Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands

Stijn Baken, Fien Degryse, Liesbeth Verheyen, Roel Merckx, Erik Smolders.*

Division of Soil and Water Management, Kasteelpark Arenberg 20 - bus 2459, 3001 Heverlee,
BELGIUM.

stijn.baken@ees.kuleuven.be

Corresponding author phone: +3216321761; fax: +3216321997; email: stijn.baken@ees.kuleuven.be.

ABSTRACT Dissolved organic matter (DOM) in surface waters affects the fate and environmental effects of trace metals. We measured variability in Cd, Cu, Ni and Zn affinity of 23 DOM samples isolated by reverse osmosis from freshwaters in natural, agricultural and urban areas. Affinities at uniform pH and ionic composition were assayed at low, environmentally relevant free Cd, Cu, Ni, and Zn activities. The C-normalized metal binding of DOM varied 4-fold (Cu) or about 10-fold (Cd, Ni, Zn) among samples. The dissolved organic carbon concentration ranged only 9-fold in the waters, illustrating that DOM quality is an equally important parameter for metal complexation as DOM quantity. The UV-absorbance of DOM explained metal affinity only for waters receiving few urban inputs, indicating that in those waters aromatic humic substances are the dominant metal chelators. Larger metal affinities were found for DOM from waters with urban inputs. Aminopolycarboxylate ligands (mainly EDTA) were detected at concentrations up to 0.14 μM and explained partly the larger metal affinity. Nickel concentrations in these surface water are strongly related to EDTA concentrations (R^2=0.96) and this is underpinned by speciation calculations. It is concluded that metal complexation in waters with anthropogenic discharges is larger than estimated with models that only take into account binding on humic substances.

BRIEFS Trace metal affinity of dissolved organic matter from different freshwaters varies up to tenfold and synthetic ligands explain metal mobilization in urban affected waters.

INTRODUCTION

Dissolved organic matter (DOM) has a profound impact on the chemistry of natural waters. Some of the functional groups on DOM have a high affinity for trace metals, and DOM may therefore strongly affect the mobility of trace metals in aquatic systems. Metal toxicity to aquatic organisms is alleviated by metal complexation with DOM. The widely used biotic ligand model (BLM) to predict trace metal toxicity [1] uses the Windermere Humic-Acid Model (WHAM) to estimate trace metal complexation by DOM. This model calculates the fraction of dissolved metal that is not complexed by DOM from the pH, DOM concentration and water hardness. Variation in DOM quality (i.e. functional properties) is usually not accounted for. Most often, it is assumed that 65% of DOM is present as fulvic acid and 35% is inert [2]. This approach is a pragmatic one and circumvents the inherently variable character of DOM: there is ample evidence showing site heterogeneity in DOM [3-5]. Metal binding by humic substances mostly occurs at carboxylic and phenolic sites. Low metal affinity sites are usually associated with carboxylic groups, whereas phenolic groups generally host high affinity sites [2-4]. Also less abundant sites like N or S containing functional groups can be important for metal binding. Moreover, anthropogenic DOM may contain additional metal binding ligands, e.g. aminopolycarboxylates and sulfides [6, 7]. Such ample site heterogeneity suggests that not only the quantity (i.e. concentration) but also the quality of DOM may have a profound impact on metal binding. Surprisingly little exploratory work has been made to assess the range of variability of DOM quality, and to relate this variability to the source of DOM which may be from the terrestrial environment (allochthonous DOM), produced by biota in water (autochthonous) or from human discharges (anthropogenic).

A few studies have previously addressed the issue of DOM quality. Lu & Allen [8] conducted Cu titrations on three different DOM samples and noted important differences in Cu binding between the samples at low Cu activity. Cheng & Allen [9] came to similar conclusions for Zn. The variability of DOM properties has also been explored in soils. The Cu affinity of DOM varied fivefold and this variation could be related to the specific UV absorbance (UV absorbance divided by DOC concentration; SUVA) of DOM [10]. The SUVA is a good measure of DOM aromaticity [11]. Several other studies on DOM quality have estimated the difference in metal complexation between DOM samples from differences in metal toxicity. An increase in EC_{50} (the dissolved metal concentration at which growth, or another endpoint, is reduced by 50% compared to the control) indicates less toxicity and more complexation by DOM if the inorganic composition is identical. De Schamphelaere et al. [12] found that Cu-EC_{50} to *Daphnia magna* varied sixfold depending on DOM source and that this variability was related to the SUVA. Schwartz et al. [13] showed around twofold variability in Cu and Pb toxicity between samples with different types of DOM, but no variability in Cd toxicity to freshwater fish. Allochthonous, more refractory DOM with high SUVA protected better against Cu and Pb toxicity than autochthonous, low SUVA DOM. Also the metal binding of wastewater organic matter (WWOM) has been examined. Higher Zn affinity [14] and Cu affinity [7] have been observed for WWOM compared to natural organic matter (NOM), whereas Pernet-Coudrier et al. [15] found that WWOM and NOM had a similar protective effect against Cu toxicity.

These limited studies show that DOM quality, and specifically the metal binding affinity, may vary more than 5-fold. This variability may be contrasted to the variability in DOM concentrations, for example a factor 18 in a European DOM surveys (10th–90th percentile range in Europe [16]). The information on variability in metal affinity of DOM is limited and is poorly related to the source of DOM. Moreover, evidence of variability in trace metal affinity is lacking for other trace metals than Cu. The objective of this study was to monitor metal complexing properties of surface water DOM from different sources. The variability in these properties was assessed and was related to the source and to structural properties of DOM. Metal affinity of DOM was measured at low, environmentally relevant metal activities. It is assumed that metal affinity variations at low metal activities are larger than at high activities where site capacity, which generally varies little among humic substances [17], becomes a determining factor.

