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Bioavailability of Cadmium–Organic Complexes to Soil Alga—An Exception to the Free Ion Model

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It is generally considered that cadmium bioavailability shows a considerable dependence on chemical speciation of Cd in solution, correlates best with the activity of free metal ion (Cd^{2+}) in solution, and is largely indifferent to soluble metal complexes. The role of soluble organic matter (DOM) and soluble metal−organic complexes in metal bioavailability and toxicity, however, is not clear. Growth studies with a soil alga (*Chlorococcum* sp.) were conducted on a growth medium and pore water of Cookes Plain soil (Paleuxeralf), spiked with Cd as $Cd(NO_3)_2$. Speciation of the Cd in pore water, and in growth medium with and without citrate, was performed using the MINTEQA2 computer model incorporating updated values of the stability constants of Cd−DOM complexes, as well as using anode stripping voltammetry. Analysis of the toxicity data showed that Cd−citrate, as well as the Cd−DOM complexes, is bioavailable and contributes toward the toxicity to alga. These data contradict the long-held notion that Cd−DOM complexes are not bioavailable to soil biota although they may increase the mobility of Cd.

KEYWORDS: Bioavailability; cadmium; soil alga; *Chlorococcum*

INTRODUCTION

The principal environmental concern over elevated concentrations of trace metals, particularly Cd, in natural ecosystems relates to toxicity. From an ecological perspective, this concern is aimed toward the problem of availability and toxicity to soil organisms and plants grown in soils. The general consensus, drawn from studies on the toxicity of soluble trace metals, is that metal speciation is more significant than total metal concentration (*1*−*3*). Metal bioavailability is reported to show a considerable dependence on chemical speciation of metal in solution (*4*) and correlates best with the activity of free metal ion (M^{2+}) in solution. Further, it is considered largely indifferent to soluble metal complexes (*5*, *6*), except in the case of Cd−Cl complexes (*7*). However, it is not clear whether the increased uptake of Cd in the presence of Cl^- is due to an increased ease of diffusion of Cd through roots or if the cadmium chloride complexes are themselves bioavailable.

It is also argued that the free ion activity model (FIAM) does not preclude biological uptake of metals as metal complexes (*6*, *8*, *9*). Recent studies (*8*, *9*) on Cd and Zn uptake by the unicellular green alga *Selenastrum capricornutum* from a synthetic medium buffered with citrate have also indicated an exception to the FIAM model. However, the role of soluble organic matter (DOM) in soil solutions, as exist under natural terrestrial systems, in Cd availability and toxicity is not clear. The objectives of this study were (1) to assess the role of soluble organic matter in the Cd speciation in soil solutions and (2) to assess the bioavailability of Cd−organic complexes using soil pore water as the growth medium for a soil alga (*Chlorococcum* sp.).

MATERIALS AND METHODS

Soil Sample. The soil used in the present study was from Cookes Plain, South Australia (Typic Palexeralf). The sandy soil sample collected from the surface horizon (0−15 cm) was air-dried, ground to pass through a stainless steel 2-mm sieve, homogenized, and stored for subsequent analysis. Selected characteristics of the soil are presented in **Table 1**. The pH of the soil in water (1:2) was measured using an Orion model 720 A pH meter (Orion Research Inc., Boston, MA). Mechanical analysis of the soils was carried out by gravity sedimentation (*10*) after dispersion of the soils with 5% sodium hexametaphosphate. Total C and inorganic C contents of the soils were determined using a Leco CNS-2000 analyzer (Leco Corp., St. Joseph, MI) and following the method of Loveday and Reeve (*11*), respectively. The organic C content was deduced from the difference between the two values. The free Fe content of the soils was determined following the methods of Mehra and Jackson (*12*). For total Cd analysis, the soils were ground to <0.1 mm in an agate mortar with a pestle and homogenized before use. The samples, 0.5 g in duplicate, were digested with $HF-HClO_4$ in 50-mL Teflon beakers (*13*).

