Environmentally relevant concentrations of aminopolycarboxylate chelating agents mobilize Cd from humic acid

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ABSTRACT

Although Cd is a pollutant of public health relevance, many dietary sources from which it can be absorbed into human tissues remain unknown. While it is well established that the biogeochemical cycle of Cd involves its complexation with environment-derived ligands (*e.g.*, humic acids, HAs) and anthropogenic ones (*e.g.*, chelating agents, CAs), the interaction of Cd with both of these ligands is less well understood. To gain insight, a HA–Cd complex was injected on a size-exclusion chromatography (SEC) column coupled on-line with a flame atomic absorption spectrometer (FAAS) using 10 mmol/L Tris buffer (pH 8.0) as the mobile phase. This approach allowed us to observe the intact HA–Cd complex and the retention behavior of Cd as a function of 2–20 μmol/L concentrations of ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) or methylglycinediacetic acid (MGDA) that were added to the mobile phase. An increase of the retention time of Cd was indicative of a partial or complete abstraction of Cd from HA. Our results revealed that all CAs abstracted Cd from the HA–Cd complex at concentrations of 5 μmol/L, while MGDA and DTPA were effective at 2 μmol/L. The bioavailability of some of the on-column formed CA–Cd complexes explains the previously reported increased accumulation of Cd in periphyton in the ecosystem downstream of wastewater treatment plants. In addition, our results imply that the use of effluents which contain CAs and Cd for the irrigation of food crops can introduce Cd into the food supply and compromise food safety.

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Introduction

The chemical element cadmium (Cd) is present in the earth's crust in the form of a variety of Cd-bearing minerals and ranks 65th in elemental abundance (Emsley, 1998). Since abiotic chemical weathering processes have been mobilizing this metal from the geosphere to the biosphere ever since life evolved, all organisms have been exposed to background concentrations of Cd throughout evolution. The realization that Cd and its compounds (*e.g.*, the yellow pigment CdS) have useful properties resulted in a steep increase in the world refinery production of this metal – which is tied to the processing of primary ores rich in Cu, Pb and Zn – in the late 1920s (Cullen and Maldonado, 2013). Today, Cd is not only a major industrial pollutant (Buchet et al., 1990), but it is also found in numerous consumer products (Guney and Zagury, 2013; Liu et al., 2013). It is therefore not surprising that Cd is inherently present in various human waste streams, such as wastewater (Karvelas et al., 2003)

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and air emitted from various industrial activities (*e.g.*, non-ferrous metal smelting, fossil fuel combustion) (Natusch et al., 1974; Pacyna and Pacyna, 2001). In fact, the anthropogenic release of this toxic metal to the environment perturbs the biogeochemical Cd cycle on a local, regional and global scale (Pacyna, 1996). Consequently, Cd pollution is becoming a major cause of environmental contamination in certain parts of the world (Hasan et al., 2009; Campbell and Gailer, 2016), where it can pose a problem for ecosystems (Larison et al., 2000; Campbell, 2006; Campbell and Gailer, 2016) and human health (Drasch, 1983; Buchet et al., 1990; Johnson et al., 2003; Nawrot et al., 2006; Campbell and Gailer, 2016). A more detailed understanding of the mechanisms by which human activities perturb the biogeochemical cycle of Cd in specific environmental compartments (*e.g.*, the hydrosphere) represents an important starting point to identify hitherto unknown dietary sources of Cd (Malmauret et al., 2002) and to develop stricter regulatory measures to curb the influx of this toxic metal into human tissues (Drasch, 1983; Campbell and Gailer, 2016).

In environmental waters and top soils, the environmental chemistry of Cd is dominated by its interactions with anions (*e.g.*, chloride) and the ubiquitous natural ligand humic acid (HA) (Campbell and Gailer, 2016). The latter is an operationally defined complex organic breakdown product of plant leaves/tissues (Malcolm and MacCarthy, 1986), which is water soluble and therefore plays an important role in the transportation of a variety of inorganic pollutants from environmental sources to sinks (Kalbitz et al., 2000). In addition, HA–Cd complexes that are present in natural waters are considered not to be bioavailable to freshwater fish, such as the common carp (Van Ginneken et al., 2001).

Anthropogenically-derived chelating agents (CAs), which are used for water softening purposes in household and industrial detergents (Pinto et al., 2014), however, are also known to form stable complexes with Cd (Gardiner, 1976), but a critical evaluation of the chemistry of CAs in natural waters is missing (Nowack, 2002). The CA ethylenediaminetetraacetic acid (EDTA) (Fig. 1), which is environmentally persistent (Nowack et al., 2006), is released into rivers at considerable quantities. It was estimated that 34,000 tons of EDTA were consumed in Europe in 1999 and a large fraction thereof was released into the environment (Knepper, 2003). It is therefore not surprising that surface waters (*e.g.*, rivers) can contain up to 4 μmol/L of EDTA (Oviedo and Rodriguez, 2003) and that effluent from municipal wastewater treatment plants (WTPs) may contain up to 19 μmol/L of EDTA (Bedsworth and Sedlak, 2001; Fuehrhacker et al., 2003). Owing to the larger complex formation constant of Cd for EDTA (log K = 16.6, 0.1 mol/L and 25°) than Mg and Ca (Knepper, 2003), Cd is predominantly present in surface waters in the form of EDTA-Cd complexes (Pinto et al., 2014). The related amino polycarboxylate CA, diethylenetriaminepentaacetic acid (DTPA) (Fig. 1), is also consumed at considerable rates (14,732 tons in Western Europe in 1999) (Knepper, 2003) and has been detected in WTP effluent from a paper recycling factory at concentrations of 7.3 μmol/L (Knepper, 2003). Methylglycinediacetic acid (MGDA) (Fig. 1), is an emerging aminopolycarboxylate CA (BASF trade name Trilon M) (Jachula et al., 2012) and its consumption is expected to increase in the near future.