MATERIALS AND METHODS

DOM Sampling and isolation. Freshwater DOM was sampled (60 L) in the autumn of 2009 at 23 different locations in Belgium and Western Germany. Samples were numbered 1-23; site description and locations are given in Table SI 1. Sampling locations were selected to obtain contrasting sources of DOM in two catchment areas (Rupel basin, Belgium, and Rur basin, Germany) in streams of different order and along different land use types. In addition, samples were taken from ponds, bogs, reservoirs, and from one agricultural drainage ditch. Samples were concentrated by reverse osmosis (RO), using a method modified from [18]. This allowed conducting the experiments at a higher DOC concentration than in the original waters. Reverse osmosis is considered the preferred method for isolation of DOM, as it has been shown to have little effect on many DOM characteristics [19]. It is assumed that this RO procedure did not significantly alter characteristics and metal affinity of DOM. Samples were filtered over two in-line filters (5-µm filter followed by 0.45-µm filter) and pumped over a Na ion exchange resin (Lewatit S1468F, Lanxess, Leverkusen, Germany) which removed higher charged cations. This prevented DOM precipitation and RO membrane fouling during concentration and made it possible to run experiments at practically equal cationic composition (see below). Samples were subsequently concentrated by RO (Filmtec TW30-2521, DOW Liquid Separations) to a final pressure of about 9.5 bar. The permeate solution was discarded and the retentate solution was recycled into the drum.

Preliminary tests showed >95% recovery of small organic molecules (acetic acid) and synthetic ligands (nitrilotriacetate) in the concentrate. After concentration, samples were stored in the dark at 4°C. The concentration factor was estimated from the DOC concentrations in the concentrate and the original sample, and ranged between 1.1 and 38 (mean 15).

Chemical analysis. Major inorganic cations and anions, trace metals, dissolved organic carbon (DOC) and total inorganic carbon (TIC) were determined both in original waters and in concentrates. The concentrations of aminopolycarboxylate ligands EDTA, NTA, DTPA, PDTA, and MGDA were determined in the concentrates. Details can be found in the Supporting Information. The UV-absorbance at 254 nm of the concentrates was measured with a spectrophotometer (Perkin Elmer, Lambda 20) in a 1 cm quartz cell.

DOM fractionation. DOM fractionation has been widely used as a tool for DOM characterization and for obtaining separate DOM fractions [20, 21]. DOM samples were fractionated into hydrophilic (HPI), hydrophobic acid (HPOA) and hydrophobic neutral (HPON) fractions according to the method of Amery et al. [22]. Briefly, an acidified (pH 2) and filtered (0.45 μm) DOM sample was injected into a DAX-8 column and subsequently eluted with 0.01 M HCl. All DOM captured at the column outlet during this stage was classified as HPI. Then, the column was backflushed with 0.1 M NaOH. All DOM captured during this stage was classified as HPOA. DOM still retained by the column was classified as HPON; this fraction was calculated from the mass balance. Hydrophobic DOM (HPO) was calculated as HPOA + HPON.

Metal affinity of DOM. A one-step resin-exchange method was used to measure trace metal affinity as described by Amery et al. [23]. A DOM solution was equilibrated with a metal loaded Chelex-100 resin at controlled inorganic composition (pH, electrolyte composition), and the metal complexed by DOM after the equilibration was determined. The resin exchange capacitity exceeded the DOM exchange capacity at least by a factor of 300, and therefore the resin buffers the metal activity of the solution. The preparation of the resin is described in the Supporting Information. This method was originally developed for Cu and was adapted here to measure complexation of Cu, Zn, Ni and Cd in a single step. The resin exchange assays were performed at practically equal cationic composition (125 mM Na and near absence of other major cations); the largest Na concentration among the concentrates was selected as the common value. An equal cationic composition excluded metal binding differences between samples due to competition effects. Na was used as background electrolyte rather than Ca (as in most natural samples) because hardly any DOM precipitates or adsorbs onto the resin, as previously found for softwater DOM exposed to larger Ca concentrations [23].

The DOM solutions used for the experiment were first purged to avoid pH changes due to degassing of CO_2 at later stages. Solutions were stripped with air and HNO_3 was gradually added while maintaining pH above 6.5 to avoid aggregation of DOM. The DOM samples were then diluted to nominal DOC concentrations of 70 mg/L or 30 mg/L. Working with high DOC concentrations resulted in easily measureable trace metal concentrations at low trace metal activities. Ionic strength was adjusted to 150 mM by adding $NaNO_3$ and pH was adjusted to 7. In order to assess the influence of ionic strength and Na competition, two additional DOM samples (22 and 23) were prepared at a lower ionic strength of 10 mM. The DOM solutions were equilibrated with the resin (25 mL solution plus 5 mL resin suspension) in polycarbonate tubes by shaking end-over-end for 17 days in an incubation room at 21°C. Samples were taken after 5, 10 and 17 days and filtered over a 0.45-µm nitrocellulose membrane. Metals and DOC concentrations were measured at all sampling times and pH after 10 and 17 days. Metal concentrations evidenced that equilibrium had been reached after 17 days. All samples were run in triplicate.

