13C-NMR spectroscopy of humic substances isolated from the agricultural soils of Puchuncavi (El Melón and Puchuncavi areas), central Chile

Ivan Alekseev*, Evgeny Abakumov

Department of Applied Ecology, Biological Faculty, Saint Petersburg State University, Saint Petersburg, Russia

*Corresponding author: alekseevivan95@gmail.com


Abstract: Chile is a well-developed agricultural country, which is faced with the problem of agricultural soil contamination with metals, such as Cu, Cd, Pb and Zn, and metalloids (As). These elements can be retained by soils through different mechanisms, i.e., complex-forming with organic matter or occlusion within organic matter. That is why studying soil contamination should also be accompanied by detailed investigations of the soil organic matter composition. Soil organic matter is crucial for plant growth since its decomposition releases nitrogen, phosphorus and other nutrients available for the plants. 13C nuclear magnetic resonance spectroscopy, providing crucial data on carbon functional structures diversity, can also be used to study changes in the soil organic matter (SOM) during decomposition and humification. This study is aimed at investigating the molecular composition of the soil organic matter in the agricultural soils of urbanised areas of central Chile using 13C nuclear magnetic resonance (NMR) spectroscopy. The studied soils were characterised by almost neutral pH values and organic carbon contents from 1.7% to 5.2%. The results showed that soils with an increased content of the total organic matter demonstrated an increased portion of aromaticity and a decreased portion of aliphaticity. Most of the investigated humic acids show the highest peaks centred at 125 ppm, which can be tentatively assigned to aromatic alkene structures. The relatively high stabilisation rate of the organic matter in the studied soils can be explained by the mineralisation of its peripheral part, which, in turn, is explained by the decreasing C/N ratio values. The humic substances of the studied agricultural soils of central Chile showed a high average content of aromatic carbon, which is also typical for subboreal soils.

Keywords: humic acids; humification; NMR spectroscopy; soil organic matter

Chile is a country with a well-developed agriculture and is the world leader in the production of copper (Gonzalez et al. 2014). The environmental problems associated with the copper mining activities in central Chile are widely known. That is why the problem of agricultural soil contamination with metals, such as Cu, Cd, Pb and Zn, and metalloids (As) has sharply risen.

The soil organic matter can retain various trace elements through different mechanisms: ion-exchange, proton displacement, and inner or outer-sphere complex formation (Schnitzer 1986). Complexes of elements with organic substances are usually characterised by a higher stability and of greater importance. While simple organic acids, such as acetic acid, are found in the soil solution phase, a

Supported by Saint Petersburg State University (Grant No. 1.42.959.2016), Research Grant of Russian Foundation for Basic Research 17-16-01030 “Soil biota dynamics in chronoseries of posttechnogenic landscapes: analyses of soil-ecological effectiveness of ecosystems restoration”. Scientific research was performed at the Center for Magnetic Resonance of Research Park of St. Petersburg State University.
large part of the soluble organic matter consists of compounds of a higher molecular weight. Organic matter will, therefore, significantly influence the behaviour of many elements, in particular Fe, Cu and Pb. That is why studying soil contamination should also be accompanied by detailed investigations of the soil organic matter.

The soil organic matter (SOM) is crucial for plant growth since its decomposition releases nitrogen, phosphorus and other nutrients available for the plants (Silva et al. 2010). The SOM promotes soil structure by holding the soil particles together as stable aggregates improve the soil’s physical properties, such as water holding capacity, water infiltration, gaseous exchange, root growth and ease of cultivation. The soil organic matter also serves as a buffer against toxic and unfavourable substances, such as heavy metals and toxins (Silva et al. 2010).

$^{13}$C nuclear magnetic resonance (NMR) spectroscopy that provides crucial data on carbon functional structures diversity can also be used to study changes in the SOM during decomposition and humification (Kogel-Knabner 1997; Zech et al. 1997). The advantage of the nuclear magnetic resonance spectroscopy method is the ability to quantify the content of the groups of the structural fragments and identify the individual structural fragments in the humic acid molecules. The application of NMR spectroscopy in the investigations of the soils and the soil organic matter has been previously discussed by Randall et al. (1997) and Preston (1996). Compared to the other methods, NMR-spectroscopy gives direct information about the structure of the studied compounds and is one of the most powerful methods for the comprehensive assessment of the structure of complex organic materials (Chukov et al. 2018). This method is also used to assess changes in the SOM during decomposition and humification. Solid-state $^{13}$C nuclear magnetic resonance spectroscopy with cross-polarisation and magic-angle spinning has been previously used for the assessment of the chemical composition of humic acids in different regions of the Earth (Novotny et al. 2007; Shiau et al. 2017; Pennock et al. 2008). Agricultural soils and their humic acids have been investigated by solid-state $^{13}$C NMR spectroscopy to reveal the humification degree and their specific molecular structure.

