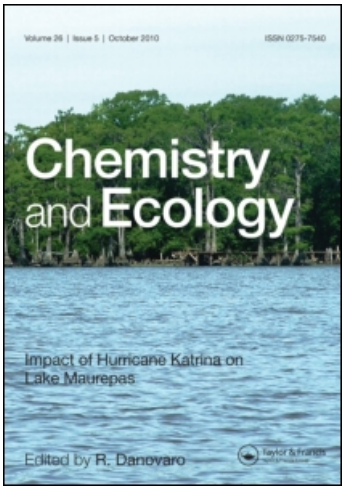

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Fate and effects of nonylphenol in the presence of the cyanobacterium *Microcystis aeruginosa*

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Interactions of nonylphenol (NP), a toxic and oestrogenic degradation product of widely used non-ionic surfactants, with the cyanobacterium *Microcystis aeruginosa* were studied. Batch cultures were incubated for 10 days with NP concentrations between 10 and 570 nM. NP was removed more quickly in the presence of *M. aeruginosa* (half-life 2.7–5.2 days) than in its absence (half-life 6.7–10.2 days) at all concentrations tested. At the end of the experiment, NP could not be found in the biomass, so the biotic removal is due to uptake and chemical transformation, and not to physical binding on the cells. The observed effective concentrations, EC_{50} and EC_{20}, were 0.45 and 0.25 μM, respectively. Therefore, NP is expected to have toxic effects on *M. aeruginosa* only in very contaminated surface waters. However, for concentrations that go far beyond environmental levels, cyanobacteria are able to cope with NP toxicity by internalising the compound in a less toxic form. Therefore, the presence of cyanobacteria may increase the rate of NP removal from the aquatic environment.

Keywords: nonylphenol; cyanobacteria; toxicity

1. Introduction

Endocrine-disrupting chemicals have been found to be ubiquitous contaminants [1]. It is known that such compounds can penetrate biological membranes and reach universal molecular systems, increasing the probability of unexpected consequences [2].

The response of phytoplankton species to these contaminants is very diverse. For example, it has been observed that the microalgae *Chlorella vulgaris* is capable of bio-concentrating natural and synthetic oestrogens [3]. Inhibition of algal growth has been caused by ethinylestradiol [4], whereas in some cases, chlorophenols at low concentrations may induce algal growth [5].

Within the phytoplankton community, the health-related relevance of the cyanobacteria is primarily linked to their ability to bloom and produce a wide range of toxins [6]. In Portugal, *Microcystis aeruginosa* represents a significant water-quality problem, with many water bodies that are used for potable and recreational purposes supporting blooms of this species during the summer months [7].

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Oestrogenicity has been detected in Portuguese surface water, mostly near the biggest urban areas [8]. Industrial surfactants, plasticisers and pesticides are most likely responsible for the observed oestrogenicity, with octylphenol (OP) and nonylphenol (NP), plus nonylphenol mono- and diethoxylates, being found [9].

A high toxic effect ($EC_{50} = 0.33\,\mu M$) of OP on a *M. aeruginosa* strain, isolated from Torrão reservoir (north of Portugal), has been reported in an *in vitro* study, although at environmental concentrations no toxic effect was found [10]. Levels of NP in the environment are usually much higher than levels of OP [1] and, if their toxicity is similar, the maximum environmental concentrations of NP will already be toxic to the *M. aeruginosa* strain used. Therefore, an investigation into the effects of NP at environmentally relevant levels on growth of the same strain of *M. aeruginosa* was carried out. The toxicity of NP was evaluated and the role of the cyanobacterium in the removal of NP was investigated.

2. Materials and methods

All material was cleaned and sterilised as explained previously [10]. Measurements were carried out in f2 algal medium [11]. Solutions of 4-nonylphenol (technical mixture, 94%, Riedel-de Haen) were prepared in ethanol (Panreac, Spain) and stored at −18 °C. NP solutions were passed through a 0.45 μm nylon filter (Whatman®) before being added to the culture media. All sample manipulations were carried out in a Class 100 laminar flow hood, in a clean room with Class 100 filtered air.

An axenic strain of *M. aeruginosa* Kütz (LEGE 05195) was isolated from the Torrão reservoir in the Tâmega River (northern Portugal) and cultured in Z8 medium [12]. The cells were then grown in fraquil medium, used to inoculate 150 mL of f2 growth medium as described previously [10] and incubated for 10 days. The final concentration of ethanol (carrier solvent) in the culture media never exceeded 0.01% (v/v). Four NP concentrations (10, 45, 230 and 570 nM), each with three replicates, were inoculated. Culture media having the same NP concentrations, but which had not been inoculated, were exposed in duplicate. Controls without NP and inoculated with *M. aeruginosa* were exposed in triplicate. The axenicity of the culture during the experiment was confirmed by microscopy examination.