Metal binding affinity (MBA, e.g. CuBA) was calculated as the concentration of metal complexed by DOM normalized to the DOC concentration (nmol/mg C). The concentration of DOM-complexed metal was calculated from the total dissolved metal concentration corrected for metal that is not complexed by DOM (i.e. free metal and inorganic complexes). The latter term was calculated with WHAM6 (National Environment Research Council, UK) from the inorganic composition of each solution and the metal activities imposed by the resin (see below) as inputs. This term accounted on average for 25% (Cd), 8%

(Ni) and 17% (Zn) of the total dissolved concentration. Metal binding affinity values calculated in this way are a good measure of conditional (i.e. at the imposed conditions) metal affinity of DOM samples.

The free metal activities at which the solution was buffered by the resin were determined in two different ways: directly from a blank (DOM-free) solution and indirectly by using a synthetic ligand (1 mM iminodiacetate) with known complexation constants. Both the blank and IDA treatment were handled in the same way and under same conditions (150 mM $NaNO_3$, pH 7) as the samples. From the equilibrium concentrations in the IDA treatment, the activities were calculated with the geochemical speciation program Geochem-EZ [24]. Activities of pCd (= -log free Cd^{2+} activity) 8.3, pCu 11.2, pNi 8.5 and pZn 7.7 were obtained. The measured concentrations in the blank solution were converted to activities and these activities were within 0.1 unit of the values obtained using IDA for Cd, Ni and Zn. No value for Cu could be derived with this approach, since the Cu concentration in the blank solution was below the ICP-OES detection limit. These metal activities are representative of different uncontaminated European surface waters [25], except pCd which is typically around 11 in uncontaminated waters. No lower pCd was selected because it would have yielded Cd concentrations in the equilibrated DOM solutions below the ICP-OES detection limit. However, speciation calculations (WHAM6) show that the binding of Cd to fulvic acid is nearly linear if pCd decreases 3 orders of magnitude, indicating that results obtained at high pCd may also be valid at lower pCd.

WHAM calculations. The MBA of DOM in the experimental solutions was also calculated with WHAM6 in order to assess the accuracy of model predictions. The inorganic composition of the equilibrated solutions was entered as input, trace metal activities were fixed at the values imposed by the resin, and fulvic acid was added under the assumption that 1 g DOC equals 2 g DOM and 65% of DOM is present as fulvic acid [2]. Both the default fulvic acid database and a version with adapted Ni parameters [26] were used.

RESULTS AND DISCUSSION

Water and DOM characteristics. The DOC concentrations in sampled waters (Table SI 1) averaged 6 mg/L and ranged between 1.3 and 20 mg/L, with the exception of a marsh water sample (sample 21) with elevated DOC (85 mg/L). Hardness (Table SI 1) averaged 150 and ranged from 3.6 to 450 mg $CaCO_3$/L. All except one sample from the Rupel basin were hard (> 200 mg/L), whereas samples from the Rur basin were softer (< 135 mg/L). The SUVA (Table SI 2) ranged >3-fold (14–46 L/g/cm), indicating considerable differences in DOM quality between different surface waters. The hydrophobic DOM fraction (%HPO DOM) (Table SI 2) ranged 62-89%. The SUVA and %HPO DOM were strongly correlated (R^2=0.84).

The waters were classified as anthropogenically influenced or natural waters. The classification was based on description of sampling location, e.g. locations in forest versus urban locations or up- versus downstream of a wastewater treatment plant (WWTP) outlet. The natural and anthropogenically influenced waters differed in their chemical characteristics. All natural waters had Na:DOC ratios below 0.22 mol/g whereas the anthropogenically influenced waters had Na:DOC ratios above 0.27 mol/g. Similarly, Cl:DOC ratios were below 0.24 mol/g in the natural samples (except sample 11) whereas anthropogenically influenced samples exceeded this value (Table SI 1). Most likely, Na and Cl concentrations originate mainly from human effluents or livestock waste.

Measured aminopolycarboxylate ligand concentrations in the original waters (Table SI 2) were up to 0.14 µM. The dominant aminopolycarboxylate was in most cases EDTA, and concentrations in river samples corresponded fairly well to earlier studies [27]. Concentrations of NTA and MGDA were important in some samples; DTPA and PDTA were negligible (< 2 nM). All anthropogenically influenced waters, except sample 14, contained higher aminopolycarboxylate concentrations than the natural waters. The DOC-normalized aminopolycarboxylate concentrations were >3 times larger in anthropogenically influenced than in natural DOM samples.

Metal binding affinity. The evolution in time of the trace metal concentrations in the DOM solutions equilibrating with the metal resin showed that after 17 days, all solutions were at or near equilibrium with the resin. The pH differences between samples were small; the pH was 6.84 ± 0.10 (average ±

standard deviation) after 17 days. The evolution of DOC concentration with time indicated no or small (< 10% in all but one sample) loss of DOM during the equilibration time. The high Na (125 mM) and low Ca in the experimental solutions differ from the natural range but could not be avoided in the present experimental set-up. This may compromise the extrapolation of our results to environmental conditions. However, we argue that the impact of these high Na and low Ca concentrations is limited. A prelimary experiment showed that metal binding of two DOM solutions prepared at 10 mM Na differed less than 20% from values measured at 125 mM Na, and this difference may partly have been related to the slightly larger equilibrium pH in the low Na solution. Moreover, absolute Cu and Zn binding at similar free metal activity agree very well with previously published data in solutions with a more environmentally relevant cationic composition (see below). Third, speciation calculations (WHAM6) show that, at the free trace metal activities in our experiment, replacing 125 mM Na with 1 mM Ca – an environmentally relevant value – yields very similar mobilized metal concentrations.

