viernes 27 de noviembre de 2009
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New insights on Fe deficiency and heavy metal (Cd, Zn and Hg) toxicity in plants

Javier Abadía

Departamento de Nutrición Vegetal, Estación Experimental de Aula Dei de Zaragoza, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), Spain
Index

Fe deficiency
Index

Fe deficiency

Progress in synthetic chelate analysis
Index

- Fe deficiency
- Progress in synthetic chelate analysis
- Fe transport molecules in plants (NA, Cit)
Index

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Cd toxicity
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- Progress in synthetic chelate analysis
- Fe transport molecules in plants (NA, Cit)
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**Cd toxicity**
- Cd effects on tomato plant physiology
- Cd effects on tomato root proteomics
Index

- Fe deficiency
  - Progress in synthetic chelate analysis
  - Fe transport molecules in plants (NA, Cit)
  - Fe metabolomics/proteomics

- Cd toxicity
  - Cd effects on tomato plant physiology
  - Cd effects on tomato root proteomics

- Zn toxicity
Index

Fe deficiency
- Progress in synthetic chelate analysis
- Fe transport molecules in plants (NA, Cit)
- Fe metabolomics/proteomics

Cd toxicity
- Cd effects on tomato plant physiology
- Cd effects on tomato root proteomics

Zn toxicity
- Zn effects on sugar beet plant physiology
Index

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  - Progress in synthetic chelate analysis
  - Fe transport molecules in plants (NA, Cit)
  - Fe metabolomics/proteomics

- **Cd toxicity**
  - Cd effects on tomato plant physiology
  - Cd effects on tomato root proteomics

- **Zn toxicity**
  - Zn effects on sugar beet plant physiology
  - Zn effects on sugar beet photosynthesis
Index

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Progress in synthetic chelate analysis
Fe transport molecules in plants (NA, Cit)
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Cd toxicity
Cd effects on tomato plant physiology
Cd effects on tomato root proteomics

Zn toxicity
Zn effects on sugar beet plant physiology
Zn effects on sugar beet photosynthesis

Hg toxicity
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Cd toxicity
- Cd effects on tomato plant physiology
- Cd effects on tomato root proteomics

Zn toxicity
- Zn effects on sugar beet plant physiology
- Zn effects on sugar beet photosynthesis

Hg toxicity
- Biothiol-Hg complexes
Metabolites: Separation by 2-D techniques
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Time (HPLC)
Metabolites: Separation by 2-D techniques

mass/charge ratio
MS (ESI/TOF)

Time (HPLC)
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mass/charge ratio
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mass/charge ratio
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Time (HPLC)

mass/charge ratio

MS (ESI/TOF)
Metabolites: Separation by 2-D techniques

mass/charge ratio
MS (ESI/TOF)

unique m/z for each compound:
molecular formula

Time (HPLC)
Metabolites: Separation by 2-D techniques

The compound can be broken further by MS/MS:
- unique m/z for each compound: molecular formula
- molecular structure

Mass/charge ratio
MS (ESI/TOF)

Time (HPLC)
Metabolites: Separation by 2-D techniques

Rellán et al. (2008)
Rapid Commun Mass Spectrom
Metabolites: Separation by 2-D techniques

Rellán et al. (2008)
Rapid Commun Mass Spectrom

Fe deficiency
Metabolites: Separation by 2-D techniques


Fe deficiency

isotopic resolution
Metabolites: Separation by 2-D techniques

Rellán et al. (2008)
Rapid Commun Mass Spectrom

Fe deficiency

isotopic resolution
molecular formula
Metabolites: Separation by 2-D techniques

Rellán et al. (2008)
Rapid Commun Mass Spectrom

isotopic resolution

molecular formula

metal signature

Fe deficiency
Metabolites: Separation by 2-D techniques

Rellán et al. (2008)  
Rapid Commun Mass Spectrom

- isotopic resolution
- molecular formula
- metal redox state
- metal signature

Fe deficiency

Plant Stress Physiology

viernes 27 de noviembre de 2009
viernes 27 de noviembre de 2009
Fe deficiency
Fe chlorosis
Fe chlorosis
Fe chlorosis
Fe deficiency

EDDHA

viernes 27 de noviembre de 2009
Progress in synthetic chelate analysis
Progress in synthetic chelate analysis
HPLC-MS(TOF) method
Progress in synthetic chelate analysis
HPLC-MS(TOF) method

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HPLC-MS(TOF) method

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Fe deficiency

Plant Stress Physiology

viernes 27 de noviembre de 2009
Fe deficiency

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Fe deficiency


Plant Stress Physiology

viernes 27 de noviembre de 2009
Progress in synthetic chelate analysis
HPLC-MS(TOF) method

