated and compared between two lakes with low ambient concentrations of metals and spectroscopically different DOM.

4.1 Lake watersheds

During the thesis research, lakes from the Rouyn-Noranda region of north-western Québec and from the Sudbury region of north-central Ontario were sampled during the summer over the course of three years (Figure 4 and Table 1).

The watershed characteristics of these lakes were found to vary between sampling regions. In general, the lakes from the more northerly Rouyn-Noranda region had a higher mean watershed-to-lake surface area (W/L) ratio (25), % vegetation (83%; dominated by coniferous forest cover) and % wetland cover (1%), fewer rock outcrops and a thicker soil layer than the lakes from the more southerly Sudbury region (mean W/L of 9; 41% vegetation; no wetland cover and 28% rock outcrops) (see Table 2 in Mueller et al. (2012b)).

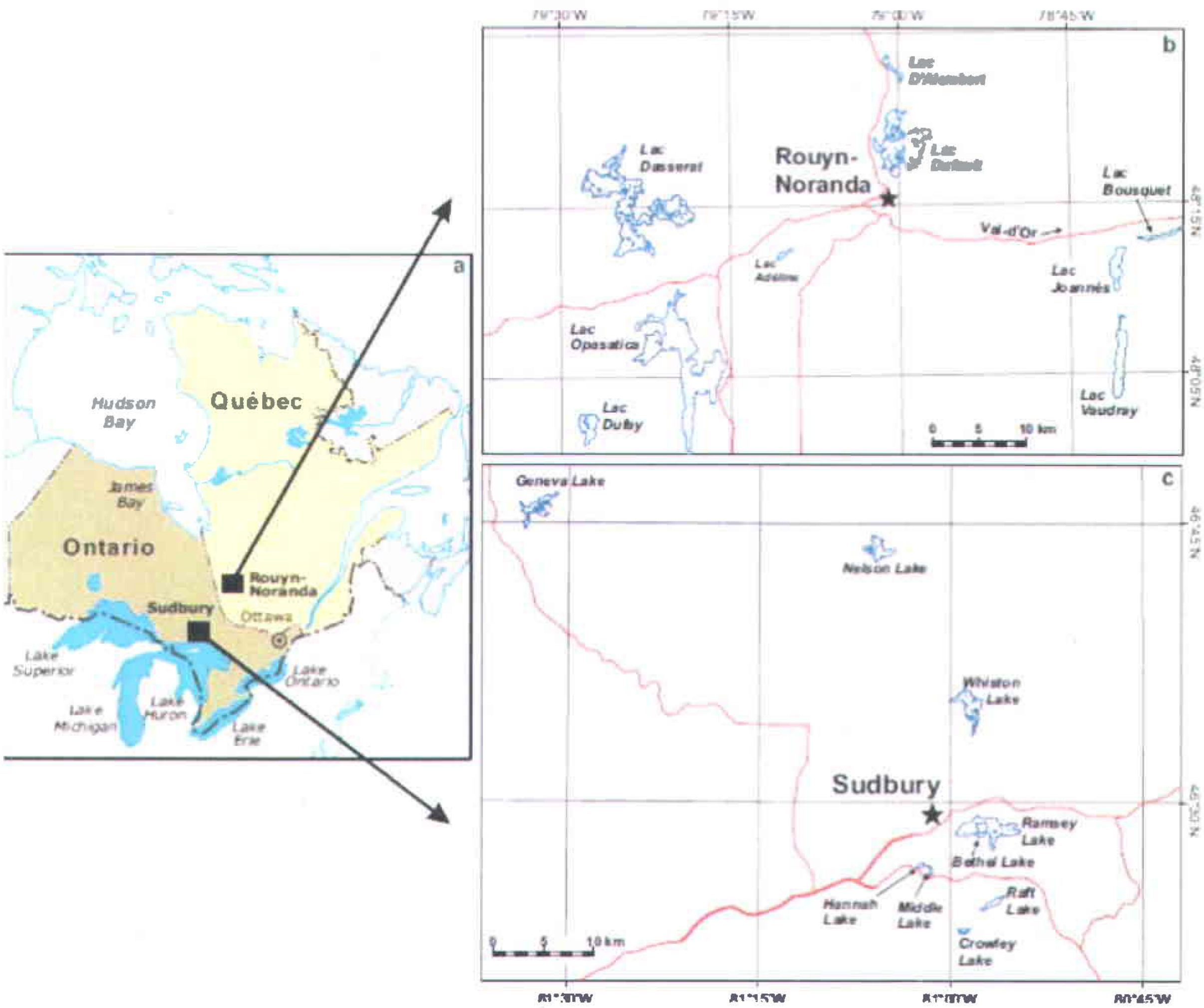

Figure 4. Map of the two study areas showing the relative position of the two regions in eastern Canada (panel a), the geographical location of the Rouyn-Noranda lakes (panel b) and the Sudbury lakes (panel c). Reproduced from Mueller et al. (2012b) / Carte de l'Est du Canada montrant les positions relatives de deux régions étudiées (A), ainsi que l'emplacement de lacs de la région de Rouyn-Noranda (B) et de la région de Sudbury (C). Reproduit de Mueller et al. (2012b).

It follows that the lakes from the Rouyn-Noranda region tend to have shorter water residence times than the lakes from the Sudbury area, and accordingly are strongly influenced by inputs from their catchments. With respect to natural organic matter, which can originate either within the lake or in the catchment, the Rouyn-Noranda lakes receive a larger proportion of DOM from their catchments than do the Sudbury area lakes.

Table 1. Lake names, sampling regions, locations and years sampled. Modified from Mueller et al. (2012b) / Noms des lacs, ainsi que les régions, sites et dates d'échantillonnage. Modifié d'après Mueller et al. (2012b).

Lake	Region	Location		Years Sampled		
				2007	2008	2009
Adeline	Rouyn-Noranda	48°12'12" N	79°10'17" W	Y		
Bousquet	Rouyn-Noranda	48°12'56" N	78°37'06" W	Y		
D'Alembert	Rouyn-Noranda	48°23'01" N	79°00'35" W	Y		
Dasserat	Rouyn-Noranda	48°15'06" N	79°24'24" W	Y	Y	
Dufault	Rouyn-Noranda	48°18'30" N	78°59'25" W	Y	Y	
Dufay	Rouyn-Noranda	48°01'42" N	79°27'33" W	Y		
Joannès	Rouyn-Noranda	48°10'53" N	78°40'02" W	Y		
Opasatica	Rouyn-Noranda	48°04'32" N	79°17'49" W	Y	Y	Y
Vaudray	Rouyn-Noranda	48°05'45" N	78°40'50" W	Y	Y	
Bethel	Sudbury	46°46'17" N	80°57'43" W	Y	Y	
Crowley	Sudbury	46°23'06" N	80°58'55" W	Y		
Geneva	Sudbury	46°45'52" N	81°32'46" W	Y	Y	Y
Hannah	Sudbury	46°26'36" N	81°02'17" W	Y		
Middle	Sudbury	46°26'22" N	81°01'26" W	Y		
Nelson	Sudbury	46°43'36" N	81°05'39" W	Y		
Raft	Sudbury	46°24'35" N	80°56'44" W	Y	Y	
Ramsey	Sudbury	46°28'57" N	80°57'00" W	Y		
Whitson	Sudbury	46°35'03" N	80°58'20" W	Y	Y	

4.2 Lake water chemistry

Differences in watershed characteristics, such as geology, hydrology and vegetation cover, between regions can play an important role in the regional variability of surface water chemistry parameters such as the pH and the concentrations of major ions and metals, as well as the quantity and quality of DOM (Sobek et al., 2007; Wetzel, 2001). However, the water chemistry, i.e. pH and major cation concentrations, varied only slightly between the two sampling regions (see Table 2). Lakes from both regions have circumneutral pHs, with low concentrations of Ca and Mg, typical of soft water lakes on the Canadian Shield.

The total dissolved concentrations of Cd, Cu, Ni and Zn did vary between regions and among the eight lakes sampled in 2008 (see Table 2), as was the aim of the experimental design. The Rouyn-Noranda region had the highest concentrations of Cd and Zn and showed the largest concentration gradients among the lakes for these metals, whereas Cu and Ni concentrations varied less. Lakes Dufault and Dasserat had the highest concentrations of total dissolved metals, while Lake Opasatica had the lowest. In the Sudbury region, Ni and Cd showed the largest concentration gradients among the lakes, whereas Cu and Zn varied less. Lakes Bethel and Geneva had the lowest concentrations of metals, while Lakes Raft and Whitson had the highest. The lake-to-lake variability in measured total metal concentrations within a given region is due to variations in atmospheric deposition down-wind, with respect to the distance from mining and smelting activities, and to local point source discharges present in individual catchments (Borgmann et al., 1998; Dixit et al., 2007; Goulet and Couillard, 2009).

4.3 Dissolved organic matter (DOM)

Dissolved organic matter plays an important role in controlling the speciation and bioavailability of many trace metals in natural aquatic systems, by complexing the metals and lowering the concentration of the free metal ion (Buffle, 1984). It is reasonable to hypothesise, however, given the recognized diversity of the origins of DOM, that its ability to complex trace metals may vary from lake to lake, due to differences in precursor material with different metal-specific functional groups. For example, the Cu binding by DOM, as well as the uptake of Cu by fish gills, was found to differ among samples with different sources of DOM (Luider et al., 2004).

Table 2. Measured pH and mean (± SD, N=3) dissolved organic matter (DOM) concentrations, total dissolved concentrations of major cations and trace metals, and % free metal values for lakes sampled from Rouyn-Noranda (QC) and Sudbury (ON) in 2008 / Valeurs de pH, de concentrations moyennes (± SD, N=3) de matière organique dissoute (MOD), de concentrations totales de cations majeurs dissous et de métaux traces ainsi que les pourcentages (%) de métal libre pour les lacs échantillonnés dans les régions de Rouyn-Noranda (Qué.) et de Sudbury (Ont.) en 2008.

	Dasserat	**Dufault**	**Opasatica**	**Vaudray**	**Bethel**	**Geneva**	**Raft**	**Whitson**
pH	6.92	7.59	7.69	6.81	8.08	6.9	6.4	6.9
DOM (mg C·L^{-1})	4.85 ± 0.03	5.2 ± 0.1	6.3 ± 0.1	8.10 ± 0.08	4.89 ± 0.03	2.9 ± 0.1	2.03 ± 0.03	3.77 ± 0.05
Ca (µM)	207 ± 1	404 ± 3	218 ± 2	83 ± 1	464 ± 3	68 ± 3	81 ± 2	143 ± 1
Mg (µM)	93.7 ± 0.3	118 ± 1	114 ± 1	37.1 ± 0.6	274 ± 3	26 ± 1	46 ± 1	78.4 ± 0.4
Cd (nM)	0.8 ± 0.2	2.0 ± 0.2	0.06 ± 0.01	0.608 ± 0.005	0.018 ± 0.009	0.021 ± 0.005	0.56 ± 0.02	0.51 ± 0.04
Cu (nM)	112 ± 3	246 ± 3	35.1 ± 0.2	43 ± 1	24.9 ± 0.1	10.5 ± 0.6	86.0 ± 0.9	165 ± 3
Ni (nM)	8 ± 2	5.1 ± 0.3	12.5 ± 0.4	12 ± 1	226 ± 2	18 ± 2	775 ± 41	1136 ± 54
Zn (nM)	464 ± 51	342 ± 60	15 ± 4	52 ± 5	12 ± 3	21 ± 8	62 ± 22	52 ± 2
Cd^{2+} (nM)	0.5 ± 0.2	1.14 ± 0.06	0.021 ± 0.008	0.26 ± 0.02	< 0.00	0.01*	0.402 ± 0.004	0.31 ± 0.05
Cu^{2+} (nM)	0.37 ± 0.04	0.44 ± 0.09	0.08 ± 0.01	0.20 ± 0.08	0.05 ± 0.01	0.48 ± 0.09	3.2 ± 0.5	1.0 ± 0.1
Ni^{2+} (nM)	0.6*	< 0.62	< 0.12	< 0.61	7.3 ± 0.9	4.0 ± 0.7	545 ± 9	489 ± 72
Zn^{2+} (nM)	279 ± 32	188 ± 23	2.3 ± 0.9	31 ± 18	2.3 ± 0.4	10.7 ± 0.9	51 ± 16	32 ± 4
% Cd^{2+}	63 ± 36	58 ± 5	37 ± 17	42 ± 4	NA	70	71 ± 2	61 ± 10
% Cu^{2+}	0.32 ± 0.04	0.17 ± 0.04	0.21 ± 0.04	0.47 ± 0.2	0.20 ± 0.05	4.6 ± 0.9	3.7 ± 0.6	0.57 ± 0.08
% Ni^{2+}	8*	NA	NA	NA	3.2 ± 0.4	22 ± 5	70 ± 4	43 ± 7
% Zn^{2+}	60 ± 10	55 ± 12	15 ± 7	60 ± 36	20 ± 6	51 ± 20	82 ± 39	62 ± 8

An asterisk * indicates only one sample above limit of quantification (LOQ; calculated as three times the standard deviation of triplicate analyses of each lake). NA indicates no samples above the LOQ. Modified from Mueller et al.(2012c).

Le symbole * dénote un réplica dont les concentrations sont au-delà de la limite de quantification (LOQ; calculée comme étant trois fois l'écart type sur une analyse en triple dans chaque lac) et le symbole NA dénote qu'aucun échantillon n'est au-delà de la LOQ. Modifié d'après Mueller et al. (2012c).

DOM Quantity

The concentration of DOM, hereafter expressed in terms of DOC (mg $C \cdot L^{-1}$), in lakes in a given region is influenced by terrestrial vegetation, soils and hydrology, which are in turn regulated by climate and topography. However at a more local scale, catchment and lake properties influence the DOM concentration in individual lakes (Sobek et al., 2007). Indeed for the eighteen lakes sampled in 2007, the lake watershed characteristics played an important role in the variability in DOM concentration among lakes (see Table 3). In general, the lakes of the Rouyn-Noranda region had higher mean concentrations of DOM (7.2 ± 2.4 mg $C \cdot L^{-1}$) and spanned a wider concentration range (3.8 to 13.4 mg $C \cdot L^{-1}$) than the lakes of the Sudbury region (mean of 3.4 ± 1.1 mg $C \cdot L^{-1}$; range from 0.8 to 6.0 mg $C \cdot L^{-1}$). Lakes Bousquet and Joannès, in the Rouyn-Noranda region, had the highest concentrations of DOM (13.0 and 10.0 mg $C \cdot L^{-1}$, respectively), whereas Lake Nelson, of the Sudbury region, had the lowest concentration (1.4 mg $C \cdot L^{-1}$). As described in section 4.1, we suspected that the lakes from Rouyn-Noranda region receive high quantities of DOM from their large catchments, whereas lakes from the Sudbury region receive lower amounts of DOM from their catchments, and relatively higher amounts from within the water column. The wide range of DOM concentrations in the lakes sampled fulfilled one of our objectives of identifying a series of lakes representing a wide gradient of DOM concentrations.

DOM Quality

In addition to differences in the quantity of organic matter, the quality of the lake DOM (as measured spectroscopically) also differed between regions and among lakes (see Table 3). The Rouyn-Noranda lakes displayed higher $SUVA_{254}$ (8.3 ± 1.5 $L \cdot m^{-1} \cdot mg\ C^{-1}$) and slightly lower FI values (1.32 ± 0.04) than the Sudbury lakes (5.3 ± 1.3 $L \cdot m^{-1} \cdot mg\ C^{-1}$ and 1.36 ± 0.08, respectively), indicative of greater inputs of allochthonous DOM to the Rouyn-Noranda lakes than the Sudbury lakes. The high watershed-to-lake area (W/L) ratios and the large proportion of surface vegetation in the catchments of the Rouyn-Noranda lakes also point to a higher proportion of DOM originating from the catchment than in the case of the Sudbury lakes. Among lakes, Lakes Bousquet, Vaudray and Joannès had the highest $SUVA_{254}$ values and the lowest FI values, while Lakes Bethel and Ramsey had the lowest SUVA$_{254}$ values and the highest FI values. Lakes Joannès and Vaudray both flow into Lake Bousquet and the prior in-lake processing of DOM in these lakes presumably contributes to the recalcitrant nature of the DOM present in Lake Bousquet. In other words, the DOM originating from the catchments of Lakes Joannès and Vaudray, and/or that generated within their water columns is exposed to degradation pathways on its way to Lake Bousquet. A lower proportion of easily degradable DOM and a higher proportion of recalcitrant DOM are therefore expected to be present in Lake Bousquet. Both Lake Ramsey and Lake Bethel, a small eutrophic lake that flows into Lake Ramsey, are likely producing a high proportion of autochthonous DOM, thus leading to their low specific absorptivities and high FI values. Previous studies have found similar relationships between the source of DOM and simple absorption and fluorescence measurements (McKnight et al., 2001; Weishaar et al., 2003). For example, Jaffé et al. (2008) found low SUVA values (0.5 to 5 L·m^{-1}·mg C^{-1}) and high FI (1.4 to 1.75) values for surface waters with a high microbial contribution found in autochthonous samples. In other cases, they found high SUVA values (1 to 7.5 L·m^{-1}·mg C^{-1}) and low FI values (1.1 to 1.5), indicative of terrestrial-derived material found in allochthonous samples. Balcarczyk et al. (2009) also found a range of stream, spring and thermokarst samples to exhibit FI values to range between 1.3 and 1.4, representative of terrestrially-derived allochthonous DOM.

Unlike the simple absorbance and fluorescence measurements that use only a small portion (two-dimensional) part of the absorbance or fluorescence spectrum, three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy incorporates data from the entire DOM fluorescence spectrum. The use of a multivariate statistical analysis tool, i.e. parallel factor (PARAFAC) analysis, on EEM fluorescence spectra identified four distinct fluorescent components contributing to the overall lake DOM fluorescence. Components 1 and 2 were ascribed to reduced quinone, humic-like fluorophores present in a wide variety of aquatic environments, component 3 to refractory, oxidized quinone, humic-like fluorophores of allochthonous origin, and component 4 to tryptophan-like protein fluorescence of

Table 3. Measured mean (± SD, N=3) dissolved organic matter (DOM) concentration, specific UV-absorbance ($SUVA_{245}$), fluorescence index (FI), and relative proportions of the C1 and C3 PARAFAC components for the eighteen lakes sampled in 2007 and the eight lakes sampled in 2008 / Concentrations moyennes (± SD, N=3) de matière organique dissoute (MOD), absorbance UV spécifique ($SUVA_{245}$), indice de fluorescence (FI) et les proportions relatives des composantes de fluorescence C1 et C3 pour les 18 lacs échantillonnés en 2007 et les 8 lacs échantillonnés en 2008.

Lake	Region	DOC (mg L^{-1})	SUVA 254 (L m^{-1} mg C^{-1})	SAC 340 (L m^{-1} mg C^{-1})	FI	C1/CT	C2/CT	C3/CT	C4/CT
Adeline	Rouyn-Noranda	4.60 ± 0.79	6.96 ± 1.27	1.46 ± 0.25	1.33 ± 0.01	0.27 ± 0.02	0.33 ± 0.02	0.26 ± 0.02	0.15 ± 0.01
Bousquet	Rouyn-Noranda	12.58 ± 0.69	11.03 ± 0.62	3.52 ± 0.20	1.29 ± 0.01	0.15 ± 0.02	0.07 ± 0.01	0.76 ± 0.05	0.02 ± 0.00
D'Alembert	Rouyn-Noranda	8.00 ± 0.80	8.67 ± 0.90	2.20 ± 0.22	1.30 ± 0.01	0.21 ± 0.03	0.24 ± 0.03	0.48 ± 0.07	0.08 ± 0.01
Dasserat	Rouyn-Noranda	5.14 ± 0.58	8.00 ± 0.40	1.98 ± 0.18	1.35 ± 0.03	0.26 ± 0.19	0.34 ± 0.25	0.30 ± 0.32	0.10 ± 0.07
Dufault	Rouyn-Noranda	5.10 ± 0.36	6.86 ± 0.70	1.48 ± 0.26	1.32 ± 0.03	0.27 ± 0.12	0.33 ± 0.14	0.27 ± 0.10	0.14 ± 0.05
Dufay	Rouyn-Noranda	8.25 ± 0.37	8.36 ± 0.44	2.11 ± 0.13	1.30 ± 0.02	0.22 ± 0.03	0.24 ± 0.03	0.45 ± 0.05	0.09 ± 0.01
Joannès	Rouyn-Noranda	10.26 ± 0.26	9.98 ± 0.25	2.90 ± 0.06	1.28 ± 0.01	0.18 ± 0.04	0.16 ± 0.03	0.61 ± 0.16	0.05 ± 0.01
Opasatica	Rouyn-Noranda	6.00 ± 0.40	7.41 ± 0.34	1.65 ± 0.14	1.36 ± 0.02	0.26 ± 0.05	0.32 ± 0.05	0.29 ± 0.05	0.12 ± 0.02
Vaudray	Rouyn-Noranda	8.07 ± 0.09	9.83 ± 0.43	2.88 ± 0.24	1.26 ± 0.01	0.27 ± 0.08	0.24 ± 0.07	0.43 ± 0.10	0.07 ± 0.02
Bethel	Sudbury	5.22 ± 0.46	4.64 ± 0.47	0.84 ± 0.25	1.49 ± 0.06	0.24 ± 0.08	0.37 ± 0.13	0.16 ± 0.06	0.23 ± 0.09
Crowley	Sudbury	2.59 ± 0.28	4.82 ± 0.44	1.02 ± 0.12	1.33 ± 0.08	0.29 ± 0.07	0.36 ± 0.08	0.16 ± 0.05	0.19 ± 0.04
Geneva	Sudbury	3.26 ± 0.39	5.23 ± 0.28	1.09 ±0.25	1.33 ± 0.06	0.29 ± 0.09	0.37 ± 0.10	0.18 ± 0.07	0.17 ± 0.06
Hannah	Sudbury	3.61 ± 0.12	5.05 ± 0.26	1.03 ± 0.08	1.31 ± 0.04	0.28 ± 0.04	0.34 ± 0.04	0.18 ± 0.03	0.20 ± 0.03
Middle	Sudbury	3.32 ± 0.40	5.17 ± 0.57	1.01 ± 0.08	1.36 ± 0.07	0.26 ± 0.03	0.34 ± 0.04	0.20 ± 0.03	0.20 ± 0.03
Nelson	Sudbury	1.36 ± 0.46	4.53 ± 2.02	0.83 ± 0.42	1.32 ± 0.03	0.28 ± 0.08	0.35 ± 0.09	0.14 ± 0.03	0.23 ± 0.07
Raft	Sudbury	2.26 ± 0.31	5.99 ± 2.32	1.35 ± 0.71	1.28 ± 0.07	0.29 ± 0.07	0.34 ± 0.08	0.15 ± 0.16	0.22 ± 0.06
Ramsey	Sudbury	3.55 ± 0.53	4.32 ± 0.60	0.74 ± 0.09	1.41 ± 0.02	0.24 ± 0.05	0.33 ± 0.06	0.17 ± 0.04	0.25 ± 0.05
Whitson	Sudbury	3.82 ± 0.12	6.87 ± 0.56	1.73 ± 0.34	1.34 ± 0.04	0.30 ± 0.06	0.36 ± 0.08	0.19 ± 0.04	0.15 ± 0.03

autochthonous origin, based on the comparison with established literature values (Coble, 1996; Cory and McKnight, 2005; Koster and Schmuckler, 1967).

DOM fluorescence from the lakes of the Rouyn-Noranda region had a higher relative proportion of allochthonous DOM fluorescence than the Sudbury lakes, whereas the Sudbury lakes had a higher proportion of autochthonous DOM fluorescence. Once again Lakes Bethel and Ramsey were found to have high proportions of protein fluorescent DOM of autochthonous origin, whereas Lakes Vaudray, Joannès and Bousquet had high proportions of humic-like fluorescent DOM of allochthonous origin. Given that DOM is an important ligand regulating the speciation of trace metals in natural waters, we hypothesised that the speciation of dissolved metals in freshwater systems would be sensitive to the different types of DOM present.

4.4 Trace metal speciation

For the purpose of the thesis, trace metal speciation is defined as the distribution of trace metals amongst their chemical forms; of particular interest to ecotoxicologists is the free metal ion. The complexation of the free metal ion in natural waters will depend on the concentration of the metal and available ligands (both organic and inorganic), as well as the influence of other cations (such as protons and major cations) competing for complexation sites on such ligands (Stumm and Morgan, 1996). Of the lakes studied, those that had the highest total dissolved metal concentrations also had the highest free metal ion concentrations (see Table 2). The relative proportion of the free metal ion in individual lakes was also considered in order to account for differences in the total metal concentrations among lakes. In the Rouyn-Noranda region, Lake Opasatica had the lowest % free metal for all metals (except Cu) and Lake Dasserat had the highest. In the Sudbury region, Lake Bethel had the lowest % free metal for all metals and Lake Raft, the highest percent metals (except Cu). The percent free metal increased as a function of increasing total metal concentration and decreasing pH, reflecting the importance of the metal-to-ligand ratio and proton competition on metal speciation, respectively.

The strength of the binding sites available on the DOM will vary as a function of the metal-to-ligand ratio. At low M/L ratios, i.e. early in the titration of available binding sites, the available sites will have a relatively high affinity for the metal. At high M/L ratios the strong binding sites will have been occupied and the remaining available sites will tend to have lower affinity for the metal than those that were occupied early in the titration. It follows that the effective binding constant will become weaker as the M/L ratio increases, and accordingly the % free metal will be higher under these conditions. It is also well documented that as the pH of natural waters decreases, the proton concentration increases and competes with metal ions for binding sites on available ligands, thus increasing the percent free metal ion (Nelson and Campbell, 1991). Compared to results found in the literature, the percent free metal was generally higher in the Quebec and Ontario lakes than in the European lakes, most likely due to lower pH and alkalinity values, as well as higher metal:NOM ratios in the Canadian lakes sampled (Kalis et al., 2006; Sigg et al., 2006; Van Laer et al., 2006).

4.5 Measured vs. modeled trace metal speciation

Chemical equilibrium speciation models, such as WHAM, are extremely useful in predicting the free-metal concentration of cations in natural systems containing DOM. WHAM assumes that DOM is best represented by fulvic and humic acids that bind cations at type A sites corresponding to carboxylic acids groups and type B sites that correspond to phenolic groups (Tipping, 2002). When applying the WHAM model to a natural water sample, the user must, however, estimate the fulvic and humic acid content of the water. Based on the analysis on the average Cu binding of 15 freshwater samples (lakes, ponds, streams and rivers), Bryan et al. (2002) suggested that an average of 65% of the DOM is actively involved in metal complexation and that it could be represented by fulvic acids only. This approach, therefore, does not take into account variations in DOM quality among samples of various origins.

In the present work, we started by predicting the free metal ion concentrations of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} using the suggested 65% of DOM active in metal complexation. For all the lakes sampled, WHAM VI predicted free Cd^{2+} concentrations that were similar to those measured (see Figure 5). Model predictions were also similar to measured values for Zn, although at low total and free Zn concentrations WHAM slightly over-predicted the concentration of free Zn^{2+}. For the Cu, however, WHAM under-predicted its free ion concentration in half of the lakes sampled, i.e., those with the lowest total Cu concentrations. Worse still, WHAM systematically over-predicted the concentration of free Ni^{2+} in all lakes for which the free Ni^{2+} concentration was within the analytically measurable window.

In an attempt to account for the variability in the nature of the DOM among lakes and its effect on their trace metal speciation, we tested the sensitivity of WHAM to the user-defined proportion of DOM active (%aFA) in metal complexation; an arbitrary range of halving (33%) and doubling (130%) the 65% value was used to calculate the free metal ion concentrations (see Figure 5). WHAM predicted a similar % free metal to that measured in most lakes for Cd and Zn, but despite the wide range of %aFA employed, agreement was still only satisfactory for half of the lakes for Cu and for none of lakes for Ni. We then calculated the optimal percent FA (%aFAopt) active in metal complexation that was required to exactly match the predicted and measured free metal ion concentrations. The %aFAopt values for each metal and each lake were calculated by repeatedly running WHAM VI and adjusting the FA concentration until the predicted free metal ion concentrations matched those measured (see Mueller et al. (2012c) for more details on how the %aFAopt values were calculated). Increasing or decreasing the %aFA changes the number of binding sites involved in metal complexation, but the intrinsic metal binding affinities at these binding sites remain the same. It should be noted that increasing the %aFA to beyond 100% simply increases the number of fulvic acid binding sites involved in metal complexation to a value higher than the default capacity assumed by WHAM. For Cu, the %aFAopt values varied between 8 and 90% of the total DOM concentration. For Cd^{2+}, Zn^{2+} and particularly Ni^{2+}, however, the %aFAopt values were all quite high, often exceeding the notional limit of 100%: 61 to 250% for Cd^{2+}; 65 to 410% for Zn^{2+}; 440 to 1900% for Ni. These results suggest that in addition to adjusting the binding capacity (i.e., the number of binding sites) of the fulvic acid, the default binding affinities for Cd, Zn and especially Ni will need to be adjusted in WHAM.

In a final step, we compared the optimised proportion of DOM active in metal complexation to the lake-to-lake variation in the spectroscopic quality of the DOM. Relationships between the calculated %aFAopt values and the relative proportion of the ubiquitous humic-like DOM fluorescence PARAFAC component (C1) were found for Cu and Ni; similarly, relationships between %aFAopt and the allochthonous fluorescence PARAFAC component (C3) were found for Cd and Zn (see Figure 6). Moreover, the trends in the relationships between %aFAopt and the C1/C_T and C3/C_T ratios for Ni were found to be opposite to those for the same relationships for Cd, Cu and Zn. These distinctly different trends suggest, for the first time, that the DOM binding sites active in Cd, Cu and Zn complexation are different from those binding Ni. Admittedly the relationships shown in Figure 6 will have to be explored over a wider range of DOM samples, but they do support our original idea that the proportion of DOM active in metal complexation could be estimated on the basis of its fluorescence signature and then introduced into chemical equilibrium models such as WHAM. Using the relationships found between the calculated %aFAopt values and the relative proportion of the allochthonous fluorescence component (C3), a C3-optimised %aFA was used to calculate, anew, the free metal Cd^{2+}, Ni^{2+} and Zn^{2+} concentrations in the sampled lakes using WHAM VI. An improvement in the WHAM VI prediction of the free metal ion concentration was observed for Ni especially (Figure 7). These results support our hypothesis that, especially for Ni, the spectroscopic quality of DOM may possibly be used as a proxy for the proportion of DOM active in metal complexation. Although this approach yielded an improvement in the WHAM VI prediction of the free metal ion concentrations, it is apparent that there is still room for improvement, especially for the lakes with low ambient metal concentrations.

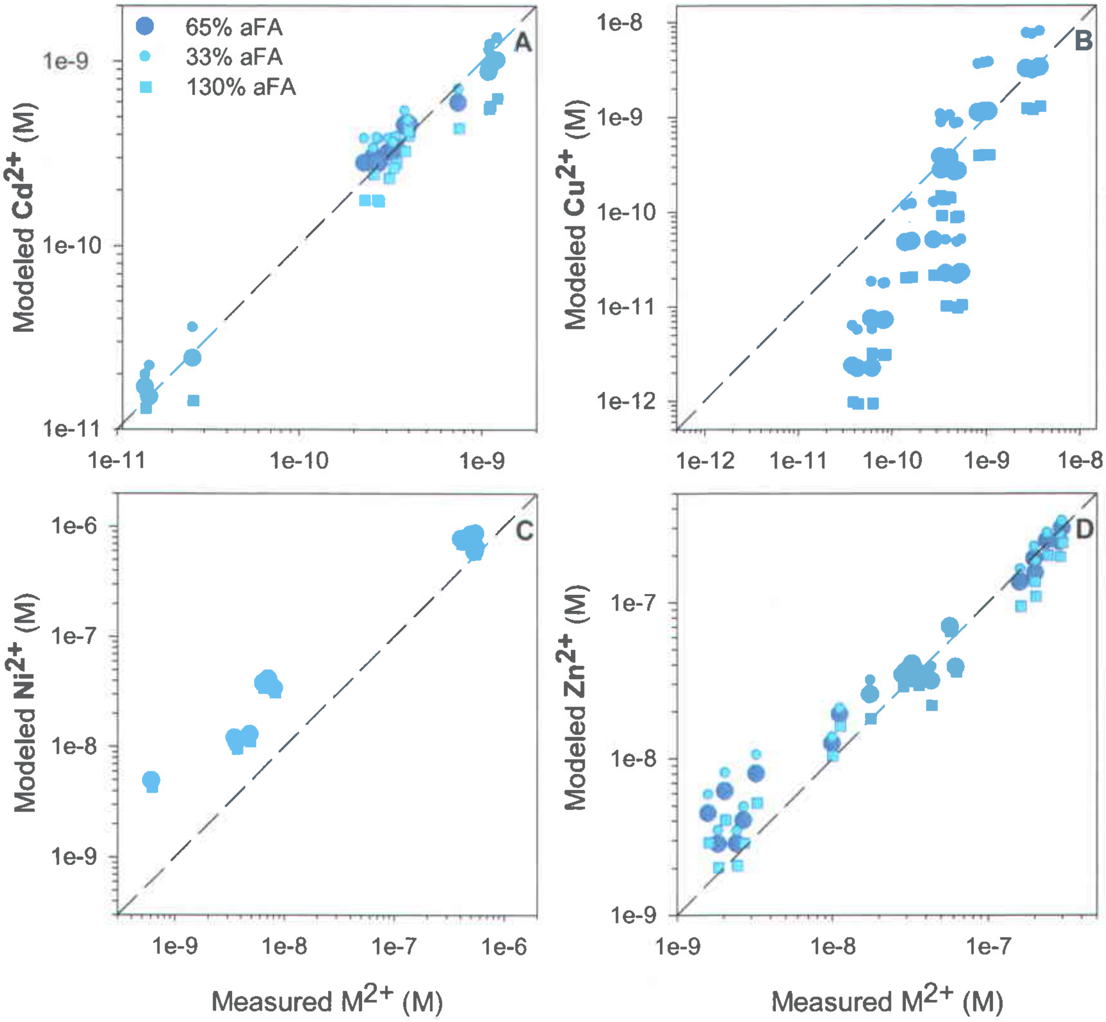

Figure 5. Comparison of the concentrations of the free metal ion [M^{2+}] measured using IET (Cd, Ni and Zn) and ISE (Cu) and those calculated with WHAM VI assuming 65% aFA (large dark circles) and a range of 33% (small light circles) to 130% aFA (small light squares). The dashed line represents the 1:1 line / Comparaison entre les concentrations d'ion métallique libre [M^{2+}] mesurées par IET (Cd, Ni et Zn) ou ISE (CU) et les concentrations prédites par WHAM VI en supposant des valeurs de % aFA de 65 % (cercles pleins) ou entre 33 et 130 % (cercles ouverts). La ligne pointillée représente l'équivalence 1 : 1.

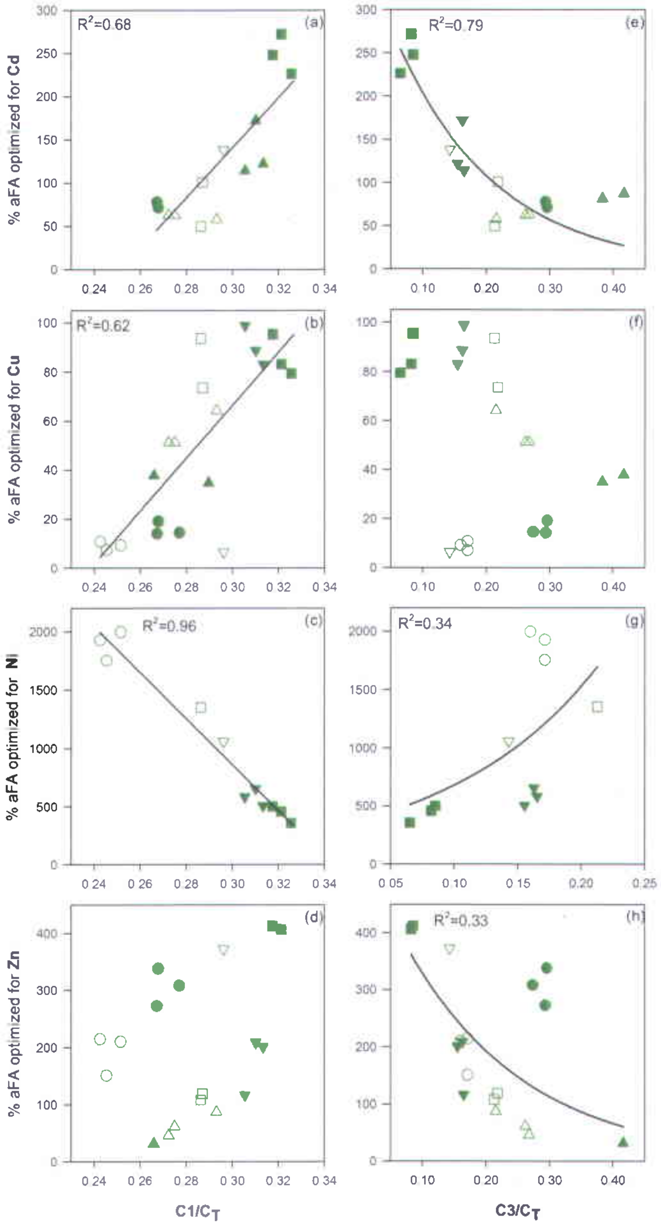

Figure 6. Relationship between the WHAM-calculated percent fulvic acid active in complexation (%aFA optimised) for Cd, Cu, Ni and Zn and the relative contribution of the PARAFAC fluorescence components 1 (panels a-d) and 3 (panels e-h) to the overall fluorescence spectrum of lake DOM. Points of the same shape and fill represent replicate samples. Solid lines represent regression equations of statistical significance at the 0.05 alpha level. Reproduced from Mueller et al. (2012c) / Pourcentage optimal d'acide fulvique actif dans la complexation (%aFA optimisé) calculé par WHAM pour Cd, Cu, Ni et Zn en fonction de la contribution, relative à la fluorescence totale de la MOD, des composantes 1 (panneaux a-d) et 3 (panneaux e-h) de fluorescence PARAFAC. Les points de même forme et de même couleur représentent des échantillons en répliqua, et les lignes continues représentent les régressions significatives au niveau statistique de alpha égal à 0.05. Reproduit de Mueller et al. (2012c).

4.6 Measured metal binding parameters

Possible reasons for discrepancies between measured and WHAM modeled free metal ion concentrations include 1) the underestimation of the concentration of metal-binding sites per unit of dissolved carbon, or 2) the underestimation of the strength of these metal-binding sites for the metals of interest. The former reason was addressed above with the calculation of the %aFAopt values for each metal and each lake. For Cd, Zn and Ni, however, the %aFAopt values exceeded the notional limit of 100%. A possible reason for the underestimation of the strength of the binding sites might be that the generic binding constants used in WHAM were generated from laboratory titrations of isolated humic and fulvic acids and often at higher metal:DOC ratios than those normally encountered in natural waters. Under these titration conditions, only the highly abundant sites with low metal-binding affinity would be probed.

The need to generate binding affinities and capacities for the binding of metals to DOM at environmentally relevant metal concentrations to naturally occurring DOM has also been expressed by Tipping and Lofts (2011) in their review of the performance of WHAM when applied to natural waters. We therefore measured the binding of Ni and Cu to DOM through titrations for two lakes with spectroscopically different DOM and low ambient dissolved metal concentrations, and then compared the resulting titration curves to those predicted by WHAM VI using default parameters and 65% of fulvic acids active in metal complexation (see Figure 8). In general, at ambient metal concentrations Lake Geneva was found to bind more Ni per unit DOC than Lake Geneva DOM. In contrast, Lake Opasatica DOM was found to bind more Cu per unit DOC than Lake Geneva DOM. The titration curves were then used to calculate conditional binding parameters for Ni and Cu binding to DOM in Lakes Opasatica and Geneva.

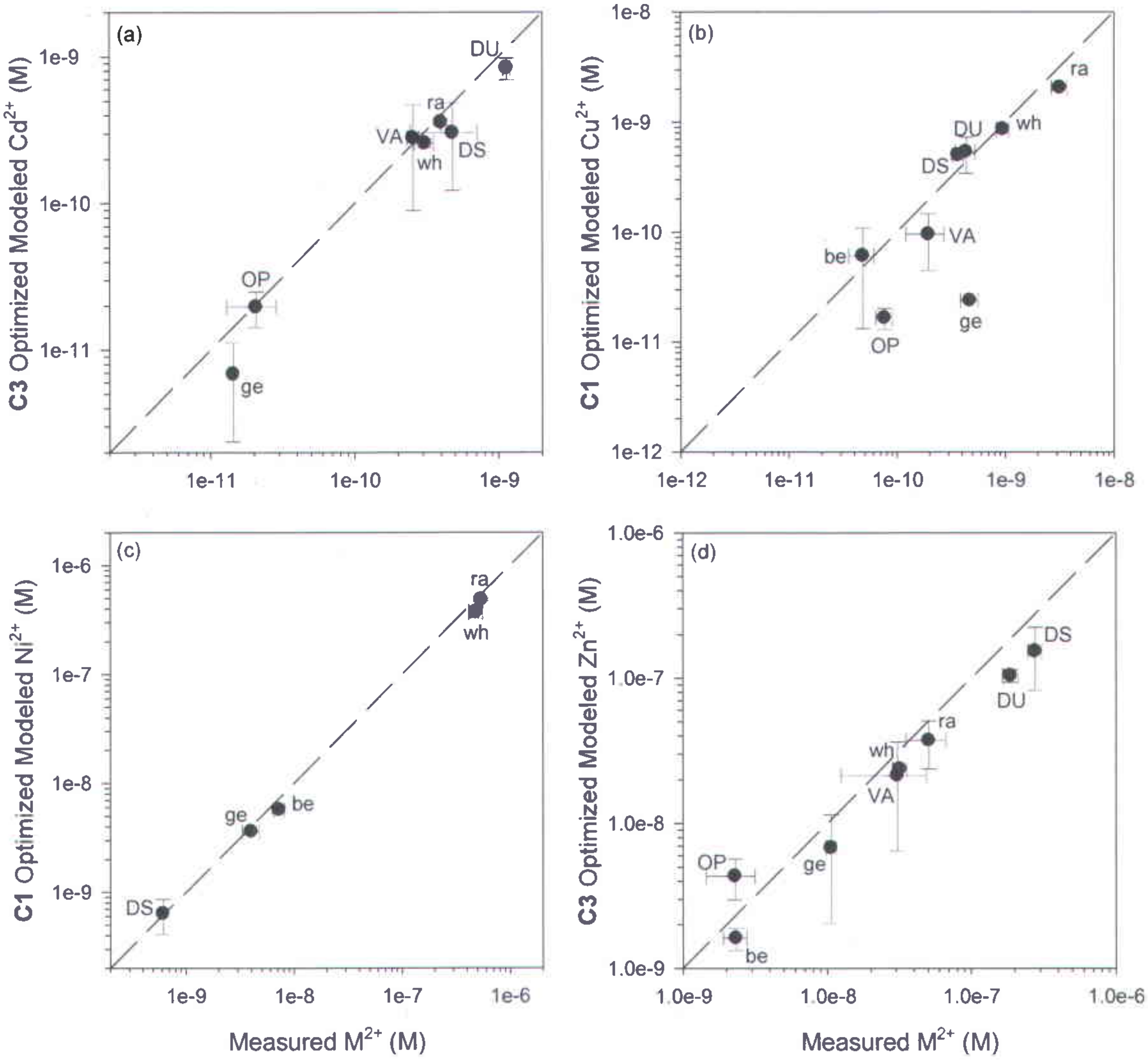

Figure 7. Comparison of the mean (± SD, n=3) concentrations of free metal ion [M^{Z+}] calculated with WHAM VI (with the percentage active fulvic acid (%aFA) estimated from Figure 6) and those measured (± SD, n=3) using the ion exchange technique (IET) (Cd, Ni and Zn) or ion selective electrode (ISE) (Cu) methods: (a) cadmium; (b) copper; (c) nickel and (d) zinc. The dashed line represents the 1:1 line / Concentrations moyennes (± ET, n=3) du métal libre [M^{Z+}] calculé par WHAM (en employant le pourcentage d'acide fulvique actif (%aFA) estimé d'après la Figure 6) comparées à celles mesurées (± ET, n=3) avec la technique d'échange d'ions (IET)(Cd, Ni, Zn) ou une électrode sélective (ISE) (Cu) : (a) cadmium; (b) cuivre; (c) nickel et (d) zinc. La ligne pointillée représente l'équivalence 1 : 1.