Measured CdBA, CuBA, NiBA and ZnBA values and standard deviations over the three replicates (Table SI 2) ranged between 1 and 40 nmol/mg C. Correspondence between the replicates was generally very good. The measured Cu affinity agrees well with previously reported values [23] at the same pH and pCu. These Cu and Zn affinities also agree with published titration curves at pH 7 [7, 8, 28]. Metal affinity was significantly different among samples and ranged by factors of 10 (Cd), 4 (Cu), 9 (Ni) and 13 (Zn). The observed range in Cu affinity agrees with previous findings [10, 12]. The DOC concentrations in sampled waters ranged by a factor of 9, if the marshwater sample (sample 21) is not considered. This shows that in our dataset, DOM quantity and DOM quality have about equal impact on trace metal mobilization. The CdBA, CuBA, NiBA and ZnBA values are highly correlated (R^2 ranges 0.76–0.91).

Effect of Fe competition. Iron was not removed by our DOM processing equipment and could thus influence metal binding through competition [2]. At equilibrium, Fe activity is governed by precipitation-dissolution of ferrihydrite and is thus strongly controlled by pH [29]. Since pH in all experimental solutions was practically equal, Fe competition is not expected to cause large differences in metal mobilization between samples. However, three DOM samples from acidic and iron rich waters (21, 22 and 23; dissolved Fe concentration > 10 µM) were presumably affected more by Fe competition. These samples did not follow the trend between metal affinity and SUVA observed for the other natural waters (see further). For these 3 samples plus 3 other samples, a dialysis experiment was set up. Membranes with a 3.5 kDa cutoff were used to exclude mineral Fe colloids according to the procedures described by Lofts et al. [29]. This confirmed large concentrations of DOM-bound Fe in these high Fe samples (16–25 µg Fe /mg C versus 0.4–4 µg/mg for reference samples). Our experimental methods were apparently unable to remove DOM-bound iron from these initially acidic and iron-rich samples, resulting in apparently lower metal affinity. We hypothesize that no equilibrium was reached in the resin experiment (pH 7.0, 17 days of equilibration) because of the slow dissociation kinetics of Fe-DOM complexes. Therefore, these samples were ignored in further considerations. The other samples had an initial pH above 7. Since the Fe(III):DOM ratio in equilibrium with $Fe(OH)_3$ increases when decreasing pH [29], no dissociation of Fe-DOM complexes is expected upon acidification to pH 7. However, it is possible that small quantities of Fe or other elements (e.g. Pb, La, Eu) are irreversibly bound to binding sites on DOM and thus are inexchangeable as described in [30].

Relation between metal binding affinity and DOM properties and source. No significant correlation was observed between MBA and SUVA (Figure 1) or between MBA and either DOM fraction. For Cu, this finding seemingly contradicts earlier studies [10, 12, 13, 23]. However, significant trends emerged when distinguishing between the natural and anthropogenically influenced waters.

For the natural DOM samples, a linear relationship was obtained between the MBA and SUVA for all metals (Figure 1) and between the MBA and %HPO DOM. This suggests that trace metal affinity of natural DOM increases with increasing aromaticity, i.e. with increasing fractions of humic substances. The largest R^2 value was found for Cu and the regression slope corresponded (difference < 10%) to the slope obtained by Amery et al. [23] for terrestrial DOM samples measured at similar pH and pCu. These results corroborate that SUVA is a suitable predictor of the metal affinity of DOM in natural waters. Similar previously reported trends [10, 12, 13, 23] were mainly based on natural DOM samples as far as can be judged from sample description. Speciation calculations (WHAM6) with the default average DOM quality, i.e. 65% of DOM is fulvic acids, generally predicted metal affinity of natural DOM within a factor of 2 (Figure 1), except for Ni affinity which was underestimated by factors 3–10 when using default parameters, and overestimated by factors 1.2–3 when using adapted [26] parameters.

Anthropogenically influenced DOM samples had markedly larger metal affinity than natural DOM samples. Average MBA values were 1.7 (Cu) or 3–4 (Cd, Ni, Zn) times larger than average values for the natural DOM samples. WHAM6 always underestimated metal binding of these DOM samples by up to a factor of 9 and, on average, by a factor of 3 (Figure 1). Sample 13 (downstream of a WWTP outlet) had a 1.5–3 times larger metal affinity than the corresponding upstream sample 7 (Table SI 2). These observations indicate that ligands other than natural humic substances are present in anthropogenically influenced waters. Previous studies have also shown larger Cu [7] and Zn [14] affinity of wastewater organic matter (WWOM) than of NOM at low metal activities.

The metal affinity of anthropogenically influenced DOM samples was unrelated to SUVA (Figure 1). The part of the MBA that was not explained by SUVA ('excess metal binding') was calculated for each sample as the summed deviations from the four regression lines in Figure 1. The excess metal binding correlated strongly ($r = 0.80$) with the aminopolycarboxylate concentration (Figure 2). This observation strongly suggests a link between anthropogenic inputs (as indicated by aminopolycarboxylates) and excess metal bound. However, on a molar basis, aminopolycarboxylates could only explain 10–43% of the excess metal binding, assuming unimolar stoichiometry. This may suggest that also anthropogenic ligands other than the ones measured may have contributed to metal mobilization, such as organophosphonates, hydroxycarboxylates, IDS (iminodisuccinic acid), and gluconic acid [6]. Sulfide and other unidentified strong, nonhumic ligands have also been shown to cause elevated Cu binding by WWOM at low Cu activity [7]. Sulfide can be stable in oxic environments for extended periods and is often associated with DOM [31]. Pernet-Coudrier [32] linked Cu binding by the hydrophilic fraction of WWOM to protein derived structures with amine and amide binding sites. Another possible explanation may be degradation of aminopolycarboxylate ligands in the 6 months time between the resin assay and the aminopolycarboxylate analysis. Samples were stored in the dark at 4ºC. EDTA is refractory and its degradation was likely limited because of its long half-life at low temperatures [33]. However, other ligands such as NTA are more readily biodegradible [34]. Degradation may have contributed to the difference between ligand concentration and excess metal mobilized in our experiment.

