Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 88 No 6, 1508 doi: 10.2166/wst.2023.291

Uptake, accumulation, and degradation of dibutyl phthalate by three wetland plants

Yaocheng Fan[a,c], Tiancui Li[b,c,*], Zihan Zhang[c], Xiaoyong Song[c], Deshou Cun[c], Baihui Cui[c] and Yuewei Wang[c]

[a] China Communications Construction Company Second Harbor Consultants Co., Ltd, Wuhan 430060, China
[b] Ecological Environment Monitoring and Scientific Research Center, Yangtze River Basin Ecological Environment Supervision and Administration Bureau, Ministry of Ecology and Environment, Wuhan 430010, China
[c] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430070, China
*Corresponding author. E-mail: litiancui1208@163.com

ABSTRACT

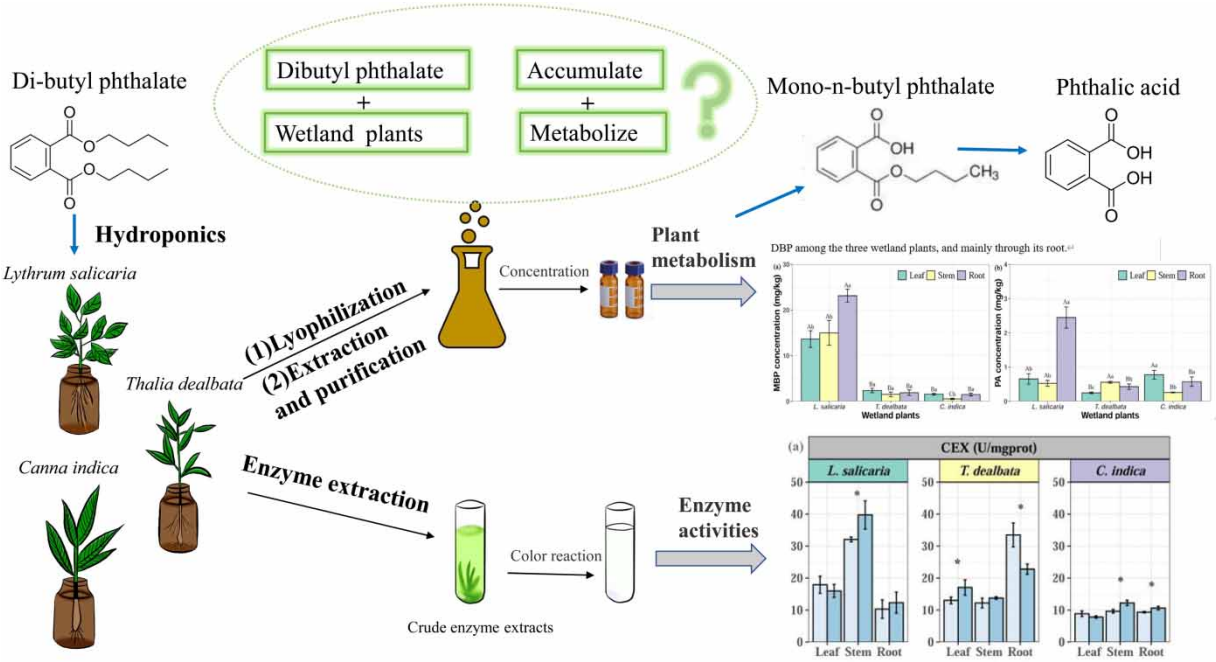
The uptake and degradation mechanisms of dibutyl phthalate (DBP) by three wetland plants, namely *Lythrum salicaria*, *Thalia dealbata*, and *Canna indica*, were studied using hydroponics. The results revealed that exposure to DBP at 0.5 mg/L had no significant effect on the growth of *L. salicaria* and *C. indica* but inhibited the growth of *T. dealbata*. After 28 days, DBP concentrations in the roots of *L. salicaria*, *T. dealbata*, and *C. indica* were 8.74, 5.67, and 5.46 mg/kg, respectively, compared to 2.03–3.95 mg/kg in stems and leaves. Mono-*n*-butyl phthalate concentrations in *L. salicaria* tissues were significantly higher than those in the other two plants at 23.1, 15.0, and 13.6 mg/kg in roots, stems, and leaves, respectively. The roots of *L. salicaria* also had the highest concentration of phthalic acid, reaching 2.45 mg/kg. Carboxylesterase, polyphenol oxidase, and superoxide dismutase may be the primary enzymes involved in DBP degradation in wetland plants. The activities of these three enzymes exhibited significant changes in plant tissues. The findings suggest *L. salicaria* as a potent plant for phytoremediation and use in constructed wetlands for the treatment of DBP-contaminated wastewater.

Key words: dibutyl phthalate, enzyme activity, hydroponics, wetland plant

HIGHLIGHTS

- The uptake and degradation mechanisms of DBP by three common wetland plants were investigated by the hydroponic experiment.
- The uptake and degradation capacities of DBP were higher in *L. salicaria*, which could well resist the oxidative damage caused by DBP and degrade it under the effect of enzymes.
- *L. salicaria* can be used as a potential plant for DBP removal in phytoremediation and the constructed wetland.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Phthalate esters (PAEs), especially dibutyl phthalate (DBP), are one of the most important additives used in the production of plastics (Becky Miriyam *et al.* 2022). In recent years, DBP has received increased attention due to its ubiquitous occurrence in the natural environment and adverse effects on animals and humans (Lu *et al.* 2019; Xu *et al.* 2019). The extensive use of plastic products in industrial manufacturing, agricultural film covering, and daily necessities has led to the detection of high concentrations of PAEs in aquatic environments, atmosphere, and soil ecosystems. DBP may have adverse effects on the human reproductive and renal systems and also cause obesity problems. DBP has been widely detected in aquatic environments, with concentrations up to 35.65 μg/L in the Yangtze River of China (Wang *et al.* 2008) and 2,705 μg/L in the Ogun River catchment in Nigeria (Adeniyi *et al.* 2011). Di (2-ethylhexyl) phthalate (DEHP) and DBP have a detrimental influence on the nitrification of black soils in China, reducing the abundance of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (NOB) (Tao *et al.* 2022). As a toxic exogenous substance to plants, DBP can inhibit the growth of some plants at excessive concentrations (Sun *et al.* 2015; Gao *et al.* 2016). DBP can also be accumulated by humans through inhalation, skin contact, and ingestion, which can disrupt the cardiovascular system and lipid metabolism and also have adverse effects on human thyroid function due to its endocrine disrupting effects (Hauser & Calafat 2005; Miodovnik *et al.* 2014).