Progress in synthetic chelate analysis

HPLC-MS(TOF) method


Fe deficiency

soil solution, irrigation water, nutrient solution, xylem

exact m/z for each compound

All 9 chelates

EDDHA  
\[ \text{HOOC} \quad \text{HNN} \quad \text{HN} \quad \text{COOH} \]

\[ \text{o,oEDDHA} \]

\[ \text{viernes 27 de noviembre de 2009} \]
Progress in synthetic chelate analysis

HPLC-MS(TOF) method


Fe deficiency

soil solution, irrigation water, nutrient solution, xylem

exact m/z for each compound

All 9 chelates

LOD µM
Progress in synthetic chelate analysis
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues were used at different plant tissue/final extract weight ratios (PTW/FEW, w/w). Data are means ± SE (n = 3).

<table>
<thead>
<tr>
<th>Plant tissue ratio (PTW/FEW, mg g⁻¹ FW)</th>
<th>racemic</th>
<th>meso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>110 ± 4</td>
<td>97 ± 1</td>
</tr>
<tr>
<td>120</td>
<td>111 ± 2</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>80*</td>
<td>103 ± 2</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>Sugar beet roots</td>
<td></td>
<td></td>
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<tr>
<td>250</td>
<td>117 ± 4</td>
<td>105 ± 1</td>
</tr>
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</tbody>
</table>

* Optimal PTW/FEW ratios.
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues and different plant tissue/final extract weight ratios were tested. Data are means ± SE (n = 3)

<table>
<thead>
<tr>
<th>Plant tissue ratio (PTW/FEW, mg g⁻¹ FW)</th>
<th>Sugar beet leaves</th>
<th>Sugar beet roots</th>
<th>Tomato leaves</th>
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</tr>
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</tbody>
</table>

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Orera et al. (2009a) Rapid Commun Mass Spectrom

Fe deficiency

good recoveries in tissue samples

plant tissues Fe(III)o,oEDDHA

viernes 27 de noviembre de 2009
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues from different plant tissue/final extract weight ratios (w/w). Data are means ± SE (n = 3).

<table>
<thead>
<tr>
<th>Plant tissue</th>
<th>Ratio (PTW/FEW, mg g⁻¹ FW)</th>
<th>Recoveries (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet leaves</td>
<td>160</td>
<td>110 ± 4</td>
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<td></td>
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* Optimal PTW/FEW ratios.

good recoveries in tissue samples

Figure 3. Ion chromatograms (at m/z 412.0) for extracts of leaves (A) and roots (B), and xylem sap (C) from tomato plants treated with a Fe(III)-EDDHA commercial fertilizer for 24 h. Racemic and meso Fe(III)-o,oEDDHA were found at 14.2 and 16.0 min, respectively. The peak at retention time 14.8 min corresponded to Fe(III)-o,oEDDHMA.
Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues were used to prepare different plant tissue/final extract weight ratios (v/w). Data are means ± SE (n = 3)

<table>
<thead>
<tr>
<th>Plant tissue ratio (PTW/FEW, mg g⁻¹ FW)</th>
<th>Sugar beet leaves</th>
<th>Sugar beet roots</th>
<th>Tomato leaves</th>
<th>Tomato roots</th>
<th>Peach leaves</th>
<th>Peach fruits</th>
</tr>
</thead>
<tbody>
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Progress in synthetic chelate analysis

Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues of different plant tissue/final extract weight ratio (w/w). Data are means ± SE (n = 3)

<table>
<thead>
<tr>
<th>Plant tissue ratio (PTW/FW, mg g⁻¹ FW)</th>
<th>Sugar beet leaves</th>
<th>Sugar beet roots</th>
<th>Tomato leaves</th>
<th>Tomato roots</th>
<th>Peach leaves</th>
<th>Peach fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td>110 ± 4</td>
<td>117 ± 4</td>
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<td>117 ± 2</td>
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<tr>
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</tr>
<tr>
<td>160*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Fe(III)-o,oEDDHA and o,oEDDHA concentrations (in nmol g⁻¹ FW for plant tissues and in nmol mL⁻¹ for xylem sap) in plants treated with a Fe(III)-EDDHA commercial fertilizer (90 μM Fe(III)-o,oEDDHA) for 24 h. Data are means ± SE (n = 3)