Extrapolation to the environment. The conditions in our resin experiment were not identical to those in the original waters, and cationic composition and Cd activity differed from natural conditions. Moreover, we only used one single set of experimental conditions (pH, ionic composition, trace metal activities), whereas these variables span wide ranges in real surface waters. Thus, the metal binding under field conditions may be very different from that under our experimental conditions. However, our finding that metal binding in the original waters is affected by anthropogenic ligands is relevant for environmental conditions.

The dissolved Ni concentrations in the original waters are strongly correlated ($R^2 = 0.96$) to EDTA concentrations (Figure 3). The Ni concentrations in the anthropogenically influenced waters were corrected for naturally occurring Ni by subtracting the average Ni concentration in natural waters (35 nM). Corrected Ni and EDTA agreed very well on a molar basis (Figure 3), and the ratio of corrected Ni to EDTA averaged 1.1. Moreover, calculations with the geochemical speciation program Visual MINTEQ 3.0 (Gustafsson, KTH, Uppsala, Sweden) showed that, at chemical equilibrium and at the imposed trace metal activities, 87% of all EDTA is Ni-bound, and this value proved very insensitive to Ni activity. These observations strongly suggest that Ni is mainly present as complex with EDTA in the anthropogenically influenced waters. It has been suggested previously that complexation by anthropogenic ligands is important for Cu and Ni speciation in wastewater effluents [35], and the importance of EDTA for Ni speciation has been demonstrated in the environment [36]. It must however be noted that in the environment, EDTA speciation may be governed by kinetic limitations rather than by chemical equilibrium if initially discharged as a slowly dissociating complex with e.g. Fe or Ni [37, 38].

Synthetic ligands may have limited effect on Zn speciation. Zinc concentrations in the anthropogenically influenced waters ranged 0.3–8 µM (except for one sample) and were, on average, more than an order of magnitude greater than Cd, Cu and Ni concentrations. This range is also far above typical concentrations of known synthetic ligands [6], so anthropogenic ligands likely have only limited effect on Zn speciation in surface waters.

Implications for the BLM. The BLM has recently been considered for regulatory purposes because of its capability to deduce site-specific water quality criteria (WQC). The Humic Ion-Binding Model VI [39], which is incorporated in WHAM6 and which is used by the BLM to calculate DOM-metal interactions, has been calibrated chiefly with NOM. The DOM quality is usually not taken into account. However, because anthropogenic inputs cause elevated metal mobilization as compared to NOM, the model is likely to underestimate metal complexation in anthropogenically impacted waters and to overestimate free metal activities and metal toxicity. It has been argued previously that the BLM be corrected for DOM aromaticity [12]. We argue that, although a UV-absorbance based correction for DOM aromaticity may improve BLM predictions, correcting for urban influences has higher priority than correcting for natural DOM quality. WHAM consistently underestimates metal affinity of urban impacted waters by a factor of up to 9, whereas metal affinity of NOM is generally predicted within a factor 2 except for Ni. A possible way to account for anthropogenic ligands in rivers could be the use of a factor reflecting the ratio of wastewater inputs to the river discharge. Based on this factor, ligand concentrations may be estimated. However, more efforts are needed to identify anthropogenic ligands and to assess their fate in surface waters and impact on trace metal speciation. This is a crucial step towards calibrating the BLM also for anthropogenically influenced surface waters, and towards development of adequate site-specific WQC.

ACKNOWLEDGEMENTS We thank Christoff Vanmoorleghem and Fien Amery for their assistance, Carlos Constantino (Brunel University) for some excellent suggestions, and Frank Sacher from the Technologiezentrum Wasser (TZW, Karlsruhe, Germany) for measuring aminopolycarboxylates. F.D. thanks the Fund for Scientific Research (F.W.O.-Vlaanderen) for a postdoctoral fellowship. This research was funded by the Onderzoeksfonds K.U.Leuven under the project number OT/08/020.

SUPPORTING INFORMATION Sampling site description and composition of each water sample (Table SI1) and major numerical results (Table SI2). Details on chemical analyses and resin preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

1. Di Toro, D. M.; Allen, H. E.; Bergman, H. L.; Meyer, J. S.; Paquin, P. R.; Santore, R. C., Biotic ligand model of the acute toxicity of metals. 1. Technical Basis. *Environmental Toxicology and Chemistry* **2001,** *20*, (10), 2383-2396.

2. Bryan, S. E.; Tipping, E.; Hamilton-Taylor, J., Comparison of measured and modelled copper binding by natural organic matter in freshwaters. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* **2002,** *133*, (1-2), 37-49.

3. Benedetti, M. F.; Milne, C. J.; Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K., Metal ion binding to humic substances: application of the non-ideal competitive adsorption model. *Environmental Science & Technology* **1995,** *29*, (2), 446-457.

4. Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena, M. J., Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **1999,** *151*, (1-2), 147-166.

5. Masini, J. C.; Abate, G.; Lima, E. C.; Hahn, L. C.; Nakamura, M. S.; Lichtig, J.; Nagatomy, H. R., Comparison of methodologies for determination of carboxylic and phenolic groups in humic acids. *Analytica Chimica Acta* **1998,** *364*, (1-3), 223-233.