This study is aimed at investigating the molecular composition of the soil organic matter in the agricultural soils of central Chile using $^{13}$C NMR spectroscopy. The objectives were (1) to study the soil organic matter contents and the main properties in the selected agricultural soils; (2) to characterise the molecular composition of the humic substances in the selected soils.

**MATERIAL AND METHODS**

Topsoil samples (0–20 cm) were collected from 8 sites currently used for agriculture in the El Melón and Puchuncavi valley that have been contaminated by copper mining and smelting activities (Figure 1). Both fields were managed with perennial *Lactuca sativa* over many years before the sampling. An expert judgment sampling approach was used (Petersen & Calvin 1996; Aguilar et al. 2011). The sampling locations have been chosen based on a previous investigation of the Cu spatial distribution (Gonzalez et al. 2014; Moya et al. 2017). The soil samples have been collected from the soils classified as entisols (Soil Survey Staff 2003). The procedure for the soil properties determination has been described previously by Moya (Moya et al. 2017). The determination of the main soil parameters has been performed by standard procedures. The values of the pH in the water and salt suspension were measured using a pH-150 meter (1 : 2.5 soil : solution ratio). The grain size distribution has been determined by the

![Figure 1. The area of investigation; 1 – El Melón; 2 – Puchuncavi](https://example.com/figure1.png)
pipette method. The carbon and nitrogen content was measured by a Vario El Cube elemental analyser (Elementar Analyse System GmbH, Germany).

The humic acids (HAs) were extracted according to the procedure recommended by the International Humic Substances Society (IHSS 1981). The HAs were extracted with 0.1 M NaOH (soil : solution ratio 1 : 10) under nitrogen gas. After 24 h of shaking, the alkaline supernatant was separated from the soil residue by centrifugation at 1 516 ×g for 20 min and acidified to pH 1 with 6 M HCl to induce the precipitation of the HAs. The supernatant contained fulvic acids (FAs), which was then separated from the precipitate (HAs) by centrifugation at 1 516 ×g for 15 min. The HAs were then re-dissolved in 0.1 M NaOH and shaken for 4 h under nitrogen before the suspended solids were removed by centrifugation. The HA solution was acidified again with 6 M HCl to pH 1.0 and the HAs were separated by centrifugation. The HAs were demineralised by shaking overnight in 0.1 M HCl/0.3M HF (solid : solution ratio 1 : 1) and then repeatedly washed with deionised water until the washing reached pH 3. The solid HAs were then freeze-dried.

The 13C NMR spectra of the air dried HAs powders are shown in Figure 2. All of the studied (excluding the HC25 soil) HAs showed the highest peaks centred at 125 ppm, which can be tentatively assigned to aromatic alkene structures and indicating that an alkyl aromatic carbon was substituted by a nitrogen or an oxygen. In general, the shifts between 112–163 ppm are aromatic and olefinic carbons (Wooten 1995). The

Table 1. The carbon species and chemical shifts (13C nuclear magnetic resonance, chemical shifts range after Chukov et al. 2018)

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>Molecular fragments: carbon species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–47</td>
<td>unsubstituted saturated alkyl carbons C</td>
</tr>
<tr>
<td>47–60</td>
<td>alkyl C substituted by oxygen and nitrogen atoms</td>
</tr>
<tr>
<td>60–110</td>
<td>alkyl C singly bonded to one oxygen atom and alkyl C bonded to two oxygen atoms</td>
</tr>
<tr>
<td>110–144</td>
<td>proton- and alkyl-substituted aromatic C</td>
</tr>
<tr>
<td>144–164</td>
<td>oxygen-substituted aromatic C</td>
</tr>
<tr>
<td>164–183</td>
<td>carboxyl, ester, and amide C</td>
</tr>
<tr>
<td>183–190</td>
<td>quinone C</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The main soil properties of studied topsoil horizons of the agricultural soils are summarised in Table 2. The fine earth of the studied soils shows a pH of almost neutral pH values (from 6.22 to 7.42). The C/N ratio usually varies quite significantly in the soils from El Melón – from 9.2 to 29.5. The soils from Puchuncavi are characterised by a narrower range of the C/N ratio values – from 8.7 to 13.4. The soils from Puchuncavi are characterised by the predominance of a sand fraction, whereas the studied soil from El Melón show the predominance of a silt fraction.

