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RESEARCH ARTICLE

Characterization of humic acids extracted from biosolid amended soils

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Abstract

The aim of this study was to evaluate the changes produced in the chemical, acid-base and structural properties of soil amended (biosolids) and humic acids HAs incubated at different times.

In the current work the amendments were conducted with biosolids at different doses (30, 90 and 180 t ha^{-1}) on Colina (CLN) soil for 1, 2, 4 and 6 months.

The acid-base characterization of extracted humic acids (HAs) showed a slight impact on Atotal (total acidity, mEq g^{-1}), at 30 and 90 t ha^{-1} while at 180 t ha^{-1} the greatest increase was observed from 10.5±1.61 (control) to 17.9±0.30 (6 months of incubation). without evolution over the incubation time. The Aphenolic (phenolic acidity, mEq g^{-1}) acidity behaves similarly while Acarboxylic (carboxylic acidity mEq g^{-1})) remains constant over time and applied doses. The application of ^{13}C solid state NMR technique revealed higher abundance of aromatic C (CLN 43.5%) over alkyl C (21.4%) compounds. Aromatic C presents a decrease at high biosolid doses, which occurs simultaneously with alkyl C increase when using the same surveyed doses. Therefore, it might be considered that through the addition of biosolids, components such as fatty acids, amino acids or paraffinic structures are incorporated. As a result of biosolids addition, carboxylic C content remains steady, which agrees with the Accarboxylic behavior previously determined.

keywords: Humic acids, soils, biosolids, Solid state, CPMAS 13CNMR

1. Introduction

Soil organic matter (OM) levels depend on the interaction between input and decomposition rates, which are controlled by factors such as climate, soil properties, drainage, and land use. In situ stabilisation and destabilisation mechanisms slow down or accelerate turnover rates (Parfitt *et al*., 1999). Organic matter content is related to several soil properties such as complexation, macro- and micronutrient reservoirs, cation exchange capacity, microorganism population, chemical and physical properties, and OM interaction with components such as N, P, and S.

The OM content of Chilean soils ranges from 4 to 36% in surface horizons (Galindo *et al.*,1992). Soil properties such as active/free iron oxide ratio, isoelectric point (IEP), cation exchange capacity (CEC), and potassium–calcium cation exchange equilibrium have been related to OM content in Chilean volcanic soils (Escudey *et al.*, 2004).

On the other hand, the application of biosolids on land has been reported to have positive effects on fertility, crop yields (Andrés and Jorba 2000), and changes in chemical, physical, biological, and microbial properties (Selivanovskaya and Latypova 2006; Vaca-Paulín *et al.*, 2006). In this context, the literature indicates that the addition of sludge provokes changes of the soil chemical characteristics, due to soil-sludge interaction, including pH, electrical conductivity, OM (%), P-Olsen and cation exchange capacity (CEC) (Singh *et al.*, 2008, Adewole and Ilesanmi 2011, Yu *et al.*, 2013). Also, background exists about substantial improvements in physical properties such as apparent density, aggregates stability, micro- and macro-pores increase, as well as significant improvement in erosion resistance (Singh *et al.*, 2008; Kladivko and Nelson 1979). These variations depend on factors such as the type of biosolid and soil and dose used (Singh *et al.*, 2008).

Considering that these amendments bring about a strong OM addition, several studies have evaluated the changes in acid-base and structural properties of HA extracted from soils that have been subjected to sludge addition and incubation, which depends on both the added dose and the time elapsed since exogenous OM incorporation took place. In literature, (Campitelli *et al.*, 2006) was reported that HAs extracted from sludge have a structure where aliphatic components and low development of acidic functional groups prevail and that soil amendment slightly modifies the properties of native HAs. On the other hand, in previous reports (Sánchez-Monedero *et al.*, 2002), has showed that incubation of organic waste produced changes in HAs increasing the acid functional groups. Moreover, amendments carried out for a longer incubation period of one year (Garcia-Gil *et al.*, 2004a) showed increase in the degree of condensation and both decrease and increase (Pedra *et al.*, 2008) of acidic functional groups. Therefore, again the heterogeneity of the material involved precludes extrapolating results from soil and sludge mixtures of different origins, and it is necessary to assess at local level the potential action of the biosolids on the characteristics of native soil OM through humic acids studies. The present work was thus conducted to investigate the characteristics of humic acids coming from soils and the effect produced by biosolid addition, associated to sludge dose and incubation, on a Mollisol soil.