In order to better understand the potentially unintended consequences that the release of large quantities of CAs into
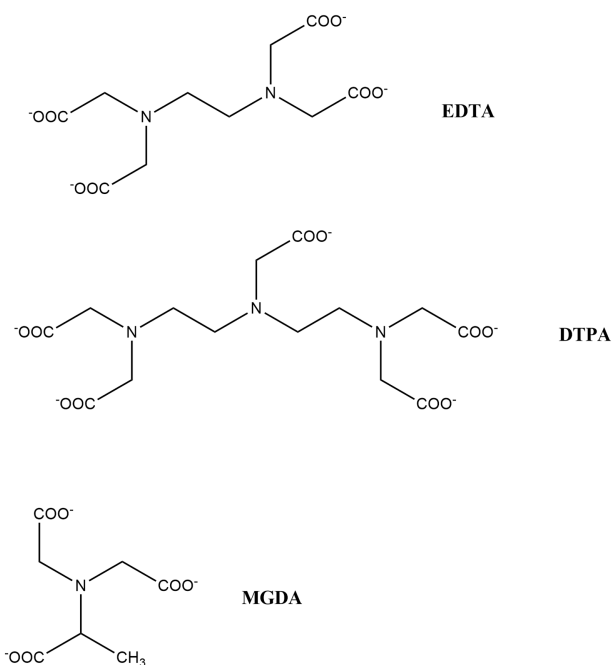
Fig. 1 – Chemical structure of the investigated chelating agents: EDTA, DTPA and MGDA.

the environment may have on the biogeochemical Cd cycle, we investigated if environmentally relevant CA concentrations can affect the stability of Cd complexed to HA (referred to as a 'HA–Cd complex' from now on). It is important to point out that while MDGA is completely biodegradable in ~2 weeks, >90% is released from WTP together with essentially all EDTA and DTPA (Knepper, 2003). To probe this chemistry, we employed a previously reported analytical approach, which is based on size-exclusion chromatography (SEC) coupled on-line to a flame atomic absorption spectrometer (FAAS) (Morris et al., 2014; Sagmeister et al., 2016). This approach allows one to detect HA–Cd complexes and to systematically investigate the comparative abstraction of Cd from HA–Cd complexes by the structurally related aminopolycarboxylate chelating agents EDTA, DTPA and MGDA dissolved in a 10 mmol/L Tris mobile phase (pH 8.0) at environmentally relevant concentrations ranging from 2 to 20 μmol/L. Although a smaller complex stability constant has been reported for Cd and HA (log K = 4.6–6.2) (Mantoura et al., 1978; Yan et al., 2011) compared to that for EDTA (log K = 16.6) (Knepper, 2003), the translocation of Cd from HA to CAs has not been experimentally investigated at environmentally relevant conditions.

The formation of CA–Cd complexes in the environment is relevant with regard to the fact that climate change induced drought conditions in certain parts of the world are associated with an increased use of municipal wastewater for the irrigation of food crops (Reemtsma et al., 2006; Khan et al., 2008). This latter practice is potentially problematic because CA–Cd complexes that can be present in WTP effluent can be absorbed into the edible part of food crops (Schaider et al.,

2006; Van Engelen et al., 2007; Khan et al., 2008). In addition, Cd in soils can be bound to HAs (as well as clays) and the treatment of such polluted soils with aqueous solutions of CAs to remove the toxic metal is referred to as soil washing (Tandy et al., 2004; Pinto et al., 2014). Humic substances that are contained in sewage sludge compost have also been shown to be effective in removing Cu and Cd from highly contaminated soils (Kulikowska et al., 2015). Accordingly, studies that aim to establish the concentration of CAs that can abstract Cd from environmentally abundant HAs to form potentially bioavailable Cd-complexes are important in terms of locating environmental sites where this environmental pollutant may inadvertently enter the human food supply (Malmauret et al., 2002). This latter problem is of increasing relevance today because the ingestion of Cd-contaminated food items is the primary source of human exposure (Campbell and Gailer, 2016). In fact, approximately 5% of the US population already have urinary Cd concentrations approaching levels that are associated with subtle adverse effects on the kidneys (Gailer, 2012).

1. Experimental

1.1. Chemicals and solutions

$CdCl_2$ (>99%), tris(hydroxymethyl)aminomethane (≥99.5%, Trizma base), ethylenediamine tetraacetic acid (EDTA) disodium salt dihydrate (99 +%), diethylenetriaminepentaacetic acid (DTPA, ≥99%) and humic acid (HA derived from natural oxidized brown coal, technical grade, Lot #BCBN1711V, pH 6.4, carbon content: 43.12%, hydrogen content: 3.95%, nitrogen content 0.99%; residue on ignition: 29.59%) were acquired from Sigma-Aldrich (St. Louis, MO, USA), while the trisodium salt of methylglycinediacetic acid (Trilon M, 84% Na_3MGDA, 3.0% NaOH) was obtained from BASF Corporation (Florham Park, NJ, USA).

