Removal of the pesticide tebuconazole in constructed wetlands: Design comparison, influencing factors and modelling

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Abstract

Constructed wetlands (CWs) are a promising technology to treat pesticide contaminated water, but its implementation is impeded by lack of data to optimize designs and operating factors. Unsaturated and saturated CW designs were used to compare the removal of triazole pesticide, tebuconazole, in unplanted mesocosms and mesocosms planted with five different plant species: *Typha latifolia, Phragmites australis*, *Iris pseudacorus*, *Juncus effusus* and *Berula erecta*. Tebuconazole removal efficiencies were significantly higher in unsaturated CWs than saturated CWs, showing for the first time the potential of unsaturated CWs to treat tebuconazole contaminated water. An artificial neural network model was demonstrated to provide more accurate predictions of tebuconazole removal than the traditional linear regression model. Also, tebuconazole removal could be fitted an area-based first order

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kinetics model in both CW designs. The removal rate constants were consistently higher in unsaturated CWs (range of 2.6–10.9 cm d^{-1}) than in saturated CWs (range of 1.7–7.9 cm d^{-1}) and higher in planted CWs (range of 3.1–10.9 cm d^{-1}) than in unplanted CWs (range of 1.7–2.6 cm d^{-1}) for both designs. The low levels of sorption of tebuconazole to the substrate (0.7–2.1%) and plant phytoaccumulation (2.5–12.1%) indicate that the major removal pathways were biodegradation and metabolization inside the plants after plant uptake. The main factors influencing tebuconazole removal in the studied systems were system design, hydraulic loading rate and plant presence. Moreover, tebuconazole removal was positively correlated to dissolved oxygen and all nutrients removal.

Capsule

System design, plant presence and species and HLR influence tebuconazole treatment in CWs, and the removal can be described by an artificial neural network model.

Keywords: Artificial neural network; Biofilm reactors; Contaminants of emerging concern; Fungicides; Phytoremediation

1. Introduction

Tebuconazole is a triazole pesticide that is widely used in agriculture for crop protection due to its broad spectrum of antifungal activities (Shikuku et al., 2014) and included as an active ingredient in wood preservatives (Miyauchi et al., 2005). Concentration levels of tebuconazole (Table S1) ranging from ng L^{-1} to µg L^{-1} have been found in both rural and urban water bodies (Bollmann et al., 2014; Casas & Bester, 2015; Shikuku et al., 2014). However, tebuconazole is reported to be toxic to aquatic life and human health at µg L^{-1} level (EFSA, 2014). In the last few decades, the occurrence of pesticides, including tebuconazole, in the aquatic environment has become a worldwide issue of increasing environmental concern. Thus, due to its wide-spread use and detection as well as its potentially harmful effects, tebuconazole was selected as the model pesticide in this study.

Constructed wetlands (CWs) have become widely used to treat pesticide contaminated wastewater as an economical, robust and sustainable technology (Vymazal & Březinová, 2015). Previous research on removal of pesticides in CWs has been conducted mostly in saturated systems, such as free water surface CWs or horizontal subsurface flow CWs, while unsaturated systems, such as vertical flow CWs, have been less studied. Vegetated and non-vegetated saturated surface flow CWs have been reported to result in removal of 45%–90% of the tebuconazole at an inflow concentration of 0.1–10 µg L^{-1} in agricultural landscapes in Europe (Passeport et al., 2013; Tournebize et al., 2013). Unsaturated CWs have usually better removal efficiency of the typical wastewater constituents BOD and ammonium, due to better oxygen transfer capability in their wetland beds (Wu et al., 2014). However, there are no results of direct comparisons in pesticide removal efficiencies among different types of CWs. Thus, the most effective CW type to treat pesticides has not yet been determined. Unsaturated CWs have different hydrological characteristics including water flow pathway and hydraulic retention time compared with saturated CWs. These features suggest possibly different contaminant removal efficiencies and mechanisms (Gregoire et al., 2009; Kadlec & Wallace, 2008; Vymazal, 2007). Thus, comparisons of pesticide removal performance, kinetics and mechanisms in different CW designs are necessary to provide better information for future applications.

To date, the factors influencing removal of the pesticide tebuconazole in different CWs have been rarely investigated. For instance, one popularly used pesticide, chlorpyrifos, has been reported that the removal efficiency and removal rate constant were negatively affected by increased influent concentrations from 100 µg L^{-1} to 500 µg L^{-1} to 1 mg L^{-1} levels through phytoremediation (Prasertsup & Ariyakanon, 2011). These concentrations are high, especially if considering that typical concentrations in urban storm water are usually below 100 µg L^{-1} (Bollmann et al., 2014; Casas & Bester, 2015). Thus, the effect of the influent concentration of pesticide on removal under real environmental levels is unknown. Different hydraulic loading rates (HLRs) affect pollutant/microbial contact time and reaction rates, which has an effect on pollutants, such as BOD, nitrogen and some pharmaceuticals, biodegradation (Lin et al., 2008; Zhang et al., 2017). Despite this, the effect of HLR on pesticide removal in CWs has not received much attention, even though removal kinetics models, such as the zero or first order kinetics models, are calculated based on pollutant removal under different HLRs. Thus, we lack the information on pesticide removal efficiencies under different HLRs that is needed to be able to determine pesticide removal kinetics. It is expected that different plant species may influence pesticide removal in CWs differently due to their different root structure, root exudate release, compound uptake ability and associated different microbial communities. Lv et al. (2016c) observed that tebuconazole removal in saturated CW mesocosms was influenced by the identity of the plant species, while plant uptake and substrate sorption made limited contributions towards tebuconazole removal. However, whether these factors also influence tebuconazole removal in unsaturated CW is unknown. Understanding the factors influencing removal of the pesticide tebuconazole in different CW designs would undoubtedly improve the design and operation of CWs for the treatment of not only tebuconazole but also other triazole pesticides.

