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FRACTIONATION OF SOIL ORGANIC MATTER

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The International Humic Substances Society (I.H.S.S.) has published a general procedure for the fractionation and isolation of FA, but crucial information is lacking. Here we give an account of a modified procedure to fractionate and isolate FA from soil using XAD-8 resin. We also present results with respect to the yield of FA and its ash content for a number of soil horizons. Our results indicate that more NaOH than previously assumed was needed to remove all adsorbed FA from the XAD-8 resin. By contrast to the IHSS procedure we combine several FA fractions prior to purification. To purify FA, two HCl/HF treatments were necessary to reduce the ash content to an acceptable level. In the four soil horizons investigated in this study, the relative importance of FA in total humic compounds increases in the order peat < podzol O < podzol BhS < podzol Bs.

Keywords: Isolation; soil organic matter; humic acid; fulvic acid; purification; XAD-8

INTRODUCTION

Organic matter plays an important role in many soil processes, including cation exchange, metal complexation and transport, acid/base reactions and water transport [1]. Comprehensive studies of the properties of soil organic matter (SOM) include investigations of specific fractions of SOM. Commonly, SOM is subdivided into humin, humic acid (HA) and fulvic acid (FA). Soil HA comprises colored high-molecular-weight organic substances in the soil that are soluble in 0.1 M NaOH but insoluble at pH 1–2. Soil FA comprises colored, moderate-molecular-weight organic substances of non-specific composition in the soil that are soluble in 0.1 M NaOH and also soluble at pH 1–2^[5].

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Unfortunately, the presence of impurities in HA and FA, as indicated by the ash content, may easily lead to erroneous results. Therefore purification of isolated HA and FA is important. However, some of the steps in the isolation and purification procedure may change the character of HA and FA. Possibly some of the steps could be omitted without having a considerable negative effect on the purity on HA and FA. Impurities not only consist of inorganic compounds (Fe, Al and Si), but also of low molecular weight organic molecules that are not structural constituents of HA and FA^[6].

Much research has been done to extract soil humic substances (HS) and several extractants have been proposed, including 0.5 M NaOH extraction under N₂ gas^[7], 0.5 M NaOH or Na₄P₂O₇ after HF treatment^[8], 0.5 M NaOH extraction after decalcification and with Na-Dowex A-1 resin without decalcification^[9], pre-treatment with 0.3 M HF and extraction first with 0.02 M Na₄P₂O₇ and then with 0.03 M NaOH^[10], as well as benzene-alcohol (1:1) extraction in a Soxhlet apparatus^[11]. Extraction with dilute aqueous NaOH is most common and quantitatively the most effective reagent for extracting HS from soils and sediments [12, 13, 14]. L  vesque^[35] found that 0.1 M NaOH solution extracted more HS than 0.5 M NaOH, but the latter gave a lower ash content and is to be preferred. Goh^[16] and Ortiz de Serra *et al.*^[9] described the ash content of the FA was much higher without treatment with HCl/HF. Only with a HCl/HF treatment, it is possible to remove amorphous clay minerals of Si, Al and Fe.

The International Humic Substances Society (IHSS) proposed procedures for fractionation of the extracted soil HS into HA and FA and for the subsequent purification of these fractions. Here we test the effectiveness of the individual steps in the purification procedures of HA and FA, with respect to the removal of metals, Si and low molecular weight (hydrophilic) organic acids. The selected procedures are based on recent literature, but several modifications were made. Samples from four soil horizons were used in the experiment, including a peat soil, organic surface layer of a forest soil (podzol) and a podzol Bh and a Bs horizon. The contribution of HA and FA to SOM are discussed with respect to their pedogenic implications.