All solutions were prepared with distilled water obtained from a Simplicity Water Purification System (Millipore, Billerica, MA, USA) and all mobile phases for the chromatography experiments were adjusted to pH of 8.00 ± 0.02 (Symphony SB20 pH Meter, Thermo Electron Corporation, Beverly, MA, USA) and filtered through 0.45 μm Whatman nylon filter membranes (GE Healthcare, Buckinghamshire, UK) before use. The mobile phases which contained the desired EDTA concentrations of 2, 5, 10 and 20 μmol/L were prepared by diluting a 1.5 mmol/L EDTA stock solution. The latter was prepared by dissolving 140 mg of $Na_2EDTA·2H_2O$ to 250 mL with 10 mmol/L Tris buffer pH 8.00 ± 0.02. An analogous procedure was used to prepare the 2, 5, 10 and 20 μmol/L solutions of DTPA and MGDA in 10 mmol/ L Tris mobile phase pH 8.0 (solutions were prepared fresh daily). After final adjustment of the pH of each mobile phase to pH 8.0 ± 0.02 (4 mol/L HCl or NaOH), the SEC column was equilibrated with each mobile phase for 1 hr (flow rate of 1.0 mL/min) before the HA–Cd complex was injected onto the SEC-FAAS system in quadruplicate.

1.2. Preparation of the HA–Cd complex

After the addition of 0.2 g HA to a 100 mL volumetric flask, 10 mmol/L Tris buffer pH 8.0 was added to the mark and the obtained slurry was stirred at room temperature for 16 hr and then kept in a refrigerator overnight and filtered through a 0.45 μm Whatman nylon filter membrane. The prepared HA solution was quantitatively transferred to a 25 mL volumetric flask and 50 μL of a 5000 mg Cd/L solution were added. The obtained mixture was stirred for 12 hr at room temperature. This solution of the HA–Cd complex contained 5 μg of Cd and 1.0 mg of HA per 0.5 mL and had a final pH of 7.11.

1.3. Preparation of CA–Cd complexes and mass spectrometry (MS)

Aqueous solutions which contained EDTA-Cd, DTPA-Cd and MGDA-Cd complexes were prepared by adding 10 μL of a 5000 mg Cd/L stock solution to 5.0 mL of a 20 μmol/L solution of each CA in 10 mmol/L Tris-buffer (pH 8.0). These aqueous solutions were analyzed by MS to detect the presence of CA–Cd 1:1 complexes using an Agilent 1200 series HPLC system at a flow rate of 0.5 mL/min. Separation of CA–Cd complexes from Tris-buffer was achieved by a C_{18} column using gradient elution with 40% methanol in water and increased to 55% over 2 min and held for 10 min. Detection of the complexes was achieved using an Accurate-Mass Q-TOF (Agilent 6520) in both positive and negative modes. The fragmentor voltage was set to 120 V, the gas temperature at 350°C with a drying gas flow rate of 4 L/min and a nebulizer pressure of 12 psi. While it was possible to detect the EDTA-Cd 1:1 complex (Fig. 2b), all attempts to detect the DTPA-Cd and the MGDA-Cd complex by this approach as well as atmospheric pressure chemical ionization (APCI)-MS were unsuccessful. This is attributed to the comparatively much lower concentration of the corresponding CA–Cd complexes compared to the Tris-buffer (10 mmol/L).

1.4. Instrumentation

The employed HPLC system consisted of a 426 HPLC Pump (Alltech Associates, Inc., Deerfield, IL, USA), a manually packed 30 × 1.0 cm (I.D.) Sephadex G-15 size-exclusion chromatography column (fractionation range: 1500–100 Da) and a Rheodyne six-port injection valve which was equipped with a 500 μL PEEK sample-loop. The flow rate was 1.0 mL/min and the void volume was determined by injecting Blue Dextran (v_0 = 542 sec). To detect the HA–Cd complex in the column effluent, the column exit was connected to the pneumatic nebulizer of the FAAS with PEEK tubing (13 cm). Cd-specific detection at 228.8 nm was accomplished with a Buck Model 200A flame atomic absorption spectrometer (FAAS; Buck Scientific, East Norwalk, CT, USA). The FAAS was operated with an air/acetylene flame (oxidant pressure: 241 kPa, fuel pressure: 83 kPa). Raw chromatographic data (i.e., the obtained Cd-specific chromatograms) were smoothed using Sigmaplot 12 software and the Cd peaks were integrated using OriginPro software (version 9.1). Although the obtained Cd-specific chromatograms are depicted only up to 750 sec, all area integrations were performed up to 900 sec. The calculation of the Cd recovery was achieved by diluting 0.5 mL of the HA–Cd complex to 5.0 mL with 10 mmol/L Tris buffer (pH 8.) and by continuously introducing this solution into the FAAS at a flow rate of 1.0 mL/min (quadruplicate). The average area that was obtained for the aforementioned direct aspirations was
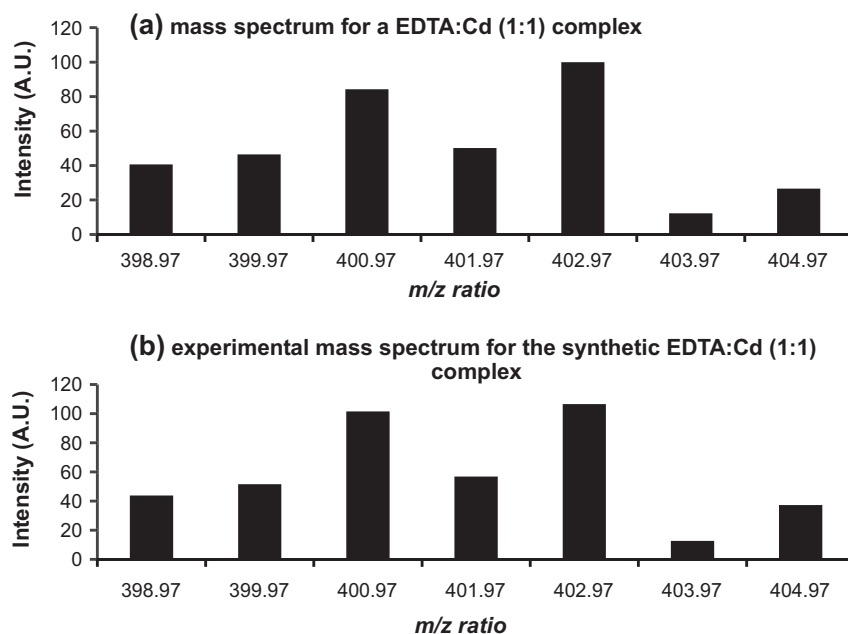
Fig. 2 – Mass spectra obtained for the Cd-EDTA complex in 10 mmol/L Tris-buffer at pH 8.0.