<table>
<thead>
<tr>
<th></th>
<th>racemic</th>
<th>meso</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>8.5 ± 0.5</td>
<td>7.4 ± 0.5</td>
<td>15.9 ± 1.0</td>
</tr>
<tr>
<td>Roots</td>
<td>4.3 ± 0.4</td>
<td>3.7 ± 0.2</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>19.9 ± 0.8</td>
<td>20.3 ± 0.8</td>
<td>40.2 ± 0.0</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar beet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>11.9 ± 0.9</td>
<td>11.7 ± 0.8</td>
<td>23.6 ± 1.8</td>
</tr>
<tr>
<td>Roots</td>
<td>6.3 ± 0.8</td>
<td>5.8 ± 0.8</td>
<td>12.1 ± 1.6</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>30.0 ± 3.0</td>
<td>33.0 ± 2.9</td>
<td>63.0 ± 5.8</td>
</tr>
<tr>
<td>Roots</td>
<td>9.5 ± 1.6</td>
<td>8.5 ± 1.3</td>
<td>18.0 ± 2.9</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

*Optimal PTW/FW ratios.

Fe deficiency

plant tissues Fe(III)-o,oEDDHA

good recoveries in tissue samples
determination possible
Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHA as Internal Standard

Fe deficiencies

Good recoveries in tissue samples

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHA as IS. Six different plant tissues or different plant tissue/final extract weight ratios (w/w). Data are means ± SE (n = 3).

<table>
<thead>
<tr>
<th>Plant tissue (PTW/FEW, mg g⁻¹ FW)</th>
<th>Racemic</th>
<th>Meso</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet leaves</td>
<td>110 ± 4</td>
<td>97 ± 1</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>111 ± 2</td>
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<tr>
<td>80</td>
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<tr>
<td>Sugar beet roots</td>
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<td>Xylem sap</td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>19.9 ± 0.8</td>
<td>20.3 ± 0.8</td>
<td>40.2 ± 0.0</td>
</tr>
<tr>
<td>Roots</td>
<td>6.4 ± 1.2</td>
<td>5.2 ± 0.8</td>
<td>11.5 ± 2.0</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>o,oEDDHA concentrations</th>
<th>Sugar beet</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>11.9 ± 0.9</td>
<td>30.0 ± 3.0</td>
</tr>
<tr>
<td>Roots</td>
<td>6.3 ± 0.8</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>
Determination of Fe(III)-EDDHA in plant tissues using o,oEDDHMA as Internal Standard

Table 1. Recoveries (in %) of racemic and meso Fe(III)-o,oEDDHA in the post-extraction assay, using Fe(III)-o,oEDDHMA as IS. Six different plant tissues of different plant tissue/final extract weight ratio (w/w). Data are means ± SE (n = 3).

<table>
<thead>
<tr>
<th>Plant tissue (PTW/FEW, mgg⁻¹ FW)</th>
<th>racemic</th>
<th>meso</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet leaves</td>
<td>110±4</td>
<td>97±1</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>111±2</td>
<td>99±1</td>
<td></td>
</tr>
<tr>
<td>80*</td>
<td>103±2</td>
<td>98±1</td>
<td></td>
</tr>
<tr>
<td>Sugar beet roots</td>
<td>117±4</td>
<td>105±1</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>102±2</td>
<td>103±1</td>
<td></td>
</tr>
<tr>
<td>160*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato leaves</td>
<td>114±4</td>
<td>98±1</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>110±1</td>
<td>97±2</td>
<td></td>
</tr>
<tr>
<td>80*</td>
<td>103±2</td>
<td>96±2</td>
<td></td>
</tr>
<tr>
<td>Tomato roots</td>
<td>114±2</td>
<td>104±1</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach leaves</td>
<td>109±2</td>
<td>98±3</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach fruits</td>
<td>117±2</td>
<td>113±1</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>119±2</td>
<td>103±1</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>113±1</td>
<td>104±3</td>
<td></td>
</tr>
<tr>
<td>60*</td>
<td>104±2</td>
<td>101±1</td>
<td></td>
</tr>
<tr>
<td>*Optimal PTW/FEW ratios.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Fe(III)-o,oEDDHA and o,oEDDHA concentrations (in nmol g⁻¹ FW for plant tissues and in nmol mL⁻¹ for xylem sap) in plants treated with a Fe(III)-EDDHA commercial fertilizer (90 μM Fe(III)-o,oEDDHA) for 24 h. Data are means ± SE (n = 3).

