Supplementary Material:

Analyzing Several Chelating Agents and Their Effect on Elemental Composition of *Lolium Perenne* and Two Growth Media by Capillary Zone Electrophoresis and Inductively Coupled Plasma Optical Emission Spectrometry

PIRKKO-LEENA HAKKARAINEN* AND ROSE MATILAINEN

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**Table S1.** Response factors determined by HEDTA for DTPA, EDTA, IDA, NTA, PDTA and TTHA.

<table>
<thead>
<tr>
<th>Chelating agent</th>
<th>Response factor</th>
<th>LOD (μmol L⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.73</td>
<td>27</td>
<td>0.998</td>
</tr>
<tr>
<td>DTPA</td>
<td>0.51</td>
<td>38</td>
<td>0.998</td>
</tr>
<tr>
<td>IDA</td>
<td>0.74</td>
<td>30</td>
<td>0.997</td>
</tr>
<tr>
<td>NTA</td>
<td>0.45</td>
<td>50</td>
<td>0.997</td>
</tr>
<tr>
<td>PDTA</td>
<td>1.05</td>
<td>20</td>
<td>0.997</td>
</tr>
<tr>
<td>TTHA</td>
<td>1.66</td>
<td>12</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The response factor was calculated by using equation $A_X/[X]=F(A_S/[S])$, where $A_X$ is the area of analyte signal, $A_S$ the area of standard signal, $[X]$ the concentration of analyte, $[S]$ the concentration of standard and $F$ the response factor.

LOD= 3*noise *(c/h), where c is concentration and h is peak height.
Table S2. The calibration parameters in the ICP-OES measurements for each element analyzed.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>R²</th>
<th>LOD (mg L⁻¹)ᵃ</th>
<th>LOQ (mg L⁻¹)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>396.153</td>
<td>0.9999</td>
<td>0.57</td>
<td>1.9</td>
</tr>
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<td>As</td>
<td>188.979</td>
<td>0.9996</td>
<td>0.80</td>
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<td>Ca</td>
<td>317.933</td>
<td>0.9999</td>
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<td>2.2</td>
</tr>
<tr>
<td>Cd</td>
<td>228.802</td>
<td>0.9998</td>
<td>0.70</td>
<td>2.4</td>
</tr>
<tr>
<td>Cu</td>
<td>327.393</td>
<td>0.9999</td>
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<td>2.2</td>
</tr>
<tr>
<td>Fe</td>
<td>238.204</td>
<td>0.9999</td>
<td>0.60</td>
<td>2.1</td>
</tr>
<tr>
<td>K</td>
<td>766.490</td>
<td>0.9999</td>
<td>0.65</td>
<td>2.2</td>
</tr>
<tr>
<td>Mg</td>
<td>285.213</td>
<td>0.9999</td>
<td>0.67</td>
<td>2.2</td>
</tr>
<tr>
<td>Mn</td>
<td>257.610</td>
<td>0.9998</td>
<td>0.74</td>
<td>2.5</td>
</tr>
<tr>
<td>Pb</td>
<td>220.353</td>
<td>0.9997</td>
<td>0.80</td>
<td>2.7</td>
</tr>
<tr>
<td>S</td>
<td>181.975</td>
<td>0.9995</td>
<td>0.90</td>
<td>3.0</td>
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<tr>
<td>Zn</td>
<td>206.200</td>
<td>0.9999</td>
<td>0.70</td>
<td>2.2</td>
</tr>
</tbody>
</table>

ᵃ limit of detection (LOD) was calculated by substituting the intercept and its standard deviation multiplier (a + 3 sₐ) in the calibration line y = bx + a.
ᵇ limit of quantification (LOQ) was calculated by substituting the intercept and its standard deviation multiplier (a + 10 sₐ) in the calibration line y = bx + a.

Table S3. Element concentrations (mg/g) in black soil (before and after cultivation) and in ryegrass. Ultra pure water, phosphate and aqua regia extractions.