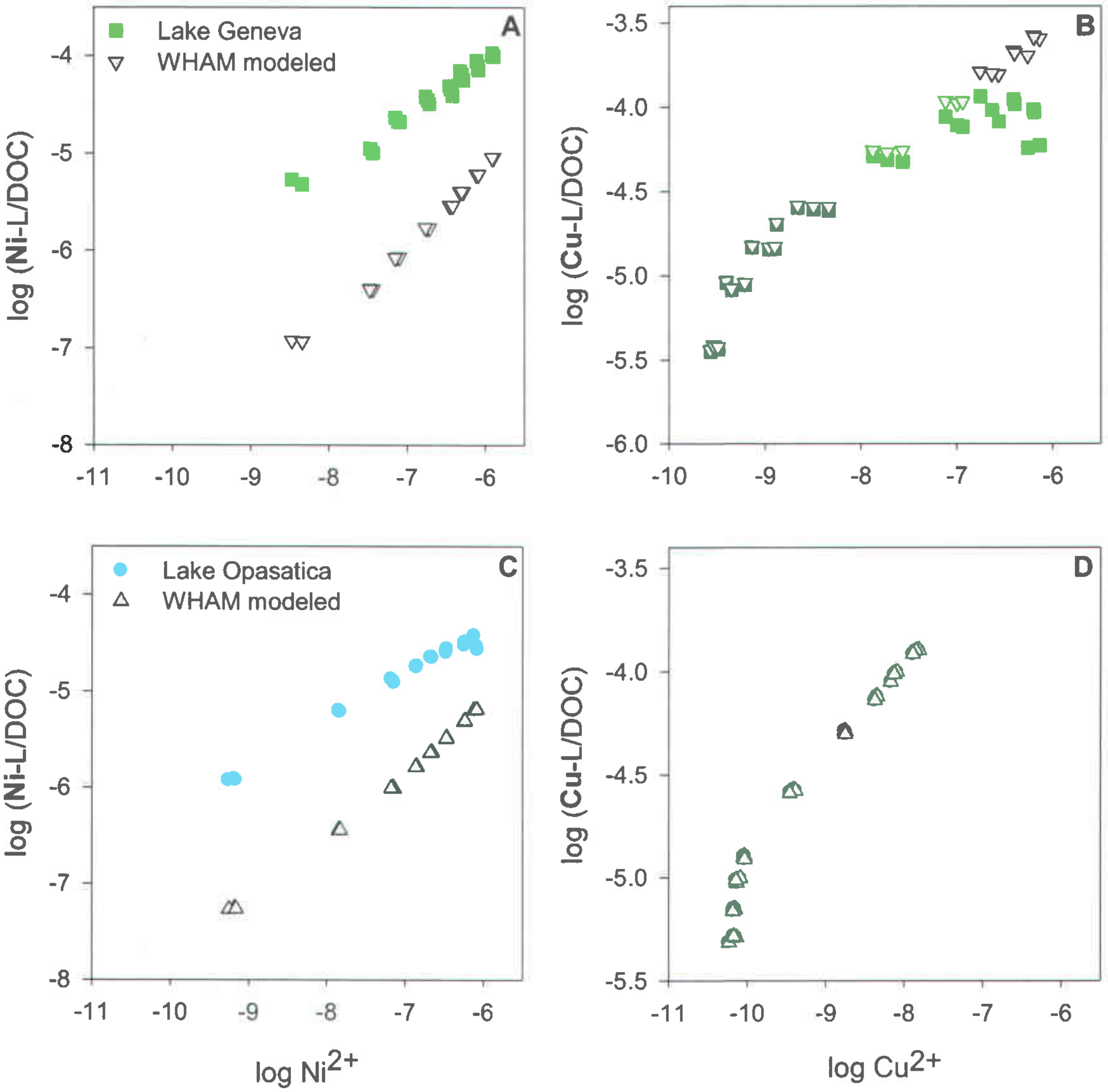

Figure 8. Normalized Ni (A and C) and Cu (B and D) complexation curves (M-DOM/DOC) for Lake Geneva (A and B) and Lake Opasatica (C and D). Measured values are shown in colour; values calculated with WHAM VI are shown in gray triangles. Modified from Mueller et al. (2012a) / Courbe de complexation normalisée (M-MOD/MOD) pour Ni (panneaux A et D) et Cu (panneaux B et D) dans les lacs Geneva (carrées; panneaux A et B) et Opasatica (cercles, panneaux C et D). Les valeurs mesurées sont montrées en couleur alors que celles prédites par WHAM VI sont représentées par des triangles gris. Modifié d'après Mueller et al. (2012a).

Considering Ni binding first, the affinity of the weak sites (Log $K_{1,Ni}$=6.98) on DOM from Lake Opasatica is significantly higher (*P*=0.009) than for Lake Geneva (Log $K_{1,Ni}$=6.30), but these weak sites are present at a concentration ($L_{1,Ni}$=34.2 nmole·mg C^{-1}) nearly four times lower (*P*=0.01) than in Lake Geneva ($L_{1,Ni}$=137 nmole·mg C^{-1}). The Ni binding affinities for the strong sites in both lakes are not significantly different (Log $K_{2,Ni}$=11.6 and 11.7 for Lakes Opasatica and Geneva, respectively), although there are nearly six times less (*P*=0.025) of these binding sites per unit DOC in Lake Opasatica ($L_{2,Ni}$=0.70 nmole·mg C^{-1}), compared to the DOM from Lake Geneva ($L_{2,Ni}$=3.98 nmole·mg C^{-1}).

Although the strength of the binding of Cu to weak sites on the DOM was not significantly different for both lakes (Log $K_{1,Cu}$=7.59 for Lake Opasatica and Log $K_{1,Cu}$=7.63 for Lake Geneva), the number of L_1 sites per unit DOC was approximately three times higher (*P*<0.001) in Lake Opasatica ($L_{1,Cu}$=244 nmole·mg C^{-1}) than in Lake Geneva ($L_{1,Cu}$=75.6 nmole·mg C^{-1}). There were significantly (*P*=0.014) stronger (Log $K_{2,Cu}$=9.46) and approximately twice as many (*P*=0.003) strong binding Cu sites per unit DOC ($L_{2,Cu}$=44.5 nmole·mg C^{-1}) on the DOM from Lake Opasatica compared to Lake Geneva (Log $K_{2,Cu}$=8.90 and $L_{2,Cu}$=27.0 nmole·mg C^{-1}). These results strongly suggest that the DOM from Lakes Opasatica and Geneva are different in terms of their Ni and Cu complexation affinity and capacity.

Based on the absorbance and fluorescence measurements described above (section 4.3), Lake Opasatica DOM was found to be largely characteristic of allochthonous sources, whereas Lake Geneva contains DOM from largely autochthonous sources. It is thus tempting to speculate that the differences in Cu and Ni binding exhibited by the DOM in the two lakes are related to the different weighting of allochthonous and autochthonous origins in the two lakes. The more aromatic humic-like DOM from allochthonous sources may have a significantly higher complexation affinity (at the strong binding sites, K_2) and capacity for Cu than the more protein-like DOM from autochthonous sources. Interestingly, a contrasting trend was found for Ni. The Ni binding affinity at the weak binding sites (K_1) was significantly lower for the lake with the more aromatic humic-like DOM from allochthonous sources. The binding capacities at both types of binding sites was significantly lower for the lake with the more aromatic humic-like DOM from allochthonous sources, again suggesting that Ni and Cu are binding to different types of binding sites on DOM. Baken et al. (2011) found higher Cu, Ni, Zn and Cd affinities for more aromatic DOM (based on SUVA measurements), although the affinity values were based on total complexed metal, not at two different binding sites. The suggestion from the titration study that Ni and Cu are binding to different DOM sites is analogous to the earlier suggestion, based on the relationships between % aFAopt and the $C1/C_T$ and $C3/C_T$ ratios, that Ni binding sites are distinct from those occupied by Cd and Zn. To our knowledge, these are among the first results to demonstrate the presence of independent metal-binding sites within the DOM mosaic. These results further indicate the importance of specifying the quality of DOM when predicting the complexation of trace metals by DOM in natural systems.

5 **Conclusions**

5.1 Improving trace metal speciation predictions

The main objective of the thesis was to demonstrate that the calculation of trace metal speciation in natural waters could be improved by incorporating a measure of the quality of DOM into existing chemical equilibrium models; we hypothesised that the spectroscopic quality of DOM might be used to this end. Each of the following sub-objectives was addressed during the course of the thesis: 1) a series of lakes representing a wide gradient of dissolved metal concentrations, DOM concentrations and physico-chemical conditions was identified; 2) we successfully determined the spectroscopic quality of the lakewater DOM, which was linked to lake and watershed characteristics; 3) the concentrations of free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} were measured in the series of lakes and compared to various environmental factors (e.g., pH, total metal concentration, quantity and quality of DOM); 4) links between the DOM conditional binding constants for Cu and Ni and the spectral properties of the lakewater DOM were explored for two lakes that exhibited different DOM characteristics and for which metal speciation also differed; and 5) WHAM predictions of the free metal ion concentrations were improved (esp. for Ni) by estimating the proportion of DOM active in metal complexation using the spectroscopic quality of the DOM. The main conclusions of the thesis are described in more detail in the following paragraphs.

Based on previous studies that showed discrepancies between measured and WHAM-predicted free metal concentrations in lakes (Fortin et al., 2010; Unsworth et al., 2006), we set out to characterize the free metal speciation in a series of lakes from two different geographical regions on the Canadian Shield. Although good agreement was found between measured and WHAM predicted free metal concentrations for Cd^{2+} and Zn^{2+} for most lakes, departures from this agreement were observed for Cu^{2+} and Ni^{2+}. Marked divergences were found for Cu^{2+} when the free Cu^{2+} concentration was below 0.4 nM (i.e., below total dissolved Cu concentrations of about 40 nM); similar low free Cu^{2+} concentrations are often found in natural waters. In the case of Ni, WHAM consistently over-predicted the free Ni^{2+} concentration in all lakes.

The presence of non-humic organic ligands of anthropogenic origin, sometimes found in natural waters (but not accounted for in chemical equilibrium models such as WHAM), has been suggested as a reason for discrepancies between measured and modeled free metal concentrations (Baken et al., 2011). This explanation was, however, discounted for the present study as remote lakes, little influenced by anthropogenic sources, were purposely chosen. Other possible reasons for discrepancies between measured and WHAM-modeled speciation values were recently summarised by Lofts and Tipping (2011). These authors noted that the following factors could influence the accuracy of WHAM predictions: 1) the precision of model input variables and model parameters, 2) the uncertainty in measured free metal concentrations, and 3) differences between the metal-complexing properties of the humic substances present in natural waters and the isolated humic and fulvic acids used to calibrate the model. It was this latter explanation that was explored in this thesis, by characterising the DOM, in addition to the metal speciation, in the study lakes.

We were interested in a DOM characterisation method that could, in principle, be applied broadly by non-specialists. We, therefore, chose to characterize natural DOM using simple spectroscopic techniques available in typical water chemistry laboratories, as opposed to the more involved humic substances isolation techniques. Moreover, it has been suggested that these humic substance isolation techniques may alter the structure and metal-complexing properties of natural organic matter (Abbt-Braun et al., 2004; De Haan, 1992). First, we showed that differences in the composition of DOM in a small set of lakes on the Precambrian Shield were detectable using various optical parameters (specific UV absorbance ($SUVA_{254}$), fluorescence indices (FI) and excitation-emission matrix (EEM) fluorescence measurements interpreted with parallel factor analysis (PARAFAC)). We then explored how these optical parameters might be used to improve metal speciation predictions in natural waters using chemical equilibrium models.

We hypothesised that the spectroscopic measurements of DOM quality could be used to estimate the proportion of DOM active in metal complexation. As described earlier (section 4.5), WHAM users must first estimate what proportion of the dissolved organic carbon present in a natural water sample (measured as mg $C \cdot L^{-1}$) is constituted of humic substances, and then estimate what proportion of this humic material is present as humic vs. fulvic acids. Current practice is to assume that humic substances are approximately 50% carbon (Thurman, 1985) and that “65% of the DOC is due to organic matter that possesses the cation-binding properties of average isolated fulvic acids” (Bryan et al., 2002). Using these assumptions (as mentioned earlier in this section) we found marked divergences between measured and WHAM modeled concentrations of free Cu^{2+} and Ni^{2+}.

The possibility of improving the agreement was then explored by calculating the optimal percent of FA actively involved in metal complexation (%aFAopt) required to exactly predict the measured free metal ion concentration for each metal and for each lake sampled. Relationships between this %aFAopt for a given lake and its DOM spectroscopic properties were investigated. The most significant relationships were found between the ubiquitous humic-like DOM fluorescence component (C1) and the %aFAopt for Cu and Ni, and between the humic-like fluorescence component of allochthonous origin (C3) and the %aFAopt for Cd and Zn (Figure 6). Given the measured fluorescence of these components for a given lake, its %aFAopt was then estimated and used to predict the speciation of Cd, Cu, Ni and Zn.

When fluorescence measurements were used to estimate the proportion of DOM actively involved in metal complexation, the WHAM predictions of free Ni^{2+} concentration were improved for all the lakes measured (up to a factor of 6), compared to the results obtained with the off-the-shelf version of WHAM and the arbitrary 65% aFA value. The WHAM predictions of the free Cu^{2+} concentration improved by a factor of 17 for Lake Bethel and by a factor of 2 for Lakes Opasatica and Vaudray. For Cd^{2+} and Zn^{2+}, however, no improvement was observed. The fluorescence signature of DOM may, therefore, potentially be used as a proxy for its metal binding activity in chemical equilibrium models such as WHAM. Ultimately, it would be of practical use to determine the EEM fingerprint of a given lake water, calculate the relative proportion of either the ubiquitous fluorescence component ($C1/C_T$) or the allochthonous fluorescence component ($C3/C_T$), and use this value to estimate the %aFA for the given lake water from a relationship similar to those shown in Figure 6. Although this approach appears to be promising, it would first have to be explored over a wider range of DOM samples and shown to hold true for other natural waters.

It was also apparent that in order to further improve the WHAM prediction of the speciation of Cu and especially Ni, adjustments to the metal binding capacity of the DOM and the affinity constants used by WHAM would be necessary. Indeed, we found that differences in the experimentally measured DOM binding capacity, i.e. the number of binding sites, for Cu and Ni could be explained by differences in the spectroscopic character of DOM. In a small number of lakes (N=2) we found between-lake and between-metal differences in the binding affinities of Ni and Cu that could also be related to differences in the spectroscopic character of the DOM. Due to the small number of samples, these relationships are speculative only and should be further investigated for a wide variety of surface waters. Nonetheless, these results support our hypothesis that the quality of DOM is important in determining its role in the speciation of trace metal in natural waters and that the fluorescence signature of DOM may be used as a proxy for its metal binding activity in chemical equilibrium models such as WHAM. However, we have calculated the conditional binding properties of DOM for Cu and Ni for only two lakes. A much larger set of water samples would be required to compare the WHAM VI Cu and Ni binding parameters to those experimentally measured here. Some previous studies have compared the conditional binding parameters for DOM measured in the sampled lake waters to the average intrinsic equilibrium constants used by WHAM VI (calibrated with data sets on the binding of metals to isolated humic substances) by adjusting WHAM model parameters for each lake until a best fit between measured and predicted free metal ion concentrations was achieved (Bryan et al., 2002; Cheng et al., 2005; Van Laer et al., 2006). Following this method, relationships between the fitted WHAM K_{MA} values and measured spectroscopic properties of DOM in natural waters could be compared.

5.2 Research perspectives

The main objective of this thesis was to enhance existing chemical equilibrium models by taking into account the spatial variability of the quality of DOM. Along these lines, we successfully demonstrated the importance of taking into account the quality of DOM when using chemical equilibrium models to predict the speciation of trace metals in natural waters. We also demonstrated that the optical properties of DOM, including its fluorescence "signature", may be used to estimate its metal binding capacity (i.e., the number of binding sites per unit of organic carbon). For example, by measuring the relative proportions of different PARAFAC fluorescent components in a given sample, the % of MOD active in the complexation of Cd, Cu, Ni and Zn in the sample may be estimated *a priori* and used as a measured input parameter in chemical speciation models, such as WHAM. Improvements in the WHAM predictions of the concentration of the free metal ranged from up to a factor of 6 for Ni and up to factor of 17 for Cu. The conditional binding constants for the metal-DOM complexation of certain trace metals may, however, need to be updated for conditions where low metal concentrations are found (i.e., where [M]/DOC ratios are low).

Enhancing chemical equilibrium models leads to improved predictions of trace metal speciation in aquatic systems. The accurate prediction of the speciation of trace metals in aquatic systems is essential for predicting their bioavailability and, thus, their toxicity to aquatic organisms. The Biotic Ligand Model (BLM) is widely used among scientific, regulated and regulatory communities to predict the toxicity of trace metals towards aquatic organisms (Paquin et al., 2002). The BLM uses WHAM to calculate the free metal ion concentration in the aqueous phase near the biotic ligand considered. Although the BLM works reasonably well for high, acutely toxic metal concentrations, improvements are needed to better predict metal toxicity at low, chronic toxicity levels (Campbell et al., 2006). Our results show improvements in WHAM speciation calculations at low metal concentrations (up to a factor of 6 for Ni) that can directly improve our ability to predict chronic metal toxicity. The wider implication of our research is that improved prediction of trace metal speciation in natural aquatic systems is needed for toxicity testing, environmental impact assessment, environmental monitoring, and ecological risk assessment and management (Luoma and Rainbow, 2008).

Although the results and conclusions of the thesis are significant and novel, more research questions were raised. First from an analytical perspective, are current analytical techniques able to measure the free metal ion accurately? Improvements are needed in the analytical measurement of many different free metal ions at low environmental concentrations (Tipping, 2002), including the measurement of free Fe and Al concentrations in natural waters, so as to improve the way chemical equilibrium models account for Fe and Al competition for complexation sites. Second, can the DOM fluorescence signature be used to estimate the metal binding activity of other types of DOM (e.g., non-lake DOM) and for metals other than Cd, Cu, Zn and Ni? In order to address this question, the comparison between measured and modeled free metal ion concentrations and DOM fluorescence should be conducted for more types of DOM (e.g., more autochthonous end-members) and for more metals. Third, do the metal-humic substances binding parameters used by WHAM sufficiently represent metal-DOM binding in natural waters? Should future research efforts move away from using isolated humic substances to represent natural DOM in chemical speciation models and work on building models using DOM as it exists in natural water samples? Again, more research into determining the binding parameters of various types of DOM with many metals, for wider concentration ranges of total and free metal and DOM, is needed. Finally, what is the best way to incorporate DOM quality into chemical speciation models such as WHAM? For now, this can be done externally by estimating the metal binding activity from the DOM fluorescence “signature”, but a new sample-specific input parameter for DOM quality would be most useful.

Deuxième partie : Articles

Article 1

Spatial variation in the optical properties of dissolved organic matter (DOM) in lakes on the Canadian Precambrian Shield and links to watershed characteristics

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Abstract

In the present study we explored the use of various optical parameters to detect differences in the composition of the dissolved organic matter (DOM) in a set of lakes that are all located on the Canadian Precambrian Shield, but within which Cu and Ni speciation predictions were previously shown to diverge from measured values in some lakes but not in others. Water samples were collected with *in situ* diffusion samplers in 2007 (N = 18 lakes) and 2008 (N = 8 lakes). Significant differences in DOM quality were identified between the sampling regions (Rouyn-Noranda, Québec and Sudbury, Ontario) and among lakes, based on dissolved organic carbon concentrations ([DOC]), specific UV absorbance ($SUVA_{254}$), fluorescence indices (FI) and excitation-emission matrix (EEM) fluorescence measurements. Parallel factor analysis (PARAFAC) of the EEM spectra revealed four components, two of which (C3, oxidized quinone fluorophore of allochthonous origin, and C4, tryptophan-like protein fluorescence of autochthonous origin) showed the greatest inter-regional variation. The inter-lake differences in DOM quality were consistent with the regional watershed characteristics as determined from satellite imagery (e.g., watershed-to-lake surface area ratios and relative percentages of surface water, rock outcrops, vegetative cover, and urban development). Source apportionment plots, built upon PARAFAC components ratios calculated for our lakes, were used to discriminate among DOM sources and to compare them to sources identified in the literature. These results have implications for other areas of research, such as quantifying lake-to-lake variations in the influence of organic matter on the speciation of trace elements in natural aquatic environments.

1 Introduction

Natural dissolved organic matter (DOM) is ubiquitous in the aquatic environment, with concentrations ranging from 0.5 mg C L^{-1} in the open ocean to more than 30 mg C L^{-1} in bog waters (Thurman, 1985). Although the quantity of DOM is important with respect to its transport and flux within an ecosystem, its role in biogeochemical processes, for example as an organic acid (Shuman et al., 1990), as a mediator of redox reactions (Aeschbacher et al., 2010), as a surfactant (Campbell et al., 1997) and as a metal-binding ligand (Tipping, 2002) will depend on its nature (i.e., source and composition). With respect to the latter role, attempts have been made to include various isolated chemical fractions of DOM, e.g. fulvic and humic acids, in chemical equilibrium models (Dudal and Gérard, 2004). These models (e.g., the Windermere Humic Aqueous Model, WHAM (Tipping et al., 1998) or the Non-Ideal Competitive Adsorption Model, NICA (Milne et al., 2003)) can reproduce laboratory titration data, but when used to predict metal speciation in natural water samples they are much less successful (Unsworth et al., 2006). One of the major problems encountered in applying these models to natural waters is the need to estimate the concentration of "active" fulvic or humic acid present in the natural water. Often the only available information is the concentration of dissolved organic carbon ([DOC]) and in such cases researchers have had to make assumptions as to the nature of the DOC in their samples and the relative contributions of the fulvic or humic acids and the other dissolved organic constituents.

The impetus for the present study was the recent observation of marked differences between measured and predicted metal speciation in lakes on the Canadian Precambrian Shield (Fortin et al., 2010; Mueller et al., 2010). On the basis of the ambient water chemistry, the WHAM and NICA chemical equilibrium models were used to predict the free-metal ion concentrations of a number of trace metals (Cd, Cu, Ni and Zn) in lakes in the Rouyn-Noranda (Québec) and Sudbury (Ontario) areas. Agreement between measured and predicted values was generally good for Cd and Zn, but for Cu the predicted free-metal ion concentrations were well below the measured values whereas for Ni^{2+} the predicted values consistently exceeded the measured free-ion concentration. In the latter two cases, the degree of divergence between measured and predicted values varied from lake to lake. We speculated that lake-to-lake differences in the composition of the DOM might be one of the major factors contributing to the differences between observed and predicted metal speciation.

Dissolved organic matter has been defined as a complex mixture of disordered, polyelectrolytic molecules, generated from plant, microbial, and animal products at various stages of decomposition (Wetzel, 2001). The DOM pool in lakes may contain some simple labile, low molecular weight organic compounds, but normally is dominated by recalcitrant, high molecular weight fulvic and humic material (Thurman, 1985). With respect to the origin of DOM, both allochthonous sources (production of DOM in the terrestrial environment from the degradation of soil and plant organic matter), and autochthonous sources (production within the water column from the excretion, decomposition and lysis of macrophytes, algae and cyanobacteria in the littoral and pelagic zones) contribute to the pool of DOM in natural aquatic environments (Thurman, 1985; Wetzel, 2001). The composition of DOM in a given lake will reflect the relative contribution of each of these sources, but it will also depend on the processes to which the organic molecules have been subjected on their path from the watershed to the lake and in the lake itself (e.g., differential sorption on inorganic soil horizons (Schumacher et al., 2006); photochemical degradation and biodegradation in the tributary streams feeding the lake and in the lake water column (Köhler et al., 2002; Yoshioka et al., 2007)). It follows that watershed characteristics, such as geomorphology, hydrologic regime, land cover, the presence of upstream lakes and land use are likely to influence the quality (i.e., the nature or composition) of DOM (Jaffé et al., 2008; Mattsson et al., 2005).

The spectroscopic character of DOM has proved useful in assessing its source and quality. Optical properties of DOM that have been used to distinguish between the types of DOM include absorbance (Gondar et al., 2008; Weishaar et al., 2003) and fluorescence (Coble, 1996; Koster and Schmuckler, 1967; McKnight et al., 2001). Absorbance and fluorescence spectroscopy are particularly useful tools in the analysis of DOM quality in that they are relatively simple, rapid, sensitive and non-destructive. Most studies, however, have discriminated among a large number of samples with DOM from very contrasting environments, for example comparing DOM from wetlands to rivers, to estuaries, and to the open ocean (Jaffé et al., 2008).

The purpose of the present study was to explore the use of various optical parameters to detect differences in the quality of the DOM in a small set of lakes that are geographically close to one another on the Canadian Precambrian Shield, and within which Cu and Ni speciation predictions have been shown to diverge from measured values. We report on the variability in DOM optical quality, as determined by simple absorbance and fluorescence measurements, in lakes from the Rouyn-Noranda and Sudbury regions on the Canadian Precambrian Shield. Parallel factor analysis (PARAFAC) is also used to identify fluorophores that contribute to the overall DOM fluorescence and to estimate their relative contributions to the spectra. Cluster analysis and principal components analysis are used to identify groups of lakes with similar DOM properties, and links to the land use within the individual catchments are explored. A source apportionment approach is then used to illustrate lake-to-lake trends and to identify the lakes that constitute "end-members" along the spatial gradient of the lakes sampled.

Methodology

1.1 Study area

The lake water quality data in this study were collected from two different regions of the Canadian Precambrian Shield: one centred on Rouyn-Noranda in north-western Québec, and the other located near Sudbury in north-central Ontario (Figure 1). In terms of the underlying geology, both regions are found on the Canadian Shield, made up of quartzite, gabbro and felsic gneisses (Carignan and Nriagu, 1985). The city of Rouyn-Noranda is found in the Abitibi sub-province of the Canadian Shield containing the Abitibi Greenstone Belt. For the most part, the bedrock is covered with a thick layer of glacial, glaciofluvial and glaciolacustrian deposits, although bedrock does occasionally break through the surface deposits (Veillette et al., 2005). While the city of Sudbury is also on the Canadian Shield, the local landscape is dominated by the presence of exposed bedrock covered by thin and discontinuous glacial deposits (Barnett and Bajc, 2002; Carignan and Nriagu, 1985).

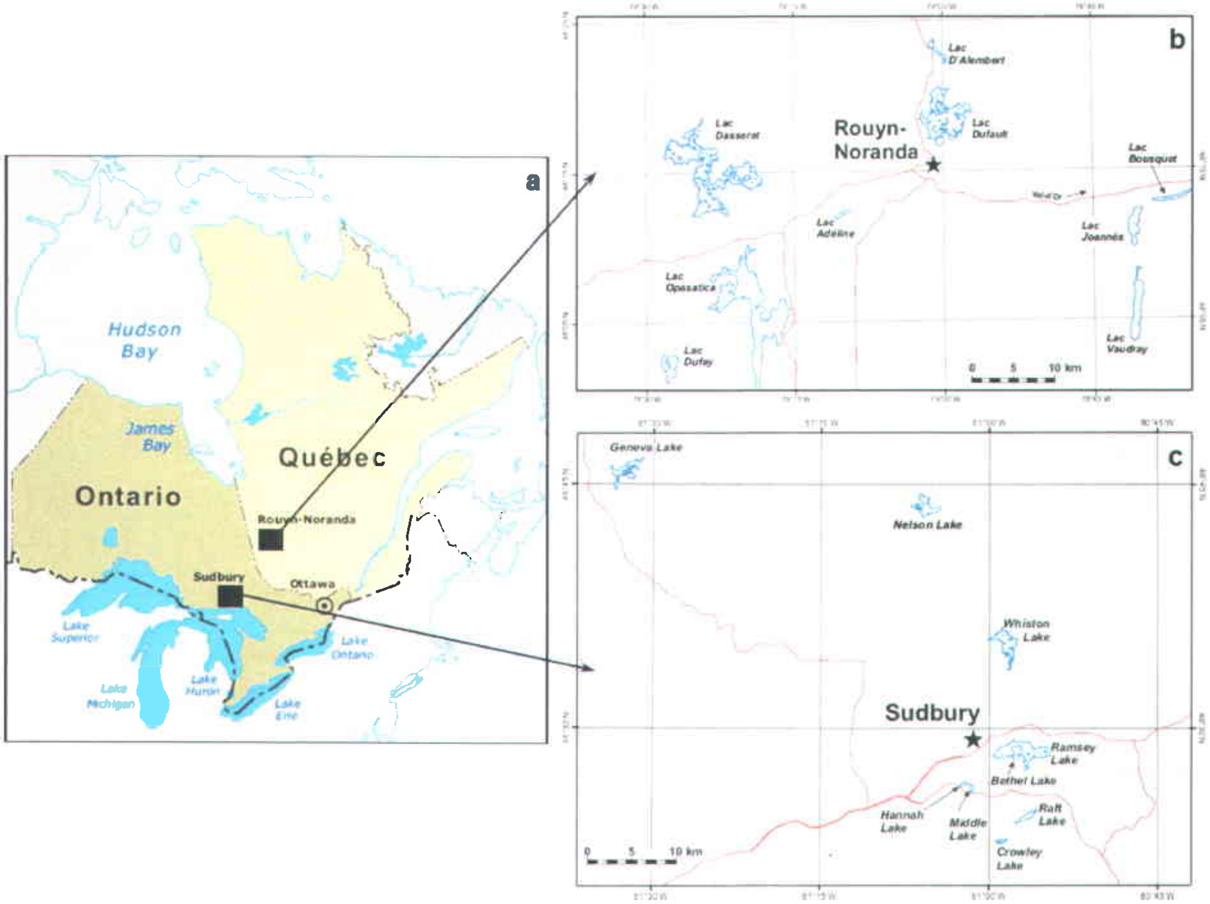

Figure 1. Map of the two study areas showing the relative position of the two regions in eastern Canada (panel a), the geographical location of the Rouyn-Noranda lakes (panel b) and the Sudbury lakes (panel c).

Both study areas are rich in ore deposits and have been greatly impacted by metal mining and smelting activities, particularly by atmospheric deposition of acid and metals (Borgmann et al., 1998; Dixit et al., 2007). The pH of the lakes tends to decrease with a decrease in the down-wind distance from the metal smelters, particularly in the Sudbury area (Yan and Miller, 1984). Acid mine drainage from point sources, such as abandoned mines or mineralized outcrops, may also decrease the pH of lake water locally. In each region, lakes were chosen to represent a gradient in water quality (pH, [DOC] and trace metal concentrations).

2.2 *Lake water sampling*

Lake water samples were taken from nine lakes in each of the study areas during July and August of 2007 and 2008 (Table 1). Sampling sites where chosen in the littoral zones of small, well mixed lakes and the sampling sites were considered to be representative of the whole lake epilimnion. The spatial variability of water quality (i.e., pH and concentrations of DOC and major cations) is known to be minimal within many of the sampled lakes (Fortin et al., 2010).

Lake water was sampled using equilibrium diffusion sampling as described in Fortin et al. (Fortin et al., 2010). Briefly, 250 mL polypropylene jars (Nalgene) topped with custom-made plastic lids fitted with a 0.2 µm filter membrane (HT Tuffryn, Pall) were filled with ultrapure water (>18 Mohms cm) and fixed to plastic rods that were anchored to the lake bottom. The samplers were installed at a depth of 1 m from the lake surface and left to equilibrate for 14 to 19 days. Following this equilibration period, the samplers were brought to the surface, the membrane surface rinsed with ultrapure water, the membranes pierced with a clean plastic pipette tip and the custom jar lids replaced with clean polypropylene screw lids. The sample jars were bagged and stored in the dark at 4°C in field coolers for a maximum of 12 days until they were transported back to the laboratory. Lake water pH, conductivity and temperature were measured in the field at the time of sample collection.

Table 1. Location, average (± SD) measured pH, dissolved organic carbon (DOC) concentration, dissolved concentrations of Ca and Mg and years the lakes were sampled (N=3-6).

Lake (code)	Region	Location		pH	DOC	Ca	Mg	Years Sampled	
					(mg·L^{-1})	(µM)	(µM)	2007	2008
Adeline (AD)	Rouyn-Noranda	48°12'12" N	79°10'17" W	7.53	4.6 ± 0.8	172.6 ± 0.8	86.7 ± 0.5	Y	
Bousquet (BO)	Rouyn-Noranda	48°12'56" N	78°37'06" W	6.35	13.0 ± 0.7	122.2 ± 0.4	54.4 ± 0.1	Y	
D'Alembert (DA)	Rouyn-Noranda	48°23'01" N	79°00'35" W	7.48	8.0 ± 0.8	180.8 ± 0.5	62.5 ± 0.1	Y	
Dasserat (DS)	Rouyn-Noranda	48°15'06" N	79°24'24" W	6.64	5.2 ± 0.7	208.9 ± 2.1	96.4 ± 3.0	Y	Y
Dufault (DU)	Rouyn-Noranda	48°18'30" N	78°59'25" W	7.41	5.1 ± 0.4	413.2 ± 10.9	122.7 ± 4.9	Y	Y
Dufay (DF)	Rouyn-Noranda	48°01'42" N	79°27'33" W	6.60	8.3 ± 0.4	75.9 ± 0.5	55.6 ± 0.2	Y	
Joannès (JO)	Rouyn-Noranda	48°10'53" N	78°40'02" W	6.82	10.0 ± 0.3	190.0 ± 1.4	77.6 ± 0.3	Y	
Opasatica (OP)	Rouyn-Noranda	48°04'32" N	79°17'49" W	7.47	6.0 ± 0.4	215.4 ± 3.0	114.2 ± 0.9	Y	Y
Vaudray (VA)	Rouyn-Noranda	48°05'45" N	78°40'50" W	6.59	8.1 ± 0.1	83.6 ± 0.8	37.9 ± 1.1	Y	Y
Bethel (BE)	Sudbury	46°46'17" N	80°57'43" W	8.93	5.2 ± 0.5	466.1 ± 3.3	282.6 ± 9.4	Y	Y
Crowley (CR)	Sudbury	46°23'06” N	80°58'55” W	6.46	2.6 ± 0.3	57.4 ± 0.2	34.2 ± 0.1	Y	
Geneva (GE)	Sudbury	46°45’52” N	81°32’46” W	6.89	3.4 ± 0.4	68.6 ± 1.9	27.3 ± 1.2	Y	Y
Hannah (HA)	Sudbury	46°26'36" N	81°02'17" W	7.40	3.6 ± 0.1	274.9 ± 1.0	156.9 ± 0.3	Y	
Middle (MI)	Sudbury	46°26'22" N	81°01'26" W	7.46	3.3 ± 0.4	246.9 ± 0.3	140.5 ± 0.4	Y	
Nelson (NE)	Sudbury	46°43'36" N	81°05'39" W	5.86	1.4 ± 0.5	58.9 ± 0.1	28.3 ± 0.1	Y	
Raft (RA)	Sudbury	46°24'35" N	80°56'44" W	6.91	2.3 ± 0.3	81.6 ± 1.1	47.7 ± 1.6	Y	Y
Ramsey (RM)	Sudbury	46°28'57" N	80°57'00" W	7.83	3.6 ± 0.5	395.0 ± 0.9	198.7 ± 0.4	Y	
Whitson (WH)	Sudbury	46°35'03" N	80°58'20" W	6.24	3.8 ± 0.1	145.1 ± 1.9	80.5 ± 2.3	Y	Y

2.3 Sample analysis

All plastic laboratory and sampling equipment was soaked in 10% (w/v) nitric acid for at least 24 h, rinsed a minimum of six times with ultrapure water and dried under a Class 100 laminar flow hood. Similarly, all glass ware was soaked in 2 N HCl solution before being rinsed with ultrapure water.

Once the polypropylene jars had arrived in the laboratory, they were opened under a clean Class 100 laminar flow hood and the following subsamples were taken. A 10-mL sample was transferred to a polypropylene vial and acidified to 2% (v/v) with concentrated ultra-trace nitric acid (BDH Aristar, VWR International) for analysis of major cations (Ca, Mg) by atomic emission spectroscopy (Vista AX CCD Simultaneous ICP-AES, Varian).

Next, 20-mL subsamples were transferred to amber borosilicate vials (I-Chem Brand) for total organic carbon analysis (TOC-V_{CPH}, Shimadzu) as well as absorption (Cary 300 Bio UV-Visible Spectrophotometer, Varian) and fluorescence (Cary Eclipse Fluorescence Spectrophotometer, Varian) measurements. All optical parameters were measured at the natural pH of the sample and without dilution. Absorption spectra were recorded using 1-cm polymethacrylate cuvettes. The spectra were measured from 240 to 700 nm at intervals of 1 nm with ultrapure water as a reference. Excitation-emission matrix (EEM) fluorescence spectra were measured using 1-cm quartz cuvettes at excitation wavelengths from 210 to 400 nm at increments of 5 nm and at emission wavelengths from 300 to 580 nm at increments of 2 nm. The protocol for data collection and treatment recommended in Stedmon and Bro (Stedmon and Bro, 2008) was followed, including spectral correction (using correction files provided by the instrument manufacturer), inner filter correction (McKnight et al., 2001; Mobed et al., 1996), calibration to Raman units (Lawaetz and Stedmon, 2009) and finally the subtraction of ultrapure water blank and removal of spectral scatter.

Absorbance measurements were used to calculate the specific UV absorbance ($SUVA_{254}$, L m^{-1} mg C^{-1}) by dividing the absorbance at 254 nm in inverse meters (m^{-1}) by the concentration of

DOC measured in milligrams of carbon per litre (mg C L^{-1}) (Weishaar et al., 2003). The fluorescence index was calculated as the ratio between the fluorescence emission intensity at 470 nm over that at 520 nm for an excitation at 370 nm (Cory et al., 2010; McKnight et al., 2001). Finally, the multivariable statistical tool, parallel factor analysis (PARAFAC), was employed using the procedure outlined by Stedmon and Bro (2008) as implemented in a MATLAB environment, using the "N-way toolbox for MATLAB" (Andersson and Bro, 2000). Excitation-emission spectra for 87 individual lake samples with excitation wavelengths from 240 to 400 nm and emission wavelengths from 350 to 550 nm were included in the PARAFAC model. The measured values at excitation wavelengths from 210 to 235 nm and emission wavelengths from 300 to 348 nm were not included in the model so as to reduce the influence of data with a low signal-to-noise ratio and to constrain the model to the wavelengths with appreciable fluorescence. The wide range of DOC concentrations in the lake samples resulted in a correspondingly wide range of fluorescence intensities (in Raman units, RU). To avoid the identification of the DOM fluorescence intensity extremes as outliers by PARAFAC and to compare all of the samples on the same scale, the normalized fluorescence intensity for a given sample was calculated by dividing the measured fluorescence intensity at each pair of excitation and emission wavelengths by the average fluorescence intensity of the entire spectrum for each lake. Following the identification of the appropriate number of PARAFAC fluorescence components, the maximum fluorescence intensities of the identified fluorescent components for each lake were then restored by multiplication by the average spectrum fluorescence intensity.

2.4 Lake watershed characteristics

Lake watershed delimitation was achieved using National Hydro Network (NHN) geospatial data from the geospatial portal Geobase and the National Topographic Data Base (NTDB) generated by Natural Resources Canada (NRCan). Using hydrographic and topographic geospatial data, the water divide line was identified and the surface area of each watershed was calculated. Watershed land cover (% surface water, % rock outcrops, % vegetation and % wetland) and land use (% agriculture and % urban development) were also calculated as percentages for each of the lake watersheds using geospatial land cover data provided by Geobase. The land cover data consisted of vectorized raster data from classified Landsat ortho-images. Data integration and surface area calculation were done using ArcGIS 10 (Environmental Systems Research Institute, Inc., Redlands, CA).

2.5 Statistical analysis

The following statistical analyses were calculated at the alpha equals 0.05 confidence level using SigmaPlot 11.0 (Systat Software Inc., Chicago, IL). Parametric correlation analyses, *t*-tests and one-way ANOVA's with pairwise multiple comparison procedures using the Holm-Sidak method were used when the normality and homogeneity of variances among datasets where confirmed; otherwise, the appropriate non-parametric tests were employed (Spearman Rank Order correlation in place of the Pearson correlation, Mann-Whitney Rank Sum test in place of the t-test; Kruskal-Wallis one-way ANOVA on Ranks with pairwise multiple comparison procedures using Dunn's method, instead of an ANOVA). Percent land cover and usage data, as well as the relative fluorescence intensities of the PARAFAC components, were arcsine square root transformed before performing these statistical tests.

A hierarchical cluster analysis based on the similarity of water chemistry (pH, the concentrations of Ca and Mg), watershed characteristics (% surface water, % rock outcrops, % vegetation cover, and % urban development) and DOM properties ([DOC], $SUVA_{254}$, FI and the relative proportions of the PARAFAC components C1, C2, C3 and C4) was conducted using the Ward linkage method with Euclidean distance. All data were standardized prior to analysis. Cluster analyses were performed using Systat 12 (Systat Software Inc., Chicago, IL). A principal component analysis (PCA) was also conducted to graphically represent the distribution of the studied lakes along the environmental gradients, using the above-mentioned input data. The PCA was performed using CANOCO version 4.5 (Plant Research International, Wageningen, NL; ter Braack and Smilauer (2002)). The results of these multivariate analyses are presented in the Supplementary Material (SM).

3 Results

The natural variability in dissolved organic matter (DOM) was assessed for the sampled lakes and the results are presented in the following four sections: 1) the general water quality of the lakes sampled, 2) the lake watershed characteristics, 3) the quantity of DOM as measured by the concentration of DOC, 4) the quality of DOM as evaluated on the basis of its absorbance and fluorescence, and 5) the statistical analysis of these latter data to identify DOM components.

3.1 General lake water quality

In general, the water quality of the lakes sampled from the two regions is quite similar (Table 1). Lakes from the Rouyn-Noranda region are circumneutral, with a mean pH of 6.79 and a narrow range between pH 6.35 and 7.53. The mean pH for the Sudbury lakes (6.53) is similar to that of the Rouyn-Noranda lakes, but they span a wider pH range (5.86 to 8.93). The lakes in both sampling regions have similarly low concentrations of calcium (Ca: 57 to 413 µM) and magnesium (Mg: 27 to 199 µM), contributing to generally soft water. Lakes Ramsey and Bethel, from the Sudbury region, had the highest pH and the highest Ca and Mg concentrations of all the lakes.

3.2 Lake watershed characteristics

The watershed characteristics of the lakes sampled were significantly different between sampling regions (Table 2). The watershed-to-lake surface area ratios (W/L) are generally higher in the Rouyn-Noranda region (mean = 25) than in the Sudbury region (mean = 9). Moreover, lake watersheds in the Rouyn-Noranda region have a significantly higher mean percent vegetation cover (83%) and wetland cover (1%), whereas percent surface water (13%) and rock outcrops (0%) are lower than in the Sudbury region (41%, 0%, 23% and 28%, respectively). Forest cover dominated the vegetative land cover for the watersheds near Rouyn-Noranda (69 – 99%) and Sudbury (96 – 100%), the contribution from shrubs being much less important. The more northerly watersheds near Rouyn-Noranda also contained a higher proportion of coniferous forests, compared with the more southerly Sudbury region watersheds.

3.3 DOM quantity

The quantity of DOM, i.e. the DOC concentration, differed significantly between the two study areas ($P<0.001$) (Figure 2a). The mean DOC concentration of Rouyn-Noranda lakes was higher (7.2 mg L^{-1}) and spanned a wider range (3.8 to 13.4 mg L^{-1}) than in the lakes from the Sudbury region (mean of 3.4 mg L^{-1}; range from 0.8 to 6.0 mg L^{-1}). Within the entire dataset, Lakes Bousquet and Joannès from the Rouyn-Noranda region had the highest [DOC], whereas Lake Nelson, from the Sudbury region, had the lowest concentration (Figure 2a). Significant differences ($P<0.001$) in [DOC] were found among most lakes. In any given year, the variability of replicate (N=3) DOC values in a given lake was low (i.e., coefficient of variation, CV < 17%), except in Lake Nelson (Sudbury), which had a CV of 33%. For those lakes that were sampled during more than one year, the inter-annual variability in the [DOC] was less than 14%.

