The Role of Natural Organic Matter in the Biodecontamination of Freshwaters from the Endocrine Disruptor Bisphenol A

G. Castellana, E. Loffredo, A. Traversa, and N. Senesi

Abstract Bisphenol A (BPA) is an endocrine disruptor compound acting on animals, especially aquatics, and humans. It can leach out of urban and industrial wastes and contaminate the environment. This study evaluated comparatively the decontamination potential of three ligninolytic fungi, *Trametes versicolor*, *Stereum hirsutum* and *Pleurotus ostreatus*, towards BPA at a concentration of 4.6 mg L^{-1} in distilled water (control) and two freshwaters, a lake water and a river water. The assessment of mycelial growth during water decontamination evidenced a good tolerance of all fungi to BPA and, in some cases, a fungal growth stimulation by the organic content of the two freshwaters, with respect to the control. In the absence of fungi, BPA persistence in water appeared negatively related to the organic matter content of water, resulting in the order: distilled water > river water > lake water. All the three fungi showed a larger removal of BPA in freshwaters with respect to distilled water. *T. versicolor* and *P. ostreatus* exerted a similar relevant capacity to remove BPA from both freshwaters, whereas *S. hirsutum* was much more effective in the decontamination of lake water with respect to river water.

Keywords Biodecontamination • Bisphenol A • Ligninolytic fungi • Water

Introduction

Endocrine disruptor compounds (EDC) are natural and xenobiotic organic molecules responsible for severe alterations of endocrine processes in animals, particularly aquatics, and humans (Staples et al. 1998; Campbell et al. 2006). EDC include natural and synthetic estrogens, pesticides, industrial chemicals, products and by-products of

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paper, paint and plastic industries. They are particularly spread in highly urbanized and industrialized areas where they can leach out of liquid and solid wastes and contaminate terrestrial and aquatic systems. Several reports are present in the literature on the environmental behaviour of some EDC (Lintelmann et al. 2003; Loffredo and Senesi 2006). Among these compounds, bisphenol A (BPA) is largely used for the production of polycarbonates, epoxy resins and flame retardants. It is also a component of food and drink packaging, electrical and electronic devices and other goods of daily human utilization. BPA is often detected in municipal and industrial wastewaters, sewage sludges and natural waters and sediments (Belfroid et al. 2002). Recently, it has been demonstrated that ligninolytic fungi can biodegrade organic contaminants, such as EDC, by means of their enzymes with low substrate specificity (Cajthaml et al. 2009). No information is available in the literature on the removal of BPA from real water systems using fungi. This work aimed to evaluate comparatively the capacity of three ligninolytic fungi to remove BPA from distilled water, used as control, and two freshwaters, a lake water and a river water. During the biodecontamination process, fungal growth was also monitored in order to assess BPA toxicity.

Materials and Methods

Two Apulian freshwaters were collected from Sassano lake (SLW) and Morelli river (MRW) and analysed for pH, electrical conductivity (EC) and total organic carbon (TOC) content. BPA at a concentration of 4.6 mg L^{-1} was dissolved in distilled water (control, C), SLW and MRW, and aliquots of 100 mL of each contaminated water were poured separately into sterile plastic pots. These were covered with a dialysis membrane and circles of PDA medium which were inoculated separately with *Trametes versicolor*, *Stereum hirsutum* and *Pleurotus ostreatus*, according to the procedure described in Loffredo et al. (2012). Pots containing only the PDA medium (without fungus) were also prepared for C, SLW and MRW. The pots were kept in an incubation chamber at a constant temperature of 23°C in the dark. All experiments were replicated five times. After 2, 7 and 21 days, the radial growth of the mycelium was evaluated, and the residual BPA was measured by high-performance liquid chromatography and ultraviolet detection (Loffredo et al. 2012). All data obtained were statistically analysed by one-way analysis of variance (ANOVA) and the means of treatments separated by the least significant differences (LSD) test.

Results and Discussion

The main differences between the two freshwaters were relative to EC and TOC. The first was about 100 times higher in MR with respect to SL and that reasonably depended on the proximity of the MR sampling site to the sea, whereas TOC

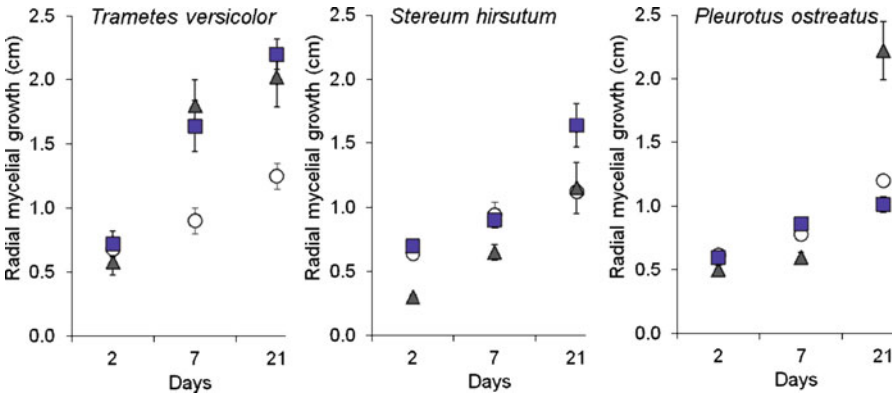

Fig. 1 Effects of the addition of BPA at 4.6 mg L^{-1} in distilled water (O), SLW (▲) and MRW (■) on the growth of each fungus. The *vertical line* on each point indicates the standard error for five replicates

