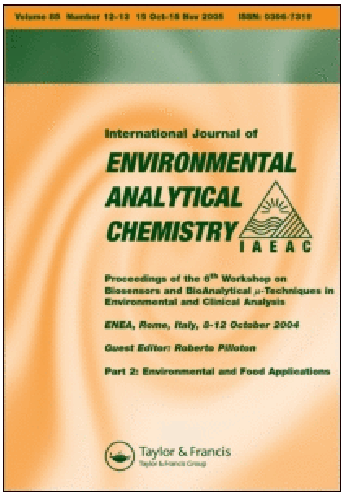

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Dissolved Organic Matter, Cadmium, Copper and Zinc in Pig Slurry- and Soil Solution- Size Exclusion Chromatography Fractions

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DISSOLVED ORGANIC MATTER, CADMIUM, COPPER AND ZINC IN PIG SLURRY- AND SOIL SOLUTION- SIZE EXCLUSION CHROMATOGRAPHY FRACTIONS

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Sephadex size exclusion chromatography was used to prepare molecular size fractions from liquid pig slurry, before and after aerobic interaction with a loamy-sand soil. In the liquid fractions organic matter was characterized and some components were identified.

The distribution of zinc and copper over MW-classes was determined. In pig slurry-liquid one third of the copper and zinc was found in the mass range > 50,000. After 3 days contact with soil, copper concentration increased and 10 % was found in the nominal molecular mass range 50,000. The zinc concentration did not change, but the MW distribution had shifted in favor of higher molecular masses.

Cation exchange experiments (4 hours) with the above fractions showed a zinc exchange rate of 50 % per hour, typically. Copper complexes in HMW-fractions did not exchange, which was tentatively explained by the measured high thermodynamic complex stability. Copper in pig slurry-liquid LMW-fractions showed an exchange rate of 25 % per hour; the reaction stopped when 50 % was left, indicating the presence of stable LMW copper species. After incubation with soil the LMW copper showed a constant dissociation rate of 20 % per hour.

KEYWORDS : Soil solution, manure, DOM, cadmium, copper, zinc, size exclusion chromatography, cation-exchange rate.

INTRODUCTION

In The Netherlands and other European regions there is a concern about a possible increase of heavy metal availability for crops and an increase in mobility leading to an excess of heavy metals leaching towards the ground water, potentially jeopardizing the drinking water quality. An integrated model was designed for nitrogen, potassium and phosphorous compounds movement through soil in the agricultural situation[1] and it is being extended to include heavy metal behavior.

In a 18-month field survey[2,3] the observed seasonal variation of the soil solution zinc and cadmium concentration was reasonably well predicted using a thermodynamic model. Soil solution pH and organic carbon concentration were shown to be the master variables. In the model[3] one type of organic ligand, although having a pH-dependent affinity for heavy metals, was postulated. Several authors[4] used two or more stability classes for heavy metal complexation with natural organic ligands in sewage sludge, bog waters, sea and surface waters.

In the present work the heavy metal binding properties of dissolved organic matter in pig slurry was studied.

Size exclusion chromatography was used to prepare nominal molecular mass fractions. In these fractions the organic matter concentration was measured and characterized using a variety of techniques. Heavy metal exchange rate was determined, and stability constants of zinc and copper complexes were calculated.

MATERIAL AND METHODS

Our laboratory has trace element laboratory facilities including HEPA filter cabinets Class 100 (Clean Air®). Precautions were taken to avoid contamination of plastic and glassware with heavy metals.

Preparation and characteristics of pig slurry-liquid, pig slurry-soil incubate liquid, and soil.

Pig slurry (P.S.) is a mixture of pig faeces and urine usually anaerobically stored for months until spread out on soil to serve as an agricultural fertilizer. Our slurries were obtained from a farm in the center of the country.

Pig slurry-liquid (P.S.-liquid) was separated from pig slurry by centrifugation (400,000 N/kg; 30 minutes), followed by pressure membrane filtration (0.4, 0.2, or 0.1 μm membranes). The P.S.-liquid had pH=8.

The acid (pH_{KCl}=4.55) loamy-sand soil from the Institute for Soil Fertility Research experimental farm used for P.S.- liquid / soil incubation experiments had 0.17 mg Cd, 2.3 mg Cu and 15 mg Zn/kg dry soil. Soil contained 3.3 % lutum (percentage <2 μm of the mineral fraction) and 1.7 % organic carbon.

Slurry/soil-liquid (S.S.-liquid) was obtained from a P.S.-liquid / soil aerobic incubation at 20 °C. 50 Grams of dry soil, prewetted during 24 hours with 5.0 grams of distilled water, was mixed with 5.0 grams of P.S.-liquid. Three days after mixing a portion of 50 ml distilled

Table 1 Conditions for the zero pressure Sephadex size exclusion chromatographic separations. G-25 (P.S.-liquid fractionation); G-75 (P.S.- and S.S.-liquid fractionation). The conditions for high pressure SEC are given in the text.

Parameter:	*Conditions*
Column size:	Ø = 16 mm, length = 400 mm
Eluent:	0.01 Mol/l KCl; pH = 7
Flow rate:	100 ml/hour
Temperature:	15 °C
Detection:	UV 215 nm
Injecting sample solution:	0.5 ml
Number of collected fractions:	20 — 80

water were added and the sample was shaken for 30 minutes; centrifuged and finally membrane filtered to remove particles.

Preparation of molecular size (mass) fractions.

In the gel permeation process (size exclusion chromatography, gel filtration) all solute species with molecular masses below the exclusion limit of a selected gel are proportionally retarded by the column. For example Sephadex G25 and G75 have molecular mass exclusion limits of, respectively, 5,000 and 50,000 Dalton, calibrated with dextrans. All molecules heavier than the limit are eluted unimpeded by a gel while lighter ones are proportionally retarded (by permeation of the gel) and so fractionated and finally collected in different elution volumes. Inorganic metal complexes are strongly retarded by the gels.

Gel permeation chromatography (GPC) was used and the conditions are summarized in Table 1. In different experiments 20 — 80 molecular mass fractions (each 10 to 2.5 ml per run) were collected. The instrument and the gels were from Pharmacia® (Sweden). For preparative separations the Sephadex gels G25 and G75 were used at 15 °C. The flow rate (peristaltic pump, model P-1) was 100 ml/hour. Samples were injected in the eluent stream using a metal free HPLC injector valve (model V-7) with a 0.5 ml sample loop. The pH of collected fractions was neutral. Blue dextran 2,000 with MW = 2.10^6 was used to define the exclusion volume (Ve; Figure 1). UV-VIS detection at 214 nm for monitoring the separation was done at the exit of the column with a UV-M monitor mounted with a zinc lamp. Fractions were collected in detergent/acid/water precleaned borosilicate glass (Pyrex ®) reagent tubes mounted in a LKB 2211-010 SuperRac.