A sufficient volume of Cd solution (as Cd nitrate) was added to 400 g of soil in an open 1-L plastic container (20-cm diameter) to achieve added soil Cd concentrations of 1, 10, 25, and 100 mg of Cd kg^{-1}. Deionized distilled water was then added to bring the soil to

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Table 1. Selected Characteristics of the Cookes Plain Soil Used in the Study

soil classification	mechanical analysis (g kg^{-1}) sand	silt	clay	pH	org C (%)	Fe[a] (%)	total Cd (mg kg^{-1})
Typic Paleuxeralf	934	16	50	6.0	0.77	0.047	0.198

[a] Fe–citrate–bicarbonate–dithionite extractable (*12*).

saturation. The soil was allowed to dry under ambient conditions for 10 days before it was again rewetted to saturation with deionized distilled water. The soil was taken through seven such wetting and drying cycles, and at the end of the last cycle, the soil was thoroughly mixed, homogenized, and stored at 4 °C for subsequent analysis.

Pore Water Extraction and Analysis. A sufficient amount of demineralized, distilled water was added to 150 g of the soil, both the natural untreated soil and the soil to which Cd was added, in duplicate, to bring the soil to saturation. The soil was mixed thoroughly to form a slurry and equilibrated for 24 h in a 250-mL plastic container. The soil solution was recovered from the slurry by filtration under vacuum using Whatman 50 filter paper. The solution was centrifuged at 15800*g* for 30 min and passed through 0.01-μm Millipore filters (*14*).

The pH of the soil solution was measured immediately using an Orion model 720 A pH meter (Orion Research Inc., Boston, MA). The concentrations of the associated cations were measured using a Spectroflame inductively coupled plasma emission spectrometer (Spectro Analytical Instruments, GMBH, Kiev) and/or a GBC 906AA atomic absorption spectrophotometer (GBC Equipment Pty Ltd., Dandenong, Victoria, Australia). The concentrations of anions were measured using a capillary ion analyzer (CIA Millipore Waters). The concentration of dissolved organic carbon (DOC) was determined on a Dohrmann DC-180 total organic carbon analyzer (Rosemount Analytical Division, Santa Clara, CA).

Cd Speciation in Soil Solution. Speciation of Cd in solution was carried out using the MINTEQA2/PRODEFA computer program (*15*), with the concentrations of major cations, anions, Cd, and other trace metals, such as Cu, Pb, and Zn, pH, and DOM as input in the database. The database of MINTEQA2 includes over 900 dissolved species, 500 dissolved mineral species, and 13 species representing complexes of Cd and other trace heavy metals with the soluble organic matter (DOM). In this model DOM was treated as a complex material consisting of various kinds of monoprotic acid sites, which were assumed to be normally distributed with respect to their log *K* values for protons and metals (*16*). The database of the MINTEQA2 program was updated using the stability constants of the metal−DOM complexes (*17*): Mg (2.90) < Ca (3.90) < Zn (5.00) < Pb (5.10) < Cd (5.80) < Cu(II) (6.50).

Free Cd Measurement by Anode Stripping Voltammetry (ASV). The free ion concentration (labile fraction) of cadmium in the solution was determined by anode stripping voltammetry (*18*). A mercury film electrode was formed on a Metrohm 3-mm-diameter glassy carbon electrode using 10.2 mL of electrolyte, $HgNO_3$ (20 μg mL^{-1} Hg) in 0.02 M KNO_3 solution. The electrolyte was initially degassed by bubbling N_2 gas through the medium for 2 min, and a Hg film was deposited for 3 min at −900 mV against a Ag/AgCl (3 M KCl) reference. A constant nitrogen flow was then maintained over the solution during each run to maintain controlled oxygen conditions. For each sample, a new Hg film was prepared as above and ASV was conducted on a blank, and then ASV measurements on a 400-μL sample were added to the blank solution. Up to three 25−100-μL aliquot additions of standard 100 μg mL^{-1} Cd^{2+} solution were then made to the sample solution for calibration. With each volume addition, a 10-s degassing was conducted before an additional ASV run. The response was found to be linear over the 0−0.5 μg mL^{-1} range. A 95% recovery was observed for Cd in standard addition runs without sample.

