**Removal mechanisms and plant species selection by
bioaccumulative factors in surface flow constructed wetlands
(CWs): In the case of triclosan**

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Abstract

Plants can bioaccumulate triclosan and bond with microbes and sediments in constructed wetlands (CWs) as well. However, little is known regarding the species-specific removal mechanism of CWs components and the selection of suitable wetland plant species for triclosan disposal. In this work, the use of bioaccumulation factors (BAFs) and biota to sediment accumulation factors (BSAFs) for choosing the best triclosan removal plant species was studied in laboratory-scale CWs. By the end of the experiment, over 80% of triclosan was removed and a specie-effect distribution was revealed in CWs with emergent, submerged and floating plants. By mass balance calculation, negative correlation between triclosan concentration in plants and degradation process was observed. The significant correlations between Log BSAFs values and triclosan concentration in plants or degradation contribution made it possible and reasonable in wetland plants selection. Introductions on plant species were provided considering the target removal process or regulation method. This work provided new information on plant species selection in CWs for triclosan removal or its emergency remediation by using bioaccumulative factors.

Keywords: CWs; Triclosan; Removal mechanism; Bioaccumulative factors; Plant species selection.

1. Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol, $C_{12}H_7Cl_3O_2$) was introduced more than 40 years ago and used as a preservative or as an antiseptic agent in pharmaceutical and personal care products (PPCPs), such as soaps, cosmetics, dental products etc. (Singer et al., 2002). The subsequent discharge of triclosan from wastewater effluent to streams or rivers was identified as a primary source of triclosan contamination, as triclosan was removed incompletely from wastewater treatment

plants (Oulton et al., 2010). It has been reported that the treated wastewater effluents contained 35-2700 ng L⁻¹ triclosan (McAvoy et al., 2002; Halden and Paull, 2005). This release brings environmental concerns as triclosan is toxic to organisms by blocking the fatty acid synthesis through inhibiting the enzyme enoyl-acyl carrier protein reductase (Levy et al., 1999). Furthermore triclosan can not only cause endocrine disruption but also interfere with thyroid hormone metabolism (Crofton et al., 2007; Gee et al., 2008). And now triclosan has been demonstrated as one of the most frequently detected organic contaminants in natural streams (Zhao et al., 2013).

CW is a cost-effective option for PPCPs removal from wastewater treatment plant effluents. Studies have indicated that 70 to 100% of triclosan can be reduced by CWs (Waltman et al., 2006; Lim et al., 2008; Park et al., 2009). PPCPs can be eliminated by biodegradation, photodegradation, sedimentation and plant uptake in CWs (Matamoros and Bayona, 2008). And wetland plants are regarded as key factors to PPCPs removal. Wetland plants are rooted in the sediment, and microbes are attached to the root surface of plant. Plant can transmit oxygen to its root and part of the oxygen will be released to the rhizosphere (Brix, 1997). This oxygen loss as well as some root exudates in plants rhizosphere form the unique physicochemical sediment environment, and also promote the microbial abundance, activity or diversity (Faulwetter et al., 2009; Meng et al., 2014).

Recent studies have indicated the importance of wetland plants in triclosan removal and their ability in triclosan bioaccumulation (Coogan et al., 2007; Park et al., 2009). Factors, i.e. bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs), are used to quantify bioaccumulation potential. According to Mackay and Fraser (2000), bioaccumulation potential by fish is species-specific, while there is a lack of knowledge of triclosan bioaccumulation ability of different

species of wetland plants. Zarate et al. (2012) conducted the first study of the species specific difference in triclosan accumulation, while they only focused on three emergent plants. Besides, our former study showed that triclosan could negatively affect the microbial abundance and community structure in CWs, and this influence also varied with plant species (Zhao et al., 2015). We also proved that microbes relating to triclosan biodegradation were species-specific. However, triclosan removal mechanisms by wetland components, i.e. microbes, plants and sediment, in CWs with different species of plants is unknown yet. Due to the relationship between plants and other wetland components, from the perspective of plants to choose index for triclosan removal may be a more comprehensive consider and an interesting topic to study.