Measurement techniques.

Several properties of P.S.-liquid GPC fractions were measured: heavy metal content, DOC, functional groups, molecular mass/particle size, several specific compounds and copper complexation capacity. The types of analysis and techniques are discussed below.

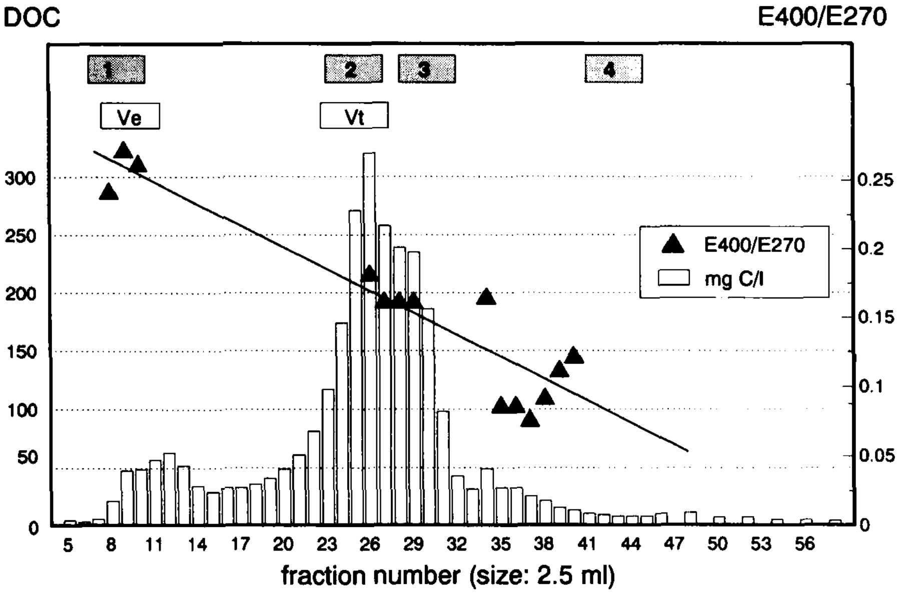

Figure 1 GPC-Fractogram showing DOM (mg C/l) in collected P.S.-liquid fractions (bars). Ve is the exclusion volume, and Vt is the total permeation volume. The UV-VIS E_{400}/E_{270} ratio of selected samples (triangles and line), and the elution volume ranges (shaded regions 1 — 4) of various test compounds are also shown. Gel: Sephadex G25. Fraction volume: 2.5 ml per run.

Cadmium and copper (copper complexation capacity excepted) concentrations were measured with graphite furnace atomic absorption spectrometry (GFAAS) using the method of standard additions and, respectively, Zeeman and deuterium correction for non-specific atomic absorption. For zinc flame atomic absorption spectrometry (FAAS) was used, using a calibration line and matrix matching of the standards. Copper concentrations in samples from complexation capacity measurements (about 2 mg Cu/l) were measured with FAAS in the same way as zinc. The recommended measurement wavelengths by the manufacturer of the instruments were used. Samples were stabilized by acidification with HNO_3 to pH 1 — 2. Both AAS instruments were from Perkin Elmer®: for FAAS model PE 2100 (copper and zinc), and for GFAAS model PE 5100 PC (Zeeman correction, Cd analysis) or model PE 5000 (deuterium correction, Cu analysis).

All pH-values were measured using a glass electrode, calibrated at pH 4 and 7 with standard pH-buffer solutions (Radiometer®).

Total and inorganic carbon were measured with a Shimadzu TC Analyzer® injecting 30 µl samples. DOC was calculated by difference. Calibration was done with potassium hydrogen phthalate standard solutions (injection of 30 µl of 0, 20, 50 or 100 mg C/l).

Molecular masses were determined using 2 techniques:

1. Ion Spray Mass Spectrometry (NERMAG R3010®D instrument) in the negative ion mode[5]. Measurements were done in several LMW (MW <5,000 D) G25 fractions, and HMW (MW >5,000 D) fractions;
2. Sephacryl F200 HR® (separation between 1,000 and 80,000 D) for high pressure

analytical size exclusion separation of G75 fractions, using 2 detection modes: multi angle laser light scattering (MALLS) at 632 nm and refractometric detection. 0.01 Mol/l KCl was used as the eluent at a flow rate of 30 ml/h and a temperature of 95 °C. Flow was controlled using a HPLC pump (HP 1084-system). 100 µl membrane filtered (consecutively at 0.2 µm and 0.1 µm) samples were injected. Calibration was done with Dextran T40 and T70 (MW standards of 40,000 and 70,000 D obtained from Pharmacia®). The MALLS detector was a DAWN from Wyatt Technology®. The limit of determination of MALLS in solutions is not fixed but at a fixed DOC concentration depends on the molecular mass to be detected. Molecular masses of about ≥ 30,000 (in the presence of 0.01 Mol/l KCl) can be determined if not less than 0.5 gC/l is present for many organic solutes. DOC concentration is measured using the refractive index increment (dn/dc) of the analyte for the calculation. For most organic solutes the dn/dc is between 0.10 and 0.20; 0.15 was assumed for the molecular mass calculation in this study. The combination of low refraction and high scattering strongly indicate the presence of colloids.

Oxalic acid was measured using a biochemical test kit (UV method, cat. no. 755699 Boehringer®, Germany, application no 4 for oxalic acid in urine). The method is based on the UV-VIS measurement of the specific production of NADH, which production is equimolar with the transformation of oxalic acid. Sample color was removed prior to analysis by treatment with active carbon.

Information about functional groups in different molecular size fractions was gathered running UV-VIS scans from 245 to 500 nm (Beckman® DU-64 spectrophotometer, scan rate of 500 nm/min). About 2.5 ml sample was used to fill the measurement cuvette.

Cu-complexing capacity was determined using the Hummel and Dreyer[6] and Yoza[7] technique with a copper saturated column. In our experiments a column (40 cm) filled with G25 gel, equilibrated with the eluent containing 0.01 Mol/l KCl and 2.15 mg/l Cu^{++} at neutral pH was used. The eluent flow was 100 ml/h. 0.5 ml test samples were injected. The test samples were 1 + 1 mixtures of several (0.01 Mol/L KCl) P.S.-liquid GPC G25 eluate fractions, and a solution of 4.30 mg/l Cu^{++} in 0.01 Mol·L KCl. Fraction numbers 9, 10, 25, 26, 34 and 35 (Figure 1) were analyzed. In the collected fractions the copper concentration was measured with FAAS. The binding ratio (Cu/C) was calculated by dividing the mass (Moles) of complexed copper by the mass of organic carbon (gram) injected with the sample.

