Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

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- Pesticides analysis in water
- Ecotoxicity bioassays
- Pesticides analysis in shellfish

RESULTS
- Pesticides in water
- Toxicity in water
- Pesticides in shellfish

CONCLUSIONS

Acknowledgements

Marine bacteria
Vibrio fischeri

Zooplancton
Daphnia magna

Alga Selenastrum
capricornutum
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

INTRODUCTION

The Ebro river

- It is Spain's most voluminous river (910 km).
- It flows through many cities before discharging in a delta on the Mediterranean Sea in the province of Tarragona.

The Ebro delta

- It is one of the largest wetland areas (320 km²) in the western Mediterranean region.
- It hosts numerous beaches, marshes and salt pans that provide habitat for over 300 species of birds.
- A network of canals and irrigation ditches are helping to maintain the ecologic and economic resources of the Ebro Delta.

- It is in intensive agricultural use for rice, fruit and vegetables.
- The rice growing areas are situated in the Delta del Ebro Natural Park where there is also shellfish farming.
- Pesticides typical of rice crops frequently used here include propanil, bentazon, MCPA and molinate. The insecticides fentrotrothion and malathion are employed occasionally if necessary.
- Recently, the seafood farmers have complained about a loss of production in the periods of rice cultivation which has raised also public concern about the toxicity of the water in this area.
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

**OJBETIVES**

**Main objective**

To investigate the role of pesticides in the mortality of shellfish in the Ebro river during the main growing season of rice

**Sub-objectives**

- To assess residual concentrations of pesticides in water and biota.
- To assess toxicity of water samples.
- To investigate potential correlations between pesticides and ecotoxicity data with shellfish mortality episodes.
- To identify possibly responsible compounds.

**SITE DESCRIPTION and SAMPLES**

A total number of 108 samples have been studied, of which 54 are from SOUTHERN hemidelta (three sample points) and the other 54 from NORTHERN hemidelta (three sample points).
SITE DESCRIPTION and SAMPLES

Physical-chemical parameters (Fangar bay)

Temperature (°C)
Conductivity (µS/cm)
Dissolved oxygen (mg/L)
pH
Turbidity (NTU)
Redox potential (mV)

CONCLUSIONS

Acknowledgements
METHODOLOGY

**Pesticides analysis in water**

**EBRO RIVER DELTA**

Water samples

Liquid Chromatography (LC-ESI(+)-MS/MS)

Filtration
(0.4 um nylon filters)

On-line Solid Phase Extraction
(sample volume = 5+5mL)

Results

### CONCLUSIONS

Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

**METHODOLOGY**

**Pesticides analysis in water**

**LC-MS/MS conditions**

- Column: Purospher STAR RP-18E (125x2 mm, 5µm, Merck)
- Mobile phase: Gradient ACN/H2O (0.2 mL/min)
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METHODOLOGY
Pesticides analysis in water

<table>
<thead>
<tr>
<th>%R (n=5)</th>
<th>LOD (ng/L)</th>
<th>LODQ (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC</td>
<td>HPLC</td>
</tr>
<tr>
<td></td>
<td>Agua superficial</td>
<td>Agua superficial</td>
</tr>
<tr>
<td>Atrazina</td>
<td>80</td>
<td>0.583</td>
</tr>
<tr>
<td>Simazina</td>
<td>80</td>
<td>0.514</td>
</tr>
<tr>
<td>Cianazina</td>
<td>91</td>
<td>0.540</td>
</tr>
<tr>
<td>Desetil-atrazina</td>
<td>80</td>
<td>0.784</td>
</tr>
<tr>
<td>Metinpropfluotrine</td>
<td>80</td>
<td>0.662</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>72</td>
<td>0.254</td>
</tr>
<tr>
<td>Fenitrotión</td>
<td>80</td>
<td>0.784</td>
</tr>
<tr>
<td>Alachlor</td>
<td>97</td>
<td>0.910</td>
</tr>
<tr>
<td>Mecoprop</td>
<td>100</td>
<td>0.944</td>
</tr>
<tr>
<td>Malathion</td>
<td>90</td>
<td>0.911</td>
</tr>
<tr>
<td>Pesticides</td>
<td>97</td>
<td>0.716</td>
</tr>
<tr>
<td>Methompr</td>
<td>99</td>
<td>0.979</td>
</tr>
<tr>
<td>Lambda</td>
<td>80</td>
<td>1.176</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>101</td>
<td>1.939</td>
</tr>
<tr>
<td>Mecoprop</td>
<td>90</td>
<td>0.776</td>
</tr>
<tr>
<td>2,4-D</td>
<td>80</td>
<td>0.482</td>
</tr>
<tr>
<td>Propamocarb</td>
<td>96</td>
<td>0.591</td>
</tr>
<tr>
<td>MCPA</td>
<td>92</td>
<td>0.277</td>
</tr>
<tr>
<td>Propamocarb</td>
<td>97</td>
<td>0.618</td>
</tr>
</tbody>
</table>
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