Reliable numerical models can be used to increase the understanding of pollutant removal processes occurring in CWs and to improve existing design criteria of CWs (Langergraber, 2007). Linear regression has been the most widely used model in CWs for predictions of pollutant removal (Rousseau et al., 2004). However, linear regression provides rather crude approximations of the complex assortment of nonlinear relationships present in environmental systems (May & Sivakumar, 2009). Artificial neural network (ANN) modelling is a technique inspired by biological neuron processing, which addresses an interconnected structure of processing elements. ANN is widely used in solving complex and nonlinear problems (Schmidhuber, 2015). In recent years, ANN has been successfully applied to predict the removal abilities of organic matter (COD and BOD_5) (Akratos et al., 2008), TSS (Naz et al., 2009), different phosphorous species (ortho-P and TP) (Akratos et al., 2009) and nitrogen (NH_4^+-N and TN) (Guo et al., 2014; Kotti et al., 2016) in various types of CWs. However, no study has been conducted on ANN model-based simulation for pesticides or other emerging organic contaminants.

Consequently, the main objectives of the present study were the following: (1) to compare the removal efficiency, kinetics and mechanism of tebuconazole removal in both unsaturated and saturated CWs with different plant species; (2) to investigate the main influencing factors (system design, HLR, initial concentration and plant species) of tebuconazole removal in both types of CW designs; and (3) to compare ANN with traditional linear regression models in order to explore a simple and robust methodology suitable for predicting tebuconazole removal in CWs.

2. Materials and methods

2.1 Mesocosm-scale CWs and experimental conditions

Each mesocosm-scale CW was made of a black plastic container with both a height and diameter of 20 cm. Each container was filled with a 4 cm layer of gravel (Ø 0.8 to 1.2 cm) on the bottom, a geotextile, a 10 cm layer of sand (Ø 0.05 to 0.1 cm with average porosity of 37%) and finally a 4 cm layer of gravel. All mesocosm-scale CWs were intermittently pulse fed by water artificially spiked with tebuconazole from the surface. The outlet height was set at 3 cm for unsaturated CWs (Fig. 1a) and 15 cm for saturated CWs (Fig. 1b). The system was setup and used for a previous experiment along summer 2014 and winter 2015 by Lv et al. (2016c). Both unsaturated and saturated CWs consisted of an influent tank and triplicates of six planting types: unplanted and planted with *Juncus effusus* (*Juncus*), *Typha latifolia* (*Typha*), *Berula erecta* (*Berula*), *Phragmites australis* (*Phragmites*) and *Iris pseudacorus* (*Iris*). In total, 36 mesocosm-scale CWs were constructed, 18 for the unsaturated and 18 for the saturated design. Artificially spiked tebuconazole water was prepared in 300 L doses and constantly mixed by a submerged centrifugal pump placed at the bottom of the influent tank. New influent was prepared every 2–5 days, and the influent load was controlled by a timer and pump. Two concentrations of tebuconazole (10 and 100 µg L^{-1}) and four hydraulic loading rates (1.7, 3.4, 6.9 and 13.8 cm d^{-1}) were used. The corresponding hydraulic retention time (HRT) for the saturated CWs were 2, 1, 0.5 and 0.25 days, respectively. The wastewater was prepared with "Pioner Grøn" (Brøste Group, Denmark) N:P:K full strength nutrient solution added to tap water (supplementary material). An additional carbon source for basic microbial community survival using acetic acid was used to simulate a 20 mg L^{-1} TOC load. The experiment lasted from July to August 2015 (57 days) after a two-month stabilization period. The air temperature ranged from 15 to 25 °C and the relative air humidity from 51 to 78% (Fig. 1c).

