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Article

Organic Matter Leached from Tropical Soils by Simulated Rain, Hard (NaOH) and Soft ($NaNO_3$) Extractions: A Realistic Study about Risk Assessment in Soils

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Alkaline extractions from humic material are used to risk assessment in soils. However, there are questions about the type of extraction most suitable for this evaluation. Thus, this work evaluated different soil extractions in search of realistic environmental conditions (simulated rain). The parameters obtained revealed significant differences between the levels and structural characteristics of organic matter, depending on the extractant used, reinforcing the importance of developing methodologies and analytical procedures that minimize structural changes and may be more representative of the molecular structures of organic matter found in soils. The results showed that the material extracted by simulated rain is similar to the fulvic acids of the soil organic matter extracted by alkaline extraction showing the importance of this type of extraction for risk assessment studies in soils.

Keywords: extraction, humic substances, chemometric analysis, characterization, soil, Brazilian soils

Introduction

Soil organic matter (SOM) is a product of plants and animal decompositions under the action of microorganisms.[1] It is a major source of soil organic carbon, pivotal for carbon balance, soil elements provision, environmental sustainability, and climatic conditions, with implications for biogeochemical processes in rivers, lakes and estuarine systems.[2,3]

Due to the heterogeneity of the SOM's chemical composition, structural characterization is essential for a better comprehension of the activity and dynamics of these materials in natural environments.[4]

Historically the extraction methodologies employed play a key role in SOM studies.[5] An optimal extraction methodology should yield a material with minimal changes in its original structure, using a reasonable extraction time, producing a high yield in organic matter and presenting a low contaminant contents.[6] Nonetheless since the first SOM extractions a large variety of extraction methodologies and reagents have been used in this procedure, creating significant difficulties in the cross-comparison of experimental results between different studies.[7,8]

In order to minimize the difficulties of interlaboratory comparison of humic substances' characteristics, the International Humic Substances Society (IHSS) was created to provide a collection of reference humic materials.[9] The IHSS keeps a webpage where it recommends the standardization of procedures for collection, extraction and purification of humic and fulvic acids from soils and natural waters.[9]

In recent years there has been some interesting arguments about the representativity of the humic matter

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regarding soil organic matter.[2,10,11] Even within the humic matter camp it is widely recognised that the current extraction procedure, especially the HF/HCl attack to destroy the resilient inorganic matrice (quartz), changes significantly the sample and that the initial extraction of 0.1 mol L^{-1} NaOH is not environmentally representative.[10] Several groups[11,12] have been testing weaker extractants with special mention to the so-called soil extraction solution (0.01 mol L^{-1} calcium chloride or nitrate).

On the other hand the whole risk assesment community uses the metal binding with humic matter data provided by the humic matter community as a model for the metal binding with natural organic matter (NOM) when performing their environmental risk assesment studies in soils and natural waters. They do this not because they consider this data better but because it is the only data available as generic parameters,[13] or included in freely available software Visual Minteq,[14] Phreeqc,[15] Orchestra.[16]

The aim of this study is to evaluate different fractions of organic matter from soils, extracted with 0.1 mol L^{-1} NaOH, 0.1 mol L^{-1} $NaNO_3$ and leaching with simulated rain, inducing a different and more realistic mechanical and chemical extraction to get even closer to environmental conditions. With the use of chemometric techniques, it is intended to obtain a better understanding of the relationships between the structural characteristics of organic matter fractions, to obtain some insights on the improvement of risk assessment studies in these systems.

Experimental

Chemicals and reagents

All reagents used were of high-purity grade unless otherwise stated. The acid and alkaline solutions necessary for extraction were prepared by dilution of 30% nitric acid (Merck, Darmstadt, Germany) and dissolution of sodium hydroxide-monohydrate (Merck, Darmstadt, Germany) in high-purity water (18.3 MΩ cm, Milli-Q systems, Millipore-Waters, Denver, USA). For calibrations and metal determinations appropriate synthetic standard (atomic absorption spectroscopy (AAS) multielement standard solution, Merck, Darmstadt, Germany) was employed.

Samples collection and preparation

Soil samples were collected between 0-20 cm depth, in the cities of Arapiraca-AL, located in a semi-arid region (S), which undergoes long periods of drought and high temperatures (9°41'59.4"S and 36°41'11.8"W) and Maceió-AL, nearby the Pratagi River (9°33'56.5"S and 35°39'08.0"W), within a region of humid climate (H) that presents long periods of rain and low temperature range. Approximately 30 kg of each soil sample was air-dried and sieved through 2 mm.