2. Materials and Methods

2.1. Soil and biosolid description

Soil samples were collected from 0 to 0.15 m depth from uncultivated areas of Colina (Mollisol) in a central region of Chile. All samples were air-dried and sifted through 2 mm. Biosolids were obtained from a domestic wastewater treatment plant with primary treatment near the city of Santiago, in central Chile. A detailed description and characterization of all samples is presented in Table 1.

2.2. Soil pots procedure

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Colina soil subjected to the different rates treatments were packed into pots with the same bulk density determined for undisturbed soil, after adding the corresponding amounts of biosolids. The total mass of undisturbed soil needed to fill the pot was weighed and the same amount of sifted soil+biosolids was packed and wetted to 50% of its water-holding capacity. The wetted soils were incubated at room temperature (25±3ºC) and weighed weekly. Double-distilled water was added to bring the soils up to 50% of their water-holding capacity (Klute 1965). The biosolids dosage applied on Mollisol soil, were equivalent to amendments of 30, 90 and 180 t ha^{-1}, in accordance with the Regulations for the Management of Non-Hazardous Sludges Generated in wastewater treatment plant with primary treatment (Conama, 2000). The incubated pots were sampled after 1, 2, 4 and 6 months. Control soils with no incubation time were also analyzed.

All experiments were performed out by triplicate.

2.3. Extraction Procedure

Briefly, the solid sample was equilibrated with 1 mol L^{-1} HCl, obtaining a suspension whose pH lies between 1 and 2 at room temperature. The volume that has a ratio of 10 mL liquid/1 g dry sample, was then adjusted with 0.1 mol L^{-1} HCl. The suspension was centrifuged to separate the supernatant (FA-1) from the sediment (R1). R1 was neutralized (pH = 7.0) with a 1 mol L^{-1} NaOH solution, and then a volume of 0.1 mol L^{-1} NaOH was added under N_2 atmosphere to give a final extractant to soil ratio of 10:1. Extract the suspension was under N_2 with intermittent stirring for a minimum of 4 h.

The alkaline suspension was left overnight and then extracted by centrifugation. Subsequently, the supernatant was acidified with 6 mol L^{-1} HCl under constant stirring to get pH 1.0 and then allowed standing for 12-16 h. Centrifuge to separate precipitated HA fractions and FA (FA-2) supernatant. The HA precipitate was re-dissolved in a minimum volume of 0.1 mol L^{-1} KOH under N_2. Solid KCl was then added to attain a 0.3 mol L^{-1} K^+ concentration and then centrifuged to remove the suspended solid. HA was re-precipitated by adding 6 mol L^{-1} HCl under constant stirring at pH 1.0. The suspension was allowed to stand another 12 to 16 h. Centrifuge and disregard the supernatant. The precipitated HA was suspended into a 0.1 mol L^{-1} HCl and 0.3 mol L^{-1} HF mixture and stirred overnight at room temperature. Centrifuge and, if necessary, repeat the acid treatment (HCl/HF), until the ash content be less than 1%. Transfer the precipitate to a dialysis tube (Visking, Co.) using distilled water until the dialysed water gives a negative test for chloride ($AgNO_3$). After liophylization, the dried HA was freezed. (de Nobili *et al.*, 1990; Watanabe and Kuwatsuka, 1991).

2.4. Chemical determinations

2.4.1. Soils and amended soils

The bulk density, organic carbon content, and exchangeable cations of the soils were determined by standard methods. The bulk density was determined by the average air dried weight of the soils in undisturbed soil cores of the 0-0.25 m soil profile in 0.05-m diameter and 0.05-m high brass rings, while the organic carbon content was determined by the Walkley-Black method, modified for Chilean volcanic soils (Sadzawka *et al*., 2004). The exchangeable cations were determined as the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} in ammonium acetate extract. The pH and electrical conductivity were measured in 1:2.5 (w/v) soil/water ratio suspensions. Available P forms were also determined by the Olsen P procedure adapted to Chilean volcanic soils with $NaHCO_3$ solution. In all the samples phosphorus in solution was determined by the sulfomolybdic acid method using ascorbic acid as the reducing agent (Sadzawka *et al*., 2004).