Heavy metal complex stability and dissociation kinetics.

Changes in metal distribution and dissociation rate. Metal and DOC concentration in MW fractions and the exchange rate of heavy metals were determined in P.S.- and S.S.-liquid fractions (before and after a 3-days incubation of pig slurry-liquid and soil). Dissolved heavy metal complex dissociation rate was determined using an approach similar to Del Castilho[2] and references therein. The cation exchange process between dissolved heavy metals and a cation from a solid phase exchanger is followed by measurement of the dissolved heavy metal concentration at different time intervals. Precleaned calcium saturated Chelex® was used as solid exchanger phase in batch experiments at pH=8. Eight GPC Sephadex G75 fractions, both from P.S.- and S.S.-liquid fractionations were incubated with the exchanger material. At the start of the incubation (t=0) an aliquot of 0.5 ml 25 % Ca-Chelex® (dry

Table 2 ^{10}Log(stability constant) of heavy metal and calcium Chelex®- and DOM-complexes used in model calculation for heavy metal DOM complex stability calculation.

	Log(Formation constant) of Metal-Chelex® complex	*Log(Formation constant) of Metal-DOM complex*
Copper	10.63*	Determined, this study
Zinc	7.27*	Determined, this study
Calcium	6**	2.4 ***

*) Iminodiacetate metal complexation constants[12]; for comparison[13] LogK = 10.4 for copper-Chelex® complexation.

**) Calculated from differences in selectivity between Ca and Zn (Bio-Rad Labora tories), and above zinc-Chelex® complexation data.

***) Reference nr 11.

weight/volume) slurry was added to each of the 20 ml GPC-fraction samples. The exchanger contributed a cation exchange site concentration of 18 mMol/l. Samples were shaken in a rotary shaker at 100 Rpm in screw cap polypropylene vials. Samples were taken (polyethylene hypodermic syringe) between 0 and 4 hours at regular intervals, and at 120 hours to check whether equilibrium was attained. Three hours is a sufficient contact time to study displacement from solution to a solid phase exchanger[8]. The time series samples were membrane filtered (0.40 μm) immediately after sampling, and transferred into polypropylene capped reagent tubes prior to analysis. For DOC determination the samples were kept in the dark at 4 °C and were measured as soon as possible.

Estimation of chemical thermodynamic equilibrium copper and zinc complex formation constants. Dissolved metal concentrations in soil extracts (prepared according to ref.[9]) were too low to allow for further fractionation. Size exclusion G75 fractions of P.S.- and S.S.-liquid from the ionexchange experiments (see preceding paragraph) were studied. The dissolved heavy metal concentrations that remained after 4 hours contact with Chelex® were used for the calculation of the stability constants of Zn or Cu complexes. It was assumed that complexation explains for the observed concentrations. A thermodynamic model for the computer program CHARON[10] was used to calculate the equilibrium metal concentrations, given measured DOC concentrations and Chelex® binding site concentration (18 mMlo/l) as model imputs. The complexation constants used in the model were: Ca-DOM[11], applying pH-dependency correction[3]; Ca-, Cu- and Zn-iminodiacetate (Chelex® monomer)[12, 13]. The model parameters are summarized in Table 2.

RESULTS AND DISCUSSION

Characterization of DOM in P.S.-liquid fractions.

A GPC Sephadex G25 fractogram of 0.40 μm membrane filtered P.S.-liquid with a DOM

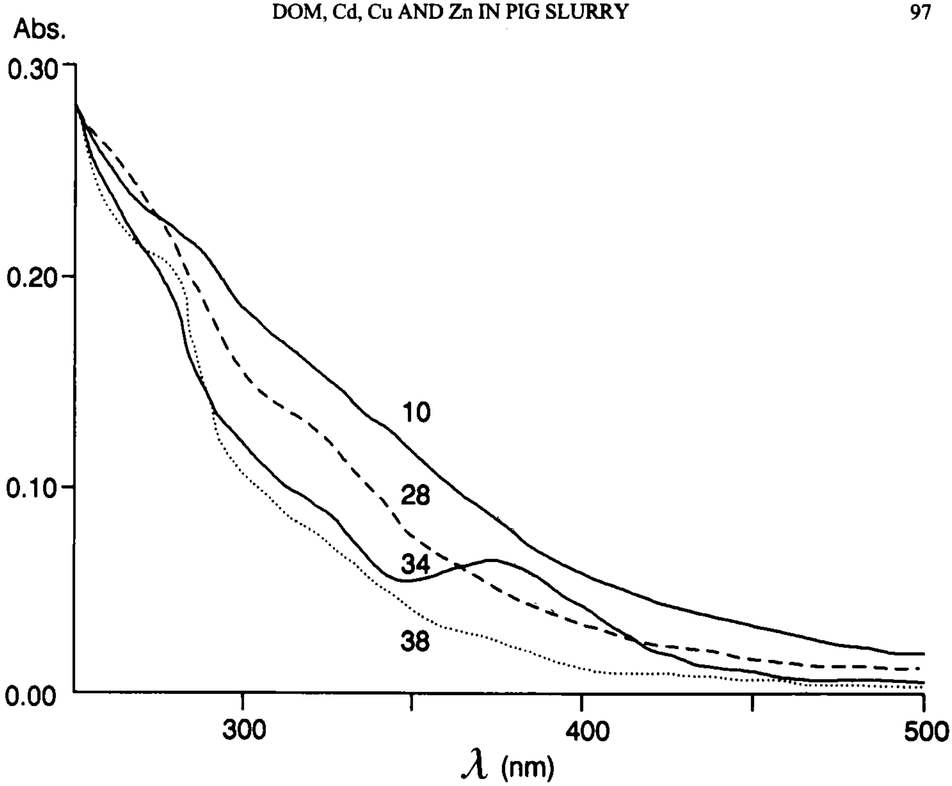

Figure 2 Spectrophotometric UV-VIS scans of four Sephadex® G-25 P.S.-liquid fractions (numbers of fractions correspond with fraction numbers of Figure 1). The light absorption (arbitrary absorbance scale) is shown on the ordinate, the wavelength (nm) on the abscissa.

of 1.44 % organic carbon (w/v) is shown in Figure 1. The dissolved organic carbon from the original P.S.-liquid sample was completely recovered in the collected eluates (found: 1.48 %).

Ideally, permeating fractions contain lower molecular size or mass molecules, and excluded fractions contain the higher molecular masses. In practice, however, the shape of the molecules, sorption and electrical charges may also determine the separation efficiency[14]. To minimize charge effects 0.01 Mol/l KCl is often used.