<table>
<thead>
<tr>
<th>Plant tissue</th>
<th>racemic</th>
<th>meso</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td>Leaves</td>
<td>8.5±0.5</td>
<td>7.4±0.5</td>
</tr>
<tr>
<td>Roots</td>
<td>4.3±0.4</td>
<td>3.7±0.2</td>
<td>8.0±0.4</td>
</tr>
<tr>
<td>Tomato</td>
<td>Leaves</td>
<td>19.9±0.8</td>
<td>20.2±0.8</td>
</tr>
<tr>
<td>Roots</td>
<td>6.4±1.2</td>
<td>5.2±0.8</td>
<td>11.5±2.0</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>0.5±0.2</td>
<td>0.4±0.2</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>Peach fruits</td>
<td>Leaves</td>
<td>11.9±0.9</td>
<td>11.7±0.8</td>
</tr>
<tr>
<td>Roots</td>
<td>6.3±0.8</td>
<td>5.8±0.8</td>
<td>12.1±1.6</td>
</tr>
<tr>
<td>Tomato</td>
<td>Leaves</td>
<td>30.0±3.0</td>
<td>33.0±2.9</td>
</tr>
<tr>
<td>Roots</td>
<td>9.5±1.6</td>
<td>8.5±1.3</td>
<td>18.0±2.9</td>
</tr>
<tr>
<td>Xylem sap</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

*Optimal PTW/FEW ratios.
Progress in synthetic chelate analysis
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates
Progress in synthetic chelate analysis

MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Fe deficiency

EDDHA

Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Plant Stress Physiology

viernes 27 de noviembre de 2009
Progress in synthetic chelate analysis

MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis

MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Fe deficiency
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Fe deficiency

Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis
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Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom
Progress in synthetic chelate analysis

MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Fe deficiency

Contaminant

EDDHA
Progress in synthetic chelate analysis

MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Fe deficiency

Contaminant
Progress in synthetic chelate analysis
MS/MS spectra of commercial chelates

Orera et al. (2009b) Rapid Commun Mass Spectrom

Fe deficiency

Contaminant

EDDHA

viernes 27 de noviembre de 2009
Fe deficiency
Fe transport molecules
Fe transport molecules
HPLC-MS: nicotianamine-metal complexes
Fe transport molecules
HPLC-MS: nicotianamine-metal complexes
Standards
Fe transport molecules

HPLC-MS: nicotianamine-metal complexes
Standards

Rellán-Álvarez et al.,
Fe transport molecules
HPLC-MS: nicotianamine-metal complexes
Standards

Rellán-Álvarez et al.,
Fe transport molecules
HPLC-MS: nicotianamine-metal complexes
Standards

Rellán-Álvarez et al.,
Fe transport molecules
HPLC-MS: nicotianamine-metal complexes
Standards

Fe transport molecules in xylem
Fe transport molecules in xylem
Beta vulgaris

Fe deficiency

Fe transport molecules in xylem

Beta vulgaris

Fe transport molecules in xylem
Beta vulgaris

Fe transport molecules in xylem
Beta vulgaris

increase after resupply

Fe transport molecules in xylem

Beta vulgaris

Fe transport molecules in xylem
Beta vulgaris


increase after resupply
Fe deficiency

Fe transport molecules in xylem
Beta vulgaris

increase after resupply

decrease in the Cit:Fe ratio

Fe transport molecules in xylem
Fe transport molecules in xylem

Cit-Fe standards
Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem

Cit-Fe standards
Fe transport molecules in xylem

Cit-Fe standards

Fe deficiency

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol

HPLC-ICP(MS)
two FeCit complexes in standards
Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol

HPLC-ICP(MS)
two FeCit complexes in standards
Fe transport molecules in xylem

Cit-Fe standards

two FeCit complexes in standards

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem

Cit-Fe standards

two FeCit complexes in standards

type of FeCit complex depends on Cit:Fe ratio

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem

Cit-Fe standards

two FeCit complexes in standards

Fe3Cit3
Fe2Cit2

type of FeCit complex depends on Cit:Fe ratio

Rellán et al. (2009)
Plant Cell Physiol

Fe deficiencies

Plant Stress Physiology

viernes 27 de noviembre de 2009
Fe transport molecules in xylem

Cit-Fe standards

Fe deficiency

Rellán et al. (2009) Plant Cell Physiol

two FeCit complexes in standards

type of FeCit complex depends on Cit:Fe ratio

viernes 27 de noviembre de 2009
Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol

Fe deficiency

HPLC-ICP(MS) two FeCit complexes in standards

type of FeCit complex depends on Cit:Fe ratio

Fe transport molecules in xylem

Cit-Fe standards

Rellán et al. (2009)
Plant Cell Physiol
Fe transport molecules in xylem
Fe transport molecules in xylem
Lycopersicum esculentum

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem
*Lycopersicum esculentum*

Rellán et al. (2009) *Plant Cell Physiol*
Fe transport molecules in xylem
Lycopersicon esculentum