Natural and constructed wetlands can capture pollutants from stormwater runoff, rivers, floods, sewage, and wastewater treatment plant effluents. Phytoremediation is an ecologically friendly treatment method for contaminated wastewater, and wetland plants are commonly used (Farid *et al.* 2014). Previous studies have demonstrated that wetland plants have high removal efficiencies for nitrogen, phosphorus, and heavy metals in water (Rai 2008; Sartori *et al.* 2016). *Lythrum salicaria*, *Thalia dealbata*, and *Canna indica* are widely planted in constructed wetlands in China due to their excellent landscape effect and high pollutant removal efficiency. Brunhoferova *et al.* (2021) found that *Phragmites australis*, *Iris pseudacorus*, and *L. salicaria* can remove 27 kinds of micropollutants (pharmaceuticals, pesticides, herbicides, fungicides, and others), with *L. salicaria* exhibiting the highest micropollutant uptake. Li *et al.* (2014) reported that *T. dealbata* and *C. indica* had high tolerance and bioaccumulation capability to triazophos. *L. salicaria* is an invasive plant in North America and occupies a significant niche in wetlands (Uveges *et al.* 2002). It can uptake and degrade fluoroquinolones, indicating its potential to be used in phytoremediation (Migliore *et al.* 2000). *C. indica* is a tolerant plant that can metabolize chlorophenols and fluorides into less toxic metabolites, making it a low-cost phytoremediator for chlorophenols in water (Enyoh & Isiuku 2021; Khandare *et al.* 2021). In a floating wetland in Jiaxing, China, *L. salicaria*, *T. dealbata*, and *C. indica* achieved

high removal efficiencies for COD, total nitrogen, and total phosphorus in urban runoff stormwater, with *T. dealbata* showing the best seasonal adaptability (Ge *et al.* 2016). Liu *et al.* (2021) found that the removal efficiency of six neonicotinoids by nine wetland plants including *T. dealbata* and *C. indica* ranged from 9.5 to 99.9%. The study by Lu *et al.* (2020) showed that the removal efficiency of levofloxacin by eight wetland plants (including *T. dealbata* and *C. indica*) was 87.29–96.69%, with no significant variation across different emergent plants. Due to the widespread use of PAEs, DBP has been frequently detected in water (He *et al.* 2019). However, there are few studies on the efficiency and mechanism of DBP accumulation and biotransformation by typical wetland plants.

An important sink for DBP in the environment is the wetland ecosystem. Constructed wetlands can efficiently remove six PAEs in wastewater (Tang *et al.* 2015). The removal rate of DBP in domestic sewage tailwater by vertical flow constructed wetlands can reach more than 90% (Li *et al.* 2020). This removal is mainly attributed to biodegradation, which comprises bacteria, fungi, and wetland plants. Many bacteria and fungi that can efficiently degrade DBP have been isolated from wetlands. Emergent macrophytes play an important role in wetlands, but the mechanisms of uptake, accumulation, and transformation of DBP in a typical wetland are poorly understood.

Hydroponics is a widely acknowledged technology for plant growth (Zhu *et al.* 2019), as a substrate or soil has been identified as a limiting factor for plant development. Furthermore, it can avoid the adsorption of pollutants by soil or substrate and reduce the impact of the microbial degradation of pollutants, allowing the effect and mechanism of phytoremediation of pollutants in aqueous solutions to be studied.

In this study, hydroponic experiments were used to investigate the growth of *L. salicaria*, *T. dealbata*, and *C. indica* in 500 mL Erlenmeyer flasks after exposure to DBP. The study also examined the uptake and metabolism of DBP, as well as the enzymatic activities in plant tissues. A DBP dose of 0.5 mg/L was chosen based on the discharge limit for DBP in the *Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant* (GB 18918-2002, China) and the results of our previous study (Li *et al.* 2020). The objectives of this study were to (1) elucidate the uptake and accumulation of DBP by the three typical wetland plants using hydroponics and (2) decipher the biotransformation mechanism of DBP through the analysis of enzyme activities and metabolites of DBP in the plants.

2. MATERIALS AND METHODS

2.1. Chemicals

DBP (analytical reagent) used in the experiment was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Standards of DBP, mono-*n*-butyl phthalate (MBP), and phthalic acid (PA) and internal standards of DnBP-d4, MBP-d4, and PA-d4 were provided by Dr Ehrenstorfer GmbH (Germany). The experimental stock solutions of PAEs were prepared in methanol and stored in amber glass vials at −20 °C before use. The organic solvents including *n*-hexane, methanol, dichloromethane, acetone, and acetonitrile were of HPLC grade and obtained from Fisher Scientific, Fair Lawn, NJ. An Ultra water purification system (Hitech-Sciencetool, China) was used for getting deionized water.