3.4 DOM quality

To probe the quality of the DOM, we calculated the specific UV absorbance ($SUVA_{254}$), measured at a wavelength of 254 nm. The $SUVA_{254}$ values varied significantly between sampling regions ($P<0.001$) (Figure 2b). The mean $SUVA_{254}$ value for the Rouyn-Noranda lakes was higher (8.3 L m^{-1} mg C^{-1}) than for the lakes from the Sudbury region (5.3 L m^{-1} mg C^{-1}), but spanned a narrower range (5.8 to 11.5 L m^{-1} mg C^{-1}) than that for the Sudbury lakes (3.2 to 10.3 L m^{-1} mg C^{-1}). Among all lake samples, significant differences ($P<0.001$) were found between Lakes Bethel and Ramsey and Lake Bousquet ($P<0.05$); Lake Bousquet had the highest $SUVA_{254}$ value, whereas Lakes Bethel and Ramsey, from the Sudbury region and which had a moderate [DOC], had some of the lowest $SUVA_{254}$ values. In any given year, the variability of replicate (N=3) $SUVA_{254}$ values in a given lake was generally similar to that of the [DOC] (i.e., CV < 18%), except in two Sudbury lakes (Nelson and Raft; CVs 45% and 57%, respectively). For those lakes that were sampled during more than one year, the inter-annual variability in the $SUVA_{254}$ values was less than 10%, except for Lake Raft for which the CV was 39%.

Table 2. Watershed-to-lake surface area ratio (W/L), percent watershed land cover (% surface water, % rock outcrops, % vegetation and % wetland) and land use (% agriculture and % urban development) for the lakes sampled.

Lake (code)	Region	W/L	Land Coverage				Land Usage	
			% surface water	% rock outcrops	% vegetation	% wetland	% agriculture	% urban development
Adeline (AD)	Rouyn-Noranda	7	16	0	82	0	2	0
Bousquet (BO)	Rouyn-Noranda	129	6	0	90	4	0	1
D'Alembert (DA)	Rouyn-Noranda	22	6	0	81	3	0	1
Dasserat (DS)	Rouyn-Noranda	19	16	0	83	0	0	1
Dufault (DU)	Rouyn-Noranda	7	21	0	69	0	2	7
Dufay (DF)	Rouyn-Noranda	11	13	0	86	1	0	0
Joannès (JO)	Rouyn-Noranda	10	11	0	87	2	0	0
Opasatica (OP)	Rouyn-Noranda	11	13	0	78	0	7	1
Vaudray (VA)	Rouyn-Noranda	8	15	0	81	3	0	1
Bethel (BE)	Sudbury	5	25	18	29	0	0	29
Crowley (CR)	Sudbury	23	19	28	52	0	0	0
Geneva (GE)	Sudbury	5	25	1	74	0	0	0
Hannah (HA)	Sudbury	6	18	63	11	0	0	7
Middle (MI)	Sudbury	11	17	70	6	0	0	6
Nelson (NE)	Sudbury	4	34	0	66	0	0	0
Raft (RA)	Sudbury	15	23	25	52	0	0	0
Ramsey (RM)	Sudbury	6	25	26	24	0	0	25
Whitson (WH)	Sudbury	7	19	21	50	1	0	8

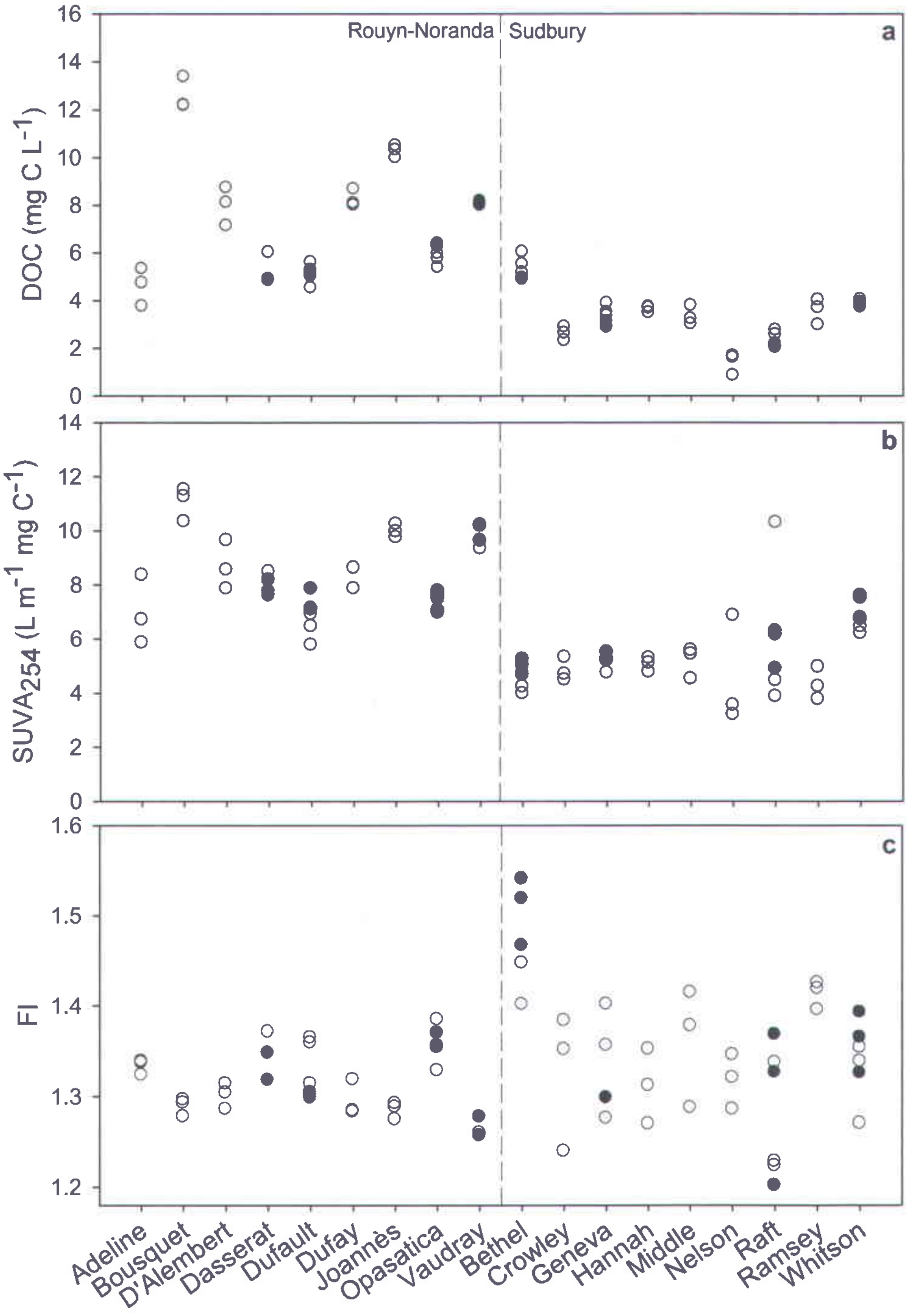

Figure 2. Replicate measures of dissolved organic carbon (DOC) concentration (panel a), specific UV absorbance ($SUVA_{254}$) (panel b) and fluorescence index (FI) (panel c) for samples taken from lakes in 2007 (open circles; N=18) and 2008 (closed circles; N=8). Lakes to the left of the dashed line are from the Rouyn-Noranda region, whereas those to the right are from the Sudbury region.

The quality of DOM was also evaluated by excitation-emission matrix (EEM) fluorescence spectroscopy. The fluorescence index (FI) ratio (inherently normalized to the [DOC]) was used to distinguish between organic matter from autochthonous sources (FI>1.4) and allochthonous sources (FI<1.4) (Cory et al., 2010; McKnight et al., 2001). The FI differed significantly between sampling regions ($P<0.05$) (Figure 2c); the mean FI for the Rouyn-Noranda lakes (1.32) was lower than that for the Sudbury lakes (1.36), and spanned a narrower range (1.26 to 1.38) than did the FI for the Sudbury lakes (1.20 to 1.54). Among all lake samples, significant differences ($P<0.001$) were found between Lakes Bethel and Vaudray only ($P<0.05$). The within-year variability of replicate ($N=3$) FI values in a given lake was minimal (CV < 7%), as was temporal variability (CV < 6%).

Further evidence for differences between the two sampling areas was found when the bivariate relationships between the DOM quantity ([DOC]), and the quality parameters $SUVA_{254}$ and FI were examined (Figure 3). A positive relationship is apparent between $SUVA_{254}$ and [DOC] for all lakes (Figure 3a). No relationship between the FI and [DOC] is apparent for the Rouyn-Noranda lakes, whereas a positive relationship between FI and [DOC] is apparent for the Sudbury lakes (Figure 3b). Note that since both $SUVA_{254}$ and FI are normalized with respect to the [DOC], it would be statistically inappropriate to calculate a correlation coefficient or regression between $SUVA_{254}$ or FI and [DOC]. A weak, but significant negative correlation ($r = -0.38$, $P = 0.001$) was found between the two DOM quality parameters, FI and $SUVA_{254}$ (Figure 4). The relationship is anchored by Lakes Bethel and Ramsey at high FI and low $SUVA_{254}$ values and by Lakes Bousquet, Joannès and Vaudray with low FI and high $SUVA_{254}$ values.

In summary, these relatively routine measurements ([DOC], $SUVA_{254}$ and FI) allowed us to identify Lakes Bousquet, Joannès and Vaudray from the Rouyn-Noranda region, and Lakes Bethel and Ramsey from the Sudbury region, as lakes that consistently emerge from the general trends and are therefore of particular interest. Further evidence for inter-lake differences in DOM quality was obtained using PARAFAC to characterize the DOM taken from the lakes sampled.

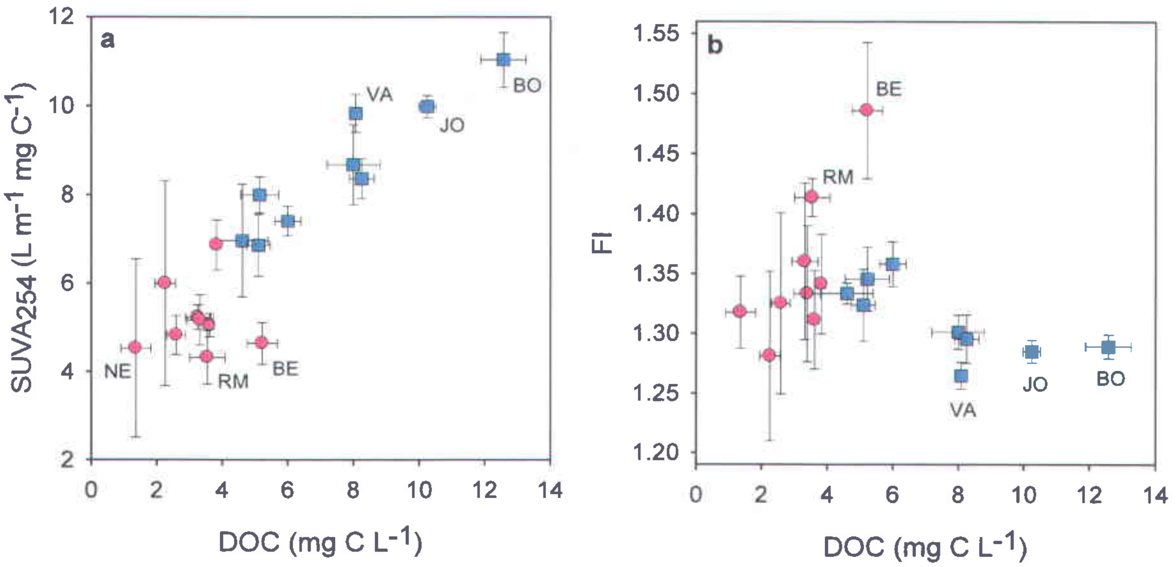

Figure 3. Relationships between $SUVA_{254}$ (panel a) and FI (panel b) with DOC concentration for samples taken from lakes in the Rouyn-Noranda (squares) and Sudbury (circles) areas. Error bars indicate the standard deviation of replicate measures (N=3-7) covering all sampling years.

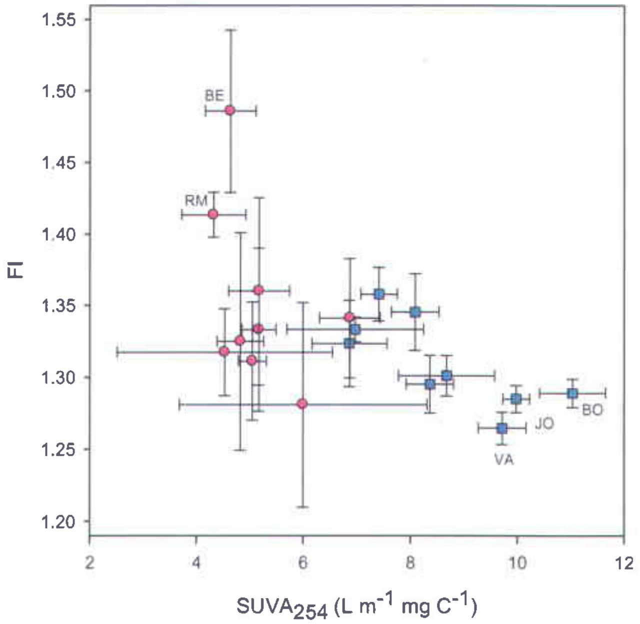

Figure 4. Relationship between FI and $SUVA_{254}$ for samples taken from lakes in the Rouyn-Noranda (squares) and Sudbury (circles) areas. Error bars indicate the standard deviation of replicate measures (N=3-7) covering all sampling years.

Four unique fluorescent components were identified using parallel factor analysis (PARAFAC) on replicate EEM spectra for each lake and sampling year (N=87) (Figure 5). Component 1 (C1) has a primary excitation peak at 250 nm and a secondary peak at 340 nm. This component also has a broad emission peak from 480 to 500 nm. Component 2 (C2) has a similar fluorescence signature with a primary excitation peak at just below 240 nm and a secondary peak at 310 nm. Its emission is, however, blue shifted to a shorter wavelength of 410 to 430 nm. Component 3 (C3) has an excitation peak below 240 nm and a very broad emission peak from 420 to 450 nm. Component 4 (C4) has a primary excitation peak at less than 240 nm and a secondary peak at 285 nm. Its emission peak occurs at 355 nm. Some residual fluorescence in the upper left corner of the spectrum was unaccounted for by the PARAFAC model. This residual fluorescence is most likely a result of unresolved secondary Rayleigh scatter. Overall, the PARAFAC model explained 99.8% of the variance in the EEM spectra.

In several of our lakes the absorbance values exceeded 0.3 at 254 nm (3 of 18 lakes in 2007 and 1 of 8 lakes in 2008), and thus the inner filter correction may have been incomplete (Miller et al., 2010). To check for the possible influence of these lakes on the PARAFAC results, we removed them from the dataset and reran the PARAFAC analysis; the same four components were identified in the smaller dataset and the % explained variance remained above 99%. We also performed a split-half validation exercise and the split data gave very similar results (see SM).

For comparison among lake samples, the maximum fluorescence of each component (F_{max}) was normalized by dividing by the total fluorescence of all four components. In general, the lakes from the Rouyn-Noranda region have a significantly higher ($P<0.001$) proportion of C3 (40 ± 16%) than do the Sudbury lakes (17 ± 5%) (Figure 6a), whereas the Sudbury lakes have a significantly higher ($P<0.001$) proportion of C4 (20 ± 4%) than the Rouyn-Noranda lakes (10 ± 4%) (Figure 6b); in particular, Lakes Bethel, Nelson, Raft and Ramsey had the highest contribution from this latter fluorescent component.

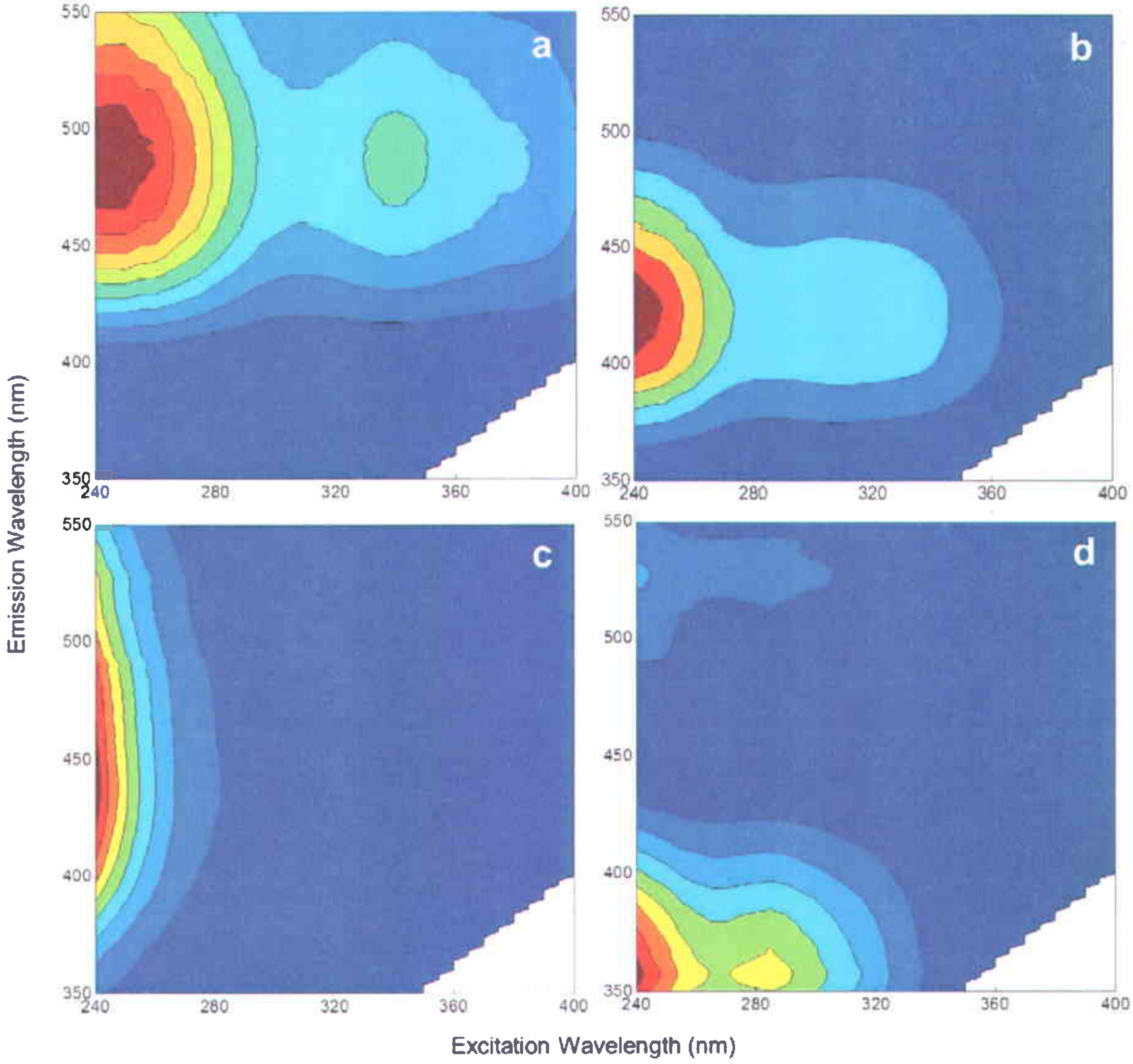

Figure 5. Parallel factor analysis (PARAFAC) components 1 (panel a), 2 (panel b), 3 (panel c) and 4 (panel d) identified from the excitation-emission matrix (EEM) fluorescence spectra of DOM from all lakes over all sampling years. Contour lines represent increasing fluorescence intensity in arbitrary units.

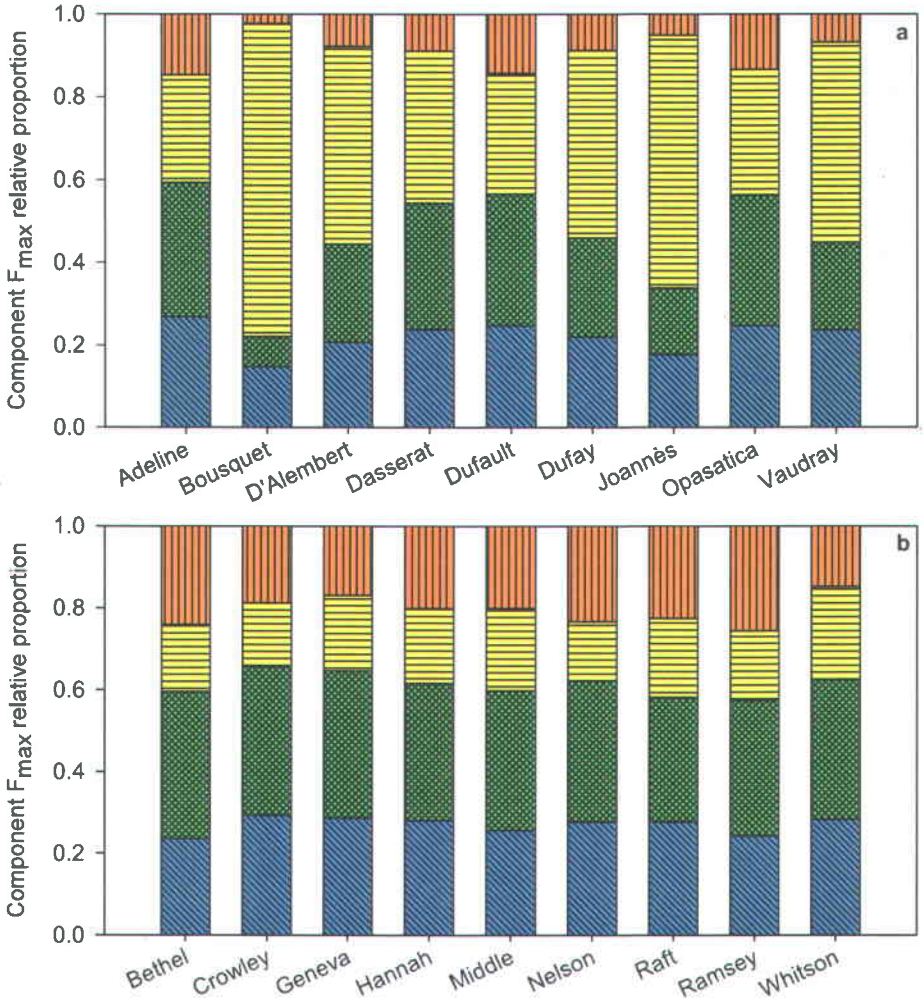

Figure 6. Proportion of maximum fluorescence (F_{max}) of PARAFAC components 1 (diagonal lines), 2 (cross-hatch), 3 (horizontal lines) and 4 (vertical lines) relative to the total component fluorescence for lakes in the Rouyn-Noranda (panel a) and Sudbury (panel b) regions sampled in 2007.

Table 3. Literature based description and position of the fluorescence maxima (secondary peak in parentheses) and description of the four fluorescent components identified by the PARAFAC model.

Component number (this study)	Excitation max (nm)	Emission max (nm)	Component assignment by Coble (1996)	Component assignment by Stedmon and Markager (2005)	Component assignment by Cory and McKnight (2005)	Description
C1	250 (340)	480 to 500	C	2	SQ1	Ubiquitous origin. Humic-like fluorescence. Reduced quinone fluorophore associated with higher plant matter.
C2	<240 (310)	410 to 430	M	4	SQ2	Ubiquitous origin. Humic-like fluorescence. Reduced quinone fluorophore associated with microbial organic matter.
C3	<240	420 to 450	A	1	Q2	Allochthonous origin. Humic-like fluorescence. Oxidized quinone fluorophore. Refractory.
C4	<250 (285)	355 to 355	T	7	Tryptophan-like	Autochthonous origin. Tryptophan-like protein fluorescence.

4 Discussion

Significant differences in the quantity and quality of DOM were highlighted in the preceding section, both among lakes and between regions. The observation of such differences is consistent with the analysis of Sobek and co-workers (2007), who concluded from their study of a very large lake database (7514 lakes from six continents) that climate and topography regulate terrestrial vegetation, soils and hydrology, and that these master variables in turn determine the range of possible DOM concentrations in lakes in a given region. The [DOC] of each individual lake within the region will be affected by the local lake and catchment characteristics, such as the proportion of wetlands in the catchment, the presence of upstream lakes, and water retention time. Using this construct, in the following sections we will first relate differences in [DOC] to the characteristics of the lake's watershed, such as the watershed-to-lake surface area ratio (hereafter noted as W/L; see Table 2) and watershed land cover. We will then apply the routinely measured optical properties ($SUVA_{254}$ and FI), along with the relative proportion of fluorescence components, as identified using PARAFAC, to characterize the DOM in the sampled lakes, identify its possible origins and discuss its upstream and in-lake processing.

Although there is a known effect of pH on the absorbance and fluorescence of humic substances over a wide range (generally 2 to 10) of pH values (Mobed et al., 1996), little effect is reported for lake natural organic matter samples within the pH range reported for our lakes (Senesi, 1990; Weishaar et al., 2003). In fact, Weishaar et al. (2003) suggest that within the pH range of 2 to 8.6 there is no need to adjust the pH to a constant value when comparing UV absorbance values among samples. Baker (2002) also found no variability in DOM fluorescence as a result of changes in pH for a series of circumneutral river sites. Therefore, all optical properties were measured at the natural pH of the sample. We found no correlations between pH and any of the optical measurements ($SUVA_{254}$, FI, PARAFAC components; data not shown), suggesting that pH effects on the optical measurements are not significant for these lakes samples and at their natural pH values reported.

4.1 Lake watershed characteristics (regional differences)

The data in this study were collected from two geographically different regions on the Canadian Precambrian Shield; the geology and hydrology of these regions play an important role in determining the inter-lake variability of surface water quality parameters such as pH, hardness, and DOM quantity and quality. Indeed, the observed difference in mean lake [DOC] between the two sampling regions (Figure 2a: $[DOC]_{R\text{-}N} > [DOC]_{Sudbury}$) is consistent with the different watershed characteristics in the two regions. The watersheds of the lakes sampled from the Rouyn-Noranda region have higher W/L ratios, higher percent vegetation cover and lower percent rock outcrops, compared to the watersheds of the lakes sampled from the Sudbury region (Table 2). It has also been reported that the Rouyn-Noranda region has greater soil cover than the Sudbury region (Veillette et al., 2005), which has very little soil cover and where bedrock outcroppings are much more frequent (Barnett and Bajc, 2002). Also, because of acidification and erosion of the soil in the Sudbury area due to past mining and smelting activities, there has been a significant loss of soil A- and B-horizons (Barnett and Bajc, 2002). Based on this information, we conclude that the Rouyn-Noranda lakes receive a larger proportion of DOM from their catchments than do their counterparts in the Sudbury region, whereas the Sudbury lakes presumably produce a higher proportion of DOM within the water column.

4.2 DOM quality

4.2.1 Specific UV Absorbance ($SUVA_{254}$) and Fluorescence Index (FI)

Optical properties have often been used in the past to characterize DOM and identify its source(s) (Coble, 1996; Koster and Schmuckler, 1967; McKnight et al., 2001). Although the absorption spectra of natural DOM are relatively featureless and similar among natural DOM environments, the calculation of the specific UV absorption ($SUVA_{254}$) can be used as a tool to estimate and compare the quality of DOM from different sources (Weishaar et al., 2003). We successfully discriminated between the two sampling regions based on the $SUVA_{254}$ values (Figure 2b) and on the trends in $SUVA_{254}$ with increasing [DOC] (Figure 3). The higher $SUVA_{254}$ values for the lakes in the Rouyn-Noranda region, compared to the Sudbury region, were expected given their higher W/L ratios and the greater amount of vegetation (i.e., forest coverage) in the region. These factors would be expected to favour greater inputs of allochthonous DOM, known to have higher aromaticity and absorptivity than autochthonous DOM (Chin et al., 1994; McKnight et al., 2001; Weishaar et al., 2003). Temperate coniferous forests of the type found in the Rouyn-Noranda region are recognized sources of leachable organic carbon with relatively high aromaticity and high $SUVA_{254}$ values (Sobek et al., 2007). In addition, given the greater soil depth in the Rouyn-Noranda region, some partitioning of the leachable organic matter likely occurs in the lower mineral soil horizons, with consequent removal of the more hydrophobic components. In their study of DOM movement in a large Alaskan catchment underlain with discontinuous permafrost, Balcarczyck et al. (2009) suggested that such selective adsorption of hydrophobic components would lead to a further enrichment of hydrophilic moieties in solution, with high aromaticity and high $SUVA_{254}$ values. Note however that in their study of the origins of dissolved organic matter in streams of the Yukon River basin, O'Donnell et al. (2010) suggested the opposite trend. During winter low flows in their system, $SUVA_{254}$ decreased and they attributed this change to selective removal of aromatic compounds, such as lignin-derived polyphenols, in the deeper soil layers.

In addition to this direct effect on the relative proportions of allochthonous and autochthonous DOM entering a lake, differences in W/L ratios would also be expected to affect DOM indirectly. For example, the higher the W/L ratio, the higher the relative input of allochthonous DOM, but also the lower the residence time of the lake and, therefore, the lower the likelihood of in-lake processing of DOM (Sobek et al., 2007). Lake Bousquet was found to have, by far, the highest W/L ratio (129) of all the lakes sampled, and the $SUVA_{254}$ value for the DOM in this lake was the highest value that we recorded. It should also be noted that Lakes Joannès and Vaudray both flow into Lake Bousquet and therefore, contribute to its DOM load. Microbial processing of the DOM in the upstream lakes, with consequent removal of the more labile fractions, would be expected to lead to an increased proportion of recalcitrant DOM in Lake Bousquet. On the other hand, lakes Nelson and Bethel had the lowest W/L ratios (4 and 5, respectively). Despite these similar low W/L ratios for the two lakes, the $SUVA_{254}$ value for Lake Bethel was lower than that for Lake Nelson. Lake Nelson is a clear, oligotrophic lake with a low [DOC], whereas Lake Bethel is a coloured, eutrophic lake with a moderate [DOC]. Lake Bethel, as well as Lake Ramsey, is found in a watershed that is highly developed (Table 2). Furthermore, Lake Bethel, a small lake that flows into Lake Ramsey, was used as a sewage lagoon until 1986 (Pearson et al., 2002), which contributed to its eutrophication. Both Lakes Bethel and Ramsey are, therefore, most likely producing a higher proportion of autochthonous DOM than Lake Nelson, thus leading to their lower specific absorptivities.

Similar specific UV absorbance values have been reported for other lake systems. For example, Miller et al. (2010) found low $SUVA_{254}$ values for alpine lakes sampled in Colorado during the summer ranging between 0.5 and 2.5 L m^{-1} mg^{-1}, indicative of autochthonous DOM produced by in-lake biogeochemical processes. They also report significantly higher $SUVA_{254}$ values for an adjacent subalpine stream during the summer months (2.5 to 4.0 L m^{-1} mg^{-1}), attributed to the inputs of allochthonous DOM.

We were also able to discriminate among lakes from the two sampling regions based on FI values (Figure 2c). Fluorescence spectra of natural DOM are more complex than absorption spectra, vary among environments and reveal structural characteristics indicative of DOM source material (Fellman et al., 2010). The FI values measured for the lakes varied from 1.3 to 1.5, enough to indicate significant differences in the source of DOM (Balcarczyk et al., 2009). The lakes from the Rouyn-Noranda region have a low mean FI, indicative of allochthonous DOM, whereas the lakes from the Sudbury region have a slightly higher mean FI, indicative of a greater contribution by autochthonous DOM. Our calculated FI values are similar to those reported for other freshwater environments in central and southern Ontario (Kelton et al., 2007).

The differences between the two sampling regions are also evident when we compare the quantity of DOM ([DOC]) to the DOM quality parameters $SUVA_{254}$ and FI (Figure 3). For the Sudbury lakes, $SUVA_{254}$ and FI increased with a higher steady-state [DOC] in the lake. The watersheds of the Sudbury area have low W/L ratios, low percent vegetation cover, high percent rock outcrops and a thin soil layer, and therefore any increase in the DOC concentration in the lakes is likely attributable to an increased contribution of autochthonous DOM in these lakes. For the Rouyn-Noranda lakes, the $SUVA_{254}$ increases with an increase in [DOC], whereas the FI appears to decrease. A shift to higher $SUVA_{254}$ values and lower FI values (indicative of allochthonous DOM) at higher [DOC] suggests an increase in the proportion of allochthonous DOM derived from the catchment. This reasoning is consistent with the high W/L ratios, higher percent vegetation cover, lower percent rock outcrops and thicker soil layer in the catchments of the lakes in the Rouyn-Noranda area as compared to the Sudbury area.

Other studies have shown that the relationship between $SUVA_{254}$ and fluorescence parameters (FI) can be used to identify autochthonous and allochthonous end members (Jaffé et al., 2008; Miller and McKnight, 2010). We therefore compared these two DOM quality parameters, which allowed us to isolate end members in terms of allochthonous and autochthonous sources of DOM within our dataset (Figure 4). Again, Lakes Bethel and Ramsey, found in the upper left hand corner of Figure 4, are isolated. Their high FI and low $SUVA_{254}$ values are indicative of autochthonous DOM. In contrast, Lakes Vaudray, Joannès and Bousquet are found in the lower right hand corner with low FI and high $SUVA_{254}$ values indicative of allochthonous DOM. These results are supported by the results of the cluster and PCA analyses which found Lakes Bousquet, Joannès and Vaudray (along with Lakes D'Alembert and Dufay) to be grouped together based on their DOM optical properties (see Supplementary Material, SM).

4.2.2 PARAFAC

Although simple absorption and fluorescence DOM quality parameters have frequently been used in the past to characterize natural DOM (Gondar et al., 2008; Green and Blough, 1994), they are not always the best tools to employ. For example, absorbance measurements are subject to interferences from particles and inorganic species such as iron and nitrate (Weishaar et al., 2003), these interferences being more important at low [DOC] than in high [DOC] situations (Green and Blough, 1994; Miller and McKnight, 2010). Furthermore, whether $SUVA_{254}$ or FI is used to describe the quality of DOM, both parameters are calculated using only a small portion of the available information in either the absorbance or fluorescence spectrum. In recent years, however, there has been a significant increase in the use of parallel factor analysis (PARAFAC) as a statistical tool to interpret excitation emission matrix (EEM) fluorescence spectra and identify specific fluorophores or groups of fluorophores (Stedmon et al., 2003) to characterize DOM sources (Jaffé et al., 2008; Miller and McKnight, 2010; Yamashita et al., 2010). Unlike the techniques described above, this approach incorporates data from the entire DOM fluorescence spectrum.

The DOM fluorescence components identified in our lakes using PARAFAC are similar to those found in the literature for similar freshwater environments (Coble, 1996; Cory and McKnight, 2005; Koster and Schmuckler, 1967). Based on these authors' findings, we ascribe C1 and C2 to reduced quinone, humic-like fluorophores that are present in a wide variety of aquatic environments, C3 to refractory, oxidized quinone, humic-like fluorophores of allochthonous origin and C4 to tryptophan-like protein fluorescence of autochthonous origin (Table 3).

The relative proportion of the allochthonous fluorescence component (C3) in the Rouyn-Noranda lakes (40 ± 16%; Figure 6a) was significantly higher than in the Sudbury lakes (17 ± 5 %), whereas the contribution from the protein fluorescence component (C4) was significantly lower (10 ± 4 compared to 20 ± 4 %) ($P < 0.001$ in both cases). These inter-regional trends are consistent with the anticipated lower inputs of DOM from the catchments of the Sudbury lakes, given their lower W/L ratios, lower percent vegetation cover, higher percent rock outcrops and thinner soil cover. When all the lakes were pooled and subjected to an analysis of variance, statistically significant differences ($P<0.001$) in the relative proportions of C3 were found between Lakes Bousquet and Bethel, and between Lakes Joannès and Bethel. Similarly, significant differences in the relative proportions of C4 were found between Lake Bousquet and Lakes Ramsey, Nelson and Bethel. Lake Bethel has the highest proportion of the protein-like fluorescent autochthonous component, C4, consistent with its eutrophic nature, as described earlier, and the consequent increase in primary production. The results of the cluster and PCA analyses support the grouping of Lakes Bethel and Ramsey as lakes with high proportions of DOM of autochthonous origin, and their separation from Lakes Bousquet and Joannès, characterized by high contributions of allochthonous DOM (see SM).

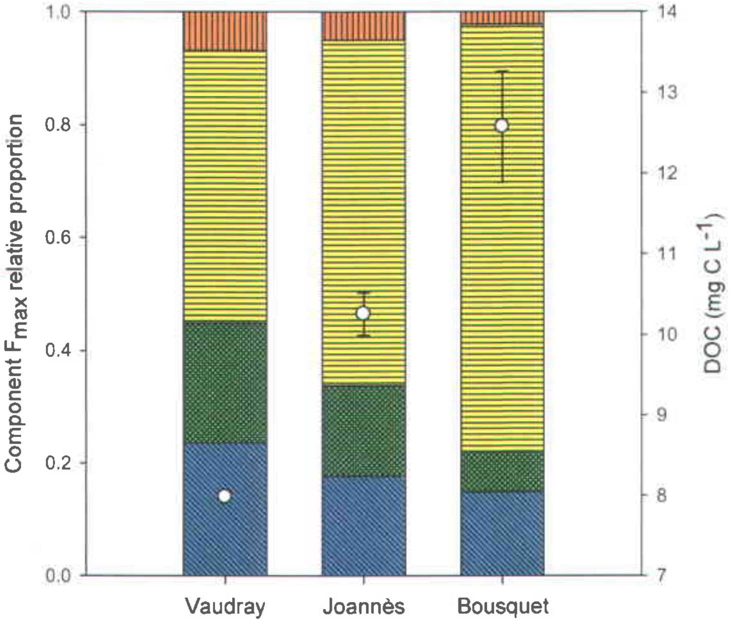

Figure 7. Proportion of maximum fluorescence (F_{max}) of PARAFAC components 1 (diagonal lines), 2 (cross-hatch), 3 (horizontal lines) and 4 (vertical lines) relative to the total component fluorescence, and DOC concentration (open circles) for three hydrologically connected lakes in the Rouyn-Noranda area sampled in 2007. Error bars indicate the standard deviation of three replicate DOC measures.

Lakes Vaudray, Joannès and Bousquet are all located in close proximity and are connected hydrologically; they are characterized by relatively high proportions of the allochthonous component C3 and low proportions of ubiquitous component C2 and the protein component C4. Figure 7 presents the trends in the relative proportions of the fluorescence components, as well as [DOC], for these three lakes. As the [DOC] increases from Lake Vaudray to Lake Bousquet, the relative proportion of the allochthonous component C3 increases. Lakes Vaudray and Joannès both independently flow into Lake Bousquet. Therefore, the increase in [DOC] in Lake Bousquet must represent inputs from its immediate watershed. Note that this reasoning is also supported by the fact that Lake Bousquet has the highest W/L ratio (129, or 83 if only its immediate watershed is considered), followed by Lake Joannès (10) and then Lake Vaudray (8).

We used source apportionment plots to further discriminate between allochthonous and autochthonous sources of DOM for our study lakes. The four fluorescence components are represented with three different component ratios and visually presented in two plots: C3/C1 vs.

C2/C1 (Figure 8a) and C4/C1 vs. C2/C1 (Figure 8b). The ratio C2/C1 was chosen for the dependent axis on the basis of its similarity to the FI (Cory and McKnight, 2005), i.e., C1 and C2 exhibit appreciable emission at 470 and 520 nm wavelengths, respectively, for an excitation wavelength of 370 nm (see Methods). Indeed, plotting C2/C1 ratios against the calculated FI values for the lakes yielded a strong positive correlation ($r=0.65$, $P<0.001$), supporting the analogy between C2/C1 and FI.

The fluorescence component ratio plots clearly discriminate between the allochthonous and autochthonous sources of DOM to the lakes studied. In Figure 8a, we compare the oxidized quinone of allochthonous origin (C3) to the reduced quinone humic-like fluorophores (C1 and C2). As the relative proportion of C2 to C1 increases, the relative proportion of C3 decreases. Lakes Bousquet, Joannès and Vaudray anchor the relationship in the upper left hand corner of the plot where there is a higher proportion of the allochthonous fluorophores, whereas Lakes Bethel and Ramsey anchor the lower right hand corner where there is less influence of the allochthonous fluorophore. In Figure 8b, we compare the relative proportion of the tryptophan-like protein fluorescence (C4) to the reduced quinone humic-like fluorophores (C1 and C2): as the relative proportion of C2 to C1 increases, the relative proportion of C4 increases. Again, lakes Bousquet, Joannès and Vaudray anchor the relationship in the lower left hand corner of the plot where there is a lower proportion of the autochthonous fluorophores, whereas lakes Bethel and Ramsey anchor the upper right hand corner where there is higher proportion of the autochthonous fluorophore.

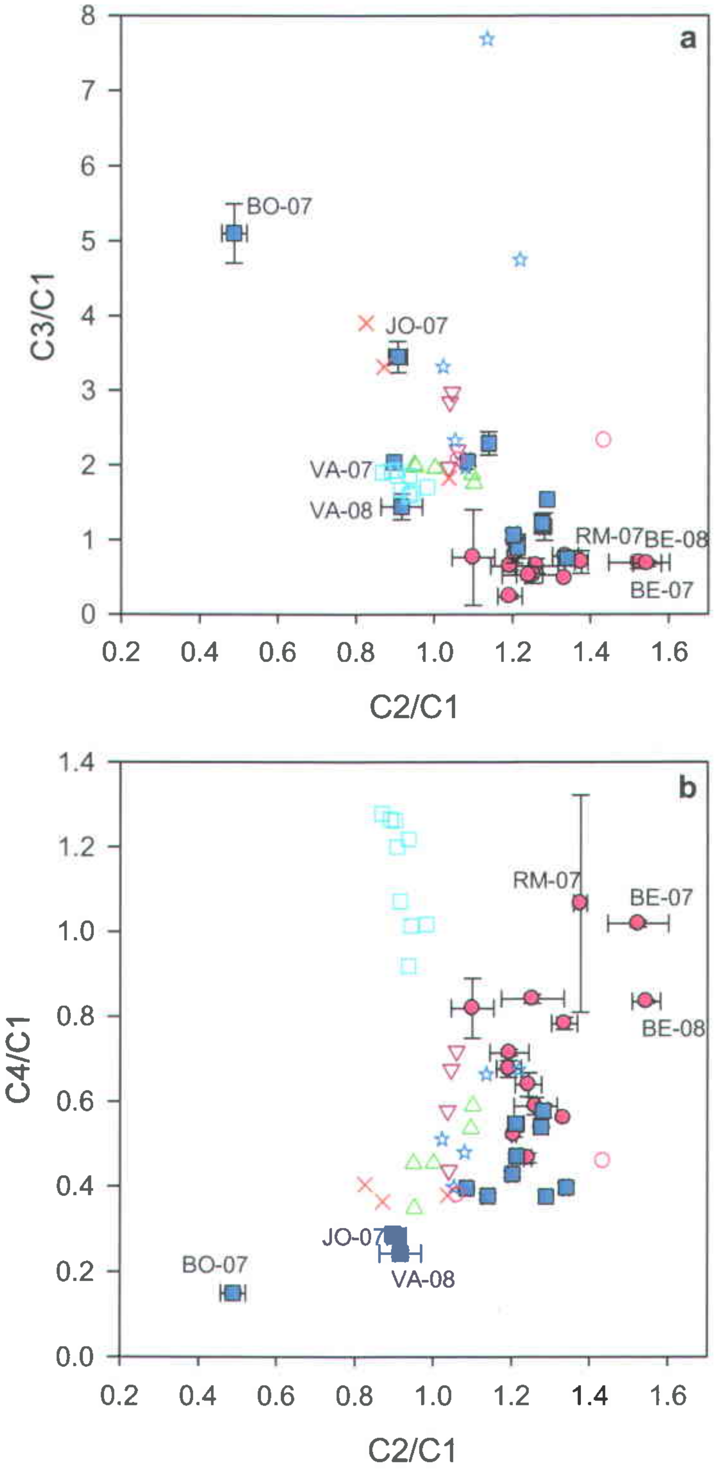

Figure 8. Variation in fluorescence component ratios for allochthonous (panel a) and autochthonous (panel b) DOM from lakes in the Rouyn-Noranda and Sudbury regions sampled in 2007 (-7) and 2008 (-8). Reference values are from Stedmon et al. 2003 (X=forest stream; upward triangle=agricultural stream; downward triangle=lake; star=wetland; circle=urban drainage; square=marine).