To determine the absorbance ratio, $Q_{2/4}$, $Q_{2/6}$ and $Q_{4/6}$, of OM extracts with NaOH from amended soils, 0.500 ± 0.001 g of sample was weighed into polypropylene centrifuge tubes and 25 mL of 0.5 M sodium hydroxide (NaOH) added. The mixture was stirred for two hours in an orbital-type shaker and then let stand overnight. After this time, the mixture was centrifuged for 25 minutes and the supernatant absorbance was simultaneously measured at 280, 472 and 664 nm on an Analytic Jena Specor 40 spectrophotometer using quartz cells. All determinations were performed out by triplicate.

2.4.2. Humic acids

The total acidity was determined by acid base titration adding a measured excess of a barium hydroxide ($Ba(OH)_2$) solution under N_2 and back-titrating the unreacted $Ba(OH)_2$ with a standard hydrochloric acid (HCl) solution (Wright and Schnitzer 1959). On the other hand, the carboxylic acid was quantified utilizing an excess of a calcium acetate ($Ca(CH_3COO)_2$) solution under N_2. The released acetic acid is titrated with a standard sodium hydroxide (NaOH) solution. Total and carboxylic acidity obtention enables the phenolic acidity to be worked out. All determinations were performed out by triplicate.

Solid-state NMR spectra were obtained using the variable amplitude cross polarization magic angle spinning (CPMAS) technique using a Bruker 600MHz Advance III wideboard with a ^{13}C resonance frecuency of 50.3 MHz. The samples were confined in a zirconium oxide rotor with an external diameter of 7 mm. Applying a contact time of 1 ms, between 5000 and 10,000 scans were accumulated using a spinning speed of 6.8 kHz and a pulse delay of 300 ms. The ^{13}C chemical shifts were referenced to external tetramethylsilane (=0 ppm) and adjusted with glycine (=176.04 ppm) as an external standard. For quantification, the spectra were subdivided into different chemical shift regions according to (Lüdemann *et al*., 1973): alkyl C (0–45 ppm), N-alkyl/methoxyl C (45–60 ppm), O-alkyl C (60–110 ppm), aromatic C (110–160 ppm), carboxyl/amide C (160–185 ppm) and carbonyl C (185–245 ppm). The relative ^{13}C intensity distribution was determined by integrating the signal intensity in different chemical shift regions with an integration routine supplied with the instrument software. Corrections concerning the intensity distribution of spinning side bands were made (Knicker *et al*., 2005). On the basis of these data, the ratio of O-alkyl C/alkyl C is calculated as indicator of the degree of OM decomposition and alteration (Knicker and Ludermann 2005).

2.4.3. Statistics

Statistics applied to the obtained data was performed using Graphpad Prism 5.0 software

3. Results and Discussion

3.1. Chemical characterization

Colina soil is classified as Mollisol, characterized by its low percentage of OM (less than 5%), negative permanent charge, due to which no variable charge is observed by IEP and mineralogy dominated by montmorillonite type phyllosilicates (Antilén 2002). Its pH-H_2O is close to neutral and exhibits high CEC.

The absorbance ratios of alkaline extracts, $Q_{2/4}$, $Q_{2/6}$ and $Q_{4/6}$, allow, in a general manner, evaluating the conditions of the organic component in soils, as well as its potential transformation in humidified material. In the case of Colina soil, $Q_{2/6}$ ratio reveals a low content of highly humidified OM, with scarce presence of condensed and aromatic groups, but with a significant presence of lignins, tannins and quinones liable to be humidified. $Q_{2/4}$ and $Q_{4/6}$ values offer a similar interpretation, with plenty of material prone to be humidified and a smaller contribution of strongly humidified material.

On the other hand, the utilized biosolid exhibits high percentage of OM, neutral pH, high conductivity and low content of ethyl ammonium extractable cations. As for absorbance ratios, they enable establishing a low presence of strongly humidified material, with a $Q_{2/4}$ ratio denoting a more balanced proportionality between material liable to humidify and that in its early stages of humification.

3.2. Soil-biosolid pots studies

Figure 1 shows pH evolution over incubation time. A slight acidification is seen for the highest dose of incorporated biosolid.
This acidification process could be directly related to dissolution of soluble bases (Ca^{2+}, Mg^{2+}), with its subsequent leaching, comparatively similar to what occurs in the presence of a high rainfall situation. At lower biosolid doses, the pH remains close to neutrality.