Simultaneously to the population growth measurements, 12 mL of culture from each flask were filtered through a glass microfibre filter (Whatman®) of 1.2 μm porosity and the concentrations of NP were determined using headspace solid-phase microextraction followed by capillary gas chromatography with a flame ionisation detector, as described previously [13].

NP measurements in *M. aeruginosa* were performed by filtering ∼50 mL of culture medium at the end of the exposure period. Filters were washed with 100 mL of deionised water and frozen at −18 °C. Prior to the analysis the filters were treated with liquid nitrogen and homogenised in 10 mL deionised water, the analysis was carried out as described previously [13]. Filters used to filter f2 media with the same NP concentration but without cyanobacteria were used as controls. Data obtained using filters spiked with 0.5 nMol of NP were used to calculate the NP recovery. For the control filters, NP recovery was $77 \pm 8\%$ ($n = 3$) and from the filters containing *M. aeruginosa* it was $35 \pm 2\%$ ($n = 8$).

Calculation of EC_{20} and EC_{50} values was performed as reported in the Organization of Economic Co-Operation and Development test guideline 201 [14]. The test endpoint was the inhibition of growth, expressed as average growth rate during the exposure period. A Student's t-test was performed to compare cyanobacteria growth rates in the presence and absence of NP.

NP removal rate from the culture medium was calculated using pseudo-first-order kinetics. A Student's t-test was performed to compare rate constants corresponding to NP removed in the

presence (k) and absence (k_{abiotic}) of cells. The overall rate constant k is a result of both abiotic and biotic (caused by cells) removal ($k = k_{\text{abiotic}} + k_{\text{biotic}}$).

3. Results and discussion

The average growth rates of *M. aeruginosa* in medium with and without ethanol were not significantly different (data not shown) and therefore both results were combined to calculate the average cyanobacteria control growth.

NP did not promote the growth of *M. aeruginosa* even at levels close to those found in the environment. A statistically significant decrease ($p < 0.05$) in the growth rate occurred for the 570 nM concentration, the effect being noticed from the fourth day onwards (Figure 1).

The initial concentrations of NP, C_0, in the medium without cyanobacteria were between 64 and 83% of the nominal concentrations. These were used to calculate the EC_{50} and EC_{20} values,which were 0.45 and 0.25 μM, respectively. Wang et al. [15] reported higher EC_{50} values, ranging from 3 to 13 μM for different strains of *M. aeruginosa*. However, high among-strain variations are very common.

NP concentrations as low as 0.25 μM (EC_{20}) can potentially decrease the growth of *M. aeruginosa*. The reported NP concentrations in surface waters reached maximum values between 115 nM [9] and 2.9 μM [1], but the maximum concentrations are most often below 10 nM [1]. Therefore, it is not likely that NP is a significant threat to *M. aeruginosa* in the aquatic environment, except in extremely polluted surface waters. However, mixture toxicity in real situations may appear even if the individual compounds are present at no observed effect concentrations (NOECs) [16].

Removal of NP followed pseudo-first-order kinetics both in the presence and in the absence of cyanobacteria (Figure 2). In addition, all experiments (abiotic and biotic) there was no preferential removal of some structural isomers of NP relative to others. During the abiotic incubations no micro-organisms were found. Therefore, the decrease in NP concentrations in the absence of cyanobacteria (Figure 2) could probably be explained by abiotic processes such as photochemical

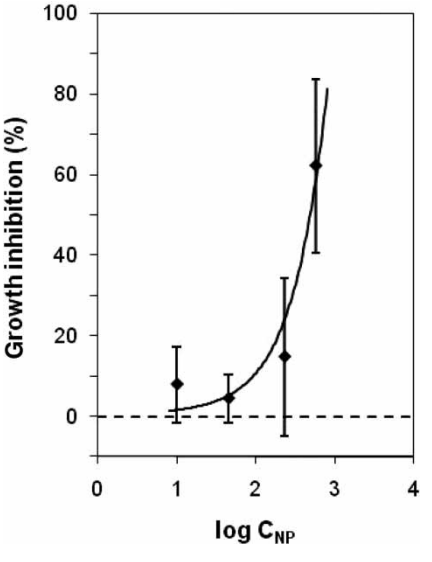

Figure 1. Concentration–effect curve of *Microcystis aeruginosa* growth in nonylphenol-containing medium. The concentration of nonylphenol (C_{NP}) is expressed in nM. Error bars represent standard deviations of three replicates.