6. Knepper, T. P., Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment. *Trac-Trends in Analytical Chemistry* **2003,** *22*, (10), 708-724.

7. Sarathy, V.; Allen, H. E., Copper complexation by dissolved organic matter from surface water and wastewater effluent. *Ecotoxicology and Environmental Safety* **2005,** *61*, (3), 337-344.

8. Lu, Y. F.; Allen, H. E., Characterization of copper complexation with natural dissolved organic matter (DOM) - link to acidic moieties of DOM and competition by Ca and Mg. *Water Research* **2002,** *36*, (20), 5083-5101.

9. Cheng, T.; Allen, H. E., Comparison of zinc complexation properties of dissolved natural organic matter from different surface waters. *Journal of Environmental Management* **2006,** *80*, (3), 222-229.

10. Amery, F.; Degryse, F.; Cheyns, K.; De Troyer, I.; Mertens, J.; Merckx, R.; Smolders, E., The UV-absorbance of dissolved organic matter predicts the fivefold variation in its affinity for mobilizing Cu in an agricultural soil horizon. *European Journal of Soil Science* **2008,** *59*, (6), 1087-1095.

11. Weishaar, J. L.; Aiken, G. R.; Bergamaschi, B. A.; Fram, M. S.; Fujii, R.; Mopper, K., Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology* **2003,** *37*, (20), 4702-4708.

12. De Schamphelaere, K. A. C.; Vasconcelos, F. M.; Tack, F. M. G.; Allen, H. E.; Janssen, C. R., Effect of dissolved organic matter source on acute copper toxicity to Daphnia magna. *Environmental Toxicology and Chemistry* **2004,** *23*, (5), 1248-1255.

13. Schwartz, M. L.; Curtis, P. J.; Playle, R. C., Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry* **2004,** *23*, (12), 2889-2899.

14. Cheng, T., Comparison of zinc complexation properties of dissolved organic matter from surface waters and wastewater treatment plant effluents. *Journal of Environmental Sciences-China* **2005,** *17*, (4), 535-539.

15. Pernet-Coudrier, B.; Clouzot, L.; Varrault, G.; Tusseau-Vuillemin, M. H.; Verger, A.; Mouchel, J. M., Dissolved organic matter from treated effluent of a major wastewater treatment plant: Characterization and influence on copper toxicity. *Chemosphere* **2008,** *73*, (4), 593-599.

16. Salminen, R., *Geochemical atlas of Europe, part one*. Geological Survey of Finland: Espoo, Finland, 2005.

17. Ritchie, J. D.; Perdue, E. M., Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. *Geochimica et Cosmochimica Acta* **2003,** *67*, (1), 85-96.

18. De Schamphelaere, K. A. C.; Unamuno, V. I. R.; Tack, F. M. G.; Vanderdeelen, J.; Janssen, C. R., Reverse osmosis sampling does not affect the protective effect of dissolved organic matter on copper and zinc toxicity to freshwater organisms. *Chemosphere* **2005,** *58*, (5), 653-658.

19. Kilduff, J. E.; Mattaraj, S.; Wigton, A.; Kitis, M.; Karanfil, T., Effects of reverse osmosis isolation on reactivity of naturally occurring dissolved organic matter in physicochemical processes. *Water Research* **2004,** *38*, (4), 1026-1036.

20. Leenheer, J. A., Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environmental Science & Technology* **1981,** *15*, (5), 578-587.

21. Aiken, G. R.; McKnight, D. M.; Thorn, K. A.; Thurman, E. M., Isolation of hydrophilic organic acids from water using nonionic macroporous resins. *Organic Geochemistry* **1992,** *18*, (4), 567-573.

22. Amery, F.; Vanmoorleghem, C.; Smolders, E., Adapted DAX-8 fractionation method for dissolved organic matter (DOM) from soils: development, calibration with test components and application to contrasting soil solutions. *European Journal of Soil Science* **2009,** *60*, (6), 956-965.

23. Amery, F.; Degryse, F.; Degeling, W.; Smolders, E.; Merckx, R., The copper-mobilizing-potential of dissolved organic matter in soils varies 10-fold depending on soil incubation and extraction procedures. *Environmental Science & Technology* **2007,** *41*, (7), 2277-2281.

24. Shaff, J. E.; Schultz, B. A.; Craft, E. J.; Clark, R. T.; Kochian, L. V., GEOCHEM-EZ: a chemical speciation program with greater power and flexibility. *Plant and Soil* **2010,** *330*, (1-2), 207-214.

25. Kalis, E. J. J.; Weng, L. P.; Dousma, F.; Temminghoff, E. J. M.; van Riemsdijk, W. H., Measuring free metal ion concentrations in situ in natural waters using the Donnan membrane technique. *Environmental Science & Technology* **2006,** *40*, (3), 955-961.

26. Van Laer, L.; Smolders, E.; Degryse, F.; Janssen, C.; De Schamphelaere, K. A. C., Speciation of nickel in surface waters measured with the Donnan membrane technique. *Analytica Chimica Acta* **2006,** *578*, (2), 195-202.

27. Schmidt, C. K.; Fleig, M.; Sacher, F.; Brauch, H. E., Occurrence of aminopolycarboxylates in the aquatic environment of Germany. *Environmental Pollution* **2004,** *131*, (1), 107-124.

28. Cheng, T.; De Schamphelaere, K.; Lofts, S.; Janssen, C.; Allen, H. E., Measurement and computation of zinc binding to natural dissolved organic matter in European surface waters. *Analytica Chimica Acta* **2005,** *542*, (2), 230-239.