METHODOLOGY

Pesticides analysis in water

Criteria for positive confirmation

Standard aqueous solution:

SRM 1: 216 → 174
SRM 2: 216 → 132
Concentration: 5 ng/L.

Sample:

SRM 1: 216 → 174
SRM 2: 216 → 132
Concentration: 2.97 ng/L.

Rt_{sample} = Rt_{standard} ± 2% 

Ratio SRM1/SRM2_{sample} = Ratio SRM1/SRM2_{standard} ± 20-50%
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

**METODOLOGY**

Ecotoxicity bioassays

**OVERVIEW**

**INTRODUCTION**

**OBJETIVES**

**SITE DESCRIPTION and SAMPLES**

**METHODOLOGY**

- Pesticides analysis in water
- Ecotoxicity bioassays
  - Toxicity assay *Selenastrum capricornutum* according to ISO 8692
  - Toxicity assay *Vibrio fischeri* according to ISO 11348-3
  - Toxicity assay *Daphnia magna* according to ISO 6341

**RESULTS**

- Pesticides in water
- Toxicity in water
- Pesticides in shellfish

**CONCLUSIONS**

Acknowledgements

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**TARGET PESTICIDES (7)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Chemical class</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicides</td>
<td>Anilides</td>
<td>Propanil</td>
</tr>
<tr>
<td></td>
<td>Thiocarbamates</td>
<td>Molinate</td>
</tr>
<tr>
<td></td>
<td>Acids</td>
<td>Bentazone</td>
</tr>
<tr>
<td>Insecticides</td>
<td>Pyrethroids</td>
<td>Cypermethrin</td>
</tr>
<tr>
<td></td>
<td>Organophosphorus</td>
<td>Malathion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maloxon (malathion TP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fenitrothion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fenitrothion oxon</td>
</tr>
</tbody>
</table>

SS: acetochlor D11, alachlor D13 y ethyl parathion D10
RESULTS

Pesticides in water

Frequency of detection and average and maximum concentration of individual pesticides

- HEIMD, draining channel in the hemidelta left; HDAD, draining channel in the hemidelta right

Mortality episodes

Acknowledgements
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

RESULTS

Pesticides in water

Mortality episodes

HEM, hemidelta left (Fangar bay); HDM, hemidelta right (Alfacs bay)

- Malathion
- Bentazone
- Molinate
- MCPA
- Fenitrothion
- Other pesticides

The five most abundant pesticides in water

MALATHION 5825.33 ng/L*
BENTAZONE 3286.38 ng/L*
MOLINATE 3589.83 ng/L*
MCPA 4196.70 ng/L*
FENITROTHION 1196.95 ng/L*

* Maximum individual concentrations.
Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