In order to associate the DOM in our lakes with sources of DOM, we have added the ratios of known components calculated from Stedmon et al. (2003) to Figure 8, as reference signature values for different DOM sources. With respect to C3 (Figure 8a), which represents the oxidized quinone of allochthonous origin, Lakes Bousquet, Joannès and Vaudray are found in the same region as DOM from forest streams. Lakes Bethel and Ramsey are found near the region for DOM from urban drainage. The lakes found between these end members exhibit signatures similar to those for DOM from lakes and from streams draining agricultural lands. For C4 (Figure 8b), representing tryptophan-like protein fluorescence of autochthonous origin, Lakes Bethel and Ramsey are found near the signature for urban drainage DOM. Lakes Bousquet, Vaudray and Joannès are once again found near the signature for forest stream DOM. The lakes lying between these end members are found near the signatures for DOM from lakes, wetlands and agricultural streams.

The relationships and end members identified in these plots are consistent with the results of the relationship between our $SUVA_{254}$ and FI values presented in Figure 3. To our knowledge, this is the first time that the discrimination between allochthonous and autochthonous derived DOM has been demonstrated using source apportionment plots built upon PARAFAC components.

5 Conclusions

In this research we set out to explore the use of various optical parameters ($SUVA_{254}$; fluorescence index; excitation-emission matrix fluorescence spectroscopy coupled to PARAFAC analysis) to detect differences in the composition of the DOM in a small set of lakes on the Canadian Precambrian Shield within which Cu and Ni speciation predictions had been shown to diverge from measured values. On a regional scale, i.e., comparing Rouyn-Noranda lakes to Sudbury lakes, all three techniques revealed distinct differences in the composition of the lake water DOM between the two sampling areas. PARAFAC components C3 ("oxidized quinone fluorophore of allochthonous origin") and C4 ("tryptophan-like protein fluorescence of autochthonous origin") were those that showed the greatest inter-regional variation, a result that is consistent with the regional watershed characteristics and the presumed routing of the DOM from the watershed to the lake. By combining the optical parameters, water quality data and percentage land cover information, using cluster analysis and principal components analysis, we were able to identify groups of lakes with similar DOM composition and relate these groupings to the local watershed characteristics of the lakes. The relative percentages of vegetative (forest) cover, rock outcrops and surface water were shown to be the key watershed characteristics.

Current metal speciation models that include dissolved organic matter, such as the Windermere Humic Aqueous Model (WHAM), incorporate the measured quantity of DOM, but users must define the variability in DOM quality (i.e., chemical character and source) by choosing the % of DOM that is actively involved in metal complexation (Bryan et al., 2002). The present results open the door to the use of optical measures of DOM quality to refine user estimates of this key parameter.

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6 Supplementary Material

6.1 PARAFAC model validation

The four-component PARAFAC model based on the excitation-emission matrix (EEM) fluorescence spectra of 87 individual lake samples was validated following the steps outlined in Stedmon and Bro (2008). Outlier tests, comparing the sum of squared residuals in the excitation and emission data, split-half analysis and random initialisation were performed. Figure S1 presents the results of the split half validation of the chosen four-component PARAFAC model. The validation procedure involved splitting the data into two halves (Figures S1a,b and S1c,d) and comparing the excitation and emission loadings for the four components. The two individual models (compare Figure S1 panels a and b with panels c and d) are nearly identical. The four-component model was, therefore, found to adequately describe the data and explained most (>99%) of the variance in the data.

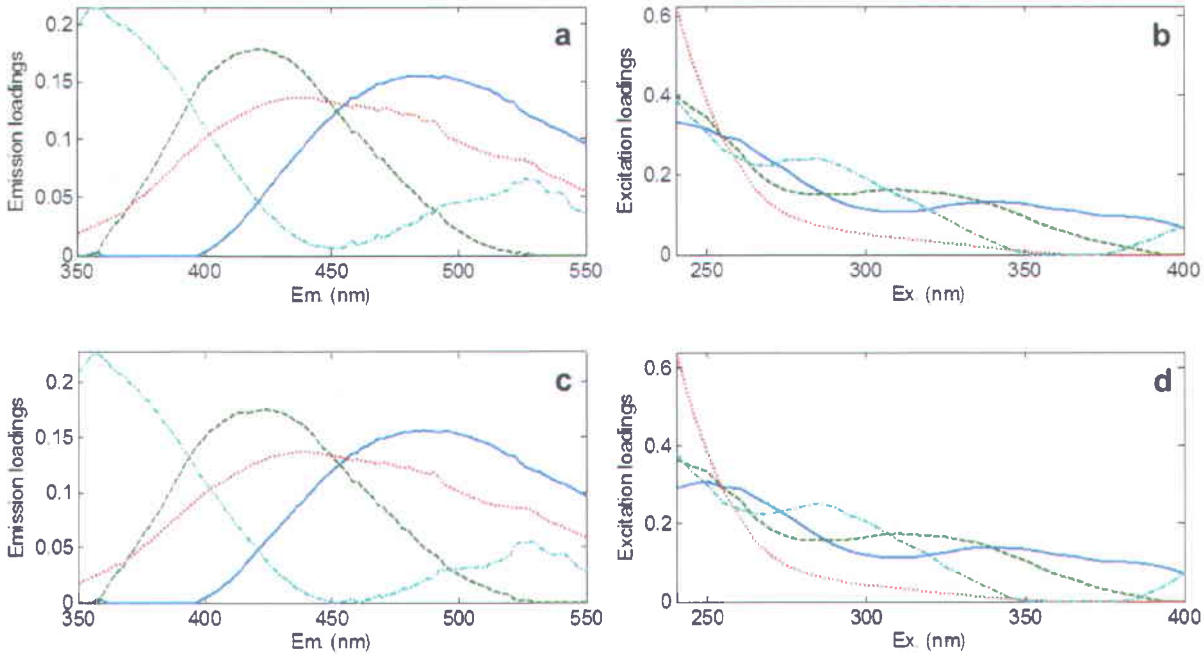

Figure S1. Split half validation of the four-component PARAFAC model. Panels a and c show the emission loadings and panels b and d show the excitation loadings of the four components for the two independent models. The solid line is for component 1, the dashed line is for component 2, the dotted line is for component 3 and the dash-dot line is for component 4.

6.2 Multivariate statistical analyses

6.2.1 Cluster Analysis

Cluster analysis was employed to identify groups of lakes with similar water quality, watershed characteristics and DOM optical properties. First, by considering water chemistry data (pH and Ca and Mg concentrations) and watershed land cover data (% surface water, % rock outcrops, % vegetation and % urban development) only, three main groups of lakes were identified (Figure S2a). All of the lakes from the Rouyn-Noranda region were grouped together (Group 3), while the lakes from the Sudbury region were separated into one group containing Lakes Whitson, Raft, Crowley, Geneva and Nelson (Group 2), and another group containing Lakes Middle, Hannah, Ramsey and Bethel (Group 1). A second cluster analysis was conducted using the DOM data only (DOC concentration, $SUVA_{254}$, FI and the relative proportions of the PARAFAC components (Figure S2b)). This second cluster analysis grouped the Rouyn-Noranda Lakes Bousquet, Joannès, D'Alembert, Dufay and Vaudray together (Group 1'). The remaining Rouyn-Noranda lakes were grouped together along with the Sudbury Lake Whitson (Group 2'). The Sudbury Lakes Raft, Geneva, Crowley, Nelson were re-grouped together with Lakes Hannah, Middle, Ramsey and Bethel (Group 3').

In the first analysis, the Sudbury Lakes Hannah, Middle, Bethel and Ramsey (Group 1, Figure S2a) were clearly distinct, reflecting the high % urban development in their watersheds, their high pH values and their high dissolved concentrations of Ca and Mg. However, if the cluster analysis was limited to the DOM optical properties, these lakes were mixed into the general Sudbury lake group (Group 3', Figure S2b). The analysis using only the DOM data identified the Rouyn-Noranda Lakes Bousquet, Joannès, Vaudray, D'Alembert and Dufay (Group 1', Figure S2b) as a distinct group. These lakes have high DOC concentrations, high $SUVA_{254}$ values and high proportions of the allochthonous fluorescence component C3. The clustering of lakes based on DOM optical properties provides additional information on the nature of lake DOM, which might otherwise have remained hidden.

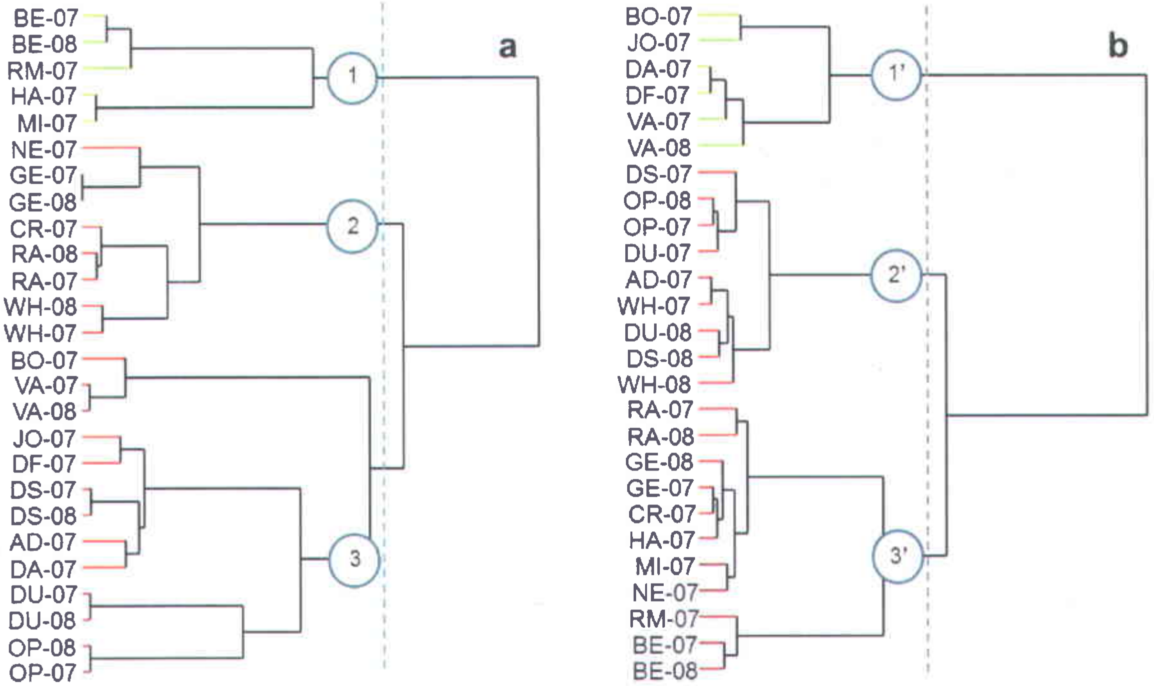

Figure S2. Dendrograms resulting from the cluster analyses. Lakes are identified by a two-letter code (see Table 1) and a two-digit year code. Results of the analysis performed using water chemistry (pH, Ca and Mg concentrations) and watershed data (% surface water, rock outcrops vegetation, and urban development) are shown in panel a). Results of the analysis using DOM properties (DOC concentration, $SUVA_{254}$, FI and the relative proportions of the PARAFAC components C1, C2, C3 and C4) are presented in panel b). The dashed line represents the level at which the clusters were established and the numbers represent the groups identified.

6.2.2 Principal Components Analysis

Principal component analysis (PCA) was employed to graphically distribute the lakes along environmental gradients (Figure S3). The first two PCA axes explained 81% of the variance in site distribution. The PCA ordination shows that the gradient of maximum variance (Axis 1) is associated with a series of environmental variables ranging from high values for DOC concentrations, the relative proportions of C3, $SUVA_{254}$ and % vegetation in quadrant I (Q-I) to high values for the relative proportions of C1, C2 and C4, as well as for % surface water and % rock outcrops in quadrant II (Q-II). The second gradient (Axis 2) spanned from high values of water chemistry variables (Ca and Mg concentrations and pH) as well as % urban development and FI in quadrant IV (Q-IV) to high values of % vegetation in quadrant II (Q-II).

The lakes were first discriminated along Axis 1, defined as an allochthonous DOM gradient spanning diagonally from Q-I (with high values for DOC concentration, the contribution from C3 and $SUVA_{254}$) to Q-III (with high contributions from C4, C2, C1, % surface water and % rock outcrops). Most of the Rouyn-Noranda lakes were found in Q-I, reflecting the importance of allochthonous sources of DOM for these lakes. In contrast, nearly all of the Sudbury lakes were found in Q-III, reflecting the lesser importance of allochthonous DOM in these lakes. On a second level, the lakes were discriminated along Axis 2, defined as an autochthonous gradient spanning from Q-IV, with high values for FI, % urban development, Mg and Ca concentration, and pH to Q-II with high % vegetation. Lakes Bethel and Ramsey were found at the top of this gradient Q-IV, indicating the importance of the autochthonous production of DOM in these lakes. This ordination graphically integrated the site distribution we achieved with the two independent cluster analyses (Figure S2a,b). General discrimination based primarily on the allochthonous gradient and secondarily on the autochthonous gradient is illustrated by the schematic in the lower right hand corner of Figure S3.

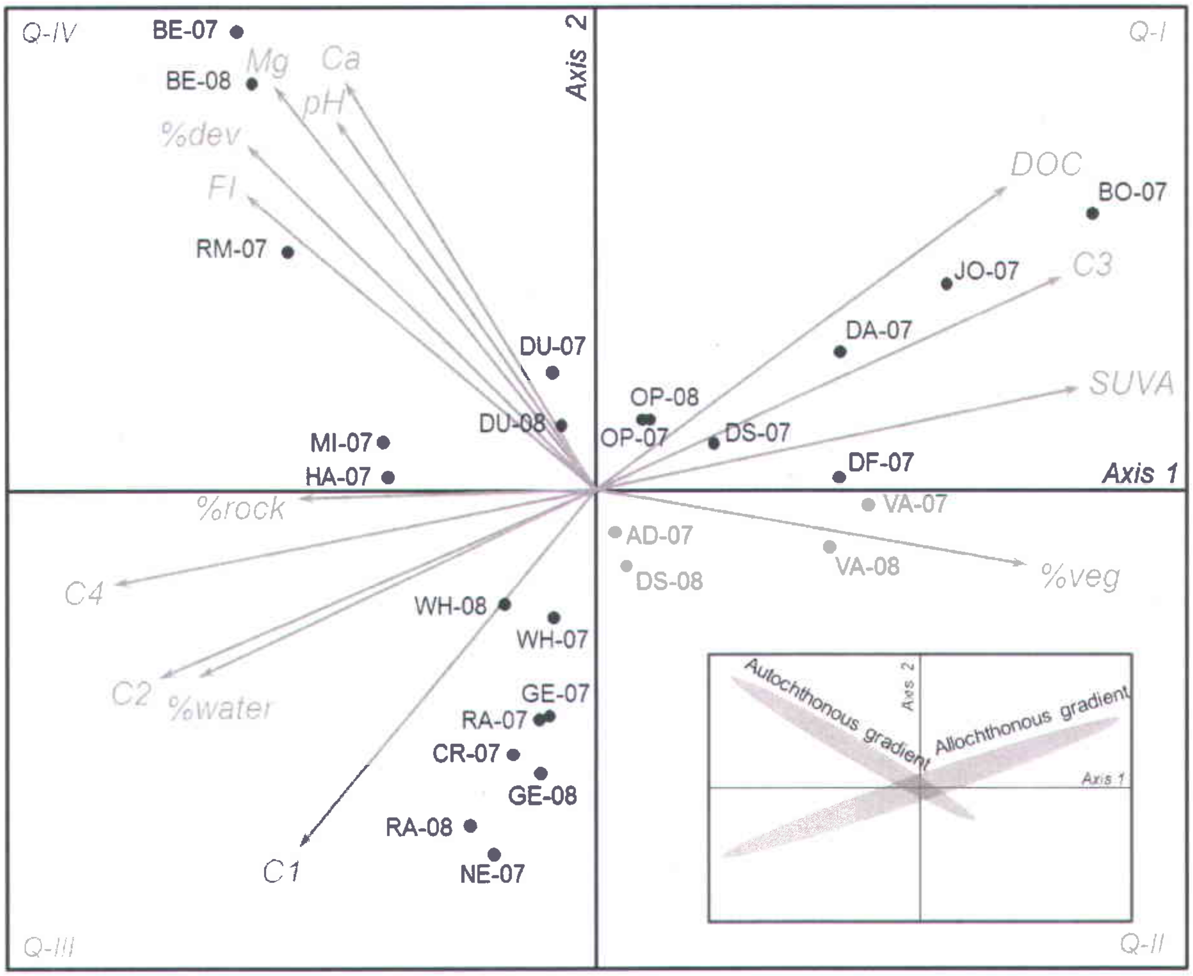

Figure S3. Principal component analysis using water chemistry (pH, Ca and Mg concentrations), watershed information (% surface water, rock outcrops, vegetation and urban development) and DOM properties (DOC concentration, SUVA, FI and the relative proportion of the PARAFAC components C1, C2, C3 and C4). The primary and secondary axes are labeled Axis 1 and Axis 2, respectively. The grey arrows represent the environmental variables contributing to the gradients and the dark circles represent the individual lake samples (identified by lake code – year sampled). Each quadrant of the figure is enumerated Q-I, Q-II, Q-III or Q-IV. The schematic in the lower right hand corner of Q-II illustrates the major environmental gradients identified.

Article 2

Trace metal speciation predictions in natural aquatic systems: incorporation of dissolved organic matter (DOM) spectroscopic quality

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Abstract

To calculate metal speciation in natural waters, modellers must choose the proportion of dissolved organic matter (DOM) that is actively involved in metal complexation, defined here as the % active fulvic acid (FA); to be able to estimate this proportion spectroscopically would be very useful. In the present study, we determined the free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations in eight Canadian Shield lakes and compared these measured concentrations to those predicted by the Windermere Humic Aqueous Model (WHAM VI). For seven of the eight lakes, the measured proportions of Cd^{2+} and Zn^{2+} fell within the range of values predicted by WHAM; the measured proportion of Cu^{2+} fell within this range for only half of the lakes sampled, whereas for Ni, WHAM systematically overestimated the proportion of Ni^{2+}. With the aim of ascribing the differences between measured and modeled metal speciation to variations in DOM quality, the % active FA needed to fit modeled to measured free metal concentrations was compared to the lake-to-lake variation in the spectroscopic quality of the DOM, as determined by absorbance and fluorescence measurements. Relationships between % active FA and DOM quality were apparent for Cd, Cu, Ni and Zn, suggesting the possibility of estimating the % active FA spectroscopically and then using this information to refine model predictions. The relationships for Ni differed markedly from those observed for the other metals, suggesting that the DOM binding sites active in Cd, Cu and Zn complexation are different from those involved in Ni complexation. To our knowledge, this is the first time that such a distinction has been resolved in natural water samples.

Running Head

Predicting metal speciation in lake waters

Environmental Context

To assess the risk posed by environmental contaminants such as metals, one needs to be able to identify the key chemical species that prevail in natural waters. One of the recognized stumbling blocks is the need to quantify the influence of heterogeneous dissolved organic matter (DOM). Here we explore the possibility of using the optical signature of DOM to determine its quality, to alleviate the need to make assumptions about its metal-binding properties, and to improve the prediction of trace metal species distributions in natural waters.

1 Introduction

Knowledge of the speciation of trace metals in natural waters is widely recognized as a key to understanding both their geochemical mobility and their ecotoxicological impacts. In natural freshwater systems, trace metals may interact with a variety of ligands, including the common inorganic anions (e.g., OH^-, HCO_3^-, CO_3^{2-}, Cl^-, F^-, SO_4^{2-}), simple monomeric organic molecules of natural or anthropogenic origin (e.g., metabolites such as citrate or glycine, or polycarboxylates such as nitrilotriacetic acid), and dissolved humic substances (fulvic and humic acids) (Batley et al., 2004). From a geochemical or ecotoxicological perspective, the key species is the free metal ion (M^{z+}), which is usually presumed to be in equilibrium with these various dissolved or particulate ligands and with the epithelial or cell surfaces of the resident aquatic organisms; it is the master species from which the concentrations of all other species of interest can in principle be calculated.

A number of approaches have been used to determine free metal ion concentrations in natural waters. For example, equilibrium ion exchange (IET) has been employed to determine Cd^{2+}, Ni^{2+} and Zn^{2+} concentrations in environmental samples (Doig and Liber, 2007; Fortin et al., 2010; Worms and Wilkinson, 2008). Various electrochemical techniques have also been used, including potentiometry with ion-selective electrodes (Rachou et al., 2007; Xue and Sunda, 1997) and cathodic stripping voltammetry (Xue and Sigg, 1993; Xue and Sunda, 1997), as has the Donnan membrane technique (Sigg et al., 2006; Weng et al., 2011). However, given the complexity of the analytical matrices in which the free metal ion is found, and the often very low ambient metal concentrations, the determination of $[M^{z+}]$ in natural waters is not a routine measurement.

A complementary approach to such analytical determinations would be the use of chemical equilibrium models to calculate the free metal ion concentration. For simple systems, containing inorganic cations and anions together with simple monomeric ligands, such an approach is reasonably straightforward. However, for waters containing natural dissolved organic matter (DOM), the chemical equilibrium calculations must deal with the challenging presence of fulvic and humic acids. These ubiquitous but poorly defined materials behave like multi-dentate ligands and poly-electrolytes in solution. Dudal and Gérard (2004) have reviewed various approaches that have been developed to account for natural organic matter in aqueous chemical equilibrium models, including two that are currently widely used: the Windermere Humic Aqueous Model (WHAM) and the Non-Ideal Competitive Adsorption (NICA) – Donnan model. In addition to fulvic and humic acids, DOM also includes biogenic molecules (amino acids, proteins, carbohydrates), the concentrations of which tend to be maintained at very low levels due to their degradation and uptake by heterotrophic microorganisms. As a result, these other components of the DOM pool normally will not affect trace element speciation (Morel and Hering, 1993). In waters affected by wastewater effluents, the DOM may also include strong chelating agents of anthropogenic origin.

Recent comparisons of free metal ion concentrations measured in natural aquatic systems with the concentrations predicted for these systems with the NICA and WHAM speciation models have demonstrated major differences between measured and predicted values for some metals, notably Cu, Ni and Pb (Fortin et al., 2010; Guthrie et al., 2005; Unsworth et al., 2006). Lofts and Tipping (2011) have discussed possible reasons for these differences, including errors in measuring the free metal ion, errors in measuring the variables that are used as input data for the model, and differences between the DOM present in the natural waters and the types of (isolated) fulvic and humic acids that have traditionally been used to calibrate or parameterize the chemical equilibrium models. In the present paper we have explored this latter factor.

One of the challenges involved in applying a chemical equilibrium model to a natural water is to estimate the fulvic and humic acid content of the water. Current speciation models that include dissolved organic matter (DOM), such as WHAM, incorporate the measured quantity of DOM, but users must define the DOM quality by choosing the percent of the DOM that is actively involved in metal complexation, and by deciding what proportions of this “active” DOM should be designated fulvic and humic acid. To be able to estimate this “% active” DOM spectroscopically would be very useful. For example, the specific ultraviolet absorbance of the DOM ($SUVA_{254}$), a measure of the aromaticity of the organic matter, has been used to estimate the proportion of DOM that is present as humic or fulvic acid in solution and that participates in the complexation of Cu in reconstituted natural water samples (Luider et al., 2004; Richards et al., 2001) and in the complexation of Cu

(Amery et al., 2008) and Cd (Cornu et al., 2009; Cornu et al., 2011) in soil extracts. A better *a priori* understanding of the quality of DOM in a given sample would alleviate the need to make assumptions about the activity of DOM and might reduce the discrepancies between predicted and measured free metal concentrations.

The purpose of this paper is thus three-fold: (i) to determine the free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations in a set of lakes located along a metal contamination gradient; (ii) to compare these measured concentrations with the free metal concentrations predicted by WHAM VI; and (iii) to explore the use of the optical signature of the DOM in each lake as a means of estimating the proportion of the natural DOM that is active in metal complexation.

2 Methodology

2.1 *Study area*

Although a detailed description of the study sites can be found in Mueller et al. (2012b); a brief description is given here. Lake water was collected from lakes in two different regions on the Canadian Shield: near Rouyn-Noranda in north-western Québec and near Sudbury in north-central Ontario. Both study areas have been greatly impacted by metal mining and smelting activities, particularly by atmospheric deposition of acid and metals (Borgmann et al., 1998; Dixit et al., 2007). The pH of the lakes tends to decrease and total metal concentrations tend to increase with a decrease in the down-wind distance from the metal smelters, particularly in the Sudbury area (Yan and Miller, 1984). Acid mine drainage from point sources, such as abandoned mines or mineralized outcrops, may also decrease the pH of lake water locally. In each region, lakes were chosen to represent a gradient in water quality (pH, dissolved organic carbon (DOC)) and trace metal concentrations.

2.2 *Lake water sampling*

Lake water samples were taken from four lakes in each of the study areas during July and August of 2008. Sampling sites were chosen in the littoral zones of small, well mixed lakes and the sampling sites were considered to be representative of the whole lake epilimnion. The spatial variability of water chemistry (i.e., pH and concentrations of DOC and major cations) is known to be minimal within many of the sampled lakes (Fortin et al., 2010).

Lake water was collected passively using equilibrium diffusion sampling as described in Fortin et al. (2010) and Mueller et al. (Mueller et al., 2012b). Briefly, 250-mL polypropylene jars (Nalgene, Nalge Nunc International Corporation, Rochester, NY) topped with custom-made plastic lids fitted with a 0.2-µm filter membrane (HT Tuffryn Membrane, Pall Canada Ltd, Ville St-Laurent, QC, Canada) were filled with Milli-Q (Millipore, EMD Millipore, Billerica, MA) ultrapure water (>18 Mohms·cm) and fixed to plastic rods that were anchored to the lake bottom. The samplers were installed at a depth of 1 m from the lake surface and left to equilibrate for 13 to 14 days. Following this equilibration period, the samplers were brought to the surface, the membrane surface rinsed with ultrapure water, the membranes pierced with a clean plastic pipette tip and the custom jar lids replaced with clean polypropylene screw lids. The sample jars were bagged and stored in the dark at 4°C in field coolers for a maximum of 12 days until they were transported back to the laboratory. Note that lake water pH, conductivity and temperature were measured in the field at the time of sample collection.

2.3 Sample analysis

All plastic laboratory and sampling equipment was soaked in 10% (v/v) nitric acid for at least 24 h and then rinsed a minimum of six times with ultrapure water and dried under a Class 100 laminar flow hood. The only exception was for the polystyrene vials used for storing the subsamples for anion analyses; these vials were rinsed three times with ultrapure water only. All glassware was soaked in a 2 N HCl solution before being rinsed with ultrapure water.

Once the polypropylene jars had arrived in the central laboratory, they were opened in a clean Class 100 laminar flow hood, and subsamples were collected as follows. A 10-mL subsample was transferred to a polypropylene vial and acidified to 2% (v/v) with concentrated ultra-trace nitric acid (BDH Aristar Ultra, VWR International, Mississauga, ON, Canada) for analysis of major cations (Ca, Mg) by atomic emission spectroscopy (ICP-OES, Varian Vista AX CCD, Agilent Technologies Canada Inc., Mississauga, ON) and total metals (Cd, Cu, Ni and Zn) by inductively coupled plasma mass spectrometry (ICP-MS, Element X Series, Thermo Scientific, Mississauga, ON, Canada). A second 10-mL subsample was transferred to a polystyrene vial for the determination of anion concentrations (F^-, Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-}) by ion chromatography (Dionex ICS-2000, Thermo Scientific).

Details concerning the quantitative and qualitative characterization of dissolved organic matter (DOM) can be found in Mueller et al. (Mueller et al., 2012b). Briefly, 20-mL subsamples were transferred to amber borosilicate vials (I-Chem Brand) for total organic carbon analysis (TOC-V_{CPH}, Shimadzu Scientific Instruments, Columbia, MD). Subsamples (1 mL) were transferred to 3-mL glass vacutainers (Kendall Monoject, Mansfield, MA) for total inorganic carbon analysis by gas chromatography (Varian 3800 GC, Agilent Technologies Canada Inc.). The absorption (Varian UV-Vis Cary 300 Spectrophotometer, Agilent Technologies Canada Inc.) and fluorescence (Varian Cary Eclipse Fluorescence Spectrophotometer, Agilent Technologies Canada Inc.) spectra of lake water DOM were also measured. The specific UV absorbance (SUVA, L m^{-1} mg C^{-1}) was also calculated by dividing the measured absorbance at 254 nm by the concentration of DOC (Weishaar et al., 2003). Excitation-emission matrix (EEM) fluorescence spectra were measured using 1-cm quartz cuvettes at excitation wavelengths from 210 to 400 nm at increments of 5 nm and at emission wavelengths from 300 to 580 nm at increments of 2 nm. The fluorescence index was calculated as the ratio between the fluorescence emission intensity at 470 nm over that at 520 nm for an excitation at 370 nm (Cory et al., 2010; McKnight et al., 2001). Finally, the multivariable statistical tool, parallel factor analysis (PARAFAC), was employed to deconvolute the EEM spectra using the procedure outlined by Stedmon and Bro (2008) as implemented in a MATLAB environment, using the "N-way toolbox for MATLAB" (Andersson and Bro, 2000).

2.4 Metal speciation measurements

All sub-samples for metal speciation measurements were filtered (0.2-µm polycarbonate filter; AMD Manufacturing Inc., Mississauga, ON) under a laminar flow hood before analysis. A precipitate was observed in a few samples, which prompted a filtration step for all samples just prior to the metal speciation analyses. WHAM metal speciation simulations were performed using the element concentrations measured on these filtered samples. All calibration solutions were prepared using ultrapure water. Stock solutions of Cd, Cu, Ni and Zn were prepared from ICP-MS standard solutions (4% HNO_3, PlasmaCAL, SPC Science, Baie D'Urfé, QC, Canada). To take into account the variable natural conditions of each lake sampled, e.g. pH, ionic strength and total metal concentrations, a lake-specific limit of quantification (LOQ) was calculated for the measurement of the free metal ion for each lake, based on the standard deviation of triplicate measurements. When the free metal value for a lake fell below its LOQ, it was discarded and the free metal ion was not reported.

2.4.1 Ion exchange technique (IET)

The concentrations of Cd^{2+}, Ni^{2+} and Zn^{2+} were measured following the methods described by Fortin and Campbell (1998) and updated by Fortin et al. (2010). Briefly, the ion-exchange technique involves the equilibration of the free metal ions in an unknown sample with a cation exchange resin (Dowex mesh, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) that has been calibrated with standard solutions according to the following reaction,

$$M^{2+} + (3-x)\cdot R_xCat \overset{K_{IE}^c}{\leftrightarrow} R_2M + (3-x)\cdot Cat^{x+} \tag{1}$$

where M^{2+} is the free metal ion, R is the resin, R_xCat is the concentration of resin binding sites filled by a mono- or divalent cation, x is the charge on the cation and R_2M is the concentration of resin binding sites filled by the metal ion of interest. The conditional equilibrium constant, K_{IE}^c, for the above reaction is calculated using the following equation,

$$K_{IE}^c = \frac{[R_2M]\cdot[Cat^{x+}]^{3-x}}{[M^{2+}]\cdot[R_xCat]^{3-x}} \tag{2}$$

Under swamping electrolyte conditions, $\lfloor Cat^{x+} \rfloor$ and $[R_xCat]$ are assumed to remain constant and equation 2 is rearranged to give the resin distribution coefficient at fixed ionic strength and pH, $\lambda_{o,i,pH}$ ($L\cdot g^{-1}$).

$$\lambda_{o,i,pH} = K_{IE}^c \cdot \frac{[Cat^{x+}]^{3-x}}{[R_xCat]^{3-x}} = \frac{[R_2M]}{[M^{2+}]} \tag{3}$$

The resin was calibrated with standard solutions with a pH range of 5.3 to 8.9 and with known concentrations of free Cd^{2+} (0.24 to 42 nM), Ni^{2+} (13 to 202 nM) and Zn^{2+} (4.9 to 148 nM) and the resin distribution coefficient (λ) was calculated for each metal before applying the method to the natural unknown samples (see Supplementary Material, Figure S1).

Experimentally, the resin was pre-equilibrated with a matrix solution that had an ionic strength ($Ca(NO_3)_2$ as the swamping electrolyte) and pH similar to the calibrating solution and the natural samples; a sufficient volume from a 25 mM $Ca(NO_3)_2$ (99.0%, Sigma-Aldrich Canada Ltd.) stock solution was also added to each natural sample so as to obtain a constant Ca concentration of 0.46 mM in all natural samples. By levelling the ionic strength of the natural samples, we ensured that the time needed to reach equilibrium between the resin and the sample was similar for all samples and that a single metal-specific distribution coefficient could be used. After a steady state had been reached between the resin and the sample (190 mL), the resin was rinsed with ultra-pure water and eluted with a volume (V, 6 mL) of 10% (v/v) ultra-trace nitric acid (BDH Aristar Ultra, VWR International). The amount of free metal originally bound to the resin at steady state (R_2M) was calculated using the following equation,

$$[R_2M] = \frac{[M_{Eluate}] \cdot V}{m_r} \tag{4}$$

where $[M_{Eluate}]$ is the concentration of metal measured in the eluate and m_r is the mass of the resin used (approximately 7 mg; weighed precisely). Finally, by combining equations 3 and 4, the free metal concentration in the sample was calculated using equation 5.

$$[M^{2+}] = \frac{[M_{Eluate}] \cdot V}{\lambda_{o,i,pH} \cdot m_r} \tag{5}$$

2.4.2 Cupric ion selective electrode (Cu ISE)

Because the IET tends to overestimate free Cu^{2+} when the lake pH is greater than 6.5 (Fortin et al., 2010), we determined Cu^{2+} in the lake samples using a cupric ion selective electrode (Cu ISE, Orion, Thermo Scientific) following the methods outlined by Rachou et al. (2007). The Cu ISE was calibrated daily with a solution containing 0.1 mM copper nitrate ($Cu(NO_3)_2$, PlasmaCAL, SPC Science), 1 mM iminodiacetic acid (IDA, Sigma-Aldrich Canada Ltd.), 2.5 mM potassium hydrogen phthalate ($KHC_8H_4O_4$, 99.95%, Sigma-Aldrich Canada Ltd.), 10 mM potassium nitrate (KNO_3, 99.995%, Fluka, Sigma-Aldrich Canada Ltd.) and 6 mM sodium hydroxide (NaOH, 98%, Sigma-Aldrich Canada Ltd.). The calibration solution was split into 5 to 11 subsamples with pH values varying from 2 to 11, adjusted by the addition small known amounts of either HNO_3 or NaOH, and allowed to equilibrate overnight. The free Cu^{2+} concentration calculated ranged from the method detection limit (three times SD of six replicate blank measurements) of 2.6×10^{-11} to 6.3×10^{-5} M. The calibration subsamples with pH values above 6 were bubbled with N_2 gas, to avoid a decrease in pH with the dissolution of atmospheric carbon dioxide, CO_2 (aq). The pH and temperature of samples were monitored using an Orion (Orion, Thermo Scientific) electrode and probe, respectively. During Cu ISE measurements, constant stirring and temperature conditions were also maintained. The calibrated response of the Cu ISE electrode was determined from the plot of the measured ISE potential against the calculated pCu for each calibration subsample. For the natural water samples, the concentration of Cu^{2+} was then calculated using the Cu ISE calibration equation and the measured electrode potential. The natural samples were amended with KNO_3 (99.995%, Fluka, Sigma-Aldrich Canada Ltd.; a specific volume of a 1.0 M stock solution was added to achieve a final concentration of 10 mM KNO_3) to ensure a constant ionic strength among the natural samples.

2.5 Metal speciation calculations

The free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations measured for each lake were compared to values calculated using the Windermere Humic Aqueous Model (WHAM, model version 6.1) (Tipping, 1998). Several assumptions were made in the application of the model to the lake water samples. First, we assumed that the DOC:DOM ratio was 2 (Buffle, 1988), that 65% of the DOM was active in the complexation of metals (Bryan et al., 2002), and that this active fraction was composed of fulvic acids only. We also assumed that both Fe(III) and Al(III) activities are controlled by the solubility of their hydroxides, as calculated using the empirical equations given by Lofts et al. (2008) and Tipping (2005), respectively. Other measured WHAM input parameters included pH and the total concentrations of Na, Mg, K, Ca, Cr, Co, Ni, Cu, Zn, Cd, Cl, NO_3, SO_4, CO_3 (measured as total inorganic carbon) and F. The measured surface temperature of the lakes sampled (used in the WHAM simulations) varied between 20 and 23 degrees Celsius. Separate WHAM simulations were run for lake samples analysed using the IET free ion measurement method (for the analysis of free Cd^{2+}, Ni^{2+} and Zn^{2+}) and for those analysed with the ISE method (for the analysis of free Cu^{2+}); the input data for these simulations corresponded exactly to the composition of the samples used for the free metal ion analyses, thus accounting for the differences in their ionic composition (additions of $Ca(NO_3)_2$ and KNO_3, respectively).

2.6 Statistical analysis

All statistical analyses were calculated at the alpha equals 0.05 confidence level using *SigmaPlot 11.0* (Systat Software Inc., San Jose, CA). Parametric t-tests and linear and non-linear regression were used when the normality and homogeneity of variances among datasets were confirmed; otherwise, the appropriate non-parametric tests were employed (Mann-Whitney Rank Sum test, in place of the t-test. Note that for the regression equations generated between the WHAM calculated percent fulvic acid active in metal complexation ($\%aFA_{optimized}$) and the relative contribution of PARAFAC fluorescence components 1 and 3 to the overall fluorescence spectrum of lake DOM (see section 3.4), the assumptions of normality and homogeneity of variance were confirmed. The only exception was for Ni, where the variance among the data sets was not constant (*P*=0.01).

3 Results and Discussion

3.1 Water quality

The speciation of dissolved metals in freshwater systems is sensitive to the concentration of DOM, which tends to complex free metal ions, but may also be affected by other water quality variables, notably the concentrations of Ca, Mg, Al, Fe and the H^+-ion, these being potential competitors for the cation binding sites on the DOM (Tipping et al., 2002). In addition, for a water of given composition, the total dissolved concentration of the metal may affect its speciation (i.e., a limited number of high affinity binding sites). In the following two subsections we describe how the water chemistry varied among the studied lakes.

3.2 Background water chemistry

The water quality (i.e. [major cations], pH) of the lakes was described in Mueller et al. (2012b) for 2007 and 2008. Only the 2008 data are considered here, since the metal speciation measurements date from that year. Wide ranges in general water chemistry (pH, Ca, Mg and DOC concentrations) were found among the eight lakes sampled (Table 1), reflecting both regional geological differences and local differences at the watershed scale (Mueller et al., 2012b). The lakes in both sampling regions have low concentrations of calcium (Ca: 68 to 464 μM) and magnesium (Mg: 27 to 274 μM), contributing to generally soft water. Lake Bethel, from the Sudbury region, had the highest pH and the highest Ca and Mg concentrations of all the lakes; for the IET measurements, all other lake water samples were thus adjusted upwards to these Ca concentrations (see section 2.4.1).

Lakes from the Rouyn-Noranda region are circumneutral, with a mean pH of 7.25 and a narrow range of values between pH 6.81 and 7.69. The mean pH for the Sudbury lakes (7.07) is slightly lower than that of the Rouyn-Noranda lakes and pH values span a wider range (6.40 to 8.08). Historically, the Rouyn-Noranda lakes were less affected by anthropogenic acidification than were those in the Sudbury area (Dupont, 1992), despite massive SO_2 emissions from the Horne smelter; the Rouyn-Noranda lakes are located in the Abitibi clay belt and are effectively better buffered against acid deposition. Although the average circumneutral pH of the lakes in the Sudbury regions is evidence of the recovery of these lakes from historical acidification in recent years (Keller et al., 2007), pH values in the lakes of this region spanned a wider range than in the Rouyn-Noranda lakes.

Based on their historical chlorophyll-a concentrations (1-4 μg chl-a L^{-1}; Campbell, unpublished data), the four studied lakes in the Rouyn-Noranda region are oligotrophic, as are Lakes Raft and Whitson in the Greater Sudbury area (2-3 μg chl-a L^{-1}; http://www.greatersudbury.ca/cms/index.cfm?app=div_lakewaterquality&lang=en&currID=619, accessed 7 August 2012). We were unable to find comparable data for Lake Geneva, which lies outside the Greater Sudbury area, but given the lack of development in its watershed, it is also likely oligotrophic. The only exception is Lake Bethel, which is much more productive (12-24 μg chl-a L^{-1}; http://www.greatersudbury.ca/cms/index.cfm?app=div_lakewaterquality&lang=en&currID=619, accessed 7 August 2012) and can be classified as eutrophic.

3.3 Dissolved organic carbon

The mean DOC concentration of Rouyn-Noranda lakes was higher (6.1 mg C L^{-1}) and spanned a wider range (4.9 to 8.1 mg C L^{-1}) than in the lakes from the Sudbury region (mean of 3.4 mg L^{-1}; range from 2.0 to 4.9 mg L^{-1}). The higher DOC concentration in the lakes in the Rouyn-Noranda region than Sudbury lakes reflects the greater soil cover and the higher watershed-to-lake area ratios for the Rouyn-Noranda lakes. As described in Mueller et al. (2012b), the quantity and the quality of DOM in the lakes sampled vary as a function of regional watershed characteristics and as well as DOM processing within the watershed and within the lake itself.

DOM optical quality ($SUVA_{254}$ and Fluorescence Index) was found to differ significantly between sampling regions and among lakes (see Table S1 in the Supplementary Material for more details) and is described elsewhere (Mueller et al., 2012b). The authors also used parallel factor analysis (PARAFAC) to extract fluorescence components from excitation-emission matrix (EEM) fluorescence spectra measured for 19 lakes in the Rouyn-Noranda and Sudbury regions, including the eight lakes retained for the present study. Of particular interest were the humic-like fluorescence components of ubiquitous origin (C1) and allochthonous origin (C3). Component C1 corresponds to a reduced quinone fluorophore associated with higher plant matter, whereas C3 corresponds to an oxidized quinone fluorophore (Mueller et al., 2012b). We used the proportion of each of these DOM fluorescence components (relative to the sum of all four PARAFAC fluorescence components, i.e., $C1/C_T$ or $C3/C_T$) as measures of the quality of DOM sampled in our lakes, and in particular as proxies for the fraction of DOM active in the complexation of trace elements (section 3.3.2).

3.4 Lake-to-lake variations in metal concentrations and metal speciation

3.4.1 Total dissolved metal ion concentrations

The total dissolved metal concentrations (Cd, Cu, Ni, Zn) varied markedly among the eight lakes sampled (Table 1). For lakes in the Rouyn-Noranda region, the concentration gradient ratios (i.e., the ratio of the maximum metal concentration ÷ minimum metal concentration, $[M]_{max}/[M]_{min}$) decreased in the sequence Cd (33) ~ Zn (31) > Cu (7) > Ni (2.5). In the Sudbury region, the $[M]_{max}/[M]_{min}$ ratios decreased in the order Ni (63) > Cd (31) > Cu (16) > Zn (5). Lakes in the Rouyn-

Noranda region had significantly higher mean total concentrations of Cd (*P*=0.004) and Zn (*P*=0.030) than the lakes in the Sudbury area, but lower concentrations of Ni (*P*<0.001). No significant difference in total dissolved Cu was found between the sampling regions.

The lake-to-lake variability in the measured total dissolved metal concentrations within a given region is largely due to variations in atmospheric loading from the local mining and smelting activities (Borgmann et al., 1998; Couillard et al., 2004). Despite the marked reductions in smelter emissions that have been achieved over the past 30 years, lakes in close proximity to and down-wind from the metal smelters (e.g., Lakes Dufault and Dasserat in the Rouyn-Noranda region, Lakes Raft and Whitson in the Sudbury area) still have considerably higher total dissolved metal concentrations than do those located upwind or far downwind.

3.4.2 Free metal ion concentrations

The free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations also varied among lakes (Table 1). The ratio of $[M^{2+}]_{max}/[M^{2+}]_{min}$ for the Rouyn-Noranda lakes decreased as follows: Zn (121) > Cd (54) > Cu (5.5). Free Ni^{2+} concentrations were only measurable in one replicate sample from one lake for the Rouyn-Noranda region and, therefore, no concentration gradient ratio was calculated. The ratio of $[M^{2+}]_{max}/[M^{2+}]_{min}$ for the Sudbury lakes decreased in the sequence Ni (136) > Cu (64) > Cd (40) > Zn (22). As anticipated, lakes that had the highest concentrations of dissolved metal also exhibited the highest free metal ion concentrations.