As an initial attempt, the removal mechanism and assessment of using bioaccumulative factors in plant species selection for triclosan removal was conducted in surface flow laboratory-scale constructed wetlands. Firstly, we studied the removal efficiency and distribution of triclosan within the wetland units with different species of plants (emergent, submerged and floating plants). Based on mass balance calculation and SPSS analysis, contributions of wetland components as well as their relationship in triclosan removal were able to be obtained. Finally, the feasibility of using bioaccumulative factors (BAFs and BSAFs) in plant species selection was assessed, and instructions on species selection of wetland plants for triclosan removal was provided. This work was crucial for developing wetland treatment technologies and understanding triclosan natural attenuation in constructed wetlands with different plants while also providing useful information on the design of constructed wetlands for triclosan disposal.

2. Materials and methods

2.1 Chemicals and laboratory-scale CWs

Triclosan and its internal standard triclosan-D3 were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma-Aldrich (Milwaukee, WI).

Polyethylene barrels (21 in total) were used to build up the laboratory-scale surface flow CWs according to Zhao et al.(2015). River sand (0-3 mm in diameter, mainly Si_2O_3 , Al_2O_3 , and Fe_2O_3) was washed in advance and used as substrate with a depth of 25 cm. An outlet was set at the bottom of each barrel to collect the effluents. All the CWs were monocultured for the plant species. Some commonly used CW plants, *Phragmites australis* (*P. australis*), *Typha angustifolia* (*T. angustifolia*) and *Zizania latifolia* (*Z.latifolia*) of emergent plants, *Cedar moss* (*C. moss*) and *Hydrilla verticillata* (*H. verticillata*) of submerged plants, and *Lemna minor* (*L. minor*) and *Salvinia natans* (*S. natans*) of floating plants, were taken and transplanted from Nansi Lake in Shandong Province and planted in each wetland unit with three replicates. All the CW units were located under the transparent rain shelter in the Baihua Park of Jinan, China. The characteristics of CW units were listed in Table 1.

2.2 Experimental procedure

The influent water was synthetic and mainly consisted of sucrose, $(\text{NH}_4)_2\text{SO}_4$, KH_2PO_4 and KNO_3 to simulate the wastewater discharged from the municipal wastewater treatment plant, which implemented the first level (B) standard of China's legislation law (GB18918-2002)(EPA, 2002). Its characteristics were shown in Table1. After acclimated in the influent water for nearly 4 months, triclosan of $60 \mu\text{g L}^{-1}$ were added in the middle of July. Then CWs were operated for six periods (5 days for each period). Wastewater effluent was homogenized in a new barrel, and then the wastewater samples (250 mL) were collected in polyethylene plastic bottles at the end of each period. For each unit, sediment samples of 25 g were taken on period 2, 4 and

6. The sediment samples were homogenized from five individuals (same height, equal amount) and then kept in polyethylene plastic bags. Plant samples were harvested at the end of the sixth period and stored in sealed bags. All samples were transported to the laboratory in the chilled insulating box.

2.3 Sample processing

Water samples were filtered through 0.45 µm nylon membranes then stored at 4°C and examined within 24 h. Sediment samples were dried by a vacuum freeze-drying machine (Uniequip, German) at -60°C for 48 h. They were then powdered to pass through a 2 mm sieve, and stored at -20°C prior to analyze. Plant samples were rinsed with distilled water and separated into root, shoot and leaf tissues. These samples were also dried by the vacuum freeze-drying machine. Samples were then weighted for biomass and stored at -20°C after powdered with the liquid nitrogen and screened through a 2 mm pore size sieve.

2.4 Water quality analysis and triclosan extraction

Laboratory analysis was performed on the water samples for chemical oxygen demand (COD), ammonium (NH₄-N), nitrite (NO₃-N) and total phosphorus (TP) according to the standard methods (Eaton et al., 2005).

