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Typha latifolia as potential phytoremediator of 2,4-dichlorophenol: Analysis of tolerance, uptake and possible transformation processes

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HIGHLIGHTS

- Cattail showed high tolerance to 2,4-DCP compared to another species.
- 99.7% of 2,4-DCP was removed when the contaminant concentration was 1.5–3 mgL^{-1}.
- Contaminant was removed by *T. latifolia* and accumulated mainly in roots.
- 2,4-DCP possible transformation take place in root tissue.
- An increment in the GST activity of plants exposed to the 2,4-DCP was observed.

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ABSTRACT

2,4-Dichlorophenol (2,4-DCP) is considered a priority pollutant due to its high toxicity. Therefore, it is urgent to develop technologies for the disposal of this pollutant. Various remediation processes have been proposed for the elimination of 2,4-DCP in contaminated water, however, most of them involve high costs of operation and maintenance. This study aimed to determine the capacity of remediation of 2,4-DCP in water by *Typha latifolia* L. wild plants. For that, the tolerance, removal, accumulation and biotransformation of 2,4-DCP by *T. latifolia* were investigated. The plants were exposed to 2,4-DCP solutions with a concentration range from 1.5 to 300 mgL^{-1} for 10 days. They exhibited a reduction in chlorophyll levels and growth rate when 2,4-DCP solutions were ≥30 mgL^{-1} and ≥50 mgL^{-1}, respectively. The removal of contaminant was dose-depended, being 99.7% at 1.5–3 mgL^{-1}, 59–70% at 10–70 mgL^{-1} and 35–42% at 100–300 mgL^{-1} of 2,4-DCP in the solution. Studies indicated that 2,4-DCP was mainly accumulated in root tissue rather than in shoot tissue. Acid hydrolysis of biomass extracts suggests 2,4-DCP bioconjugates formation in root tissue as a response mechanism. Additionally, an increment in glutathione S-transferase (GST) activity could indicate a 2,4-DCP conjugation with glutathione as a detoxification mechanism of *T. latifolia*.

1. Introduction

Chlorophenols are an important class of organic environmental contaminants that are widely used as pesticides and wood preservative products (Favaro et al., 2008). These compounds are also generated by pesticides degradation and organic trash burning (Ahlborg and Thunberg, 1980; Hoekstra et al., 1999). Additionally, they are precursors of the highly toxic dibenzo-*p*-dioxins and dibenzofurans during incineration processes (Tuppurainen et al., 2003). 2,4-dichlorophenol (2,4-DCP) is used in the production of the herbicide 2,4-dichlorophenoxiacetic acid (2,4-D) and several germicides and soil fertilizers (Talano et al., 2012). Moreover, it is applied as seed disinfectant and in the pulp paper production (Stoilova et al., 2006). The Environmental Protection Agency (EPA) has classified 2,4-DCP as a toxic, carcinogenic and persistent chemical compound and a priority environmental contaminant

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(ATSDR, 2015). This compound has been detected in high levels in fresh water sources, marine environment, industrial water discharges, soils and atmospheric emissions during trash burning (Kot-Wasik et al., 2004; Dimou et al., 2006; Gao et al., 2008; Talano et al., 2012).

Therefore, efforts to develop 2,4-DCP removal strategies from water are valuable. In this sense, various physicochemical and biological methods have been reported in the literature. (e.g. activated carbon adsorption, chemical oxidation, photocatalytic and biological degradation) (Chaliha and Bhattacharyya, 2008; Shin et al., 2010; Shaarani and Hameed, 2011). However, physicochemical methods are expensive and cannot always be applied. On the other hand, the use of microorganisms in practical remediation carries some difficulties when concentrations of pollutants are low (Shi et al., 2012).

A potential process for organic contaminants removal is phytoremediation. This is an innovative technology involving the use of plant species to remove a variety of contaminants such as metals, pesticides and hydrocarbons from soil, sediment as well as surface and groundwater (Glick, 2003; Pilon-Smits, 2005). The destiny of the pollutant in the phytoremediation process depends on its properties, for example, organic pollutants are frequently taken up by plants, metabolized or degraded by enzymes, and incorporated into plant tissues (McCutcheon and Schnoor, 2003; Talano et al., 2012). Phytoremediation is an aesthetic technique, cost-effective and has a minimal impact on the environment (McKinlay and Kasperek, 1999; Suresh and Ravishankar, 2004). Different alternatives of phytoremediation have been developed (e.g. enzymes or hairy root culture), however, these have not been scaled up to date (González et al., 2013; Wang et al., 2015b; Arriel-Torres et al., 2016). The use of whole plants has many advantages, since plants are robust in growth, are renewable resources, and can be used for *in situ* remediation (Suresh and Ravishankar, 2004). In this sense, plants used for phytoremediation purposes should be fast growing, have high biomass, deep roots and be easy to harvest (Garcinuño et al., 2006). Based on these multiple requirements, macrophyte-based wastewater treatments have been developed and show several advantages as compared to conventional treatments (Dhir et al., 2009; Guittonny-Philippe et al., 2015).