2.2. Hydroponic cultivation experiment

The seeds of *L. salicaria* and *T. dealbata* were purchased from Jiangsu Tangfeng Agricultural Technology Co. Ltd. After sterilization in H_2O_2, the seeds were germinated in the culture dish on the filter paper moistened with deionized water. After 2 weeks, the seedlings of *L. salicaria* and *T. dealbata* were transferred to a glass crystallization dish (diameter 18 cm, height 8 cm) equipped with sterilized silver sand. For seedling development, 200 mL of sterilized 1/4 Hoagland nutrient solution was added to the crystallizing dish. After 1 month of cultivation, the seedlings were removed from the silver sand and washed carefully with deionized water before being planted in a 100 mL glass jar containing 1/2 Hoagland nutrient solution. Because of the poor germination rate of *C. indica* seeds, seedlings of *C. indica* purchased at this stage and equivalent in height to those of *L. salicaria* and *T. dealbata* at this stage were used for the experiment.

After 4 weeks, the plants were cultivated in 500 mL Erlenmeyer flasks, and experimental treatments were performed. Briefly, in the DBP exposure group, the plants were suspended in a Hoagland nutrient solution containing 0.5 mg/L DBP (DBP_0.5). To negate the possible cross-contamination, a no-spiked control group with plant hydroponics cultivation was also undertaken (DBP_0). To study the effects of microbial degradation and photodegradation on DBP, a no-plant control group spiked with 0.5 mg/L DBP was conducted simultaneously. Plant cultivation was carried out in an artificial growth chamber with a 14 h light/10 h dark cycle, constant 80% relative air humidity, a temperature of 25 °C, and a photosynthetic

photon flux density of 350 mmol/($m^2 \cdot s^{-1}$). Every other day, plants were transferred to Erlenmeyer flasks with fresh nutrient solution containing DBP to restore nutritional levels and lower microbial load.

Plants were harvested after 28 days of growth and divided into three groups for the determination of growth indicators (the dry biomass and height of the three wetland plants), DBP and its metabolites, and enzymatic activities.

2.3. Analysis of DBP and its metabolites

After 28 days of exposure, the concentration of DBP and its metabolites in the roots, stems, and leaves of all the three wetland plants were determined. Following the blot with tissue paper, the roots were washed three times with Milli-Q water and then rinsed with methanol to assess DBP adsorbed on the root surface. Subsequently, the plants were separated into roots, stems, and leaves. These samples were freeze-dried and ground homogeneously for analysis. DBP and its metabolites were extracted and purified from plant tissues following the methods of Sun *et al.* (2015) and (Zhu *et al.* 2019) with some modifications, and details are presented in Supplementary Material, S1 and S3. In this study, DBP was detected by GC–MS (Agilent 7890A-5975C), while its metabolites (MBP and PA) were determined by the Waters Xevo TQ LC–MS/MS system. The instrument operational parameters for GC–MS and LC–MS/MS are presented in Supplementary Material, S2 and S4.

2.4. Enzyme activity assays in plants

Carboxylesterase (CXE) activity was determined according to the method of Lin *et al.* (2017). Briefly, 1.0 g of fresh plant tissue was homogenized in an ice bath with 10 mL of phosphate buffer (0.1 M, pH 7.0) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA) and 1% (w/v) polyvinyl pyrrolidone. The homogenate was then centrifuged at 15,000 g for 10 min at 4 °C, with the supernatant reserved for the enzymatic assay. About 0.2 mL of the enzyme extract was added into 2.5 mL of phosphate buffer (0.1 M, pH 7.0) containing 0.3 mM 1-naphthyl acetate and 1 μM physostigmine as a substrate. After incubation in a water bath at 25 °C for 30 min, 0.5 mL of Fast Blue B salt-sodium and dodecyl sulfate solution was added. After 10 min of chromogenic reaction, the absorbance was measured at 600 nm using an ultraviolet spectrophotometer (UV3600,Thermo Fisher Scientific). One unit of enzyme activity (U) is defined as the amount of enzyme releasing 1 μmol/L 1-naphthol per minute.

Polyphenol oxidase (PPO) activity was determined by the method of Zhu *et al.* (2019). In brief, 0.2 mL of the enzyme extract was mixed with 2.0 mL of phosphate buffer (0.01 M, pH 6.0) and 1.0 mL of o-dihydroxybenzene (0.1 M). Absorbance values were read continuously at 420 nm for four times at 1-min intervals. An enzyme activity unit (U) is defined as the amount of enzyme that causes an increase in absorbance (optical density) per minute.

The activities of superoxide dismutase (SOD) and peroxidase (POD) were measured using kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

The total protein content of each fresh plant tissue was also assessed using kits procured from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All enzymatic activities are expressed as units of enzyme activity per milligram of protein (U/mgprot).

2.5. Data analysis

The bioconcentration factor (BCF) and the translocation factor (TF) values were calculated using Equations (1) and (2), respectively:

$$\mathrm{BCF} = C_p/C_0 \tag{1}$$

$$\mathrm{TF} = C_l/C_r \tag{2}$$

where C_p is the concentration of the target compound in the plant tissue and C_0 is the concentration of chemicals in the growth medium. C_l and C_r are the concentrations of the target compound in leaves and roots, respectively.

The mean and standard deviation of three replicates were computed using R-4.1.2 for Windows. All statistical analyses and graphics were created in R-4.1.2 for Windows. A one-way ANOVA with a significance threshold of 0.05 was performed to compare differences in target chemical concentrations between various plants and treatments.

3. RESULTS AND DISCUSSION

3.1. Growth response of plants on exposure to DBP

DBP is a toxic exogenous substance to plants that is tolerated variably by different plants, and it can inhibit the growth of some plants at excessive concentrations (Sun *et al.* 2015; Gao *et al.* 2016). For *L. salicaria* and *C. indica*, ANOVA tests revealed no significant differences ($P > 0.05$) in plant height and dry biomass between the DBP-exposed group (DBP_0.5) and their corresponding control groups (DBP_0) (Table 1). However, for *T. dealbata*, although there was no substantial difference in plant height, the dry biomass was significantly lower in DBP_0.5 ($P = 0.01$). DBP dosing reduced *T. dealbata* dry biomass by 24.2% as compared to the control. This indicated that *L. salicaria* and *C. indica* were more tolerant to DBP as their growth was not significantly affected at a DBP concentration of 0.5 mg/L. In contrast, *T. dealbata* was susceptible to the significant inhibition of growth by DBP. Hence, *T. dealbata* was less suitable for treating wastewater containing DBP in constructed wetlands as compared to the other two wetland plants.