Calibration with known compounds was done to check for the separation achieved. The elution volume ranges of various dissolved test compounds in separate runs are indicated in Figure 1. Humic acid (Fluka ®) yielded 95 % of its carbon in the exclusion volume (Ve) range 1, indicating a MW > 5,000 D. The complement was found in permeation range 3. Acetate appeared in range 2. Benzoate (molecular size about 5 nm), and LUDOX® (according to the manufacturer: 10 nm size spherical silica particles) appeared in range numbers 2 and 3. Catechol (molecular size about 5 nm) had a large permeation volume (range 4).

Changes in the UV-VIS spectra from one fraction to the next appeared to be gradual. Scans of selected P.S.-liquid fractions are shown in Figure 2. The shoulder at 370 nm for P.S. fraction number 34 might be ascribed to e.g. porphyrines, chinones and unconjugated polyenes[15]. These types of substances are likely to be present in manure. However, more work should be done to identify the substances.

The relatively high extinction values at the wavelengths > 400 nm in near Ve-fractions may be ascribed to e.g. polycyclic aromatic compounds[15]. Humic acids are often regarded as such. The near Ve P.S.-liquid fractions and Fluka® showed comparable E_{400}/E_{270} ratios. This E_{400}/E_{270} ratio (Figure 1) indicates that these fast eluting fractions contain relatively much polynuclear aromatic compounds. However, differences in E_{400}/E_{270} ratios may also be caused by differences in colloidal size[16]. Colloids were detected with a high level of probability in low and high MW fractions using MALLS/refractometry measurements. This was shown in two separate experiments where freshly membrane filtrated (0.1 or 0.2 μm) GPC G75 P.S.- and S.S.-liquid fractions were measured. This was done in flow injection mode without further size separation, and after high pressure size exclusion chromatographic separation over Sephacryl F200 HR prior to detection. Both experiments showed low refraction signals, combined with high scattering signals, strongly indicating the presence of colloids in both nominal LMW and HMW fractions of P.S.- and S.S.-liquid fractions.

Concentrations of acetic acid, other small chain fatty acids and many other compounds present in common animal manures have been compiled[17]. We determined using Ion Spray Mass Spectrometry (IS-MS) in which GPC fractions the molecular masses from fatty acids and other masses up to 1000 D are found. It should be mentioned that IS-MS determines molecular mass and does not identify a specific compound[5]. Throughout the whole GPC-fractogram a variety of molecules with a molecular mass < 300 D were found. In addition, in fractions 14 and 15, masses up to 1000 were detected. The relatively high KCl-concentration of the samples, which was chosen to obtain acceptable GPC separation, had raised the detection limits of IS-MS. In spite of this drawback, certain fatty acids could be identified tentatively in several fractions. G25 fractions No. 25, and at lower concentrations No. 26, showed the molecular masses of acetic, propionic, butyric, and valeric acid. The mass peaks of acetic acid were found in the same fraction as in separate test runs with injection of a 0.5 promille (w/v) sodium acetate solution (range 2; Figure 1). The fractions, containing fatty acids, showed low E_{400}/E_{270} ratios, suggesting that these fatty acid fractions were separated quite well from polyaromatic substances or colloids during these runs.

In fraction No. 15 mass peaks ascribable to oxalic acid and/or lactic acid (identical masses) were found. Subsequent analysis (Boehringer test) of P.S.-liquid confirmed the presence of oxalate (30 mg/l); lactic acid concentration was below the detection limit (<< 20 mg/l.). Other findings confirm the suspicion oxalic acid instead of lactic acid to be present in fraction 15. The permeation in this fraction, instead of number ≥ 24 as anticipated from its small molecular mass, may be caused by the molecule's double electrical negative charge promoting exclusion from the gel, despite the presence of 0.01 Mol/l KCl in the eluent. Lactate is single charged and is not expected to elute early because of charge effects. For sake of the spectrophotometric measurement of NADH (Boehringer test) the P.S.-liquid had been treated with active carbon to remove color. Because possible sorption losses on the active carbon were not determined, the sample oxalic and lactic acid concentrations may have been higher.

Some DOM (fraction numbers 35-40) with E_{400}/E_{270} ratios between 0.07 — 0.13 eluted later then the total permeation volume. The low ratio suggests polycyclic aromatic compound concentrations to be low. Monocyclic aromatic test compounds (benzoic acid and catechol) had ratios close to zero. The reason for the late elution might be the reversible sorption of solutes on the column material, or the on-column dissociation of larger molecules. Because we determined a high retention volume for catechol (Figure 1), while elution of benzoic acid was normal, it is tempting to ascribe the difference in elution to the first mechanism: reversible sorption of the electrically neutral charged catechol molecule. The retention of electrically neutral charged molecules, in the worst case, can even be irreversible (strong sorption by hydrophobic interaction). The second mechanism, on-column dissociation is discussed with the combined IS-MS / MALLS results in a following paragraph.

It was stated[18] that, generally speaking, the presence of two, or more peaks in GPC analysis of humic substances (like around fractions Nos. 11 and 25; Figure 1) is a common occurrence that might be an artifact of the method. These authors believe that the true molecular mass distribution of humic acids is gradually changing from a high occurrence of molecular masses in the order of hundreds to thousands to a lower occurrence of molecular masses in the order of 10,000 — 25,000. Only a minor part of the humic acids would have a MW > 25,000. In contrast with this, other authors[19, 20] concluded that humic acids have masses around 50,000.

In our experiments the observed bimodal distribution is probably real: both high molecular mass humic substances, and high fatty acid concentrations were anticipated, the large difference in molecular mass leading to the observed bimodal size distribution.

The IS-MS/ MALLS results are not fully understood. How can both nominally low, and high MW Sephadex fractions contain molecules < 300 D as well as colloids? One explanation could be that association / dissociation processes occur: part of the (fast eluting) macromolecules cleaving into smaller molecules, and (slowly eluting) smaller molecules associating to larger, ultimately colloidal forms.

As the Sephadex chromatographic material is a rather inert material, and temperature was quite low (15 °C), it is not likely that dissociation / association of stable molecules is promoted. More studies should be done to understand what is happening exactly, especially because mass peaks were determined only in a qualitative way.

Dissolved Cd, Cu, Zn and DOM concentration levels in GPC fractions.

Complete recovery of dissolved organic carbon was obtained after elution. Recoveries of copper and zinc were respectively about 65 and 75 %. These findings were in accordance with separate radiochemical experiments where ^{109}Cd and ^{65}Zn equilibrated P.S.-liquids were chromatographed over G75. The γ-spectrometry results showed 28 ± 3 % of zinc or cadmium was retained on the column material. Above findings fit the general observation that inorganic metal species may be strongly retained on the gel.