Rellán et al. (2009) Plant Cell Physiol

xylem sap
Fe transport molecules in xylem
*Lycopersicum esculentum*

Rellán et al. (2009) *Plant Cell Physiol*
Fe transport molecules in xylem
Lycopersicum esculentum

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem
Lycopersicum esculentum

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem
Lycopersicum esculentum

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem 
*Lycopersicum esculentum*

**HPLC-ICP(AMS)**

Rellán et al. (2009) *Plant Cell Physiol*

---

xylem sap

---

54Fe

56Fe

---

viernes 27 de noviembre de 2009
Fe transport molecules in xylem
*Lycopersicum esculentum*

In xylem only one FeCit complex: Fe$_3$Cit$_3$

---

Rellán et al. (2009) *Plant Cell Physiol*

---

Plant Stress Physiology

viernes 27 de noviembre de 2009
Fe transport molecules in xylem
Lycopersicum esculentum

in xylem only one FeCit complex: Fe$_3$Cit$_3$

current LOD 20 µM (for synthetic chelates 0.01 µM)

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem
Fe transport molecules in xylem
Hordeum vulgare
Fe transport molecules in xylem
Hordeum vulgare with FeSO₄
Fe transport molecules in xylem
*Hordeum vulgare* with FeSO₄
Fe transport molecules in xylem
*Hordeum vulgare* with FeSO₄

**HPLC-MS**
Fe transport molecules in xylem
Hordeum vulgare with FeSO₄
Fe transport molecules in xylem

*Hordeum vulgare* with FeSO₄

HPLC-MS

\[ [\text{Fe}_3\text{OCit}_3\text{H}_3]^{2-} \]
Fe transport molecules in xylem

Hordeum vulgare

with FeSO₄

Fe deficiency
Fe transport molecules in xylem

_Lycopersicum esculentum_

Rellán et al. (2009) Plant Cell Physiol
Fe transport molecules in xylem

_Lycopersicum esculentum_

Fe deficiency

Fe transport molecules in xylem

*Lycopersicium esculentum*

Rellán et al. (2009) *Plant Cell Physiol*
Fe transport molecules in xylem

*Lycopersicum esculentum*

Rellán et al. (2009) *Plant Cell Physiol*
Fe transport molecules in xylem

Lycopersicium esculentum

Rellán et al. (2009) Plant Cell Physiol
Fe deficiency

Fe metabolomics/proteomics (root tips)
Fe deficiency

Fe metabolomics/proteomics (root tips)

Beta vulgaris

viernes 27 de noviembre de 2009
Fe deficiency
Fe metabolomics/proteomics (root tips)
Beta vulgaris

Proteomics, EEAD-CSIC
Fe deficiency

Fe metabolomics/proteomics (root tips)

Beta vulgaris

+Fe

-Fe

viernes 27 de noviembre de 2009
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Rellán et al. (2009) in preparation
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Proteomics, EEAD-CSIC

+Fe

-Fe

Rellán et al. (2009) in preparation

viernes 27 de noviembre de 2009
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Proteomics, EEAD-CSIC

Rellán et al. (2009) in preparation

+Fe

-Fe

IEF

SDS-PAGE
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Proteomics, EEAD-CSIC

Rellán et al. (2009) in preparation
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Proteomics, EEAD-CSIC

+Fe

-Fe

IEF

SDS-PAGE

150 proteins
61 change
22 identified

Rellán et al. (2009) in preparation

viernes 27 de noviembre de 2009
Fe deficiency

Fe metabolomics/proteomics (root tips)

Beta vulgaris
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Metabolomics, Fiehn's lab, UC Davis

viernes 27 de noviembre de 2009
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Metabolomics, Fiehn's lab, UC Davis

Rellán et al. (2009) in preparation

viernes 27 de noviembre de 2009
Fe deficiency

Fe metabolomics/proteomics (root tips)
Beta vulgaris

Metabolomics, Fiehn’s lab, UC Davis

300 resolved
77 identified
26 change

Rellán et al. (2009) in preparation
Fe metabolomics/proteomics
Beta vulgaris

Rellán et al. (2009) in preparation
Fe metabolomics/proteomics
Beta vulgaris
Fe metabolomics/proteomics

Beta vulgaris

Rellán et al. (2009) in preparation

increase DMRL
Fe metabolomics/proteomics
Beta vulgaris

Fe deficiency

Rellán et al. (2009) in preparation

increase RFOs (raffinose, etc)
increase DMRL
Cd toxicity
Cd effects on tomato plant physiology
Cd effects on tomato plant physiology

<table>
<thead>
<tr>
<th>Control</th>
<th>10 µM Cd</th>
<th>100 µM Cd</th>
</tr>
</thead>
</table>