<table>
<thead>
<tr>
<th></th>
<th>water</th>
<th>phosphate</th>
<th>aqua regia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
<td>grass</td>
</tr>
<tr>
<td>Al</td>
<td>0.72±0.04</td>
<td>0.020±0.002</td>
<td>0.017±0.008</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.44±0.04</td>
<td>0.016±0.008</td>
<td>0.017±0.005</td>
</tr>
<tr>
<td>DTPA</td>
<td>0.211±0.010</td>
<td>0.023±0.003</td>
<td>0.021±0.008</td>
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<tr>
<td>NTA</td>
<td>0.40±0.03</td>
<td>0.036±0.013</td>
<td>0.010±0.009</td>
</tr>
<tr>
<td>IDA</td>
<td>0.043±0.003</td>
<td>0.010±0.006</td>
<td>0.0317±0.0015</td>
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<tr>
<td>PDTA</td>
<td>0.16±0.04</td>
<td>0.021±0.002</td>
<td>0.167±0.010</td>
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<tr>
<td>mix1</td>
<td>0.65±0.02</td>
<td>0.04±0.02</td>
<td>1.08±0.07</td>
</tr>
<tr>
<td>mix2</td>
<td>0.57±0.04</td>
<td>0.062±0.006</td>
<td>0.41±0.03</td>
</tr>
<tr>
<td>clean</td>
<td>0.006±0.002</td>
<td>0.0073±0.0012</td>
<td>0.060±0.011</td>
</tr>
<tr>
<td>Element</td>
<td>Compound</td>
<td>Sample</td>
<td>As (μg/g)</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>TTHA</td>
<td></td>
<td>0.013±0.002</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td></td>
<td>0.019±0.014</td>
</tr>
<tr>
<td></td>
<td>DTPA</td>
<td></td>
<td>0.026±0.04</td>
</tr>
<tr>
<td></td>
<td>NTA</td>
<td></td>
<td>0.13±0.04</td>
</tr>
<tr>
<td></td>
<td>IDA</td>
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<td>0.08±0.02</td>
</tr>
<tr>
<td></td>
<td>PDTA</td>
<td></td>
<td>0.0077±0.0015</td>
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<tr>
<td></td>
<td>mix1</td>
<td></td>
<td>0.029±0.005</td>
</tr>
<tr>
<td></td>
<td>mix2</td>
<td></td>
<td>0.027±0.002</td>
</tr>
<tr>
<td></td>
<td>clean</td>
<td></td>
<td>0.008±0.003</td>
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</tbody>
</table>