Algal Assay. Algal growth inhibition tests (*19*) were performed by exposing the axenic culture of a soil alga *Chlorococcum* sp. to the pore water or Bold's basal medium as growth medium (*20*) placed in sterile flasks. Axenic culture of *Chlorococcum* sp., isolated from an uncontaminated soil (*21*), was maintained under continuous illumination in Bold's basal medium at 26 °C (*20*). Portions (10 mL) of growth medium treated with $Cd(NO_3)_2$, at final Cd concentrations in the medium ranging between 0 and 2 μg mL^{-1}, with and without 0.001 M citric acid, or of soil pore water placed in sterile flasks (50-mL glass flasks with Teflon-lined screw caps) were inoculated with exponentially growing culture (0.5 mL) of *Chlorococcum* sp. Controls containing only growth medium and alga were included in the test in addition to the pore water from untreated soil. The test flasks were placed in a temperature-controlled (26 °C) orbital shaker set at 100 rpm under continuous illumination (200 μE m^{-2} s^{-1} PPFD) provided by cool white fluorescent lamps. At the end of 96 h, growth of the alga was estimated in terms of cell count in a Neubaur hemocytometer using a phase contrast microscope (*19*). Growth inhibition of the alga was used as an endpoint in this bioassay. All the assays were conducted in triplicate.

Cd Uptake by the Alga. The concentration of Cd in the algal cells was determined as follows. A 5-mL portion of the algal culture from each of the experimental flask was centrifuged (7000 rpm, 10 min), and the cell pellets were washed with Na_2EDTA (5 mL; 100 μmol L^{-1}) for 10 min and subsequently twice with sterile distilled water. The EDTA wash was aimed at removing the surface-bound Cd so that the intracellular Cd can be estimated. The washed algal pellets were digested with concentrated HNO_3 by heating at 200 °C for 2 h, and the digests were diluted to appropriate levels with deionized water for Cd analysis.

Cadmium Analysis. The Cd content in the soil solutions and algal digest was determined on a Varian Spectraa 400 plus graphite furnace atomic absorption spectrophotometer (GFAAS) at 228.8 nm with a pyrolytically coated tube and L'vov platform, using background correction with Pd−Cl in citric acid and/or NH_4NO_3 as a modifier, and/or on a GBC 906AA atomic absorption spectrophotometer (GBC Equipment Pty Ltd., Dandenong, Victoria, Australia) with suitable dilution (*22*). The quality assurance of Cd analyses was checked by routinely analyzing a certified reference sediment sample. Analyses of sediment sample (BCSS-1; certified Cd concentration, 0.25 ± 0.04 mg/kg) gave a Cd concentration of 0.23 ± 0.02 mg/kg. The certified standard samples were included at every stage of analysis.

Deionized distilled water was used in preparing stock solutions of all the reagents, which were of analytical reagent grade. All glassware used was previously soaked overnight in 2 M HCl and rinsed with deionized distilled water.

RESULTS AND DISCUSSION

Solution Cd Speciation. The data on soil pore water of the soil spiked with different amounts of Cd are presented in **Table 2**. Soil solutions are obtained either by extraction with a dilute salt solution (soil:solution = 1:2) or from field-moist soils, using centrifugation and/or filtration (e.g., refs *23*, *24*). In the present study, the soil solutions were obtained using the saturated paste technique (*14*) and were assumed to represent the soil water at the soil−water interface (interstitial water).