![Image of tomato plants under different Cd treatments]
Cd effects on tomato plant physiology
Cd effects on tomato plant physiology

López-Millán et al. (2009) Environ Exp Bot
Cd effects on tomato plant physiology

López-Millán et al. (2009) Environ Exp Bot

viernes 27 de noviembre de 2009
Cd effects on tomato plant physiology
Cd effects on tomato plant physiology

Table 2
Modulated chlorophyll fluorescence parameters in control and Cd-treated (10 and 100 μM Cd) tomato plants. The incident PPFD was between 130 and 170 μmol m⁻² s⁻¹. Data are means ± SD of 30 replications (3 batches of plants with 10 replicates per treatment). Data followed by the same letter within the same column are not significantly different (Student’s test) at the p < 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>F_v/F_m</th>
<th>Φ_PsII</th>
<th>Φ_exc</th>
<th>qP</th>
<th>NPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.81 ± 0.01 a</td>
<td>0.66 ± 0.05 a</td>
<td>0.68 ± 0.04 a</td>
<td>0.98 ± 0.01 a</td>
<td>0.21 ± 0.01 a</td>
</tr>
<tr>
<td>10 μM Cd</td>
<td>0.81 ± 0.01 a</td>
<td>0.62 ± 0.05 a</td>
<td>0.65 ± 0.04 a</td>
<td>0.96 ± 0.02 a</td>
<td>0.25 ± 0.04 ab</td>
</tr>
<tr>
<td>100 μM Cd</td>
<td>0.79 ± 0.02 a</td>
<td>0.60 ± 0.08 a</td>
<td>0.62 ± 0.08 a</td>
<td>0.96 ± 0.02 a</td>
<td>0.34 ± 0.10 b</td>
</tr>
</tbody>
</table>

López-Millán et al. (2009) Environ Exp Bot
Cd effects on tomato plant physiology

López-Millán et al. (2009) Environ Exp Bot

Table 2
Modulated chlorophyll fluorescence parameters in control and Cd-treated (10 and 100 μM Cd) tomato plants. Data are means ± SD of 30 replications (3 batches of plants with 10 replicates per treatment). Data followed by the same letter are not significantly different (Student’s test) at the p < 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>$F_v/F_m$</th>
<th>$\Phi_{PSII}$</th>
<th>$\Phi_{exc}$</th>
<th>qP</th>
<th>NPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.81 ± 0.01 a</td>
<td>0.66 ± 0.05 a</td>
<td>0.68 ± 0.04 a</td>
<td>0.98 ± 0.01 a</td>
<td>0.21 ± 0.01 a</td>
</tr>
<tr>
<td>10 μM Cd</td>
<td>0.81 ± 0.01 a</td>
<td>0.62 ± 0.05 a</td>
<td>0.65 ± 0.04 a</td>
<td>0.96 ± 0.02 a</td>
<td>0.25 ± 0.04 ab</td>
</tr>
<tr>
<td>100 μM Cd</td>
<td>0.79 ± 0.02 a</td>
<td>0.60 ± 0.08 a</td>
<td>0.62 ± 0.08 a</td>
<td>0.96 ± 0.02 a</td>
<td>0.34 ± 0.10 b</td>
</tr>
</tbody>
</table>

Little changes in photosynthesis
Cd effects on tomato plant physiology
 Cd effects on tomato plant physiology

López-Millán et al. (2009) Environ Exp Bot
Cd effects on tomato plant physiology

López-Millán et al. (2009) Environ Exp Bot

moderate chlorosis
Cd effects on tomato plant physiology
Cd effects on tomato plant physiology

control  10 \(\mu M\) Cd  100 \(\mu M\) Cd
Cd effects on tomato root proteomics
Cd effects on tomato root proteomics

control 10 μM Cd 100 μM Cd

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Cd effects on tomato root proteomics

control | 10 µM Cd | 100 µM Cd

A. 194 spots
B. 192 spots
C. 164 spots

Rodríguez-Celma et al., in preparation
Cd toxicity

Cd effects on tomato root proteomics
Cd effects on tomato root proteomics

10 µM Cd

100 µM Cd

Rodríguez-Celma et al., in preparation
Cd effects on tomato root proteomics

10 μM Cd

100 μM Cd

33 change
27 identified
Cd effects on tomato root proteomics

10 µM Cd

33 change
27 identified

100 µM Cd

41 change
33 identified

Rodríguez-Celma et al., in preparation
Cd effects on tomato root proteomics
Cd effects on tomato root proteomics