considered to be 100% and all areas that were obtained for each Cd-specific chromatogram were expressed as a percentage thereof (Table 1). Simultaneous multi element-specific detection of C (193.091 nm) and Cd (214.441 nm) in the SEC column effluent was achieved with a Prodigy, high-dispersion, radial-view ICP-AES (Teledyne Leeman Labs, Hudson, NH, USA) at an Ar gas-flow rate of 19 L/min, an RF power of 1.3 kW and a nebulizer gas pressure of 35 psi. The nebulizer gas flow rate was 1.4 L of Ar min^{-1}. The raw data (Salsa Software) were imported into Sigmaplot 13.0 and smoothed using the bi-square algorithm.

2. Results and discussion

Humic acids are widely distributed in the environment (Mantoura et al., 1978) and represent an intrinsically complex mixture of small molecules, oligomers, and some polymers that form labile aggregates that can change their overall size (reversible self-association) depending on the pH and the nature of the buffer salts (Conte and Piccolo, 1999; Hertkorn et al., 2008). Owing to the fact that HA is water soluble and contains a diverse array of functional groups (e.g., carboxylate, thiol, phenolic groups) that can bind to metal ions (Ravichandran, 2004; Vasilevich et al., 2014), it is not surprising that HA-metal interactions also play an important role in the environmental chemistry of potentially toxic metals, such as Cd (Van Ginneken et al., 2001). The analysis of HAs that were extracted from different geographical locations by SEC revealed its average molecular weight (MW) to be in the range 0.3–29 kDa, while an Aldrich HA was previously characterized with an average MW of 4.1 kDa (Perminova et al., 2003). It is impossible to verify if the HA preparation used in that previous study is similar to that used for the present study (although both were from Sigma Aldrich no lot numbers were available for the previously used HA). Considering that CAs

(e.g., EDTA, DTPA) are introduced by various human activities into the environment in considerable quantities (Knepper, 2003) and have long been known to interact with metals in sewage effluents (Bender et al., 1970), we decided to investigate if three structurally related aminopolycarboxylate CAs, namely EDTA, DTPA or MGDA will affect the integrity of a HA–Cd complex at environmentally relevant CA concentrations at pH 8.0. To be able to observe the translocation of Cd from HA to a CA, we employed a SEC-FAAS based approach, which has been previously used to directly observe an abstraction of Cd from a human serum albumin (HSA)–Cd complex by small molecular weight thiols dissolved in the mobile phase (Morris et al., 2014; Sagmeister et al., 2016). In an analogous manner, the retention time of Cd from an injected HA–Cd complex (MW ~ 4 kDa) is expected to increase when a CA is added to the mobile phase since a comparatively much smaller CA–Cd complex (MW ~ 270–390 Da) should eventually form on the column as the CA concentration is increased. This approach hinges on selecting a SEC stationary phase with an appropriate fractionation range. Sephadex G-15 has been used previously to study interactions of metals with HA (Mantoura and Riley, 1975). In addition, its fractionation range (MW 1500–100 Da) should allow one to observe a translocation of Cd from HA (MW ~4000 Da) to a CA (MW ~270–390 Da). The utilization of Sephadex G-15 was therefore deemed to be well suited to probe the complex chemical equilibrium that exists between HA, Cd and individual CAs in a practical manner. Although this approach should allow one to directly observe the effect of increasing μmol/L concentrations of CAs in the mobile phase on the integrity of a HA–Cd complex to possibly observe a partial or complete abstraction of Cd from HA, it cannot provide information on the kinetics of the abstraction event. To enhance clarity it is instructive to describe the results that were obtained for each CA at all investigated concentrations first. Thereafter, the results that were obtained for 2 and 20 μmol/L of all investigated CAs will be discussed in a comparative manner in order to evaluate which CA is least

Table 1 – Results obtained for the analysis of a HA–Cd complex by SEC-FAAS using a mobile phase comprised of 10 mmol/L Tris-buffer (pH 8.0) without or with different chelating agents.								
EDTA			DTPA			MGDA		
Conc. μmol/L	RT (sec)	Peak area (% [c])	Conc. μmolL	RT (sec)	Peak area (%)	Conc. μmol/L	RT (sec)	Peak Area (%)
0	526 ± 1 [a]	5.056 ± 0.338 (86)	0	526 ± 1 [a]	5.056 ± 0.338 (86)	0	526 ± 1 [a]	5.056 ± 0.338 (86)
2	529 ± 2 [a]	5.995 ± 0.663 (102)	2	534 ± 1 [b]	5.236 ± 0.202 (89)	2	517 ± 1 [a]	3.904 ± 0.775 (66)
5	577 ± 13 [a]	4.473 ± 0.533 (76)	5	538 ± 3 [a]	4.738 ± 0.543 (80)	5	546 ± 9 [a]	3.929 ± 0.133 (67)
10	566 ± 8 [b]	3.543 ± 0.256 (60)	10	553 ± 6 [a]	3.985 ± 0.205 (68)	10	551 ± 8 [a]	3.735 ± 1.285 (63)
20	556 ± 1 [b]	3.792 ± 0.299 (64)	20	542 ± 2 [b]	4.802 ± 0.205 (81)	20	543 ± 6 [a]	3.843 ± 0.733 (63)
EDTA-Cd	571 ± 5 [b]	3.342 ± 0.465 (57)	DTPA-Cd	542 ± 1 [a]	3.770 ± 0.597 (64)	MGDA-Cd	641 ± 10 [a]	3.993 ± 0.325 (68)