EXPERIMENTAL

The soil horizons investigated in this study include the O, Bhs and Bs of a sandy podzol, and the upper layer of a peat soil. The podzol is at Tongbersven, the Netherlands and has developed in well drained coversand and is classified as a Typic Haplorthod^[15]. The site is dominated by Scots pine (*Pinus sylvestris*) and

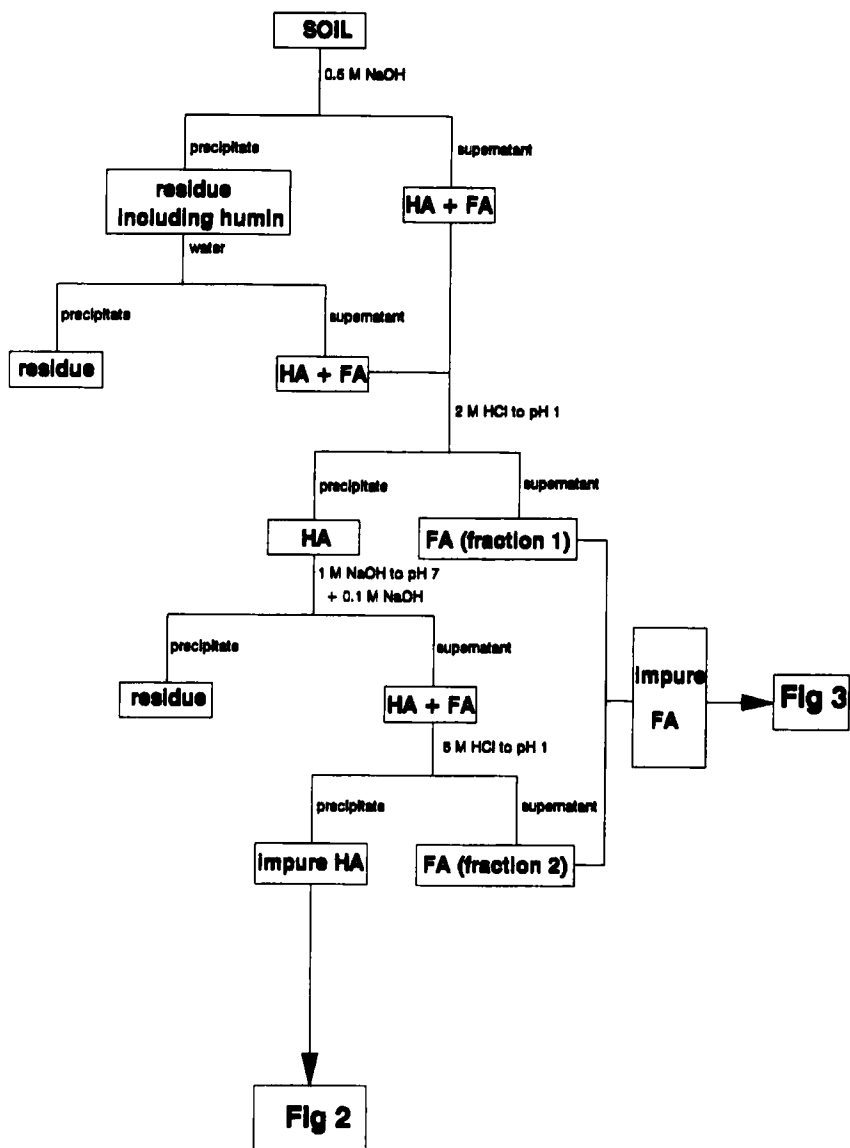


FIGURE 1 Separation scheme for humic fractions

ground vegetation is lacking^[17]. The peat soil is used as pasture is near Veenendaal, the Netherlands^[18].

Humic substances were extracted (24 h) from 75 g of field moist sample in 750 ml 0.5 M NaOH under N₂ atmosphere^[19] (Figure 1). After centrifugation (3260 g, 1 hour) the extract was stored in a refrigerator (4°C). The residue was washed (1 h) with 250 ml ultra pure water and centrifuged (3260 g, 1 h). The water extract was added to the first supernatant and the combined supernatant was acidified slowly to pH 1–2 using 2 M HCl and left overnight. The resulting precipitate is impure HA and the supernatant is impure FA (fraction 1). Impure FA solution was stored in the refrigerator until purification.