Triclosan in water was extracted by liquid/liquid extractions with dichloromethane according to the recommendations of the U.S. Environmental Protection Agency (EPA) Method 3510. Triclosan-D3 (100 ng) was added before the extraction as surrogate standard. The extraction procedure was repeated twice and extracts were evaporated by the rotary vacuum evaporator system then dried under a gentle nitrogen stream. The extracts were resolved in methanol and filtered through 0.22 µm nylon membrane filter before kept in 4°C for further analysis.

Sediment (18 g) samples were extracted by soxhlet extraction for 6 h using

dichloromethane (90 mL) as extracting solvent (Coogan et al., 2007). Anhydrous sodium sulfate was mixed with the samples to improve the extraction efficiency. Before the extraction, samples were spiked with triclosan-D3 (100 ng) as surrogate standards. Extracts were concentrated by rotary vacuum evaporation then dried under nitrogen gas. Plant samples (0.15 g) were analyzed following the modified procedure for sediment samples. Before drying with nitrogen gas, silica gel was added to their extracts to remove chlorophyll. After centrifugation at 4000 rpm for 8 min, the supernatant was collected and underwent the following steps.

The extracts of sediment and plant samples were then prepared using the modified method by Ying and Kookana (2007). Extracts were resolved in methanol/water (50%, v: v; 10 mL) and subsequently cleaned with a C18 cartridge (50mg, 6mL; CNW, German) using the SPE apparatus Visiprep Solid Phase Extraction Vacuum Manifold (12-port, Supelco, Bellefonte, USA). The C18 cartridges were preconditioned by methanol (5 mL) and Milli-Q water (5 mL). Samples were then pulled through the cartridge. The cartridges were then washed with methanol/water (10%, v: v; 50 mL). After that, C18 cartridges were dried under vacuum for 20 min. Target compounds were eluted from the cartridges with methanol (2 × 4 mL). The extracts were blown to dryness by a gentle stream of nitrogen gas, and re-dissolved in methanol. The extracts were filtered through 0.22 µm filter membrane before kept in 4°C for further analysis.

2.5 Triclosan determination

The determination of triclosan and triclosan-D3 was conducted by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; Waters AcquityTM and Quattro Premier XE). The compounds were separated by BEH C18 column (2.1×50 mm i.d., 1.7 µm; Waters) using the mobile phase with aqueous 10mmol ammonium acetate (NH₄OAC) in deionized water (A) and 0.1% of Formic

acid and 1 mmol of NH_4OAc in methanol (B) at a flow rate of 0.2 mL min^{-1} . The conditions were firstly 45% B for 2.5 min, gradient to 100% B and sustained for 1.1 min, dropped to 45% B at 3.6 min, and then hold for 1.4 min.

The electrospray negative ionization (ESI-) source had the following settings: capillary voltage of 3 kV, cone voltage of 30 V, source temperature of 120°C , desolvation temperature of 300°C , cone gas flow rate of 50 L h^{-1} , desolvation gas flow rate of 650 L h^{-1} . Parent ions of 286.80 and 289.80 (m/z) for triclosan and triclosan-D3 were produced the daughter ion of 35.2 (m/z) under the MRM mode. All the data were analyzed by Waters Masslynx software (v 4.1).

2.6 Data calculation and analysis

The mass balance of triclosan was calculated by using the following equation,

$$\text{Total mass} = \text{adsorption in sediments} + \text{absorption in plants} + \text{residual in water} + \text{triclosan loss} \quad (1)$$

The total mass of triclosan and the mass of triclosan residual in water were calculated from the influent and effluent water, respectively. Triclosan mass in each component of equation (1) was calculated as shown in equation 2.

$$M = CV \quad (2)$$

Where,

M = the mass of triclosan in influent and effluent water, sediment or plants (μg)

C = triclosan concentration in influent and effluent water, sediment or plants ($\mu\text{g g}^{-1}$ or $\mu\text{g L}^{-1}$)

V = water volume of CW units (L), or the weight of sediment and plants of CW units (g)

