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Cyanobacterium *Microcystis aeruginosa* response to pentachlorophenol and comparison with that of the microalga *Chlorella vulgaris*

Paulo de Morais [a,b], *Teodor Stoichev* [b,*], *M. Clara P. Basto* [a,b], *V. Ramos* [b], *V.M. Vasconcelos* [a,b], *M. Teresa S.D. Vasconcelos* [b]

[a] *CIIMAR/CIMAR and Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal*
[b] *Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal*

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form
26 December 2013
Accepted 27 December 2013
Available online 8 January 2014

Keywords:
Pentachlorophenol
Cyanobacteria
Microalgae
Toxicity
Removal

ABSTRACT

Pentachlorophenol (PCP) effects on a strain of the cyanobacterium *Microcystis aeruginosa* were investigated at laboratory scale. This is the first systematic ecotoxicity study of the effects of PCP on an aquatic cyanobacterium. The microalga *Chlorella vulgaris* was studied in the same conditions as the cyanobacterium, in order to compare the PCP toxicity and its removal by the species. The cells were exposed to environmental levels of PCP during 10 days, in Fraquil culture medium, at nominal concentrations from 0.01 to 1000 μg L^{-1}, to the cyanobacterium, and 0.01 to 5000 μg L^{-1}, to the microalga. Growth was assessed by area under growth curve (AUC, optical density *vs* time) and chlorophyll *a* content (chl_a). The toxicity profiles of the two species were very different. The calculated effective concentrations EC_{20} and EC_{50} were much lower to *M. aeruginosa*, and its growth inhibition expressed by chl_a was concentration-dependent while by AUC was not concentration-dependent. The cells might continue to divide even with lower levels of chl_a. The number of *C. vulgaris* cells decreased with the PCP concentration without major impact on the chl_a. The effect of PCP on *M. aeruginosa* is hormetic: every concentration studied was toxic except 1 μg L^{-1}, which promoted its growth. The legal limit of PCP set by the European Union for surface waters (1 μg L^{-1}) should be reconsidered since a toxic cyanobacteria bloom might occur. The study of the removal of PCP from the culture medium by the two species is an additional novelty of this work. *M. aeruginosa* could remove part of the PCP from the medium, at concentrations where toxic effects were observed, while *C. vulgaris* stabilized it.

1. Introduction

Pentachlorophenol (PCP), a broad-spectrum pesticide, is the most toxic of the chlorophenols (CPs) due to its higher number of chlorine atoms, hydrophobicity and acidity. It is considered a priority pollutant since it has a very long half-life in the environment and is harmful at very low concentrations (Tao et al., 2012; Xing et al., 2012). It can be an endocrine

* *Corresponding author*. Tel.: +351 22 340 1805; fax: +351 22 339 0608.
E-mail address: tstoichevbg@yahoo.com (T. Stoichev).

0043-1354/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2013.12.036

disruptor and cause high toxicity to all kinds of organisms (Hong et al., 2010), including fetotoxic, embryotoxic and teratogenic effects in wildlife (Lawrence and Poulter, 2001) and carcinogenicity in humans (Longoria et al., 2008).

The aquatic environment is particularly sensitive to PCP, where it can accumulate in the sediment and be released back to the water through desorption. Its deprotonated form dominates at high pH (pKa 4.7) (Muir and Eduljee, 1999) and exhibits lower toxicity (Xing et al., 2012). In order to predict potential adverse effects of PCP in an aquatic ecosystem, the knowledge of the level and the mechanisms of toxicity to aquatic organisms is important (Hong et al., 2010). The European Union legislation has set 1 $\mu g\,L^{-1}$ of PCP as the maximum admissible concentration in inland and other surface waters (De Morais et al., 2012). The concentrations of PCP in environmental freshwaters are usually very low, in the ng L^{-1} level, but highly variable, from ng L^{-1} to low mg L^{-1} level (De Morais et al., 2012). Therefore, the freshwater phytoplankton might be exposed to different PCP concentrations, possibly leading to toxic responses.