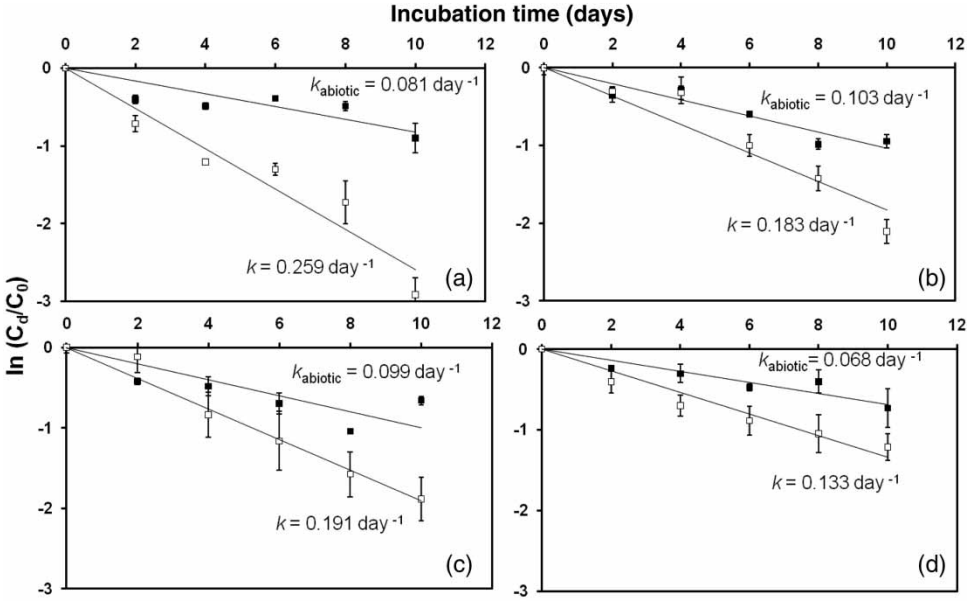

Figure 2. Comparison of nonylphenol (NP) removal over time in (a) 10 nM, (b) 45 nM, (c) 230 nM and (d) 570 nM NP from culture media without *Microcystis aeruginosa* (■) or inoculated with *M. aeruginosa* (□). Error bars represent the standard deviations of three replicates for cultures with *M. aeruginosa* and mean deviations of two replicates for the abiotic experiment. C_0 is the initial NP concentration and C_d is the NP concentration measured over time.

transformation, adsorption on the glass walls and/or volatilisation loss. Under abiotic conditions, degradation of NP by photolysis may occur, especially at low NP concentrations and in the presence of nitrates and iron [17], which are constituents of the medium used in the current study. However, the results of our study do not permit to us quantify the role of the different abiotic methods of NP removal from the medium. Values of $k_{abiotic}$, between 0.068 and 0.103 day^{-1}, were higher than those obtained previously for OP under the same conditions [10], indicating that NP was less stable than the OP.

Figure 2 shows that NP removal was significantly ($p < 0.05$) faster in the presence of cells than in their absence, and that the biotic effect was more extensive at lower concentrations of NP. A previous study on the toxicity of OP to *M. aeruginosa* [10] demonstrated that the cyanobacteria helped to remove OP from the cultures at low concentrations, whereas at levels $>1.25\,\mu M$ the ability was lost.

In principle, biotic removal of NP may be due to either physical binding on the cell surfaces or uptake/metabolism. In analysis of the biomass following cell destruction with liquid nitrogen, NP was not detected. Therefore, internalisation of NP, followed by formation of another sub-product (which could not be measured in this study) probably took place. This result is very different from that reported by Wang and Xie [18] for *M. aeruginosa* binding of NP, at concentrations from 0.09 to 9 μM, who have found binding as high as 61% of the compound. Even so, results similar to ours have been reported in another work in which the amount of oestrogen that partitioned to the algae *Chlorella vulgaris* did not exceed 9% of the total [3].

Other studies have reported that freshwater microalgae were able to metabolise bisphenol A to glycosides [19], and marine microalgae were able to use glucosyl and malonyl transfer to detoxify xenobiotics, such as dichlorophenols [20]. Wang and Xie [18] have found that NP at concentrations ranging from 4.5 to 9.0 μM was biotically removed from cultures of *M. aeruginosa* by glutathione conjugation. We can hypothesise, but not prove using our data, that a similar mechanism might occur when *M. aeruginosa* was exposed to the lower NP concentrations used in the current study.

4. Conclusion

In very contaminated surface waters, NP might have toxic effects on some strains of *M. aeruginosa*. However, for concentrations that go far beyond environmental levels, the cyanobacteria are able to cope with NP toxicity by internalising the compound as a less toxic form. Therefore, the presence of cyanobacteria may increase removal of NP from the aquatic environment.