Soil samples characterization

Samples were characterized for the organic matter content (by muffle furnace calcination), carbon and nitrogen (by elemental analysis), pH, granulometry, cation exchange capacity and bases saturation.[10,17]

Extraction of SOM's fractions

Extraction with NaOH

Humic substances from semi-arid (SHS) and humid (HHS) soils, as well as the fulvic acids (SFA, HFA) and humic acids (SHA, HHA) fractions, were extracted and purified following the procedure from IHSS,[9] by using 1:10 (m/v) soil:0.1 mol L^{-1} NaOH, with stirring for 4 h in inert atmosphere. Humic acids were purified using a Visking Dialysis Tube (London, UK) (molecular weight cut-off (MWCO) = 7 kDa), according to Town *et al.*[18]

Extraction with $NaNO_3$

Soil solutions from semi-arid (SSS) and humid (HSS) soils were obtained using 0.1 mol L^{-1} $NaNO_3$, at a ratio of 1:10 soil:extractant, stirring for 4 h, followed by decantation (for 2-16 h) and centrifugation to separate the supernatant from the soil residue. The extracts obtained were filtered and stored under refrigeration.[11,12]

Organic matter leached by simulated rain

A rain simulator was used, according to specifications by de Oliveira *et al.*,[19] equipped with a Veejet 80/150 sprinkler nozzle, internal diameter of 12.7 mm, at 2.70 m above the soil surface, operating at a constant pressure of 41.0 kPa, for 30 min, with an average intensity of 96.6 mm h^{-1}, measured through a set of 10.0 rain gauges. For each soil, two plastic trays whose dimensions were 40 × 30 × 6 cm (length × width × depth) were used. The trays were filled up to 5.00 cm with soil, and a drain to collect solutions leached from each fraction (SLS and HLS), adjusted in the support of the rain simulator with 9% of the slope. Afterward, leached solutions were centrifuged and stored under refrigeration.

Structural characterization of SOM fractions

The total organic carbon (TOC) was determined by catalytic combustion, with infrared detection and oxygen atmosphere, in a Shimadzu TOC-VCSH Analyzer.[10]

Characterization by UV-Vis spectroscopy

The UV-Vis absorption spectra, from 700 to 200 nm, were obtained in a double-beam spectrophotometer UV-Vis Shimadzu 1650 PC, equipped with 1.0 cm quartz cuvettes. The analyses were carried out by using 10 mg L^{-1} of TOC from each fraction in 0.05 mol L^{-1} $NaHCO_3$ at pH 8.0.[20] From the values of absorbance, the parameters E_{465}/E_{665} (condensation degree of aromatic constituents), E_{250}/E_{365} (molecular aggregation) and E_{270}/E_{407} (amount of lignin derivatives) were obtained.

Characterization by molecular fluorescence spectroscopy

Molecular fluorescence spectra were obtained in a Shimadzu spectrofluorophotometer, RF-5301PC (Tokyo, Japan) equipped with a 150 W xenon lamp, 1.0 cm quartz cuvette, excitation and emission window (slit) adjusted at 5 nm and scanning speed of 500 nm m^{-1}. Samples were prepared in the same way as for the UV-Vis analysis, following methodologies proposed by Zsolnay *et al.*,[21] Milori *et al.*[22] and the calculated parameters A_4/A_1 (ratio between the area of the last emission quarter (from 570 to 641 nm) and the area of the first emission quarter (from 356 to 432 nm)) and A_{465} (total area of the emission spectrum obtained with excitation at 465 nm) used as indicative of the humification index.[23]

Determination of carboxylic and phenolic groups

The determination of carboxylic (–COOH) and phenolic groups (C_6H_5OH) contents was carried out in triplicate by neutralization titration, following an adaptation of the system proposed by Ritchie and Perdue,[24] using a controlled system with stirring and $N_{2(g)}$ purging. Into 10 mL of each sample, 2.5 mL of 0.1 mol L^{-1} KCl was added, then the pH was adjusted to 3 and the titration carried out up to the pH 11, with a 0.02 mol L^{-1} NaOH solution. The pH values were considered as directly representative of the activity of H^+ ions in solution and the numbers of moles of carboxylic and phenolic functional groups (Q_1 and Q_2) calculated according to Ritchie and Perdue.[24] For the carboxylic (–COOH) groups, the number of mol of base consumed up to pH 8 is considered, whereas, for the phenolic (C_6H_5OH) groups, it has been considered two times the number of mol consumed between pH 8 and 10.

Determination of metals in the soil fractions

The determination of metals (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, Pb and Zn) was made, in triplicate, by microwave plasma atomic emission spectrometry (MPAES), after acid digestion of 5 mL aliquot from the extracted fraction with 20 mL of 65% (v/v) HNO_3 and 35% (v/v) H_2O_2 until the mixture remained translucent. After cooling, samples volumes were adjusted to 50 mL with deionized water. The ionic lines emission (in nm) for the metal species were: 396.152 (Al), 649.690 (Ba), 616.217 (Ca), 228.802 (Cd), 340.512 (Co), 357.868 (Cr), 324.754 (Cu), 372.993 (Fe), 766.491 (K), 383.829 (Mg), 403.076 (Mn), 379.825 (Mo), 352.454 (Ni), 405.781 (Pb) and 213.857 (Zn).

