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ABSTRACT

Size-exclusion chromatography is a powerful tool for distinguishing dissolved organic matter in relation to its molecular size. However, problems may arise in the choice of the most appropriate eluent composition, regarding the gel used. In the present work, soil solution samples from four Brazilian topsoils were analyzed by high-performance liquid chromatography (HPLC) using a size-exclusion polymer-based column (Superdex 75 HR 10/30, Pharmacia). Water and $0.01 \, \text{mol} \, L^{-1}$ NaCl were investigated as mobile phases. The analysis of the UV peak distribution as well as their corresponding Fe, Mn, and Al concentrations suggested that the salt solution should be preferred since it minimizes unwanted interactions between the sample and the support material. Fe, Mn, and Al speciation calculated by the Geochem-PC program involving the Mattigod and Sposito Mixed Model was compared with the Fe, Mn, and Al-organic complex concentrations determined by SEC-HPLC/GFAAS. No correlation was found for Mn and Fe. For Al a significant non-linear correlation was observed.

Keywords: SEC-HPLC; Geochem-PC

INTRODUCTION

The characteristics and amount of humic substances affects the environmental fate of metals in the soil (Sposito, 1989; Wolt, 1994; Alloway, 1995; Tack and Verloo, 1995). In this context, many authors have claimed that knowledge on the molecular mass (MM) of the humic substances (HS) is an essential parameter to be determined (Swift, 1989; Stevenson, 1994; Zhou *et al*., 1994; Cabaniss *et al*., 2000). Among the several methods to characterize HS, the recent literature demonstrates a renewed interest on the use of size-exclusion chromatography – SEC (Swift, 1999; Trubetskoj *et al*., 1999; Kudryavtsev *et al*., 2000; Zhou *et al*., 2000; Piccolo, 2002). However, many contradictory results still arise due to the choice of the calibration standards as well as

that of other analytical parameters (Hongve *et al*., 1996; Perminova *et al*., 1998; Piccolo *et al*., 2001).

In the absence of absolute HS standards, SEC can be employed to obtain MM profiles to be used as a distinctive criterion for extracts of the same soil under different managements (Ceccanti *et al*., 1989; Rottman and Heumann, 1994; Nobili and Chen, 1999). In that case, any arbitrary MM standard could be used (Conte and Piccolo, 1999).

Despite the large number of works on SEC applied to soil humic extracts, only a few SEC studies have been reported for soil solutions (Table 1). Several eluents have been used, most frequently water or aqueous solutions with ionic strengths (I) close to that of the soil solution (Table 1). Moreover, the eluent composition and the corresponding gel used in SEC are key factors for an accurate interpretation of the results (Nobili and Chen, 1999; Specht and Frimmel, 2000). This is especially true in speciation analysis, since any modification of the original

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Table 1 Metal speciation studies in soil solution samples using HPLC-SEC

Column	Source	Element	Eluent
Sephadex G-50/G-100	Gregson and Alloway, 1984	Pb	$0.01 \, mol \, L^{-1}$ KCl
Superose 12	Gardiner *et al.*, 1987	Al	$0.2 \, mol \, L^{-1}$ CH_3COONa
TSK G3000 SW	Morrisson *et al.*, 1990	Cu	$(Na_2HPO_4 + NaH_2PO_4)$ $0.0058 \, mol \, L^{-1}$
TSKgel G2000SWXL	Berdén and Berggren, 1990	ND	$NaNO_3$ and $CH_3COONH_4/NaNO_3$ 0.05, 0.10, 0.15, $0.20 \, mol \, L^{-1}$
Sephadex G-75	Castilho *et al.*, 1993	Cd, Zn, Cu	$0.01 \, mol \, L^{-1}$ KCl
Fractogel TSK HW40(S)	Kerven *et al.*, 1995	Al, Fe, Mn	$0.02 \, mol \, L^{-1}$ KCl
Sephadex G-10/Bio-Gel P2	Hees *et al.*, 1996	Al, Si	$0.1 \, mol \, L^{-1}$ NaSCN
Shodex Asahipac-320HQ	Tsunoda *et al.*, 1997	Al	$0.1 \, mol \, L^{-1}$ $NaNO_3$
Bio-Sil SEC 250	Romkens and Dolfing, 1998	Ca, Cu	$0.05 \, mol \, L^{-1}$ $NaNO_3$ or H_2O
Superdex 75 HR 10/30	Hils *et al.*, 1999	Al	H_2O
Superdex 75 HR 10/30	Mitrovic and Milacic, 200	Al	$0.025 \, mol \, L^{-1}$ TRIS-HCl $+ 0.15 \, mol \, L^{-1}$ NaCl

ND = Not determined.

species composition of the sample must be avoided (Hills *et al.*, 1999; Emos, 2001).