The impure HA slurry was weighed and neutralized to pH 7 with 1 M NaOH. Next, 0.1 M NaOH solution was added until reaching a liquid : solid ratio (v/w) of 10 : 1^[1]. The resulting suspension was shaken for 4 h, left overnight and centrifuged. Subsequently this suspension was slowly acidified to pH 1–2 using 6 M HCl, left overnight and centrifuged. The supernatant (FA fraction 2) was combined with FA fraction 1 and stored in the refrigerator. The HA precipitate was further purified using 0.1 M KOH (v/w = 10/1) and 2.5 M KCl solution to obtain an estimated concentration of K of 0.3 M^[20]. This suspension was shaken overnight (Figure 2). After centrifugation (3260 g, 1 h), the supernatant was slowly acidified with 6 M HCl to pH 1–2 and left overnight to allow HA to precipitate. The supernatant, containing polyvalent ions and small amounts of FA (Figure 2), was discarded. The treatment of the HA precipitate was repeated once to remove additional silicon and polyvalent cations. Subsequently, the precipitate (HA) suspended in 0.1 M HCl/0.3 M HF solution using liquid : solid ratio (v/w) of 10 : 1^[10], was shaken overnight, left for 1 h to precipitate and centrifuged (3260 g, 1 h). The clear supernatant was discarded and the ash content in the precipitate was determined. This treatment with HCl/HF was repeated until the ash content was less than 1%^[16]. At that stage, the precipitate (HA) was transferred to dialysis tubing (Visking, R. Arlow & Co Ltd, Lower Hutt, New Zealand^[16]) and HA was dialyzed against distilled water until the conductivity was below 5 $\mu\text{S}\cdot\text{cm}^{-1}$. Dialyzed HA was freeze-dried (vacuum capacity below 10 micron), weighed and stored in a desiccator until further use^[1, 21].

For the purification of the FA, XAD-8 resin (Rohm and Haas, 20–50 mesh) was used. XAD-8 was pre-cleaned by means of successive Soxhlet extractions for 24 hours with methanol, acetonitrile and diethyl ether and kept in methanol^[22]. Three 240 ml inert borosilicate glass columns (e.g. Biorad, art. no 737–2551) were packed with 220 ml pre-cleaned XAD-8 resin. Next, the XAD-8 filled columns were rinsed with (a) 20 liter ultra pure water until the effluent contained less than 0.3 mg/l DOC, (b) 2 liter 0.1 M NaOH solution, (c) 4 liter 0.1 M HCl solution and (d) 4 liter ultra pure water. All solutions in the FA purification

