Toxic effects of copper and cadmium on fertilization potency of gametes of Pacific oyster (Crassostrea gigas)

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Introduction

In the present study, the effects of copper (Cu) and cadmium (Cd) to the Pacific oyster Crassostrea gigas gametes were investigated. Exposure to metals concentrations of 1, 5, 10, 20, 40 μg.L⁻¹ for Cu and 100, 200, 400, 800, 1600 μg.L⁻¹ for Cd for 30 min in seawater caused significant adverse effects on the fertilization success of sperm [effective concentration (EC)50=20 μg.L⁻¹ for Cu, EC50=830 μg.L⁻¹ for Cd] and oocytes (EC50=57 μg.L⁻¹ and 1350 μg.L⁻¹, respectively). Lowest observed effect concentrations (LOEC) for spermototoxicity assay were 1 μg.L⁻¹ for Cu and 100 μg.L⁻¹ for Cd. However, LOEC values for fertilization success of Cu-exposed and Cd-exposed oocytes were obtained at higher concentrations: 10 μg.L⁻¹ and 400 μg.L⁻¹ for Cu and Cd respectively. It can therefore be concluded that Cu was more toxic than Cd to oyster C. gigas gametes where spermatozoids were seemingly more sensitive than oocytes. The study showed that these metals could adversely affect reproduction at environmentally realistic concentrations.

Materials and Methods

Analytical grade of copper sulfate, cadmium chloride and formalin solution were purchased from Sigma-Aldrich Chemical (St. Quentin Fallavier, France). Seawater was collected from Arcachon Bay (SW of France), an area where oysters reproduce naturally. Immediately after sampling, seawater was filtered using 0.2 µm pore membrane filter. Filtered seawater (FSW) was stocked at 4°C in the dark and was used within 3 days. Mature oysters (Crassostrea gigas) came from a commercial hatchery specialized in the year-round production of mature oysters (Guernsey Sea Farms, UK). All oysters were used within 3 days.

Stock solutions of metals (500 mg.L⁻¹ for Cd and 250 mg.L⁻¹ for Cu) were prepared in Milli-Q water. Test concentrations of either metal were prepared by diluting the stock solution in FSW. The test concentrations were 1, 5, 10, 20, 40 μg.L⁻¹ for Cu and 100, 200, 400, 800, 1600 μg.L⁻¹ for Cd. In each experiment, FSW was used as negative control. All glasswares were acid-washed before the experiments. Experimental solutions were acidified with 1% HNO₃ acid-washed before the experiments. Experimental solutions were acidified with 1% final v/v 65% nitric acid and were then analyzed for Cd or Cu content by ICP-AES (Varian Vista ProAxial, Aglient Technologies, USA) using standard conditions (Table 1). Detection limits were 1 μg.L⁻¹ for water samples.

The spermototoxicity and oocyte toxicity tests have been described in details previously. Briefly, sperms cells and oocytes were exposed to metals for 30-min before they were used for fertilization. Two fertilization assays were then conducted. For Assay (1), 1.0 mL of exposed sperm solution was added to 10 mL of FSW containing unexposed oocytes. For Assay (2) 1.0 mL of unexposed sperm solution was added to 10 mL of FSW containing exposed oocytes. Embryos were incubated at 24°C for 2 h until the 2-4 cell-stage was attained in the control. To calculate the fertilization rate (FR), unfertilized oocytes were scored under an inverted microscope (Olympus, magnification x 200) among 100 oocytes. Data are expressed as mean±SEM (standard error mean). Differences in fertilization success were assessed for significance by one-way analysis of variance and Tukey post hoc test. The EC50 defined here as the toxicant concentration tested for both Cu and Cd.

Results and Discussion

Nominal and measured concentrations of Cu and Cd for the different applied treatments were determined (Table 1). Measured concentrations were within 10-23% of the nominal concentrations. Therefore, nominal concentrations were used for presentation and calculation of toxicity parameters. The background level of unfertilized eggs in the controls was low as well as the response variability between replicates (ca 7%). A significant decrease of the fertilization rate was observed after sperm exposure (Assay 1) to the lowest Cu and Cd concentrations tested (1 µg.L⁻¹ and 100 µg.L⁻¹, respectively) as compared to the control (P<0.05) (Figure 1). The fertilization success was reduced to 20% at the highest concentration tested for both Cu and Cd. Calculated EC50 were 20 µg.L⁻¹ for Cu and 827 µg.L⁻¹ for Cd (Table 2). The non-observed effect concentrations (NOECs) for Cu and Cd.
in this assay was below 1 and 100 µg.L⁻¹ respectively. For oocyte exposure (Assay 2), Cu inhibited fertilization from 10 µg.L⁻¹ concentration and Cd impaired fertilization at concentration of 400 µg.L⁻¹ (Figure 1). The EC₅₀ and NOEC data for fertilization rate are summarized in Table 2.