Therefore, the purpose of the present study is to assess the influence of the mobile phase's ionic strength on the chromatogram profile of soil solutions analyzed by SEC-HPLC. In all cases, both UV peaks and coeluted contents of Fe, Al, and Mn were examined.

METHODOLOGY

Soils

Brazilian soil samples, each one composed of six subsamples, were collected from the top 15 cm of two different study sites. The first study site was located in a Research Station (Embrapa-Agrobiologia) in the state of Rio de Janeiro (Seropédica county). This soil was classified as kaolinitic Typic Kanhapludult. From this site one kind of soil management was investigated. It represents a three-year corn crop cultivated only with organic amendments (**CS**). The soil sample was collected on 17 December 2001, 1 day after a rain event.

The other study site was located in another Research Station (Embrapa-Meio Ambiente) in the state of São Paulo (Jaguariúna county). The soil was classified as oxidic Eutric Acrustox. In this site, three kinds of soil management were studied. One represents a small fragment of a secondary forest (**FJ**). The second one was located in a three-year field experiment and represents a conventional tillage management of corn crop (**CCJ**). The last sample was taken in the same experiment and represents a sewage-sludge-amended $(12 \, t \, ha^{-1}y^{-1})$ corn crop (**SSJ**). These soil samples were collected on 20th December 2001, 1 day after a rain event.

The relevant properties of the soil samples are provided in Table 2. These soils had contrasting clay mineralogies and are representative of the most typical Brazilian soils (Santos and Pérez, 1996).

Soil Solution Extraction

The soil solution extraction was carried out using a Maxi-Spin® centrifugal filter device. It consists of two polypropylene units: a sample chamber with a 0.45 µm PVDF membrane at its bottom, and a 50 mL graduate, screw-cap conical-ended centrifuge tube. Centrifugation time and speed were 60 min and 2,260 g, respectively (Pérez *et al.*, 2002). All results concerning the soil solution composition refer to three replicates.

To avoid a possible adulterating effect of the PVDF filter on the soil solution composition, the

Table 2 Selected characteristics of the four Brazilian soil samples studied

Sample	pH_{H2O} (1 : 2.5)	Organic carbon	Clay	FM[a]	WHC[b]	CEC[c]
		g kg^{-1}				cmol$_c$ kg^{-1}
CS	6.8	7.0	240	219	253	5.5
FJ	4.5	17.0	460	228	382	9.1
CCJ	5.7	11.9	420	202	360	8.3
SSJ	6.1	18.2	420	245	358	10.3

[a]Field moisture; [b]Water holding capacity (0.033 MPa); [c]Cation exchange capacity.

filters were pre-washed (Jay, 1985; Jardine *et al*., 1986) three times with ultrapure water. Field-moist samples were extracted less than 24 h after collection. All centrifugations were carried out at 25°C in an IEC B-22M high-speed refrigerated centrifuge equipped with an 877 rotor and a 72310F-tube adapter.

Fractionation of humic substances in soil solution and the detection of Al, Fe, and Mn

The high-performance liquid chromatography size-exclusion separation (SEC-HPLC) was carried out with a Superdex 75 HR 10/30 column (Pharmacia) which has a nominal molecular-mass exclusion range between 3,000 and 70,000 Da (protein and peptide standards). The HPLC apparatus consisted of a 200LC pump, a 235C diode array detector and a 1022LC Plus controller, all manufactured by Perkin Elmer Nelson (USA). Exactly 500 μL of soil solution were injected via a 7125 Rheodyne injection valve fitted with a 100 μL PEEK injection loop. All tubes and connections were made of plastic material, mainly PEEK, to avoid metal contamination. After UV detection, fractions of the eluate (300 μL) were subsequently collected with an automatic fraction collector (LKB-RediFrac, Pharmacia) in 6 mL polypropylene tubes (Sarstedt), acidified with HNO_3 up to 0.2% m/v, and kept in refrigerator (4°C) until further analysis. The determination of Al, Mn and Fe in the collected fractions was conducted in a Zeenit 60 graphite furnace atomic-absorption spectrometer (GFAAS) (Analytik Jena, Germany). This spectrometer was fitted with an MPE 60 autosampler and automatically controlled by the WinAAS v.3.30® software. The furnace conditions employed are given elsewhere (Pérez *et al*., 2004).