Rodríguez-Celma et al., in preparation
Cd effects on tomato root proteomics

Fe deficiency-like changes

A
Plant Stress

Others
Glycolysis
Respiration
TCA Cycle

10 μM Cd

B
Plant Stress

Others
Glycolysis
Respiration
TCA Cycle

100 μM Cd

 Cd toxicity

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Cd effects on tomato root proteomics

Fe deficiency-like changes

other changes

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viernes 27 de noviembre de 2009
Zn toxicity
Zn effects on sugar beet plant physiology
Zn effects on sugar beet plant physiology

Sagardoy et al. (2009) Plant Biology
Zn effects on sugar beet plant physiology

Sagardoy et al. (2009) Plant Biology

Zn toxicity

growth decreases
Zn effects on sugar beet plant physiology

1.2 µM Zn

100 µM Zn

300 µM Zn
Zn effects on sugar beet plant physiology
Zn effects on sugar beet plant physiology

Sagardoy et al. (2009) Plant Biology
Zn effects on sugar beet plant physiology

Sagardoy et al. (2009) Plant Biology

Zn toxicity

Sagardoy et al. (2009) Plant Biology
Zn effects on sugar beet plant physiology

Sagardoy et al. (2009) Plant Biology

- Moderate chlorosis
- Changes in VAZ cycle at low Zn

Zn toxicity
Zn effects on sugar beet photosynthesis
Zn effects on sugar beet photosynthesis

Sagardoy et al. (2009) Plant Biology

Zn toxicity
Zn effects on sugar beet photosynthesis.

Sagardoy et al. (2009) Plant Biology
Zn effects on sugar beet photosynthesis

<table>
<thead>
<tr>
<th>Zn treatment</th>
<th>1.2 μM</th>
<th>100 μM</th>
<th>300 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_N$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>$21.4 \pm 1.2^a$</td>
<td>$12.4 \pm 1.4^b$</td>
<td>$11.1 \pm 1.8^b$</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>$0.821 \pm 0.001^a$</td>
<td>$0.807 \pm 0.005^a$</td>
<td>$0.802 \pm 0.008^a$</td>
</tr>
<tr>
<td>ETR (μmol e$^{-}$ m$^{-2}$ s$^{-1}$)</td>
<td>$143.8 \pm 3.1^a$</td>
<td>$114.0 \pm 5.8^a$</td>
<td>$120.0 \pm 17.3^a$</td>
</tr>
<tr>
<td>$g_s$ (mol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>$0.231 \pm 0.033^a$</td>
<td>$0.070 \pm 0.014^b$</td>
<td>$0.055 \pm 0.010^b$</td>
</tr>
<tr>
<td>$g_m$ (mol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>$0.389 \pm 0.091^a$</td>
<td>$0.243 \pm 0.055^{ab}$</td>
<td>$0.204 \pm 0.048^b$</td>
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<tr>
<td>$C_{a}$ (μmol CO$_2$ mol$^{-1}$ air)</td>
<td>$286 \pm 8^a$</td>
<td>$200 \pm 11^b$</td>
<td>$176 \pm 17^b$</td>
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<tr>
<td>$C_{c}$ (μmol CO$_2$ mol$^{-1}$ air)</td>
<td>$221 \pm 19^a$</td>
<td>$143 \pm 14^b$</td>
<td>$115 \pm 9^b$</td>
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<tr>
<td>$V_{max}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
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<tr>
<td>$J_{max}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
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<td>$122.6 \pm 1.0$</td>
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Sagardoy et al. (2009) in preparation

Zn toxicity decreases in stomatal conductance

Plant Stress Physiology

viernes 27 de noviembre de 2009
Zn effects on sugar beet photosynthesis

Zn toxicity

Sagardoy et al. (2009), in preparation

Zn treatment | 1.2 µM | 100 µM | 300 µM
---|---|---|---
$A_N$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$) | 21.4 ± 1.2$^a$ | 12.4 ± 1.4$^b$ | 11.1 ± 1.8$^b$
$F_{v}/F_{m}$ | 0.821 ± 0.001$^a$ | 0.807 ± 0.005$^a$ | 0.802 ± 0.008$^a$
ETR (µmol e$^{-}$ m$^{-2}$ s$^{-1}$) | 143.8 ± 3.1$^a$ | 114.0 ± 5.0$^b$
SII | 0.218 ± 0.005$^a$ | 0.173 ± 0.08
$g$ (mol CO$_2$ m$^{-2}$ s$^{-1}$) | 0.231 ± 0.033$^a$ | 0.070 ± 0.014$^b$ | 0.055 ± 0.010$^b$
$g_m$ (mol CO$_2$ m$^{-2}$ s$^{-1}$) | 0.389 ± 0.091$^a$ | 0.243 ± 0.055$^a$ | 0.204 ± 0.048$^b$
$C_4$ (µmol CO$_2$ mol$^{-1}$ air) | 286 ± 8$^a$ | 200 ± 11$^b$ | 176 ± 17$^b$
$C_c$ (µmol CO$_2$ mol$^{-1}$ air) | 221 ± 19$^a$ | 143 ± 14$^b$ | 115 ± 9$^b$
$V_{max}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$) | 104.4 ± 4.5 | 102.8 ± 5.0 | 126.5 ± 19.1
$J_{max}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$) | 128.7 ± 6.7 | 122.6 ± 1.0 | -