To date, most bioassays using bivalves have been performed using either embryos and larvae or adults, while the sensitivity of spermatozoa and oocytes to toxicants are less understood. Most of the existing data on the effects of metals on sperm and eggs have been obtained with the sea-urchin¹¹,¹² and to a lesser extend with blue mussel.¹³ Herein it is shown that Cu and Cd can induce deleterious effects in C. gigas gametes. Cu pre-exposure did significantly decrease fertilization capacity of oyster spermatozoa at very low concentrations (1 µg.L⁻¹) consistent with those measured in harbor environment. Sperm cells of Pacific oyster seem to be more sensitive to Cu than spermatozoa from other marine invertebrates such as blue mussel¹³ and sea squirt¹⁴ with LOEC at 100 µg.L⁻¹ and 1,024 µg.L⁻¹, respectively. In the present study, the deduced EC₅₀ of Cu for oyster sperm is 20 µg.L⁻¹. This EC₅₀ value is very close to that reported for spermatozoa of various sea urchins.¹⁵ Indeed, they reported an EC₅₀ values of 25 µg.L⁻¹ Cu for purple sea urchin and 59 µg.L⁻¹ Cu for green sea urchin. Cd was less toxic to Pacific oyster sperm cells than Cu with significant adverse effects observed after 30-min Cd exposure at 100 µg.L⁻¹ Cd. The Cd concentration causing 50% unsuccessful fertilization on spermatozoa was 830 µg.L⁻¹ in this study (Table 2), whereas EC₅₀ values for several sea urchin species¹⁵ ranged from 8000 to

![Figure 1. Percentages (mean±SEM) of fertilization success after gamete exposure to (A) copper or (B) cadmium for 30 min. Asterisks indicate statistical differences between control and exposed treatments at *P<0.05, **P<0.01, ***P<0.001.](image)

Table 1. Copper and cadmium concentrations (µg.L⁻¹) determined for exposure solutions.

<table>
<thead>
<tr>
<th></th>
<th>Copper</th>
<th>Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal</td>
<td>0*</td>
<td>1</td>
</tr>
<tr>
<td>Measured</td>
<td>&lt;1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>12.6</td>
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<td></td>
<td>21.1</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td>&lt;1</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td>910</td>
<td>1610</td>
</tr>
</tbody>
</table>

*Control seawater.

Table 2. Mean effective concentrations (EC₅₀) and their 95% coefficients of variation, and non-observed effect concentration (NOEC) values for different toxicity assays with C. gigas for cadmium and copper. Less than (<) values are given for cases where NOEC could not be calculated.

<table>
<thead>
<tr>
<th>Bioassays</th>
<th>EC₅₀ (µg.L⁻¹)</th>
<th>Cd (µg.L⁻¹)</th>
<th>NOEC</th>
<th>EC₅₀ (µg.L⁻¹)</th>
<th>Cu (µg.L⁻¹)</th>
<th>NOEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatoxicity</td>
<td>827 (681-1005)</td>
<td>&lt;100</td>
<td></td>
<td>20.3 (17.8-23.1)</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Oocyte toxicity</td>
<td>1346 (1258-1440)</td>
<td>200</td>
<td>57.1 (37.1-88.0)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryotoxicity*</td>
<td>212 (182-277)</td>
<td>&lt;100</td>
<td>12.5 (11.0-14.2)</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cd, cadmium; Cu, copper; EC₅₀ effective concentrations; NOEC, non-observed effect concentration. *Embryotoxicity data were previously published.¹⁶

[page 24]
26,000 µg.L⁻¹. Unlike spermiotoxicity, eggs fertilization was only altered after exposure to high exposure concentrations: 10 µg.L⁻¹ for Cu and 400 µg.L⁻¹ for Cd. This is consistent with previous studies in which acute exposure of oocytes had no, or limited, effect on fertility.¹⁰,¹¹,¹³ A likely explanation is that the complex envelop of oocyte may act as a protective barrier against metals or other contaminants accumulation into eggs.¹⁴ As previously reported,⁹ Pacific oyster embryos are also very sensitive to Cu and Cd inducing embryotoxicity at 0.1 µg.L⁻¹ for Cu and 10 µg.L⁻¹ for Cd. The EC₅₀ values for embryotoxicity reached 12 µg.L⁻¹ for Cu and 210 µg.L⁻¹ for Cd (Table 2). The results are in good agreement with existing data on EC₅₀ values of embryotoxicity assays of those metals in other marine invertebrate species.²,¹⁵ The results confirm previous studies¹¹ demonstrating that Pacific oyster embryos were more sensitive than sperm or oocytes to pollutant exposure. Gametes and embryos were relatively resistant to Cd toxicity compared to Cu. The impact of Cd has apparently no ecological relevance as the effects on oyster early life stages only appear at environmentally unrealistic concentrations. We can, however, expect that Cu could represent a threat for the reproduction of wild or cultivated Pacific oysters in particular in the Arcachon Bay where concentrations exceeding 0.7 µg L⁻¹ are currently measured.¹⁶

References