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References

[1] G.-G. Ying, B. Williams, and R. Kookana, *Environmental fate of alkylphenols and alkylphenol ethoxylates – a review*, Environ. Inter. 28 (2002), pp. 215–226.
[2] C.G. Daughton and T.A. Ternes, *Pharmaceuticals and personal care products in the environment: agents of subtle change?* Environ. Health Persp. 107 (1999), pp. 907–938.
[3] K.M. Lai, M.D. Scrimshaw, and J.N. Lester, *Biotransformation and bioconcentration of steroid estrogens by* Chlorella vulgaris, Appl. Environ. Microb. 68 (2002), pp. 859–864.
[4] B.I. Escher, N. Bramaz, R.I.L. Eggen, and M. Richter, In vitro *assessment of modes of toxic action of pharmaceuticals in aquatic life*, Environ. Sci. Technol. 39 (2005), pp. 3090–3100.
[5] E. Sahinkaya and F.B. Dilek, *The growth behavior of* Chlorella vulgaris *in the presence of 4-chlorophenol and 2,4-dichlorophenol*, Ecotoxicol. Environ. Safe. 72 (2009), pp. 781–786.
[6] C. Wiegand and S. Pflugmacher, *Ecotoxicological effects of selected cyanobacterial secondary metabolites – a short review*, Toxicol. Appl. Pharm. 203 (2005), pp. 201–218.
[7] V.M. Vasconcelos, *Freshwater cyanobacteria and their toxins in Portugal*, in *Cyanotoxins: Occurrence, Causes and Consequences*, I. Chorus, ed., Springer, New York, 2001, pp. 62–67.
[8] R. Céspedes, M. Petrovic, D. Raldúa, U. Saura, B. Piña, S. Lacorte, P. Viana, and D. Barceló, *Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC–ESI-MS*, Anal. Bioanal. Chem. 378 (2004), pp. 697–708.
[9] L. Quirós, R. Céspedes, S. Lacorte, P. Viana, D. Raldúa, D. Barceló, and B. Piña, *Detection and evaluation of endocrine-disruption activity in water samples from Portuguese rivers*, Environ. Toxicol. Chem. 24 (2005), pp. 389–395.
[10] M.S. Baptista, T. Stoichev, M.C.P. Basto, V.M. Vasconcelos, and M.T.S.D. Vasconcelos, *Fate and effects of octylphenol in a* Microcystis aeruginosa *culture medium*, Aquat. Toxicol. 92 (2009), pp. 59–64.
[11] R.R.L. Guillard, *Culture of phytoplankton for feeding marine invertebrates*, in *Culture of Marine Invertebrate Animals*, W.L. Smith and M.H. Chanley, eds., Plenum Press, New York, 1975, pp. 26–60.
[12] M.L. Saker, J. Fastner, E. Dittmann, G. Christiansen, and V.M. Vasconcelos, *Variation between strains of the cyanobacterium* Microcystis aeruginosa *isolated from a Portuguese river*, J. Appl. Microbiol. 99 (2005), pp. 749–757.
[13] T. Stoichev, M.S. Baptista, M.C.P. Basto, P.N. Carvalho, and M.T.S.D. Vasconcelos, *Application of SPME for determination of alkylphenols and bisphenol A in cyanobacteria culture media*, Anal. Bioanal. Chem. 391 (2008), pp. 425–432.
[14] Organisation for Economic Co-operation and Development (OECD), *Guidelines for Testing of Chemicals 201: Alga, Growth Inhibition Test*, OECD, Paris, 2002.
[15] J. Wang, P. Xie, and N. Guo, *Effects of nonylphenol on the growth and microcystin production of* Microcystis *strains*, Environ. Res. 103 (2007), pp. 70–78.
[16] H. Walter, F. Consolaro, P. Gramatica, M. Scholze, and R. Altenburger, *Mixture toxicity of priority pollutants at no observed effect concentrationjs (NOECs)*, Ecotoxicology 11 (2002), pp. 299–310.
[17] M. Neamţu and F.H. Frimmel, *Photodegradation of endocrine disrupting chemical nonylphenol by simulated solar UV-irradiation*, Sci. Total Environ. 369 (2006), pp. 295–306.
[18] J. Wang and P. Xie, *Antioxidant enzyme activities of* Microcystis aeruginosa *in response to nonylphenols and degradation of nonylphenols by* M. aeruginosa, Environ. Geochem. Health 29 (2007), pp. 375–383.
[19] N. Nakajima, T. Teramoto, F. Kasai, T. Sano, M. Tamaoki, M. Aono, A. Kubo, H. Kamada, Y. Azumi, and H. Saji, *Glycosylation of bisphenol A by freshwater microalgae*, Chemosphere 69 (2007), pp. 934–941.
[20] D. Petroutsos, P. Katapodis, M. Samiotaki, G. Panayotou, and D. Kekos, *Detoxification of 2,4-dichlorophenol by the marine microalga* Tetraselmis marina, Phytochemistry 69 (2008), pp. 707–714.