Chemometric analysis

The statistical treatment of the experimental data related to the characteristics of the different fractions of SOM was made using the Minitab program,[25] hierarchic cluster analysis (HCA) and principal components analysis (PCA), with data normalized by auto-scaling and Euclidean distance.

Results and Discussion

Characteristics of the soil samples

The soil is quite important for the environmental balance, pivotal in the carbon cycle, as a carbon reservoir utilizing the SOM; therefore, the characteristics of each soil may influence the leaching, quantity and composition of the organic material. Furthermore, soils have physical and chemical properties, which are indicators of its capacity to supply nutrients.[26] Thus, quantifying these main indicators is relevant for the characterization of the soils. Table 1 brings results referring to parameters of quality of the analyzed soils.

Table 1. Physicochemical parameters for the soils from semi-arid (SS) and humid (HS) climates: OM (n = 3), C/N (n = 2), pH in H_2O, 1 mol L^{-1} KCl, $Ca(C_2H_3O_2)_2$ at pH 7 (n = 3), CEC, effective and total, V and granulometry

	OM / %	C/N	pH			CEC		V / %	Granulometry		
			H_2O	KCl	$Ca(C_2H_3O_2)_2$	Effective / (cmol dm^{-3})	Total / (cmol dm^{-3})		Sand / %	Silt / %	Clay / %
SS	4.58^a	10.3^a	6.43^a	5.76^a	3.15^a	3.73^a	4.86^a	77^a	74^a	16^a	10^a
HS	11.6^b	16.5^b	6.10^b	5.58^b	2.74^b	8.58^b	11.97^b	72^b	64^b	13^b	23^b

^{a,b}Differ statistically, with a significance level of 5%. OM: organic matter; CEC: cation-exchange capacity; V: base saturation.

Considering the *t*-test for the two samples, it is possible to infer that they statistically differ in all parameters, with a significance level of 5%. The values of OM (in %) are in agreement with the intrinsic conditions of each soil: the semiarid soil (SS) is used for cultivation of vegetables (cassava and coriander are the most cultivated in this region), being its organic matter content up to 5%, whereas the humid soil is not used for agriculture and, due to being of hydromorphic origin (because of the location), with vegetation cover, the amount of organic matter exceeds 5% in this case.[27]

The C/N ratio (Table 1) allows inferring that the organic matter amount diminishes since the higher ratio C/N indicates a decrease in the nitrogen amount and, consequently, an increase of the source material's degradation. Thus, the SS sample presents a lower degree of decomposition, which is in agreement with its soil's management that propitiates a more frequent renewal of SOM than in the case of the HS sample, which presents a higher possibility of decomposing, due to the intrinsic conditions of the matrix.

The unit particles of the soil act together with the SOM in the aggregation process. Different studies[28-30] point out that higher clay contents favor the formation of stable aggregates, whereas sandy soils hamper the physical protection of SOM and, consequently, the formation of aggregates. As clay amount values (Table 1) are relatively low for both soil samples, when compared to sand and silt amounts, there is a greater possibility of particles disaggregation, especially in the semi-arid soil, within the surface limits from which samples were collected.

Characteristics of the different fractions of SOM

The determination of TOC has been used to quantitatively estimate the organic fraction of the soil since the SOM contains about 58% of C, which helps to understand its chemical, physical and biological properties.[19]

Based on the *t*-test, the TOC amounts differ significantly among the samples, depending on the extractant employed and on the kind of soil, with a significance level of 5%. As expected the fractions extracted with NaOH showed higher amounts of TOC (Table 2). This extraction is indicated when a higher yield of organic material is needed. The differences observed are related to the extractants' mechanism of action: $NaNO_3$ reacts more mildly with the soil and with its organic matter, as in the simulated rain, which is only capable of leaching the more soluble organic contents from the soils. The TOC values obtained from our extraction with $NaNO_3$ corroborated by works of Chito *et al.*[11] and Ponthieu *et al.*,[12] in which $CaCl_2$ and KNO_3 were used for extraction of metals, finding out that the amounts of organic carbon in different soil and depth extracts varied between 17.8 and 21.4 mg L^{-1} and 23 and 101 mg L^{-1}, respectively.