The pH increased from 5.6 to 6.5 with and increase in the amount of Cd added to the soil (**Table 2**). The increase in the pH might be due to the increased amounts of Cd as $Cd(NO_3)_2$ added to the soil. The amount of Cd in the pore water, ranging from 0.003 to 2.028 mg L^{-1}, was observed to be only 0.3−2.0% of the Cd added to the soil. Even though large amounts of Cd were added to the soil, most of the Cd was bound to various sorption sites in the soil, such as exchangeable, specifically sorbed, and bound to both organic and inorganic sorption sites. More than 98% of Cd was retained in solid phase.

The dissolved organic carbon content (DOC) of the soil solutions in the present study varied between 68 and 47 mg L^{-1} (**Table 2**). The DOC values of interstitial waters of soils are reported to range from 65 to 830 mg L^{-1} (*14*). The nature of DOM in soil interstitial water (pore water) is poorly established and poorly understood (*25*). The DOM may consist

Table 2. Speciation of Cd in Soil Solution in Cookes Plain Soil Spiked with Different Amounts of Cd as $Cd(NO_3)_2$, As Determined Using MINTEQA2 Computer Model with Updated Database

amount of Cd added[a]	soil solution (mg L^{-1})			Cd species in solution (μg L^{-1})				
	Cd	org C	pH	Cd^{2+}	$CdCl^{+}$	$CdSO_{4(aq)}$	$CdNO_3^{+}$	Cd–DOM
0	0.003	68	5.6	0.1				2.9
1	0.013	64	5.8	0.6				12.4
10	0.115	62	6.0	5.3	0.3	0.1		109.3
25	0.479	58	6.2	49.7 (73)	1.8	0.6	0.1	426.8
100	2.028	47	6.5	503.9 (486)	21.3	7.0	4.6	1491.2
ASE[b]	±5%	±5	±0.1					

[a] Cd added as $Cd(NO_3)_2$ in milligrams of Cd per kilogram of soil. [b] Average standard error. The values in parentheses are the free Cd^{2+} concentration in the solutions determined by anodic stripping voltammetry.

Table 3. Speciation of Cd in Soil Solution of Cookes Plain Soil Spiked with 25 mg of Cd kg^{-1} of Soil, Using Different log *K* Values for the Cd–DOM Complexes in the MINTEQA2 Computer Model

log K_{Cd-DOM} value	reference citation	Cd species in solution (μg L^{-1})		
		Cd^{2+}	Cd–DOM	Cd–inorg[a]
4.10	(*41*)	391.9 (81.8) [b]	40.7 (8.5)	46.4 (9.7)
5.30	(*23*)	149.7 (31.3)	308.7 (64.4)	20.6 (4.3)
5.80	(*40*)	49.7 (10.4)	426.8 (89.1)	2.5 (0.5)

[a] Cd–inorg: Cd–inorganic ligand complexes, such as $CdCl^{+}$, $CdSO_4$, and $CdNO_3^{+}$. [b] Values in parentheses are percent distribution of the species.

of a wide range of organics, including fulvic and humic acids, amino acids, and other low-molecular-weight organic acids.

Studies on metal speciation in soil solution indicate that complexes with DOM are significant for metals such as Fe, Al, and Cu (*26*). Many authors report Cd^{2+} speciation using chemical equilibrium models (*27*−*29*) and assume the concentration of soluble organic complexes with metals to be insignificant (*30*). The reported high values of pCd^{2+} ranged between 8 and 5, and the proportion of Cd−organic complexes is usually reported as being negligible (*30*−*34*). Nevertheless, the association of Cd with organics in soil solution is not insignificant (*35*, *36*).