The carbon content in the ashless HAs in the soils of El Melón varied from 34.58% to 48.92% whereas the carbon content was less (Table 3) in the HAs in the soils of Puchuncavi. This gives us the possibility to classify the substances investigated as containing relatively high carbon in El Melón. The oxygen is subsequently higher in the HAs of the Puchuncavi soils. A high oxygen content indicates an increased degree of oxidation, this corresponds well with the oxidation coefficient values. The molecular ratios were revealed as normal HA materials.

The 13C NMR spectra from the studied humic acid powders are shown in Figure 2. All of the studied (excluding the HC25 soil) HAs showed the highest peaks centred at 125 ppm, which can be tentatively assigned to aromatic alkene structures and indicating that an alkyl aromatic carbon was substituted by a nitrogen or an oxygen. In general, the shifts between 112–163 ppm are aromatic and phenolic carbons and the peaks in this region mainly correspond to the aromatic and olefinic carbons (Wooten 1995). The
HC25 soil shows the highest peak at 173 ppm, which refers to the carbonyl (ester, acid) structure. According to Hatcher et al. (1981), the carboxyl groups could be partly derived from the polyuronic acid moieties associated with the polysaccharides. Moreover, HC12 and HC84 show relatively sharp peaks centred at quite the same chemical shift (175 ppm). It should be noticed that the HC76 soil shows an atypical molecular composition of the humic acids for sub-boreal zone soils.

The HC12 and HC23 soils show a few quite intensive peaks at 175 ppm (ester, R(C=O)OR, carboxyl C), 125 ppm (alkene, aromatic), 72 ppm (RCH2OH, RCH2OR) and 34 ppm (quaternary alkyl, R₄C). At the same time, the lower peaks in HC12 were identified at 148 ppm (oxygen-substituted aromatic C), 108 ppm, 85 ppm, 67 ppm and 60 ppm (alkyl C singly bonded to one oxygen atom), 42 ppm and 22 ppm (unsubstituted saturated alkyl carbons C). HC23 is also characterised by lower peaks at 50 and 25 ppm. HC37 shows the most intense peaks in the aromatic area – the highest peaks at 150, 125 ppm. The peaks of the chemical shifts in HC76 are shifted to the aliphatic portion – only one very intensive peak at 130 ppm (aromatic), the other peaks are much lower and refer to the chemical shifts of 105, 90, 75 and 73 ppm; 60 and 40 ppm (aliphatic). HC01 is characterised by the two most intense peaks at 175 and 125 ppm. HC29 shows the highest peak in the aliphatic area – 71 ppm (alkyl C singly bonded