Abbreviations: CA : chelating agent; RT : retention time.
[a] $n = 3$
[b] $n = 4$
[c] Cd-recovery.

prone to abstract Cd from HA at conditions that are found in the environment.

Fig. 3a depicts a representative Cd-specific chromatogram that was obtained when a HA–Cd complex was analyzed using 10 mmol/L Tris buffer (pH 8.0) as the mobile phase. While the detection of a single Cd peak in the void volume (t_r = 526 ± 1 sec, Table 1) is consistent with the elution of a HA–Cd complex, it is important to point out that not all HA molecules were necessarily 'labeled' with Cd. Based on the comparatively recently advanced view of the behavior of HA in aqueous solution (Conte and Piccolo, 1999), it is likely that the detected HA–Cd complex actually corresponds to Cd ions being bound to one 'HA molecule' *via* several functional groups, possibly carboxylate and/or thiol groups (Perminova et al., 1999; Vasilevich et al., 2014). When a mobile phase containing 2 μmol/L of EDTA was used, the injection of the HA–Cd complex resulted in the elution of a single Cd peak (t_r = 529 ± 2 sec; Fig. 3, line b) with a similar retention time and peak shape as the parent HA–Cd complex (Fig. 3, line a). The slightly elevated Cd recovery of 102% obtained with 2 μmol/L EDTA (Table 1) can be rationalized in terms of the mobilization of a small amount of Cd that had adsorbed onto the stationary phase during the quadruplicate injection of the HA–Cd complex using 10 mmol/L Tris buffer (pH 8.0). A mobile phase which contained 5 μmol/L EDTA resulted in the detection of an exceedingly broad Cd peak (t_r = 577 ± 13 sec) with considerable tailing (Fig. 3, line c) with the main Cd peak eluting almost 50 sec after the parent HA–Cd complex. This retention behavior can be rationalized in terms of the on-column formation of a putative EDTA-Cd complex which had some affinity for the Sephadex G-15 stationary phase. When the mobile phase concentration of EDTA was increased to 10 μmol/L, the chromatographic analysis of a HA–Cd complex revealed a Cd double peak (main peak at t_r = 566 ± 8 sec, Fig. 3, line d), while the 20 μmol/L EDTA mobile phase produced a single Cd peak (t_r = 556 ± 1 sec, Fig. 3, line e) which eluted 30 sec later than the parent HA–Cd complex. Since the latter results (Fig. 3, line d and e) indicated that Cd was completely abstracted from HA, we attempted to corroborate these findings by two different approaches. The first approach involved the injection of a synthetic EDTA-Cd 1:1 complex with 10 mmol/L Tris buffer (pH 8.0), which produced a Cd-peak (t_r = 569 ± 1 sec; Fig. 3, line f) that had a similar peak shape and intensity as the Cd-peak that was observed when a HA–Cd complex was