Simultaneously, it is important to consider the decomposition of organic matter that entails the biosolid system, producing CO_2, acidifying the soil at the end of the experiment (de Melo *et al.*, 2002).

This may explain why incubation with doses of 180 t ha^{-1} (higher OM percentage) decreases the pH to “slightly acid.”

Table 1. Soil and biosolid characterization.

Characteristics	*Colina*	*Biosolids*
Soil order	Mollisol	Water treatment plant
Soil class	Mixed, thermic, Fluventic Haploxerolls	Solid waste
Sampling location		
Latitude	33°10´ S	33°10´ S
Longitude	70°45´ W	70°45 ´W
Altitude (m)	500-600	500-600
Rainfall (m year^{-1})	0.3	0.3-0.4
Mean annual Temperature (°C)	14.5	14.5
Bulk density (g mL^{-1})	1.3±0.0	n.d.
Electrical Conductivity(dS m^{-1})	0.1±0.0	1.9±0.1
Organic carbon (wt %)	2.2±0.2	18.0±0.5
pH (H_2O 1:2.5)	6.9±0.0	6.9±0.0
C/N analysis (%)	9.9	6.3
Exchangeable cations (cmol$_{(+)}$ kg^{-1})	42.1±0.7	24.9±1.3
Absorbance rates		
$Q_{2/4}$	9.3±0.2	1.6±0.1
$Q_{2/6}$	68.7±7.1	12.1±1.1
$Q_{4/6}$	7.4±0.7	7.4±0.3
Isoelectric point	n.d.	6.4±0.2
Mineralogical composition >50%	Montmorrillonite	n.d.

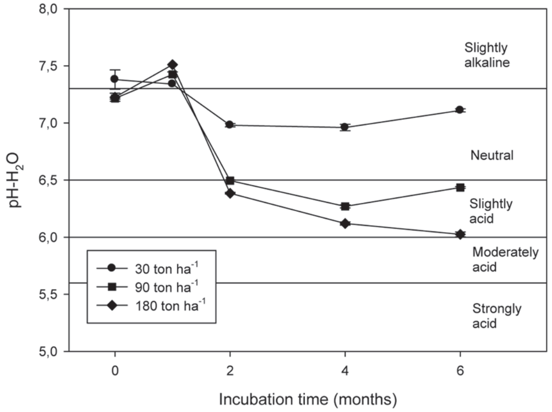


Figure 1. pH of biosolids-amended soils using different doses over incubation time.

On the other hand, the EC underwent a steady increase over time, from normal to high values in relation to the dose of biosolid used, as depicted in Figure 2. The large number of ionic species coming from the biosolid would be responsible for the immediate EC increase, product of the soil-biosolids interaction during the incubation process.

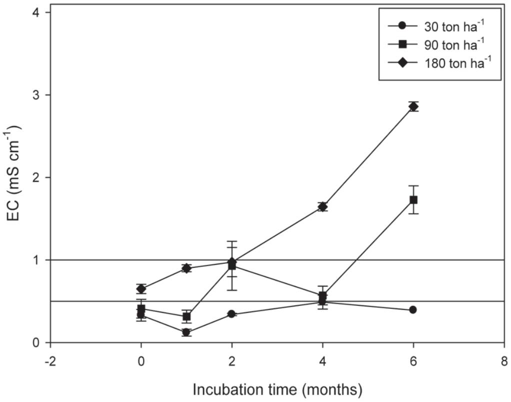


Figure 2. Electrical conductivity of amended soils (30, 90 180 ton ha^{-1}) in the course of incubation time.

Figure 3 shows the OM content in terms of incubation. The CLN soil exhibits a moderately low percentage of OM (2.99 ± 0.14%), reaching normal levels (5-10%) at the 180 t ha^{-1} dose. The observed OM content can be related to variations of the average incubation temperature (Connant *et al*., 2011), due to the generation of increased microbial respiration (Curiel *et al*., 2007), a phenomenon that might also occur in soils treated with this kind of organic wastes (Shimizu *et al*., 2009).