29. Lofts, S.; Tipping, E.; Hamilton-Taylor, J., The Chemical Speciation of Fe(III) in Freshwaters. *Aquatic Geochemistry* **2008,** *14*, (4), 337-358.

30. Bryan, N. D.; Jones, D. L. M.; Keepax, R. E.; Farrelly, D. H.; Abrahamsen, L. G.; Pitois, A.; Ivanov, P.; Warwick, P.; Evans, N., The role of humic non-exchangeable binding in the promotion of metal ion transport in groundwaters in the environment. *J. Environ. Monit.* **2007,** *9*, (4), 329-347.

31. Kramer, J. R.; Bell, R. A.; Smith, D. S., Determination of sulfide ligands and association with natural organic matter. *Applied Geochemistry* **2007,** *22*, (8), 1606-1611.

32. Pernet-Coudrier, B. Influence de la matière organique dissoute sur la spéciation et la biodisponibilité des métaux: cas de la Seine, un milieu sous forte pression urbaine. Université Paris-Est, France, 2008.

33. Tiedje, J. M., Microbioal degradation of EDTA in soils and sediments. *Applied Microbiology* **1975,** *30*, (2), 327-329.

34. Larson, R. J.; Clinckemaillie, G. G.; Vanbelle, L., Effect of temperature and dissolved oxygen on biodegradation of nitrilotriacetate. *Water Research* **1981,** *15*, (5), 615-620.

35. Sedlak, D. L.; Phinney, J. T.; Bedsworth, W. W., Strongly complexed Cu and Ni in wastewater effluents and surface runoff. *Environmental Science & Technology* **1997,** *31*, (10), 3010-3016.

36. Bedsworth, W. W.; Sedlak, D. L., Sources and environmental fate of strongly complexed nickel in estuarine waters: The role of ethylenediaminetetraacetate. *Environmental Science & Technology* **1999,** *33*, (6), 926-931.

37. Xue, H. B.; Jansen, S.; Prasch, A.; Sigg, L., Nickel speciation and complexation kinetics in freshwater by ligand exchange and DPCSV. *Environmental Science & Technology* **2001,** *35*, (3), 539-546.

38. Xue, H. B.; Sigg, L.; Kari, F. G., Speciation of EDTA in natural waters - exchange kinetics of Fe-EDTA in river water. *Environmental Science & Technology* **1995,** *29*, (1), 59-68.

39. Tipping, E., Humic ion-binding model VI: An improved description of the interactions of protons and metal ions with humic substances. *Aquatic Geochemistry* **1998,** *4*, (1), 3-48.