Table 1. Measured pH and mean (± SD, n=3) dissolved organic carbon (DOC), total dissolved concentrations of major cations and trace metals, as well as measured free metal concentrations and % free metal values for lakes sampled from Rouyn-Noranda (QC) and Sudbury (ON) in 2008. * indicates only one sample above limit of quantification (LOQ; calculated as three times the standard deviation of triplicate analyses of each lake). NA indicates no samples above the LOQ.

		Rouyn-Noranda region lakes				Sudbury region lakes			
		Dasserat (DS)	Dufault (DU)	Opasatica (OP)	Vaudray (VA)	Bethel (be)	Geneva (ge)	Raft (ra)	Whitson (wh)
Measured	pH	6.92	7.59	7.69	6.81	8.08	6.9	6.4	6.9
	DOC (mg·L^{-1})	4.85 ± 0.03	5.2 ± 0.1	6.3 ± 0.1	8.10 ± 0.08	4.89 ± 0.03	2.9 ± 0.1	2.03 ± 0.03	3.77 ± 0.05
	Ca (μM)	207 ± 1	404 ± 3	218 ± 2	83 ± 1	464 ± 3	68 ± 3	81 ± 2	143 ± 1
	Mg (μM)	93.7 ± 0.3	118 ± 1	114 ± 1	37.1 ± 0.6	274 ± 3	26 ± 1	46 ± 1	78.4 ± 0.4
	Cd (nM)	0.8 ± 0.2	2.0 ± 0.2	0.06 ± 0.01	0.608 ± 0.005	0.018 ± 0.009	0.021 ± 0.005	0.56 ± 0.02	0.51 ± 0.04
	Cu (nM)	112 ± 3	246 ± 3	35.1 ± 0.2	43 ± 1	24.9 ± 0.1	10.5 ± 0.6	86.0 ± 0.9	165 ± 3
	Ni (nM)	8 ± 2	5.1 ± 0.3	12.5 ± 0.4	12 ± 1	226 ± 2	18 ± 2	775 ± 41	1136 ± 54
	Zn (nM)	464 ± 51	342 ± 60	15 ± 4	52 ± 5	12 ± 3	21 ± 8	62 ± 22	52 ± 2
	Cd^{2+} (nM)	0.5 ± 0.2	1.14 ± 0.06	0.021 ± 0.008	0.26 ± 0.02	NA	0.01*	0.402 ± 0.004	0.31 ± 0.05
	Cu^{2+} (nM)	0.37 ± 0.04	0.44 ± 0.09	0.08 ± 0.01	0.20 ± 0.08	0.05 ± 0.01	0.48 ± 0.09	3.2 ± 0.5	1.0 ± 0.1
	Ni^{2+} (nM)	0.6*	NA	NA	NA	7.3 ± 0.9	4.0 ± 0.7	545 ± 9	489 ± 72
	Zn^{2+} (nM)	279 ± 32	188 ± 23	2.3 ± 0.9	31 ± 18	2.3 ± 0.4	10.7 ± 0.9	51 ± 16	32 ± 4
	% Cd^{2+}	63 ± 36	58 ± 5	37 ± 17	42 ± 4	NA	70	71 ± 2	61 ± 10
	% Cu^{2+}	0.32 ± 0.04	0.17 ± 0.04	0.21 ± 0.04	0.47 ± 0.2	0.20 ± 0.05	4.6 ± 0.9	3.7 ± 0.6	0.57 ± 0.08
	% Ni^{2+}	8	NA	NA	NA	3.2 ± 0.4	22 ± 5	70 ± 4	43 ± 7
	% Zn^{2+}	60 ± 10	55 ± 12	15 ± 7	60 ± 36	20 ± 6	51 ± 20	82 ± 39	62 ± 8

In the present context, where we are comparing measured and predicted metal speciation in different lakes, it is important to consider the relative proportion of the free metal ion in each lake, as this measure takes into account the differences in total dissolved metal concentrations among lakes. As anticipated, copper was much more strongly complexed than the other three metals – the mean percent free metal for copper was only 1.3%, whereas the mean percentages for Cd^{2+} (54%), Ni^{2+} (29%) and Zn^{2+} (50%) were much higher (Table 1). For all four metals, and in both the Rouyn-Noranda and Sudbury regions, the percent free metal tended to decrease as the pH increased, pointing to complexation competition by protons as an important factor affecting metal speciation. A general decline in the percent free metal was also observed for Cd, Cu and Ni as the DOC concentration increased, presumably reflecting an increase in the ligand-to-metal ratio (see Figs. S3 and S4 in the Supplementary Material for these pH and DOC relationships).

3.4.3 Comparison of the observed metal speciation with literature values

In comparing metal speciation in different freshwater environments, the reported free metal ion concentrations are not particularly useful, since they will vary according to the metal loadings affecting the water body. Comparisons of free metal ion proportions are a more meaningful way of evaluating how metal speciation varies from one lake or river to another. In Table 2, we have compiled free metal ion percentages that have been reported in the literature for Cd, Cu, Ni and Zn (the recent review paper by Lofts and Tipping (2011) was used as a starting point to identify papers where free metal ion concentrations for natural waters had been reported). Table 2 cannot claim to be comprehensive, as the representation of surface waters from Switzerland and Canada is abnormally high, but the compilation does offer some insight into metal-to-metal differences in metal speciation.

Copper is the metal for which the Table 2 database is the most extensive and it is also consistently the most strongly complexed metal, followed by Ni > Cd ≥ Zn. For a given metal, however, there are distinct regional differences; the percent free metal is virtually always lower in the alkaline Swiss lakes than in more acidic and more dilute lakes on the Canadian Precambrian Shield. On a relative scale, this difference is particularly notable for Cu^{2+}, where the percentage can be less than 0.001% in many of the Swiss lakes but consistently above 1% in the Canadian surface waters. Factors contributing to this trend include the aforementioned differences is pH and alkalinity (carbonate and hydroxide complexation), but also the metal loadings. Total dissolved metal concentrations in the Canadian lakes are much higher than in the Swiss surface waters (Table 2), presumably reflecting inputs from the mining and smelting activities that are prevalent on the Canadian Shield. Higher total metal concentrations, and the resulting higher metal:DOM ratios, would be expected to lead to lower degrees of complexation.

3.5 Lake-to-lake variations in predicted metal speciation and comparison with measured values

3.5.1 Initial WHAM simulations

Free metal ion concentrations. The WHAM VI chemical equilibrium model has been described in detail by Tipping (2002; Tipping et al., 2011). In comparisons with its predecessor (Model V), Model VI does a better job of simulating metal binding to fulvic and humic acids, particularly at low metal concentrations, for low [M]/DOC ratios, and for metals that tend to bind to the strong metal-binding sites (putative N and S functional groups) that were expressly introduced into Model VI. In brief, fulvic and humic acids are assumed to be rigid spheres of uniform size (radii of 0.8 and 1.7 nm, respectively) with two classes of ion-binding groups positioned randomly on their surface: type A sites correspond to monoprotic carboxylic acid groups, whereas type B sites are modeled as weaker (e.g. phenolic) acid groups. Metal ions and their first hydrolysis products ($MOH^{(n-1)+}$) compete with each other, and with protons, for the type A and type B groups. Bi-dentate metal-binding sites are generated by combining pairs of proton-binding sites (A-A or A-B combinations). Only proton-binding sites that are sufficiently close to each other are able to form bi-dentate sites (≤ 0.45 nm apart; estimated statistically). Similar rules are used to generate tri-dentate metal-binding sites.

Table 2. Free metal ion percentages in various natural water samples.

Metal	Sample description	n	Total dissolved [M] (nM)	Percent free metal ion Mean	SD	Reference
Cd	Canadian Shield	7	0.06-2.0	54	13	(this study)
	Canadian Shield	13	0.07-9.3	72	11	Fortin et al.(2010)
	Canadian Shield	4	0.9-8.0	20	16	Guthrie et al.(2005)
	Swiss lake, UK river	4	0.07-0.20	3.7	3.5	Sigg et al.(2006)
	Swiss lake, UK river	5	0.06-0.87	4.9	3.5	Kalis et al.(2006)
Ni	Canadian Shield	5	8-1140	29	28	(this study)
	Canadian Shield	6	490-1760	11	6	Gopalapillai et al. (2008)
	Swiss lake, UK river	4	6-20	6.6	1.4	Sigg et al.(2006)
	Swiss lake, UK river	7	6-59	3.0	1.4	Kalis et al.(2006)
	Belgian surface	6	44-75	22	16	Van Laer et al.(2006)
Cu	Canadian Shield	8	11-246	1.3	1.8	(this study)
	Canadian Shield	2	42-150	1-53[a]	-	Guthrie et al.(2005)
	Swiss lake, UK river	4	19-30	0.25	0.12	Sigg et al.(2006)
	Swiss lake, UK river	7	13-76	0.21	0.22	Kalis et al.(2006)
	Swiss lakes (neutral)	25	4-17	<0.001[b]	-	Xue and Sigg(1996)
	Swiss lakes (acidic)	5	3-71	4	5	Xue and Sigg(1996)
	Swiss rivers	20	10-68	<0.001[b]	-	Xue and Sigg(1996)
	Swiss lake	2	65-71	0.3	0.2	Xue and Sunda(1997)
Zn	Canadian Shield	8	12-464	50	23	(this study)
	Canadian Shield	10	10-2010	86	15	Fortin et al.(2010)
	Canadian Shield	4	46-1300	25	4	Guthrie et al.(2005)
	Swiss lake, UK river	4	28-282	10	6	Kalis et al.(2006)
	Swiss lakes	6	11-25	8	1	Xue and Sigg(1994)

[a] Concentration range (N=2) rather than the mean value. [b] These very low values were obtained by competitive ligand exchange and cathodic stripping voltammetry. Lofts and Tipping (2011) have recently suggested that this technique may underestimate free Cu^{2+} concentrations.

When using the WHAM model to predict the free metal concentrations in the studied lakes, we initially assumed that the components of the DOC pool that participate in metal complexation reactions could be represented entirely by fulvic acid (100% FA, 0% HA), and that this FA portion corresponds to 65% of the DOC; these choices were based on the recommendation of Bryan et al.(Bryan et al., 2002) for copper complexation in natural waters. Good agreement was observed between predicted and measured values of [Cd^{2+}] (Figure 1a), over a 100-fold concentration range (0.01 to 1.1 nM). Agreement for [Zn^{2+}] was also reasonable (Figure 1d), over a similar 100-fold concentration range but for free Zn^{2+} concentrations (2 to 280 nM) that were considerably higher than for Cd^{2+}. In the least contaminated lakes (Geneva, Bethel and notably Opasatica), the predicted free Zn^{2+} concentrations were somewhat higher than the measured values, suggesting that WHAM VI may underestimate Zn complexation at low dissolved Zn:DOC molar ratios.

At high free Cu^{2+} concentrations (> 0.4 nM) our ISE measurements and the free Cu^{2+} values predicted by WHAM VI were in very good agreement (Figure 1b), but below this threshold, in Lakes Vaudray, Geneva, Opasatica and Bethel, our measured values were 4 to 22 times higher than the predicted values. In their study of Cu speciation in three natural waters with similar low total dissolved Cu concentrations (19-30 nM), Unsworth et al. (2006) also reported measured values of Cu^{2+} that exceeded WHAM VI predictions; in their study, free Cu^{2+} as estimated by two independent techniques (Donnan membrane; Hollow-fibre permeable liquid membrane) was 1 to 3 orders of magnitude higher than the WHAM VI predictions.

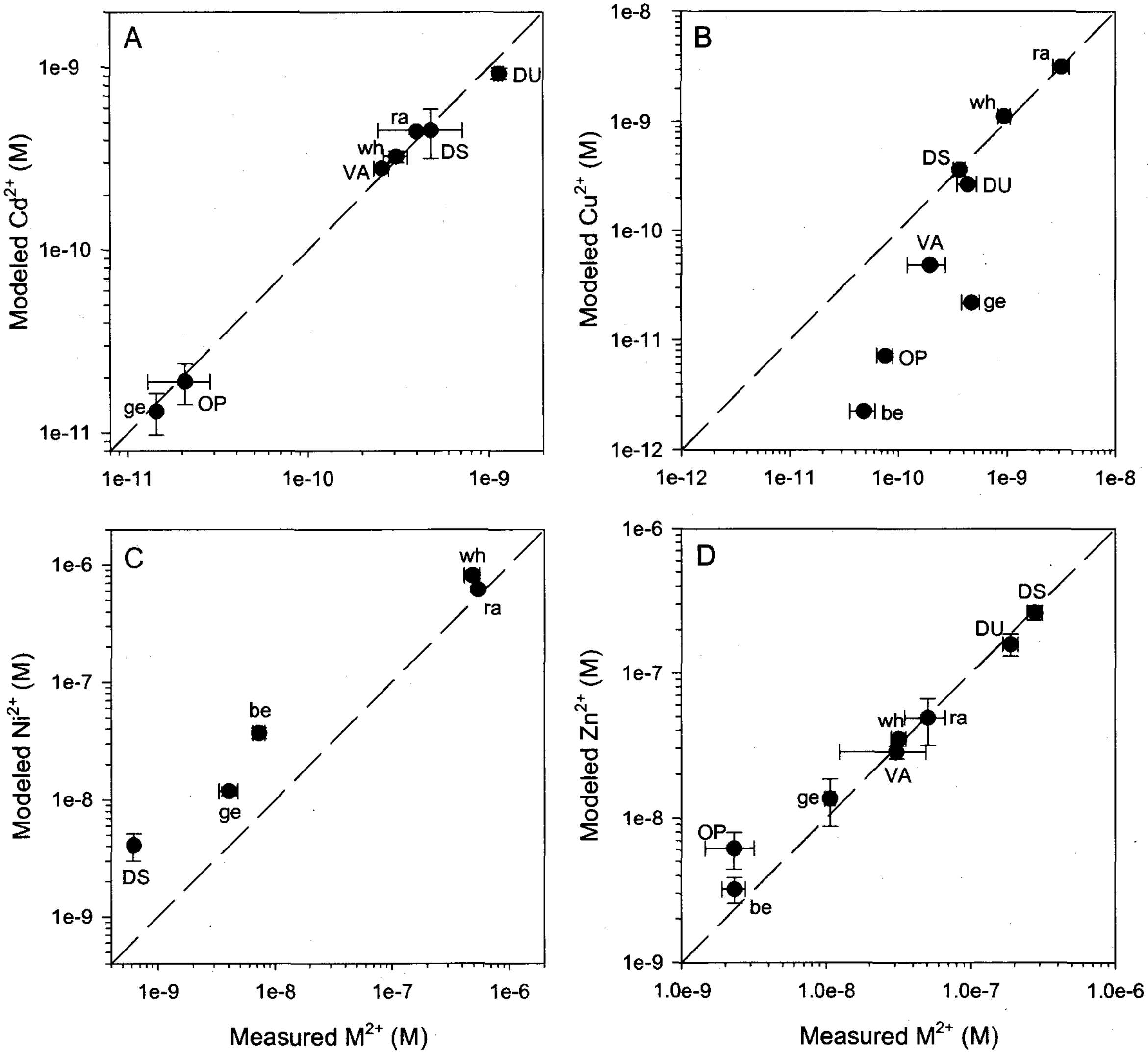

Figure 1. Comparison of mean (± SD, n=3) concentrations of the free metal ion [M^{Z+}] calculated with WHAM (with the 65% aFA assumption) and those measured using IET (Cd, Ni and Zn) and ISE (Cu). The dashed line represents the 1:1 line.

The generic constants used in WHAM VI have been generated from laboratory titrations of isolated humic and fulvic acids. Copper/DOC ratios in these titrations are very often higher than those encountered in natural water samples, and thus one might have expected calculations with the generic constants to underestimate copper complexation. However, we observe the opposite result (overestimation of Cu complexation, as was the case for Unsworth et al. (2006)). Clearly more experiments to determine the conditional equilibrium constants for the complexation of Cu to DOM should be carried out under conditions approaching those found in natural waters (e.g., low Cu/DOC ratios; natural rather than isolated organic matter).

In our experiments, but not in those of Unsworth et al. (2006) there is a second possible explanation of an analytical nature for the discrepancy between measured and modeled free Cu^{2+} concentrations. In the four lakes with low total Cu concentrations, the total dissolved Cu concentration may be below that which is necessary to properly measure the free Cu^{2+} concentration using an ion-selective electrode (~10^{-7} M) in a partially buffered system (Buffle, 1988; Mota and Correia Dos Santos, 1995; Rachou et al., 2007). In such systems, if the buffering capacity is insufficient, Cu contamination from the electrode during the potentiometric measurement may artificially increase the apparent $[Cu^{2+}]$ value. However, if this were the case, all four lakes might be expected to exhibit similar Cu^{2+} concentrations, which is not the case. Note too that in all our lakes, the free Cu^{2+} concentration is buffered by the DOM naturally present in the samples.

Finally, WHAM VI consistently over-predicted the free Ni^{2+} concentration in all the lakes where the ambient concentration was above the method detection limit (Figure 1c), i.e. the opposite result from that with Cu, suggesting that the default equilibrium constants for the complexation of Ni to natural DOM are too low for the environmental conditions found in our lakes. Van Laer et al. (2006) came to a similar conclusion in their study of the complexation of Ni by the DOM present in six Belgian surface waters. At their background Ni concentrations (40 to 80 nM), the measured free-ion fraction, as measured using the Donnan membrane technique, varied from 4 to 45%; WHAM VI overestimated the free-ion proportion more than 2-fold, even if it was assumed that all the DOM was present as fulvic acid and actively participating in Ni complexation.

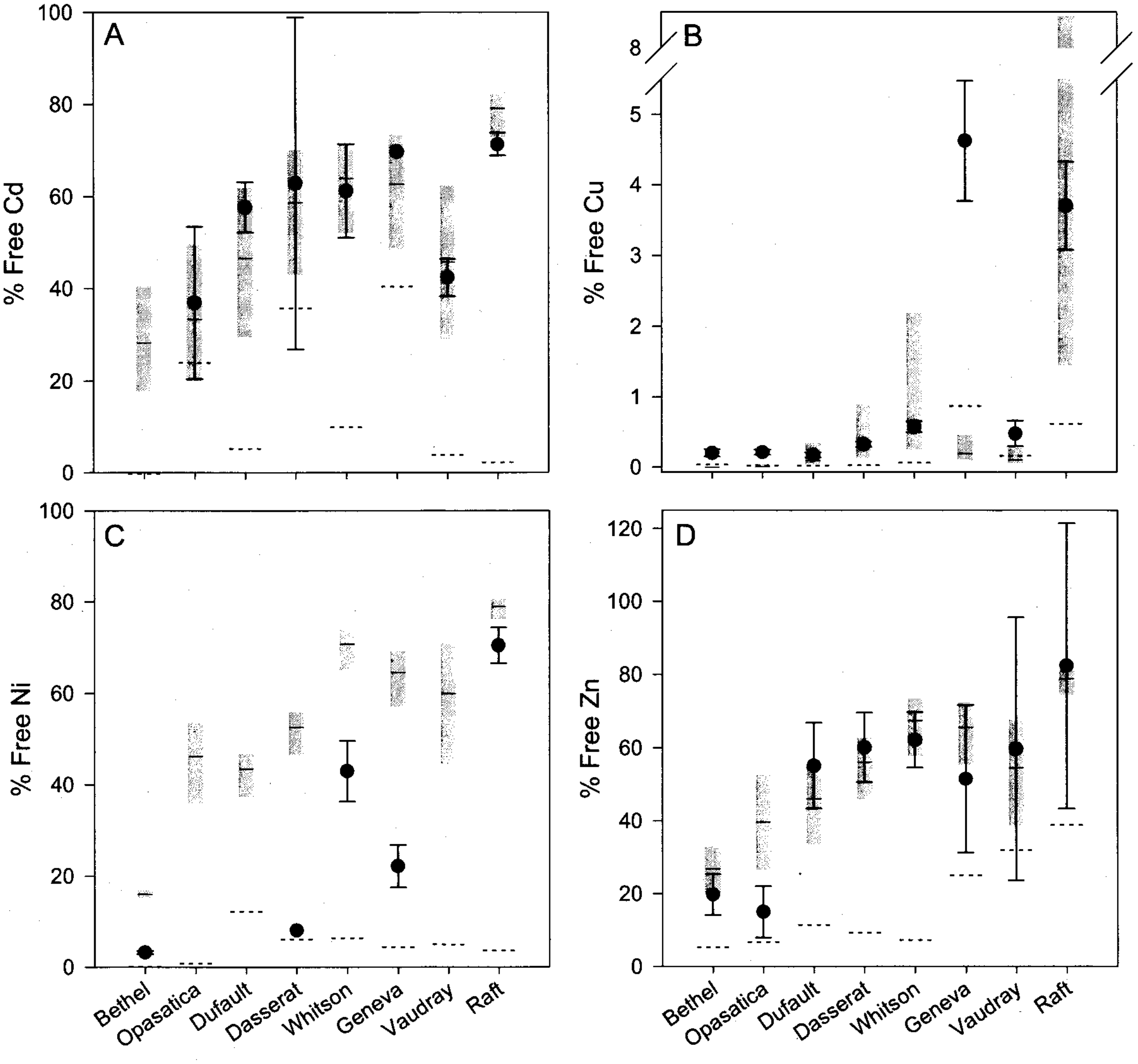

Figure 2. Comparison between measured percent free Cd^{2+} (A), Cu^{2+} (B), Ni^{2+} (C) and Zn^{2+} (D) and WHAM predicted percent free M^{Z+} for eight lakes sampled from the regions of Rouyn-Noranda, QC and Sudbury, ON. Measured points below the method limit of quantification (LOQ) are not shown. WHAM predictions were calculated for a range of percent active fulvic acid (%aFA) input values. Lakes on the x-axis are arranged from high pH on the left to low pH on the right. Solid circles are measured % free M^{Z+} values with error bars represent standard deviation of triplicate measures. Grey vertical bars represent the range of WHAM predicted % free M^{Z+} values using 33% to 130% aFA. Solid horizontal lines are WHAM predicted % free M^{Z+} values using 65% aFA. Dashed horizontal lines represent the LOQ.

Free metal ion percentages. A comparison was also made between measured and modeled "percent free metal" values to take into account differences in the total metal concentrations specific to each lake (Figure 2). A lake-specific percent free metal limit of quantification (LOQ) was calculated for each lake based on three times the standard deviations of triplicate measurements of both the free metal and total metal concentrations measured for each lake. Any percent free metal value calculated for a lake that yielded a value below its LOQ was discarded.

In addition to using the default assumption that 65% of the DOM is present as fulvic acid (FA) and is active in metal complexation, we also calculated a range of model percent free metal predictions by arbitrarily halving (33%) and doubling (130%) this value. It should be noted that increasing the %aFA to a value higher than 100% simply increases the number of fulvic acid binding sites involved to a value that exceeds the complexation capacity (μmol binding sites per unit carbon) of the default fulvic acid. In the presentation of the results of these simulations (Figure 2), the calculated percent free metal is depicted as an envelope of values for each lake: the middle value corresponds to the default value obtained with the assumption of 65% aFA, whereas the upper and lower values correspond to the percent free metal calculated with 33% and 130%, respectively, as the proportions of DOM active in metal complexation. The bracket of upper and lower % free metal values included the measured percent free metal values for both Cd and Zn in most lakes (7 of 8). The lakes for which the measured value fell outside the predicted envelope were those with the lowest total and free metal concentrations. Even with the assumption of only 33% aFA, i.e., minimum complexation, WHAM VI still predicted a lower percentage free Cu^{2+} than the measured value for the lakes with the lowest total Cu concentrations (4 of 8). Similarly, even with the assumption of 130% aFA, i.e., maximum complexation, the measured values of percent free Ni^{2+} all fell below the minimum predicted value.

3.5.2 Optimization of WHAM VI predictions

The chemical equilibrium simulations discussed to this point were run with WHAM VI in its default mode, using the assumptions that were outlined earlier. Among the adjustable WHAM input parameters are (i) the user-defined proportion of DOM active in metal complexation and (ii) the relative proportions of humic and fulvic acids (FA and HA) making up the DOM that is active in metal complexation. The latter parameter varies as a function of the type of organic matter, e.g., whether it is derived from soil water, peat water, lake water, etc. (Bryan et al., 2002). The proportion of DOM that is active in metal complexation is usually unknown and must be estimated by the user of WHAM.

At this point, we retained the assumption that the DOM actively involved in metal complexation could be represented as fulvic acid, but we removed any constraint on the "percent active FA" and ran multiple simulations for each lake and each metal, individually. The optimal percent of FA actively involved in metal complexation ($\%aFA_{opt}$) required to exactly predict the measured free metal ion was calculated by repeatedly running WHAM/Model VI, adjusting the FA concentration until the predicted free metal ion concentration equalled the observed value. A computer code was used to automatically run WHAM and optimise the values of %aFA. The $\%aFA_{opt}$ was calculated in this manner for each metal and for each lake as

$$\%\mathrm{aFA}_{\mathrm{opt}} = \frac{100 \cdot [\mathrm{FA}]_{\mathrm{opt}}}{2 \cdot [\mathrm{DOC}]}$$

where $[FA]_{opt}$ is the optimised FA concentration (mg L^{-1}) and [DOC] is the DOC concentration (mg L^{-1}). In a given lake and for a particular metal, an optimal %aFA for each of the triplicate samples was calculated by finding the FA concentration that best described all the measured free ion concentrations for each sample simultaneously (Table 3). This was done by adjusting the model FA concentration, as before, and minimising the error term

$$\sum_{1}^{i} ([\mathrm{M}]_{\mathrm{obs},i} - [\mathrm{M}]_{\mathrm{calc},i})^2$$

where $[M]_{obs,\,i}$ and $[M]_{calc,\,i}$ are the observed and calculated free ion concentrations for metal *i*.

By increasing or decreasing the %aFA, the number of FA binding sites involved in metal complexation is either increased or decreased, whereas the intrinsic metal binding affinities at these binding sites remain constant. In other words, the ligand concentration is adjusted. As can be seen in Table 3, the optimal %aFA values obtained in this manner varied from lake to lake, presumably reflecting inter-lake differences in the quality of the DOM. For Cu^{2+} the $\%aFA_{opt}$ values all fell in the 8 to 90% range, but for Cd^{2+}, Zn^{2+} and particularly Ni^{2+}, the values were all much higher, often exceeding the notional limit of 100%: 61 to 250% for Cd^{2+}; 65 to 410% for Zn^{2+}; 440 to 1900% for Ni.

3.5.3 Possible reasons for the divergence between WHAM calculations and measured metal speciation

Although increasing the %aFA, and thus augmenting the number of binding sites on the FA (as described in the preceding section), is an acceptable adjustment, increasing this value up to three orders of magnitude higher than the default value is clearly unrealistic. This result suggests that in addition to adjusting the binding capacity of the fulvic acid, it will likely also be necessary to adjust metal binding affinities (K_{MA}) (Tipping, 2002) for Cd, Zn and especially Ni. There may well be natural ligands present in the lake water that have a higher affinity for Ni than does fulvic acid. Further experiments to determine the binding constants of these metals with natural DOM, at environmentally realistic metal concentrations, are clearly needed.

Discrepancies between WHAM predictions and measured free-metal ion concentrations could also arise if ligands other than fulvic and humic acids were involved in metal complexation. For example, Baken et al. (2011) detected amino-polycarboxylate anions in some urban Belgian surface waters and suggested that these ligands of anthropogenic origin were responsible for the greater than expected metal complexation in these samples (as seen here for Ni). However, such an explanation seems unlikely in the present case, given the remote location of our lakes and the absence of urban wastewater inputs.

Table 3. Mean (± SD, n=3) calculated %aFA$_{opt}$ for lakes sampled from Rouyn-Noranda (QC) and Sudbury (ON) in 2008.

	%aFA$_{opt}$			
Lake (code)	**Cd**	**Cu**	**Ni**	**Zn**
Dasserat (DS)	93 ± 41	80 ± 12	1400[a]	95 ± 33
Dufault (DU)	61 ± 3	56 ± 8	NA[b]	65 ± 21
Opasatica (OP)	75 ± 5	16 ± 3	NA	310 ± 33
Vaudray (VA)	92 ± 14	32 ± 7	NA	95 ± 90
Bethel (be)	NA	9 ± 2	1900 ± 130	190 ± 36
Geneva (ge)	140[a]	8 ± 1	890 ± 170	290 ± 120
Raft (ra)	250 ± 23	86 ± 8	440 ± 74	410 ± 4
Whitson (wh)	140 ± 31	90 ± 8	580 ± 75	180 ± 51

[a] indicates only one sample above limit of quantification (LOQ; calculated as three times the standard deviation of triplicate analyses of each lake).

[b] NA indicates values not available.

3.6 Relationships between %aFA$_{opt}$ and DOM spectroscopic properties

Given the marked lake-to-lake differences in %aFA$_{opt}$, we explored possible relationships between this value and the optical properties of the DOM in the original lake water samples. Initially we looked for relationships with SUVA$_{254}$ values, given the promising results obtained by soil scientists working on Cu and Cd speciation in soil solutions (Amery et al., 2008; Cornu et al., 2011). However, plots of %aFA$_{opt}$ against SUVA$_{254}$ or the fluorescence index failed to yield useful relationships (see Figs. S5 and S6 in the Supplementary Material). Our interest then turned to the fluorescence spectrum, to determine if it could be used to estimate the proportion of DOM that is active in metal complexation. We plotted the relative fluorescence of each of the four PARAFAC fluorescence components against the %aFA needed to force the WHAM-calculated free metal concentration to equal the measured free metal ion concentration (%aFA$_{opt}$) (see Figs. S7 and S8 in the Supplementary

Material). The most statistically significant relationships with %aFAopt for the four metals emerged for C1/C_T and C3/C_T (Figure 3). In seeking such relationships, we are using the fluorescence excitation-emission matrix as a "proxy" for the composition of the DOM. Although spectrofluorimetry does not necessarily probe the functional groups or the exact sites involved in metal complexation by the DOM, it is nevertheless a useful and easy way to track changes in the quality of DOM in natural aquatic samples (Fellman et al., 2010; Mueller et al., 2012b).

For Cd, the strongest (R^2=0.79) and most statistically significant (P<0.0001) relationship (y=380e$^{(-6.4x)}$) emerged between %FA_{opt} and the relative proportion of C3 (Figure 3e). A statistically significant (P<0.001) positive linear relationship (y=1100x – 260; R^2=0.62) was found between Cu and C1/C_T (Figure 3b). For Ni a strong (R^2=0.96) and significant (P<0.001) linear relationship (y= –20 000x+6800) was observed with C1/C_T (Figure 3c). Finally, a weak (R^2=0.33), but statistically significant (P=0.01) decreasing exponential relationship (y=560e$^{(-5.3x)}$) was found between Zn and C3/C_T (Figure 3h).

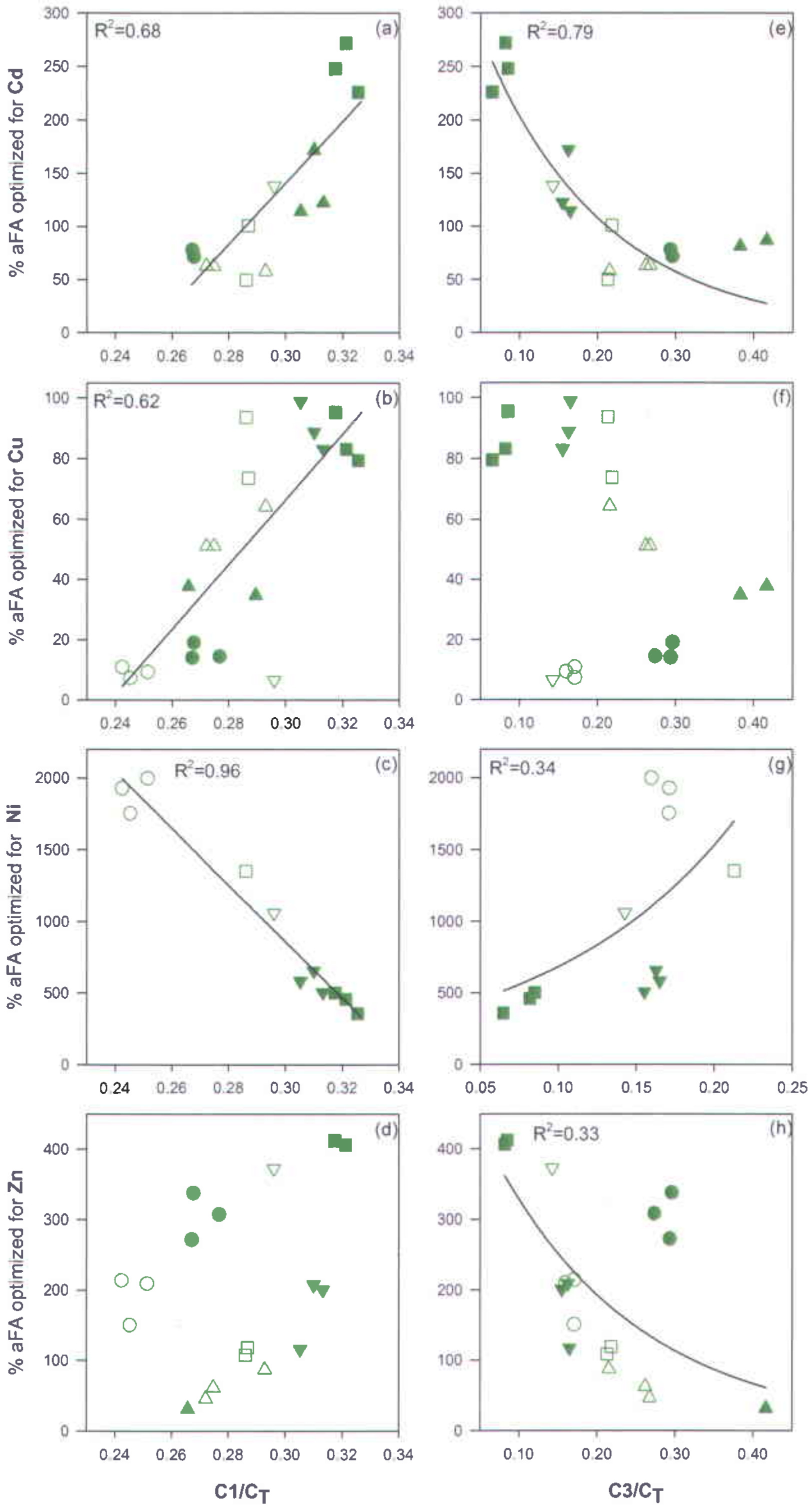

Figure 3. Relationship between the WHAM calculated percent fulvic acid active in complexation (%aFA$_{optimized}$) for Cd, Cu, Ni and Zn and the relative contribution of PARAFAC fluorescence components 1 (panels a – d) and 3 (panels e – h) to the overall fluorescence spectrum of lake DOM. Points of the same shape and fill represent replicate samples. Solid lines represent regression equations of statistical significance at the 0.05 alpha level.

In terms of the ubiquitous humic-like DOM fluorescence component (C1), the positive linear relationships between %aFA$_{opt}$ and C1/C$_T$ for Cd and Cu (Figures 3a and 3b) suggest that with an increase in the relative contribution of this component in the lakewater samples, the proportion of the DOM that is involved in Cd and Cu complexation increases. In contrast, a negative linear relationship was found for Ni (Figure 3c), indicating a decrease in the binding capacity of the DOM for Ni. With respect to the allochthonous-like DOM component (C3), the negative exponential relationships between %aFA$_{opt}$ and C3/C$_T$ for Cd and Zn (Figures 3e and 3h) suggest that with an increase in the allochthonous signature of the DOM in the lakewater samples, the proportion of the DOM that is involved in Cd and Zn complexation decreases. In contrast, a weak but positive relationship with the C3/C$_T$ ratio was found for Ni – as the allochthonous signature of the DOM in the lake samples decreases, the binding activity of the DOM for Ni increases. This singular behaviour of Ni, as demonstrated by the contrast between Figures 3c and 3g and the comparable plots for the other metals, suggests that the DOM binding sites active in Cd, Cu and Zn complexation are different from those involved in Ni complexation. To our knowledge, this is the first time that such a distinction has been resolved in natural water samples.

Admittedly the relationships shown in Figure 3 will have to be explored over a wider range of DOM samples, but they do support our original idea that the proportion of DOM active in metal complexation could be estimated on the basis of its fluorescence signature and then introduced into chemical equilibrium models such as WHAM. To test this idea, we incorporated each lake's optimized %aFA value, as calculated from the relationship between %aFA optimized and C1/C$_T$ or C3/C$_T$ for each metal and lake (i.e., the regressions shown in Figure 3), into WHAM and re-ran the chemical equilibrium simulations to predict the free M^{2+} concentrations; this was done for each metal, since the optimized %aFA value is metal-specific. Figure 4 depicts the comparison between these "improved" WHAM calculations of free M^{2+} and the measured free M^{2+} concentrations. Compared to the results presented in Figure 1, the greatest improvement using the C1/C$_T$ ratio was found for nickel, for which the modeled free Ni^{2+} decreased to within at most a factor of 1.3 difference from the measured free Ni^{2+} concentrations. For Cd^{2+} and Zn^{2+}, metals for which the original WHAM predictions were already good, no improvement was noted (indeed the agreement was slightly worse than with the arbitrary value of 65% active fulvic acid). Although the WHAM calculation of free Ni^{2+} in our lakes was improved by estimating the proportion of fulvic acids active in Ni complexation from the spectroscopic quality of DOM, the estimated %aFA was still unrealistically high, as mentioned earlier. Nevertheless, the idea of using the spectroscopic quality of DOM as a proxy for the proportion of DOM that is active in metal complexation remains promising.

4 Conclusions

The main goal of this study was to explore the possibility of incorporating a measure of the quality of DOM into chemical speciation models, such as WHAM, to improve their predictions of trace metal speciation in natural aquatic systems. For Cd, Cu, Ni and Zn, we have shown that such an approach is feasible, using simple fluorescence measurements on lakewater DOM to estimate the proportion of DOM that is actively involved in metal complexation (as was done in generating Figure 4). However, our results have also indicated that in addition to adjustments to the metal-binding capacity of the DOM, it may also be necessary to adjust the affinity constants that are used by WHAM to calculate the speciation of some metals, especially Ni; the possibility of using the spectroscopic properties of DOM to predict its metal-binding affinity should be explored. The possible involvement of ligands other than fulvic and humic acids in metal complexation should also be considered, particularly for samples with low total trace metal concentrations. Improved trace metal speciation predictions, especially at low, environmentally significant metal concentrations, will be important not only for geochemical studies, but also for the prediction of chronic toxicity in natural aquatic systems (e.g., as part of models such as the Biotic Ligand Model or BLM (Slaveykova and Wilkinson, 2005)).

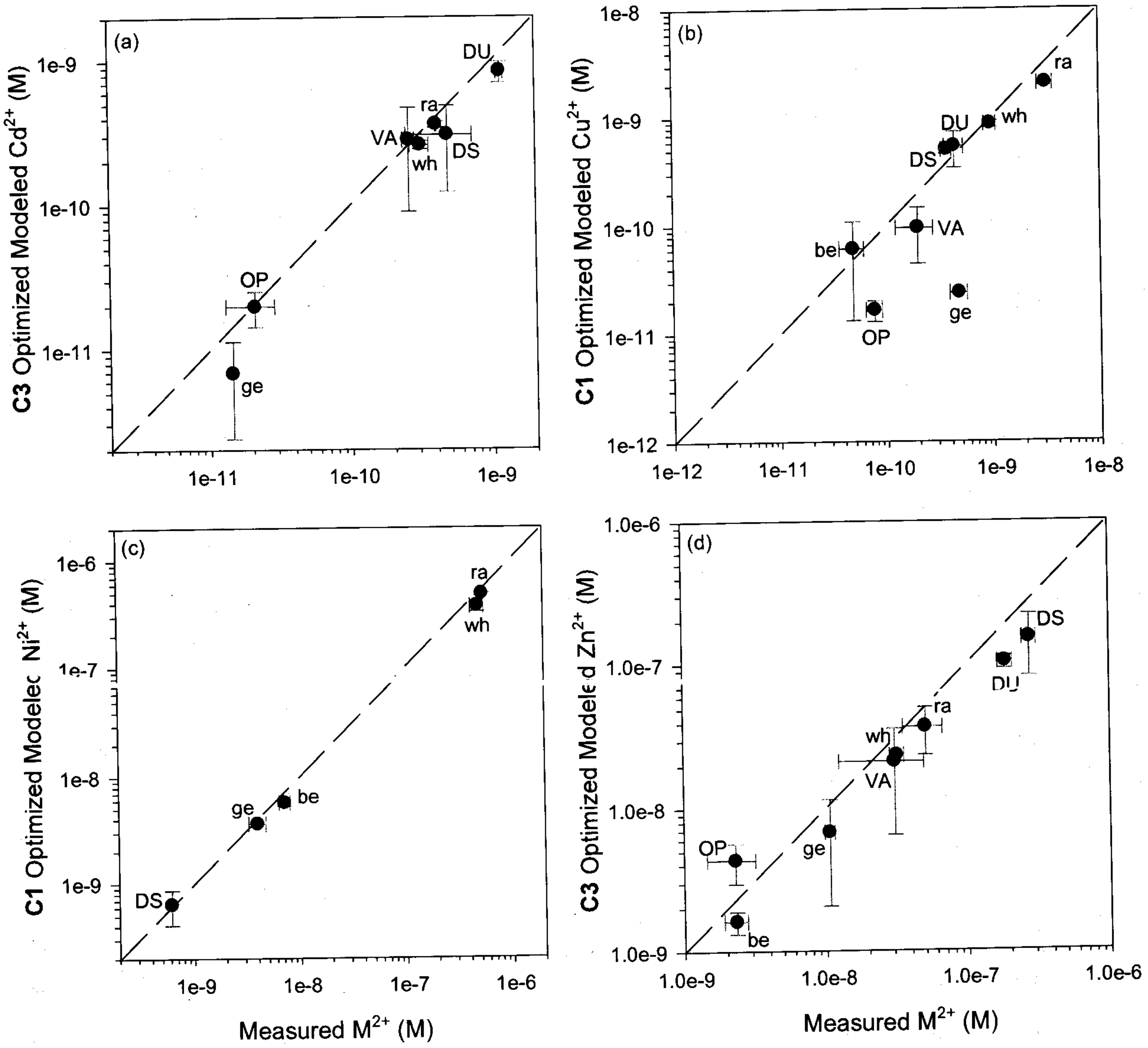

Figure 4. Comparison of mean (± SD, n=3) concentrations of the free metal ion [M^{Z+}] calculated with WHAM (with the %aFA estimated from Figure 3) and those measured (± SD, n=3) using the IET (Cd, Ni and Zn) or ISE (Cu) methods. The dashed line represents the 1:1 line.

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5 Supplementary Material

5.1 Ion Exchange Technique (IET) calibration results

The calibration of the IET for Cd^{2+}, Ni^{2+} and Zn^{2+} was performed with standard solutions in a pH range of 5.29 to 8.86 and with known concentrations of free Cd^{2+} (0.24 to 405 nM), Ni^{2+} (12.8 to 1750 nM) and Zn^{2+} (4.86 to 1310 nM). Figure S1 depicts the changes in the distribution coefficient (λ) for Cd, Ni and Zn as a function of the free metal concentration.

Across this pH and concentration range, the distribution coefficients were generally stable. The λ values calculated at the highest pH value of 8.86 (closed squares in Figure S1), however, are much higher than the remaining λ values. At high pH values, metal hydroxo complexes tend to interact with the resin(Fortin et al., 2010), creating a positive interference and increasing the λ values for all three metals. For the remaining pH range (5.29 to 7.88) the λ values can be considered to be constant. For Ni, the λ values were found to be stable across the whole concentration range (N=56, CV<18%). For Cd and Zn, the lowest concentration (0.24-0.26 nM for Cd and 4.86-5.42 nM for Zn) introduced a considerable amount of variability in the λ values. At the lowest free metal concentrations, trace amounts of an unidentified (strong) ligand may have complexed the metal, yielding an artificially low value for λ. The λ values for Cd and Zn were, however, found to be stable across the free metal concentration of range of 4.08 to 405 nM Cd^{2+} (N=46, CV<23%) and 14.6 to 1310 nM Zn^{2+} (N=46, CV<34%) and these values were used for the calculation of the free-metal ion concentrations.