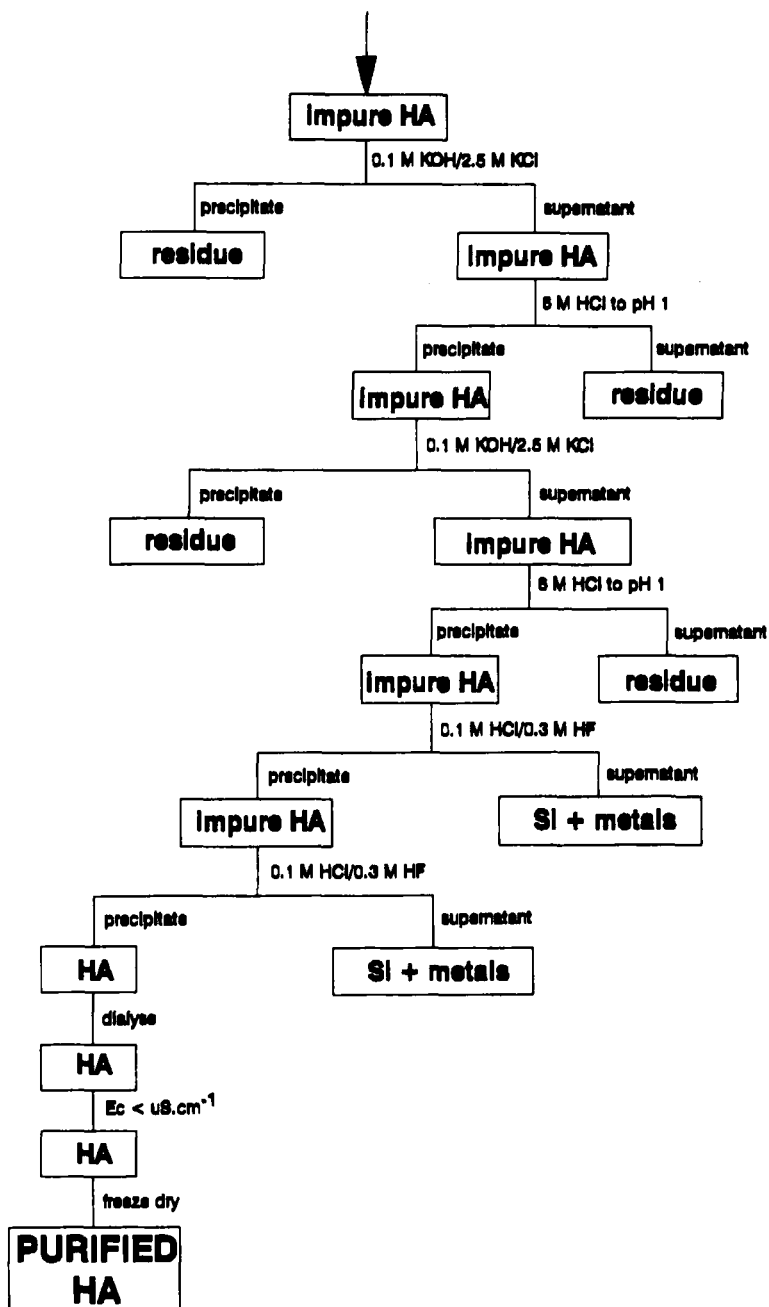


FIGURE 2 Purification of humic acids

procedure were percolated at a speed of 10–15 bed volumes/hour^[23]. At least 75–100 bed volumes ultra pure water are necessary to remove methanol. The FA containing suspension was passed over a cleaned XAD-8 column at a speed of 10–15 bed volumes/hour, and the concentration of DOC was measured after each void volume (= 156 ml) to prevent column breakthrough^[24]. The effluent, commonly containing minor amounts of DOC that did not adsorb on XAD-8, was discarded. After percolation the XAD-8 column was rinsed with one void volume ultra pure water and the effluent was discarded.

The adsorbed FA was leached by applying three void volumes of 0.1 M NaOH solution^[3] and one void volume of ultra pure water. The leachates were collected in a 1 liter polypropylene cylinder, containing 12 ml HCl (6 M) and 9 ml HF (48%) solution. Subsequently, the dissolved FA in the HCl/HF mixture was passed over a second XAD-8 column. Again breakthrough of the column was avoided by frequent checking the concentration of DOC in the effluent (after every 156 ml). After percolation of the FA containing solution the XAD-8 column was rinsed with one void volume ultra pure water prior to desorption of FA with NaOH. This procedure was repeated until nearly all Si was removed from the FA. The final 0.1 M NaOH leachate of the XAD-8 column was passed over a pre-cleaned H⁺ saturated 150 ml cation exchange column. The cation exchange resin (BioRad, AG-MP-50, 20–50 mesh) was pre-cleaned by Soxhlet extraction with methanol for 24 hours, methanol was replaced and extraction was repeated. Prior to use the 150 ml cation exchange column (e.g. Biorad, art. no 738–2532) was rinsed with 10 liter ultra pure water until the DOC of the effluent was below 0.3 mg/l DOC. Next, the column was rinsed with (a) 1 liter 1 M NaOH solution, (b) 2 liter 2 M HCl solution and (c) approximately 2 liter ultra pure water. Percolation with water was continued until Cl was absent from the effluent.

The FA containing NaOH leachate from the XAD-8 column was passed over the cation exchange column. Next, the cation exchange resin was rinsed with one void volume (100 ml) ultra pure water. The obtained effluent, containing purified FA, was freeze-dried and the quantity of FA weighed. Freeze-dried FA was stored in a desiccator. The entire procedure is schematically shown in Figures 1, 2 and 3. The chemical composition of the freeze-dried HA and FA were determined using a Philips PW1404 XRF-spectrophotometer. Organic carbon was measured using an element-analyzer (Interscience, EA 1108). Aluminum, Fe and Si in the different liquid fractions were measured using atomic absorption spectrometry (Varian Spectra AA-20 Plus) with acetylene and N₂O. Dissolved organic carbon (DOC) was measured using a T.O.C. analyzer (Shimadzu 5000). Full analytical details are given by Buurman *et al*^[25].

