



Insights into the molecular mechanism of the responses for *Cyperus alternifolius* to PhACs stress in constructed wetlands



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HIGHLIGHTS

- *C. alternifolius* can tolerate multiple PhACs and be useful for phytoremediation.
- Over time, the photosynthetic pigments were not inhibited.
- ROS could be effectively counteracted by the enhanced antioxidant enzyme activities.
- It is the first time to study comparative proteomic of plant exposed to PhACs.
- The proteins associated with antioxidant defense and stress showed up-regulation.

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ABSTRACT

Cyperus alternifolius has been widely reported to be an effective phytoremediation plant in constructed wetland systems (CWs). In this context, an integrated biochemical and proteomic analysis of *C. alternifolius* leaves exposed to pharmaceutically active compounds (PhACs) in CWs was conducted to understand the mechanism of phytoremediation. The obtained results showed the antioxidant enzyme activities were induced throughout the experiment; however over time, the malondialdehyde content is not significantly different from the control and the photosynthetic pigment contents in plant were subsequently slowly recovered. Therefore, we concluded that reactive oxygen species could be effectively counteracted by the enhanced antioxidant enzyme activities, and therefore the photosynthetic pigments were ultimately restored. Leaf extract proteome maps were obtained through 2-DE, and an average of 55, 49, and 24 spots were significantly altered by 30, 100, and 500 µg/L of PhACs over the control, respectively. Protein expression patterns showed that proteins in *C. alternifolius* leaves are associated with photosynthesis, energy metabolism, defense, and protein synthesis. Moreover, the most relevant pathways modulated by PhACs were photosynthesis and energy metabolism. The protein expression involved in antioxidant defense and stress response generally increased in all the PhAC treatments. The regulated proteins may favor PhAC degradation in CWs; however, the role of these proteins in degrading PhACs remains unknown; further biochemical studies should be conducted. This study indicated that *C. alternifolius* can tolerate multiple PhACs.

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1. Introduction

Various kinds of pharmaceutically active compounds (PhACs) have been detected in samples from aquatic environments, such as wastewater, coastal water, surface water, and even drinking water,

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and their main pathway is known as the effluent of wastewater treatment plants (Verlicchi et al., 2012; Liu and Wong, 2013; Godoy et al., 2015). The “pseudo-persistence” of PhACs in water environments can result in potentially adverse effects on aquatic communities and humans, even at trace environmental concentrations (Fent et al., 2006; Corcoran et al., 2010; Gutowski et al., 2015). Consequently, the control of such emerging pollutants in effluent has been one of the major global concerns. Advanced technologies, such as activated carbon adsorption, advanced oxidation processes,

and membrane separation, have shown good performance in the removal of selected PhACs from wastewater. However, these treatments are costly; as such, large-scale applications are usually limited in developing countries. Thus, effective and low-cost alternative technologies should be developed to further remove residual PhACs from municipal wastewater effluent, especially in less developed areas. For this purpose, constructed wetland systems (CWs), which are low cost in terms of construction, operation, and maintenance, are attracting considerable attention for their application in the removal of PhACs from wastewater effluent, although CWs have some constraints in their application under natural conditions (Li et al., 2014; Verlicchi and Zambello, 2014).

Plants are one of the three important components (substrate, plants, and microbes) of CWs. Plants play a very significant role in the direct uptake of various pollutants and stimulate the development and activities of microbial communities (Verlicchi and Zambello, 2014; Xu et al., 2014). CW plants can accumulate PhACs in CWs; PhACs affect the growth, photosynthesis, antioxidant system, and lipid peroxidation of CW plants under hydroponic conditions (Dordio et al., 2009, 2011). However, the ability and the molecular mechanism of CW plants to accumulate and tolerate PhACs remain unknown. Therefore, the physiological and molecular regulatory mechanisms involved in PhAC hypertolerance and detoxification should be understood to determine the key features of phytoremediation and then to completely optimize the phytoremediation technique. The related molecular mechanisms in cells in response to various stimuli can be revealed through protein analysis because proteins are the main effectors of most cellular functions (Tian et al., 2004; Semane et al., 2010). Previous studies have also shown the proteomic response of plants under salt, drought, and heavy metal stress (Rose et al., 2004; Semane et al., 2010). Nevertheless, information on the plant proteome under exogenous organic pollutant stress has only recently started (Chen et al., 2011; Zhang et al., 2013). The protein responses to increased PhAC levels in plants in CWs have yet to be described.