3.2. Uptake and accumulation of DBP

The DBP concentration accumulated in roots was significantly greater than that in stems and leaves ($P < 0.05$) in all three plants (Figure 1). For the same plant species, there was no significant variation in the concentration of DBP between

Table 1 | The height and dry biomass of plants after the experiment (mean ± SD)

Wetland plants	Groups	Plant height (cm)	Dry biomass (g)
L. salicaria	DBP_0	38.60 ± 2.07a	8.36 ± 0.33a
	DBP_0.5	39.40 ± 1.52a	7.48 ± 0.16a
T. dealbata	DBP_0	75.2 ± 12.77a	7.72 ± 0.31a
	DBP_0.5	65.20 ± 8.26a	5.85 ± 0.23b
C. indica	DBP_0	51.80 ± 3.27a	8.63 ± 0.51a
	DBP_0.5	49.80 ± 4.66a	8.65 ± 0.25a

Note: Different letters indicate significant differences between the two groups of each plant ($P < 0.05$). DBP_0, no-spiked control group; DBP_0.5, 0.5 mg/L DBP exposure group.

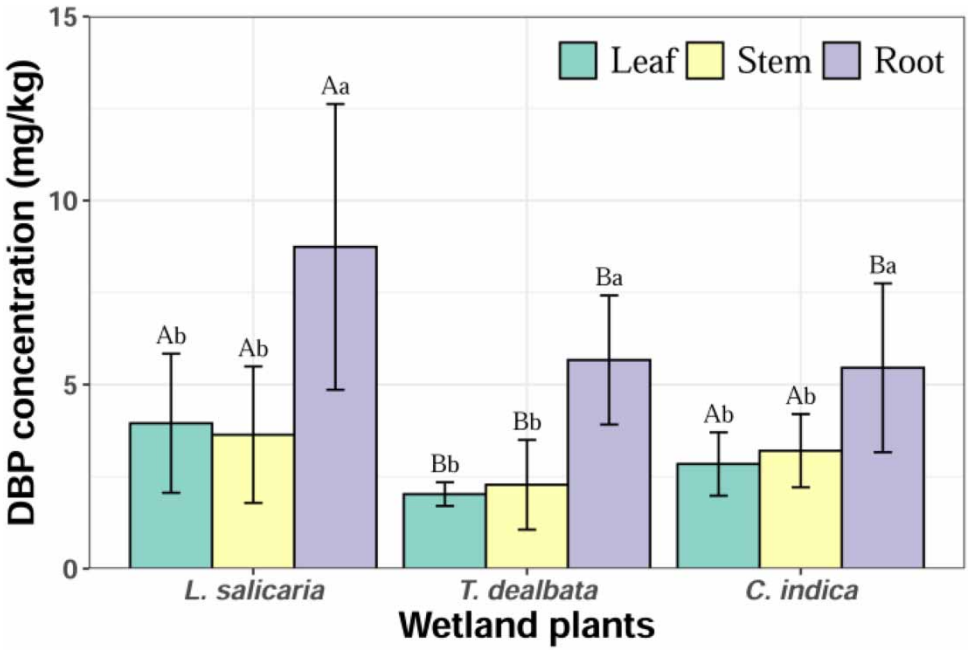


Figure 1 | DBP concentrations in different plant tissues after 28 days of hydroponics. (Different capital letters indicate significant differences in uniform tissues of different plants, and different lowercase letters denote significant differences among different tissues of each plant.)

stems and leaves (Figure 1). This was also supported by the fact that the BCF values of DBP were significantly higher in the roots when compared to the stems and leaves ($P < 0.05$) (Table 2). This could be attributed to direct contact and the uptake of DBP by plant roots. These findings were in agreement with the outcomes reported for plants such as *Oryza sativa* (Zhu *et al.* 2019), *Cucurbita moschata* (Lin *et al.* 2017), and *P. australis* (Li *et al.* 2020).

The DBP concentration in the roots of different plants also varied significantly (Figure 1). It was 8.47 ± 3.88 mg/kg in the roots of *L. salicaria*, which was significantly higher than that in the roots of *T. dealbata* (5.67 ± 1.75 mg/kg) and *C. indica* (5.45 ± 2.29 mg/kg). However, there was no significant difference between the roots of *T. dealbata* and *C. indica* ($P > 0.05$). The lowest concentration of BDP was accumulated in the stems and leaves of *T. dealbata*, while no significant variations were found in the stems or leaves between *L. salicaria* and *C. indica* ($P > 0.05$). Additionally, the total amount of DBP accumulation from the entire plant was 35.6 ± 10.4 μg in *L. salicaria* and 29.7 ± 6.3 μg in *C. indica*, both of which were remarkably higher than 16.5 ± 4.8 μg in *T. dealbata* ($P < 0.05$). BCF and TF values among the three wetland species reflected a pattern similar to that of plant accumulation (Table 2). In general, plants take up pollutants mainly through their root system, and thus, the more developed the root system, the better the absorption of pollutants (Li *et al.* 2014). The study findings indicated that the root system of *L. salicaria* was particularly well developed, which contributed to the uptake of DBP. Although *T. dealbata* had a rich root system, a considerable amount of DBP was adsorbed on the root surface (3.12 ± 0.82 mg/kg) (Table 3), instead of direct absorption. All these findings suggest that the uptake and accumulation capacity of DBP is the highest for *L. salicaria*, followed by *C. indica*, and the lowest for *T. dealbata*.