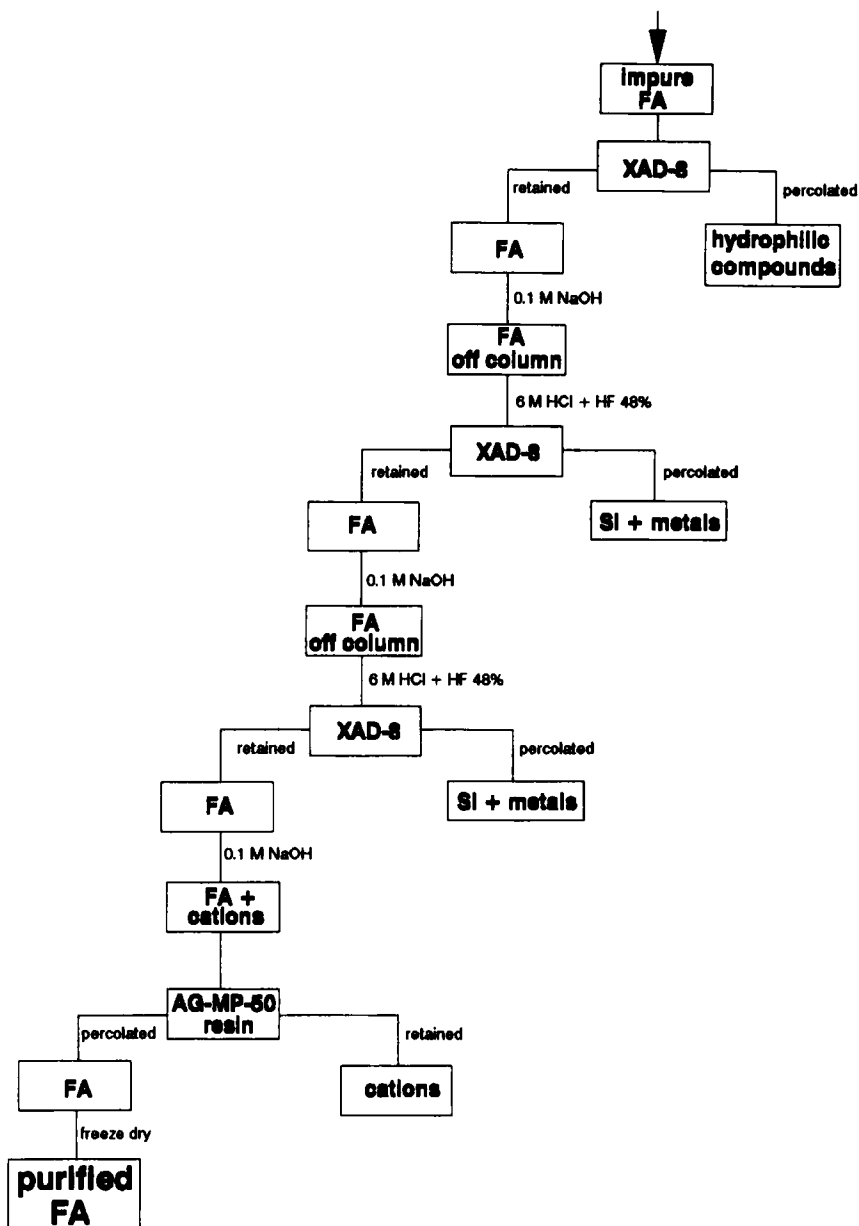


FIGURE 3 Purification of fulvic acids

RESULTS AND DISCUSSION

The total chemical composition of the four soil samples is given in Table I. Loss on ignition is highest in the O horizon, and lowest in the Bs and Bhs. On the contrary, SiO₂ is the lowest in the O horizon, while Bs and Bhs of the sandy podzol have about 95% SiO₂. With respect to SOM and SiO₂ content the peat soil is intermediate. The contents of Al₂O₃ varies from 1.3% in the O horizon to 3.2% in the peat soil. The Fe₂O₃ contents of the fine earth fraction increases from 0.5% in the Bhs to 0.9% in the peat soil (Table I).