Table 2. The main soil properties of the studied soils

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>pH</th>
<th>Total organic matter (%)</th>
<th>N</th>
<th>C</th>
<th>C/N</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Melón</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC01</td>
<td>6.96</td>
<td>4.20</td>
<td>0.08</td>
<td>2.50</td>
<td>29.50</td>
<td>34</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>HC12</td>
<td>7.42</td>
<td>5.30</td>
<td>0.27</td>
<td>3.10</td>
<td>11.40</td>
<td>35</td>
<td>22</td>
<td>43</td>
</tr>
<tr>
<td>HC23</td>
<td>7.23</td>
<td>5.00</td>
<td>0.47</td>
<td>3.60</td>
<td>7.70</td>
<td>36</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>HC25</td>
<td>7.23</td>
<td>4.30</td>
<td>0.47</td>
<td>3.50</td>
<td>7.50</td>
<td>36</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>HC29</td>
<td>6.30</td>
<td>2.70</td>
<td>0.10</td>
<td>1.60</td>
<td>15.20</td>
<td>47</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>HC37</td>
<td>7.13</td>
<td>3.50</td>
<td>0.22</td>
<td>2.00</td>
<td>9.10</td>
<td>40</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>Puchuncavi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC76</td>
<td>6.92</td>
<td>2.90</td>
<td>0.12</td>
<td>1.70</td>
<td>13.40</td>
<td>82</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>HC84</td>
<td>6.22</td>
<td>3.40</td>
<td>0.23</td>
<td>2.00</td>
<td>8.70</td>
<td>74</td>
<td>4</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 3. The elemental composition of the studied humic acids from the surface soil horizons.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>C (%)</th>
<th>H (%)</th>
<th>N (%)</th>
<th>O (%)</th>
<th>C/N</th>
<th>H/C</th>
<th>O/C</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Melón</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC01</td>
<td>34.58</td>
<td>4.32</td>
<td>5.90</td>
<td>55.20</td>
<td>5.86</td>
<td>0.12</td>
<td>1.60</td>
<td>5.00</td>
</tr>
<tr>
<td>HC12</td>
<td>36.12</td>
<td>4.78</td>
<td>6.91</td>
<td>52.19</td>
<td>5.23</td>
<td>0.13</td>
<td>1.44</td>
<td>5.00</td>
</tr>
<tr>
<td>HC23</td>
<td>48.92</td>
<td>4.96</td>
<td>6.45</td>
<td>39.67</td>
<td>7.58</td>
<td>0.10</td>
<td>0.81</td>
<td>5.00</td>
</tr>
<tr>
<td>HC25</td>
<td>39.85</td>
<td>5.12</td>
<td>5.84</td>
<td>49.19</td>
<td>6.82</td>
<td>0.13</td>
<td>1.23</td>
<td>5.00</td>
</tr>
<tr>
<td>HC29</td>
<td>42.13</td>
<td>5.35</td>
<td>4.78</td>
<td>47.74</td>
<td>8.81</td>
<td>0.13</td>
<td>1.13</td>
<td>5.00</td>
</tr>
<tr>
<td>HC37</td>
<td>40.89</td>
<td>4.89</td>
<td>4.56</td>
<td>49.66</td>
<td>8.97</td>
<td>0.12</td>
<td>1.21</td>
<td>5.00</td>
</tr>
<tr>
<td>Puchuncavi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC76</td>
<td>35.15</td>
<td>3.90</td>
<td>4.25</td>
<td>56.70</td>
<td>8.27</td>
<td>0.11</td>
<td>1.61</td>
<td>5.00</td>
</tr>
<tr>
<td>HC84</td>
<td>33.54</td>
<td>4.78</td>
<td>4.36</td>
<td>57.32</td>
<td>7.69</td>
<td>0.14</td>
<td>1.71</td>
<td>5.00</td>
</tr>
<tr>
<td>Standards</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leonardite HA standard (1S104H)</td>
<td>63.81</td>
<td>3.70</td>
<td>1.23</td>
<td>31.27</td>
<td>51.88</td>
<td>0.06</td>
<td>0.49</td>
<td>2.60</td>
</tr>
<tr>
<td>Elliot soil HA standard (1S102H)</td>
<td>58.13</td>
<td>3.68</td>
<td>4.14</td>
<td>34.08</td>
<td>14.04</td>
<td>0.06</td>
<td>0.59</td>
<td>0.90</td>
</tr>
</tbody>
</table>

The gravimetric concentrations are given for the C, H, N and O contents
The distribution of the peaks in HC25 is quite smoothed out – the two most intense peaks (170 and 28 ppm) are observed together with less intense peaks in the areas of 155–120 ppm and 75–50 ppm. HC84 shows the strict predominance of the aromatic peaks – the highest are at 170 and 125 ppm.

The resonance within the chemical shift region between 105 and 144 ppm (aromatic region) may originate from the unsubstituted and alkyl-substituted aromatic C (Abakumov et al. 2015). The 13C-signals in the 148–160 ppm region are assigned to the aromatic carbon bound to the strongly electron-withdrawing OH group and the other heteroaromatic carbons. In the range of 129–148 ppm, the sp2 hybridized carbons with the aliphatic carbon substituents and the internal carbons of the condensed aromatic compounds can be observed (Gonzalez et al. 2014). The signals in the 118–129 ppm region are assigned to the aromatic C-H resonances and those in the 108–118 ppm region are assigned to the aromatic C-H ortho- to aromatic C-O groups. The carboxyl region (164–183 ppm) is dominated by a peak with a maximum at approximately 175 ppm, largely attributed to carboxyl C, but also to carbonyl C from the amides and polypeptides (Lodygin & Beznosikov 2003; Keeler et al. 2006). The carbonyl region is between 160 and 220 ppm, and there is a considerable overlap of the chemical shifts in this region due to the huge number of chemical components that contain a carbonyl group (Wershaw et al. 1998).

In all the samples, there was a statistically significant predominance of aromatic carbons (57.29 ± 4.05%) over the aromatic ones (42.71 ± 4.05%); t-test, P < 0.001; Table 4). The standard deviation of all the measured 13C-NMR spectra showed that the highest variability among the studied samples is observed at the intervals of 70–80 (the alkynes structure) ppm and 125–135 (the aromatic structures) ppm (Figure 3).