Triclosan distribution in CWs was calculated by using the following equation.

$$\text{Triclosan distribution} = (M / \text{total mass}) \quad (3)$$

Bioaccumulation factor (BAFs) and biota to sediment accumulation factors (BSAFs) were calculated as shown in equation 4 and 5 (Harrad and Smith, 1997).

$$\text{BAFs} = (C/C_w) \quad (4)$$

$$\text{BSAFs} = (C/C_s) \quad (5)$$

Where,

C = triclosan concentration in plants or their organs ($\mu\text{g g}^{-1}$);

C_w = triclosan concentration in water phase of wetland unit ($\mu\text{g L}^{-1}$);

C_s = the organic carbon-normalized triclosan concentration in sediment of wetland unit (ng goc^{-1}).

Data analyses were carried out with ORIGIN 8.5 software package. Statistical significant of difference was determined with a one-way analysis of variance (ANOVA) by using SPSS 13.0.

2.7 Quality control

No contaminants showed in anhydrous sodium sulfate blanks. The correlation coefficient of the standard curve for triclosan and triclosan-D3 was 0.9996 and 0.9995, respectively. Instrument detection limits (IDL) for triclosan and triclosan-D3 were 20 and 25 ng mL^{-1} . The method detection limit (MDL) was defined as 3 times of the IDL value, giving MDL values of triclosan and triclosan-D3 were 0.15 and 0.19 ng mL^{-1} in water, 1.67 and 2.08 ng g^{-1} in sediment, 200 and 250 ng g^{-1} in plant tissues, respectively. Uncontaminated sediment, plant root, shoot and leaf samples ($n=3$) spiked with triclosan and triclosan-D3 standard were extracted and examined for recovery efficiencies. Recovery efficiencies in different matrices were from $79.24\% \pm 5.62\%$ to $85.91\% \pm 3.93\%$ and from $87.09\% \pm 5.09\%$ to $91.95\% \pm 4.69\%$ for triclosan and triclosan-D3, respectively (Table S1).

3. Results and discussion

3.1 Pollutants removal efficiency in CW units

Removal efficiencies of common contaminants in CW units revealed species effect (Fig. S1). Units with emergent plants can effectively remove those contaminants. And they revealed significantly higher removal efficiency than units with submerged and floating plants, especially in the removal ability of $\text{NH}_4\text{-N}$ and TP ($p<0.05$). This species effects could probably be due to the different adaptation processes of microbial communities in CWs with different species of plants under the presence of triclosan (Zhao et al., 2015).

Triclosan removal efficiencies are shown in Fig S2. All the units revealed stable removal efficiencies since the fourth period and more than 80% of triclosan was removed from these units by the end of the sixth period. Apparently, different species of wetland plants had different removal efficiencies in our study. Units with emergent plants had the fastest stable process and revealed the highest triclosan removal efficiency at the end of the sixth period (96.42% to 96.78 %), while it was the lowest in the units with floating plants (83.89% and 90.00%). This is probably because emergent plants have relatively larger rhizome volume and thus it could be helpful in their direct uptake of organic pollutants i.e. triclosan, or biodegradation process of attached microbes (Verlicchi and Zambello, 2014).

3.2 Triclosan distribution and removal mechanism in CW units

3.2.1 Triclosan adsorption by sediment

The concentration of triclosan in sediment is shown in Fig.1. Triclosan concentration of the second period in sediment was the lowest for all the units. Since then, units with emergent plants, particularly *T. angustifolia* and *Z. latifolia*, indicated a stable adsorption process. Units with floating plants showed a rise then decreasing trend. It kept rising in units with submerged plants. In conclusion, adsorption of

triclosan to sediment for all the plants was in the range of 16.55 - 23.48 ng g⁻¹ in the fourth and sixth period and was not significantly different ($p > 0.05$). Therefore, taking both the concentration of triclosan in water (Fig.S2) and sediment into consideration, all the CW units were under a stable condition before the sixth period.