TABLE I Total analysis (XRFS) of original samples

Sample	Na ₂ O %	K ₂ O %	SiO ₂ %	TiO ₂ %	Al ₂ O ₃ %	Fe ₂ O ₃ %	MgO %	CaO %	Loss on ignition%
O-hor.	0.06	0.35	52.67	0.16	1.37	0.60	0.09	0.10	44.76
Bhs-hor.	0.04	0.53	94.77	0.18	1.62	0.56	0.11	0.01	5.36
Bs-hor.	0.04	0.65	95.98	0.17	2.08	0.64	0.13	0.01	2.34
Peat	0.35	0.99	77.60	0.18	3.26	0.96	0.23	0.61	16.04

TABLE II Absolute and relative amounts of organic C, Si, Al and Fe in the different fractions obtained during isolation and purification of HA and FA

O-horizon Tongbersven	org. C mg	% total org. C	Si mg	% total Si	Al mg	% total Al	Fe mg	% total Fe
Soil sample 75 gram (field-moist)	10854	100	7975	100	337	100	194	100
Residues (humins + residues)	6555	60.4	7759	97.3	257	76.3	164	84.5
Silicon + metals	97	0.9	162	2.0	50	14.8	5	2.6
Hydrophilic compounds	729	6.7	49	0.6	30	8.9	24	12.4
HA	2710	25.0	0	0.0	0	0.0	0	0.0
FA	763	7.0	5	0.1	0	0.0	1	0.5

A summary of the results of the isolation and purification procedures of HA and FA for all four soil horizons is given in Tables II to V. The contribution of humic substances (HA and FA) to soil organic carbon increases in the order O (32%) < peat (38%) < Bhs (56%) < Bs (58%) (Tables II to V). Particularly in the

O horizon and to a lesser extent in the peat soil, a considerable portion of SOM consists of partly decomposed organic matter. In the podzol Bhs and Bs the amount of non-humified material (e.g. fresh and dead roots) is limited. In the podzol O, Bhs and Bs horizon most of the Si, Al and Fe occurred in the residues, remaining after extracting HS 97%-99%, 74%-79%, 84%-98%, for Si, Al and Fe respectively. The same holds through the peat soil, albeit that the relative amount of Al and Fe occurring in this residue fraction is slightly different (90% and 78% respectively). The high proportion of Si, Al and Fe in the residues after extraction of HS contain all mineral particles. Besides Si, Al and Fe does the residue fraction, contain one-third to about a half of the total organic carbon (33%, 34%, 50% and 60%) in the Bs, Bhs, peat and O horizon, respectively. This fraction is probably dominated by humin and non-humified organic matter.

TABLE III Absolute and relative amounts of organic C, Si, Al and Fe in the different fractions obtained during isolation and purification of HA and FA

<i>Bhs-horizon Tongbersven</i>	<i>org. C mg</i>	<i>% total org. C</i>	<i>Si mg</i>	<i>% total Si</i>	<i>Al mg</i>	<i>% total Al</i>	<i>Fe mg</i>	<i>% total Fe</i>
Soil sample 75 gram (field-moist)	2033	100	25037	100	522	100	266	100
Residues (humin + residues)	710	34.9	24981	99.8	388	74.3	246	92.5
Silicon + metals	71	3.5	54	0.2	10	1.9	3	1.1
Hydrophilic compounds	112	5.5	2	0.0	124	23.8	16	6.0
HA	595	29.3	0	0.0	0	0.0	0	0.0
FA	545	26.8	0	0.0	0	0.0	1	0.4

TABLE IV Absolute and relative amounts of organic C, Si, Al and Fe in the different fractions obtained during isolation and purification of HA and FA

<i>Bs-horizon Tongbersven</i>	<i>org. C mg</i>	<i>% total org. C</i>	<i>Si mg</i>	<i>% total Si</i>	<i>Al mg</i>	<i>% total Al</i>	<i>Fe mg</i>	<i>% total Fe</i>
Soil sample 75 gram (field-moist)	1182	100	28399	100	637	100	311	100
Residues (humin + residues)	396	33.5	28376	99.9	504	79.1	307	98.7
Silicon + metals	18	1.5	17	0.1	3	0.5	1	0.3
Hydrophilic compounds	84	7.1	4	0.0	130	20.4	3	1.0
HA	216	18.3	0	0.0	0	0.0	0	0.0
FA	468	39.6	2	0.0	0	0.0	0	0.0

TABLE V Absolute and relative amounts of organic C, Si, Al and Fe in the different fractions obtained during isolation and purification of HA and FA