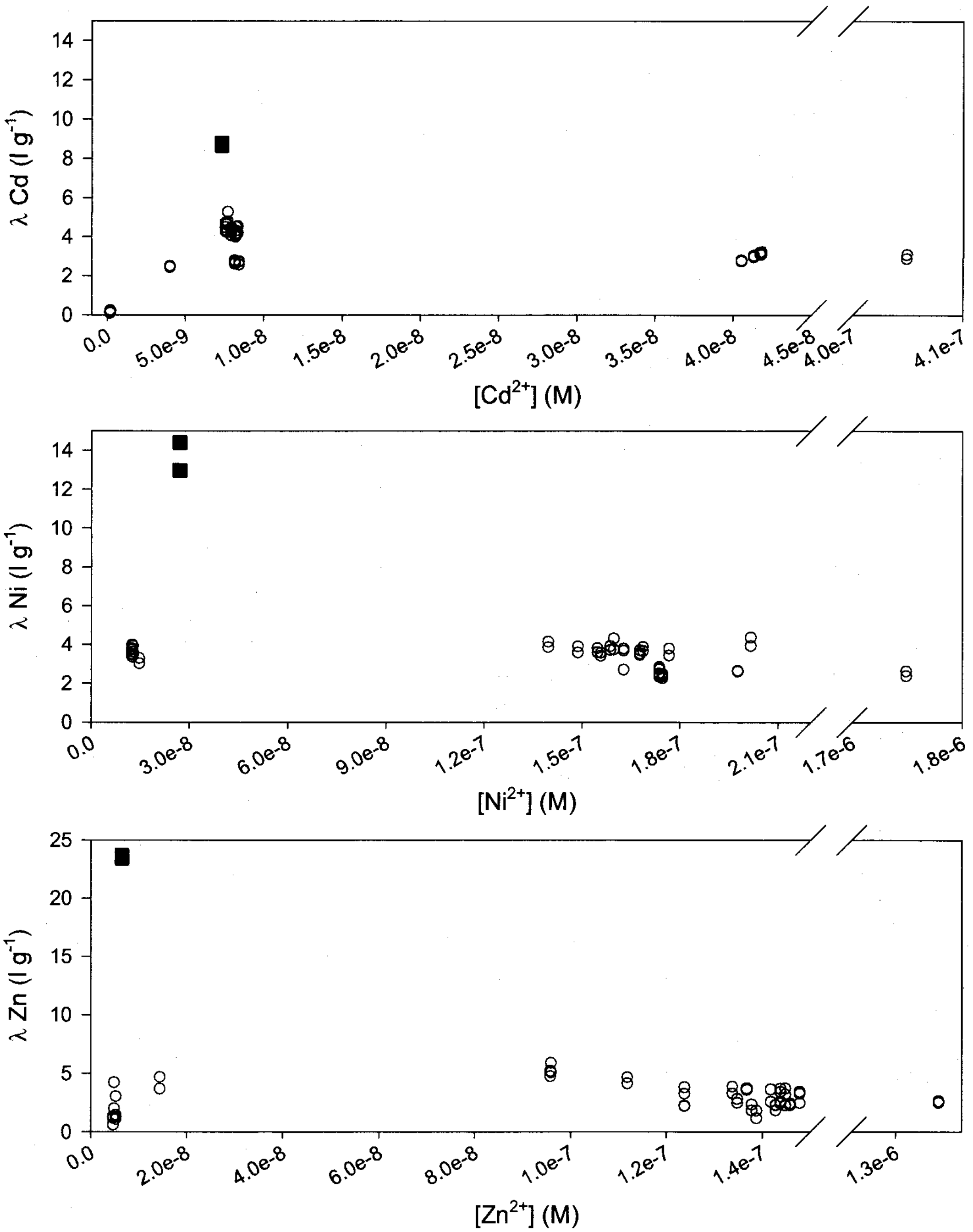

Figure S1. IET distribution coefficients (λ) for Cd, Ni and Zn as a function of free metal concentration. Open circles represent λ calculated at pH 5.29 to 7.88, whereas closed squares were calculated at pH 8.86.

5.2 Ion Exchange Technique (IET) calibration results (Rachou et al., 2007)

Although the calibration curve (Figure S2) was calculated based on measured free Cu^{2+} concentrations as low as 4.5×10^{-15} M, a method detection limit of 2.6×10^{-11} M Cu^{2+} was calculated based on the three times the standard deviation of six replicate method blank measurements.

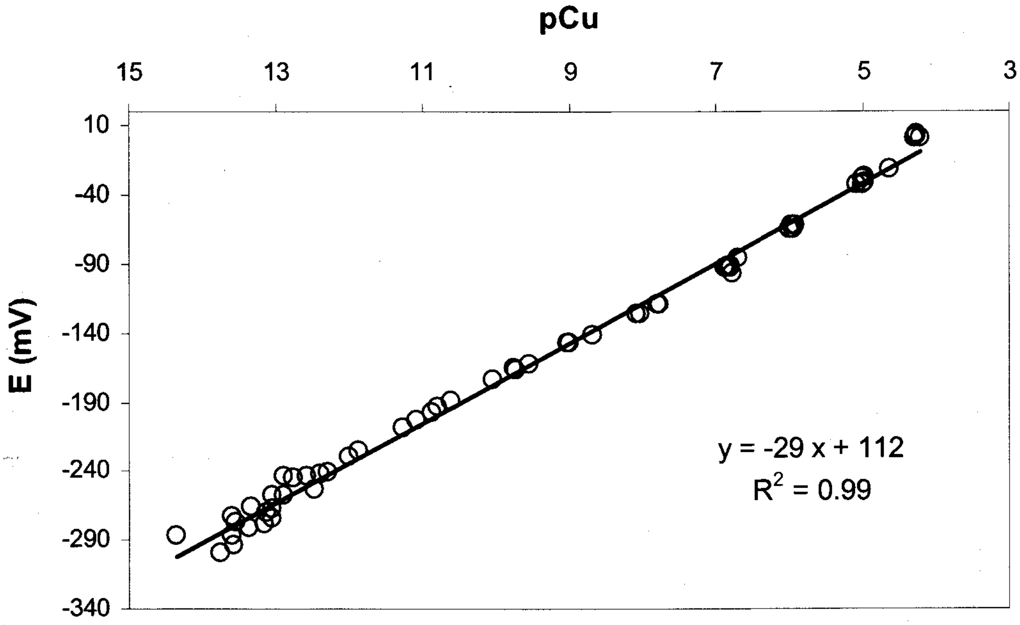

Figure S2. ISE potential response as a function of Cu activity (pCu). N=66.

5.3 Relationships between percent free metal and pH and DOC concentrations

For all four metals (Cd, Cu, Ni and Zn), and in both the Rouyn-Noranda and Sudbury regions, the percent free metal as determined by the IET and ISE methods tended to decrease as the lake pH increased, pointing to complexation competition by protons as an important factor affecting metal speciation.

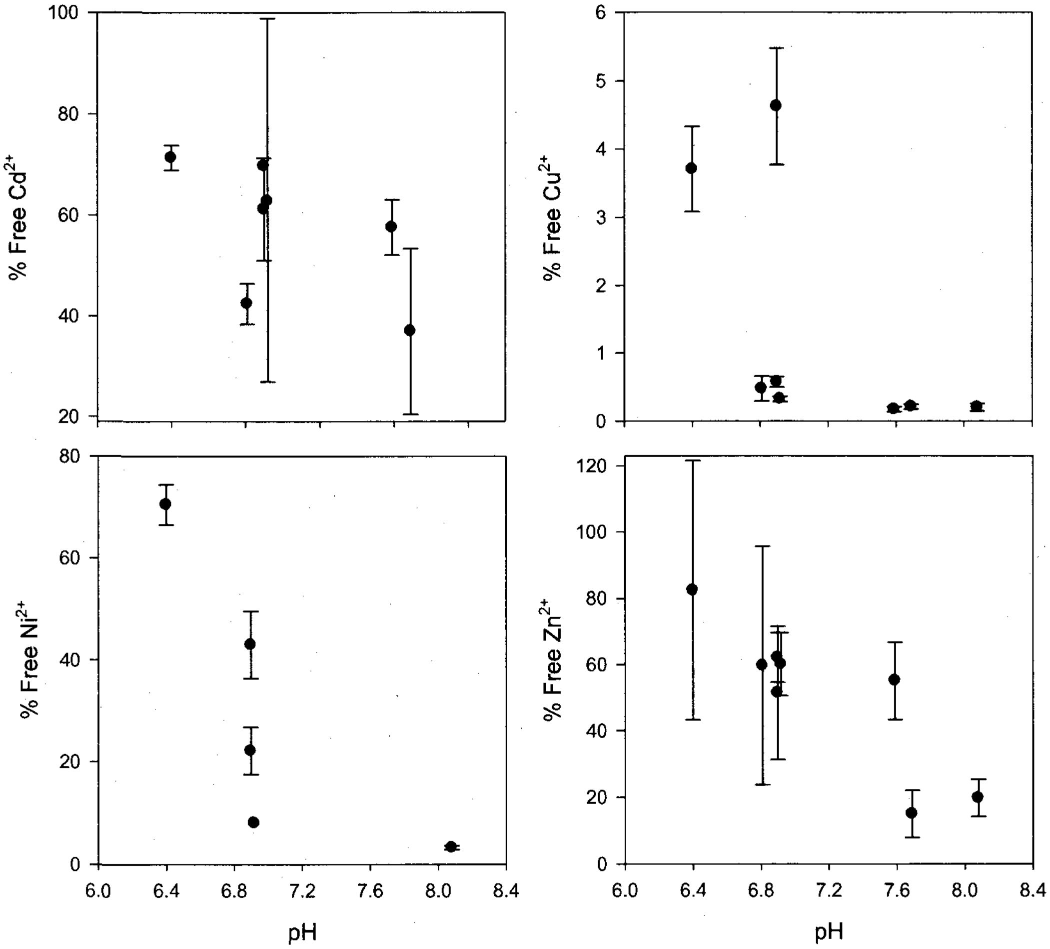

Figure S3. Measured % free metal (±SD, n=3) as a function of lake pH.

A general decline in the measured percent free metal was also observed for Cd, Cu and Ni as the DOC concentration increased, presumably reflecting an increase in available metal binding sites on the DOM with an increase in the ligand-to-metal ratio.

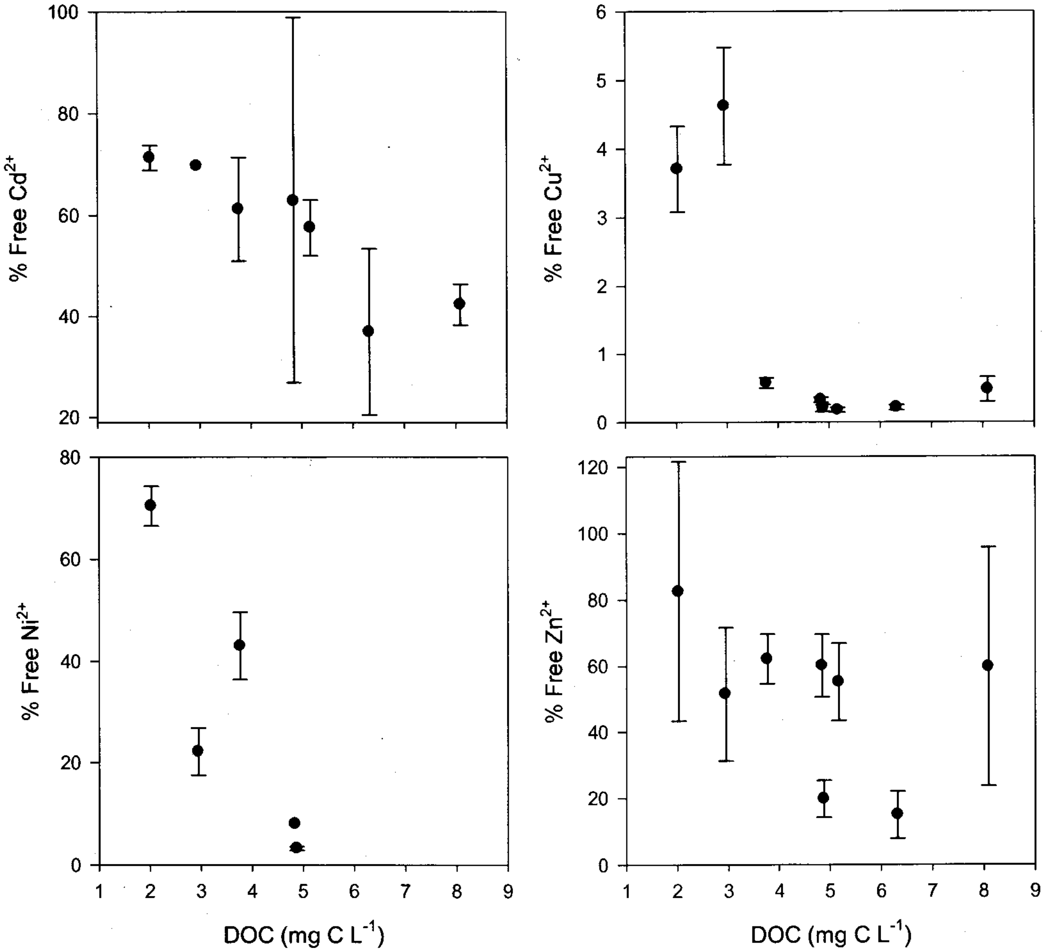

Figure S4. Measured % free metal (±SD, n=3) as a function of lake DOC concentration.

5.4 Relationships between the optimized %aFA$_{opt}$ and DOM optical properties

The measured optical quality of the DOM in the lakes sampled for this study is presented below (Table S1) and is described in detail elsewhere (Mueller et al., 2012b).

Table S1. Measured (±SD, n=3) DOM optical quality ($SUVA_{254}$, Fluorescence Index (FI) and the relative proportion of PARAFAC fluorescence components 1 to 4) for lakes sampled from Rouyn-Noranda (QC) and Sudbury (ON) in 2008.

Lake	$SUVA_{254}$ ($L\ m^{-1}\ mg\ C^{-1}$)	FI	$C1/C_T$	$C2/C_T$	$C3/C_T$	$C4/C_T$
Dasserat	7.8 ± 0.3	1.33 ± 0.02	0.287 ±	0.384 ±	0.216 ±	0.114 ±
Dufault	7.3 ± 0.4	1.301 ±	0.28 ± 0.01	0.34 ± 0.01	0.25 ± 0.03	0.133 ±
Opasatica	7.5 ± 0.4	1.361 ±	0.271 ±	0.325 ±	0.29 ± 0.01	0.116 ±
Vaudray	10.0 ± 0.3	1.27 ± 0.01	0.28 ± 0.02	0.254 ±	0.40 ± 0.02	0.068 ±
Bethel	5.0 ± 0.3	1.51 ± 0.04	0.246 ±	0.380 ±	0.167 ±	0.206 ±
Geneva	5.3 ± 0.2	1.30[a]	0.30[a]	0.39[a]	0.14[a]	0.17[a]
Raft	5.8 ± 0.8	1.30 ± 0.09	0.321 ±	0.384 ±	0.08 ± 0.01	0.218 ±
Whitson	7.3 ± 0.5	1.36 ± 0.03	0.310 ±	0.385 ±	0.161 ±	0.144 ±

[a] indicates only one replicate sample measured.

We explored possible relationships between the optimal percent of FA actively involved in metal complexation ($\%aFA_{opt}$) and the optical properties of the DOM in the lake water samples, in order to estimate the proportion of DOM that is active in metal complexation. The most statistically significant relationships with $\%aFA_{opt}$ for the four metals emerged for $C1/C_T$ and $C3/C_T$ (see Figure 3 in the manuscript). The following figures (S5 to S8) show the lack of significant bivariate relationships between $\%aFA_{opt}$ and the other optical properties.

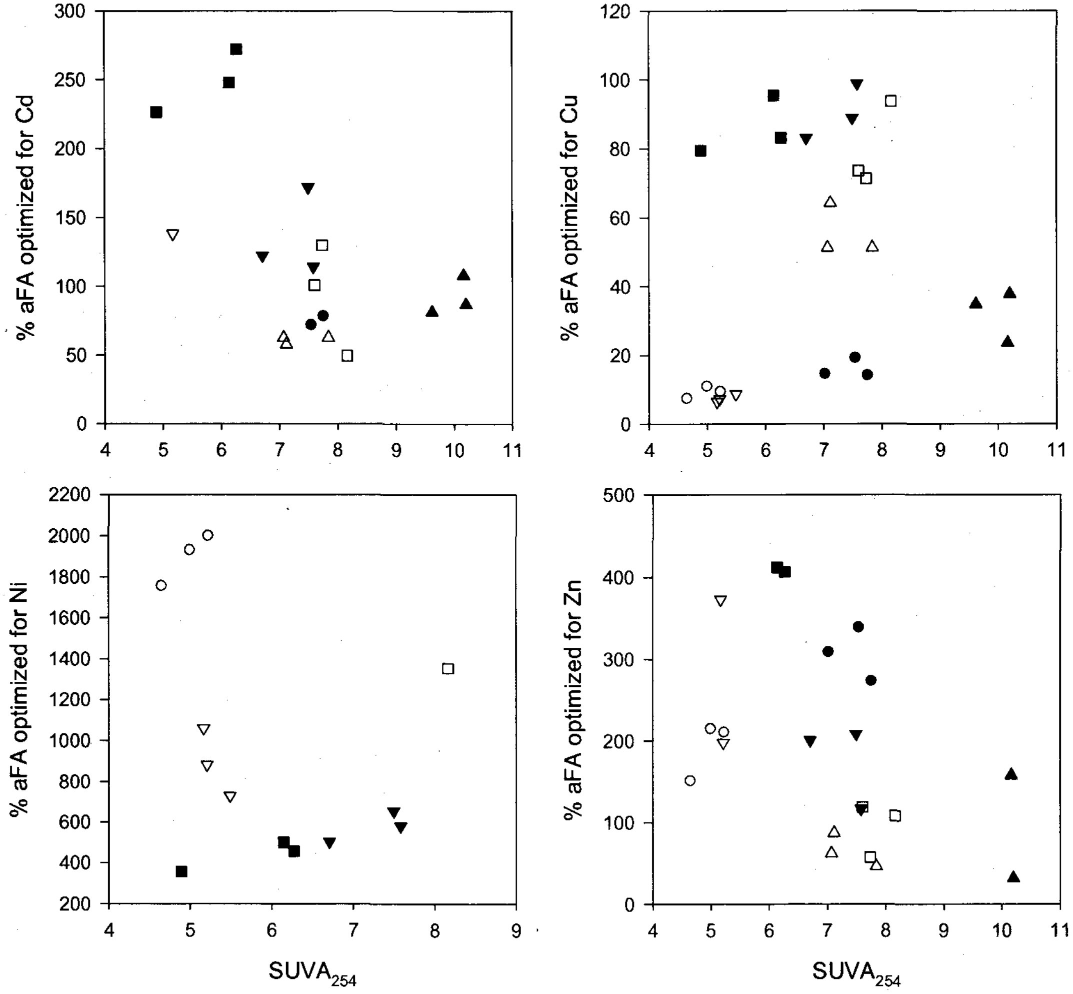

Figure S5. Relationship between the WHAM calculated percent fulvic acid active in complexation (%aFA$_{opt}$) for Cd, Cu, Ni and Zn and the specific UV absorbance (SUVA$_{254}$) of lake DOM. Points of the same shape and fill represent replicate samples.

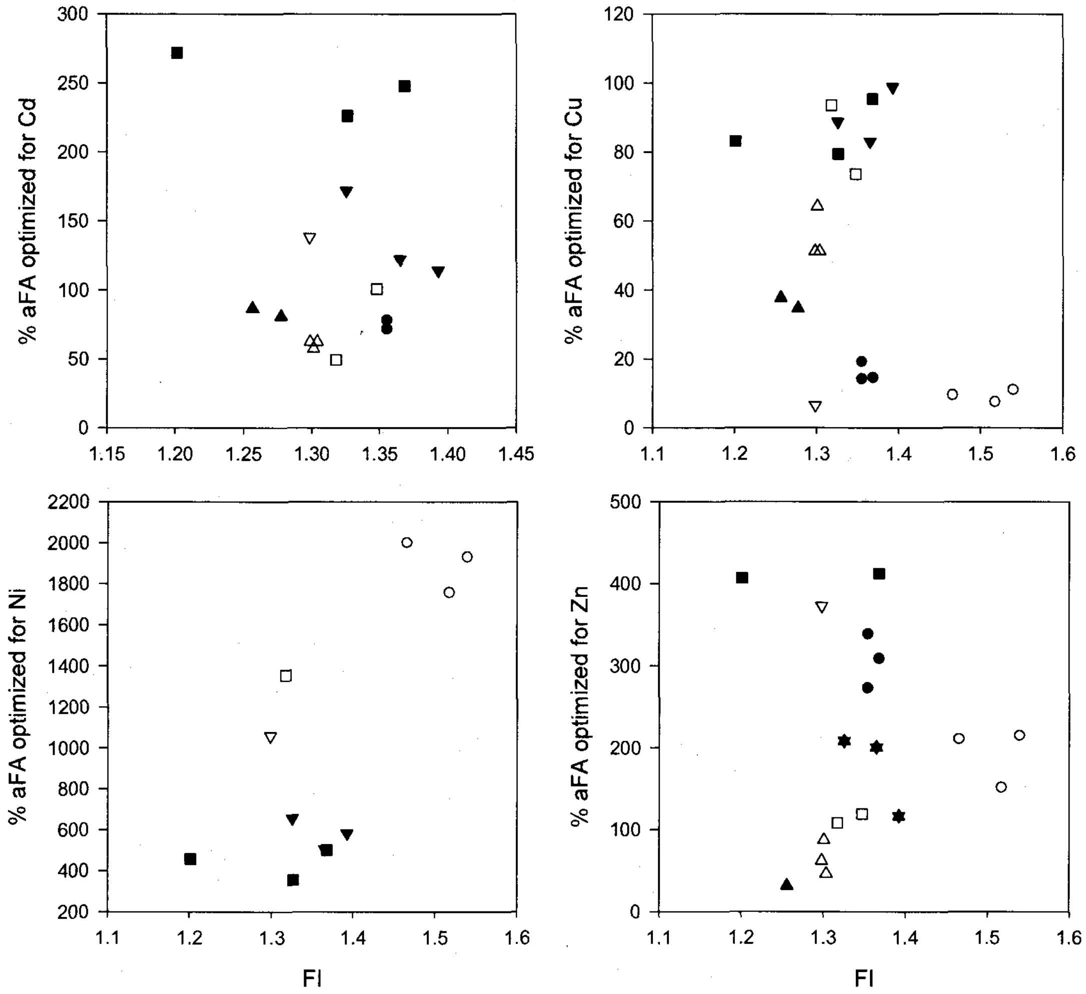

Figure S6. Relationship between the WHAM calculated percent fulvic acid active in complexation (%aFA$_{opt}$) for Cd, Cu, Ni and Zn and the fluorescence index (FI) of lake DOM. Points of the same shape and fill represent replicate samples.

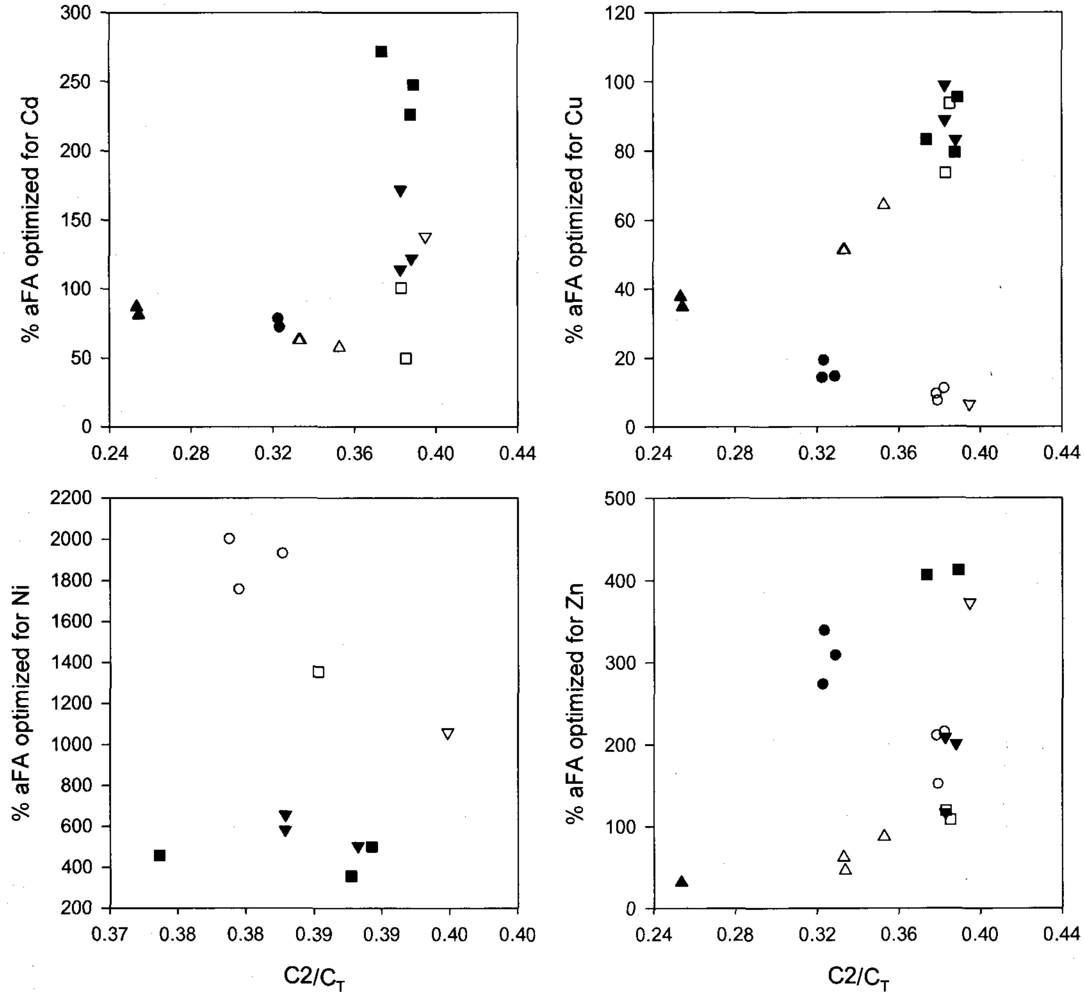

Figure S7. Relationship between the WHAM calculated percent fulvic acid active in complexation (%aFA$_{opt}$) for Cd, Cu, Ni and Zn and the relative contribution of PARAFAC fluorescence component 2 to the overall fluorescence spectrum of lakewater DOM. Points of the same shape and fill represent replicate samples.

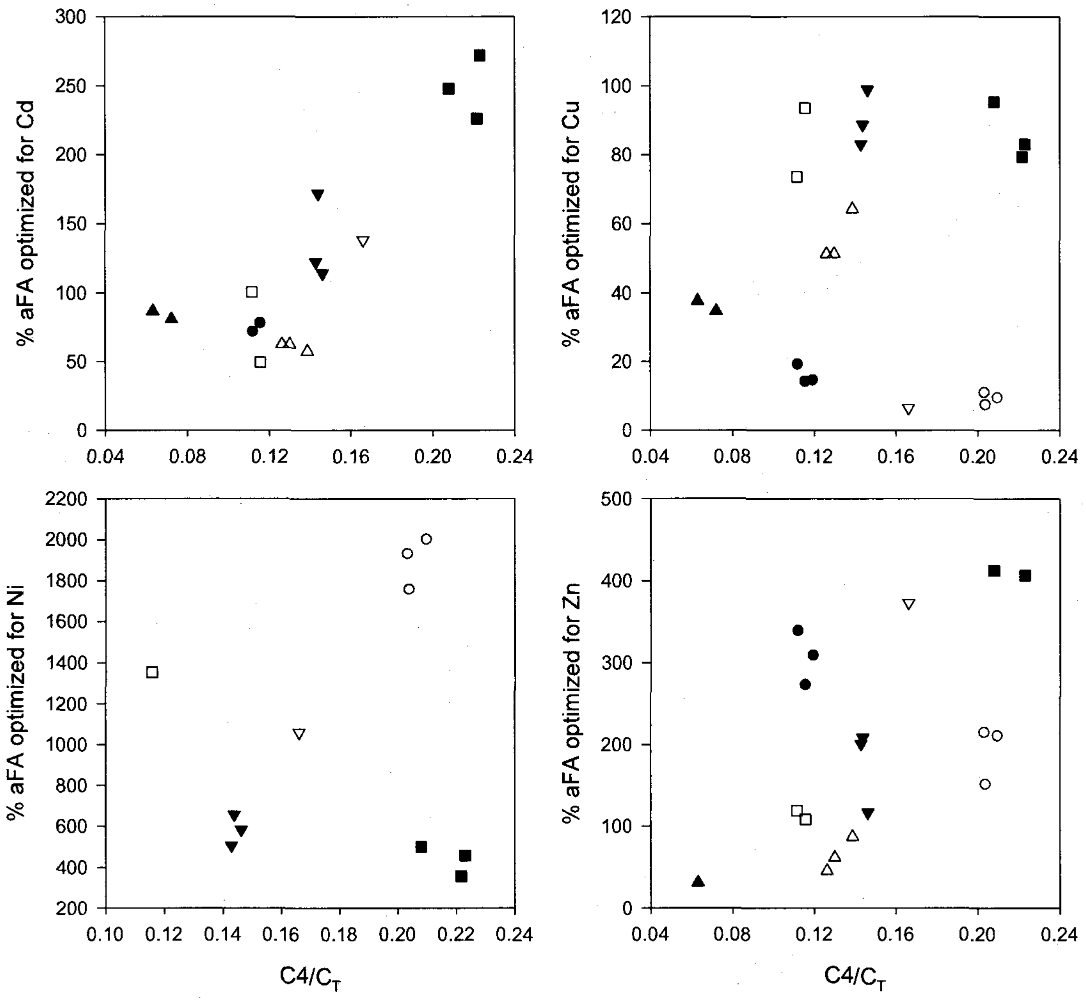

Figure S8. Relationship between the WHAM calculated percent fulvic acid active in complexation (%aFA$_{opt}$) for Cd, Cu, Ni and Zn and the relative contribution of PARAFAC fluorescence component 4 to the overall fluorescence spectrum of lakewater DOM. Points of the same shape and fill represent replicate samples.

Article 3

Nickel and copper complexation by natural dissolved organic matter in lake waters

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En préparation

Abstract

The Windermere Humic Aqueous Model (WHAM), used to estimate the binding of cations by dissolved organic matter (DOM) in natural aquatic systems, is calibrated using the results of titration experiments on isolated and purified humic and fulvic acids, at DOM and total metal concentrations that are often unrealistic for natural aquatic systems. Using well-characterized natural water samples, we demonstrate, for realistic environmental conditions, that the conditional binding parameters for the complexation of Ni and Cu with natural DOM vary as a function of the spectroscopic quality of the DOM. Waters from two lakes with contrasting types of DOM were titrated with Cu and Ni and the calculated conditional binding parameters were compared between lakes and between metals, and the titration curves for each lake were compared to those predicted by the off-the-shelf version of WHAM 6.1. Binding affinities and capacities of DOM for Ni and Cu were found to be significantly different not only between lakes, but also between metals. WHAM was found to underestimate the amount of DOM-bound Ni, while the opposite trend was found for DOM-bound Cu. Overall, the titration results suggest that the more aromatic humic-like DOM from allochthonous sources may have a significantly higher complexation affinity and capacity for Cu than the more protein-like DOM from autochthonous sources, whereas the opposite trend was found for Ni, indicating that Ni and Cu are binding to different types of binding sites within the DOM matrix.

1 Introduction

The complexation of trace metals by natural dissolved organic matter (DOM), an important organic complexing agent in most natural waters, affects their speciation, chemical reactivity, and bioavailability. Chemical equilibrium models, such as the Windermere Humic Aqueous Model (WHAM), are often used to predict the complexation of metals by DOM in natural aquatic systems based on established thermodynamic equilibrium reactions. The heterogeneous nature of DOM, however, makes it difficult to assign specific thermodynamic data to its complexes. Nevertheless, with the use of some reasonable assumptions based on current knowledge of the characteristics of DOM, the complexation of cations with DOM has been modeled with some success (Tipping, 2002; Tipping et al., 2011).

The WHAM model is parameterised based on information gained from laboratory studies on isolated humic substances, but it is often used to predict the complexation of metals to organic matter in natural water samples. Unfortunately, discrepancies have been found between the WHAM-predicted speciation of some metals, especially for Cu and Ni, and that measured in natural freshwater samples (Fortin et al., 2010; Mueller et al., 2012c; Unsworth et al., 2006; Van Laer et al., 2006). The discrepancies between measured and modeled free metal concentrations in natural freshwater systems may be due to differences in metal-binding character between the DOM in natural waters and the isolated humic substances used to parameterise the model (Lofts and Tipping, 2011).

In the present study, we have titrated two lake water samples with Cu and Ni and compared the resulting titration curves with those predicted by WHAM. The two lakes were chosen because earlier work had shown that their DOM differed spectroscopically, and because their ambient metal levels were low (thus allowing us to explore a wide range of metal:DOM ratios). The objective of the work was to compare the metal-binding properties of two contrasting types of DOM. Conditional binding parameters for the complexation of Cu and Ni with the natural DOM have been calculated, compared between lakes and between metals, and related to the optical quality of the lake DOM.

2 Materials and Methods

2.1 *Site description and sampling*

The Canadian areas of Rouyn-Noranda, Québec and Sudbury, Ontario have been impacted by past and present copper/nickel mining, smelting and refining activities. A detailed description of these study areas can be found in Mueller et al. (2012b). Two lakes with low total metal concentrations were selected for the titration experiments: Lake Opasatica (48°04'32" N; 79°17'49" W) from the Rouyn-Noranda region and Lake Geneva (79°17'49" W; 81°32'46" W) from the Sudbury region. Based on the findings reported by Mueller et al. (2012b; 2012c), Lake Opasatica has a moderate concentration of DOC (6.0 ± 0.4 $mg\cdot L^{-1}$), a near-neutral pH (7.47), very low total dissolved concentrations of Ni and low concentrations of Cd, Cu and Zn (see Table 1). Lake Geneva has a low concentration of DOC (3.4 ± 0.4 $mg\cdot L^{-1}$), a near-neutral pH (6.89), and the total dissolved concentrations of Cd, Cu, Ni and Zn are all very low (see Table 1). Differences in the spectroscopic quality of DOM between the lakes were also reported (Mueller et al., 2012b).

Grab samples were taken from the littoral zones of Lakes Opasatica and Geneva using two 4-L amber glass jugs with Teflon®-lined, polypropylene closures (I-CHEM Brand) for each lake in June and July of 2009, respectively. The samples were then transported back to the laboratory within 24 h and vacuum filtered under a clean laminar flow hood using polycarbonate filter holders (Advantec MFS, Inc.) and a series of 47-mm polycarbonate filters (5, 2, 0.4 and 0.2 µm; Millipore and AMD Manufacturing Inc.). Before filtering the lake water samples, 1 L of ultrapure water and a small volume of sample were filtered through the filtration apparatus and discarded. Following filtration, the samples were stored in the dark and at 4°C until analysis (storage time < 72 d).

2.2 *Analyses*

All plasticware used for sampling and sample analyses was soaked in a solution of 10% (v/v) nitric acid for at least 24 h, rinsed several times with ultrapure water (>18 Mohms·cm) and dried under a laminar flow hood. Polystyrene vials used for storing the subsamples for anion analyses were, however, rinsed three times with ultrapure water only. All glassware was soaked in a solution of 2 N HCl and rinsed with ultrapure water.

Following sample filtration, subsamples were taken under a laminar flow hood to measure the ambient water chemistry for Lakes Opasatica and Geneva. Subsamples (10-mL) were transferred to polypropylene vials and acidified to 2% (v/v) nitric acid (ultra-trace nitric acid, BDH Aristar) for analysis of major cations (Ca, Mg, Na, K) by atomic emission spectroscopy (Vista AX CCD Simultaneous ICP-AES, Varian) and total trace metals (Cd, Cu, Ni, Zn) by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Element X Series). Similar 10-mL subsamples were also transferred to polystyrene vials (VWR International) for major anions (Cl, NO_3, SO_4) analysis by ion chromatography (ICS-2000 Ion Chromatography System, Dionex), and 20-mL subsamples were transferred to amber glass vials (I-CHEM Brand) for total organic carbon analysis (TOC-V_{CPH}, Shimadzu). Finally, 1-mL subsamples were transferred to 3-mL glass vacutainers (Kendal Monojet) for total inorganic carbon analysis by gas chromatography (3800 Varian).

For details on the calibration and measurement of the free Ni^{2+} concentrations using an ion exchange technique (IET) and the free Cu^{2+} concentrations using a cupric ion selective electrode (ISE), the reader is referred to a previous paper by Mueller et al. (2012c). Briefly, when using the IET, the free Ni^{2+} ion in a lake water sample was equilibrated in a flow-through system with a cation exchange resin (Dowex 50W-X8, 50-100 mesh) that had been calibrated using standard solutions with a pH range of 5.3 to 7.9 and with a known concentration of free Ni^{2+} ranging from 13 nM to 1.8 μM (see Supplementary material in Mueller et al. (2012c)). The concentration of Ca in the calibration and natural lake samples was adjusted using a stock solution of 25 mM $Ca(NO_3)_2$ (99%, Sigma-Aldrich) to a final concentration of 0.46 mM in order to attain a constant ionic strength among samples and to ensure that the time needed to reach equilibrium between the resin and the sample was similar for all samples. Following the elution of the resin with an acidic solution and determination of the amount of Ni that had been retained by the resin, the concentration of free Ni^{2+} in the original unknown sample was then calculated using the resin distribution coefficient (λ) for Ni (Fortin and Campbell, 1998). For the analysis of the concentration of Cu^{2+} in the lake water samples, the electrode potential response of the Cu ISE, was calibrated over a free Cu^{2+} concentration ranging from the method detection limit (three times the standard deviation of six replicate blank measurements) of 0.03 nM to 63 μM following the method of Rachou et al. (2007) – see the Supplementary material for Mueller et al. (2012c)). The calibration and lake samples were amended with KNO_3 (99.995%, Fluka); a known amount of a 1.23 M stock solution was added to achieve a final concentration of 10 mM KNO_3 and thus ensure a constant ionic strength and a similar electrode response among all samples analysed.

2.3 *Titration experimental set-up*

Samples from Lakes Opasatica and Geneva were individually titrated with Cu and Ni. For the Cu titrations, known amounts of a stock solution of 0.2 mM copper nitrate ($Cu(NO_3)_2$, PlasmaCal) were added to eight 200-mL aliquots of the sample (polypropylene container, BD Falcon), which had been amended with KNO_3. The pH of the titration aliquots was adjusted to the natural lake pH using small volumes of either HNO_3 or NaOH, and the solutions were left to equilibrate overnight under a laminar flow hood and in the dark before measuring the free Cu^{2+} concentration using a cupric ion selective electrode. An equilibration time of approximately 24 hours was considered a conservative estimate of the time needed for the added metal to reach equilibrium with the DOM, based on literature values presented by Tipping (2002). The pH of the aliquots was checked before measuring the free Cu^{2+} concentration and re-adjusted to the natural lake pH if necessary. An initial sample aliquot with no added Cu was also analysed. Triplicate Cu titrations were carried out for a total of 27 Cu titration aliquots analysed. After the measurement of the free Cu^{2+} concentration, subsamples of each titration aliquot were taken for the measurement of total dissolved Cu (5 mL in a 13-mL polypropylene vial, Sarstedt) and DOC concentrations (10 mL in a 40-mL amber glass vial, I-CHEM Brand).

For the Ni titrations, known amounts of a stock solution of 1 mM nickel nitrate ($Ni(NO_3)_2$, PlasmaCal) were added to seven 220-mL aliquots of sample (polypropylene container, BD

Falcon), which had been amended with $Ca(NO_3)_2$. Again, the pH of the titration aliquots was adjusted to the natural lake pH and the solutions were left to equilibrate overnight before measuring the free Ni^{2+} concentration using the IET (the pH was re-adjusted to the natural lake pH if necessary just prior to IET analysis). An initial sample aliquot with no added Ni was also analysed. Triplicate Ni titrations were carried out for a total of 24 Ni titration aliquots analysed. In the same manner as for the Cu titrations, subsamples of each titration aliquot were taken for the measurement of total dissolved Ni (5 mL in a 13-mL polypropylene vial, Sarstedt) and DOC concentrations (10 mL in a 40-mL amber glass vial, I-CHEM Brand).

2.4 Calculations

Triplicate titrations curves of measured free Ni^{2+} and Cu^{2+} as a function of added total metal were generated for each lake and for each metal. Binding parameters were calculated from each replicate titration curve and then the averages of these triplicate binding parameters were calculated along with their associated variability. The equilibrium formation of a 1:1 complexes between metal (M^{2+}) and a two-site ligand (L_i), with an associated conditional stability constants (K_i) was assumed.

$$\mathrm{M^{2+} + L_i = ML_i} \qquad \mathrm{K_i = \frac{[ML_i]}{[M^{2+}][L_i]}} \qquad \textbf{(1)}$$

The binding parameters, L_1, K_1, L_2 and K_2, were calculated from titration curves using the following two-site Langmuir-type model equation:

$$[ML] = \frac{L_1K_1[M^{2+}]}{1+K_1[M^{2+}]} + \frac{L_2K_2[M^{2+}]}{1+K_2[M^{2+}]} \qquad (2)$$

where [ML] is the molar concentration of bound metal. Using SigmaPlot (version 11.0), non-linear regressions were performed on triplicate titrations for each lake and each metal for a total of 12 titration curves.

For comparison with the measured concentrations of bound metal, [ML], in the sampled lakes, [ML] was also calculated using the off-the-shelf version of WHAM 6.1 and measured values for pH, DOC and the total concentrations of Na, Mg, K, Ca, Ni, Cu, Zn, Cd, Cl, NO_3, SO_4, CO_3 (measured as total inorganic carbon) and F. We assumed that the DOC:DOM ratio was 2

(Buffle, 1988), that 65% of the DOM was active in the complexation of metals (Bryan et al., 2002), and that this active fraction was composed of fulvic acids only. We also assumed that both Fe(III) and Al(III) activities are controlled by the solubility of their hydroxides, as calculated using the empirical equations given by Lofts et al. (2008) and Tipping (2005), respectively. Statistical analyses were all conducted in SigmaPlot (version 11.0) at the alpha equals 0.05 confidence level.

3 Results and Discussion

3.1 *Lake water and DOM characteristics*

The regions of Rouyn-Noranda and Sudbury have been impacted by past and present metal mining and smelting activities, giving rise to lakes with a wide range of metal concentrations and pH values according to their location up- or down-wind of the smelters. Lakes Opasatica and Geneva are located upwind from the smelters and were chosen for their naturally low total dissolved metal concentrations and differing DOM concentrations (Table 1). The spectroscopic quality of the DOM was also found to differ between the two lakes (Mueller et al., 2012b); lake Opasatica was found to have DOM largely characteristic of allochthonous sources, whereas the characteristics of Lake Geneva DOM suggested a marked contribution from autochthonous sources. By titrating natural samples with low ambient total dissolved metal concentrations, fewer DOM binding sites are already occupied by metals and the strong binding sites, of low abundance, can be probed. Although both lakes have circumneutral pH values, Lake Geneva is slightly more acidic than Lake Opasatica. Lake Geneva also has a lower concentration of major cations (Ca, Mg, Na and K), major anions (Cl, NO_3, SO_4 and F), total dissolved metal (Cd, Cu, Ni and Zn) and DOC than Lake Opasatica (Table 1).

3.2 *Titration curves*

The titration curves for Ni and Cu complexation with natural ligands in Lakes Opasatica and Geneva are plotted in Figure 1. The complexation of Ni and Cu by inorganic ligands in the presence of DOM for the lakes sampled was estimated using WHAM. For Lake Opasatica, approximately 4.8-5.2% of total dissolved Ni and 1.7-3.9% of Cu was bound in carbonate, sulfate and chloride species. Although a similar 1.1-4.3% of total dissolved Cu was bound by these inorganic ligands for Lake Geneva, 44-47% of Ni was calculated to be present as inorganic complexes with these ligands. Therefore the concentration of bound metal to DOM, [ML], was assumed to be equal to the total dissolved metal concentration minus the concentration of free metal and that bound to inorganic complexes.

