Antibiotics are prescribed to both the human and farm animal populations of many countries in both Europe and the rest of the world. Up to 50% of the antimicrobial use in humans is considered inappropriate (Peacock et al., 2000). Antibiotics are finding their way back into the environment. This may impact the rate at which organic matter in the environment is broken down, or the effectiveness of sewage treatment works. At low concentrations, antibiotics can lead to the development of antibiotic-resistant strains of the microbes responsible for disease in populations of many countries in both Europe and the rest of the world. Such activity could arise both from antibiotics in effluent compounds, and also from the products of their metabolisation.

Introduction

- Antibiotics are prescribed to both the human and farm animal populations of many countries in both Europe and the rest of the world. Up to 50% of the antimicrobial use in humans is considered inappropriate (Peacock et al., 2000).
- Antibiotics are finding their way back into the environment. This may impact the rate at which organic matter in the environment is broken down, or the effectiveness of sewage treatment works.
- At low concentrations, antibiotics can lead to the development of antibiotic-resistant strains of the microbes responsible for disease in populations of many countries in both Europe and the rest of the world. Such activity could arise both from antibiotics in effluent compounds, and also from the products of their metabolisation.

Method

Premitest

Premitest (GSM food industries, Netherland), is commercially available in kit form and has been shown to be responsive to all antibiotics tested with it (God et al., 2004). It provides a simple yes/no response to presence of antibiotics, using Bacillus subtilis starch-fermenting, a thermophilic bacterium, which is resistant to all of the most common used antibiotics and provides a measure of the concentration of active antibiotic material in the sample.

Aims

The aim of this research was to establish a screening method for the detection of antibiotics in effluent and environmental samples. The ABC assay was selected as a standard as a representative of the totally non-ionic antibiotics. A selection of representative antibiotics and disinfectants (Chlorine, Zinc and Diuron) were also screened to establish whether they could cause false positive. False positives were found, but at environmental relevant concentrations.

Results

Direct application

Early testing was carried out using standards directly applied to the vials. Two antibiotics were tested using this methodology at a range of dilutions to produce simple dose-response curves. Erythromycin and sulfadimethoxine were selected as the antibiotics to be present in dilution series and re-analysed including an equivalent concentration of each antibiotic in the lower Tyne catchment. Science of The Total Environment, Volume 356, Issues 1-3, Pages 143-153.

Extracted spiked samples

Antibiotic-spiked samples were made up in distilised water, extracted and eluted with methanol and transferred into nutrient medium at a concentration factor of 1000:1. The antibiotics, erythromycin and sulfadimethoxine, were tested at a range of concentrations, and in simple binary mixtures. Zn and 4,4’-DCT have also been tested as anthropogenic toxicants, but were not found to be active at all.

Table 1: ABC assay results from directly applied spiked stocks. The number in brackets indicates the number of hours incubation.

<table>
<thead>
<tr>
<th>Antibiotic/Concentration</th>
<th>ABC 1</th>
<th>ABC 2</th>
<th>ABC 3</th>
<th>ABC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulamyte (1000 μg/l)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin (100 μg/l)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zinc (5000 μg/l)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Environmental samples

A selection of environmental samples were analysed. Samples that showed strong antibiotic activity were diluted and re-analysed including an equivalent concentration of each antibiotic in simple binary mixtures. Zn and 4,4’-DCT have also been tested as anthropogenic toxicants, but were not found to be active at all.

Discussion

This assay is screened as a rapid way to identify the presence of any antibiotic. In environmental samples, including compounds that may not be identified using directed chemical analysis.

Conclusions

1. Although still a new technique with further validation to carry out, the ABC assay shows promise as a tool for the assessment of antibiotic activity in the environment.
2. The assay works as a rapid, inexpensive yes/no test for antibiotic activity in environmental samples.
3. False positives can be avoided by incorporating cytotoxicity data from other bioassays and by further research into assay behaviour in the presence of non-antibiotic contaminants.
4. Toxic metals which could lead to false positives are not retained during the water sample extraction process, reducing the false positive responses.
5. More statistically useful responses, including EC50 and antibiotic equivalents can be determined by using a ‘score’ for the colour change.
6. Directly applied standards, extracted water and sediment samples can all be assessed using this assay.

The 96-well plate method is on its way…


Acknowledgements

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References

Davies, D. et al. “The development of a rapid screening technique to measure antibiotic activity in effluents and environmental samples.”

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