3.2.2 Triclosan accumulation by plants

Previous studies have found that triclosan can be quickly (within 2-3 days) removed from CWs with floating plants (Matamoros et al., 2012; Reinhold et al., 2010). Although they did not measure triclosan concentration in plants, they believed that these floating plants contributed in triclosan removal through passive plant-associated processes, specifically sorption. As shown in Table 2, triclosan accumulation in plants varied among plant species. And triclosan concentration in floating plants was the highest among the three types of wetland plants. Triclosan concentration in *L. minor* and *S. natans* was 16.80 ± 1.10 and 19.66 ± 1.12 $\mu\text{g g}^{-1}$, respectively. Therefore, the uptake of triclosan by plants was an important pathway for triclosan removal in wetlands with floating plants.

Triclosan was only detected in the root of emergent plants with an order of *T. angustifolia* > *Z. latifolia* > *P. australis*. This result was in accordance with a former study by Zarate et al.(Matamoros et al., 2012) and they believed that the 4-6 layered hypodermises of *T. angustifolia* can favor the sorption of lipophilic compounds, including triclosan (Seago et al., 1999). In some terrestrial plants, the transmission of triclosan from below to above ground tissue is limited (Prosser et al., 2014). Although wetland plants differ in living conditions with terrestrial plants, the limitation of triclosan transmission from root to shoot and leaf also happened in emergent plants.

Unlike emergent plants, the leaf and shoot of submerged plants can directly uptake the organic contaminants from water. Therefore, the root, leaf and shoot of submerged

plants were all detected with triclosan. Triclosan concentration in shoot and leaf of *C. moss* were much higher than the root, while triclosan concentration in *H. verticillata* showed a different trend of root > shoot > leaf. This different trend was probably caused by their different accumulation abilities.

3.2.3 Degradation assessment by mass balance calculation

Apart from the sediment adsorption and plant accumulation, triclosan can also be removed by other mechanisms, *i.e.* biodegradation and photodegradation. CW is a “black box”. The contribution of degradation process cannot be evaluated directly. By a mass balance calculation (Eq.1, Eq.2 and Eq. 3), the estimated distribution and removal contribution by CW components were analyzed (Fig.2). And by subtracting the amount of triclosan in water, sediment and plants, and total loss due to other mechanisms, *i.e.* biodegradation and photodegradation, can be obtained.

According to Fig.2, sediment adsorption and total loss due to other mechanisms contributed a lot in triclosan removal. The estimated percentage of triclosan removed by these two processes was 28.67%~61.00% and 33.21%~47.13%, respectively. Similar result was obtained by Karnjanapiboonwong et al. (2011) in sand and soil systems with bean plant. Microbial degradation process is regarded as the primary way for the removal of most pollutants (Cui et al., 2013). Apart from biodegradation, triclosan is also susceptible to photodegradation under aerobic conditions. Li et al. (2014) reviewed that triclosan elimination can be enhanced by high temperature and strong sunlight irradiation in summer. Considering the density and different types of wetland plants (Table 1), the cover area by plants may differ with plant species. Floating plants covered all the surface area of the barrel, thus had the least sunlight through. The branches and leaves of emergent plants can also block the sunlight. Thus photodegradation in CWs with submerged plants could probably be stronger than

others. The contribution of degradation process in wetland units followed the order of emergent plants > submerged plants > floating plants. And in our previous study (Zhao et al., 2015), we also found that microbial abundance and diversity under triclosan addition were in accordance with this trend, and the dominant biodegradation bacteria differed with the type of plants. Thus we can deduce that biodegradation contributed more with the whole degradation part and revealed a species-specific effect.

In order to investigate the correlation between wetland plants and degradation process, further analysis by SPSS was conducted (Fig 3A). Triclosan concentration in wetland plants had significant negative correlation with removal contribution by degradation ($r=-0.783$; $p<0.05$). Therefore, wetland plant can not only bioaccumulate triclosan from wetland system, but also highly connect to the microbial degradation and photodegradation process. In consideration of these findings, factors from the perspective of plant may be used as index for plant selection for triclosan removal in CWs.