For a given total dissolved metal concentration, Lake Geneva had a higher free metal concentration than Lake Opasatica (Figure 1). Because Lake Geneva has a lower DOC concentration, presumably less DOM is available to bind Ni and Cu. In addition, at the (slightly) lower pH in Lake Geneva, the proton would be expected to compete somewhat more successfully for cation binding sites on the DOM. As Ni is known to be more weakly bound to DOM than Cu (Buffle, 1988), the effect of proton competition would be expected to be greater for Ni than for Cu. This was in fact observed for both lakes where the concentration of free Ni was generally higher than free Cu for a given total metal concentration. The presence of strong Cu-binding sites on the DOM is suggested by Figure 1B, where with the first few additions of total Cu result in little increase in the free Cu^{2+} concentration. This behaviour is characteristic of Cu filling up the strong DOM binding sites first, followed by the weaker sites (Sunda and Hanson, 1979).

Alternatively, the observed asymptote at low total Cu concentrations may be a result of reaching the detection limit of the Cu ISE method. In four of the eight lakes sampled in 2008 by Mueller et al. (2012b), i.e. in those with the lowest total ambient dissolved Cu concentrations (Opasatica, Vaudray, Bethel and Geneva), the measured free Cu^{2+} concentrations were higher than those predicted by WHAM, possibly suggesting that the measured concentrations were affected by dissolution of Cu from the ISE. However, when the free Cu^{2+} activity in solution is well buffered with organic ligands, an ISE detection limit as low as 10^{-14} M can be achieved (Rachou et al., 2007). In the present study, as well as in the Mueller et al. (2012c) study, the free Cu^{2+} activities in the calibration solutions were buffered with iminodiacetic acid (IDA) and with DOM in the natural lake water samples.

Table 1. Total dissolved concentrations of major cations, anions and trace metals for Lakes Opasatica and Geneva under IET and ISE experimental conditions

	Lake Opasatica		Lake Geneva	
	IET	**ISE**	**IET**	**ISE**
pH	7.69	7.71	7.02	6.97
DOC (mg/L)	6.92	7.47	3.36	3.54
Na (mM)	0.13	0.13	0.027	0.027
Mg (mM)	0.11	0.11	0.026	0.026
K (mM)	0.023	10	0.0070	10
Ca (mM)	0.46	0.21	0.46	0.066
Ni (nM)	16	16	22	23
Cu (nM)	39	38	11	13
Zn (nM)	33	20	31	12
Cd (nM)	0.062	0.050	0.012	0.053
Cl (µM)	89	89	7.0	7.0
NO_3 (mM)	0.93	10	0.93	10
SO_4 (mM)	0.058	0.058	0.044	0.044
CO_3 (mM)	0.52	0.52	0.05	0.05
F (µM)	2.6	2.6	2.8	2.8

The nickel and copper titration curves were simulated with WHAM VI and compared to the observed values (Figure 2). Early in the Ni titrations, i.e., for low Ni-DOM ratios, WHAM overestimated the free Ni^{2+} concentration in both lakes (Figures 2A and 2C), but for total dissolved Ni concentrations greater than about 10^{-6} M the agreement between observed and predicted values improved. In the case of Cu, the two lake waters behaved differently. For Lake Geneva, WHAM underestimated the free Cu^{2+} concentrations throughout the titration (Figure 2B), whereas for Lake Opasatica the agreement between WHAM predictions and the ISE measurements was reasonably good early in the titration (up to about $10^{-6.7}$ M Cu); for higher total dissolved Cu concentrations, however, WHAM again underestimated the free Cu^{2+} concentrations (Figure 2D).

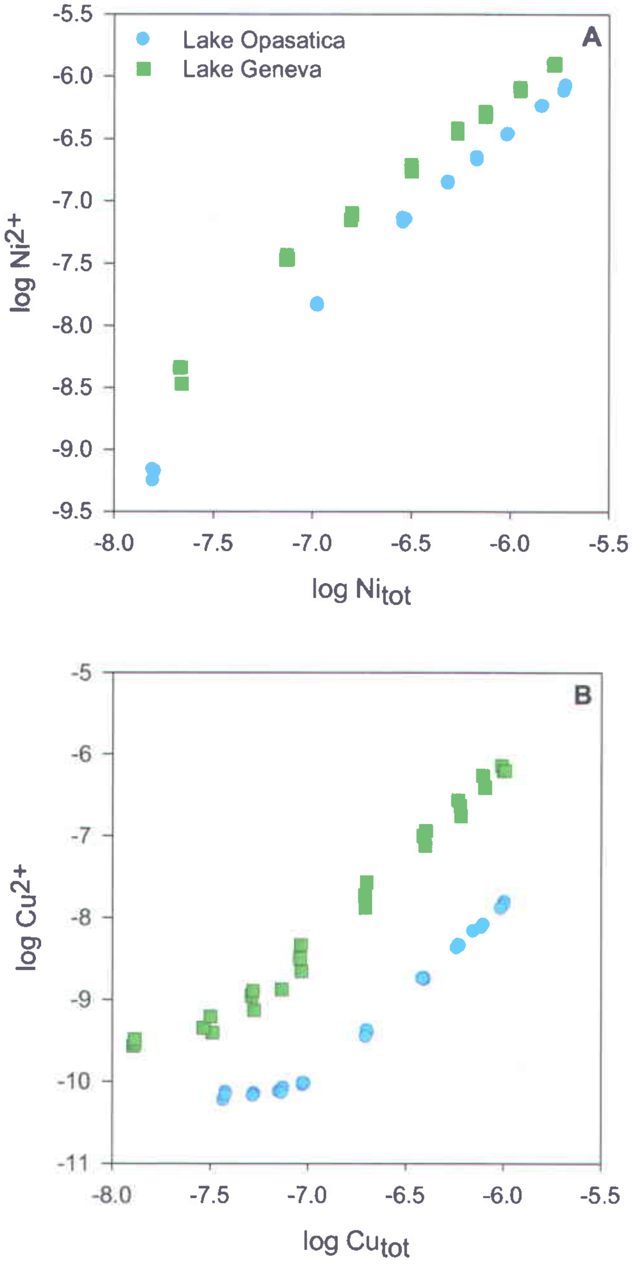

Figure 1. Comparison of titration curves for Ni (A) and Cu (B), for Lake Opasatica (circles) and Lake Geneva (squares).

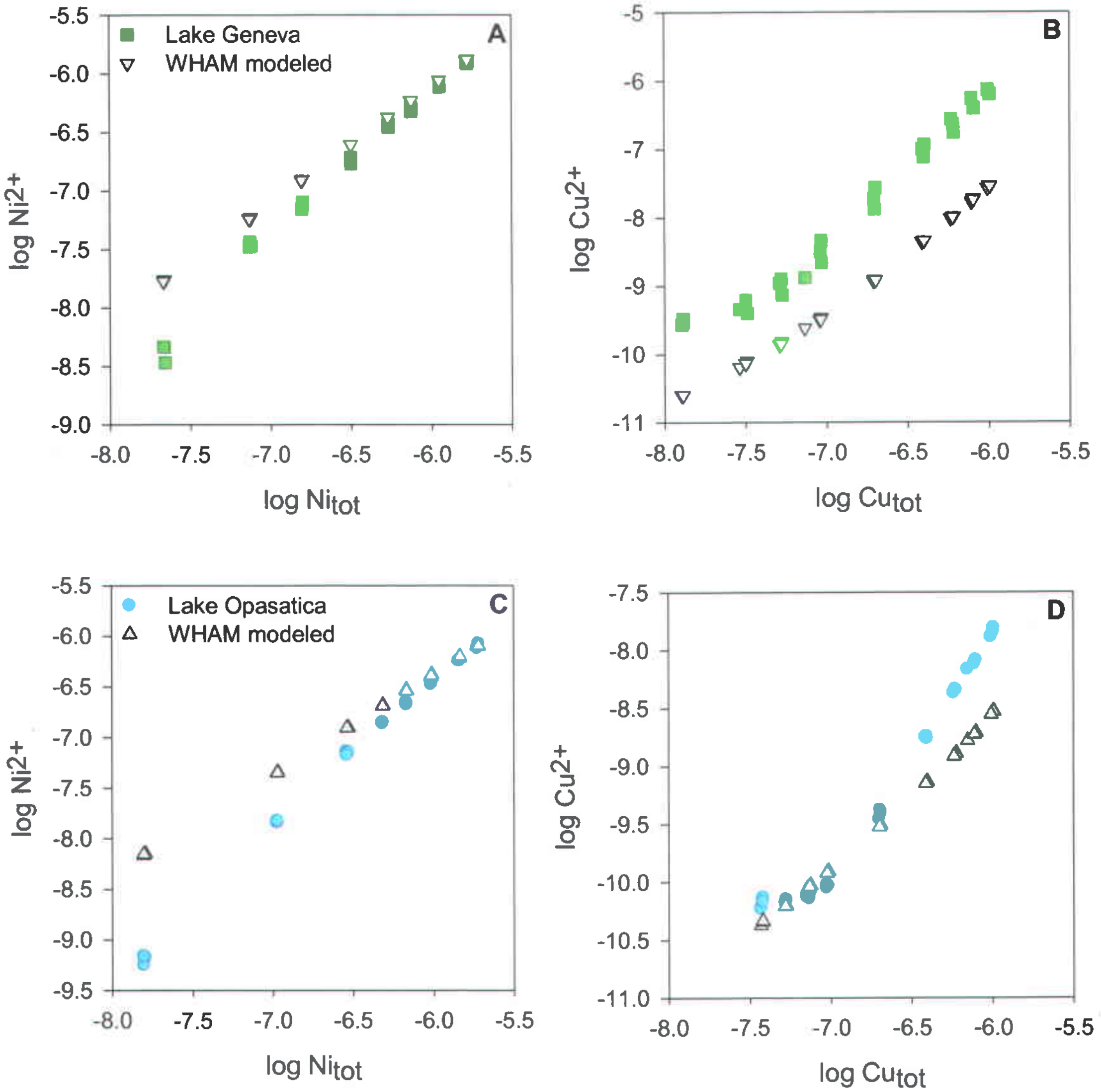

Figure 2. Nickel (A and C) and copper (B and D) titration curves for Lake Geneva (A and B) and Lake Opasatica (C and D). Measured values are shown in colour; values calculated with WHAM VI are shown in gray triangles.

3.3 Metal binding parameters

The complexation curves for Ni and Cu with natural ligands in Lake Geneva and Opasatica are presented in Figure 3 and their associated calculated binding parameters can be found in Table 2. The binding parameters were derived using a two-site Langmuir-type model (Tipping, 2002). It should be noted that a successful fit using two or more discrete sites does not constitute evidence for the true existence of such sites. As pointed out by Tipping, it simply means that within a given range of conditions ([DOM], pH, pM), the effects of site heterogeneity can be accounted for to some acceptable degree.

For the range of free Ni concentrations in the titrations, the concentrations of bound Ni (M) normalized to the concentration of DOC (g/L) are slightly higher in Lake Geneva than in Lake Opasatica (Figure 3A). The calculated binding parameters reflect the results of the titration curves; the Ni binding affinity of the weak sites (Log $K_{1,Ni} = 6.98$) on DOM from Lake Opasatica is significantly higher ($P = 0.009$) than for Lake Geneva (Log $K_{1,Ni} = 6.30$). In contrast, the concentration of these weak sites ($L_{1,Ni} = 34.2$ nmole·mg C^{-1}) is nearly four times lower ($P = 0.01$) than in Lake Geneva ($L_{1,Ni} = 137$ nmole·mg C^{-1}) (Table 2). The Ni binding affinities for the strong sites in both lakes are not significantly different (Log $K_{2,Ni} = 11.6$ and 11.7 for Lakes Opasatica and Geneva, respectively), although there are nearly six times less ($P = 0.025$) of these binding sites per unit DOC in Lake Opasatica ($L_{2,Ni} = 0.70$ nmole·mg C^{-1}), compared to the DOM from Lake Geneva ($L_{2,Ni} = 3.98$ nmole·mg C^{-1}). These results point to the difference between the DOM in Lakes Opasatica and Geneva, with respect to their Ni-binding characteristics, discussed further in section 3.4.

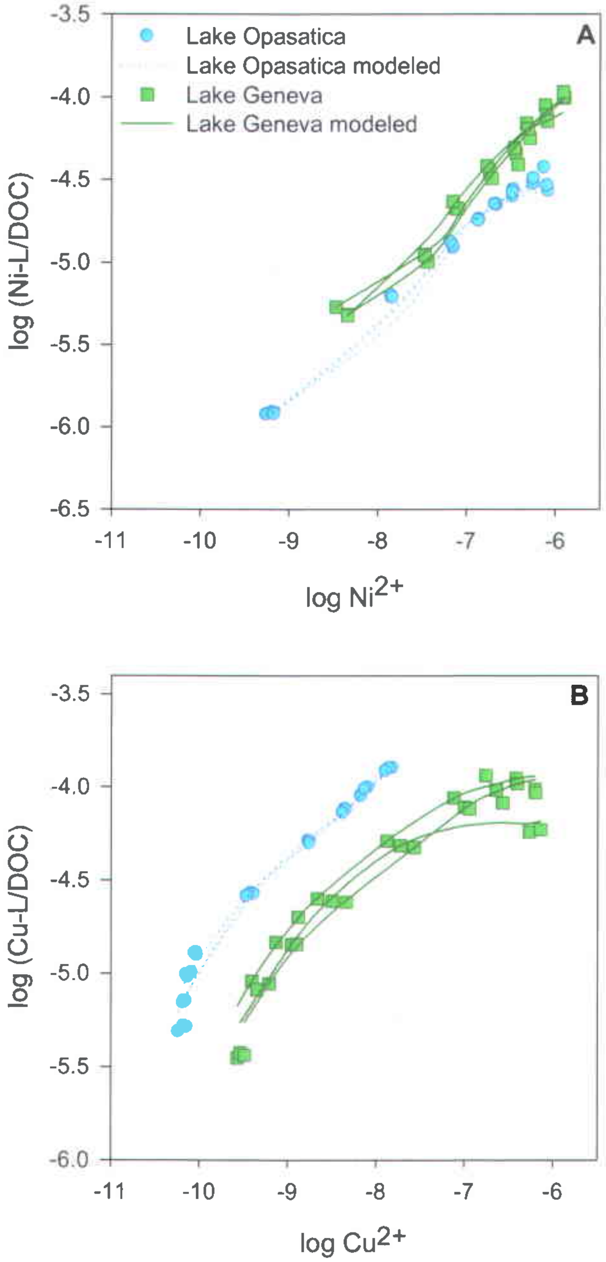

Figure 3. Comparison of complexation curves for Ni (A) and Cu (B), for Lake Opasatica (circles) and Lake Geneva (squares). The modelled curves were calculated with the two-site Langmuir binding model (see text for details).

Table 2. Conditional stability constants (log K_i) and carbon normalized site densities (L_i, µmole/g C) of Ni and Cu complexation by DOM for Lakes Opasatica and Geneva modeled using a 2-site Langmuir model. The statistical significance (*P*) values for the non-linear regressions ranged from 0.001 to 0.024.

	Lake Opasatica	**Lake Geneva**
IET		
Log $K_{1,Ni}$	6.98 ± 0.12	6.30 ± 0.23
$L_{1,Ni}$ (nmole·mg C^{-1})	34.2 ± 3.2	137 ± 24
Log $K_{2,Ni}$	11.6 ± 0.6	11.7 ± 0.5
$L_{2,Ni}$ (nmole·mg C^{-1})	0.70 ± 0.40	3.98 ± 0.11
ISE		
Log $K_{1,Cu}$	7.59 ± 0.12	7.63 ± 0.46
$L_{1,Cu}$ (nmole·mg C^{-1})	244 ± 10	75.6 ± 19.4
Log $K_{2,Cu}$	9.46 ± 0.13	8.90 ± 0.04
$L_{2,Cu}$ (nmole·mg C^{-1})	44.5 ± 7.4	27.0 ± 8.1

A higher concentration of Cu was bound per gram of DOC in Lake Opasatica than in Lake Geneva, at all free Cu^{2+} concentrations (Figure 3B). Furthermore, for Lake Geneva, the concentration of bound Cu appears to tend towards an asymptote with an increase in the concentration of free Cu^{2+}, indicating binding site saturation. Although the strength of the binding of Cu to weak sites on the DOM was not significantly different for both lakes (Log $K_{1,Cu}$ = 7.59 for Lake Opasatica and Log $K_{1,Cu}$ = 7.63 for Lake Geneva), the number of L_1 sites per unit DOC was approximately three times higher ($P < 0.001$) in Lake Opasatica ($L_{1,Cu}$ = 244 nmole·mg C^{-1}) than in Lake Geneva ($L_{1,Cu}$ = 75.6 nmole·mg C^{-1}). There were significantly ($P = 0.014$) stronger (Log $K_{2,Cu}$ = 9.46) and twice as many ($P = 0.003$) strong binding Cu sites per unit DOC ($L_{2,Cu}$ = 44.5 nmole·mg C^{-1}) on the DOM from Lake Opasatica compared to Lake Geneva (Log $K_{2,Cu}$ = 8.90 and $L_{2,Cu}$ = 27.0 nmole·mg C^{-1}). Clearly these results for Ni and Cu complexation demonstrate that the DOM in Lake Opasatica differs from that in Lake Geneva, with respect to their affinity for these metals and their complexation capacity. These differences are explored in section 3.4.

We compared our calculated concentrations of bound metal as a function of the measured free metal concentration to those predicted by WHAM. Throughout the entire Ni^{2+} concentration range, the measured amount of bound Ni in both lakes is much higher than that predicted by WHAM (Figures 4A and 4C). A contrasting trend was observed for Cu. The concentration of bound Cu predicted by WHAM is very similar to that measured for Lakes Opasatica and Geneva, over nearly the entire free Cu^{2+} concentration range (Figures 4B and 4D). Only at free Cu^{2+} concentrations above 10^{-7} M do the WHAM predictions diverge from the measured values, in Lake Geneva, where the amount of bound Cu predicted by WHAM is higher than that measured.

Note that since the binding of Cu to DOM is much more important than is the binding of Ni, the plots of bound metal ([M-DOM]) versus $[M^{2+}]$ for Cu appear to be very close to the WHAM predictions. The earlier plots of $[M^{2+}]$ versus $[M]_{dissolved}$, are more sensitive and more useful for comparing predicted and measured values (e.g., compare Figures 3A and 5A). In addition, from an ecotoxicological perspective, the free metal ion concentration is much more important than is the concentration of bound metal. From this point of view, the type of DOM involved in metal complexation, i.e., the contrast between Lakes Geneva and Opasatica, is important for both Cu and Ni.

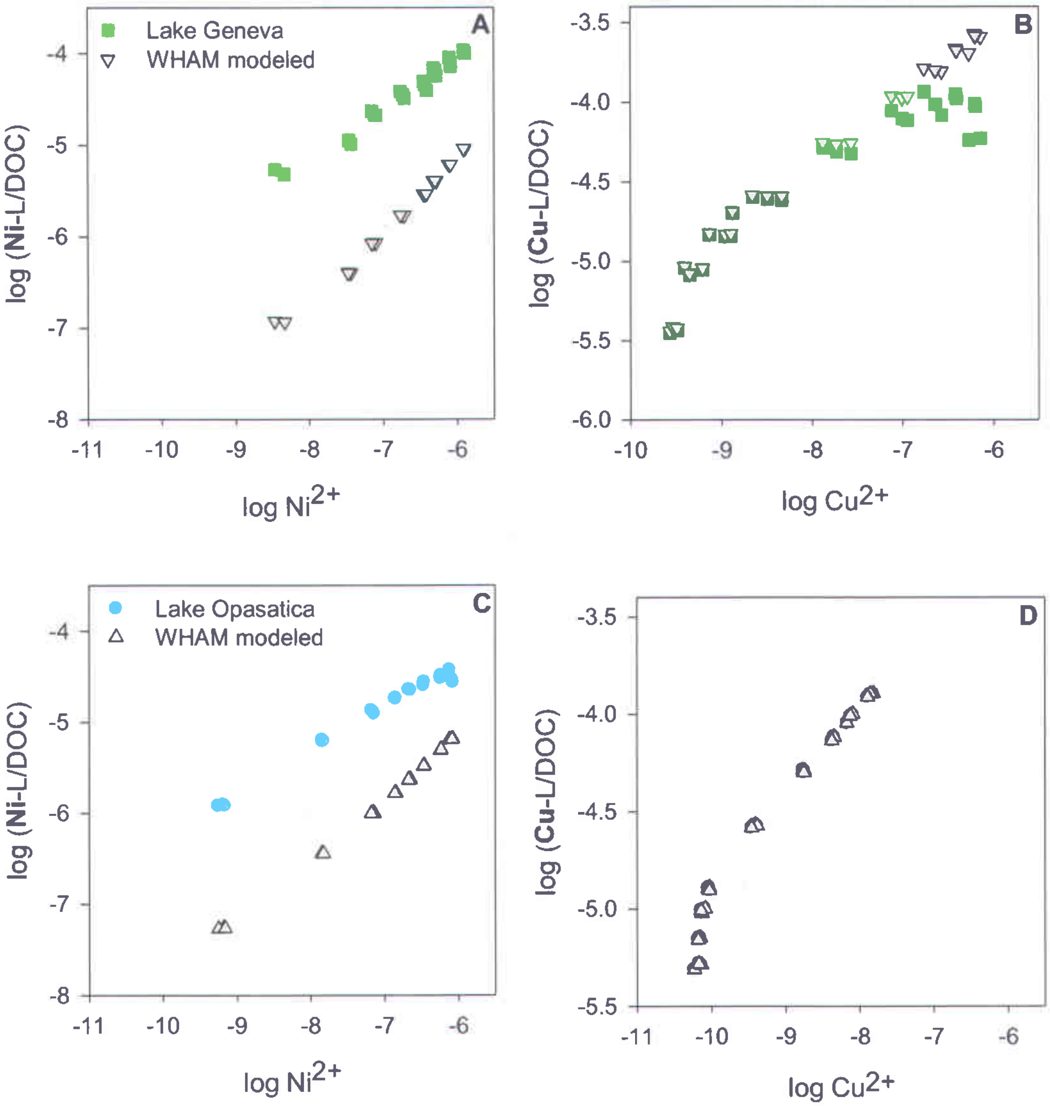

Figure 4. Normalized Ni (A and C) and Cu (B and D) complexation curves (Ni-DOM/DOC) for Lake Geneva (A and B) and Lake Opasatica (C and D). Measured values are shown in colour; values calculated with WHAM VI are shown in gray triangles.

Although the calculated Ni and Cu binding parameters are conditional and apply only to the experimental conditions under which they were determined, it is useful to compare them to other parameters found in the literature. Unfortunately there are few studies that report the measured binding properties of metals to natural organic matter, as opposed to isolated humic substances, in freshwater systems (e.g. (Antelo et al., 1998; Sunda and Hanson, 1979; Xue and

Sunda, 1997)). In natural lake waters and at low, environmentally significant concentrations, Ni was found to bind to strong ligands with log K values from 12 to 14 (Xue et al., 2001). Xue and Sigg (1999) reported similarly strong binding of Cu to natural ligands in lake water (log K values from 10 to 16). It has, however, been suggested that these values calculated from competitive ligand exchange-adsorptive stripping voltammetry (CLE-AdSV) measurements under-estimated the free metal concentrations and therefore over-estimated the complexation of metals with natural ligands (Van Leeuwen and Town, 2005). Nevertheless, it is apparent that over a wide range of Ni concentrations in natural waters WHAM overestimates the free Ni^{2+} concentration (Figures 2A and 2C; see also Mueller et al. (2012c) and Van Laer et al. (2006)). The opposite trend is observed for Cu, i.e., an underestimate of the free Cu^{2+} concentration, but the absolute differences between measured and predicted values are smaller than for Ni^{2+}, and in addition the differences are more important in Lake Geneva than in Lake Opasatica (Figures 2B and 2D), suggesting that the binding of Cu to natural ligands may depend of the type of DOM present.

3.4 Relationship between metal binding and DOM characteristics

For the ambient conditions prevailing in the two lakes, Lake Opasatica DOM was found to bind more Ni and Cu than Lake Geneva DOM (Figure 1). When the concentration of metal binding sites was normalized to the total organic carbon concentration, Lake Opasatica DOM had a higher apparent number of metal binding sites for Cu but a lower number of sites for Ni (Table 2). The conditional stability constants also differed between the two lakes. Based on the spectroscopic quality of the DOM sampled from the same lakes in 2007 and 2008, Mueller et al. (2012b) found Lake Opasatica to have DOM largely characteristic of allochthonous sources, whereas the characteristics of Lake Geneva DOM suggested a marked contribution from autochthonous sources. This was evidenced by a higher specific UV absorbance at 254 nm ($SUVA_{254}$ values), a higher relative proportion in Lake Opasatica DOM of the PARAFAC fluorescence component attributed to reduced-quinone, humic-like fluorophores of allochthonous origin, and a lower proportion of the component attributed to tryptophan-like protein fluorescence of autochthonous origin. The authors supported their finding with watershed characteristic data that showed that Lake Opasatica lies in a region with a thicker soil layer and has a much larger watershed-to-lake surface area ratio than Lake Geneva, contributing to higher inputs of DOM from the lake's watershed as opposed to being produced within the lake itself. These results suggest that the more aromatic humic-like DOM from allochthonous sources may have a higher complexation capacity for Cu than does the protein-like DOM from autochthonous sources, whereas for Ni the opposite trend is apparent. A positive relationship between the Ni and Cu binding affinity and the optical quality ($SUVA_{254}$) was also found for natural surface waters by Baken et al. (2011). Similar positive relationships for Cu have been reported by Al-Reasi et al. (2012). This is, however, the first time that the parallel factor analysis (PARAFAC) of the fluorescence character of DOM has been compared to its metal binding affinity and capacity.

3.5 Environmental implications

Currently, chemical equilibrium models, such as WHAM, are used to estimate the binding of cations by DOM in natural aquatic systems (Tipping, 2002). These models are, however, calibrated using the results of titration experiments on isolated and purified humic and fulvic acid at high DOC and total metal concentrations that are often environmentally unrealistic for natural aquatic systems. Here we demonstrate, for low, environmentally realistic concentrations, that the conditional binding parameters for the complexation of Ni and Cu with natural DOM vary as a function of the quality of the DOM, as revealed by previous fluorescence measurements.

This result has important consequences for those interested in estimating the speciation of these trace metals in natural aquatic systems. For example, the Biotic Ligand Model (BLM), used to predict the toxicity of trace metals to aquatic organisms, incorporates WHAM to calculate metal speciation in the exposure medium (Paquin et al., 2002). Improved binding parameters for Ni and Cu would improve the prediction of the bioavailability and toxicity of these metals at low, chronic level exposures.

Acknowledgments

The authors acknowledge the technical assistance provided by M.G. Bordeleau, S. Duval, P. Fournier and J. Perreault in the laboratory. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada and by the Metals in the Human Environment Research Network (www.mithe-rn.org). P.G.C. Campbell and C. Fortin are supported by the Canada Research Chair Program.

Troisième partie : Annexes

Appendix A: Effect of sample storage on metal speciation and DOM quality / Annexe A : Effet de l'entreposage des échantillons sur la spéciation de métaux et la qualité de la MOD

1 Introduction

Once a natural aqueous sample is taken from the environment, possible changes in trace metal speciation may occur due to abiotic changes (e.g. pH, adsorption and chemical reactions) or biotic changes (e.g. microbial activity) occurring in the sample during storage. Although very few studies on the stability of samples during storage can be found in the literature, filtration through 0.2-µm filters coupled with storage at 4°C and in the dark is generally accepted as a means to minimize these changes (Batley et al., 2004)[5]. Here we tested the effect of sample storage on trace metal speciation and DOM spectroscopic quality. For this experiment we identified two lakes (Lake Geneva and Lake Vaudray) that exhibit marked differences in DOM (quantity and quality) and in total and free metal concentrations.

2 Methods

Large volume (10 L, polypropylene, Nalgene) surface water samples were taken from lakes Geneva and Vaudray in July 2009, returned to the lab and sequentially filtered through a series of acid-cleaned polycarbonate filters (5 µm, 2 µm, 0.4 µm and 0.2 µm; AMD Manufacturing Inc.). Subsamples were taken for analyses of pH, major ion concentrations, DOM optical properties (absorbance and fluorescence) as well as total and free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} concentrations immediately after filtration and at regular intervals (approximately 3 months) over an 8-month period. Between sample analyses, samples were stored in the dark at 4°C, as was the case for the lake water samples collected with the *in situ* diffusion samplers.

For a more detailed description of the sample analyses, the reader is referred to Mueller et al. (Mueller et al., 2012a; Mueller et al., 2012b; Mueller et al., 2012c) found in Part II of the thesis. Briefly, subsamples were taken for determination of total metal concentrations. The concentrations of Cd^{2+}, Ni^{2+} and Zn^{2+} in the lake samples were also measured using an ion

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[5] All references can be found on page 205 of the thesis.

exchange technique (IET), whereas the concentration of Cu^{2+} was measured using a cupric ion selective electrode (ISE). Finally, dissolved organic carbon (DOC) concentration, as well as absorbance ($SUVA_{254}$) and fluorescence (FI and PARAFAC components) measurements were also made.

The following statistical analyses were calculated at the alpha=0.05 confidence level using SigmaPlot 11.0 (Systat Software Inc., Chicago, IL). Linear regressions and one-way ANOVA's with pairwise multiple comparison procedures using the Holm-Sidak method were used when the normality and homogeneity of variances among datasets were confirmed; otherwise, the appropriate non-parametric tests were employed (Kruskal-Wallis one-way ANOVA on Ranks with pairwise multiple comparison procedures using Dunn's method, instead of a parametric ANOVA). All proportional data were arcsine square root transformed before performing these statistical tests. Dates were converted to Julian days.

3 Results and discussion

Statistically significant differences ($P<0.05$) in the total metal concentrations and relative proportion the free metal ion to the total metal concentration as a function of time were found for some metals in both lakes (Figures A1 and A2). Statistically significant, although small, increases in the relative proportion of the free metal ion to the total metal concentration over 8 months were found for all metals in both lakes except for Zn in Lake Vaudray and Cu in Lake Geneva (Table A1). It should be noted that the total and free Cd concentrations for Lake Geneva at the two final time points were below the detection limits of the measurements.

No statistically significant differences ($P>0.05$) in DOC concentrations or any DOM spectroscopic parameters were found for Lake Geneva. However, significant differences ($P<0.05$) in DOC concentration, $SUVA_{254}$ and $C3/C_T$ as a function of time were noted for Lake Vaudray (Figure A3). Despite the variability in these parameters, no statistically significant trends in the spectroscopic quality of the DOM sampled from Lakes Vaudray and Geneva as a function of time were observed (Table A1).

Although some variability in the total dissolved metal concentrations, the free metal ion concentrations and DOM spectroscopic quality was observed over the 8-month sample storage time, the temporal trends (when observed) were modest. The Ni^{2+} concentration appeared to change more over time than did the other free metal ion concentrations (Figure A2), which again may point to the binding of Ni to different sites or different ligands within the DOM mosaic. We speculate that these Ni-binding sites may be more labile or susceptible to change over time than the ligands involved in complexing the other metals. Despite the possible liberation of Ni^{2+} ions as ligands are degraded over time, our measured free Ni^{2+} concentrations were, nevertheless, much lower than those values predicted by WHAM. We therefore have confidence that there was no significant bias, due to sample degradation, in the trace metal speciation and DOM quality results reported in the thesis.

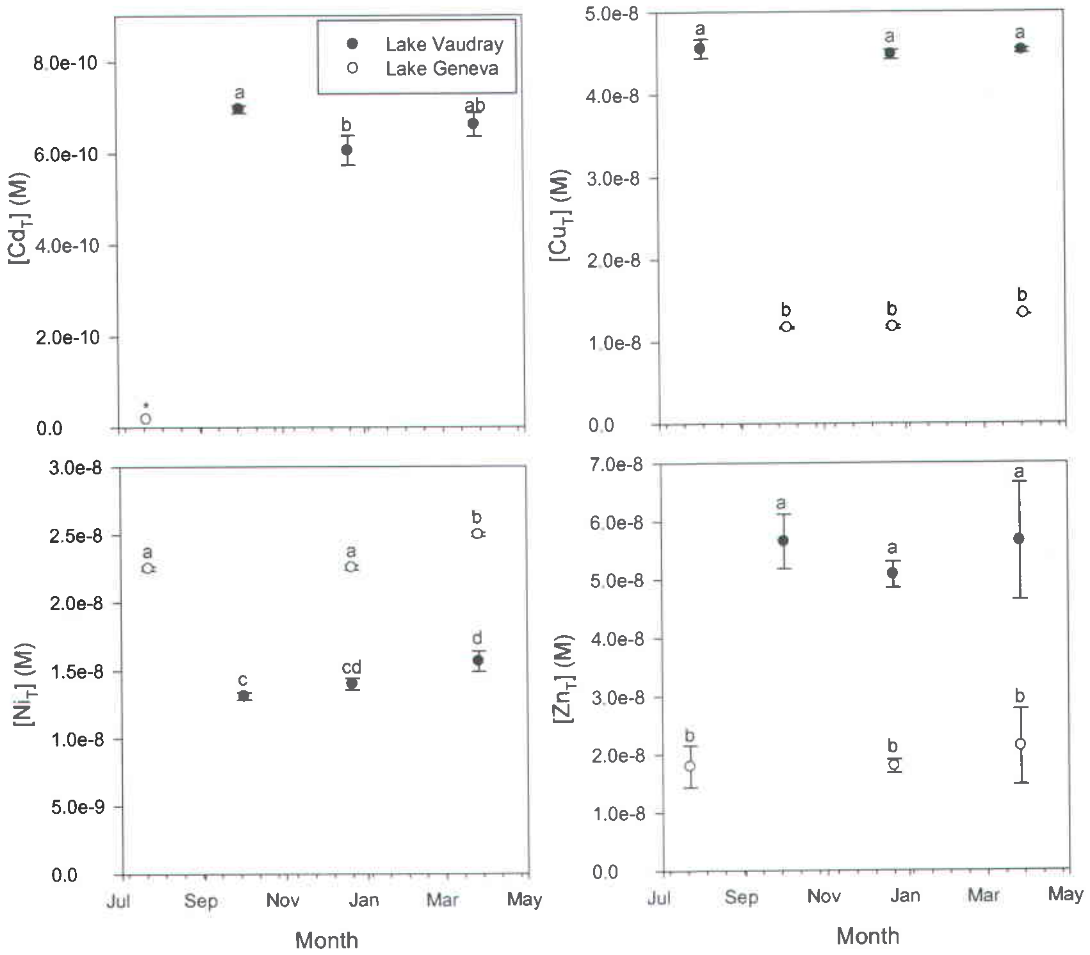


Figure A1. Changes in total metal (M_T) concentrations in filtered samples of lakes Vaudray (filled circles) and Geneva (open circles) over time (2009-2010). Points with similar letters (a-d) indicate no significant difference ($P>0.05$) between the points. An asterisk indicates only one replicate above the detection limit / Changement dans les concentrations totales de métaux (M_T) dans les échantillons filtrés des lacs Vaudray (cercles pleins) et Geneva (cercles ouverts) en fonction du temps (2009-2010). Les lettres identiques (a-d) indiquent qu'il n'y a pas de différence significative ($P>0.05$) entre les points. Le symbole * dénote un réplica dont les concentrations sont au-delà de la limite détection.

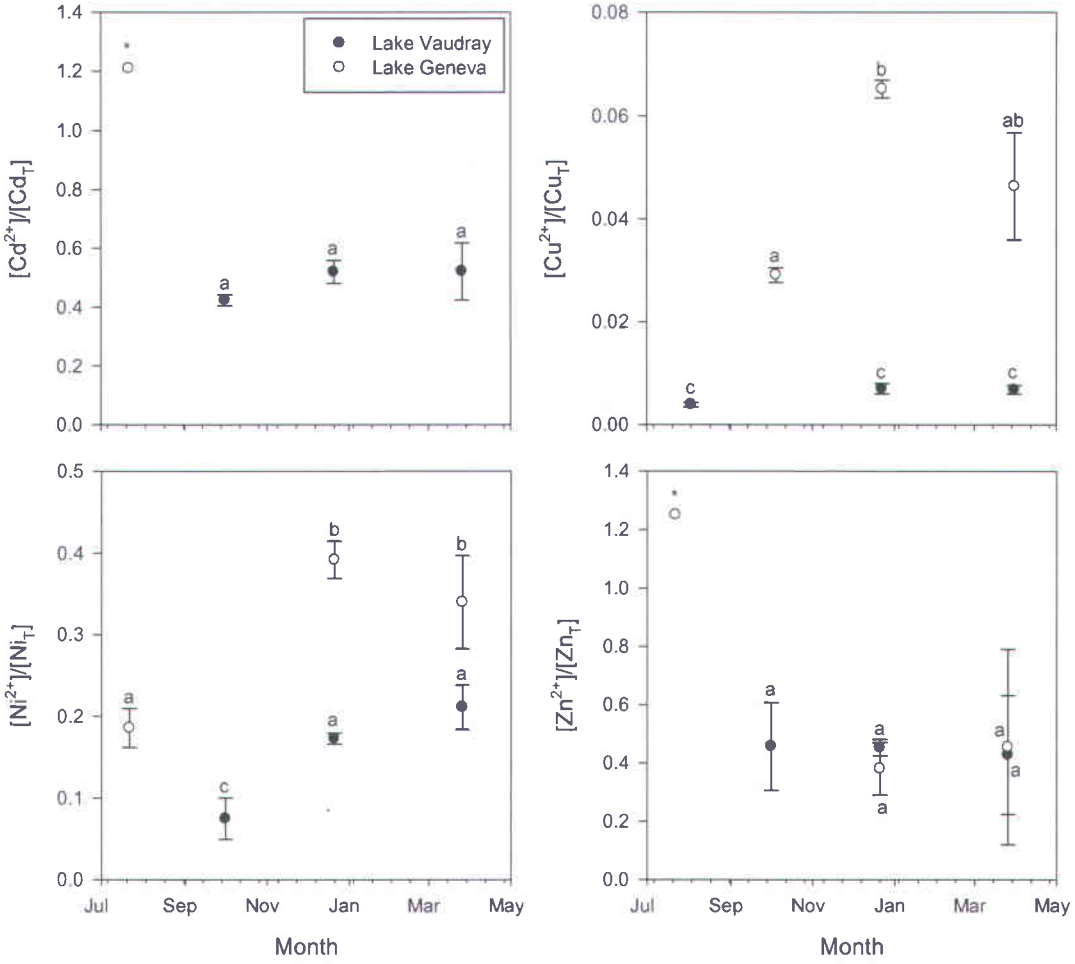


Figure A2. Changes in the ratio of the free metal Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} to their total metal (M_T) concentrations in filtered samples of lakes Vaudray (filled circles) and Geneva (open circles) over time (2009-2010). Points with similar letters (a-c) indicate no significant difference (P>0.05) between the points. An asterisk indicates only one replicate above the detection limit / Évolution du rapport entre les concentrations de métaux libres Cd^{2+}, Cu^{2+}, Ni^{2+} et Zn^{2+} et les concentrations de métal totale (M_T) dans échantillons filtrés des lacs Vaudray (cercles pleins) et Geneva (cercles ouverts) au cours du temps (2009-2010). Les lettres identiques (a-c) indiquent qu'il n'y a pas de différence significative (P>0.05) entre les points. Le symbole * dénote un répliqua dont les concentrations sont au-delà de la limite détection.

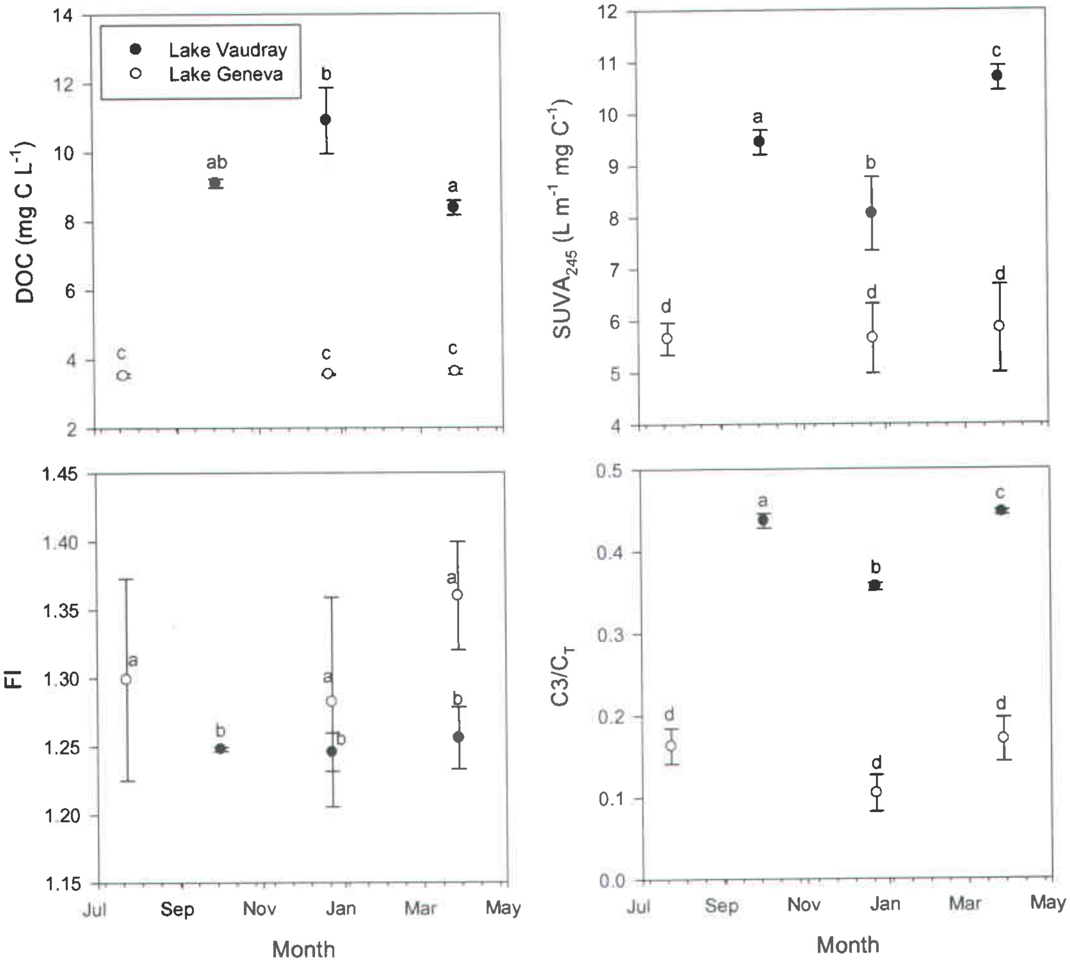


Figure A3. Changes in the DOC concentration and the DOM spectroscopic properties, SUVA$_{254}$, FI and C3/C$_T$, in filtered samples of lakes Vaudray (filled circles) and Geneva (open circles) over time (2009-2010). Points with similar letters (a-d) indicate no significant difference (P>0.05) between the points / Évolution des concentrations de COD et des propriétés spectroscopiques de la MOD (SUVA$_{254}$, FI et C3/C$_T$) dans des échantillons filtrés des lacs Vaudray (cercles pleins) et Geneva (cercles ouverts) au cours du temps (2009-2010). Les lettres identiques (a-d) indiquent qu'il n'y a pas de différence significative (P>0.05) entre les points.

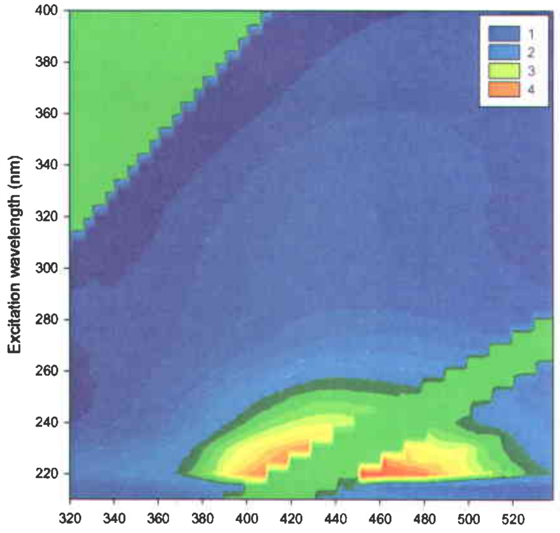
Table A1. Regression statistics (regression coefficient R^2; F statistic, *P* value) for changes in metal speciation and DOM quality in filtered samples of lakes Vaudray and Geneva over the 8-month sample storage time. *P* value less than 0.05 indicates a slope not equal to zero. NA indicates not enough data to perform the test / Régression statistique (coefficient de régression R^2, statistique F et valeurs de *P*) pour les changements de la spéciation des métaux et de la qualité de la MOD dans des échantillons filtrés des lacs Vaudray et Geneva pendant 8 mois d'entreposage. Les valeurs de *P* plus petite que 0.05 indiquent une pente non égale à zéro, tandis que le symbole NA indique qu'il n'y avait pas assez de données disponibles pour effectuer le test statistique.

