Mixture Toxicity of Some Antibiotics to Fish, Mosquito and Cyanobacteria

El-Nahhal Yasser*, EL-dahdouh Nabila, Alshanti Adli

Department of Enverionmental and Earth science, Faculty of science, The Islamic University-Gaza, Gaza Strip, Palestine

The Islamic University-Gaza
14-11-2015

introduction

• Antibiotics are pharmaceuticals widely used not only for human and veterinary medication but also for livestock and aquaculture growth promotion (Sarmah et al., 2006).
• After normal application of the antibiotics, 50 to 90% of them and/or their metabolites are eliminated from the body, mainly through urine and feces, which then enter the environment indirectly through sewage treatment plants or directly through fertilizers application to agricultural land (Schlusener & Bester, 2006).
objectives

1. To investigate the toxicity to fish and mosquitoes using concentrations far below the U.S. official tolerance for amoxicillin/erythromycin (10 mg Kg\(^{-1}\)) in milk and uncooked edible tissue of cattle.

2. To characterize the phytotoxicity of Penicillin G, Tylosin tartrate, and Ciprofloxacin hydrochloride, as individuals and mixtures to cyanobacterial mats as an aquatic model of vegetation and

3. To study the toxico-dynamic of certain concentrations on the toxicity to cyanobacteria.

Materials and Methods

1. Tested Chemicals used Amoxicillin (AM), Erythromycin (ER), Ciprofloxacin hydrochloride (cipro), Penicillin, and Tylosin were purchased from Birzeit-Palestine Pharmaceutical company.

2. Standard toxic chemicals: Diuron and Endosulfan (EN) were purchased from Sigma, Germany and used in this study to as standard toxic substances.
Tested organisms (Fish, Mosquitoes and cyanobacteria)
Breading and acclimatization of fish and mosquito (2 weeks breading under laboratory conditions 12/12 light dark cycles).

Figure 1. Chemical structure of the tested compounds
Table 1. Physicochemical properties of the tested chemicals

<table>
<thead>
<tr>
<th>Properties</th>
<th>AM</th>
<th>ER</th>
<th>EN</th>
<th>CI</th>
<th>PE</th>
<th>TY</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>365.4</td>
<td>733.9</td>
<td>406.9</td>
<td>331.3</td>
<td>334.4</td>
<td>917.1</td>
<td>233.1</td>
</tr>
<tr>
<td>Solubility mg L⁻¹</td>
<td>3430</td>
<td>1.44</td>
<td>0.32</td>
<td>1.1</td>
<td>22</td>
<td>5000</td>
<td>36.4</td>
</tr>
<tr>
<td>Kₐw</td>
<td>0.9</td>
<td>2.8</td>
<td>3.5</td>
<td>2.3</td>
<td>1.67</td>
<td>13</td>
<td>2.85</td>
</tr>
<tr>
<td>pKa</td>
<td>3.39</td>
<td>8.9</td>
<td>-</td>
<td>5.64</td>
<td>2.62</td>
<td>3.41</td>
<td>na</td>
</tr>
</tbody>
</table>

**Toxicity tests to cyanobacterial mats:**

This test based on evaluation of population growth density using the changes of optical density of bacterial growth media.

1. **Collection of cyanobacteria from Wadi Gaza**
2. **Preparation of growth media**
3. **Breading of cyanobacterial mats under lab conditions.**
4. **Single and mixture toxicity tests**
   - The following concentrations 0, 0.2, 1, 2, 3, and 4 mg/L of the antibiotics and 0, 0.4, 4, 8, 12, 16 mg/l. diuron were prepared and added to round bottom flasks containing 1ml of cyanobacterial mat and growth media of total volume of 50 ml.
Calculation of toxicity parameters

Toxicity Tests on Fish and mosquitoes based on calculating mortality % viruses tested concentrations using equation 1

\[ \text{Mortality} \% = 100 \times \frac{(L_c - L_t)}{L_c} \quad (\text{Eq 1}) \]

where \( L_c \) and \( L_t \) are the number of live organisms in the control and the treated samples respectively.

The dynamics of Cyanobacterial mats growth (relative growth) in the control samples evaluated by plotting time versus the \( \frac{OD_{\text{max}}}{OD_0} \) ratio, where \( OD_{\text{max}} \) and \( OD_0 \) are the optical densities at maximum growth and growth at time zero respectively.

Relative toxicity (RT) was calculated according to equation 2.

\[ \text{RT} = \frac{LCt_{50}}{LCs_{50}} \quad (\text{Eq 2}) \]

Where \( LCt_{50} \) and \( LCs_{50} \) are the lethal concentration of the tested and the standard compounds, respectively.

LC50 values were calculated from the log scale of Mortality (%) - Concentration relationship.
Mixture toxicity index (MTI)

To estimate the synergetic and/or the antagonistic effects of antibiotics mixtures we calculated the mixture toxicity index (MTI) (Table 3) proposed by Konemann, (1985).

\[ \text{MTI} = 1 - \left( \frac{\text{Log } M}{\text{Log } n} \right), \text{ where } M = \sum C / EC_{50} \]

at 50% effect in the mixture, and \( n = \text{ total number of compounds in the mixture} \).