Of the aquatic plants, *Typha* genus (cattail) have the advantage of growing under various climatic conditions, function as biofilters to protect lakes, estuaries and groundwater as well as aquatic fauna and flora (Milam et al., 2004). They have the required characteristics to be used in phytoremediation because of their rapid growth, easy spreading and harvesting. However, although it has been shown that *Typha* plants have the ability to tolerate and remove different xenobiotics (e.g. atrazine, metalaxyl, simazine, methyl parathion, chlorinated benzenes, carbamazepine, diazinon, permethrin, chlorpyrifos and metformin). (Langan and Hoagland, 1996; Wilson et al., 2000; Amaya-Chávez et al., 2006; Ma and Havelka, 2008; Dordio et al., 2011; Moore et al., 2013; Wang et al., 2013; Cui et al., 2015); there is very little information regarding the transformation of organic compounds by *Typha* plants. This data is crucial for phytoremediation implementation since it should be ensured that highly toxic metabolites are not released into the environment.

Based on the above, the objective of the present study was to determine the removal efficiency of 2,4-DCP in water using wild plants of *T. latifolia* L. (cattail). The specific objectives of this research were focused on the following points: (i) determining the effect of 2,4-DCP concentration on the index growth and chlorophyll content of *Typha latifolia*; (ii) quantifying 2,4-DCP uptake; (iii) determining the plant distribution of xenobiotic; and (iv) elucidating the possible mechanism involved in the detoxification process; through acid hydrolysis and determination of GST activity.

2. Materials and methods

2.1. Plant material

T. latifolia L. individuals were collected from a site unpolluted with chlorophenols; then, the plants were transported in plastic bags to the laboratory, washed with running tap water and maintained in a greenhouse at 25–28 °C and 60% of humidity. The plants were grown in commercial soil Pro-Mix® (Premier Horticulture Inc., Red Hill, PA). The carefully selected *T. latifolia* plants with similar weight (51.61 ± 8.9 g/plant) and length (94.9 ± 9.7 cm) were used for the experiments described below.

2.2. 2,4-DCP tolerance and removal by T. latifolia

Plants (n = 3, per treatment) of similar weight and length were exposed during 10 days to single solutions of 2,4-DCP at concentrations varying from 10 to 300 mgL^{-1} (Sigma-Aldrich; St. Louis, MO, USA). Based on a previous report, the pH range for the optimal growth of *T. latifolia* was established as 6.0–6.5 (Brix et al., 2002). The ionic strength (I) was adjusted to 0.01 M with KNO_3. The final volume for each experimental unit was 300 mL. Previously, plants were acclimated to a hydroponic medium at the conditions described above without the addition of 2,4-DCP and maintained under the same conditions during 10 days. Samples of experimental solutions were collected at 0, 1, 3, 5, 7 and 10 days to determine the remaining amount of 2,4-DCP in the medium. The 2,4-DCP removal efficiency (RE, %), was calculated according the equation: $RE(\%) = [(C_0\text{-}C_t)/C_0]\cdot 100$. Where C_0 and C_t are the concentration of 2,4-DCP in solution at time 0 and time *t*, respectively.

Control solutions without 2,4-DCP were added to cattail plants under the same experimental conditions. Reference solutions at the 2,4-DCP concentrations and conditions mentioned above were prepared to verify non-biological losses of 2,4-DCP during experimental time (n = 3, per treatment). All experiments were performed under hydroponic conditions employing ambar glass material to avoid photodegradation or adsorption onto the container.

Evapotranspiration losses were replaced with deionized water in order to maintain the nominal contaminant concentrations. In addition, the 2,4-DCP concentrations tested in this work exceed the maximum concentrations permitted by the USA and the European Community in effluents, established in 0.2 mgL^{-1} (Liu et al., 2001).

The effect of 2,4-DCP on the growth of *T. latifolia* was followed by measuring the chlorophyll *a*/b ratio (Bruinsma, 1963), and the growth index (GI); biomass ratio at 0 and 10 days, fresh weight (Flocco et al., 2002).

2.3. Chemical analysis

The quantification of 2,4-DCP in test solutions was performed by High Performance Liquid Chromatography (HPLC). Samples of experimental solutions were filtered through Millipore 0.22 μM nylon filters and analyzed with a UV–vis detector (HPLC 600E Waters). A Novapack-Phenyl column of 4 μM, 3.9 mm × 150 mm dimensions was selected and detector was tuned at 242 nm. The mobile phase was prepared as a mixture of 60% aqueous solution (11 mM citric acid/0.055 mM EDTA) and 40% methanol. Flow rate of mobile phase was 1 mL/min. Standards of 2,4-DCP were analyzed at same HPLC conditions to compare retention times with samples (15.7 min).

2.4. Plant tissue extraction

After the experimental period of 10 days, the plants were