Phytoplankton composition in freshwater ecosystems is varied and oftentimes includes cyanobacteria (formerly known as blue-green algae, now recognized as prokaryotes) and green microalgae as major components (El-Dib et al., 2000; Mostafa and Helling, 2002). The phytoplankton species are primary producers of organic matter on which the higher levels in the food chain rely (El-Dib et al., 2000). Their sensitivity to toxic compounds is highly variable and depends on the species, the toxicant and the experimental conditions. At low concentrations, some pesticides are known to stimulate the growth of phytoplankton. However, low or no effects are usually observed at small levels, and growth inhibition occurs at higher concentrations (Mostafa and Helling, 2002). In the environment, the behaviour of pollutants and their interaction with phytoplankton is complex due to the interplay between abiotic and biotic processes (Abrahamsson and Ekdahl, 1996), which hampers the extrapolation of laboratory data. On the other hand, experiments based on pure cultures, despite their drawback in terms of ecological relevance, separate the response of one species to changes in a particular variable from interactions with other organisms and/or chemical compounds found in the environment (Mostafa and Helling, 2002). The identification to the organism level is important since the toxicity profile can be very different for closely related species (Mostafa and Helling, 2002). This is particularly relevant for cyanobacteria, since only some species, or even some strains within a same species, may produce toxins (Wang et al., 2007).

The overall toxic effect of CPs is caused by a combination of several distinct mechanisms, most of which interfere with energy transduction, mainly in mitochondria, chloroplasts and bacterial cytoplasmic membranes, where phenols may act as uncouplers, inhibitors or merely as narcotic agents (Ertürk and Saçan, 2013; Escher et al., 1997). PCP interferes with the oxidative phosphorylation process and inhibits ATP synthesis (Mäenpää et al., 2008) as well as the electron flow process in photosynthesis (Sharma et al., 1997). Therefore, chlorophyll *a* content (chl_a) can be an indicator of PCP toxicity (Mostafa and Helling, 2002).

The potential of some cyanobacteria and microalgae in the removal of CPs by biodegradation and biosorption has been recognized (Hirooka et al., 2003; Klekner and Kosaric, 1992). The uptake of pesticides by phytoplankton involves adsorption on the cell surface followed by absorption within the cell, which can lead to the entry of toxic chemicals into the food chain (El-Dib et al., 2000). Abiotic mechanisms of PCP removal include photodegradation, oxidation and evaporation (Czaplicka, 2004).

There are two articles about the toxicity of PCP to cyanobacteria, carried out at relatively high concentrations of PCP: one at the phytoplankton community level, without taxonomic identification details (El-Dib et al., 2000), and one for a soil species (Mostafa and Helling, 2002). The toxicity of sodium pentachlorophenolate (Na-PCP) was also studied for aquatic cyanobacteria, in a plate incubation test during 9 days, by measuring only optical density (OD) (Ando et al., 2007). That article, however, focused on the development of toxicity tests for veterinary antimicrobial products, and used Na-PCP as a model substance. Apart from data on the median effective concentration (EC_{50}), non-observed effect concentration and minimum inhibitory concentration, no other results about the effects of PCP were demonstrated. Conversely, several studies treat toxicity of PCP to *Chlorella vulgaris*, at very different PCP concentrations from 0.01 to 40 mg L^{-1}, with growth (growth rate or inhibition) as endpoint (Burrell et al., 1985; Gokcen, 1998; Olivier et al., 2003; Repetto et al., 2001; Shigeoka et al., 1988; Tikoo et al., 1997, 1996; Yen et al., 2002). The aforementioned studies on the toxicity of PCP to *C. vulgaris* and cyanobacteria, further discussed, used nominal concentrations of PCP and do not measure its initial concentrations and possible removal during the tests.

The objective of this work was to research the fate and effects of PCP at environmental levels on two common freshwater phytoplankton species, one prokaryotic and the other eukaryotic. The results reported for *Microcystis aeruginosa* are among the first for the toxicity of CPs on cyanobacteria. For the first time, the toxicity of PCP on a single aquatic cyanobacterial species was evaluated using data for both OD and chl_a measurements. The results were compared with those for the chlorophyte *C. vulgaris*, obtained in the same conditions, as well as with data from the literature. An additional novelty of this work was the study of the PCP removal ability by the two species.

2. Experimental conditions

2.1. Materials and decontamination

All material was cleaned with a solution of detergent (Teepol N, Tensoquímica, Portugal), washed with tap water, and rinsed several times with deionized water (conductivity $<0.1\ \mu S\ cm^{-1}$) before being acid cleaned (HCl 1 M) for 24 h, again rinsed with deionized water, and air dried in a sterile atmosphere. Microwave sterilisation at 700 W was performed during 10 min for the plastic material (polycarbonate bottles, Nalgene), and for 5 min for the pipettes' tips. Glass Erlenmeyers, plugged with cotton stoppers (cotton wrapped in cheesecloth), were dry heat sterilized in an oven at 170 °C, for 1 h. The cotton stoppers were immediately covered with two layers of aluminium foil, before cooling. Unless otherwise