Toxicity of Amoxicillin and Erythromycin to Fish and Mosquitoes

EL-NAAHAL YASSER & EL-DAHDOUH NABILA

Department of Environmental and Earth Science. Faculty of Science. The Islamic University-Gaza, Gaza Strip, Palestine.

(Received November 27, 2014; Accept April 22, 2015)

Abstract

This study characterized the toxicity of Amoxicillin (AM), Erythromycin (ER) and Endosulfan (EN) to fish and mosquito larvae obtained from a certified fish farm and from Wadi Gaza. The acute toxicity tests were determined by calculating mortality percentage of fish and mosquitoes through a gradient concentration of the tested compounds. Mortality percentage, exposure time, \( LC_{50} \) and \( LT_{50} \) were taken as indicators of toxicity. The results showed that AM was the potent compound against fish with \( LC_{50} \) value lower that EN. Toxicity to fish is in the following order: AM > EN > ER whereas the toxicity to mosquitoes is in the following order: ER > EN > AM. Fish are more sensitive to AM than mosquitoes whereas mosquitoes are more sensitive to ER than fish. The interesting outcome of the study is the calculated \( LC_{50} \) values are far below the concentration found in different water systems. Mixture toxicity of the tested antibiotics indicates antagonistic effect on both fish and mosquitoes.
Results
Single toxicity test on fish

Figure 2. Concentration response relationships of AM, ER and EN on fish and mosquito mortality. % mortality was recorded after 48 and 96 h for mosquito and fish respectively.

Figure 3. Time response relationships of diluted concentrations ER, AM and EN on fish and mosquito Mortality. Mortality percentage was recorded at a certain concentration of each compound (0.08 mg L⁻¹) overtime.
Table 2: toxicity parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fish</th>
<th>Mosquito</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>ER</td>
</tr>
<tr>
<td>LC$_{50}$ (µg L$^{-1}$)</td>
<td>35.72</td>
<td>242.7</td>
</tr>
<tr>
<td>RT</td>
<td>0.4</td>
<td>2.72</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Re Eq</td>
<td>y=0.14, x+1.48</td>
<td>y=0.17, x+1.29</td>
</tr>
<tr>
<td>LT$_{50}$ (h)</td>
<td>69</td>
<td>132</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.84</td>
<td>0.94</td>
</tr>
<tr>
<td>Re Eq</td>
<td>Y=0.6X+8.95, X-0.55</td>
<td>Y=0.38, X+1.48</td>
</tr>
</tbody>
</table>

Toxicity of Single and Mixtures of Antibiotics to Cyanobacteria

EL-Nahhal Yasser, Alchtani Adji
Department of Environmental and Earth Sciences, Faculty of Science, The Islamic University-Gaza, Palestinian Territory

Abstract

The present study was designed to investigate the disappearance of cyanobacterial mat from fish breeding lakes and to evaluate the phytotoxic effects of Penicillin, Ciprofloxacin, and Tylosin as single and as mixtures to cyanobacterial mats. Phytotoxic effects were measured as growth inhibition of cyanobacteria using spectrophotometer at 680 nm at low concentrations of antibiotics. Results showed potential phytotoxicity of the tested antibiotics with EC$_{50}$ values of Penicillin, Ciprofloxacin and Tylosin of 0.13, 0.71, 5.28 mg/L respectively. Relative toxicity indicated that Penicillin and Ciprofloxacin were more toxic to cyanobacterial mats than Diuron (standard toxic material). EC$_{50}$ values of binary mixtures are 0.077, 0.103, 0.292 TU for (Penicillin+Tylosin), (Ciprofloxacin+Tylosin) and (Ciprofloxacin+Penicillin) respectively, whereas EC$_{50}$ of the tertiary mixture is 0.034 TU. Statistical analysis of the results indicated significant differences between the toxic effects of compounds and their mixtures to cyanobacterial mats. Observation of toxicity over time indicated that cyanobacterial mats were able to overcome the toxic effects after approximately 72 h of exposure time. It can be concluded that antibiotics exert dangerous toxic effects to cyanobacterial mat, an important organism in the eco-system. These results are considered the first of its kind in Palestine.

Keywords: Cyanobacteria; Penicillin; Tylosin; Ciprofloxacin; Toxicity

Number of studies investigated the toxicity of antibiotics to various organisms. For instance toxicity on: Bacterial growth [11], Daphnia magna [20], soil bacteria [21], and algae [22].

Introduction
Figure 4: Population growth of cyanobacterial mats under laboratory conditions.