The accuracy of the computed estimate of free Cd^{2+} depends on the stability constants used for metal−DOM complexes (*36*) used in the computer models. Early work on stability constants of metal complexes with fulvic acids (FAs) was done using the ion-exchange equilibrium method and continuous variation method (*37*). Even at the same pH, a wide range of values was evident. The extent to which the results reflect differences in the nature or source of FA is unknown. Using ion-selective electrodes (*38*) and the Scatchard plot technique (*39*), conditional stability constants of Cd, Cu, and Pb with natural organic matter isolated from a number soil water samples were produced (*40*). The reported value for log *K* of Cd−DOM was 5.83 ± 0.35. To assess the effect of log *K* values (stability constants) of Cd−DOM complexes on the proportion of free Cd^{2+}, we speciated soil solution Cd using the MINTEQA2 computer program. The database was updated using different log *K* values, as reported in the literature. To assess the effect of log *K* values of Cd−DOM complexes on the proportion of free Cd^{2+}, we speciated soil solution Cd using different log *K* values as reported in the literature. As shown in Table 3, the proportion of free Cd^{2+} and Cd−DOM complexes in soil solution varies from 10.4 to 81.8% and from 8.5 to 89.1%, respectively, depending on log *K* values for Cd−DOM complexes. These results highlight the need for (a) caution in the interpretation of geochemical speciated data for metals in the absence of defined log *K* values and (b) laboratory-based analytical techniques for metal speciation.

Both the individual chemical compounds (aliphatic acids, amino acids, etc.) and FA-type constituents are involved in the formation of mobile complexes, with the FAs being the most effective in complexing metals in soils (*42*). The ability of FAs to form stable complexes can be attributed to their high content of O-containing functional groups, with COOH and phenolic OH dominant (*43*, *44*). Factors influencing the metal−FA interaction include pH, ionic strength, molecular weight, and functional group content, with the trivalent ions bound to a greater extent than divalent ions (*17*). Fulvic acid consists of phenolic and benzene carboxylic acids held together through H-bonds to form polymeric structures of considerable stability. The log *K* values of the Cd−low-molecular-weight organic acid complexes, reported in the literature and used in the computer speciation models, viz., 1.9−5.3 (*46*) and 2.6−5.8 (*4*), are in the same range as those reported for Cd−fulvate complexes: 2.7−3.5 (*47*) and 4.1−5.8 (*17*). The structure of DOM was assumed to resemble that of fulvic acid, and the stability constants of metal−fulvates were used in the database of the model. The Scatchard plot approach for obtaining the stability constants of metal−DOM complexes has been the method of choice in most of the recent studies of metal binding by organic acids (*17*). The stability constants of the metal−DOM complexes, as reported by Stevenson and Fitch (*17*), were used in the revised database in the present study (see Materials and Methods section). Almas et al. (*23*) also arrived at a conditional stability constant of around 5.30 for Cd−fulvate using MINEQL+ (*47*).

A number of structural models were proposed for FAs (*48*−*51*) to explain the hydrophobic xenobiotic interactions. The most exhaustively studied FA was the one obtained from the Suwannee River (Georgia, U.S.A.), and a number of models were proposed which differ in minor detail. The model of FA from the Suwannee River, as detailed by Stevenson (*52*), was used in arriving at the molar concentration of the DOM in the soils used in the present study.

Data on the speciation of Cd in pore water of Cookes Plain soil spiked with different amounts of Cd, as measured using the MINTEQA2/PRODEFA computer model (*45*) with updated database, are presented in **Table 2**. The data indicated that the Cd−DOM complex species were the dominant soluble species (73.5−96.7%), followed by the free metal Cd^{2+} species (3.3−24.9%), with low amounts of Cd−inorganic complexes (1.2−5.3%). These findings are consistent with the recent differential pulse anodic stripping voltammetry Cd speciation studies that showed the presence of dominant amounts of Cd−organic complex in most of the soil solutions (*23*, *24*).

