Application of antibiotic sensitive bioassays to assess water and waste water quality

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INTRODUCTION

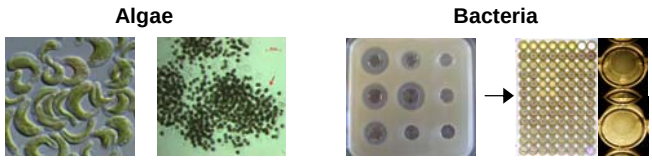
The potential toxicity of surface waters in The Netherlands is monitored with a battery of bioassays. This test battery is responding well to compounds causing narcotic effects [1], but more attention should be paid to compounds with a specific mode of action, like antibiotics. The aim of the present study was therefore to develop and apply antibiotic sensitive bioassays. To this purpose we compared the sensitivity of three bioassays (with a green alga, a cyanobacterium and bacteria) to six antibiotics.

METHODS

Algae tests: a 96-wells application of the PAM test. Measures photosynthetic efficiency of green algae and cyanobacteria with Pulse Amplitude Modulated fluorescence (Yield).

Bacteria test: a 96-wells application of the multi-bacteria screening test. Measures growth inhibition of 5 bacterial strains (sensitive to specific antibiotic groups) using optical density in 5 separate plates.

Test compounds: Sulfamethoxazole, Trimethoprim, Flumequine, Tylosin, Streptomycin, Oxytetracycline.



Green alga (PAM)
Pseudokirchneriella subcapitata
24 h
Fluorescence photosystem
EC_{50}

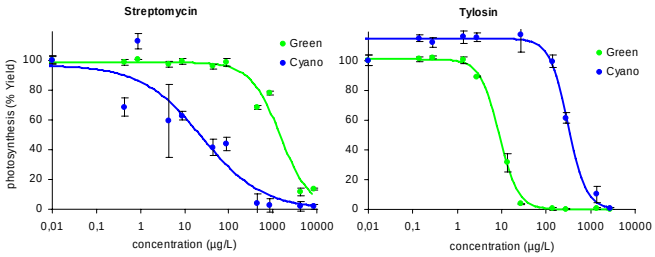
Cyanobacteria (PAM)
Microcystis aeruginosa
24 h
Fluorescence photosystem
EC_{50}

Waterscan[6][2]
Multi-bacteria test
(5 species)
20 h
Bacterial growth reduction
NOEC

96 wells Waterscan[3]
Multi-bacteria test
(5 species)
20 h
Bacterial growth reduction
EC_{50}

RESULTS

Algae



Dose-response relationships for the effects of Tylosin (left panel) and Streptomycin (right panel) on the photosynthesis of the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Microcystis aeruginosa* after 24 h of exposure.

Using photosynthetic efficiency as an endpoint, the cyanobacterium was more sensitive than the green alga to Streptomycin (left panel) and Sulfamethoxazole (as expected). In contrast, Tylosin (right panel) and Oxytetracyline were more effective to the green algae.

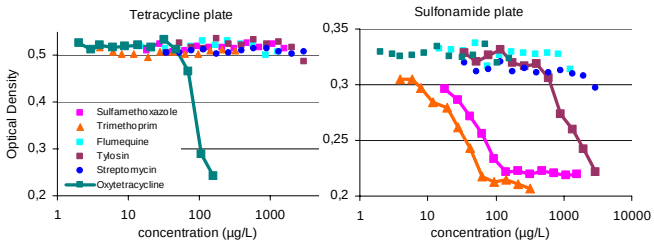
Comparison of sensitivities

EC_{50} (µg/L)	Green	CL	Cyano	CL	Bacteria	CL
Sulfamethoxazole	**> 9000**		**440**	(290 – 670)	**37**	(29 - 47)
Trimethoprim	**> 9000**		**6800**	(5500 – 8300)	**22**	(16 - 31)
Flumequine	**> 8000**		**> 8000**		**170**	(120 - 240)
Tylosin	**8.7**	(7.6 - 9.9)	**310**	(240 – 390)	**560**	(530 - 590)
Streptomycin	**1500**	(1000 - 2200)	**25**	(5.7 – 110)	**-**	
Oxytetracycline	**620**	(590 - 650)	**6200**	(3700 – 10400)	**81**	(78 - 84)

EC_{50} values (µg/L) with 95% confidence limits (CL) for the effects of the six antibiotics on the test species

For all test compounds the multi-bacteria screening test was more sensitive than the PAM test (green and cyano), except for Tylosine.

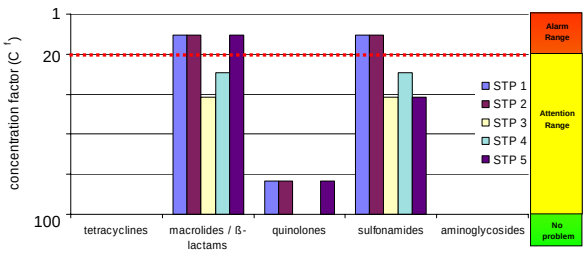
Bacteria



Dose-response relationships for the effects of the six antibiotics on bacterial growth on the plate sensitive to tetracyclines (left panel) and the plate sensitive to sulfonamides (right panel) after 20 h of exposure (measured with the 96-well application at O.D. 600 nm).

All bacterial plates were sensitive to their target antibiotics, except for the plate sensitive to aminoglycosides (Streptomycin). Four bacterial plates were specifically sensitive to their target antibiotics, but the plate sensitive to sulfonamides also responded to Tylosin (from the macrolides group).

Application



Effects of five sewage treatment plant effluent concentrates on bacterial growth on the five antibiotic group specific plates using the classical 9-well application. Effects are expressed in concentration factor of sample (C^f). The red dotted line is the lower limit of the alarm range, representing potential risk of effects of antibiotics.

Effluent samples caused growth inhibition in bacteria sensitive to antibiotics from the groups of macrolides / ß-lactams and sulfonamides.

CONCLUSIONS

1. The PAM algae test not always responded conform the expected algal species specific sensitivities.
2. The bacterial test was more sensitive than the PAM test.
3. The bacterial test is a promising tool to screen water samples for the presence and effects of specific groups of antibiotics.

Acknowledgements

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References

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