chromatographed using 20 μmol/L EDTA (Fig. 3, line e). The synthetic EDTA-Cd 1:1 complex, however, had a 15 sec longer retention time. This apparent discord can be rationalized by a small affinity of the synthetic EDTA-Cd complex for the stationary phase (also evident from Fig. 3, line c), which was somewhat reduced in the presence of 20 μmol/L EDTA in the mobile phase. The second approach involved the injection of the HA–Cd complex onto the column using the 20 μmol/L EDTA mobile phase and simultaneously monitoring the emission lines of carbon and Cd in the column effluent using an inductively coupled plasma atomic emission spectrometer (see inset of Fig. 3). These results clearly demonstrated that the retention time of the carbon specific peak that corresponded to HA was smaller than that of the Cd-peak (see vertical dotted line in inset of Fig. 3), which implied that Cd was not bound to HA. Altogether, these results indicate that 5 μmol/L of EDTA in the mobile phase noticeably affected the integrity of the HA–Cd complex, while 20 μmol/L EDTA completely abstracted Cd from HA to form a EDTA-Cd complex.
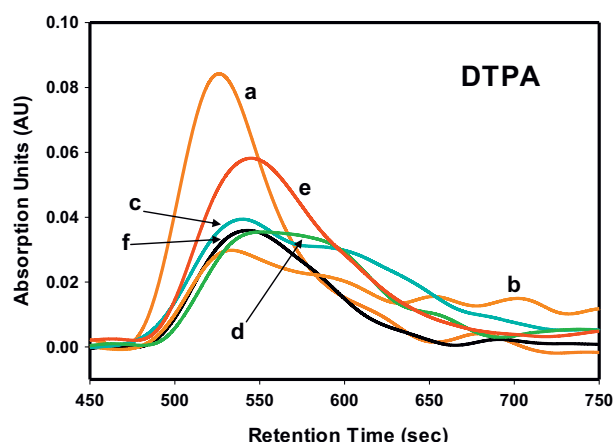
Representative Cd-specific chromatograms that were obtained with mobile phases that contained increasing concentrations of DTPA are depicted in Fig. 4. In contrast to the results that were obtained with 2 μmol/L EDTA (Fig. 3, line b), the corresponding results for DTPA (Fig. 4, line b) revealed a comparatively less intense Cd-peak, which had a slightly but significantly increased retention time (t_r = 534 ± 1 sec) and displayed several Cd peaks on its long retention end. With a 5 μmol/L DTPA containing mobile phase, the retention time of the major Cd-peak (Fig. 4, line c) slightly increased (t_r = 538 ± 3 sec) and resulted in fewer Cd peaks at its long retention end. Increasing the DTPA concentration in the mobile phase to 10 μmol/L resulted in one broad Cd-peak (t_r = 553 ± 6 sec, Fig. 4, line d), which displayed less tailing compared to the 5 μmol/L DTPA mobile phase (Fig. 4, line c). Employing a 20 μmol/L DTPA mobile phase produced a single Cd-peak (t_r = 542 ± 2 sec, Fig. 4, line e), which eluted 16 sec later than the Cd-peak corresponding to the parent HA–Cd complex. The injection of a synthetic DTPA-Cd complex produced a Cd-peak (t_r = 542 ± 1 sec) with a peak shape (Fig. 4, line f) that strongly resembled that of the HA–Cd complex with a mobile phase that contained 20 μmol/L DTPA (Fig. 4, line e) and essentially identical Cd retention times (Table 1). Although the Cd recovery for the DTPA-Cd complex
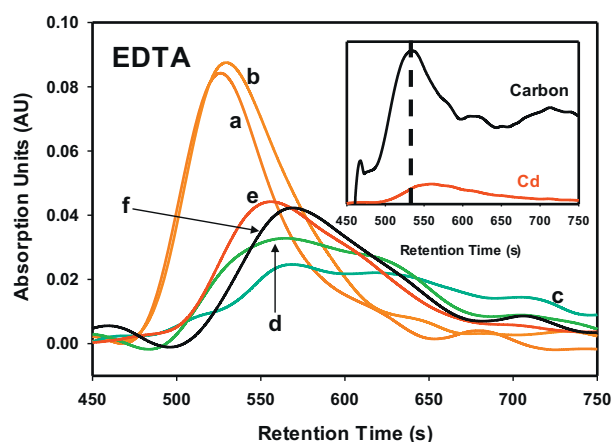
Fig. 3 – Representative Cd-specific chromatograms obtained for the analysis of a HA–Cd complex with either 10 mmol/L Tris buffer (pH 8.0) (line a) and 10 mmol/L Tris-buffer containing either 2 μmol/L EDTA (line b), 5 μmol/L EDTA (line c), 10 μmol/L EDTA (line d), 20 μmol/L EDTA (line e) or a synthetic EDTA–Cd complex (line f). The inset pertains to the results obtained when the HA–Cd complex was analyzed with a 20 μmol/L EDTA mobile phase using an inductively coupled plasma atomic emission spectrometer to detect the emission lines of carbon (from HA) and Cd (from the on-column formed EDTA-Cd complex). The increased baseline that was observed for the carbon-specific chromatogram stems from the utilization of 10 mmol/L Tris-buffer (pH 8.0) as the mobile phase. SEC column: Sephadex G-15 (30 × 1.0 cm); flow rate: 1.0 mL/min; injection volume: 0.5 mL (5.0 μg of Cd); detector: FAAS at 228.8 nm.

Fig. 4 – Representative Cd-specific chromatograms obtained for the analysis of a HA–Cd complex with either 10 mmol/L Tris buffer (pH 8.0) (line a) and 10 mmol/L Tris-buffer containing either 2 μmol/L DTPA (line b), 5 μmol/L DTPA (line c), 10 μmol/L DTPA (line d), 20 μmol/L DTPA (line e) or a synthetic DTPA–Cd complex (line f). SEC column: Sephadex G-15 (30 × 1.0 cm); flow rate: 1.0 mL/min; injection volume: 0.5 mL (5.0 μg of Cd); detector: FAAS at 228.8 nm.

was reduced (64%, Table 1), these results imply that a 20 μmol/L DTPA mobile phase completely abstracted Cd from HA, which is reminiscent of the results that were obtained for EDTA (Fig. 3, lines b–f). In contrast to EDTA, however, 2 μmol/L of DTPA noticeably affected the integrity of the HA–Cd complex.

Fig. 5 summarizes the results that were obtained with increasing concentrations of MGDA in the mobile phase. With the 2 μmol/L MGDA mobile phase, the retention time of the detected Cd-peak was decreased (t_r = 517 ± 1 sec) and its peak shape was noticeably affected compared to that of the parent HA–Cd complex. In addition, a small Cd peak was detected past the inclusion volume (t_r = 665 sec, Fig. 5, line b), which may correspond to a MGDA-Cd species. These results indicate that MGDA (the smallest of the investigated CAs which also contained a methyl-group) affected the hydrodynamic assembly of the HA–Cd complex, which in turn resulted in the abstraction of a small fraction of Cd from HA. Using a 5 μmol/L MGDA containing mobile phase, a comparatively much less intense major Cd-peak was observed (t_r = 546 ± 9 sec; Fig. 5, line c), which was followed by two Cd-peaks of lower intensity (total Cd recovery 67%). The 10 μmol/L MGDA mobile phase produced somewhat similar results (t_r = 551 ± 8 sec, total Cd recovery 63%, Fig. 5, line d), as did the 20 μmol/L MGDA mobile phase (t_r = 543 ± 6 sec, total Cd recovery 63%, Fig. 5, line e). The injection of a synthetic MGDA-Cd complex resulted in the elution of a rather broad Cd-peak (t_r = 641 ± 10 sec, Fig. 5, line f), which