decreases in stomatal conductance
Zn toxicity

Zn effects on sugar beet photosynthesis

Sagardoy et al. (2009), in preparation

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decreases in stomatal conductance

decreases in mesophyll conductance

decreases in stomatal conductance

Sagardoy et al. (2009) Plant Biology

viernes 27 de noviembre de 2009

Plant Stress Physiology

CSIC
Zn effects on sugar beet stomata
Zn effects on sugar beet stomata

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<tr>
<td>Density (stomata mm⁻²)</td>
<td>218 ± 7ᵃ</td>
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<td>156 ± 12ᵇ</td>
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<td>6-15</td>
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Sagardoy et al. (2009), in preparation

1.2 µM Zn

300 µM Zn

Plant Stress Physiology

viernes 27 de noviembre de 2009
Zn effects on sugar beet structure
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1.2 µM Zn

300 µM Zn

Sagardoy et al. (2009), in preparation

Zn toxicity
Zn effects on sugar beet structure
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1.2 µM Zn
Zn toxicity

Zn effects on sugar beet structure

1.2 μM Zn

300 μM Zn
Zn effects on sugar beet structure

1.2 µM Zn

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300 µM Zn
Zn and Cd in xylem
### Zn and Cd in xylem

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<td>Control</td>
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<td>Zn 10 μM</td>
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Zn and Cd forms of transport
Hg toxicity
Hg toxicity

Biothiol-Hg complexes in standards
Hg toxicity
Biothiol-Hg complexes in standards

HPLC-MS(ToF)
Hg toxicity
Biothiol-Hg complexes in standards

HPLC-MS(ToF)

![Graph showing Hg toxicity and Biothiol-Hg complexes in standards]
Hg toxicity
Biothiol-Hg complexes in standards
HPLC-MS(ToF)
Fig. 4. HPLC-ESI-MSTOF mass spectra of a Hg₂P₂C₆ mixture standard solution (25:50 μM in 0.1% formic acid) acquired in the 50-1000 (A) and 900-3000 (B) m/z ranges. Experimental and theoretical isotopic signatures of the identified ions are shown in insets.
Hg toxicity
Biothiol-Hg complexes in standards

Fig. 4. HPLC-ESI-MSTOF mass spectra of a Hg,hpc₃ mixture standard solution (25:50 μM in 0.1% formic acid) acquired in the 50-1000 (A) and 900-3000 (B) m/z ranges. Experimental and theoretical isotopic signatures of the identified ions are shown in insets.
Fig. 4. HPLC-ESI-MSTOF mass spectra of a Hg-hPC₃ mixture standard solution (25:50 μM in 0.1% formic acid) acquired in the 50-1000 (A) and 900-3000 (B) m/z ranges. Experimental and theoretical isotopic signatures of the identified ions are shown in insets.
Fig. 4. HPLC-ESI-MSTOF mass spectra of a Hg$_2$PC$_2$ mixture standard solution (25:50 μM in 0.1% formic acid) acquired in the 50-1000 (A) and 900-3000 (B) m/z ranges. Experimental and theoretical isotopic signatures of the identified ions are shown in insets.
Fig. 4. HPLC-ESI-MSTOF mass spectra of a Hg-hPC$_2$ mixture standard solution (25:50 μM in 0.1% formic acid) acquired in the 50-1000 (A) and 900-3000 (B) m/z ranges. Experimental and theoretical isotopic signatures of the identified ions are shown in insets.
Hg toxicity
Hg toxicity

Biothiol-Hg complexes in plant extracts
Hg toxicity

Biothiol-Hg complexes in plant extracts
Hg toxicity

Biothiol-Hg complexes in plant extracts

Hg(Glu-Cys)_2

HPLC-MS(TOF)
Hg toxicity

Biothiol-Hg complexes in plant extracts

Hg(Glu-Cys)$_2$

Hg(Glu-Cys)$_2$-Gly

HPLC-MS(ToF)
Thank you for your attention!
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