To obtain information on the chemical structures present in the fractions, UV-Vis analysis was carried out in the samples and the ratios between absorbances at certain wavelengths were investigated.[17] The E_{465}/E_{665} ratio has been related to the condensation degree of aromatic constituents, where a ratio lower than 4 indicates the presence of condensed ring aromatic hydrocarbons, whereas a ratio higher than 4 corresponds to a high proportion of aliphatic structures.[31] The E_{250}/E_{365} ratio is inversely proportional to the molecular aggregation, while the E_{270}/E_{407} ratio is directly proportional to the amount of lignin derivatives with the highest contribution of porphyrin structures.[30] As lignin is a precursor for humic substances formation, the latter parameter may be applied as indicative of the material's humification.[32]

Another way of characterizing the organic material is through fluorescence measurements since those materials have fluorophoric groups, which might be related to the material's stability. In this context, molecular fluorescence has been used as indicative of the humification degree when characterizing soil organic matter.[20,21]

From the fluorescence intensities, it was possible to calculate the humification index, according to the proposal by Milori *et al.*,[22] which takes into account the total area of the emission spectrum obtained with excitation at 465 nm (A_{465}). A higher value of A_{465} indicates a great humification index of the material. The humification index proposed by Zsolnay *et al.*[21] consists of the ratio between the area of the last emission quarter (from 570 to 641 nm) and the area

Table 2. Total organic carbon (TOC) amounts, determined by catalytic combustion of the fractions: SHS, HHS, SFA, HFA, SHA, HHA (extracted with 0.1 mol L^{-1} NaOH), SSS, HSS (extracted with 0.1 mol L^{-1} $NaNO_3$) and SLS, HLS (obtained by simulated rain)

	NaOH						$NaNO_3$		Simulated rain	
	SHS	HHS	SFA	HFA	SHA	HHA	SSS	HSS	SLS	HLS
TOC / (mg L^{-1})	246.9 ± 6.2	438 ± 17	172.9 ± 2.9	291.9 ± 2.5	803.8 ± 1.0	1177.0 ± 1.0	19.03 ± 0.88	22.70 ± 0.03	9.02 ± 0.08	34.91 ± 0.09

TOC limit of detection: 0.1 mg L^{-1}. SHS: semi-arid humic substances; HHS: humid humic substances; SFA: semi-arid fulvic acids; HFA: humid fulvic acids; SHA: semi-arid humic acids; HHA: humid humic acids; SSS: semi-arid soil solution; HSS: humid soil solution; SLS: semi-arid leaching solution; HLS: humid leaching solution.

of the first emission quarter (from 356 to 432 nm), called A_4/A_1 (Table 3).

The E_{465}/E_{665} ratios for the SHS, HHS, SHA and HHA fractions were higher than 4 (Table 3), indicating a lower degree of aromatic condensation in the order: SHS < SHA < HHS < HHA. The other fractions presented zero absorbance at wavelengths above 518 nm, making it impossible to calculate this parameter.

Sanches *et al.*[33] carried out a study on humic substances' molecular size and concluded that, the smaller the molecular size, the higher the E_{465}/E_{665} ratio. Thus, a possible explanation for the high values of this parameter for SHS, HHS, SHA and HHA, as well as the zero absorbance of the SFA, HFA, SSS, HSS, SLS and HLS fractions at higher wavelengths, could be a smaller molecular size of the structures, consequently leading to a lesser aromatic condensation.

The E_{250}/E_{365} ratio is, in turn, inversely proportional to the molecular size. The rain leached fractions (SLS and HLS) values are quite similar to the fulvic acid fractions extracted by NaOH (SFA and HFA). As observed in Table 3, the HSS and SSS fractions presented higher values, followed by SFA, HLS, HFA and SLS fractions. The SHS, HHS, SHA and HHA fractions presented relatively low values, indicating larger molecular sizes of the structures composing these fractions, corroborating the results of the E_{465}/E_{665} ratio. The presence of lignin-derived structures, including porphyrin structures, also follows the same order of molecular size.

At 465 nm, fluorophores from more complex structures are excited, whose concentrations increase during the humification process; consequently, the area under the emission curve is proportional to the humification degree.[22] Thus, the increasing order of humification, based on the emission, with excitation at 465 nm, for the fractions were: SFA < SLS ≅ HFA < SSS ≅ HSS < HLS < SHS < HHS < HHA < SHA.

Again the values for the rain leached fraction are quite similar to the NaOH fulvic material, and in this case to the extracted by $NaNO_3$ (values between 4.6 and 7.2) while the other humic acids from NaOH show significantly higher values (larger than 20).

At 240 nm, fluorophores from simpler structures are excited, but since the ratio A_4/A_1 stands for the quotient between the area referring to more complex structures and that corresponding to simpler structures, it is directly proportional to the humification index. Therefore, the higher the A_4/A_1 ratio, the higher the humification degree.[21] Thus, fractions extracted with NaOH from the humid climate soil presented a higher humification index when compared to the samples from the semi-arid climate soil, while for saline extractions and leaching through simulated rain, an opposite behavior was observed.