eluted ~100 sec after the major Cd-peak that was observed with the 5–20 μmol/L MGDA mobile phases (Fig. 5, line c-line e). These results indicate that the on-column formed MGDA-Cd complex had a comparatively larger affinity for the stationary phase than the EDTA-Cd complex (Fig. 3, line b and d). The retention time of the synthetic MGDA-Cd complex also coincided with some of the low intensity Cd-peaks that were detected after the main Cd-peak when 5–20 μmol/L MGDA mobile phases were used (Fig. 5, line c-e). Since 20 μmol/L MGDA in the mobile phase resulted in ~50% of Cd still eluting as the parent HA–Cd complex
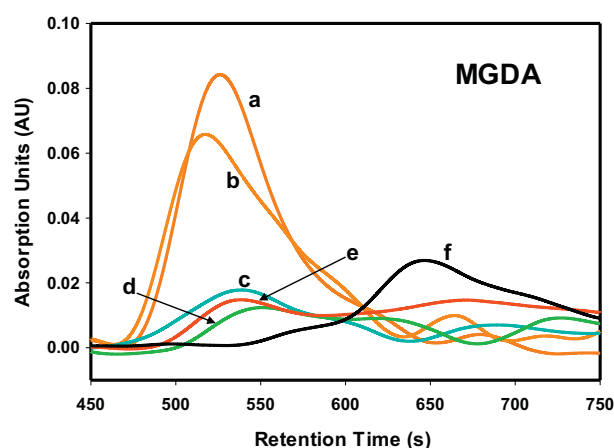
Fig. 5 – Representative Cd-specific chromatograms obtained for the analysis of a HA–Cd complex with either 10 mmol/L Tris buffer (pH 8.0) (line a) and 10 mmol/L Tris-buffer containing either 2 μmol/L MGDA (line b), 5 μmol/L MGDA (line c), 10 μmol/L MGDA (line d), 20 μmol/L MGDA (line e) or a synthetic MGDA–Cd complex (line f). SEC column: Sephadex G-15 (30 × 1.0 cm); flowrate 1.0 mL/min; injection volume: 0.5 mL (5.0 μg of Cd); detector: FAAS at 228.8 nm.

followed by a MGDA-Cd complex, MGDA was unable to abstract all Cd from HA. It is useful to point out here that despite the inherently limited chromatographic resolution of the employed Sephadex G-15 stationary phase, we were able to gain insight into the different effect of structurally related EDTA, DTPA and MGDA on the integrity of the HA–Cd complex (Figs. 3–5).

In order to more clearly visualize the differences between the investigated CAs, it is instructive to depict the results that were obtained for the lowest CA concentration (2 μmol/L) (Fig. 6) because these results are also most environmentally significant as rivers have been reported to contain similar concentrations (e.g., 4 μmol/L EDTA (Oviedo and Rodriguez, 2003)). While EDTA clearly exerted only a marginal to negligible effect on the integrity of the HA–Cd complex (Fig. 6 line b), MGDA resulted in an asymmetric Cd-peak shape followed by the elution of a small MGDA–Cd complex (Fig. 6 line d). DTPA clearly had the most profound effect in terms of abstracting Cd from the HA–Cd complex (Fig. 6 line c) since a considerable fraction of the formed DTPA-Cd peak had an affinity for the stationary phase that eluted beyond the inclusion volume. Based on the corresponding complex formation constant of Cd (log K) which decreases in the order DTPA (19.0), EDTA (16.4) and MGDA (10.6) (Knepper, 2003), one would expect a decreasing effect of these CAs on the integrity of the HA–Cd complex. While DTPA was indeed most effective, EDTA, however, was not and MGDA was somewhat effective (Fig. 6). The results obtained for MGDA clearly indicate that in addition to thermodynamic consideration, structural factors must also be considered in terms of explaining the effect of this CA on the integrity of the HA–Cd complex. In support of this notion, the altered peak shape of the HA–Cd peak induced MGDA (Fig. 6 line d) implies that the hydrodynamic radius of the HA–Cd complex (i.e., its structure) was altered by MGDA and that this subtle rearrangement may have contributed to the abstraction of some Cd from HA (see small Cd peak in Fig. 6 line d).
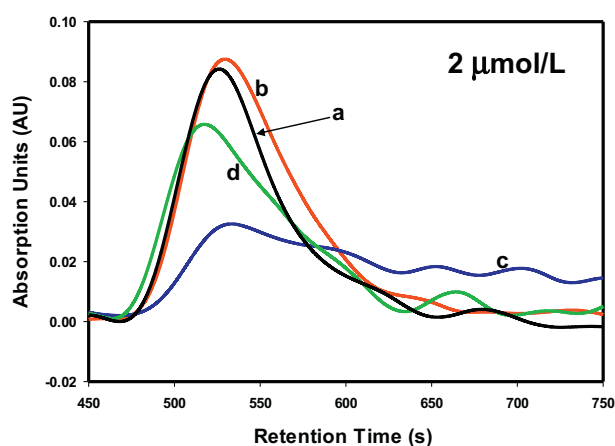
Fig. 6 – Representative Cd-specific chromatograms obtained for the analysis of a HA–Cd complex with either 10 mmol/L Tris buffer (pH 8.0) (line a) and 10 mmol/L Tris-buffer containing either 2 μmol/L EDTA (line b), 2 μmol/L DTPA (line c) or 2 μmol/L MGDA (line d). SEC column: Sephadex G-15 (30 × 1.0 cm); flowrate 1.0 mL/min; injection volume: 0.5 mL (5.0 μg of Cd); detector: FAAS at 228.8 nm.