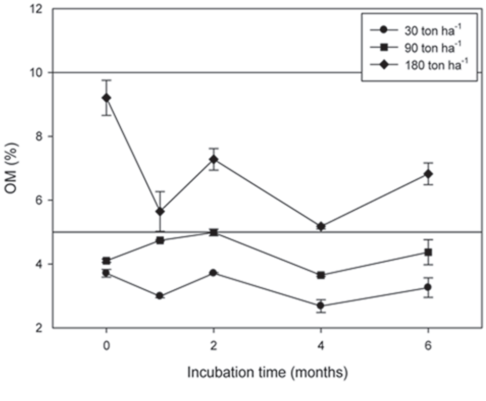


Figure 3. Organic matter (OM) content of amended soils (30, 90 180 ton ha^{-1}) during incubation time.

Figure 4 displays the effect of biosolid application on the content of ammonium acetate extractable cations, in which a moderate increase, biosolid dose-dependent, is observed. Since the presence of both inorganic components and OM are responsible for the CEC value, the increase is related to the organic component of the waste, as a result of the high number of acidic functional groups that are capable of interacting with soil cations (Kladivko and Nelson, 1979); on the other hand, the major mineralogical component contributed by the soil is montmorillonite (Besoain, 1985). From an agricultural standpoint, soils with high CEC tend to be more fertile, because the probability for nutrient losses by leaching is low. A larger storage and supply capacity of the cultures is thus obtained.

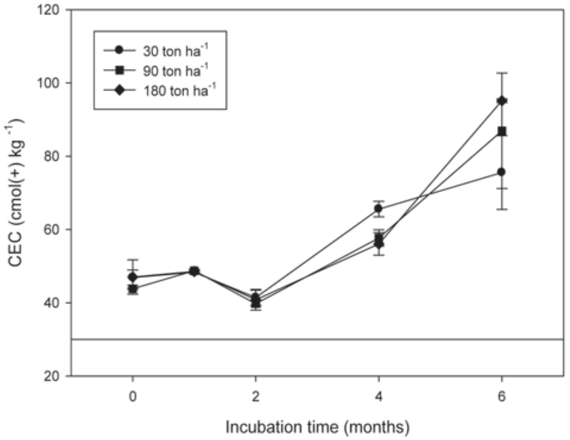


Figure 4. Cation exchange capacity CEC of amended soils (30, 90 180 ton ha^{-1}) over incubation time.

The absorbance ratios $Q_{2/4}$, $Q_{2/6}$ and $Q_{4/6}$ are useful parameters to assess the quality of organic matter in the soil, as it allows studying its degree of humification and the material that is in early stages of conversion to organic compounds of higher degree of condensation of the system.

Three important spectral ranges exist wherein exact absorbance is measured: about λ=280, 400–500 nm and above 600 nm. UV–Vis analysis of alkaline extracts usually relies on the assumption that absorbance at 260–280 nm is due to lignin and quinone moieties, i.e. the material at the very beginning of transformation. The absorbance at 460–480 nm reflects the organic matter at the humification onset and the absorbance at 600–670 nm is said to be indicative of strongly humidified material with a high degree of aromatic, condensed groups (Kononova, 1968; Gieguzynska *et al*., 1998). The following absorbance ratios can be then calculated: $Q_{2/6} = A_{280}/A_{664}$, $Q_{4/6} = A_{472}/A_{664}$, $Q_{2/4} = A_{280}/A_{472}$. Using these absorbance ratios, the degree of maturation (humification) of organic matter can be determined. The absorbance ratios ($Q_{2/6}$, $Q_{4/6}$, $Q_{2/4}$) of NaOH extracts reflect the degree of organic matter maturity in bulk compost (Sapek and Sapek, 1999). In this study the ratios tended to remain constant, as in the case of $Q_{2/4}$,in Figure 5A that represents the potentially humificable material with regard to that whose process is just starting, with values close to those of CLN soil. On the other hand, $Q_{2/6}$ and $Q_{4/6}$ ratios tend to increase over time of incubation, particularly with the highest dose of biosolid, as shown in Figure 5B and 5C, respectively. This trend could be interpreted as a relative increase in compounds with phenolic and benzene-carboxylic groups in the structure of the humic substances (Zbytniewski and Buszewski 2005). At the end of incubation the $Q_{4/6}$ values are greater than 5, which in the literature has been reported as a characteristic of mature humic acids. This fact suggests that differences exist between the added organic matter and the one already existing in the soil under survey.