	Lake Vaudray			**Lake Geneva**		
	R^2	F	*P*	R^2	F	*P*
Cd^{2+}/Cd_T	0.417	5.713	0.044	NA	NA	NA
Cu^{2+}/Cu_T	0.720	20.527	0.002	0.202	1.774	0.225
Ni^{2+}/Ni_T	0.807	29.311	<0.001	0.642	14.357	0.005
Zn^{2+}/Zn_T	0.031	0.253	0.629	0.587	7.110	0.045
DOC	0.084	0.640	0.450	0.290	2.857	0.135
$SUVA_{254}$	0.225	2.029	0.197	0.015	0.105	0.755
FI	0.057	0.423	0.536	0.117	0.924	0.368
$C3/C_T$	0.016	0.115	0.744	0.001	0.009	0.928

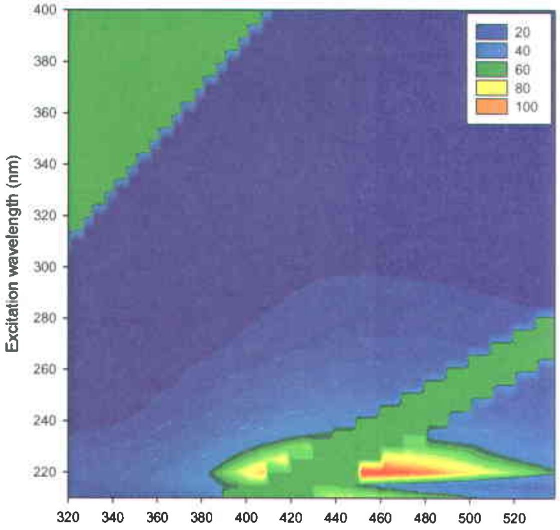
Appendix B: Lake DOM spectroscopic quality results / Annexe B : Résultats de l'analyse spectroscopique de la MOD lacustre

The following section is a compilation of the spectroscopic results for the eighteen lakes sampled from the regions of Rouyn-Noranda, QC and Sudbury, ON. Figure B1 consists of the excitation-emission matrix (EEM) fluorescence spectra for the lakes sampled in 2007 (presented on the left side of the page) and for the lakes sampled in 2008 (presented on the right side of the page). Table B1 consists of all of the spectroscopic results calculated for the lakes sampled.

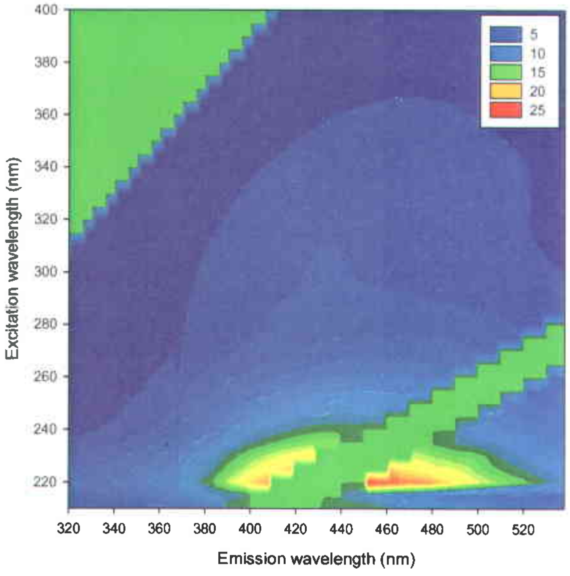
Lake Adeline 2007

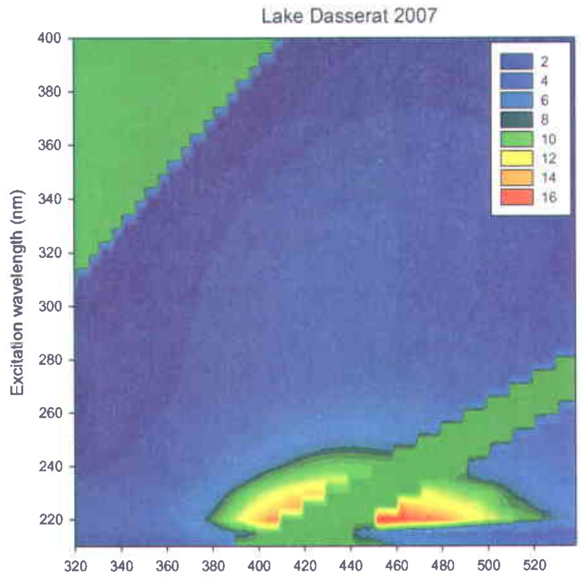


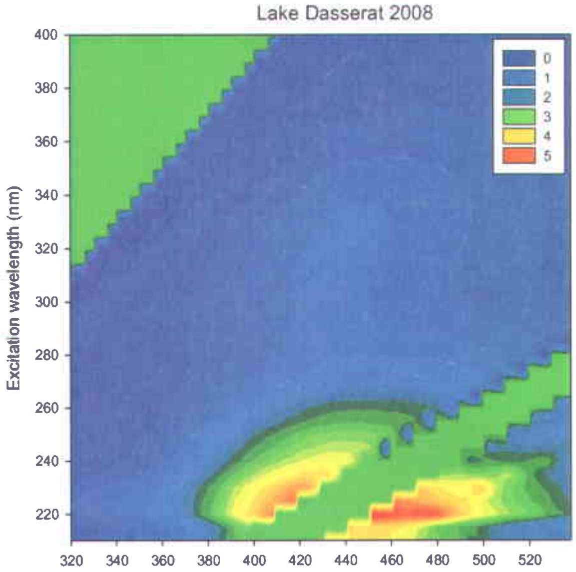
Lake Bousquet 2007

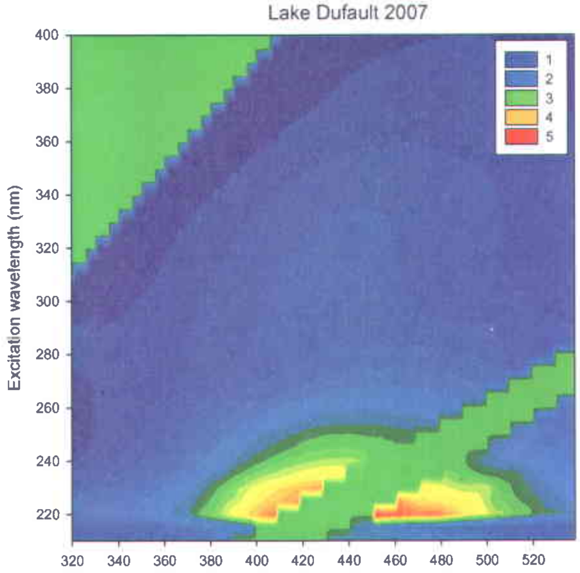


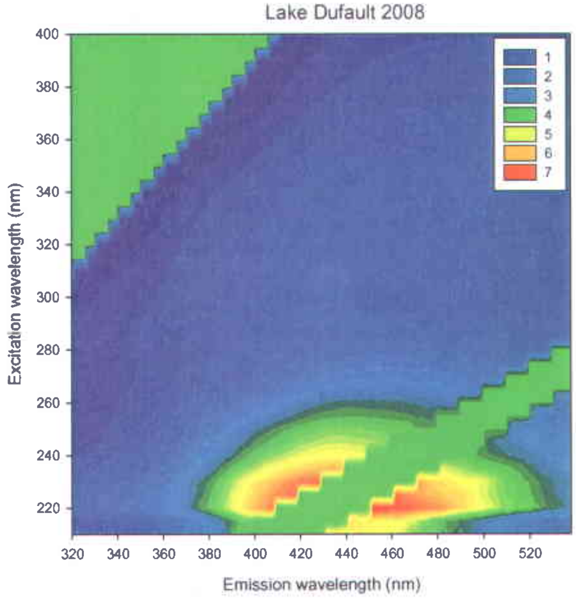
Lake D'Alembert 2007

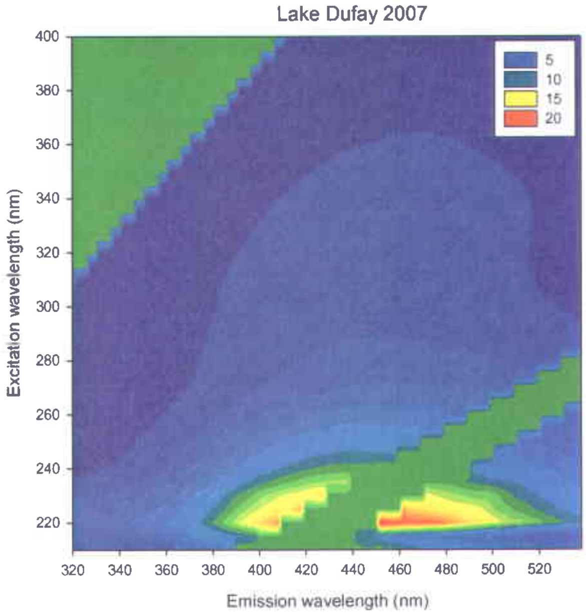



Lake Dasserat 2007

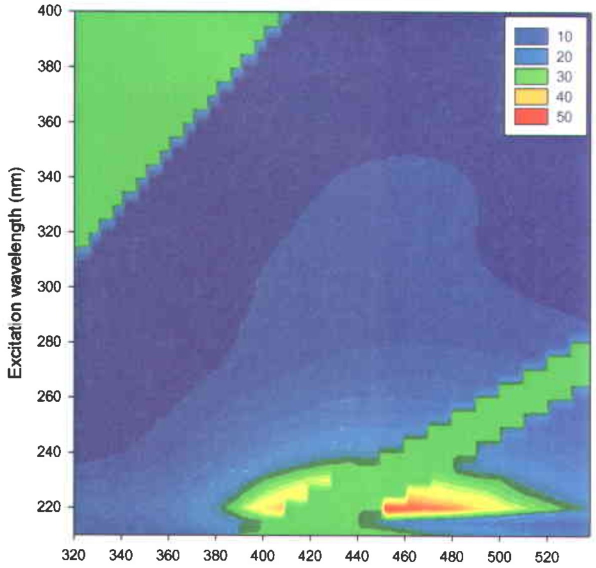

Lake Dasserat 2008


Lake Dufault 2007

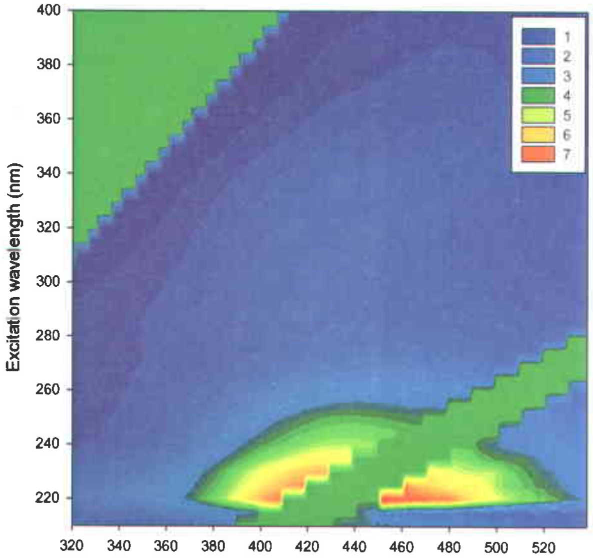

Lake Dufault 2008


Lake Dufay 2007

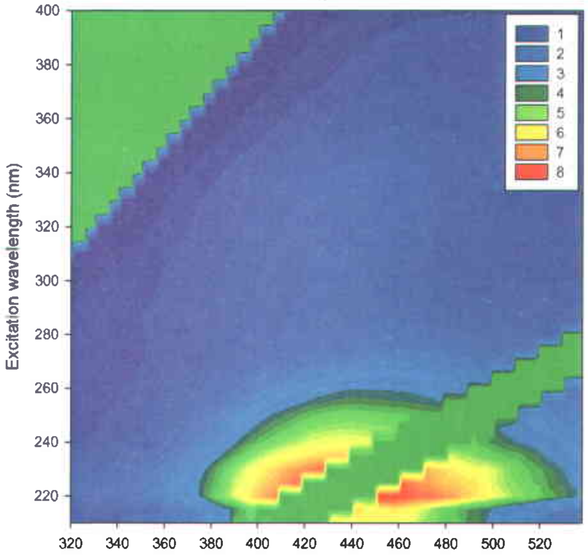
Lake Joannès 2007



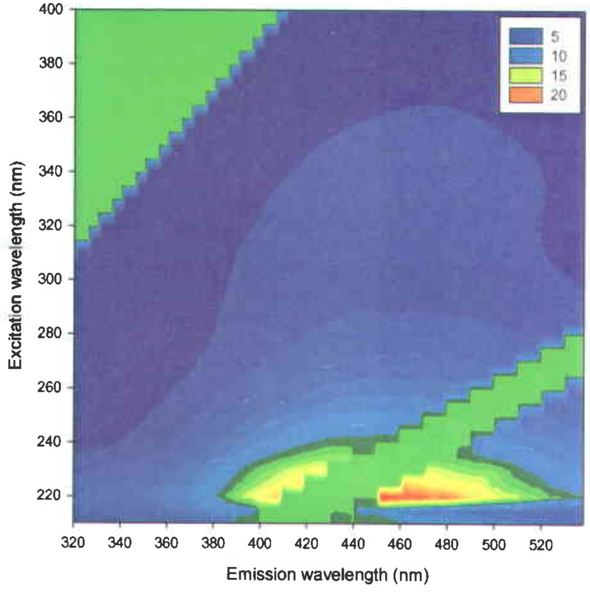
Lake Opasatica 2007



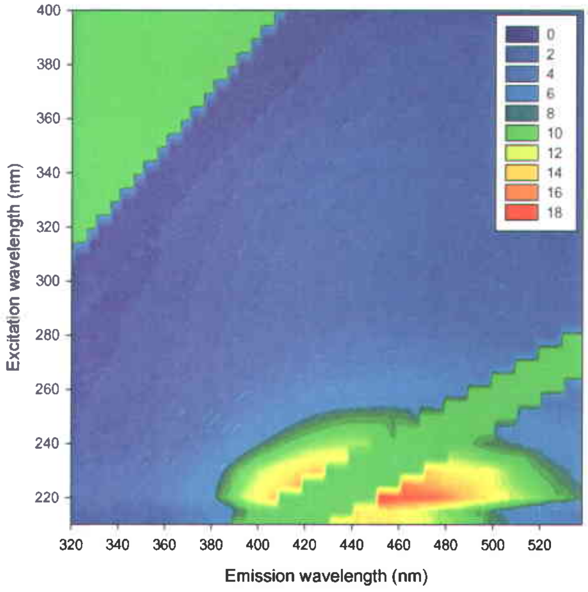
Lake Opasatica 2008



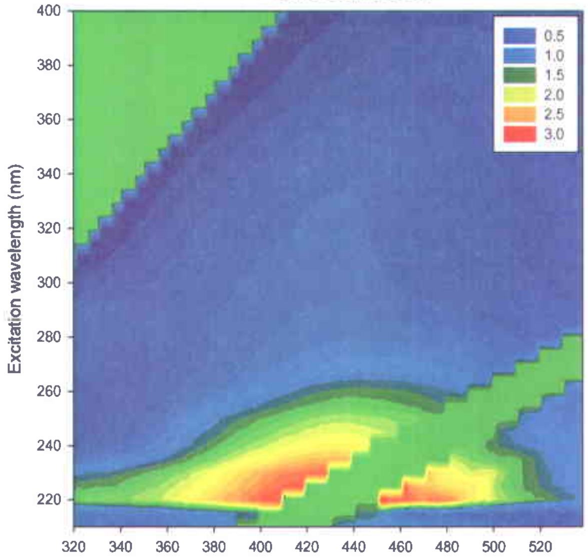
Lake Vaudray 2007



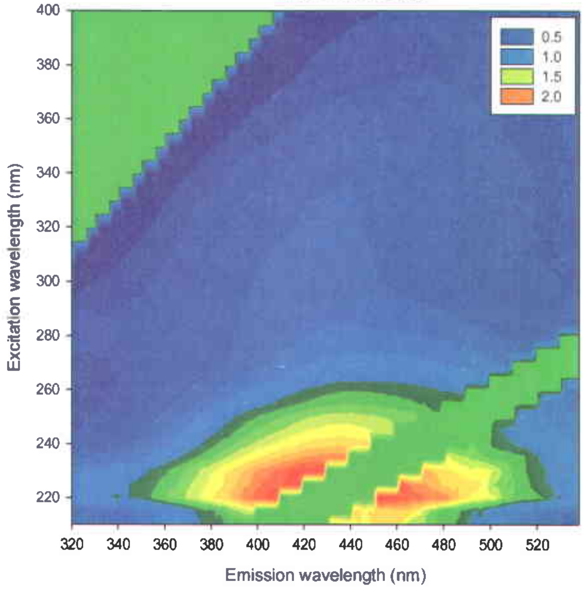
Lake Vaudray 2008



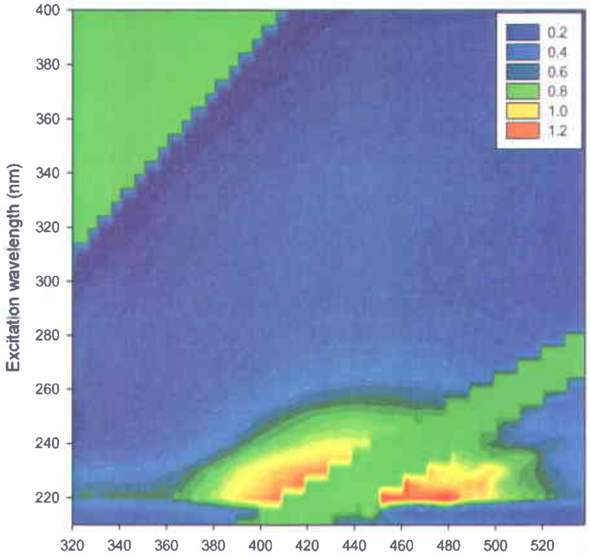
Lake Bethel 2007



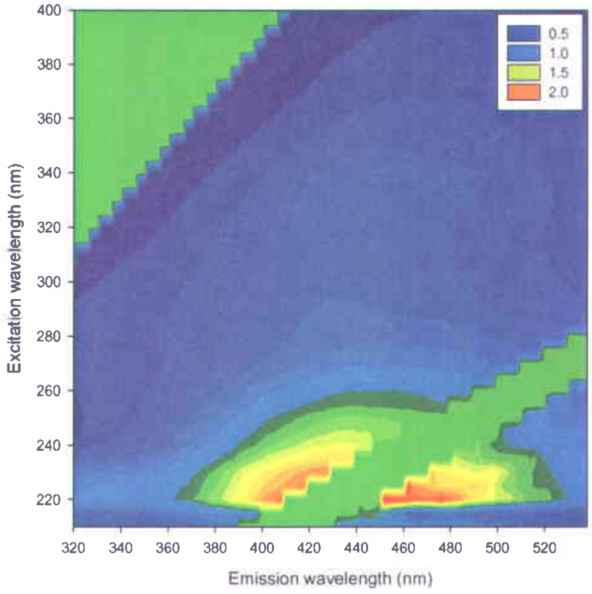
Lake Bethel 2008



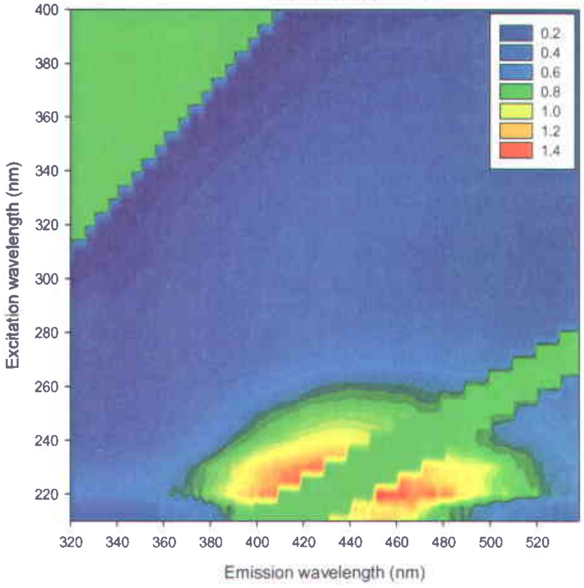
Lake Crowley 2007

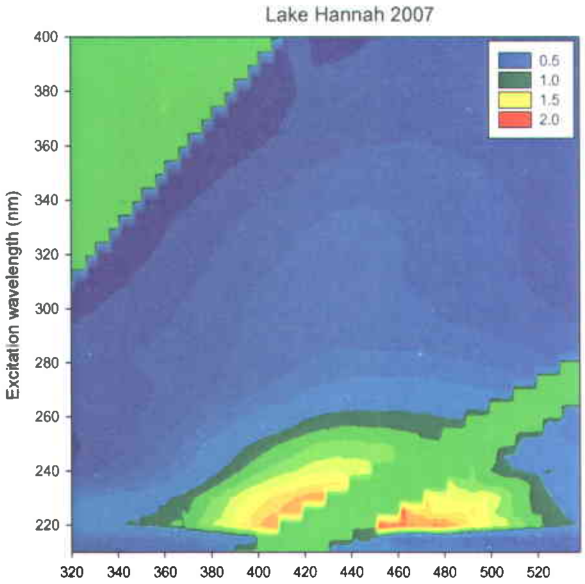


Lake Geneva 2007



Lake Geneva 2008




Lake Hannah 2007
0.5
1.0
1.5
2.0
Excitation wavelength (nm)
400
380
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340
320
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440
460
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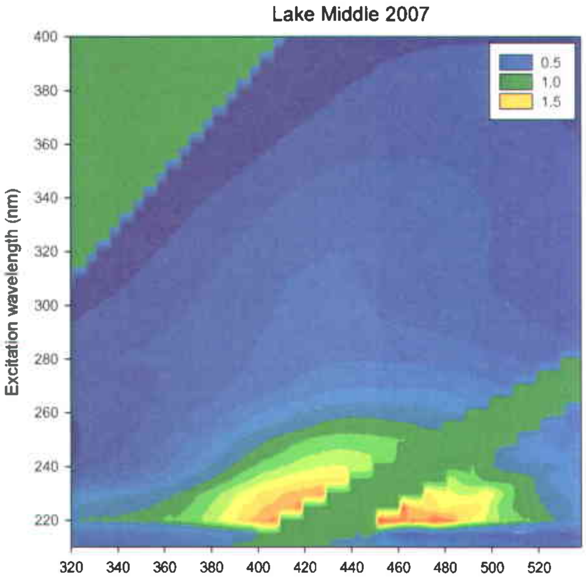
500
520


Lake Middle 2007
0.5
1.0
1.5
Excitation wavelength (nm)
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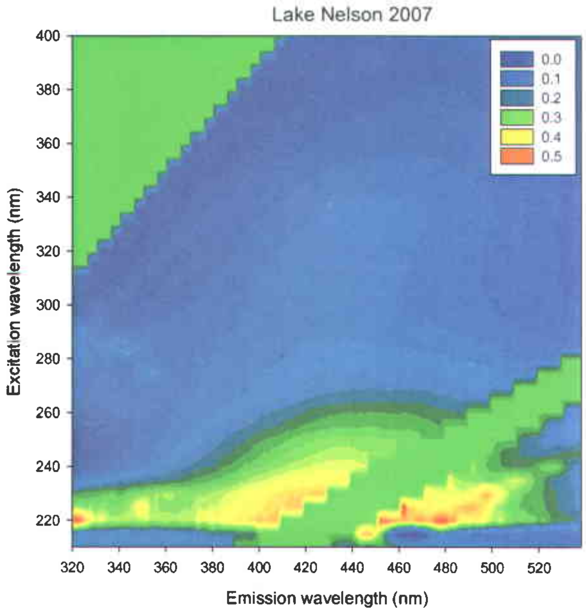
Lake Nelson 2007
0.0
0.1
0.2
0.3
0.4
0.5
Excitation wavelength (nm)
400
380
360
340
320
300
280
260
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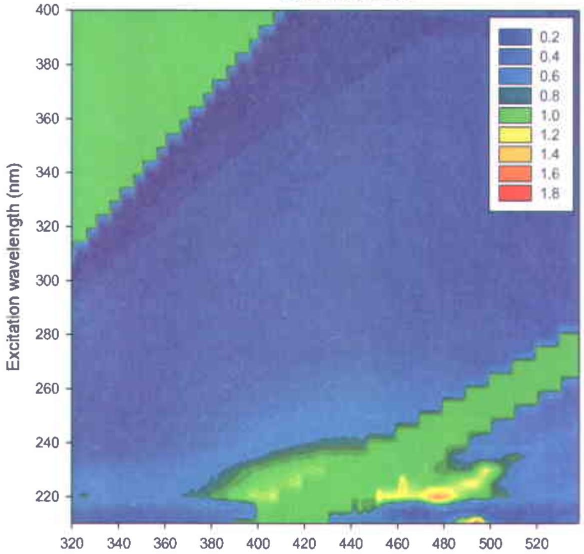
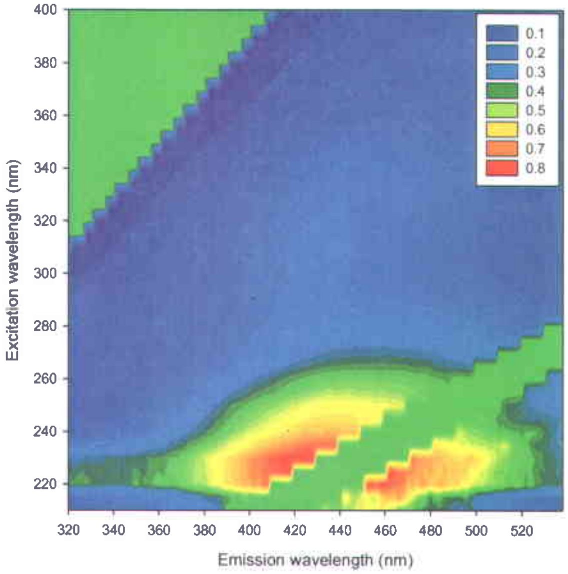
380
400
420
440
460
480
500
520
Emission wavelength (nm)

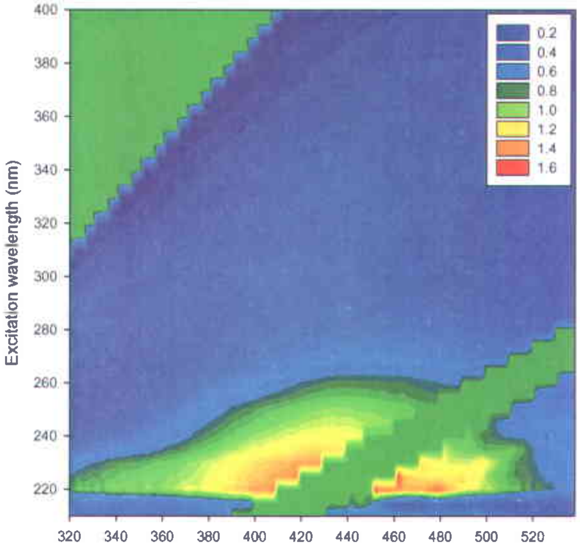
Lake Raft 2007



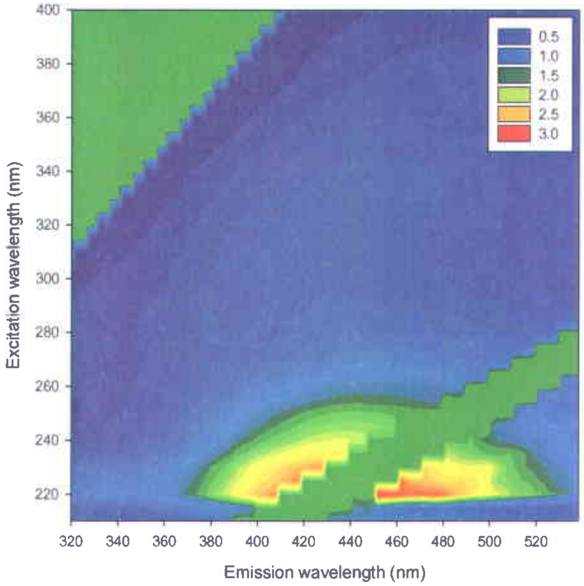
Lake Raft 2008



Lake Ramsey 2007



Lake Whitson 2007



Lake Whitson 2008

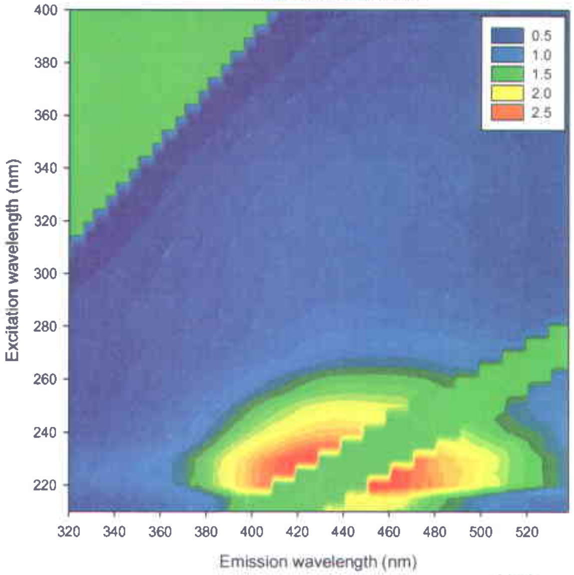


Figure B1. Average (N=3) 3D excitation-emission matrix (EEM) fluorescence spectra of lake DOM from the eighteen lakes sampled in 2007 (left) and the eight lakes sampled in 2008 (right). Each spectrum was corrected for instrument and inner filter effects, normalised to Raman units and blank subtracted. Jagged regions represent regions where the Raman scatter was removed. / Moyenne (N=3) des spectres de fluorescence d'excitation-émission en 3D de la MOD provenant des dix-huit lacs échantillonnés en 2007 (à gauche) et les huit lacs échantillonnés en 2008 (à droite). Chaque spectre a été corrigé pour des effets d'instruments et des effets liés à l'atténuation de l'émission par la MOD elle-même (« inner filter effects»), normalisé par rapport aux unités de Raman, et corrigé par soustraction d'un blanc. Les régions embrouillées représentent des régions où la dispersion a été enlevée.

Table B1. Measured mean (± SD, N=3) dissolved organic matter (DOM) concentration, specific UV-absorbance (SUVA$_{245}$), specific absorbance coefficient (SAC$_{340}$), fluorescence index (FI), and relative proportion of the PARAFAC components (C1, C2, C3 and C4) for each of the eighteen lakes sampled in 2007 and the eight lakes sampled in 2008 / Concentrations moyennes (± SD, N=3) de matière organique dissoute (MOD), l'absorbance UV spécifique (SUVA$_{245}$), le coefficient d'absorbance spécifique (SAC$_{340}$), l'indice de fluorescence (FI) et la proportion relative des composantes de fluorescence (C1, C2, C3 et C4) pour chacun des 18 lacs échantillonnés en 2007 et les 8 lacs échantillonnés en 2008.

Lake	DOC (mg L^{-1})	SUVA 254 (L m^{-1} mgC^{-1})	SAC 340 (L m^{-1} mgC^{-1})	FI	C1/CT	C2/CT	C3/CT	C4/CT
Adeline	4.60 ± 0.79	6.96 ± 1.27	1.46 ± 0.25	1.33 ± 0.01	0.27 ± 0.02	0.33 ± 0.02	0.26 ± 0.02	0.15 ± 0.01
Bousquet	12.58 ± 0.69	11.03 ± 0.62	3.52 ± 0.20	1.29 ± 0.01	0.15 ± 0.02	0.07 ± 0.01	0.76 ± 0.05	0.02 ± 0.00
D'Alembert	8.00 ± 0.80	8.67 ± 0.90	2.20 ± 0.22	1.30 ± 0.01	0.21 ± 0.03	0.24 ± 0.03	0.48 ± 0.07	0.08 ± 0.01
Dasserat	5.14 ± 0.58	8.00 ± 0.40	1.98 ± 0.18	1.35 ± 0.03	0.26 ± 0.19	0.34 ± 0.25	0.30 ± 0.32	0.10 ± 0.07
Dufault	5.10 ± 0.36	6.86 ± 0.70	1.48 ± 0.26	1.32 ± 0.03	0.27 ± 0.12	0.33 ± 0.14	0.27 ± 0.10	0.14 ± 0.05
Dufay	8.25 ± 0.37	8.36 ± 0.44	2.11 ± 0.13	1.30 ± 0.02	0.22 ± 0.03	0.24 ± 0.03	0.45 ± 0.05	0.09 ± 0.01
Joannès	10.26 ± 0.26	9.98 ± 0.25	2.90 ± 0.06	1.28 ± 0.01	0.18 ± 0.04	0.16 ± 0.03	0.61 ± 0.16	0.05 ± 0.01
Opasatica	6.00 ± 0.40	7.41 ± 0.34	1.65 ± 0.14	1.36 ± 0.02	0.26 ± 0.05	0.32 ± 0.05	0.29 ± 0.05	0.12 ± 0.02
Vaudray	8.07 ± 0.09	9.83 ± 0.43	2.88 ± 0.24	1.26 ± 0.01	0.27 ± 0.08	0.24 ± 0.07	0.43 ± 0.10	0.07 ± 0.02
Bethel	5.22 ± 0.46	4.64 ± 0.47	0.84 ± 0.25	1.49 ± 0.06	0.24 ± 0.08	0.37 ± 0.13	0.16 ± 0.06	0.23 ± 0.09
Crowley	2.59 ± 0.28	4.82 ± 0.44	1.02 ± 0.12	1.33 ± 0.08	0.29 ± 0.07	0.36 ± 0.08	0.16 ± 0.05	0.19 ± 0.04
Geneva	3.26 ± 0.39	5.23 ± 0.28	1.09 ±0.25	1.33 ± 0.06	0.29 ± 0.09	0.37 ± 0.10	0.18 ± 0.07	0.17 ± 0.06
Hannah	3.61 ± 0.12	5.05 ± 0.26	1.03 ± 0.08	1.31 ± 0.04	0.28 ± 0.04	0.34 ± 0.04	0.18 ± 0.03	0.20 ± 0.03
Middle	3.32 ± 0.40	5.17 ± 0.57	1.01 ± 0.08	1.36 ± 0.07	0.26 ± 0.03	0.34 ± 0.04	0.20 ± 0.03	0.20 ± 0.03
Nelson	1.36 ± 0.46	4.53 ± 2.02	0.83 ± 0.42	1.32 ± 0.03	0.28 ± 0.08	0.35 ± 0.09	0.14 ± 0.03	0.23 ± 0.07
Raft	2.26 ± 0.31	5.99 ± 2.32	1.35 ± 0.71	1.28 ± 0.07	0.29 ± 0.07	0.34 ± 0.08	0.15 ± 0.16	0.22 ± 0.06
Ramsey	3.55 ± 0.53	4.32 ± 0.60	0.74 ± 0.09	1.41 ± 0.02	0.24 ± 0.05	0.33 ± 0.06	0.17 ± 0.04	0.25 ± 0.05
Whitson	3.82 ± 0.12	6.87 ± 0.56	1.73 ± 0.34	1.34 ± 0.04	0.30 ± 0.06	0.36 ± 0.08	0.19 ± 0.04	0.15 ± 0.03

Appendix C: Effects of aluminum (Al) and iron (Fe) on WHAM VI predictions of Cd, Cu, Ni and Zn speciation / Annexe C : Effets de l'aluminium (Al) et du fer (FE) sur les prédictions de la spéciation du Cd, du Cu, du Ni et du Zn

The competition of trivalent cations, such as Al^{3+} and Fe^{3+}, with trace elements for binding sites on DOM is accounted for in WHAM VI (Tipping 2002). The following section describes how we chose to deal with the effects of Al and Fe on WHAM VI predictions of Cd, Cu, Ni and Zn speciation in the sampled lakes.

Although the total dissolved concentrations of Al and Fe were measured in the lakes sampled (see Table C1, it is unknown what fraction of the measured total dissolved Al and Fe concentrations is colloidal Al and Fe and what fraction is truly dissolved and available to compete with trace metals for binding sites on the DOM. First, following suggestions by Ed Tipping and Steve Lofts, we calculated the activity of Al^{3+} and Fe^{3+} in the samples using equations presented in Tipping (2005) and Lofts et al. (2008) where the concentrations of Al^{3+} and Fe^{3+} are calculated as a function of pH and the solubility of $Al(OH)_3$ (s) and $Fe(OH)_3$ (s), respectively. These values were then inputted directly into WHAM. In the second approach, we assumed that all of the total measured Al and Fe was in the truly dissolved form, and that all the iron was present as Fe(III). In this case, WHAM VI allows the user to input the total dissolved Al(III) and Fe(III) concentrations, and then calculates the Al^{3+} and Fe^{3+} concentrations internally. The solubility equations used by WHAM are slightly different from the empirical relations suggested in Tipping (2005) and Lofts et al. (2008). Figure C1, demonstrates that the WHAM calculated Cd, Cu, Ni and Zn free metal concentrations are similar to those calculated by WHAM when using the external pH-solubility equations.

Table C1. Comparison of measured total dissolved Al and Fe concentrations, Al^{3+} and Fe^{3+} activities calculated using WHAM VI assuming all measured Al and Fe is truly dissolved, and Al^{3+} and Fe^{3+} activities calculated using the external pH-solubility relations for Al and Fe hydroxides / Comparaison de concentrations totales dissoutes mesurées pour l'Al et le Fe, les activités d'Al^{3+} et de Fe^{3+} calculées en utilisant WHAM VI et en considérant que tout l'Al et Fe mesuré est dissous, et les activités d'Al^{3+} et de Fe^{3+} calculées à partir des relations pH-solubilité externes pour les hydroxydes d'Al et de Fe.

Lake	Measured $[Al]_T$ (M)	WHAM VI calculated Al(III)a	Calculated* Al(III)a	Measured $[Fe]_T$ (M)	WHAM VI calculated Fe(III)a	Calculated^ Fe(III)a
Bethel	7.6E-08	7.3E-18	1.2E-16	NA	NA	5.7E-20
Dasserat	4.9E-07	4.1E-13	6.7E-12	2.6E-07	4.4E-17	7.4E-17
Dufault	8.1E-08	6.0E-16	1.8E-14	1.2E-07	2.6E-19	1.5E-18
Geneva	1.6E-08	1.1E-15	7.9E-12	3.9E-08	2.1E-19	6.3E-17
Opasatica	3.7E-07	1.2E-15	6.6E-15	1.5E-07	9.4E-20	7.0E-19
Raft	NA	NA	3.3E-10	1.6E-08	4.6E-18	1.7E-15
Vaudray	3.4E-06	1.9E-11	1.6E-11	5.0E-07	3.5E-16	1.2E-16
Whitson	1.5E-07	7.5E-14	7.9E-12	2.2E-07	2.7E-17	9.6E-17

*indicates Al activity calculated using the equation outlined in Tipping et al. (2005).

^indicated Fe activity calculated using the equation outlined in Lofts and Tipping (2008).

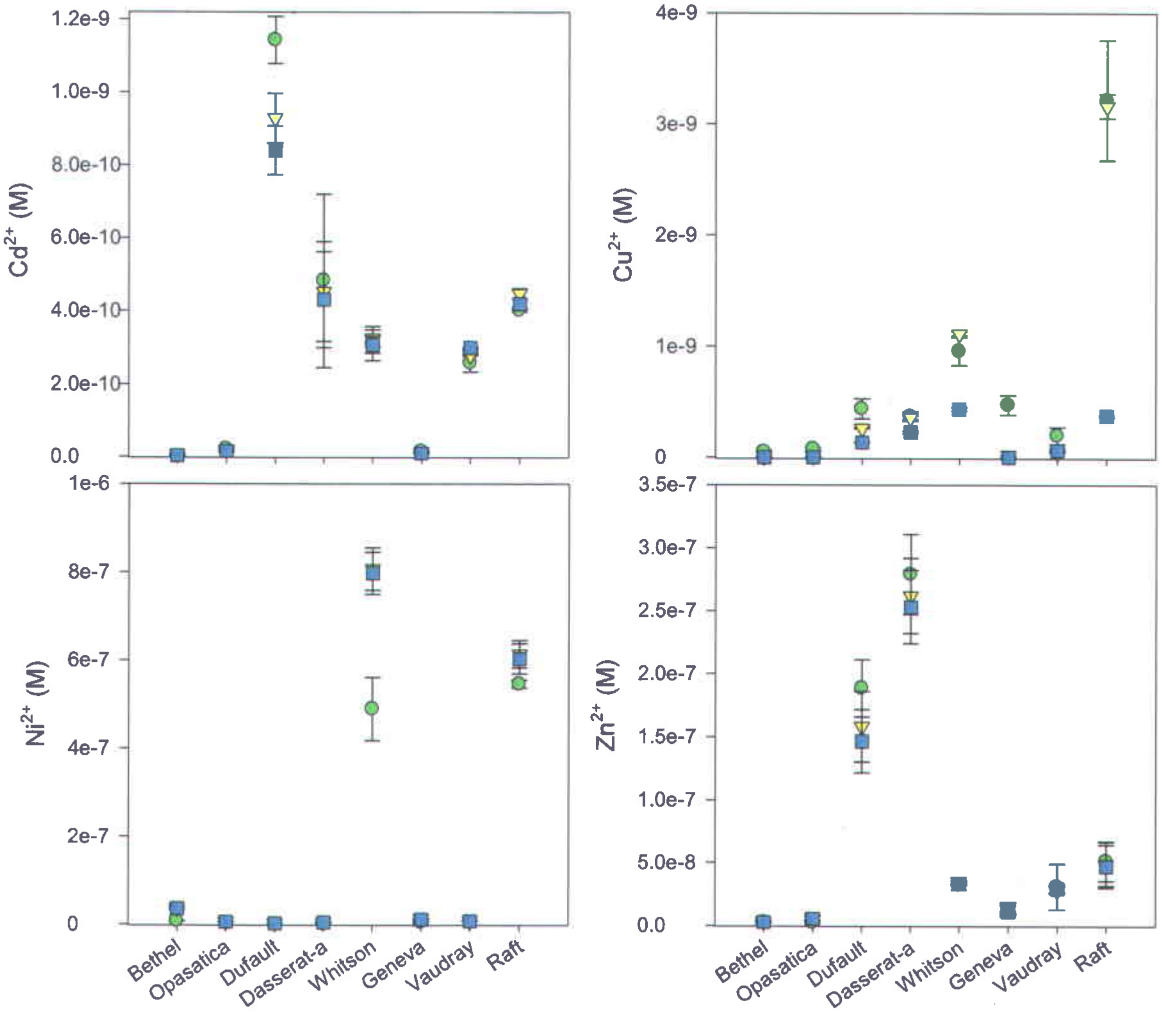


Figure C1. Comparison between measured concentrations of free Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} (circles) and free M^{2+} concentrations predicted by WHAM VI (i) assuming all measured Al and Fe is truly dissolved (squares), or (ii) using the Al^{3+} and Fe^{3+} activities calculated from empirical pH-solubility relations for Al and Fe hydroxides (triangles). Results for eight lakes sampled from the regions of Rouyn-Noranda, QC and Sudbury, ON. / Comparaison entre les concentrations de Cd^{2+}, Cu^{2+}, Ni^{2+} et Zn^{2+} libres mesurées (cercles) et les concentrations des métaux libres M^{2+} prédites par WHAM VI (i) en considérant que tout l'Al et Fe mesuré est dissous (carrés), ou (ii) en utilisant les activités d'Al^{3+} et de Fe^{3+} calculées à partir de relations pH-solubilité empiriques pour les hydroxydes d'Al et de Fe (triangles). Résultats pour les huit lacs échantillonnés dans les régions de Rouyn-Noranda, QC et Sudbury, ON.

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