The relative abundance of DOM, copper and zinc in size fractions is summarized in Table 3. The G25 fractograms showing DOM and heavy metal concentrations are shown in Figure 1 and 3. Of the three metals copper was the most evenly distributed metal over the DOM size fractions.

During the separating process cation exchange may occur by contact with dissolved KCl (0.01 Mol/l). Because of this, the fast eluting compounds may actually have been associated

Table 3 Molecular mass (MW) distribution of DOM and copper or zinc complexes in pig slurry-liquid (P.S.-L), and in pig slurry / soil incubate-liquid (S.S.-L).

Component	*MW < 5,000*	*MW 5,000—50,000*	*MW > 50,000*
Zn in P.S.-L	20 %	45 %	35 %
Cu in P.S.-L	33 %	30 %	37 %
DOM in P.S.-L	80 %	15 %	5 %
	MW < 50,000		MW > 50,000
Zn in S.S.-L	45 %		55 %
Cu in S.S.-L	90 %		10 %
DOM in S.S.-L	95 %		5 %

with more heavy metals than found in the collected fractions and so the LMW-compounds in reality may have been associated with a smaller amount of heavy metals.

The copper-binding capacity of GPC-fractions.

Hummel and Dreyer experiments[6] for the measurement of the binding ratio between dissolved copper and DOM yield a copper fractogram. The fractogram from freshly membrane filtered (0.4 µm) P.S.-liquid fraction number 9 (a total exclusion fraction) is shown in Figure 4. The copper concentration in each collected fraction is shown (numbering differs from that in the preparative fractionations). The surface under the positive peak (coinciding with the total exclusion volume) represents the copper removed from the column by solution complexation; the negative peak represents the copper replenished to the column by the eluent. Calculations showed 3.4 mMol Cu was associated per gram C (one atom Cu per 125 C-atoms) in fraction number 9. For the fractions 10 and 26 an estimated binding ratio was obtained: 1.5 mMol Cu/g C (one Cu-binding for 245 carbon atoms). The above binding ratios compare well with compiled literature data[11] for other types of DOM where on the average 2 mMol sites /g C are reported. Based on the measured copper complexation capacity, and the copper concentration in the samples, the relative site saturation with copper could be calculated: in the order of 1 %. This leaves much room for additional complexation. For a successful H&D experiment the positive and negative peak must be well separated, and of equal surface area. Other prerequisites are found in Yoza[7]. Unfortunately, H&D complexation capacity measurement of other fractions failed. Abnormal fractograms were obtained: the area of the negative peak was larger than from the positive peak. This suggests on-column losses by sorption of complexed copper. This might be caused by phenomena associated with the long storage time of these samples (1 month in the refrigerator).

P.S-, and S.S.-liquid incubation experiment.

Sephadex G75 was used to fractionate soil liquid, P.S.-liquid, and the S.S.-liquid. In the soil liquid low copper, zinc, and cadmium concentrations were found (sub µg to µg/l region), not permitting a meaningful size exclusion separation (below the detection limit).

Sephadex G75 was used to fractionate P.S.-liquid, before and after contact with soil. In

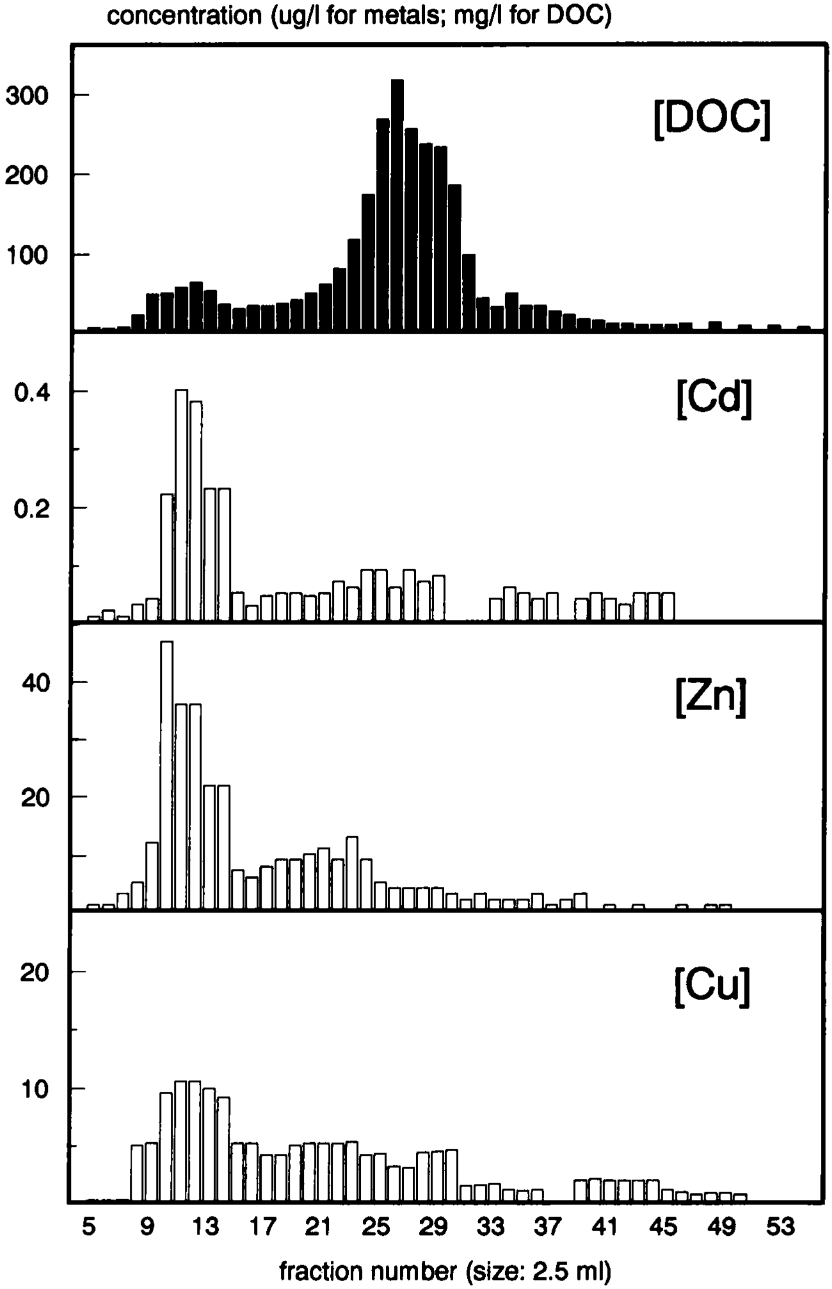

Figure 3 Fractograms (Sephadex® G-25) of P.S.-liquid: dissolved organic carbon (the same as Fig.1), Cd, Zn and Cu concentrations.