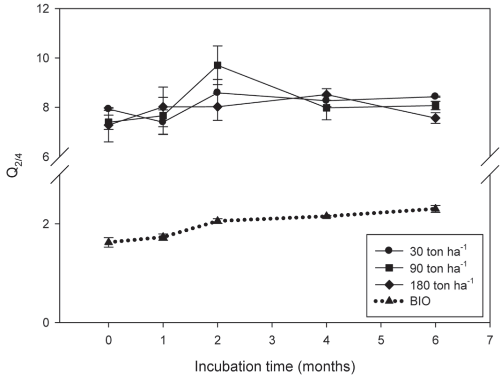


Figure 5 A. Absorbance ratio of 0.5 M NaOH solution for the amended soils and biosolids (BIO): Q2/4 (280/472 nm) through incubation time.

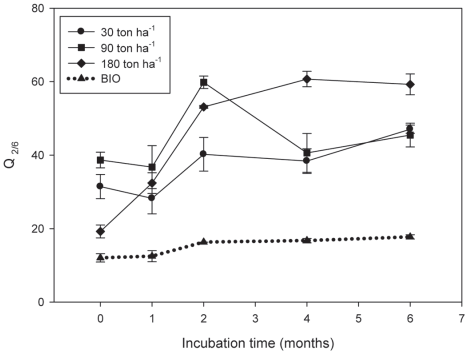


Figure 5B. Absorbance ratio of 0.5 M NaOH solution for the amended soils and biosolids (BIO): Q2/6 (280/664 nm) during incubation time.

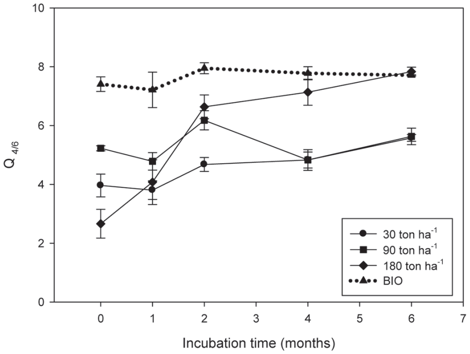


Figure 5C. Absorbance ratio of 0.5 M NaOH solution for the amended soils and biosolids (BIO): Q4/6 (472/664 nm) over incubation time.

Absorbance ratios $Q_{2/4}$, $Q_{2/6}$ and $Q_{4/6}$ show the dynamics of the organic matter and its transformation into humidified material. However, they are only a qualitative approach to the HA characteristics, considering they are the outcome of a single alkaline extraction.

Table 2. Acid-Base properties of amended soils.

Sample	A_{Total}	A_{COOH}	$A_{Phenolic}$
CLN	8.5 ± 1.09	3.4 ± 0.01	5.1 ± 1.01
CLN – B30			
0m	9.7 ± 0.1	2.0 ± 0.2	7.7 ± 0.0
1m	15.5 ± 0.4	2.7 ± 0.1	12.7 ± 0.2
2m	10.0 ± 1.3	3.2 ± 0.4	6.8 ± 1.7
4m	14.8 ± 2.4	2.6 ± 0.1	12.1 ± 2.2
6m	11.8 ± 0.5	2.5 ± 0.2	9.3 ± 0.3
CLN – B90			
0m	10.3 ± 1.3	2.1 ± 0.0	8.3 ± 1.2
1m	11.6 ± 1.1	2.7 ± 0.0	8.9 ± 1.1
2m	8.8 ± 1.6	2.4 ± 0.1	6.4 ± 1.5
4m	16.2 ± 0.1	2.1 ± 0.2	14.1 ± 0.1
6m	13.2 ± 0.2	2.0 ± 0.2	11.3 ± 0.5
CLN – B180			
0m	10.5 ± 1.6	2.5 ± 0.1	10.5 ± 0.3
1m	15.8 ± 0.8	2.9 ± 0.0	12.9 ± 0.8
2m	14.9 ± 1.3	3.0 ± 0.3	11.9 ± 1.6
4m	16.7 ± 0.8	2.4 ± 0.0	14.3 ± 0.8
6m	17.9 ± 0.3	2.5 ± 0.1	15.5 ± 0.1

3.3. Humic acids

The acid-base properties of humic acids extracted from amendments and sludge were evaluated. The results can be found in Table 2.