<i>Peat Veenendaal</i>	<i>org. C mg</i>	<i>% total org. C</i>	<i>Si mg</i>	<i>% total Si</i>	<i>Al mg</i>	<i>% total Al</i>	<i>Fe mg</i>	<i>% total Fe</i>
Soil sample 75 gram (field-moist)	2600	100	11557	100	571	100	242	100
Residues (humins + residues)	1302	50.1	11471	99.3	515	90.2	190	78.5
Silicon + metals	27	1.0	70	0.6	16	2.8	8	3.3
Hydrophilic compounds	294	11.3	14	0.1	39	6.8	43	17.8
HA	771	29.7	0	0.0	0	0.0	0	0.0
FA	206	7.9	2	0.0	1	0.2	1	0.4

The application of HCl/HF has a pronounced effect on the purity of HA (Table VII) and is a necessary step to reduce the ash content. After one HCl/HF treatment the ash content was 1.9 – 2.0%. After the second HCl/HF treatment the ash content in all the samples was < 1% (not shown). The same applies to FA. Here we determined Si, Al and Fe, because the impure FA is only available in liquid form. Before this treatment the impure FA solutions of the four samples contained 0.02 – 0.5% Si, 3 – 11 % Al and 1 – 20% Fe (Table IX). After the first HCl/HF step the impure FA solution contained < 0.1% Si, <0.1% Al and < 1.2% Fe. After the second HCl/HF step the Fe content was reduced to < 1%, while silica showed a small increase to 0.1 – 0.2%. This could be caused by releasing silica from the XAD-8 column (Table VI). Nevertheless the second HCl/HF treatment was necessary, because the Fe content was too high after the first treatment. To avoid Si release the second HCl/HF step may be replaced by a HCl step only.

TABLE VI Release of elements from XAD-8 resin by reagents

<i>Sample</i>	<i>Si mg/L</i>	<i>Al mg/L</i>	<i>Fe mg/L</i>
HCl/HF solution (+ ultra pure water) (blank)	2	0	0
HCl/HF solution + XAD-8 resin	100	1	2
XAD-8 resin (+ ultra pure water)	2	0	0
NaOH (0.1 M) solution (+ ultra pure water) (blank)	1	0	0
NaOH (0.1 M) solution + XAD-8 resin	2	0	1
HCl (0.1 M) solution (+ ultra pure water) (blank)	1	0	0
HCl (0.1 M) solution + XAD-8 resin	2	0	1

Samples shaken overnight, centrifuged and measured with A.A.S. (C_2H_2 and N_2O).

Hydrophilic acids, present in non-purified FA, contribute significantly to the total organic C in the soils, its contribution increasing from 5% in the Bhs to 11% in the peat soil. This means that non-purified FA, contains 15% (Bs), 17% (Bhs), 49% (O) and 59% (peat) hydrophilic acids (Tables II to V). In the hydrophilic fraction Si is virtually absent. However, a significant proportion of Al and Fe is associated with this fraction, accounting for 6% to 24% of the total Al and 1% to 18% of the Fe. Note that the Al content of the hydrophilic acid fraction is particularly high in the podzol Bhs and Bs, whereas the Fe content is highest in the podzol O and peat soil (Tables II to V). At this point it is unclear whether the hydrophilic acid fraction really exists as such in the soil solid phase, or if it is an experimental artifact, i.e. merely resulting from the repeated acid and base treatments. Humic material in the O horizon and in the peat sample were dominated by HA, whereas in the Bhs horizon HA and FA were present in about equal concentrations. Only in the Bs horizon was HS dominated by FA (Tables II to V). FA in mineral soils is believed to result from accumulation after transport of DOC from the organic surface layer. Because much of HA and FA is produced at the soil surface, the higher mobility of the smaller FA molecules is expected to result in increased contributions of FA to HS with depth. Table VII shows the carbon contents (corrected for moisture content) in the samples and fractions. XRFS scans showed small amounts of Fe, Al, Si and Ca after one KOH step. No Fe, Al, Si or Ca were found after the second step. After purification HA had an organic C content of 53% for all four soil horizons. Si, Al and Fe were virtually absent. Purified FA contained 53% to 55% organic C and minor amounts of Fe and Si were present (Tables II to V, VII).