3.3. Degradation of DBP

Previous studies have confirmed that DBP taken up by plants can be degraded to MBP and PA (Sun *et al.* 2015; Lin *et al.* 2017; Zhu *et al.* 2019). In the present work, MBP and PA were observed in all tissues of plants (Figure 2), and none were detected in the plants or solution of controls (no-spiked control and no-plant control). This demonstrated that MBP and PA were derived from the degradation of DBP by wetland plants. The concentration of PA was nearly one order of magnitude, which was lower than that of MBP in the tissues of all three plants (Figure 2), suggesting that PA was derived from the degradation of MBP. These findings were similar to the previously reported studies of DBP degradation by rice (Zhu *et al.* 2019) and pumpkin (Lin *et al.* 2017).

As shown in Figure 2(a), MBP concentration in all tissues of *L. salicaria* was significantly higher than that in the other two species ($P < 0.05$), which was 23.1 ± 1.4, 15.0 ± 2.7, and 13.6 ± 1.8 mg/kg in roots, stems, and leaves, respectively. Except for the stems (the stems of *C. indica* contained only 0.5 ± 0.1 mg/kg MBP), the MBP concentrations in the tissues of *T. dealbata* and *C. indica* were comparable. Additionally, the MBP concentration in *L. salicaria* was significantly higher than the DBP content in its equivalent tissues, but not in *T. dealbata* and *C. indica.* Likewise, for PA (Figure 2(b)), the highest content was also found in the roots of *L. salicaria*. These results confirmed that among the three wetland plants, *L. salicaria* was the most efficient in degrading DBP, mostly through its roots.

Table 2 | BCF and TF values of DBP in the three plants

Plants	BCF (root)	BCF (stem)	BCF (leaf)	TF
L. salicaria	17.48 ± 7.76	7.28 ± 3.71	7.90 ± 3.78	0.49 ± 0.05
T. dealbata	11.34 ± 3.50	4.56 ± 2.43	4.05 ± 0.64	0.33 ± 0.07
C. indica	10.91 ± 4.58	6.41 ± 3.17	5.69 ± 1.72	0.31 ± 0.08

Table 3 | Concentrations and total mass of DBP absorbed on the surface of three plants

Plants	DBP concentration (mg/kg, dry weight)	DBP adsorbed mass (μg)
L. salicaria	1.72 ± 0.39	12.94 ± 2.67
T. dealbata	3.12 ± 0.82	18.34 ± 5.42
C. indica	1.00 ± 0.22	8.71 ± 2.11

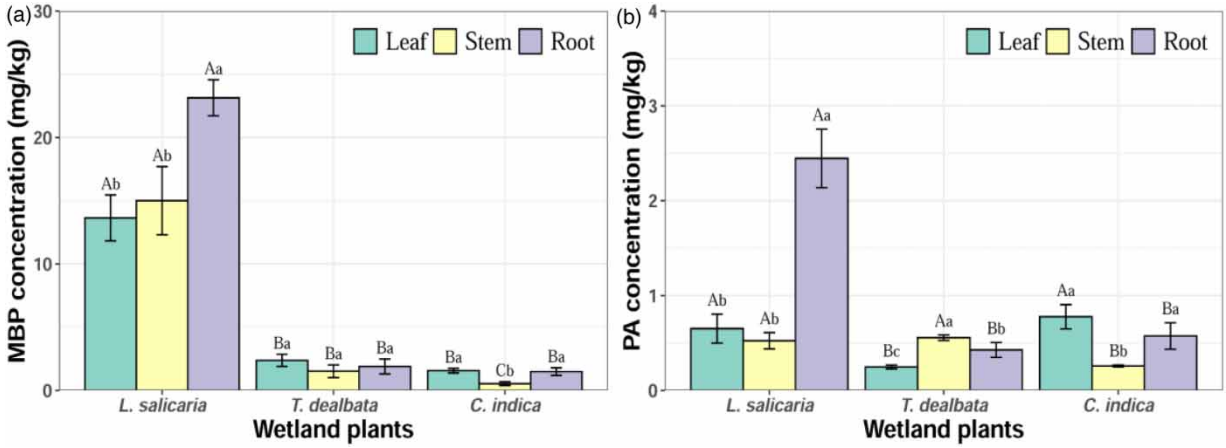


Figure 2 | MBP and PA concentrations in different plant tissues.

3.4. Enzymatic activities in plant tissues

Studies have revealed that the metabolism and tolerance of PAEs vary amidst different plants (Cai *et al.* 2008; Sun *et al.* 2015; Zhao *et al.* 2015). These variations are related to the differences in metabolic and tolerance enzyme activities in plants (Huang *et al.* 2018; Zhu *et al.* 2019). In the present study, four DBP metabolizing antioxidant enzymes were detected in the no-spiked control group (DBP_0) and the DBP exposure group (DBP_0.5) at the end of the experiment (Figure 3).