Cyperus alternifolius, with the common names of umbrella papyrus, umbrella sedge or umbrella palm, is a perennial herb and grows in humid areas or swamp land. It grows fast with strong root system and it can form a good landscape. *Cyperus* spp. has been widely used in various CWs in many parts of the world for landfill leachate and for municipal and industrial wastewater treatment (Chen et al., 2006; Hadad et al., 2006; He et al., 2012; Leto et al., 2013; Vymazal, 2013). Some studies have shown that *Cyperus* spp. has great tolerance of hyper-eutrophic conditions and salinity (Chan et al., 2008; Thongtha et al., 2014; Tao et al., 2015). Cui et al. (2009) investigated the uptake and removal of total nitrogen (TN) by *C. alternifolius* and results showed that a linear correlation existed between the aboveground biomass and its TN content and an increase in total biomass by 100 g resulted in an increase in TN accumulation in the aboveground biomass by 2.4 g. Soda et al. (2012) reported that *C. alternifolius* can accumulate and translocate Cr, Mn, Fe, Ni, Cu, Zn, Zr, Ag, In, Sn, Au, Pb, and Bi metals. However, the ability of *C. alternifolius* to tolerate PhACs has yet to be investigated. Therefore, efforts to elucidate the molecular mechanisms of *C. alternifolius* tolerance for PhACs are of importance to lay the basis for its potential in phytoremediation of PhACs in contaminated waters. Based on the analysis above, the present study was conducted to provide integrated biochemical and proteomic information on PhAC-stressed *C. alternifolius*. PhAC-induced protein changes in *C. alternifolius* were investigated by 2-dimension electrophoresis and (2-DE) matrix-assisted laser desorption/ionization time-of-flight/time-of-flight high-resolution tandem mass spectrometer (MALDI TOF/TOF MS) analyses. The main goals of this study were (1) to evaluate the degree of oxidative stress by investigating the effects of the targeted PhACs on the

antioxidant system and photosynthetic pigments of *C. alternifolius* and (2) to derive new insight into the molecular mechanism governing PhAC tolerance through a proteomic approach. As far as we know, this is the first study to use proteomics to analyze responses of CW plants to PhACs. We hope that the results of this research will deepen our understanding of plants in CWs for wastewater treatment. To our knowledge, this is the first report on the molecular mechanisms (biochemical and proteomic responses) involved in the tolerance of *C. alternifolius* to PhAC.

2. Methods

2.1. Plant and CW microcosms

In August 2013, four experimental CW microcosms planted with pre-grown *C. alternifolius* were constructed in stainless steel containers. *C. alternifolius* with a height of 0.7–0.8 m was selected and transplanted into the simulated CWs at a density of 30 plants/m², 6 tillers per plant.

All CWs were located in a controlled greenhouse environment (photoperiod: 12 h, temperature: 28 ± 2 °C, illumination: 2800 ± 250 lux) in Chongqing University, China. Fig. 1 shows the layout of the experimental CWs. Details of the microcosm design are illustrated in Supplementary Materials (Text S1).

The synthetic water simulating municipal wastewater treatment plant (WWTP) secondary effluent was fortified with a mixture of all the studied PhACs, and the concentrations of individual PhACs in the influent of CW-0, CW-30, CW-100, and CW-500 were 0, 30, 100, and 500 µg/L, respectively. The synthetic water simulating municipal WWTP secondary effluent was prepared with the following composition (in mg/L): 5 MgSO₄, 3.8 CaCl₂, 1.1 KH₂PO₄, 1.85 K₂PHO₄, glucose, 10.1 NH₄Cl, 36.11 KNO₃, 54.16 NaHCO₃, 5 FeSO₄, 0.75 H₃BO₃, 5 EDTA-Na₂, 0.09 KI, 0.03 Na₂MoO₄·2H₂O, 0.075 CoCl₂·6H₂O, 0.05 MnSO₄·H₂O, 0.06 ZnSO₄·7H₂O, 0.015 CuSO₄·5H₂O. The average values of influent pH, dissolved oxygen (DO), oxidation reduction potential (ORP), and conductivity (Cond) were 7.5 ± 0.2, 7.0 ± 1.5 mg/L, 150 ± 20 mV, and 1200 ± 50 µS/cm, respectively. The wastewater was maintained in the CWs for 12 h and replaced every 12 h by freshly enriched wastewater. A set of the two replicates with a total of 8 simulated CWs was established for each PhACs concentration (0, 30, 100, and

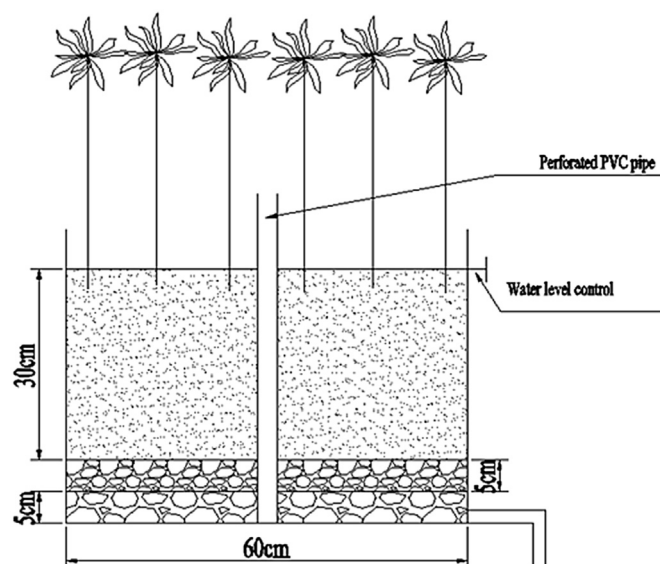


Fig. 1. Schematic representation of the experimental CW used in this study.

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