3.3 Assessment of using bioaccumulative factors in wetland plant selection

3.3.1 Plant bioaccumulation ability assessed by bioaccumulative factors

By using Eq. (4) and (5), BAFs and BSAFs values can be obtained and their Log values are shown in Fig.4. Log BAFs were all higher than 3.1, which represented the same order of magnitude to those have been reported in aquatic environment. For instance, the reported BAFs values for triclosan in algae and snail (Coogan et al., 2007; Coogan and Point, 2008) of a stream receiving effluents from the wastewater treatment plant were higher than 1000 L kg⁻¹. BSAFs were much smaller than BAFs with its Log values were all lower than 3.0. According to the European Commission (Rogers, 2003), if Log BAFs was higher than 3.30 or 3.70, it can be categorized as

“bioaccumulative” or “very bioaccumulative” ones. As to the BSAFs, the classification brought up by Dallinger indicated that those with $\text{Log BSAFs} > 0.3$ were regarded as macro concentrator, $0 < \text{Log BSAFs} < 0.3$ as micro concentrator and $\text{Log BSAFs} < 0$ as deconcentrator. Based on these standards, although these two factors revealed different trend, most of the wetland plants were categorized as bioaccumulative plants or macro concentrators in triclosan accumulation (Fig.4).

3.3.2 Correlations between bioaccumulative factors and triclosan distribution in CWs

The correlations between bioaccumulative factors and triclosan distribution in CWs were analyzed by SPSS (Fig 3 B and C). BAFs and BSAFs are commonly used factors for plant accumulation ability assessment, thus both their Log values had significant correlation with triclosan concentration in plants ($|r| > 0.4, p < 0.05$). There was no significant correlation between Log BAFs values and triclosan distribution in other components of CWs ($p > 0.05$). However, it is worth noting that Log BSAFs values were significantly connected to triclosan degradation process (including microbial degradation and photodegradation) with r values of -0.665 ($p < 0.05$). This result indicated that the factor BSAFs was the index that we were looking for. BSAFs factor was calculated from wetland plants and sediments, and it also had significant correlation with degradation process. These made it a successful link in CWs with triclosan addition. By BSAFs factors, we can deduce the contribution of bioaccumulation and degradation, thus to give instructions on plant selection based on the removal mechanism.

3.3.3 Instructions on plant selection by using bioaccumulation factors

To achieve the best removal through degradation process, a recommendation based on Log BSAFs values can be obtained. Considering the significant negative correlation between Log BSAFs and degradation process, it can be inferred that the

bioaccumulation process in plants is disadvantageous to triclosan degradation in CWs. Thus wetland plant with lower Log BSAFs values is recommended for thoroughly triclosan degradation. In this way, emergent plants are more preferable than submerged plants and floating plants for triclosan removal in CWs.

Plant harvest in winter or summer is a common used regulation in CW. By harvesting, pollutants will also be removed from the system, thus plant harvest is also used in phytoremediation (Ji et al., 2011). Unlike floating or submerged plants, the roots of emergent plants will not be harvested, thus the sink of triclosan in emergent plants will still be in the CWs. Therefore, when under emergency remediation, submerged plants and floating plants are recommended rather than emergent plants, as they can not only grow quickly but also have higher Log BSAFs values. When CWs are well managed and can be harvested regularly, a combination of these plants could be more appropriate.

Triclosan is lipophilic with a log Kow of 4.8, which makes it easily bioaccumulate in aquatic organisms and sufficiently persistent in environment (Coogan et al., 2007). Therefore the plants accumulation could be important in triclosan removal. And this could also be the reason that BSAFs can be used as index for plant selection for triclosan removal. While whether this method could be used in the wetland plants selection for other persistent contaminants needs to be further studied. Furthermore, CWs are complex systems, thus further studies are still needed in the application of BASFs for plant species selection, especially some field scale investigations. In addition, half-life of triclosan was affected by oxygen condition (Ying and Kookana, 2007), the estimation based on the concentration of triclosan in the root zone would need further consideration. Moreover, further studies on the transmission and

metabolism within the plants, as well as the release to the surrounding environment need are necessary.