After DBP exposure, the CXE activity increased significantly in the stems of *L. salicaria* ($P < 0.05$) while numerically elevated and decreased in the roots and leaves, respectively (Figure 3(a)). For *T. dealbata*, compared to DBP_0, CXE activity in the roots of DBP_0.5 was significantly reduced by 31.9% ($P < 0.05$). However, in the leaves, it was remarkably elevated by 30.7% ($P < 0.05$), while it was not significantly altered in the stems. A marked activity increase was also observed in both

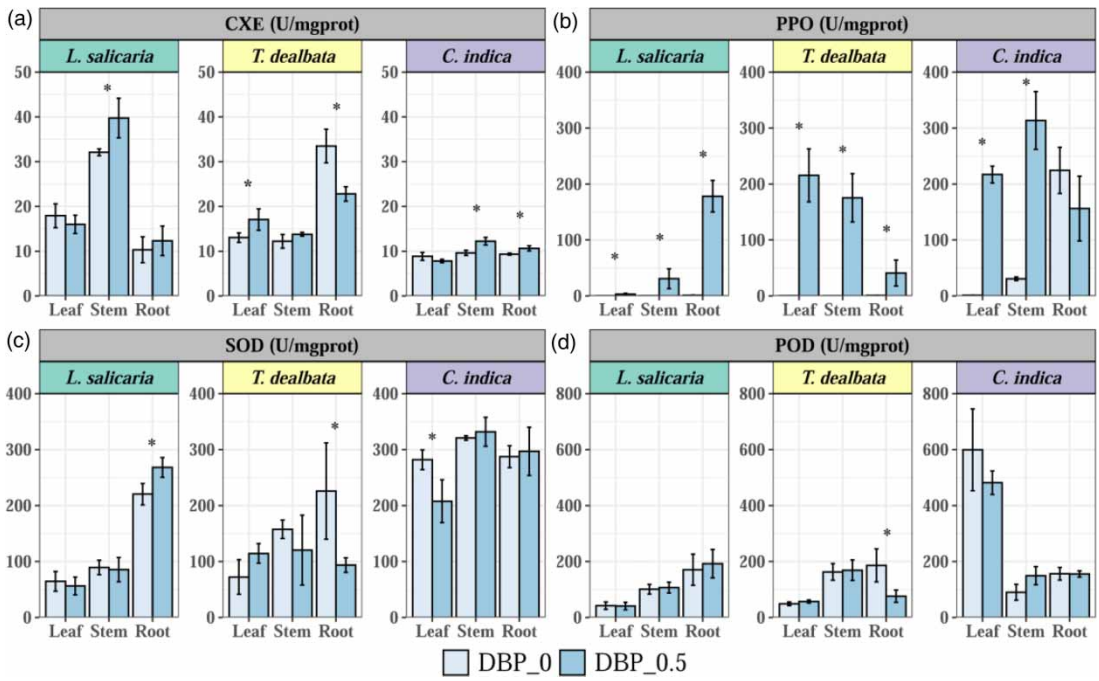


Figure 3 | Enzyme activities in different plant tissues at the end of the experiment. (Asterisks above the columns indicate significant differences between the no-spiked control group (DBP_0) and the DBP exposure group (DBP_0.5) at the level of $P < 0.05$. CEX, carboxylesterase; PPO, polyphenol oxidase; SOD, superoxide dismutase; POD, peroxidase.).

roots and stems of *C. indica* after DBP exposure ($P < 0.05$), but no significant change was observed in the leaves. CXE has been proved to hydrolyze PAEs, especially short-side-chain PAEs (e.g. DBP) (Ozaki *et al.* 2017; Zhu *et al.* 2019), and has the active site required to participate in DBP catabolism (Mahajan *et al.* 2019). Compared to rice (Zhu *et al.* 2019) and pumpkin (Lin *et al.* 2017), relatively higher CXE activities were detected in the tissues of the three wetland plants, indicating that all three plants are capable of metabolizing DBP.

In addition to *C. indica*, the PPO activity detected in the roots, stems, and leaves of both *L. salicaria* and *T. dealbata* was very low in the DBP_0 group (below 1.2 U/mgprot) but was significantly elevated in the DBP_0.5 group ($P < 0.05$) (Figure 3(b)). For roots, stems, and leaves, the increase was 155-, 101-, and 18-fold in *L. salicaria*, and 43-, 841-, and 520-fold in *T. dealbata*, respectively. No substantial increase was observed in the roots of *C. indica* ($P > 0.05$), while the stems and leaves exhibited a significant increase ($P < 0.05$). PPO is an enzyme that is involved in the catabolic conversion of aromatic organic compounds. Research has shown that PPO can catalyze the oxidation of polycyclic aromatic hydrocarbons to open their rings and convert them into more easily degradable intermediates, such as quinones, thus accelerating their degradation (Gao *et al.* 2012; Taranto *et al.* 2017). Therefore, the enhanced PPO activity in the tissues of the three plants after DBP exposure (apart from the roots of *C. indica*) indicated that all three plants can further open the ring to degrade DBP.

Figure 3(c) and 3(d) depicts the changes in the enzyme activities of SOD and POD in the plant tissues of DBP_0 and DBP_0.5, respectively. Compared to DBP_0, a significant increase in SOD activity was observed in the roots of *L. salicaria* ($P < 0.05$), while a significant reduction was observed in the roots of *T. dealbata* and the leaves of *C. indica* ($P < 0.05$) (Figure 3(c)). There were no significant alterations in any of the other tissues of the three plants ($P > 0.05$). Nevertheless, there was a significant difference in POD activity between DBP_0 and DBP_0.5 only in the roots of *L. salicaria* ($P < 0.05$), which decreased from 186.3 ± 58.8 to 76.2 ± 21.7 U/mgprot after DBP exposure (Figure 3(d)). SOD and POD are two important classes of antioxidant enzymes that eliminate reactive oxygen species from cells, thereby maintaining the oxidation–reduction balance in the cells and protecting plants from damage (Mascher *et al.* 2002; Gao *et al.* 2017). The SOD and POD activities in the roots of *T. dealbata* decreased dramatically, suggesting that the roots may have been damaged by DBP, so that their growth was somewhat inhibited (Table 1).