resulted more than five times higher in SLW (4.31 mg L^{-1}) with respect to MRW (0.80 mg L^{-1}), probably for the organic contribution of water runoff from the near town into SLW. Fungal growth on PDA medium laying on C, SLW and MRW added with BPA is shown in Fig. 1. *T. versicolor* was the most stimulated by the organic content of both freshwaters, whereas *S. hirsutum* and *P. ostreatus* grew better on MRW and SLW, respectively. The residual BPA found in the control, SLW and MRW at the three sampling times in the absence or presence on each fungus is reported in Fig. 2. In the absence of fungi, after 21 days, reductions of about 20, 40, and 24% of BPA were observed, respectively, in C, SLW and MRW, which could be attributed to degradation by microorganisms naturally occurring in non-sterile water, diffusion of BPA from water to PDA medium through the membrane and absorption of BPA on the membrane. In general, in the presence of each fungus at any sampling time, the residual BPA in both freshwaters resulted significantly lower than in the absence of the fungus and in distilled water (Fig. 2). All the three fungi contributed markedly to BPA removal from SLW with a similar capacity and trend, whereas *S. hirsutum* was less effective in MRW. The high initial removal capacity of all the three fungi can be ascribed to the high initial concentration of BPA triggering the expression of ligninolytic enzymes, whereas possible stress conditions occurring later, such as the decrease of nutrients in the growth medium, would reduce this capacity. At the end of experiments, the residual BPA resulted 62% for all the three fungi in distilled water, whereas it was 26, 25, and 15% in SLW and 32, 65, and 34% in MRW in the presence of *T. versicolor*, *S. hirsutum* and *P. ostreatus*, respectively (Fig. 2). In conclusion, results obtained in the present study are very encouraging because they indicate that when biodecontamination from BPA occurs in waters with normal endowment of organic matter, results are even better than those obtained in artificial aqueous media such as distilled water. Further, the experimental protocol used in this work avoids the

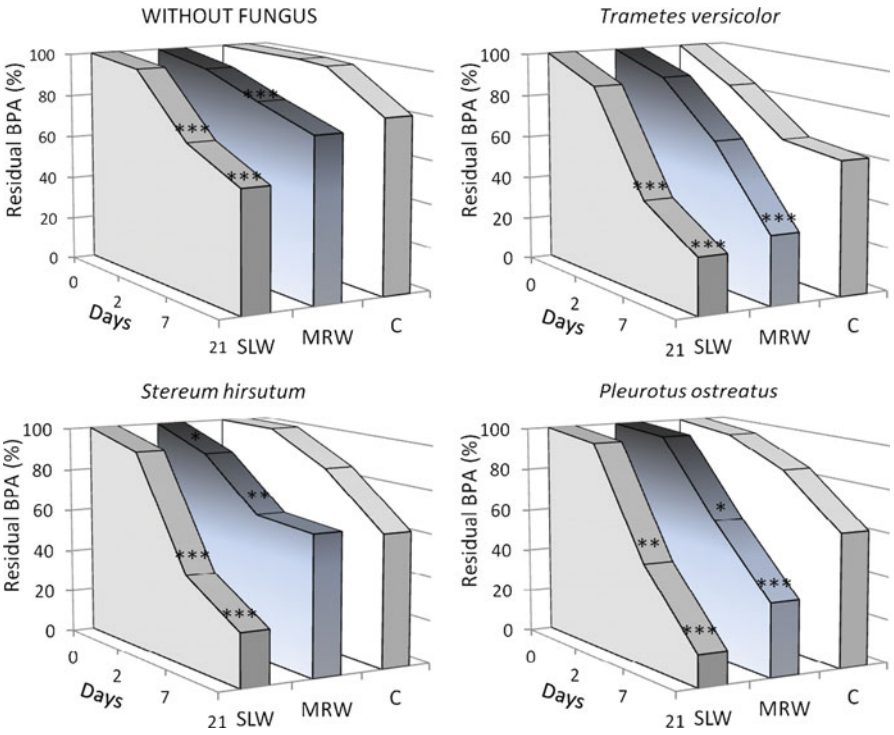

Fig. 2 Residual BPA in distilled water (*C*), Sassano lake water (*SLW*) and Morelli river water (*MRW*) in the absence and in the presence of each fungus growing on the PDA medium. Means of MRW and SLW were compared to means of C (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$)

fungal presence or its migration in the BPA-contaminated water, thus the fungus can be easily removed at the end of the decontamination process.

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