The eluent flow rate was 1 mL min^{-1} for both demineralized water and the 0.01 mol L^{-1} NaCl solution. This concentration was chosen with the purpose of suppressing any kind of negative ionic groups in the gel (Muller *et al*., 2000; Specht and Frimmel, 2000) and also of matching more closely the ionic strength of the studied soil solutions (Table 3). This last statement is a very important

condition since the conditional stability constants, which control the distribution of a given element among its possible chemical forms, also vary with the ionic strength of the solution (Sposito, 1989; Das *et al*., 2001). All elutions were performed in duplicate and, in order to avoid any kind of cross contamination, blanks were injected between different soil solution samples.

The column's void volume (5.3 and 6.8 mL for water and NaCl, respectively) was determined using Blue Dextran 2000 (Pharmacia) and the total permeation volume (18.6 and 17.8 mL for water and NaCl, respectively) with acetone (Merck). The linear size-exclusion calibration range of the column was determined with solutions of ovalbumin, albumin, ribonuclease A, chymotrypsinogen A (Pharmacia), aprotinin (Sigma), and vitamin B12 (Sigma-Aldrich).

All solutions were prepared using Milli Q® water with an electrical resistance ≥ 18 MΩ cm. The nitric acid and NaCl were of Suprapur grade (Merck).

After centrifugation the soil solutions were kept in refrigerator until analysis, for a period never longer than 24 h.

RESULTS AND DISCUSSION

The influence of the eluent composition on the SEC chromatograms of a soil solution sample from a Kanhapludult topsoil can be seen in Figure 1. Using water as eluent, most of the detected compounds were eluted close to the exclusion limit (void limit). The cation exchange capacity of Superdex 75 HR is reported to be low (Kagedal *et al*., 1991; Hils *et al*., 1999). However, it seems to be large enough to cause electrostatic repulsion between the gel and the organic compounds of the sample. This ion exclusion phenomenon is well known for humic substances extracted from soils (Saito and Hayano, 1979; Mori *et al*., 1987; Ceccanti *et al*., 1989; Hongve *et al*., 1996; Specht and Frimmel, 2000) and may be also present in soil solutions. Thus, the use of a neutral salt (such as NaCl) solution as eluent is supposed to reduce ionic interactions, since the Na$^+$ cations should surround

Table 3 Soil solution composition of the 4 Brazilian topsoil samples studied

Sample	Na	Mg	K	Ca	Mn	Fe	Al	F	Cl	NO$_3$	SO$_4$	DOC	pH	I
	mg L^{-1}													$\times 10^{-2}$ mol L^{-1}
CS	22.53	5.64	29.82	11.13	0.04	10.53	15.62	0.49	27.40	14.12	34.21	47.5	6.96	0.28
FJ	5.07	8.67	9.76	16.47	0.39	0.16	0.52	<LD	6.55	92.88	5.17	51.6	4.28	0.28
CCJ	1.73	4.64	3.59	8.92	0.01	3.86	1.18	<LD	2.18	40.12	5.37	33.9	5.32	0.14
SSJ	4.12	35.08	37.50	65.89	0.01	0.41	0.80	<LD	11.16	274.36	74.89	61.3	6.20	1.02

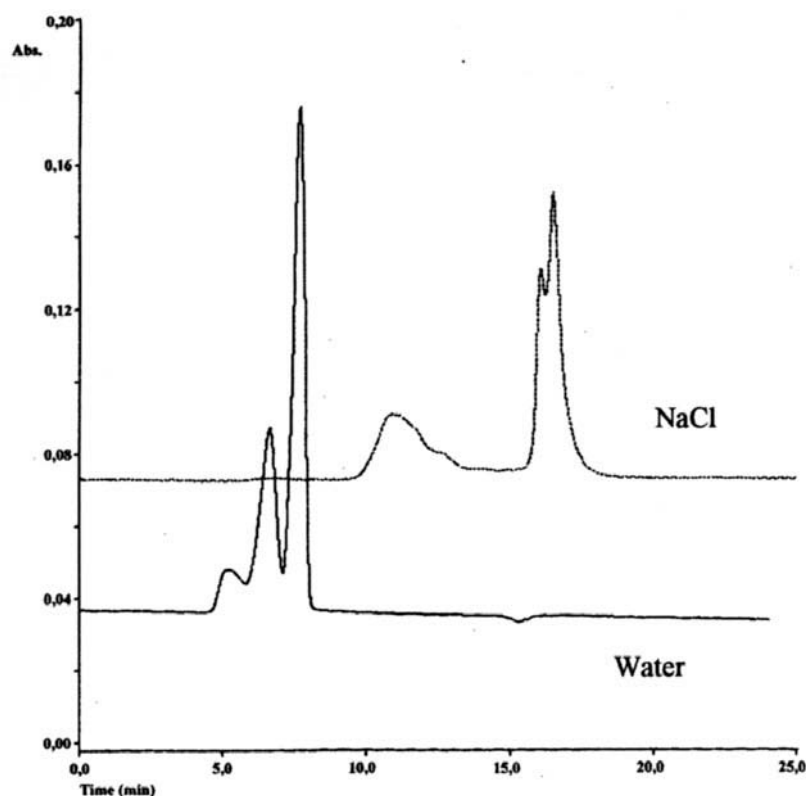[a]Limit of detection (0.080 mg L^{-1} F; 0.370 mg L^{-1} NO$_3$).