TABLE VII Carbon contents in different fractions (105°C dry basis)

<i>Sample</i>	<i>O-hor % C</i>	<i>Bhs % C</i>	<i>Bs % C</i>	<i>Peat % C</i>
Original sample	42.88	3.83	2.31	15.85
Humin (residue)	20.38	1.17	0.58	4.74
Residue	32.93	23.33	12.86	1.00
Residue after KOH/KCl treatment	14.78	15.63	8.53	12.03
Impure HA after 1st. HCl/HF treatment	42.23	42.20	43.68	38.33
Impure HA after 2nd. HCl/HF treatment	52.24	50.82	n.d.	49.02
HA	53.78	52.87	53.33	53.34
FA	53.59	54.98	55.64	53.72

n.d. = not determined

TABLE VIII Carbon losses with HCl/HF treatments

<i>Horizon</i>	<i>impure HA mg C</i>	<i>Carbon loss after 1 HCl/HF treatment mg C</i>	<i>Carbon loss after 2 HCl/HF treatments mg C</i>	<i>losses after both HCl/HF treatments % C</i>
O	7055	39.1	30.8	1.0
Bhs	1737	39.6	31.2	4.1
Bs	746.7	10.2	7.9	2.4
Peat	4300	14.0	12.7	0.6

TABLE IX Iron, aluminum and silica losses with HCl/HF treatments

<i>Sample</i>	<i>Fe %</i>	<i>Al %</i>	<i>Si %</i>
O-horizon Tongbersven			
Impure FA solution	20.13	9.45	0.51
1st HCl/HF step	1.23	0.08	0.05
2nd HCl/HF step	0.96	0.04	0.20
Pure FA	0.07		0.28
Bh-Horizon Tongbersven			
Impure FA solution	6.35	11.46	0.03
1st HCl/HF step	0.56	0.02	0.01
2nd HCl/HF step	0.23	0.08	0.18
Pure FA	0.10		0.22
Bs horizon Tongbersven			
Impure FA solution	1.06	10.79	0.02
1st HCl/HF step	0.06	0.01	0.01
2nd HCl/HF step	0.09	0.06	0.16
Pure FA	0.03		0.25
Peat Veenendaal			
Impure FA solution	21.53	3.27	0.20
1st HCl/HF step	0.72	0.05	0.02
2nd HCl/HF step	0.08	0.02	0.06
Pure FA	<0.04	0.03	0.07

In the O-horizon 60% of the organic carbon is lost in the residue fraction. For Si, Al and Fe this is 97%, 76% and 84% respectively. This suggests that most of SOM in the podzol O horizon consists of humin and non-humified organic matter. In addition, the large fraction of total Al, Fe and Si occurring in residues is probably present as mineral particles. In this horizon 25% of the organic matter is present as HA and only 7% as pure FA. In the Bhs horizon 35% of the organic matter is present in the residue fraction, almost 100% of the Si, 74% of the Al and 92% of the Fe is found in these fractions. Almost 30% of the organic matter of the original sample is pure HA and 26% is pure FA. In the Bs horizon 33% of the organic matter stays in the residue. Almost 100% of Si and Fe is in this fraction and almost 80% of Al. Only 18% of the organic matter in this horizon is pure HA and almost 40% is pure FA. Finally in the peat sample 50% of the organic matter is lost in the residue fraction. Nearly all Si, 90% of Al and about 80% of Fe occurs in this fraction. In peat 29% of the organic matter is pure HA and only 8% is pure FA. Of total Al and Fe in the soil, about 7% and 18% respectively are in the hydrophilic fraction. No Si, Al and Fe was found in the pure HA. In pure FA only small amounts of Si, Al and Fe were detected. The ash content of the pure samples did not exceed 1%.

CONCLUSIONS

Here we described a purification and isolation procedure to obtain soil HA and FA with low ash content (<1%). To our knowledge such a procedure for FA has never been tested rigorously before. In this contribution we give a detailed account of all steps involved. Throughout the procedure we recorded the fate of organic C, Fe, Al and Si in order to obtain quantitative information about the distribution of these elements in different organic matter fractions. The study was conducted using material from surface- and sub-soils in the Netherlands. From the original soil samples, the carbon content increases with every purification step. On the other hand, the aluminum content decreases to < 0.05%, iron to <0.1% and silica to < 0.2% (Table IX). All described steps are necessary to obtain pure HA and FA with low ash content. Following our procedure the pure HA of all samples contain 53% C, for the pure FA the organic C content varies from 53–55% C.

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