The sp2/sp3 carbon ratio was calculated according to equation:

\[
\frac{\text{sp}^2}{\text{sp}^3} = \frac{\text{area (108–220 ppm))}}{\text{area (0–108 ppm))}}
\]

(Mao et al. 2000)

where:

\[
\text{sp}^2 - \text{sp}^2 \text{ hybridized carbons}
\]

\[
\text{sp}^3 - \text{sp}^3 \text{ hybridized carbons}
\]
The results of the $sp^2/sp^3$ carbon ratio calculation are presented in Table 5. The ratio of the samples investigated was generally higher than 1. The highest value was observed for the HAs of the HC84 sample from Puchuncavi (2.174), the lowest value was observed for the HAs of the HC12 sample from El Melón (0.971). Our data are principally in the scope of those published earlier by Mao et al. (2000). The authors found the $sp^2/sp^3$ carbon ratio in the HA models ranged from 1.1 to 6.8. Hence, the $sp^2/sp^3$ carbon ratio in the HAs investigated here did not match with any previously reported results.

The quality of the precursor material has been identified as one of the main drivers of the soil humic acids’ composition (Andersson et al. 2012). This factor is followed by the climatic conditions (i.e., temperature and moisture) which shape the biophysical conditions necessary for the humification (Zech et al. 1997).

The humic substances of the studied agricultural soils of the urbanised areas of central Chile showed a high average content of aromatic carbon. This is quite typical for soils with well-humified organic matter, e.g., Mollisols of Umbric Luvisols (Abakumov et al. 2009; Kholodov et al. 2011) (Table 2). Such type of humus with a pronounced portion of aromaticity is also typical for subboreal soils.

The predominance of quite the same SOM composition in the studied soil might be explained by the

Table 4. The percentage of carbon in the main structural fragments of the humic acids (according to the $^{13}$C nuclear magnetic resonance) ($n = 3$)

<table>
<thead>
<tr>
<th>Site</th>
<th>Chemical shifts (ppm)</th>
<th>Aromatic groups (%)</th>
<th>Aliphatic groups (%)</th>
<th>$P$ (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–45</td>
<td>45–110</td>
<td>110–160</td>
<td>160–220</td>
</tr>
<tr>
<td><strong>El Melón</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC01</td>
<td>18.27</td>
<td>19.21</td>
<td>47.12</td>
<td>16.23</td>
</tr>
<tr>
<td>HC12</td>
<td>22.35</td>
<td>32.23</td>
<td>33.20</td>
<td>13.02</td>
</tr>
<tr>
<td>HC23</td>
<td>19.21</td>
<td>20.07</td>
<td>44.10</td>
<td>17.05</td>
</tr>
<tr>
<td>HC25</td>
<td>20.05</td>
<td>24.23</td>
<td>40.05</td>
<td>15.12</td>
</tr>
<tr>
<td>HC29</td>
<td>12.07</td>
<td>29.02</td>
<td>46.12</td>
<td>13.12</td>
</tr>
<tr>
<td>HC37</td>
<td>22.09</td>
<td>17.08</td>
<td>44.04</td>
<td>17.92</td>
</tr>
<tr>
<td><strong>Puchuncavi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC76</td>
<td>14.12</td>
<td>36.07</td>
<td>43.17</td>
<td>7.12</td>
</tr>
<tr>
<td>HC84</td>
<td>17.23</td>
<td>17.09</td>
<td>48.12</td>
<td>18.06</td>
</tr>
</tbody>
</table>

Figure 3. The stacked spectra of the humic acids of the studied soils

CONCLUSION

13C-NMR spectroscopy was used to characterise the molecular composition of humic acids from 8 agricultural soils in central Chile. All the samples were slightly different with some common peaks. The similar SOM composition in the studied soil might be explained by the cultivated state of the studied soils, which is expressed in the sufficient equalisation of the soil water regimes and the erasure of the signature of the differential accumulations of the plant polymers.

Most of the investigated humic acids show the highest peaks centred at 125 ppm, which can be tentatively assigned to the aromatic alkene structures. The relatively high stabilisation rate of the organic matter in the studied soils can be explained by the mineralisation of its peripheral part, which, in turn, is explained by the decreasing C/N ratio values. The soils of urbanised landscapes are characterised by intense humification, which leads to the increased aromatisation of the organic matter in the topsoil horizons due to the intense aeration, oxidation and accumulation of nitrogen in the aromatic compounds.

This work revealed that the studied agricultural soils with an increased content of the total organic matter demonstrated an increased portion of aromaticity and a decreased portion of aliphaticity. High aromaticity rates were found in the soils with the increased SOM content, the decreased values of the C/N ratio (and correspondingly the low aliphaticity rate).

Acknowledgements. The authors would like to acknowledge A. Neaman, the research assistants from Pontificia Universidad de Católica de Valparaíso, School of Agronomy for helping with organisation of the field studies.

REFERENCES


Received: July 4, 2019
Accepted: December 2, 2019
Published online: January 20, 2020