RESULTS Pesticides in water

Malathion levels in water

RESULTS
- Pesticides in water
- Toxicity in water
- Pesticides in shellfish

CONCLUSIONS

Acknowledgements
### RESULTS Pesticides in water and their THEORETICAL toxicity

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Chloroacets</th>
<th>Triazines</th>
<th>Anilides and Chloroacetamides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bioassays</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical toxicity - V. Fischeri ( T_{\text{theoretical}} = \sum C_i \times T_{\text{UI}} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesticide</td>
<td>Chloroacets</td>
<td>Triazines</td>
<td>Anilides and Chloroacetamides</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
<td>-----------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>malathion</td>
<td>100</td>
<td>0.002</td>
<td>0.015</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>6600</td>
<td>0.010</td>
<td>23</td>
</tr>
<tr>
<td>desethylatrazine</td>
<td>50</td>
<td>0.022</td>
<td>16</td>
</tr>
<tr>
<td>terbutylazine</td>
<td>10</td>
<td>0.015</td>
<td>10</td>
</tr>
<tr>
<td>( C_{\text{UI}} = \frac{C_{\text{EC50}}}{100} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>100</td>
<td>0.002</td>
<td>0.015</td>
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<tr>
<td>Fenitrothion</td>
<td>6600</td>
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<tr>
<td>Terbutylazine</td>
<td>10</td>
<td>0.015</td>
<td>10</td>
</tr>
</tbody>
</table>

**The most representative organism:**

Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation.
RESULTS Toxicity in water

Mortality episodes occurred often 1-3 days after water toxicity tests indicated toxicity.

Ecotoxicological responses of tests & shellfish mortality episodes

Samples with %I > 30% are considered TOXIC SAMPLES

Toxic samples: for Selenastrum indicates possible residues of herbicides and metals.

% Inhibition measured and toxicity units calculated as a simple additive approach.
RESULTS Pesticides in shellfish

Levels (mg/kg) of pesticides in biota

<table>
<thead>
<tr>
<th>Samples</th>
<th>Molinate</th>
<th>Propanil</th>
<th>Fenitrothion</th>
<th>Malathion</th>
<th>Bentazone</th>
<th>Cipermetrine</th>
<th>Maloxon</th>
<th>Fenitrothion Oxon</th>
</tr>
</thead>
</table>

* mortality episodes

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Water toxicity and pesticides loads in water and seafood in the Ebro river delta during rice cultivation

RESULTS Pesticides in shellfish

<table>
<thead>
<tr>
<th>Pesticide aquatic species (scientific name)</th>
<th>LC50 (µg/L)</th>
<th>classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion Mussel (Mytilus galloprovincialis)</td>
<td>6000</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Malathion Mussel (Mytilus edulis)</td>
<td>123000</td>
<td>No acute toxicity</td>
</tr>
<tr>
<td>Maloxon Fish Medaka (Oryzias latipes)</td>
<td>365</td>
<td>Highly toxic</td>
</tr>
<tr>
<td>Maloxon Carpa (Cyprinus carpio)</td>
<td>3.46 (fenitrothion), 3.62 (maloxon)</td>
<td></td>
</tr>
<tr>
<td>Maloxon Mussel (Mytilus galloprovincialis)</td>
<td>9090</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Maloxon Carpa (Cyprinus carpio)</td>
<td>25138</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Fenitrothion Mussel (Mytilus edulis)</td>
<td>123000</td>
<td>No acute toxicity</td>
</tr>
<tr>
<td>Fenitrothion Fish Medaka (Oryzias latipes)</td>
<td>4911</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Fenitrothion Carpa (Cyprinus carpio)</td>
<td>3938</td>
<td>Moderately toxic</td>
</tr>
</tbody>
</table>

* mortality episodes
** big mortality episode (around 60 %)
CONCLUSIONS

- With respect to the Maximum Allowable Concentration (MAC) stipulated by the EU for 5 of the 22 pesticides in inland and other surface waters (Directive 2008/105/EC), only alachlor was found to exceed the proposed limit of 700 ng/L in two of the samples investigated.

- The northern bay is more contaminated than the southern bay (larger in size) and in both bays the inner sites are more contaminated than the outer sites (dilution).

- Most of toxicity responses were explained using an additive approach with concentrations of pesticides measured by LC-MS/MS and TU of each pesticide (100/EC50).

- Modulation of the toxicological responses can be attributed to synergic effects, matrix effects and possible presence of metals.

- The most representative organism was, as expected, Daphnia magna, a micro crustacean.

- Among the compounds analyzed in water discharges from rice, the most problematic compounds were:
  - in terms of abundance and ubiquity: bentazone, MCPA, propanil, malathion and molinate
  - taking into account toxicity: malathion, diazinon, molinate and fenitrothion
This work has been supported by the Catalan Water Agency and by the Spanish Ministry of Science and Innovation through the projects CEMAGUA [CGL2007-64551/HID] and SCARCE [CSD2009-00065], and reflects the author’s view.

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