For amendments, in general an increase of Atotal and Aphenolic, proportional to the sludge dose, is observed at the end of the incubation process. However, an analysis regarding the incubation time indicates that biosolid addition in doses of 30 or 90 t ha^{-1} exhibits a maximum phenolic acidity increase of four months incubation; in spite of that, the addition of 180 t ha^{-1} causes this maximum to be reached in a shorter incubation time. On the other hand, A_{COOH} presents no significant changes as a function of biosolid load ($P = 0.1394$, two-way ANOVA test).

In previous studies, the increase of both Acarboxylic and Atotal has been explained by the occurrence of chemical reactions typical of the humification process (Garcia-Gil *et al*, 2004a; Garcia-Gil *et al*., 2004b; Senesi *et al*., 2007). Thus, oxidation of methoxy groups, and alcohols located on the side chains of lignins, as well as bacterial degradation of carbohydrates to quinones, ketones and carboxylic groups, would increase these parameters.

Hence, the results observed for the amendments would suggest that the humification processes are not yet stable to be reflected in changes of the humic fraction, and that the incubation time was less than required for the processing of the organic matter present in the biosolid.

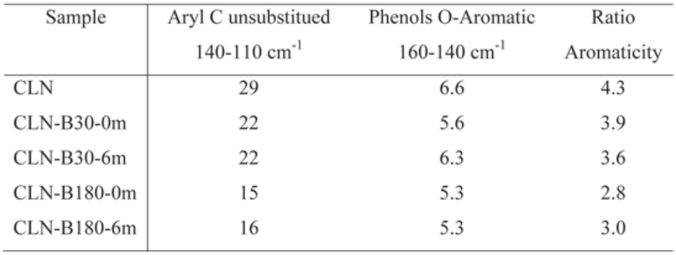
3.4. Solid state CPMAS ^{13}C NMR

The solid state NMR allows developing qualitative and quantitative assessment of the OM existing in soils and sediments, utilizing no previous extraction processes. Figure 6 shows HA spectra from CLN soil samples, CLN with biosolids at 30 and 180 t ha^{-1} incubated during 0 and 6 months. In general, these spectra illustrate the high heterogeneity that HAs, originated from the OM present in soils, possess. Furthermore, due to the definition of regions that exhibit a specific chemical assignment, quantification is possible. Table 3A shows the areas associated with each zone. For CLN soil the largest abundance appears between 160-110 cm^{-1}, corresponding to the C sp^2 resonance, mainly ascribed to aryl C or olefin C. In addition, between 160-140 cm^{-1}, signals corresponding to aryl C or olefins C substituted by O, N or C are seen, whereas between 140-110 ppm, unsubstituted aryl C exists. No incubation time effect was observed for both doses. In Table 3B, the aryl C region is 4 times larger than phenols O-Aromatic groups for CLN soil, suggesting that each phenol would be attached to 4 different alkanes. Besides, a significant aromaticity ratio decrease, from 4.0 to 3.0, at 180 t ha^{-1} dose takes place. This decrease is observed simultaneously with C alkyl increase, in a higher doses increase, 180 t ha^{-1}. This increase would suggest that the addition of biosolids would incorporate components associated to C alkyl such as fatty acids, amino acids and/or paraffin structures. Therefore, the O/N alkyl abundance observed between 110-45 cm^{-1} usually presents a slight increase in the presence of the largest dose of biosolid. This region exhibits carbohydrates, alcohols and ethers signals, while in the more specific 60 to 45 ppm region N-alkyl C or methoxyl C show up. From Table 3C, the organic fraction (HAs) humification state is obtained, wherein the greater the value, the lesser the degradation, pointing to the existence of less carbohydrates as a result of the large content of microbes (Baldock *et al*., 1989). Hence, the lipids ratio increases as the biosolid dose does, in which each carboxylic acid group would be attached to other three different alkanes. Finally, with regard to the presence of carboxyl C, no significant differences were observed that evidenced an impact of the biosolids or the incubation process.

Table 3A. Relative intensity distribution (%) in the solid state ^{13}C NMR spectra.