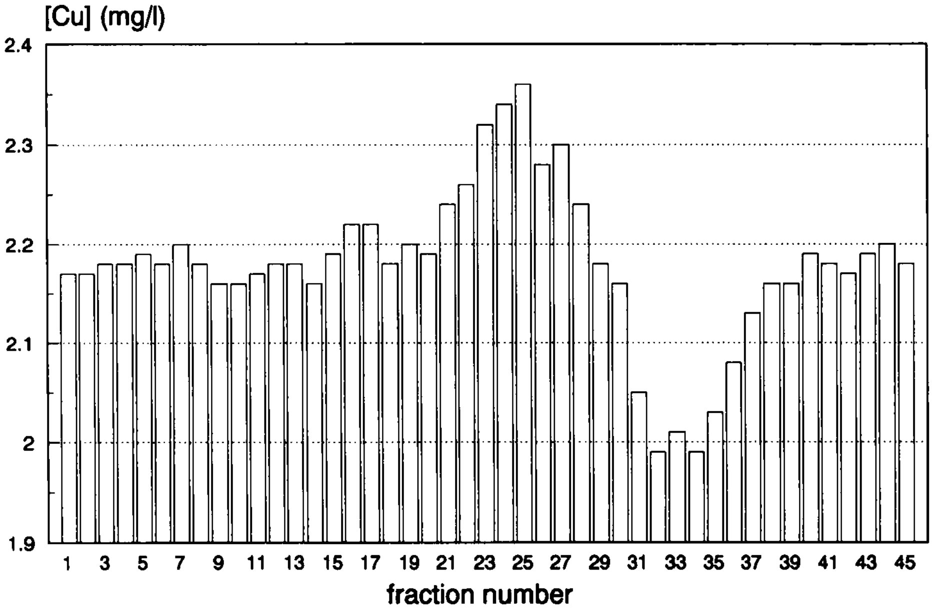

Figure 4 Fractogram of a Hummel and Dreyer[6] experiment with P.S.-liquid sample no 9. The amount of complexed copper around fraction 26 (positive peak) is equal to the copper missing in the eluent around fraction 33 (negative peak). For explanation see the text. Note: the fraction numbers from this experiment are not comparable with the ones from the preparative fractionations.