The humic acids presented higher values for the A_4/A_1 ratio, therefore, they are the most humified, whereas the fulvic acids, which showed the lowest values are the least humified. The ascending order of the humification degrees for all fractions is: SFA < HFA < HSS < HLS < SLS < SSS < HHS < SHS < SHA < HHA.

Humic acid fractions (HHA and SHA) had the highest proportions of carboxylic and phenolic groups, whereas lowest proportions were found in fractions SSS, HSS, SLS

Table 3. Parameters values: E_{465}/E_{665}, E_{250}/E_{365} and E_{270}/E_{407}, A_{465} and A_4/A_1, carboxylic (Q_1) and phenolic (Q_2) groups concentrations in different fractions of SOM

Extractant	Fraction	E_{465}/E_{665}	E_{250}/E_{365}	E_{270}/E_{407}	A_{465} (× 10^3)	A_4/A_1	Q_1 / (mmol L^{-1})	Q_2 / (mmol L^{-1})
NaOH	SHS	9.60	3.28	5.50	12.9	0.123	7.76	6.80
	HHS	6.50	3.14	4.72	14.2	0.0973	10.7	12.3
	SFA	nd	5.89	10.4	4.64	0.0445	5.74	6.80
	HFA	nd	5.27	8.82	5.38	0.0579	9.89	15.3
	SHA	7.70	2.88	4.01	25.4	0.285	110	76.4
	HHA	5.40	2.16	2.99	22.3	0.325	117	115
$NaNO_3$	SSS	nd	10.5	8.76	6.49	0.080	1.10	0.636
	HSS	nd	11.3	10.1	6.58	0.0655	1.17	1.02
Simulated rain	SLS	nd	5.03	6.22	5.29	0.0779	1.17	1.53
	HLS	nd	5.59	8.75	7.19	0.0710	1.62	2.93

E_{465}/E_{665}: condensation degree of aromatic constituents; E_{250}/E_{365}: molecular aggregation; E_{270}/E_{407}: amount of lignin derivatives; A_{465}: total area of the emission spectrum obtained with excitation at 465 nm; A_4/A_1: ratio between the area of the last emission quarter (from 570 to 641 nm) and the area of the first emission quarter (from 356 to 432 nm); SHS: semi-arid humic substances; HHS: humid humic substances; SFA: semi-arid fulvic acids; HFA: humid fulvic acids; SHA: semi-arid humic acids; HHA: humid humic acids; SSS: semi-arid soil solution; HSS: humid soil solution; SLS: semi-arid leaching solution; HLS: humid leaching solution; nd: not detected.

and HLS; fulvic acid (SFA and HFA) and humic substances (SHS and HHS) fractions presented intermediate proportions of those groups. A possible explanation for the higher levels of Q_1 and Q_2 in alkaline extraction fractions might be the stronger interaction between extractant and SOM, since the predominant events occurring during the extraction with NaOH are: (*i*) rupture of hydrogen bonds among organic molecules and in the mineral's surface and (*ii*) binder exchange reactions, involving hydroxyls from the medium and carboxylic and phenolic groups from SOM molecules, through which they are coordinated on mineral's hydroxylated surface.[34]

Chemometric analysis

HCA revealed clusters in a hierarchical structure, in which samples are grouped and sub-grouped, depending on their similarities. The dendrogram, illustrated in Figure 1, shows the grouping of the SOM fractions studied, according to their chemical similarities.

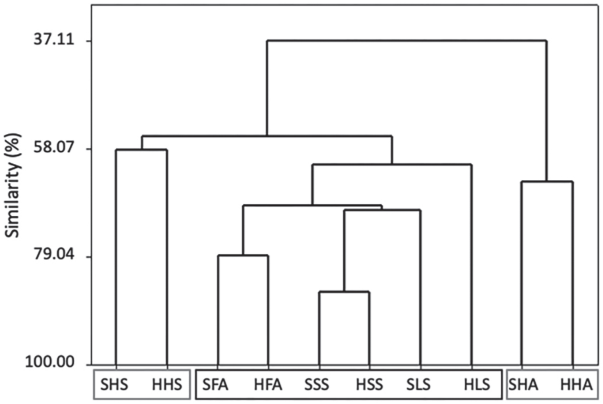

Figure 1. Dendrogram from hierarchical cluster analysis (HCA) for the SOM fractions studied, based on the parameters: E_{465}/E_{665}, E_{207}/E_{470}, E_{250}/E_{365}, A_4/A_1, A_{465}, Q_1, Q_2 and metal species.