In this study, the enzyme activities in different plant tissues responded differentially to DBP exposure. Since the TF values of all three plants to DBP were less than 1 (Table 2), the transport ability of BDP was poor in all three plants, resulting in that the majority of the absorbed DBP accumulated in the roots (Figure 1). Hence, roots' capacity to metabolize and tolerate DBP is crucial. The roots of *L. salicaria* had a strong metabolic and tolerant ability to DBP, with the enzymatic activities of CEX, PPO, SOD, and POD increased by 19.4%, 155-fold, 21.7%, and 12.6%, respectively. Consequently, *L. salicaria* absorbed a large amount of DBP in its roots and hydrolyzed it to produce large amounts of MBP and PA (DBP, MBP, and PA concentrations were highest in the roots of *L. salicaria* (Figures 1 and 2)). For *C. indica*, the CXE activity of the roots was significantly higher, but its PPO activity was reduced and its antioxidant capacity against DBP was normal (SOD and POD did not change dramatically). Thus, the amount of DBP absorbed was small and its metabolic capacity was limited. After DBP exposure, although there was a significant increase in PPO activity in *T. dealbata* (Figure 3(b)), the significant decrease in root SOD and POD activities indicated that the roots might have been oxidatively damaged by DBP. This process resulted in a significant decrease in root CXE activity and a poor capacity for DBP uptake and initial hydrolytic metabolism.

4. CONCLUSIONS

This study investigated the uptake, accumulation, and degradation of DBP by three wetland plants, as well as the impact of DBP on enzyme activities and plant growth. The results showed that:

(1) DBP at 0.5 mg/L exhibited no significant effect on the short-term growth of *L. salicaria* and *C. indica* but dramatically inhibited the growth of *T. dealbata*.
(2) DBP could be absorbed and accumulated by the three wetland plants, with most of the DBP accumulating in roots and low transfer to stems and leaves. Among the three species, *L. salicaria* had the highest DBP uptake and accumulation capacity, followed by *C. indica* and the lowest by *T. dealbata*.
(3) DBP could be degraded to MBP and further metabolized to PA in all three wetland plants. However, *L. salicaria* had the highest DBP degradation ability due to its well-developed root system and the elevated activity of metabolic and antioxidant enzymes in the roots after DBP exposure.

Summarily, the findings of this study suggested that among the three wetland plants, *L. salicaria* could be the most suitable for the phytoremediation of DBP-contaminated water in constructed wetlands. Moreover, in the future, further research is needed to investigate the practical effectiveness of *L. salicaria* in constructed wetlands for treating DBP-contaminated water.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (No. 51578538).

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Adeniyi, A. A., Okedeyi, O. O. & Yusuf, K. A. 2011 Flame ionization gas chromatographic determination of phthalate esters in water, surface sediments and fish species in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environmental Monitoring and Assessment* **172** (1–4), 561–569.

Becky Miriyam, I., Anbalagan, K. & Magesh Kumar, M. 2022 Phthalates removal from wastewater by different methods – a review. *Water Science & Technology* **85** (9), 2581–2600.

Brunhoferova, H., Venditti, S., Schlienz, M. & Hansen, J. 2021 Removal of 27 micropollutants by selected wetland macrophytes in hydroponic conditions. *Chemosphere* **281**, 130980.

Cai, Q. Y., Mo, C. H., Wu, Q. T., Katsoyiannis, A. & Zeng, Q. Y. 2008 The status of soil contamination by semivolatile organic chemicals (SVOCs) in China: a review. *Science of the Total Environment* **389** (2–3), 209–224.

Enyoh, C. E. & Isiuku, B. O. 2021 Competitive biosorption and phytotoxicity of chlorophenols in aqueous solution to *Canna indica* L. *Current Research in Green and Sustainable Chemistry* **4**, 100094.

Farid, M., Irshad, M., Fawad, M., Ali, Z., Eneji, A. E., Aurangzeb, N., Mohammad, A. & Ali, B. 2014 Effect of cyclic phytoremediation with different wetland plants on municipal wastewater. *International Journal of Phytoremediation* **16** (6), 572–581.

Gao, Y., Li, H. & Gong, S. 2012 Ascorbic acid enhances the accumulation of polycyclic aromatic hydrocarbons (PAHs) in roots of tall fescue (*Festuca arundinacea* Schreb.). *PLoS One* **7** (11), e50467.

Gao, M., Qi, Y., Song, W. & Xu, H. 2016 Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings. *Chemosphere* **151**, 76–83.

Gao, M., Dong, Y., Zhang, Z., Song, W. & Qi, Y. 2017 Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. *Chemosphere* **172**, 418–428.

Ge, Z., Feng, C., Wang, X. & Zhang, J. 2016 Seasonal applicability of three vegetation constructed floating treatment wetlands for nutrient removal and harvesting strategy in urban stormwater retention ponds. *International Biodeterioration & Biodegradation* **112**, 80–87.

Hauser, R. & Calafat, A. M. 2005 Phthalates and human health. *Occupational and Environmental Medicine* **62** (11), 806–818.

He, Y., Wang, Q., He, W. & Xu, F. 2019 The occurrence, composition and partitioning of phthalate esters (PAEs) in the water-suspended particulate matter (SPM) system of Lake Chaohu, China. *Science of the Total Environment* **661**, 285–293.

Huang, Y. H., Huang, X. J., Chen, X. H., Cai, Q. Y., Chen, S., Mo, C. H., Lu, H. & Wong, M. H. 2018 Biodegradation of di-butyl phthalate (DBP) by a novel endophytic bacterium *Bacillus subtilis* and its bioaugmentation for removing DBP from vegetation slurry. *Journal of Environmental Management* **224**, 1–9.

Khandare, R. V., Watharkar, A. D., Pawar, P. K., Jagtap, A. A. & Desai, N. S. 2021 Hydrophytic plants *Canna indica*, *Epipremnum aureum*, *Cyperus alternifolius* and *Cyperus rotundus* for phytoremediation of fluoride from water. *Environmental Technology & Innovation* **21**, 101234.

Li, Z., Xiao, H., Cheng, S., Zhang, L., Xie, X. & Wu, Z. 2014 A comparison on the phytoremediation ability of triazophos by different macrophytes. *Journal of Environmental Sciences* **26** (2), 315–322.