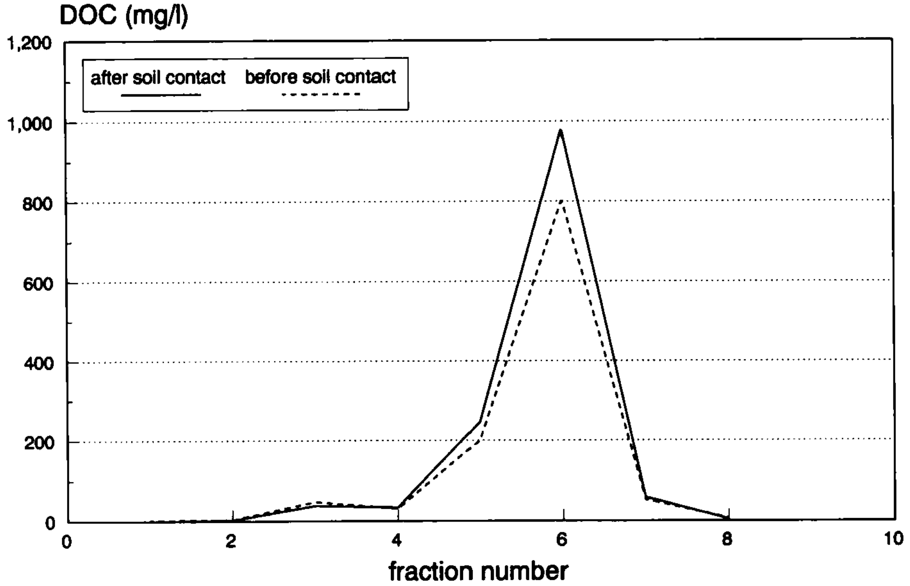

Figure 5 a) DOC-,

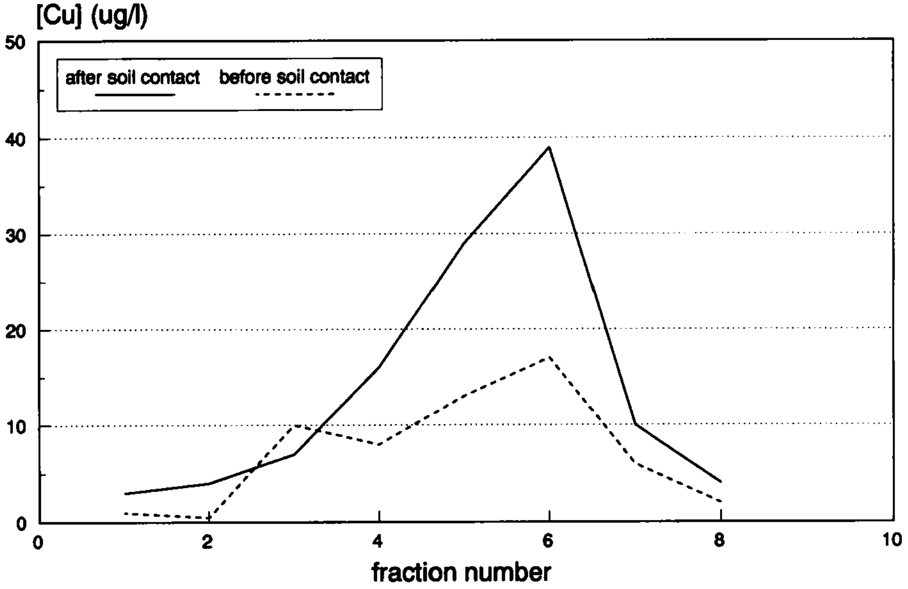

Figure 5 b) Cu-

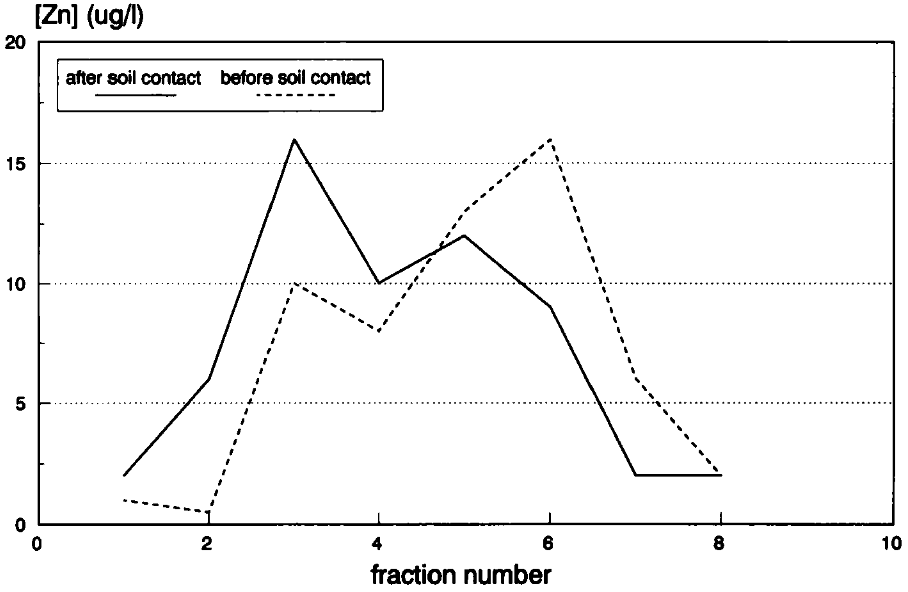

Figure 5 c) Zn-fractograms (Sephadex® G75; P.S.- and S.S.-liquid). See text for explanations.

the collected fractions heavy metal and organic carbon concentration were measured (Figures 5 a, b, c). In these fractograms the fractions numbers 3 and 4 represent nominal masses > 50,000 and numbers 5 and 6 masses < 50,000. Total DOM concentration and carbon size distribution had not significantly changed after the incubation of P.S.-liquid with soil. However, dissolved copper concentration had increased, and the LMW fractions were favored (Figures 5 a and b). The copper concentration increase represents an extraction of 1% of total soil copper. Total dissolved zinc concentration remained constant. However,

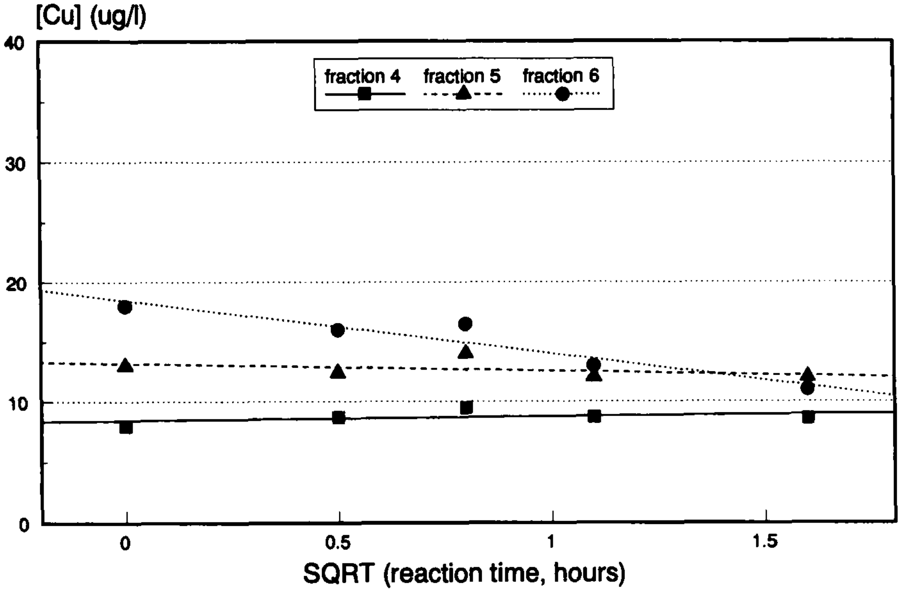

Figure 6 Dissolved Cu and Zn concentrations in fractions number 4, 5 and 6 after addition of Chelex® to P.S.- and S.S.-liquid fractions (G75); pH = 8. On the x-axis the SQRT of the time interval (hours) after Chelex® addition is shown; on the y-axis measured copper and zinc concentrations are given (μg/l). a) Cu from P.S.-liquid;

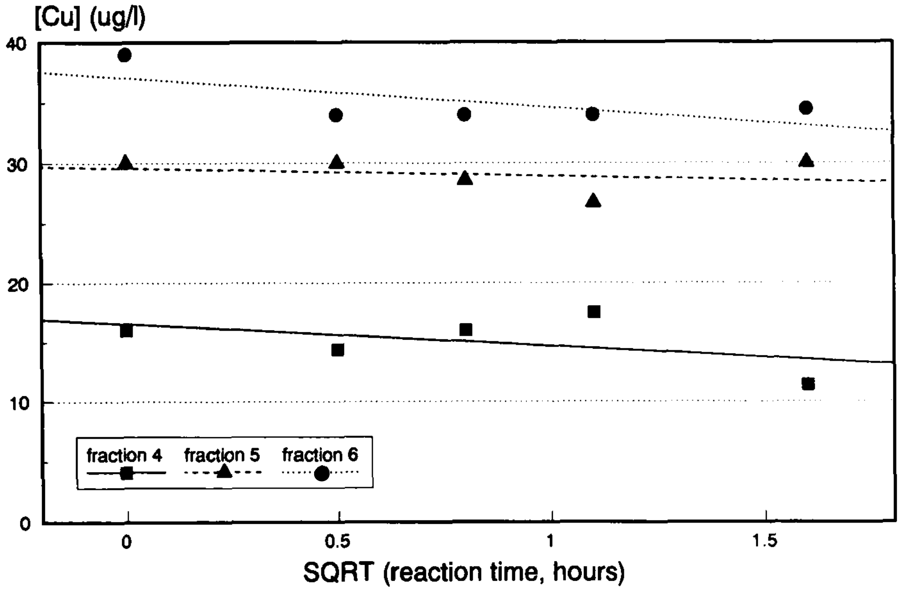

Figure 6 b) Cu from S.S.-liquid;

zinc increased in fraction number 3 (HMW) and decreased in fraction 6 (LMW) (Figures 5 a and c). The disappearance of zinc from the low molecular size fraction may tentatively be ascribed to the removal from inorganic zinc species by specific sorption of $ZnOH^+$ on soil amorphous Fe, Mn and Al oxides[21] at the experimental pH=7.