Conclusion

In this work, using BAFs and BSAFs to choose the best wetland plant species for triclosan removal was studied. By monitoring triclosan concentration in water, sediment and plants, a specie-effect distribution trend of triclosan was found in CWs units with emergent, submerged and floating plants. By mass balance calculation and further SPSS analysis, the significant correlations between Log BSAFs values and triclosan concentration in plants or degradation contribution were indicated. Therefore based on this factor, introductions on plant species can be provided. As CWs are complex systems, further studies, especially some field scale investigations, are still needed to understand the detailed mechanisms for triclosan removal, *i.e.* the transmission and metabolism within the plants.

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Figure Captions:

Fig 1. Triclosan concentration in wetland sediments.

Fig 2. Estimated triclosan distribution in CWs.

Fig 3. Pearson's correlation coefficient (r) and p value for triclosan concentration in wetland plants (A) or the bioaccumulative factors (B: Log BAFs; C: Log BSAFs) with triclosan distribution in CWs.

Fig 4. Bioaccumulative factors for triclosan in CWs.

Fig 1. Triclosan concentration in wetland sediments

Fig 2. Estimated triclosan distribution in CWs.

Root for floating plants represents the whole plants.

Fig 3. Pearson's correlation coefficient (r) and p value for triclosan concentration in wetland plants (A) or the bioaccumulative factors (B: Log BAFs; C: Log BSAFs) with triclosan distribution in CWs. Data using to calculate the correlation with triclosan removal by degradation was from Fig 3. The two red lines ($r = \pm 0.4$) indicated the critical values in between the correlation strengths are considered insignificant. The bars with $|r| > 0.4$, $p < 0.05$ are regarded as statistical significance.

Fig 4. Bioaccumulative factors for triclosan in CWs

Table Captions:

Table 1 Characters of wetland units and its influent water.

Table 2 Triclosan concentration ($\mu\text{g g}^{-1}$) in plants.

Table 1 Characters of wetland units and its influent water.

Wetland unit	Location	36°40'36"N, 117°03'42"E
	Height × diameter	52cm × 25 cm
	Hydraulic load	0.287 m ³ m ⁻² batch ⁻¹
	Hydraulic Retention Time (HRT)	5 days
	Average porosity	35%
	Ambient temperature	20.4 ± 2.3 to 30.0 ± 2.3 °C ^a
Influent water (mg L ⁻¹)	COD	62.79±3.24
	Total nitrogen (TN)	18.45±1.22
	Ammonium (NH ₄ -N)	8.23±0.47
	pH	7.34±0.22
Plant density (plant per barrel)	Emergent plants	8~10
	Submerged plants	16~18
	Floating plants	Covered all the surface area

^a Zhao et al., 2015

Table 2 Triclosan concentration ($\mu\text{g g}^{-1}$) in plants.
BL: below detection limit.

		Emergent plant			Submerged plant	
	Location	<i>P. australis</i>	<i>T. angustifolia</i>	<i>Z. latifolia</i>	<i>C. moss</i>	<i>H. varticilla</i>
triclosan ($\mu\text{g g}^{-1}$)	Root	4.43 $\pm$ 0.21 ^a	8.83 $\pm$ 0.47 ^b	5.46 $\pm$ 0.33 ^a	4.71 $\pm$ 0.33 ^a	13.68 $\pm$ 0.79
	Shoot	BL	BL	BL	11.82 $\pm$ 0.74 ^c	13.15 $\pm$ 0.78
	Leaf	BL	BL	BL	10.60 $\pm$ 0.62 ^c	10.96 $\pm$ 0.64

Floating plant was regarded as a whole.

Analyzed with Duncan's multiple range test, different letters (a-f) represent significant difference ($p < 0.05$, $n=3$).

Graphical Abstract