Figure 1 Composed SEC-chromatograms (diode array detection) of a soil solution sample eluted with water and 0.01 mol L^{-1} NaCl from a Typic Kanhapludult topsoil cultivated with corn and amended with organic compost (CS).

the negative sites formed at the gel (Mori *et al.*, 1987). This assumption is confirmed in Figure 1, where one can see that using 0.01 mol L^{-1} NaCl as eluent the peaks shift towards larger elution times. A similar behavior was observed for the Eutric Acrustox soil samples (FJ, CCJ, SSJ), as shown in Figure 2. The effect of the eluent composition on the metal distribution in the soil solution samples was also investigated. Special attention was given to the metal fraction that elutes simultaneously with the dissolved organic matter. This fraction was determined by identifying those metal peaks (detected by GFAAS) with retention times similar to those of the UV detected peaks (Figure 3).

As can be seen in Table 4, the association between the dissolved organic matter and the studied metals in soil solutions became clear only if 0.01 mol L^{-1} NaCl is used as eluent. These results corroborate the supposed electrostatic repulsion, as they explain the chromatogram patterns obtained using water as eluent: if the gel of the column is negatively charged, a competition for the cations originally bonded to the humic substances is established, thus displacing the original equilibrium situation. Mori and Barth (1999) have observed that adding an electrolyte to the mobile phase in a range of

10^{-2} mol L^{-1} should eliminate both the ion-exchange and the ion-exclusion effects. This way, the significant drop in the coeluted metal-UV peaks observed when water is used as eluent can be explained.