The free Cd^{2+} concentration of the pore water samples, containing 0.479 and 2.028 mg L^{-1} of total Cd, was measured using anodic stripping voltammetry. The values 0.073 and 0.486 mg L^{-1} obtained were close to the values of 49.7 and 503.9 μg

Table 4. Influence of Cd on Growth of Alga (*Chlorococcum* sp.), Both in the Growth Medium Spiked with Different Concentrations of Cd Added as $Cd(NO_3)_2$ and in Soil Pore Water of Cookes Plain Soil Equilibrated with Different Amounts of Cd Added as $Cd(NO_3)_2$

growth medium		soil pore water	
Cd (mg L^{-1})	algal growth[a]	Cd (mg L^{-1})	algal growth[a]
0	207.0 (0)	0.003	216.6 (0)
0.1	258.0 (–25)	0.013	237.6 (–10)
0.5	99.4 (54)	0.115	194.3 (11)
1.0	64.1 (69)	0.479	44.6 (79)
2.0	0 (100)	2.028	0 (100)

[a] Number of cells $\times 10^4$ mL^{-1}; % inhibition of growth in parentheses. Negative numbers indicate promotion of growth at low concentration of Cd in solution. Average standard error was ± 5%.

L^{-1} obtained from the MINTEQA2 model (**Table 2**). The direct measurement of Cd^{2+} concentration using anodic stripping volammetry validated the speciation data obtained by using the MINTEQA2 computer model.

The present work showed Cd−DOM complexes as the dominant species in soil solution, which could be arrived at using the MINTEQA2 computer program with updated log *K* values of metal−DOM complexes in the database.

Cd Bioavailability to Soil Alga. The relative importance of the free Cd^{2+} and Cd−DOM complex species of the pore water in Cd bioavailability was assessed using data on the growth of soil alga (*Chlorococcum* sp.) in growth medium and in soil pore water. The use of soil pore water in algal growth studies has not been previously attempted to assess the role of toxic metals in algal growth.

Data on algal growth with the soil pore water obtained from the soil spiked with different amounts of Cd, and the growth medium spiked with different amounts of Cd (0, 0.1, 0.5, 1, and 2 mg Cd L^{-1}), were presented in **Table 4**. The algal growth decreased with increasing amounts of Cd in solution and/or medium. However, at low amounts of Cd in soil pore water (0.013 mg L^{-1}) and/or medium (0.1 mg L^{-1}), a slight stimulation of growth (25% and 10%, respectively) was observed. The data presented in **Table 4** indicated 79% inhibition of algal growth at a total Cd concentration of 0.479 mg L^{-1} in the soil pore water. The free Cd^{2+} concentration of the pore water, corresponding to a total Cd concentration of 0.479 mg L^{-1}, was 0.050 mg L^{-1} (MINTEQ computer program) or 0.073 mg L^{-1} (ASV) (**Table 2**). In contrast, 25% stimulation in algal growth was observed at a Cd concentration of 0.100 mg L^{-1} in the growth medium (**Table 4**). The free Cd^{2+} concentration at a Cd concentration of 0.100 mg L^{-1} of growth medium was 0.090 mg L^{-1}, as obtained using the MINTEQA2 computer program (**Table 6**, below). If Cd^{2+} species are the only bioavailable species of Cd in solution, the free Cd^{2+} concentration of 0.050−0.073 mg L^{-1} in soil pore water is expected to cause stimulation in algal growth. On the contrary, 79% inhibition to algal growth was observed. The toxicity at this level of Cd in solution must be due to the bioavailability of organically complexed Cd species (0.427 mg L^{-1}, concentration of Cd−DOM species, **Table 2**) in soil pore water to alga.

The data on algal growth (**Table 4**) fitted best ($r^2 > 0.95$) with a polynomial model, $y = ax^2 + bx + c$, where *y* is the total concentration of Cd in the growth medium or soil pore water and *x* is the percent inhibition in growth of alga. The LC_{50} (concentration of Cd for 50% of inhibition in algal growth, mg L^{-1}) values for the algal growth as calculated from the polynomial model were 0.413 mg L^{-1} for the growth culture medium and 0.302 mg L^{-1} for Cookes Plain soil pore water.