In Figure 1 it is possible to observe the formation of a group relating the characteristics of SHS and HHS with a similarity of 61.12% (A). These fractions were a result of the same type of extraction and not submitted to any purification treatment, which justifies their similarity. A second group (B) joins the fractions SFA, HFA, SLS, HLS, SSS and HSS with 69.66% of similarity. The third group (C) gathers, with a similarity of 78.91%, SHA and HHA, which are fractional samples from their respective humic substances (HS) submitted to purification, leading to so different characteristics when compared with the other fractions.

The similarity between leachate solutions (SLS and HLS) and fulvic acid fractions (SFA and HFA) corroborated by the results of Schaefer *et al.*,[29] which evaluated the loss of organic matter and other parameters through simulated rain. In that work, a material resulting from soil erosion was submitted to chemical fractionation, as proposed by the IHSS,[9] revealing a selective loss of more soluble forms (fulvic acids) through the simulated rain.

Ren *et al.*[35] used a soil column-Donnan membrane technique (SC-DMT) and 2 mmol L^{-1} $Ca(NO_3)_2$, to obtain soil solutions. Their results showed that, for the 26.2% of organic matter in soil solution, the majority corresponded to fulvic acids. According to López *et al.*[36] and Cooke *et al.*,[37] fulvic acids are the mobile fraction of the organic matter, rather found in the dissolved form in aquatic environments, which justifies the similarity of the leachate (SLS and HLS) and soil (SSS and HSS) solutions with the fulvic acid fractions (SFA and HFA) studied in this work.

Furthermore, it may be observed that the HS' group (A) and the other fractions' group (B), with exception of HA (C), present relation of 38.54%, whereas this new group does not present similarity with the HA's group. It may be justified by the fact that HAs were purified using an HCl/HF treatment, which might lead to structural alterations of precursor compounds.[38]

The PCA revealed differences in structural characteristics of the fractions (Figures 2a and 2b). In Figure 2a, three different groups containing the 10 SOM fractions studied are identified. Such distribution is explained by 75.3% of total data variance, which is the sum of the two principal components (PC1 61% and PC2 14.3%). This result corroborates the clustering previously seen in HCA, thus confirming that humic acids fractions (SHA and HHA) presented higher amounts of condensed aromatic structures, larger molecular size and few lignin derivatives, therefore, being more humified, with higher amounts of carboxylic and phenolic groups.

The fractions SFA, HFA, SSS, HSS, SLS and HLS have lower quantities of condensed aromatic structures, smaller molecular size and higher amount of lignin derivatives, thus being less humified and with lower amounts of phenolic and carboxylic groups concerning the humic acid fractions. The humic substances from each soil (SHS and HHS), therefore, present intermediate characteristics.

Comparing the principal component analysis of samples and chemical parameters (Figures 2a and 2b), it is possible to notice that the distinction of the groups formed by the fractions: SFA, HFA, SSS, HSS, SLS, HLS is strongly influenced by E_2/E_4 and E_2/E_3 and, to a lesser extent, by the Ca and Mg contents. The groups formed by fractions: SHS, HHS and SHA, HHA were influenced by Zn, Mn and Al, and Cu, Fe and Cd, respectively. The fractions HA and HS presented higher affinity for potentially toxic metals, corroborating results of complexation capacity from several

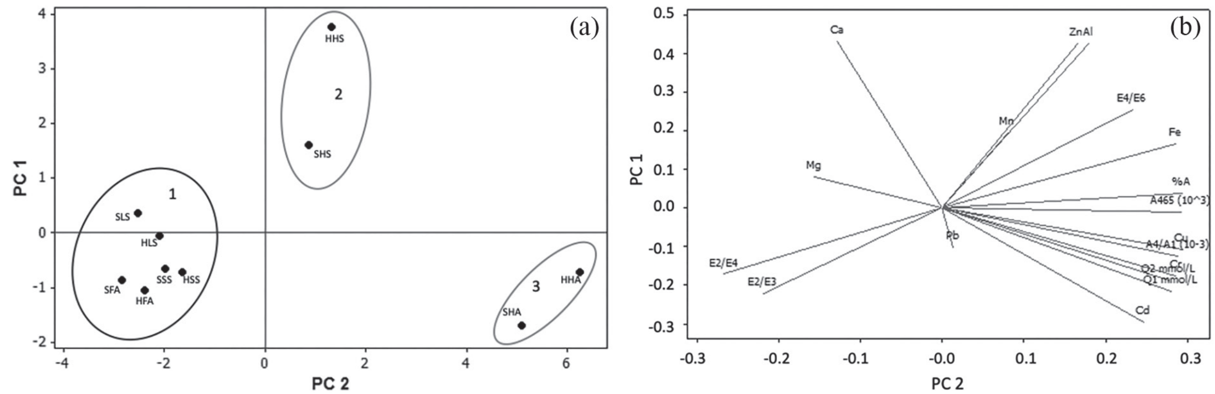

Figure 2. Principal component analysis (PCA) for samples (a) and parameters (b) from the chemical characterization of different SOM and contribution of the parameters: E_{465}/E_{665}, E_{270}/E_{407}, E_{250}/E_{365}, A_4/A_1, A_{465}, Q_1, Q_2 and metal species to groups' distributions along PC1 *versus* PC2.

authors,[17] who also observed such a higher affinity for potentially toxic metals, when compared to macronutrients, evidencing the selectivity of those fractions.