Figure 5: Acute toxicity profiles of Penicillin, Tylosin, Cipro, and Diuron to cyanobacterial mats under laboratory conditions.
Figure 6
Toxicodynamic effect of penicillin (40 mg/l), Tylosin (20 mg/l) on Cipro (0.2mg/l) and Diuron (4mg/l) on cyanobacterial mats growth after 96 h.

Figure 7 Effect of binary and tertiary of Penicillin, Cipro and/or Tylocin mixtures on cyanobacterial mats growth.
Figure 8
Toxicodynamic binary and tertiary mixtures: B1 (Penicillin + Tylosin), B2 (Tylosin + Cipro), B3 (Penicillin + Cipro) and T (Penicillin + ciprofloxacin + Tylosin) mixture (0.33: 0.33: 0.33) on the growth of cyanobacterial mats after 96h. Exposure time measured at concentration equals to 0.025 TU after 72 h.

Table 3. Toxicity parameters to CB

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC\textsubscript{50}</th>
<th>R\textsuperscript{2}</th>
<th>Reg. Eq</th>
<th>MTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuron</td>
<td>1.92 mg/l</td>
<td>0.82</td>
<td>Y=0.23X+1.6</td>
<td>Nd</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.71 mg/l</td>
<td>0.98</td>
<td>Y=0.73X+1.8</td>
<td>Nd</td>
</tr>
<tr>
<td>Tylosin</td>
<td>5.28 mg/l</td>
<td>0.98</td>
<td>Y=0.19X + 1.6</td>
<td>Nd</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.13 mg/l</td>
<td>0.96</td>
<td>Y=0.1X + 1.8</td>
<td>Nd</td>
</tr>
<tr>
<td>Penicillin 0.5: Tylosin 0.5 = B1</td>
<td>0.077*</td>
<td>0.92</td>
<td>Y = 19.9X +72</td>
<td>-3.701</td>
</tr>
<tr>
<td>Cipro 0.5: Tylosin 0.5 = B2</td>
<td>0.103*</td>
<td>0.97</td>
<td>Y = 41.6X + 91</td>
<td>-2.294</td>
</tr>
<tr>
<td>Cipro 0.5: penicillin 0.5 = B3</td>
<td>0.292*</td>
<td>0.83</td>
<td>Y = 43.9X + 73.5</td>
<td>-0.792</td>
</tr>
<tr>
<td>Penicillin 0.33: Tylosin 0.33:</td>
<td>0.034 *</td>
<td>0.99</td>
<td>Y = 25.1X + 86.7</td>
<td>-2.325</td>
</tr>
<tr>
<td>Cipro 0.33= T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values in toxic unit

CB= cyanobacterial mats
Conclusion

The study ranked amoxicillin the most toxic antibiotic to fish with LC$_{50}$ value equals to 35.72 µg L$^{-1}$. Endosulfan and erythromycin have LC$_{50}$ values for fish 89.32 and 242.7 µg L$^{-1}$ respectively.

The relative toxicity of amoxicillin is lower than 1 indicating extreme toxicity, whereas the relative toxicity of erythromycin is 2.6 indicating lower toxicity. Erythromycin is more toxic than amoxicillin to mosquito, LC$_{50}$ values are 60.2 and 107.6 µg L$^{-1}$ respectively. The relative toxicity of erythromycin is 0.95 whereas that of amoxicillin is 1.7.

Time response relationships are not similar and LT$_{50}$ values ranged from 33.72-63.47 h indicating different exposure times required to produce the toxic effect.

The study provides evidence of the toxic effects of Penicillin, Tylosin, and Ciprofloxacin to cyanobacterial mats. Single toxicity tests demonstrated high potential toxicity against cyanobacterial mats. EC$_{50}$ of individual tests were penicillin 0.13 < Ciprofloxacin 0.71 < Tylosin 5.28 mg/l.

Relative toxicity values indicate Penicillin, and Ciprofloxacin, are more toxic than Diuron to cyanobacterial mats.

The interesting outcome of this study is that Penicillin is the most toxic compound to the cyanobacteria mat followed by Ciprofloxacin and Tylosin was less toxic.

The effects of time indicated that cyanobacteria masts were able to overcome the toxic effects after 72h of exposure time.

The mixture B1 was the most toxic one among all showed antagonistic effect. EC$_{50}$ of mixtures are: B1 0.077 < B2 0.103 < B3 0.292 and T1 0.034 TU /l.

The study also demonstrates the sensitive cyanobacterial mats as aquatic microorganisms to antibiotics.
Recommendation

1. To reduce the application of antibiotics for human and animal health care.
2. Waste residues of antibiotics in hospitals, manufacturing houses and or pharmacies should be treated on site before transferring to the eco-system.
3. Develop organo-clay modified filters to reduces the contamination of ground water.

Acknowledgement

Dr Y. El-Nahhal acknowledges Alexander von Humboldt Stiftung Foundation Fellowship Grant no IV-PAL/1104842 STP, Germany. Special thanks go to Prof Dr G.lagaly at Kiel University, Prof Shourrmman Lypsch University Germany.