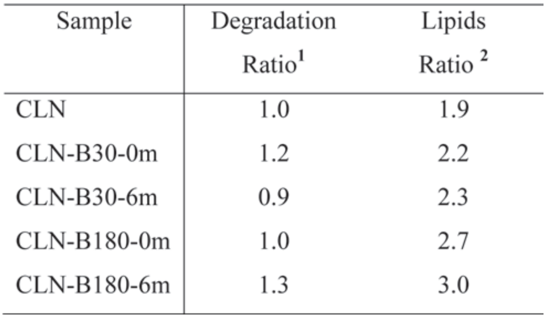
Sample	Carboxyl 220-160 cm^{-1}	Aromatic 160-110 cm^{-1}	O/N Alkyl 110-45 cm^{-1}	Alkyl C 45-0 cm^{-1}
CLN	11	43	24	22
CLN-B30-0m	11	37	27	25
CLN-B30-6m	10	39	29	22
CLN-B180-0m	11	29	30	30
CLN-B180-6m	10	31	29	30

Table 3B. Relative intensity distribution (%) of aromatic compounds and aromaticity ratio.



Sample	Aryl C unsubstitued 140-110 cm^{-1}	Phenols O-Aromatic 160-140 cm^{-1}	Ratio Aromaticity
CLN	29	6.6	4.3
CLN-B30-0m	22	5.6	3.9
CLN-B30-6m	22	6.3	3.6
CLN-B180-0m	15	5.3	2.8
CLN-B180-6m	16	5.3	3.0

Table 3C. Degradation ratio and Lipid ratio.



Sample	Degradation Ratio[1]	Lipids Ratio [2]
CLN	1.0	1.9
CLN-B30-0m	1.2	2.2
CLN-B30-6m	0.9	2.3
CLN-B180-0m	1.0	2.7
CLN-B180-6m	1.3	3.0

[1] (O/N Alkyl/ Alkyl C) [2] (Alkyl C / Carboxyl C)

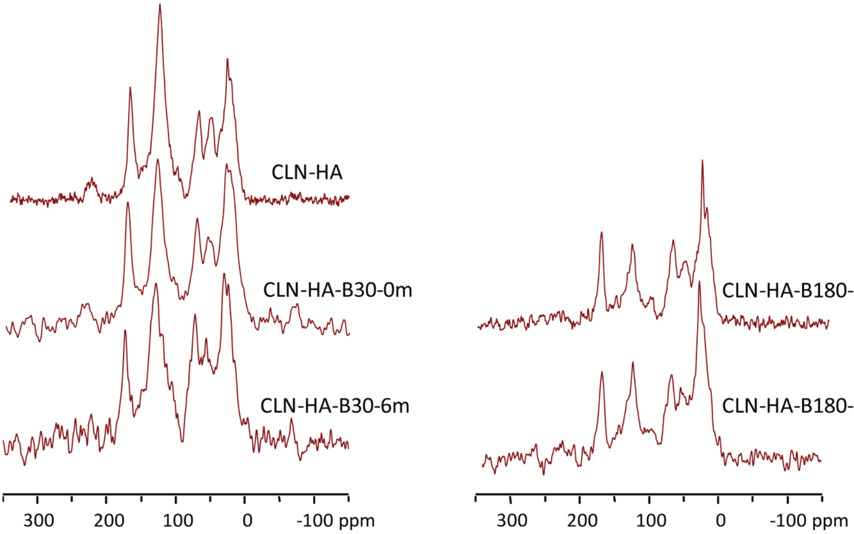


Figure 6. 13C CP/NMR solid state spectra of humic acids extracted from CLN soil, CLN amended soils with biosolids at differents doses and incubation time.

4. Conclusions

According to the progress of amendment incubation process, significant pH decrease was recorded, while electrical conductivity, organic matter and cation exchange capacity increase. The observed variations are proportional to the applied biosolid dose, demonstrating thus the direct effect of this organic material on soil.

$Q_{2/4}$, $Q_{2/6}$ and $Q_{4\ 6}$ absorbance ratios reflect the differences of the OM and its components in both soil and amendments. These parameters could be indicative of the state of biosolid humification, revealing the low quality of the OM of this material with respect to the original soil. However, should be analyzed carefully.

The addition of biosolids caused little impact on the total acidity, whereas no effect of incubation time was observed. These parameters reveal that a longer time is required for the exogenous HA coming from low quality MO be incorporated into the native soil HA.

The use of solid-state ^{13}C NMR enabled the following sequence, relative to the abundance of the different areas of the spectrum, to be established: Aromatic > O/N Alkyl ~ Alkyl C > Carboxyl of HA from CLN soil and amendments. Furthermore, the addition of high doses of biosolids incorporates fatty acids, amino acids and/or paraffinic structures. However, the carboxylic groups undergo no significant changes during this process.

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