### Notes:
- All values are in μg/g.
- Standard deviations are listed with each measurement.
<table>
<thead>
<tr>
<th></th>
<th>mix1</th>
<th>mix2</th>
<th>clean</th>
<th>mix1</th>
<th>mix2</th>
<th>clean</th>
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<td>0.37±0.05</td>
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<td>28.55</td>
<td>24±3</td>
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<td>28±4</td>
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<td>27.0</td>
<td>28±4</td>
<td>9±4</td>
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<td>1.45±0.05</td>
<td>1.39±0.06</td>
<td>1.26±0.06</td>
<td>1.85±0.06</td>
<td>1.92±0.06</td>
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<td>0.56±0.011</td>
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<tr>
<td>TTHA</td>
<td>0.97±0.03</td>
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<td>0.016±0.008</td>
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<tr>
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<td>0.0007±0.0006</td>
<td>0.06±0.02</td>
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<td>0.071±0.012</td>
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<td>3.4±0.3</td>
<td>1.12±0.02</td>
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<td></td>
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<td>--------</td>
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<tr>
<td>NTA</td>
<td>0.068±0.009</td>
<td>0.020±0.004</td>
<td>0.057±0.008</td>
<td>0.06±0.003</td>
<td>0.018±0.004</td>
<td>0.04±0.003</td>
</tr>
<tr>
<td>IDA</td>
<td>0.044±0.004</td>
<td>0.014±0.004</td>
<td>0.16±0.04</td>
<td>0.052±0.010</td>
<td>0.011±0.002</td>
<td>0.005±0.002</td>
</tr>
<tr>
<td>PDTA</td>
<td>0.045±0.005</td>
<td>0.011±0.003</td>
<td>0.109±0.008</td>
<td>0.057±0.010</td>
<td>0.007±0.006</td>
<td>0.026</td>
</tr>
<tr>
<td>NTA</td>
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<td>0.038±0.003</td>
<td>0.0107±0.0006</td>
<td>0.051±0.003</td>
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</tr>
<tr>
<td>IDA</td>
<td>0.029</td>
<td>0.063</td>
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<td>0.34±0.11</td>
<td>0.050±0.006</td>
<td>0.073±0.002</td>
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<tr>
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<td>0.036±0.011</td>
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<td>0.05±0.02</td>
<td>0.056±0.011</td>
<td>0.035±0.006</td>
<td>0.006±0.005</td>
</tr>
<tr>
<td>mix1</td>
<td>0.055±0.005</td>
<td>0.021±0.007</td>
<td>0.042</td>
<td>0.047±0.005</td>
<td>0.017±0.005</td>
<td>0.015</td>
</tr>
<tr>
<td>mix2</td>
<td>0.052±0.013</td>
<td>0.044±0.003</td>
<td>0.04±0.03</td>
<td>0.055±0.005</td>
<td>0.035±0.006</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>clean</td>
<td>0.034±0.004</td>
<td>0.035±0.003</td>
<td>0.059±0.002</td>
<td></td>
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<td></td>
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</tbody>
</table>

Notice! if empty spaces are left, the result obtained is <LOD; if there are no error limits after the result, the result obtained is from only one replicate
Table S4. Element concentrations (mg/g) in clay (before and after cultivation) and in ryegrass. Ultra pure water, phosphate and *aqua regia* extractions.

<table>
<thead>
<tr>
<th></th>
<th>water before</th>
<th>water after</th>
<th>grass</th>
<th>phosphate before</th>
<th>phosphate after</th>
<th>grass</th>
<th>aqua regia before</th>
<th>aqua regia after</th>
<th>grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>1.3±0.2</td>
<td>0.29±0.10</td>
<td>0.50±0.03</td>
<td>1.44±0.07</td>
<td>0.29±0.04</td>
<td>0.428±0.011</td>
<td>2.6±0.3</td>
<td>2.8±0.13</td>
<td>2.5±0.02</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.71±0.07</td>
<td>0.028±0.005</td>
<td>0.273±0.006</td>
<td>1.28±0.14</td>
<td>0.046±0.003</td>
<td>0.248±0.008</td>
<td>2.34±0.02</td>
<td>3.00±0.04</td>
<td>2.5±0.03</td>
</tr>
<tr>
<td>DTPA</td>
<td>1.28±0.08</td>
<td>0.06±0.03</td>
<td>0.28±0.08</td>
<td>1.29±0.05</td>
<td>0.08±0.04</td>
<td>0.24±0.07</td>
<td>2.50±0.12</td>
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Notice! if empty spaces are left, the result obtained is <LOD; if there are no error limits after the result, the result obtained is from only one replicate
Figure S1. Trends of chelating agent effect on to the solubility and extractability of Fe in water and phosphate solutions. a) Black soil extracted with ultra pure water, b) Black soil extracted with phosphate solution, c) Clay extracted with ultra pure water, d) Clay extracted with phosphate solution.
Figure S2. The cumulative results of elemental concentrations and chelating agents measured in phosphate solution. a) Black soil before cultivation, b) Black soil after cultivation, c) Ryegrass grown in black soil, d) Clay before cultivation, e) Clay after cultivation, f) Ryegrass grown in clay.