With the 20 μmol/L mobile phase concentration, both EDTA and DTPA completely abstracted Cd from the HA–Cd complex (Fig. 3 line e and inset, Fig. 4 line e), while MGDA was only partially able to do so (Fig. 5 line e). These results are in accord with the corresponding complex formation constants of Cd (log K) for DTPA to EDTA and MGDA. Therefore, it appears that at 20 μmol/L CA concentrations thermodynamic factors predominantly govern the abstraction of Cd from HA, whereas at 2 μmol/L concentrations structural factors significantly contribute as well.

Overall, the most relevant finding of our investigations is that the integrity of a HA–Cd complex can be affected by CA concentrations that are encountered in rivers and WTP effluents. For example, Cd was partially abstracted by 2 μmol/L of MGDA/DTPA (Fig. 6) and by 5 μmol/L of EDTA (Fig. 3), while 20 μmol/L of EDTA/DTPA were required for a complete abstraction (Figs. 3 and 4). The observed on-column formation of CA–Cd complexes under environmentally relevant conditions at pH 8.0 (the pH of rivers) suggests that these CA–Cd complexes are also likely to be present in surface waters even though they may contain HAs (Hertkorn et al., 2008). These findings are particularly relevant from an environmental pollution point of view because of the bioavailability of CA–Cd complexes to certain plants (Schaider et al., 2006; Van Engelen et al., 2007), including durum wheat (Berkelaar and Hale, 2003; Harris and Taylor, 2013). Accordingly, our results suggest that the aqueous ecosystem downstream of WTPs – which contains small concentrations of chelating agents and Cd (as well as other metals (Bedsworth and Sedlak, 2001)) – is a likely environmental site where human activities can perturb the biogeochemical Cd cycle. The resulting increased bioaccumulation of Cd in periphyton in rivers downstream of WTPs is important because it acts as a conduit for the movement of Cd through the aquatic food ecosystem. Since it has been reported that the presence of nitrilotriacetic acid (NTA) – which is structurally related to EDTA – enhances the uptake of Cd by periphyton (Bradac et al., 2009), our findings also directly relate to the emerging practice of utilizing WTP effluent to irrigate food crops (e.g., in areas that suffer from severe drought), which can result in an increased unwitting dietary ingestion of Cd (Khan et al., 2008). From a food safety perspective, jurisdictions which utilize WTP effluent for the irrigation of food crops should therefore ascertain that the latter do not inadvertently accumulate CA–Cd complexes into the edible part to compromise food safety. Alternatively strict regulatory guidelines could be defined to establish maximum permissible concentrations of either free CAs (Jimenez, 2013) and/or CA-metal complexes in wastewater (Bedsworth and Sedlak, 2001). If WTP effluent is used for the irrigation of food crops, one has to also consider that excess free CAs may mobilize Cd that is already present in the soil (e.g., fertilizers can contain up to 225 mg Cd/kg of Cd as a contaminant (Mortvedt, 1996)) into the edible part of a food crop, if the mineral goethite – which is known to adsorb EDTA-metal complexes – is largely absent (Nowack and Sigg, 1996). Last but not least, the utilization of river water that contains low concentrations of CA–Cd complexes as a source of drinking water (e.g., by inhabitants of one city downstream of another) could also be potentially problematic in the long term since labile complexes of Cd can enhance the

bioavailability of Cd to intestinal cells (Verheyen et al., 2012) and therefore contribute to an increased absorption of Cd into human tissues (Drasch, 1983).

3. Conclusion

We have investigated the effect of 2–20 μmol/L concentrations of EDTA, DTPA and MGDA on the integrity of a HA–Cd complex. Mobile phases which contained ≥5 μmol/L of all investigated CAs affected the integrity of the HA–Cd complex, while DTPA and MGDA were already effective at 2 μmol/L. The observed CA-mediated abstraction of Cd from HA is relevant with regard to the chemistry that unfolds in the confluence zone where rivers that may contain HA–Cd complexes (e.g., downstream of mining operations) mix with the effluent plume of WTPs which frequently contain CAs at elevated concentrations (e.g., up to 19 μmol/L of EDTA (Bedsworth and Sedlak, 2001; Fuehrhacker et al., 2003) and 7.3 μmol/L of DTPA (Knepper, 2003)), as well as low concentrations of Cd (up to 2 μg/L). The bioavailability of EDTA–Cd complexes implies that aquatic ecosystems downstream of WTPs may be affected by increased Cd concentrations in periphyton as has already been reported (McCauley and Bouldin, 2016). Another issue that our results directly relate to is the emerging practice of using WTP effluent for the irrigation of food crops in areas that are affected by drought. This practice is potentially problematic because certain CA–Cd complexes can be absorbed into the root system of plants (Schaider et al., 2006) and the accumulated Cd can be subsequently translocated to the edible parts (Khan et al., 2008). Taken together, our results suggest that CAs which are omnipresent in many surface waters may have a much more profound effect on the global biogeochemical cycles of toxic metals, such as Cd than previously thought as they can potentially exacerbate contamination problems. Future studies should be directed toward studying the comparative bioavailability of CA-Cd complexes and to identify which food crops can safely be grown with WTP effluents that contain CA–Cd complexes without unintentionally compromising food safety (Khan et al., 2008).

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