The present speciation results were compared to those obtained by modeling (Ross, 1994; Mattigod, 1995; Mattigod and Zachara, 1996). Geochem-PC was selected as the equilibrium code since its development was oriented to soil solution and related topics (Parker *et al.*, 1995). In order to quantitatively describe the equilibrium between trace metals and the soluble organic fraction in the studied soil solution samples, the Mattigod and Sposito Mixed Model was employed (Mattigod and Sposito, 1979; Sposito, 1981; Sposito *et al.*, 1982). The analytical soil solution data used are shown in Table 3 and their determination is described elsewhere (Pérez *et al.*, 2002). No significant correspondence was found between the experimental results obtained using water as eluent and those arising from the modeling. However, considering results obtained with 0.01 mol L^{-1} NaCl as eluent, a significant nonlinear agreement was observed for Al (Figure 4). The fact that neither Fe nor Mn showed a significant correlation between experimental and modeling data
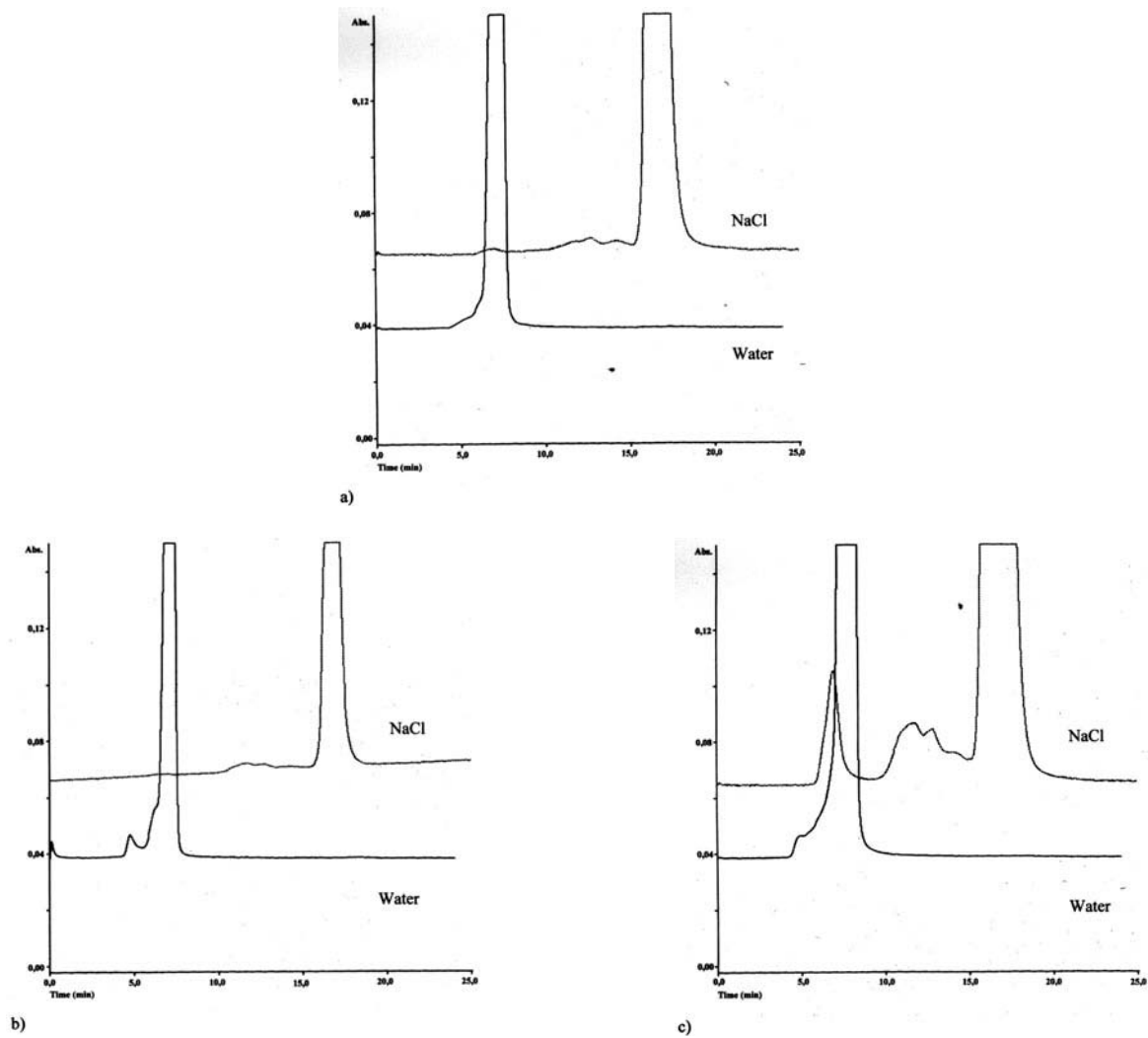
Figure 2 Composed SEC-chromatograms (diode array detection) of soil solution samples eluted with water and 0.01 mol L^{-1} NaCl from an Eutric Acrustox topsoil in a: (a) small fragment of a secondary forest (FJ), (b) conventional tillage management of corn crop (CCJ), (c) sewage sludge amended (12 t ha^{-1}y^{-1}) corn crop (SSJ).
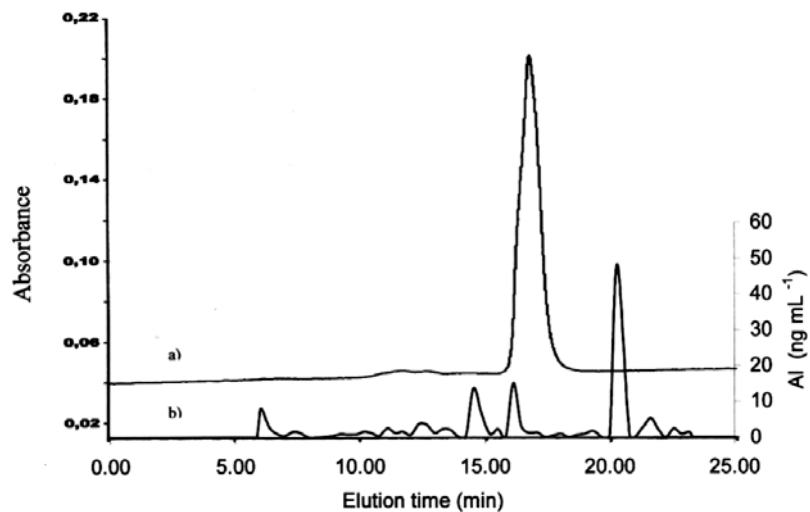

Figure 3 Association between dissolved organic matter (a) and Al (b) in CCJ soil solution as obtained by SEC-HPLC diode array detection (a) and GFASS (b) sequential detection. Similar retention times was interpreted as metal-organic matter association. Similar experiments for other metals and samples led to Table 4 data.