Li, T., Fan, Y., Cun, D., Song, X., Dai, Y., Wang, F., Wu, C. & Liang, W. 2020 Treatment performance and microbial response to dibutyl phthalate contaminated wastewater in vertical flow constructed wetland mesocosms. *Chemosphere* **246**, 125635.

Lin, Q., Chen, S., Chao, Y., Huang, X., Wang, S. & Qiu, R. 2017 Carboxylesterase-involved metabolism of di-n-butyl phthalate in pumpkin (*Cucurbita moschata*) seedlings. *Environmental Pollution* **220**, 421–430.

Liu, H., Tang, X., Xu, X., Dai, Y., Zhang, X. & Yang, Y. 2021 Potential for phytoremediation of neonicotinoids by nine wetland plants. *Chemosphere* **283**, 131083.

Lu, L., Rong, H., Wu, C., Cui, B., Huang, Y., Tan, Y., Zhang, L., Peng, Y., Garcia, J. M. & Chen, J. A. 2019 Levels of phthalate acid esters and sex hormones and their possible sources in traffic-patrol policemen in Chongqing. *Environmental Science and Pollution Research* **26** (9), 9005–9013.

Lu, H., Wang, H., Lu, S., Li, J. & Wang, T. 2020 Response mechanism of typical wetland plants and removal of water pollutants under different levofloxacin concentration. *Ecological Engineering* **158**, 106043.

Mahajan, R., Verma, S., Kushwaha, M., Singh, D., Akhter, Y. & Chatterjee, S. 2019 Biodegradation of dinbutyl phthalate by psychrotolerant *Sphingobium yanoikuyae* strain P4 and protein structural analysis of carboxylesterase involved in the pathway. *International Journal of Biological Macromolecules* **122**, 806–816.

Mascher, R., Lippmann, B., Holzinger, S. & Bergmann, H. 2002 Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. *Plant Science* **163** (5), 961–969.

Migliore, L., Cozzolino, S. & Fiori, M. 2000 Phytotoxicity to and uptake of flumequine used in intensive aquaculture on the aquatic weed, *Lythrum salicaria* L. *Chemosphere* **40** (7), 741–750.

Miodovnik, A., Edwards, A., Bellinger, D. C. & Hauser, R. 2014 Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence. *Neurotoxicology* **41**, 112–122.

Ozaki, H., Sugihara, K., Watanabe, Y., Moriguchi, K., Uramaru, N., Sone, T., Ohta, S. & Kitamura, S. 2017 Comparative study of hydrolytic metabolism of dimethyl phthalate, dibutyl phthalate and di(2-ethylhexyl) phthalate by microsomes of various rat tissues. *Food and Chemical Toxicology* **100**, 217–224.

Rai, P. K. 2008 Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an ecosustainable approach. *International Journal of Phytoremediation* **10** (2), 131–158.

Sartori, L., Canobbio, S., Fornaroli, R., Cabrini, R., Marazzi, F. & Mezzanotte, V. 2016 COD, nutrient removal and disinfection efficiency of a combined subsurface and surface flow constructed wetland: a case study. *International Journal of Phytoremediation* **18** (4), 416–422.

Sun, J., Wu, X. & Gan, J. 2015 Uptake and metabolism of phthalate esters by edible plants. *Environmental Science & Technology* **49** (14), 8471–8478.

Tang, X., Wang, S., Yang, Y., Tao, R., Dai, Y., D, A. & Li, L. 2015 Removal of six phthalic acid esters (PAEs) from domestic sewage by constructed wetlands. *Chemical Engineering Journal* **275**, 198–205.

Tao, Y., Feng, C., Xu, J., Shen, L., Qu, J., Ju, H., Yan, L., Chen, W. & Zhang, Y. 2022 Di(2-ethylhexyl) phthalate and dibutyl phthalate have a negative competitive effect on the nitrification of black soil. *Chemosphere* **293**, 133554.

Taranto, F., Pasqualone, A., Mangini, G., Tripodi, P., Miazzi, M. M., Pavan, S. & Montemurro, C. 2017 Polyphenol oxidases in crops: biochemical, physiological and genetic aspects. *International Journal of Molecular Sciences* **18** (2), 377.

Uveges, J. L., Corbett, A. L. & Mal, T. K. 2002 Effects of lead contamination on the growth of *Lythrum salicaria* (purple loosestrife). *Environmental Pollution* **120** (2), 319–323.

Wang, F., Xia, X. & Sha, Y. 2008 Distribution of phthalic acid esters in Wuhan section of the Yangtze River, China. *Journal of Hazardous Materials* **154** (1–3), 317–324.

Xu, P., Xiao, E., Wu, J., He, F., Zhang, Y. & Wu, Z. 2019 Enhanced nitrate reduction in water by a combined bio-electrochemical system of microbial fuel cells and submerged aquatic plant *Ceratophyllum demersum*. *Journal of Environmental Sciences* **78**, 338–351.

Zhao, H. M., Du, H., Xiang, L., Chen, Y. L., Lu, L. A., Li, Y. W., Li, H., Cai, Q. Y. & Mo, C. H. 2015 Variations in phthalate ester (PAE) accumulation and their formation mechanism in Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars grown on PAE-contaminated soils. *Environmental Pollution* **206**, 95–103.

Zhu, T. K., Du, P. P., Zeng, L. J., Lu, H., Zhao, H. M., Li, Y. W., Mo, C. H. & Cai, Q. Y. 2019 Variation in metabolism and degradation of di-n-butyl phthalate (DBP) by high- and low-DBP accumulating cultivars of rice (*Oryza sativa* L.) and crude enzyme extracts. *Science of the Total Environment* **668**, 1117–1127.

First received 9 May 2023; accepted in revised form 16 August 2023. Available online 7 September 2023