Table 5. Speciation of Cd in Soil Pore Water and Growth Medium at LC_{50}, As Determined Using MINTEQA2 Computer Program with Updated Database[a]

	Cd species in solution (μg L^{-1})					
	LC_{50}	Cd^{2+}	$CdCl^+$	$CdSO_4$ (aq)	$CdNO_3^+$	Cd–DOM
growth medium	413	372.0	8.0	20.3	17.9	1.9
soil pore water	302	31.1	1.2	0.4		269.3

[a] LC_{50}, Cd concentration (μg L^{-1}) at 50% inhibition of algal (*Chlorococcum* sp.) growth. Ionic concentration (mg L^{-1}) of the solutions: for growth medium, Ca 7, Mg 8, Na 77, K 83, NO_3 170, Cl 40, SO_4 35, pH 7.0; for soil pore water, Ca 22, Mg 4, Na 16, K 27, NO_3 79, Cl 28, SO_4 14, pH 6.0.

Table 6. Speciation of Cd in Growth Medium with and without Citrate, and Uptake of Cd by Alga (*Chlorococcum* sp.)[a]

Cd (μg L^{-1})	citric acid (M)	pH	Cd species in solution (μg L^{-1})					Cd uptake (μg L^{-1})
			Cd^{2+}	$CdCl^+$	$CdSO_4$ (aq)	$CdNO_3^+$	Cd–citrate	
100	0	7.0	90.5	4.8	4.2	0.5		75.4
100	0.001	6.5	9.2	0.5	0.4	0.1	89.8	64.7

[a] Speciation of Cd in solution was carried out using the MINTEQA2 computer program.

The speciation of the solutions at a Cd concentration corresponding to the LC_{50} value, for both growth medium and pore water, is presented in **Table 5**. The data on algal growth presented in Table 4 indicated that 50% reduction in algal (*Chlorococcum* sp.) growth occurred at a Cd concentration (LC_{50} value) of 302 μg L^{-1}, which could be termed as bioavailable Cd. The speciation study showed the concentration of free Cd^{2+} and Cd−DOM complexes to be 31 μg of Cd L^{-1} and 269.3 μg of Cd L^{-1} at the LC_{50} value (**Table 5**). Thus, we can conclude that the observed toxicity to alga must be due to the availability of Cd−DOM complexes (269 μg of Cd L^{-1}). The 50% reduction in algal growth could not have occurred at the low free Cd^{2+} concentration (31 μg L^{-1}) of the soil pore water, assuming that only Cd^{2+} species are bioavailable and the Cd−organic complexes are not bioavailable. The reduction in algal growth must be due to the availability of the Cd present as soluble Cd−DOM complexes that had been taken up by the algae.

It is generally accepted that the free Cd^{2+} in soil solution is the major bioavailable species (*5*). It is also argued that although the Cd−organic complexation increases metal mobility, it actually reduces Cd toxicity (*53*). However, the data presented above clearly showed that the Cd−DOM complexes were bioavailable and caused Cd toxicity, as indicated by the 50% decrease in algal growth.

Direct evidence for the bioavailability of Cd−organic acid complexes was obtained using citric acid, a common organic metabolite present in soil solutions, in the uptake of Cd by the soil alga (*Chlorococcum* sp.). In the absence of citric acid in the growth medium, it is apparent that the Cd present as Cd^{2+} in the medium was bioavailable (algal Cd concentration of 75.4 μg L^{-1}, Table 6). However, the speciation of Cd in algal medium with citric acid showed that 89.8% of the total Cd is present as Cd−citrate complexes (**Table 6**). Assuming that the entire amount of Cd^{2+} (9.2 μg L^{-1}) present in the medium was bioavailable, the remaining amount of bioavailable Cd (55.5 μg L^{-1}) must be due to the availability of Cd−citrate complexes. The data clearly demonstrated the uptake of Cd as Cd−citrate complexes by the alga (**Table 6**). An increase in the availability of Cd to green alga, *Selenastrum caprocornutum*, in the presence