Table 4 Contents of Mn, Fe, and Al associated with the UV detected organic compounds determined after elution of the soil solutions using a Superdex 75 HR 10/30 SEC column

| Sample	Eluent	Mn	Fe	Al
			%[a]	
CS	Water	22	15	23
CS	NaCl	67	27	66
FJ	Water	5	3	1
FJ	NaCl	61	60	73
CCJ	Water	34	16	24
CCJ	NaCl	17	83	53
SSJ	Water	13	5	12
SSJ	NaCl	74	91	72

[a]This fraction of the metal was calculated by rationing its concentration associated to the UV peak to the total concentration of the eluted metal.

could be possibly related to the species and the conditional stability constants assumed by the model, which may be incorrect or inadequate for soils, as suggested by Sposito (1989).

CONCLUSIONS

For speciation purposes, the optimization of the mobile-phase composition must consider the minimization of unwanted interactions of the soil solution's humic substances with the support material of the SEC column. In comparison to water, $0.01\,mol\,L^{-1}$ NaCl is recommended for the polymer-based studied column, Superdex 75 HR 10/30.
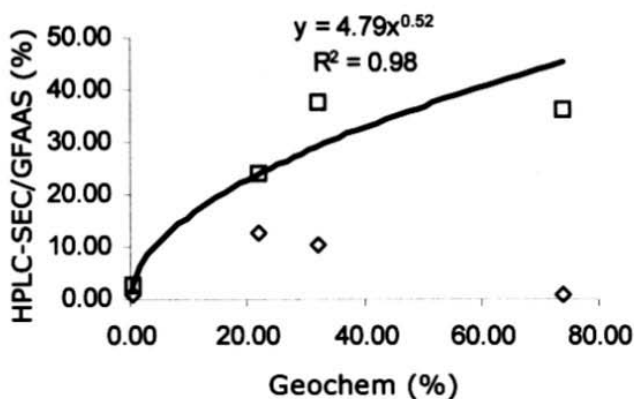
Despite the small number of samples studied, the significant agreement between predicted and determined Al-organic complex fractions suggests that the Mixture Model can be used as a first approximation for a qualitative evaluation of this fraction.
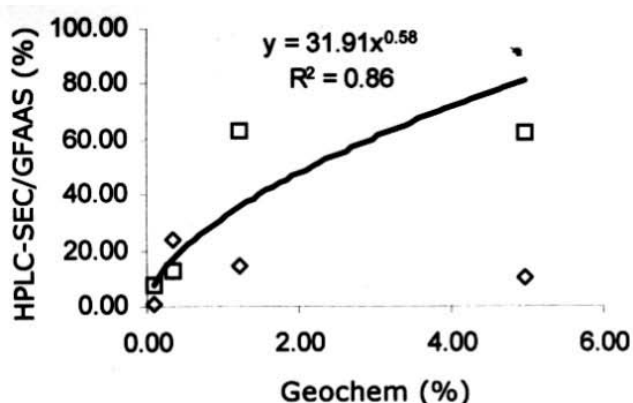
(a)
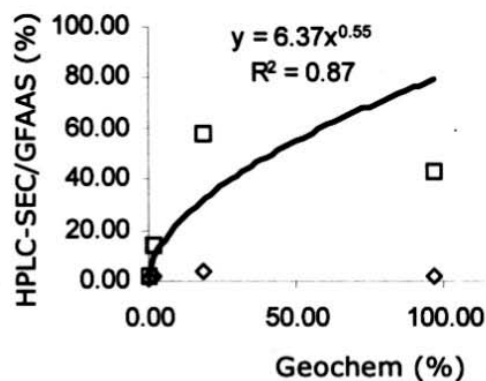
(b)

(c)

Figure 4 Relationship between the percentage of Mn (a), Fe (b), and Al (c) related to humic substances calculated by the Geochem-PC and determined by the association of HPLC-SEC (◇ for water and □ for NaCl eluent) and GFAAS.

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