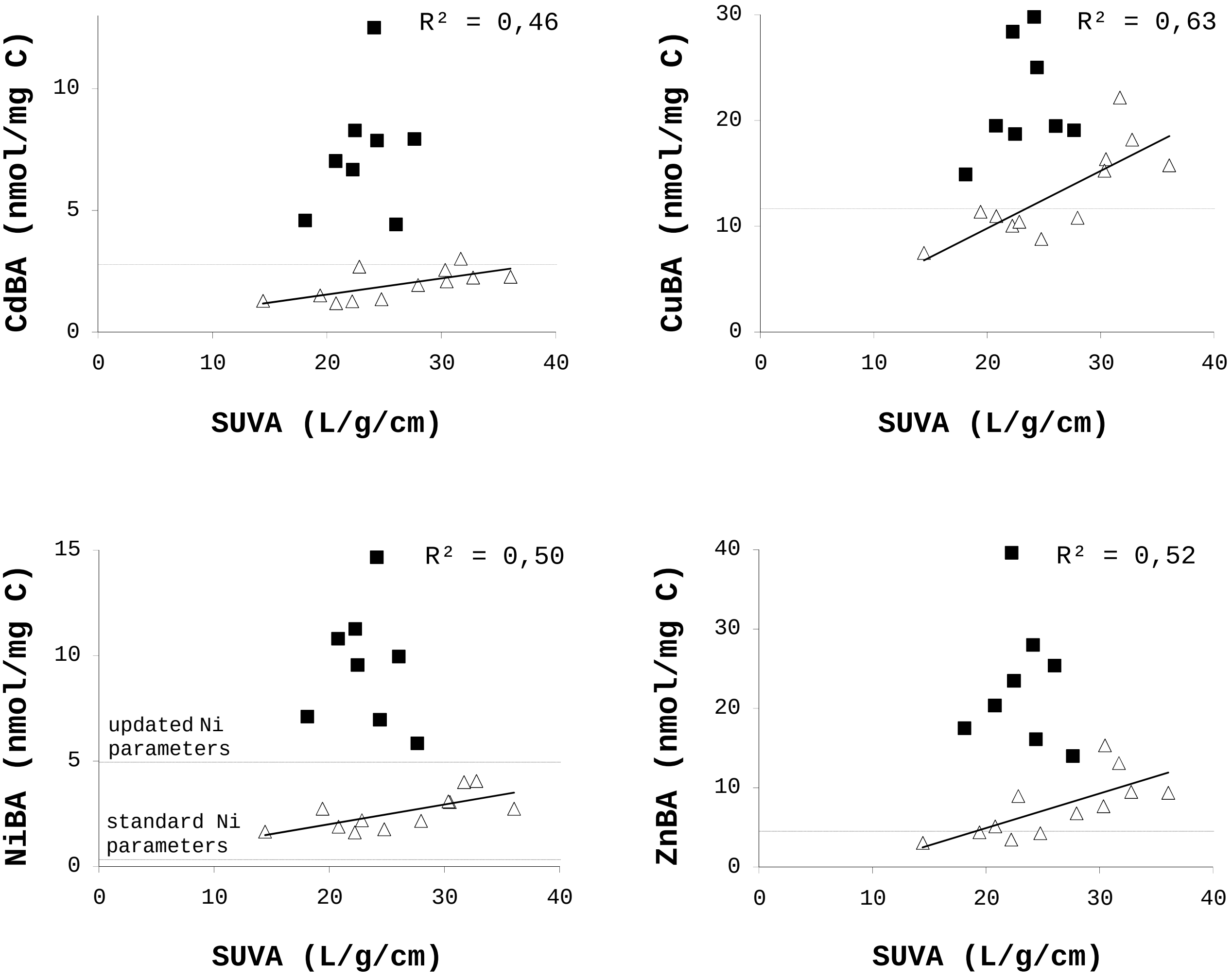

FIGURE 1. The organic-C normalized metal binding affinity (MBA) of isolated DOM as related to the Specific UV Absorbance (SUVA) which indicates the aromaticity of DOM (■ anthropogenically influenced samples; Δ natural samples). The full line is the linear regression line obtained for natural DOM samples (R^2 given in the graph). The dashed horizontal line is the average MBA calculated by WHAM6 under the assumption that 65% of DOM is present as fulvic acid. For Ni, calculation was made with standard parameters and with adapted parameters as proposed by [26].

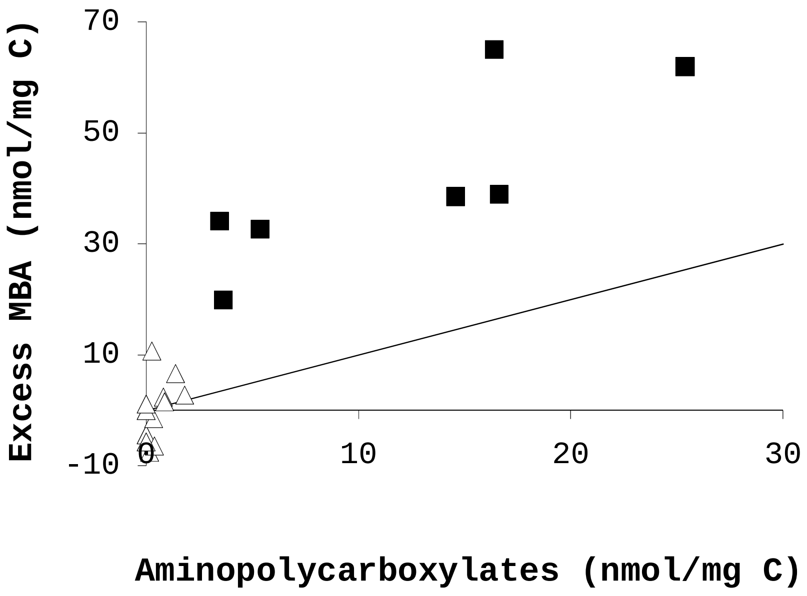

FIGURE 2. The metal binding affinity of DOM that cannot be explained by the relationship with SUVA (excess MBA) relates to the organic-C normalized aminopolycarboxylate ligand concentrations (■ anthropogenically influenced samples; Δ natural samples). The excess MBA was calculated as the difference in measured MBA and the value predicted with the regression line relating MBA to SUVA, summed for all four metals (Figure 1). The full line is the 1:1-line.

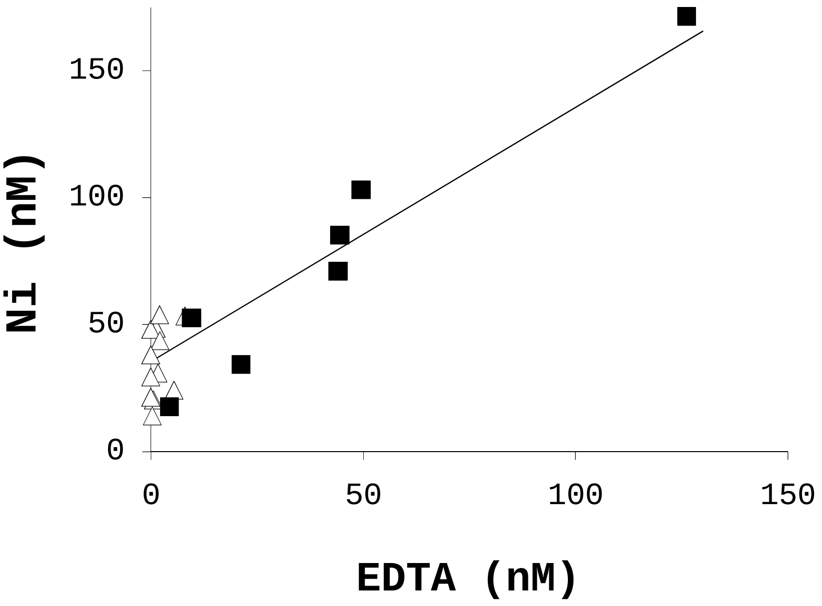

FIGURE 3. Ni concentrations in the original waters plotted against EDTA concentrations (■ anthropogenically influenced samples; Δ natural samples). Both variables are correlated ($R^2 = 0.96$) in anthropogenically influenced waters. The solid line is the 1:1-line augmented by the average Ni concentration in natural samples, resulting in an intercept of 35 nM Ni. Ni and EDTA in anthropogenically influenced samples agree very well on a molar basis as indicated by their proximity to this line.