Thus, the type of extraction influences, not only the fraction of organic matter obtained, with different structural characteristics, but also the metal species availability, through the fractions selectivity, independently of the soil.

Despite the controversy regarding the studies[38] of humic substances and their representativeness, the results show the similarity of the organic matter leached with fulvic acids and reinforce the need to study these fractions to better understand their role in the availability of nutrients and contaminants in different environmental compartments.

Conclusions

The organic matter leached by simulated rain from two soils was compared with one extracted by both 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} $NaNO_3$.

The extractions yielded five fractions from each soil, with different TOC values and structural characteristics, based on analysis by UV-Vis, molecular fluorescence and acid-base volumetry. Chemometric analysis evaluated the similarity of the fractions leading to the formation of three groups. The first group is composed of humic acids with higher aromatic condensation, larger molecular size and higher content of carboxylic and phenolic groups, hence the most humified. A second and larger group combines the rain leachates, the soils solutions and the fulvic acids, showing lower aromatic condensation, smaller molecular size and lower contents of carboxylic and phenolic groups, hence the least humified. Moreover, they did not present similarity with the humic acids, which may be indicative of structural alterations of the source material during the extraction, especially in steps related to humic acid purification. The third group consists on the humic substances fractions that as expected showed intermediate characteristics being a mixture of both humic and fulvic acids.

Based on the aforementioned, it is clear that the rain leached organic matter is predominantly similar to the fulvic fraction of the SOM. Such a result is important from the environmental point of view, since it allows a better understanding of the rain leaching processes of organic matter in soils and thus leads to more realistic results, regarding the most active fraction of SOM and its role in the bioavailability of different species to the environment, helping in both risk assessment and molecular modeling.

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Author Contributions

Maria C. Tavares was responsible for the conceptualization, data curation, formal analysis, funding acquisition, investigation, writing original draft and review; Luciana C. Oliveira for the conceptualization, investigation, project administration, resources, software, validation, visualization and writing original draft; José P. Pinheiro for the conceptualization, data curation, resources, software, writing original draft, review and editing; Alexandre D. M. Cavagis for the software, validation, visualization, writing original draft, review and editing; Andrea P. Fernandes for the software, validation, visualization, writing original draft, review and editing; Vinicius Del Colle for the conceptualization, data curation, resources, software, writing original draft, review and editing; Wander G. Botero for the conceptualization, investigation, project administration, resources, software, validation, visualization, writing original draft, review and editing.