Table 7. Influence of Cd and Cd Species, As Determined Using MINTEQA2 Computer Model, on Growth of Alga (*Chlorococcum* sp.), Both in the Growth Medium and in Soil Pore Water

growth medium				soil pore water			
total Cd (μg L^{-1})	Cd species, μg L^{-1}		inhibition of algal growth (%)[a]	total Cd (μg L^{-1})	Cd species, μg L^{-1}		inhibition of algal growth (%)[a]
	Cd^{2+}	Cd–DOM			Cd^{2+}	Cd–DOM	
100	91	0	–25	115	5	109	11
413	372	2	50	302	31	269	50
500	452	2	54	479	50	427	79

[a] Negative numbers indicate promotion of growth at low concentration of Cd in solution.

of citrate in well-defined synthetic medium (*8*, *9*) was cited as an exception to the FIAM model suggested earlier (*4*−*6*).

It appears that plants can take up metals that may have been initially complexed with ligands, be they organic or inorganic, but it is less clear whether the complex always dissociates at the root surface or the metal is adsorbed by the root as the complex. The algal growth studies showed increased reduction of algal growth in soil pore water in comparison to algal growth medium at a similar value of total Cd concentration (**Table 4**). Further, a 50% decrease in algal growth was observed (**Table 5**) at a total Cd concentration of 0.413 mg L^{-1} (Cd^{2+} concentration of 0.372 mg L^{-1}) in algal growth medium, whereas a relatively lower total Cd concentration of 0.302 mg L^{-1} (Cd^{2+} concentration of 0.031 mg L^{-1}; Cd−DOM complexes concentration of 0.269 mg L^{-1}) present in soil pore water resulted in a 50% decrease in algal growth (**Table 5**). It appears that Cd−DOM complexes were taken up by algae and resulted in the 50% decrease in algal growth at a significantly lower Cd concentration in comparison to the growth studies in algal growth medium. It is probable that Cd−DOM complexes are more toxic than Cd^{2+} species. The mechanism of Cd uptake, free Cd^{2+} vis-à-vis Cd complexes, is yet to be unraveled.

The data on algal growth, both in growth medium and in soil pore water, at different amounts of total Cd are presented in **Table 7**. Speciation of Cd as determined by the MINTEQA2 computer model with the updated database is also included in **Table 7**. It is established that free Cd^{2+} species are bioavailable and cause toxicity. Increasing amounts of Cd in the medium result in the inhibition of algal growth (**Table 7**). It is possible that Cd is taken up by alga as Cd^{2+} ion, with the Cd−DOM complex providing a constant source of Cd^{2+} as long as the rate of dissociation of Cd−DOM complexes is greater than the rate of free Cd^{2+} ion uptake. However, it can be seen that Cd present as Cd−DOM complexes, in the soil pore water, resulted in greater inhibition of algal growth as compared to the case where Cd is present as Cd^{2+} in algal growth medium (**Table 7**). This clearly demonstrates that Cd−DOM complexes are taken up intact by alga and result in increased Cd toxicity. These data contradict the long-held notion that Cd−DOM complexes are not bioavailable to soil biota although they may increase the mobility of Cd.

The data presented for soil pore water and growth medium, spiked with Cd and citrate, clearly demonstrate the bioavailability of Cd−organic complexes, as indicated by the growth studies with a soil alga (*Chlorococcum* sp.). The results in the present study contradict the long-held notion that the Cd−DOM complexes are not bioavailable to soil biota although they increase the mobility of Cd. This is the first report to show the bioavailability of Cd−organic complexes to soil alga (*Chlorococcum* sp.) in soil pore water as growth medium and has significant implications in the understanding of Cd bioavailability and toxicity in natural terrestrial ecosystems.

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