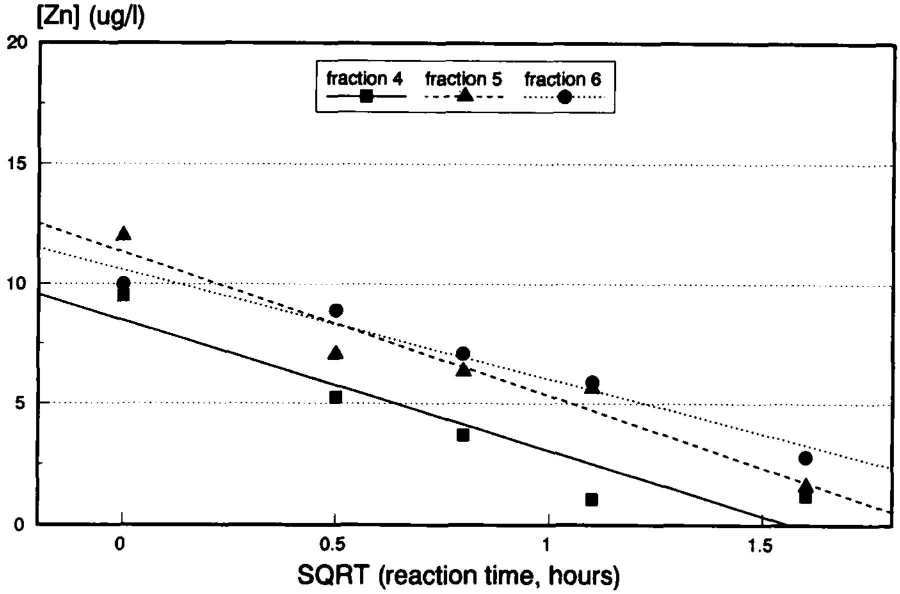

Figure 6 c) Zn from P.S.-liquid;

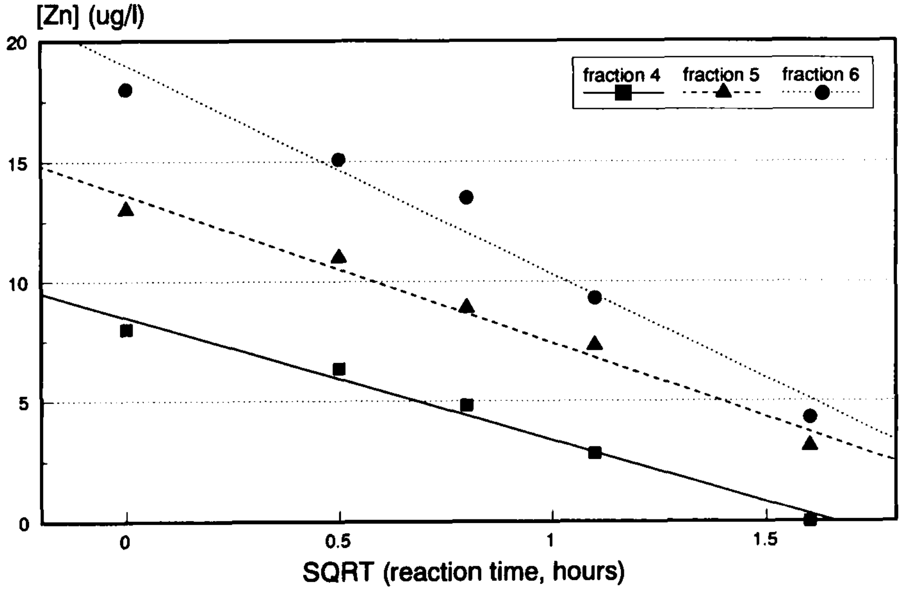

Figure 6 d) Zn from S.S.-liquid.

Cation exchange rate and binding strength of dissolved Cd, Zn and Cu species in GPC fractions.

The G-75 P.S.- and S.S.-liquid fractions, discussed in the preceding paragraph were used to determine the heavy metal exchange kinetics. After Chelex® addition the cadmium concentration approached the detection limit already at the second or third sampling, and were not used for calculations. The copper and zinc concentrations were followed over 4 hours. From most P.S.- and S.S.-fractions copper concentration remained constant (Figures 6a and b). However, P.S.-fraction No. 6 (LMW) showed a loss of 50 % of the copper, at a rate of 25 %/h (Figure 6a). Zinc concentration went down gradually in all fractions (Fig. 6c and d): rate 50 %/h finally reaching the detection limit. No changes in concentration were observed between 4 and 120 hours, after the start of the ion exchange experiment.

The chemical thermodynamic calculation indicated that the measured metal concentrations from the above kinetic experiments, after 4 h, could be explained if metal organic stability constants are log $K_{ZnDOM} \leq 4.6$ and log $K_{CuDOM} = 9.5$, at pH 8 of the experiment. The results are consistent with published stability constants for humic and fulvic acids[22]. Complexation constants of log $K \geq 10$ are as strong as covalent bonds and chelates.

If the rapid ion exchange of cadmium and zinc complexes also occur in-situ, the behavior of cadmium and zinc should be described as "metal-like". Distinction between "metal-like" and "ligand-like" behavior might be useful when describing the metal behavior at living and non-living surfaces and, therefore, on availability and mobility of heavy metals in soils. Biological surfaces with specific binding sites for cadmium or zinc are able to strip the "metal-like" behavior complexes. Copper, in contrast, would at least in part react "ligand-like": copper ion specific binding sites would be unable to extract copper from the kinetic stable copper complexes, but might bind copper, e.g. by sorption of the complex according to some distribution quotient instead.

CONCLUSIONS

Cadmium and zinc complexes in pig slurry-liquid molecular size fractions showed rapid dissociation. The same was found for zinc and cadmium complexes after 3-days contact of the pig slurry-liquid with a sandy-loam soil. This means that assuming chemical equilibrium for the thermodynamic model calculations is not unreasonable for these metals in the solution phase.

HMW copper complexes in pig slurry-liquid GPC fractions did not dissociate noticeably in the presence of a solid exchanger. Part of the copper is probably chelated, or strongly sorbed. Copper from one LMW-fraction showed some dissociation: in 4 hours the copper concentration decreased from 36 μg/l to 21 μg/l. After contact between pig slurry-liquid and soil (3 d), the copper concentration had increased, and the molecular size distribution of copper had changed in favor of the lower molecular sizes. For zinc the distribution changed in favor of the high molecular sizes; total zinc concentration had not changed.

It is not certain that complete separation between colloidal and dissolved matter from membrane filtrated pig slurry-liquids or slurry/soil-liquids was achieved when using gel permeation chromotography. Large molecules/colloids and their complexation with heavy metals deserve further attention in order to improve our understanding of the behavior of heavy metals in soils.

Cadmium and zinc from pig slurries are expected to behave mainly metal-like. On the contrary part of pig slurry copper is expected to behave ligand-like when in contact with (biological) surfaces.

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