References

1. Stevenson, F. J.; *Humus Chemistry: Genesis, Composition, Reaction*, 1st ed.; Wiley Interscience: New York, USA, 1994.
2. Lehmann, J.; Kleber, M.; *Nature* **2015**, *528*, 60.
3. Lal, R.; *Science* **2004**, *304*, 1623.
4. Enya, O.; Heaney, N.; Iniama, G.; Lin, C.; *Sci. Total Environ.* **2020**, *724*, 138223.
5. Thurman, E. M.; Malcolm, R. L.; *Environ. Sci. Technol.* **1981**, *15*, 463.
6. Tipping, E.; *Cation Binding by Humic Substances*, 1st ed.; Cambridge University Press: Cambridge, 2012.
7. Swift, R. S. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Sparks, D. L.; Page, A. L.; Helmke, P. A.; Loeppert, R. H., eds.; Soil Science Society of America, Inc., American Society of Agronomy, Inc.: Madison, 1996, p. 1018-1020.
8. Hayes, M. H. B.; *Soil Sci. Soc. Am. J.* **2006**, *70*, 986.
9. *Isolation of IHSS Samples*; available at http://humic-substances.org/isolation-of-ihss-samples/, accessed in October 2020.
10. Botero, W. G.; Pineau, M.; Janot, N.; Domingos, R. F.; Mariano, J.; Rocha, L. S.; *Environ. Chem.* **2017**, *14*, 417.
11. Chito, D.; Weng, L.; Galceran, J.; Companys, E.; Puy, J.; Riemsdijk, W. H. V.; Leeuwen, H. P. V.; *Sci. Total Environ.* **2012**, *238*, 421.
12. Ponthieu, M.; Pourret, O.; Marin, B.; Schneider, A.; Morvan, X.; Conreux, A.; Cancès, B.; *J. Geochem. Explor.* **2016**, *165*, 1.
13. Milne, C.; Kinniburgh, D.; Riemsdijk, W.; Tipping, E.; *Environ. Sci. Technol.* **2003**, *37*, 958.
14. *Visual MINTEQ ver. 3.1*; available at http://vminteq.lwr.kth.se, accessed in October 2020.
15. *Description of Input and Examples for PHREEQC Version 3-A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations*; available at http://pubs.usgs.gov/tm/06/a43/, accessed in October 2020.
16. Meeussen, C. L.; *Environ. Sci. Technol.* **2003**, *37*, 1175.
17. Botero, W. G.; Oliveira, L. C.; Rocha, J. C.; Rosa, A. H.; Santos, A.; *J. Hazard. Mater.* **2010**, *177*, 307.
18. Town, R. M.; Kipton, H.; Powell, J.; *Anal. Chim. Acta* **1992**, *256*, 81.
19. de Oliveira, L. C.; Botero, W. G.; Santos, F. A.; Sargentini Jr., E.; Rocha, J. C.; dos Santos, A.; *J. Braz. Chem. Soc.* **2012**, *23*, 1711.
20. dos Santos, C. H.; Romano, R. A.; Nicolodelli, G.; Carvalho, C. M.; Villas-Boas, P. R.; Martin-Neto, L.; Montes, C. R.; Melfi, A. J.; Milori, D. M. B. P.; *J. Braz. Chem. Soc.* **2015**, *26*, 775.
21. Zsolnay, A.; Baigar, E.; Jimenez, M.; Steinweg, B.; Saccomandi, F.; *Chemosphere* **1999**, *38*, 45.
22. Milori, D. M. B. P.; Martin-Neto, L.; Bayer, C.; Mielniczuk, J.; Bagnato, V. S.; *Soil Sci.* **2002**, *167*, 739.
23. Rodríguez, F. J.; Schlenger, P.; García-Valverde, M.; *Sci. Total Environ.* **2014**, *476*, 718.
24. Ritchie, J. D.; Perdue, M. E.; *Geochim. Cosmochim. Acta* **2003**, *67*, 85.
25. *Minitab*; available at https://www.minitab.com, accessed in October 2020.
26. Schoenholtz, S. H.; Van Miegroet, H.; Burger, J. A.; *For. Ecol. Manage.* **2000**, *138*, 335.
27. Manahan, S. E.; *Introduction to Chemistry - Fundamentals of Environmental Chemistry*; CRC Press LLC: Boca Raton, 2001.
28. Rosa, A. H.; Pinheiro, J. P.; Monteiro, A. S. C.; Janot, N.; Groenenberg, B. J.; *Quim. Nova* **2017**, *40*, 1191.
29. Schaefer, C. E. R.; Silva, D. D.; Paiva, K. W. N.; Pruski, F. F.; Albuquerque Filho, M. R.; Albuquerque, M. A.; *Pesqui. Agropecu. Bras.* **2002**, *37*, 669.
30. Beach, T. A.; Kinabalu, K.; Effendy, K.; Natin, P.; *Trans. Sci. Technol.* **2016**, *3*, 209.
31. Rosa, A. H.; Rocha, J. C.; Furlan, M.; *Quim. Nova* **2000**, *23*, 472.
32. Santos, O. S.; Lourenço, R.; Sato, A.; Oliveira, F.; Santos, H.; Lourenço, S.; Oliveira, A. F.; *Rev. Tecnol.* **2008**, *17*, 1517.
33. Sanches, S. M.; Campos, S. X.; Vieira, E. M.; *Ecletica Quim.* **2007**, *32*, 49.
34. Dick, D. P.; Gomes, J.; Rosinha, P. B.; *Rev. Bras. Cienc. Solo* **1998**, *22*, 603.
35. Ren, Z. L.; Tella, M.; Bravin, M. N.; Comans, R. N. J.; Dai, J.; Garnier, J. M.; Sivry, Y.; Doelsch, E.; Straathof, A.; Benedetti, M. F.; *Chem. Geol.* **2015**, *398*, 61.
36. López, R.; Gondar, D.; Antelo, J.; Fiol, S.; Florencio, A.; *Geoderma* **2011**, *164*, 249.
37. Cooke, S.; Peacock, M.; Evans, C. D.; Page, S. E.; Whelan, M. J.; Gauci, V.; Kho, L. K.; *Water Res.* **2017**, *115*, 229.
38. Hayes, M. H. B.; Swift, R. S.; *Adv. Agron.* **2020**, *163*, 1.

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