2.2 Sampling and analysis

Before each sampling, the mesocosms were allowed to stabilize for three complete hydraulic cycles (calculated by the saturated mesocosms), after which the effluent quality was assumed to be representative. The triplicates samples of the influent were collected directly from the influent tank using a 1 L amber flask. Similar 1 L amber flasks were connected to the CW effluent flow valve and left *in-situ* for 2-10 hours, in order to collect a minimum of 800 mL of composite water samples. In total, eight sampling campaigns were conducted. For each campaign, a total of 42 samples were collected: the influent (3) plus effluent samples (3 x 6) for each design (x2). The volume of each effluent was noted to calculate water loss by evapotranspiration (Equation S1, supplementary materials). Dissolved oxygen (DO), pH, temperature and electrical conductivity (EC) were measured *in-situ*. The nutrients of total nitrogen (TN), total organic carbon (TOC) NH_4-N, NO_3-N and PO_4-P were analysed within 12 hours. A detailed description of the measurements can be found in supplementary materials. The pesticide tebuconazole was pre-concentrated by solid-phase extraction (SPE) prior to further analysis using an HPLC system (Thermo Scientific Ultimate 3000) equipped with a diode array detector (Lv et al., 2016c). Tebuconazole removal efficiencies were corrected for water loss due to evapotranspiration according to Equation S2. The removal of tebuconazole from the water samples was simulated to fit both area-based (Equation S3) and volume-based (Equation S4) first order kinetics models.

The weight of each CW mesocosm was measured at the end of the experiment to roughly estimate the fresh biomass of the different plant species according to Equation S5. Approximately 100 g of plant aerial tissue and 100 g of substrate (1 cm Ø cores at 4–14 cm depth) were collected at the end of experiment. Substrate and plant tissue samples were extracted by ultrasonication, and the substrate extracts were analyzed directly while the plant extracts were further cleaned by two-stage saponification and SPE prior to analysis by HPLC (Lv et al., 2017b). Total substrate organic carbon content (SOC) was estimated by the loss on ignition method (LOI) using a muffle furnace and calculated by Equation S6. All the equations for the calculations are described in detail in the supplementary materials.

2.3 Modelling

All measured variables, besides the data of tebuconazole removal, influent water quality (tebuconazole influent concentration, HLR, water temperature, pH, EC, DO and evapotranspiration) and nutrient removal (TOC, TN, TP, NH_4-N and NO_3-N) in the two studied CW designs were used in the principal component analysis (PCA). Moreover, one more parameter labelled as “plant” was included in the PCA. The value was set as 0 for the unplanted CWs and 1 for planted CWs. The loading factors, corresponding to the principal components that explained most of the variation in the original data were extracted to be used in the ANN and linear regression models as input parameters.

The multi-layer perceptron (MLP) type of ANN, the most popular method used in hydrological modelling (Govindaraju, 2000a; Govindaraju, 2000b), was used. In MLP, the artificial neurons are arranged in a layered configuration (Fig. 2) containing a single input layer, a single processing (hidden) layer and a single output layer. For the linear regression analysis, tebuconazole removal efficiency was simulated as the summation of each selected variable multiplied by a factor. For the saturated CWs, all the input data from the present study was used for model training (144 data points). Moreover, data from the previous experiment by Lv, et al. (2017) for tebuconazole removal was used to validate the saturated CW model. For the unsaturated CWs, since this is the first study reporting tebuconazole removal under unsaturated conditions, the input data was randomly split into two subsets (2:1 ratio), with the larger subset used for training and the other subset used for validation. Thus, 96 and 48 data points were used to train and validate the models, respectively. The Mean Absolute Error (Equation S8) was used to evaluate the precision of both ANN and linear regression models during model validation in the present study. The detailed data training and simulation of the ANN and linear regression models are described in the supplementary materials.

2.4 Statistical analysis and software

Statistical analyses were carried out using the XLStat Pro® statistical software (XLStat, Paris, France). Analysis of variance (ANOVA) followed by Tukey's HSD test was used to identify significant differences in water quality (pH, EC, and DO), influencing factors (plant species, HLR, influent concentration and system designs) on tebuconazole removal, reaction rate constants, tebuconazole concentration by substrate sorption and plant uptake, and nutrient (TOC, TN, NH_4-N and TP) removal at the 0.05 significance level ($p<0.05$). Plant height and leaf chlorophyll differences at the beginning and end of the experiment were compared with the student-T test. The data were checked for normality and homogeneity of variance prior to statistical analysis. If variables were not normally distributed, they were log-transformed. Composite values of water quality (pH, EC, and DO) and nutrient removal (TOC, TN, NH_4-N and TP) from each mesocosm throughout the whole study were visualized using beanplots by the program BoxPlotR. PCA, ANN and linear regression models were performed in StatSoft Statistica version 7 (StatSoft). Visualized co-occurrence network figures were illustrated using the Gephi platform (Bastian et al., 2009).

3. Results

3.1 Plant vitality and water quality

The height and leaf chlorophyll of each wetland plant species were not significantly different at the end of the experiment when compared with that at the beginning of the experiment (Fig. S1). Thus, the tebuconazole concentration levels tested in the present setup did not reveal toxic effects on the plants, which is in agreement with previous studies (Lv et al., 2016a; Lv et al., 2016c).

The values of DO, pH and EC in the effluent under different HLRs and influent concentration levels during the whole experiment were not significantly different between the planted mesocosms for either unsaturated or saturated CWs (Table S2). Thus, for easier visualization of the results, these parameters for all influent and effluent of unplanted mesocosms and planted mesocosms were integrated and displayed in beanplots for the comparison (Fig. 3). The DO values were significantly higher in the effluent (2.1–9.2 mg/L) than in the influent (0.2–3.8 mg/L), and significantly higher in planted (4.2–9.2 mg/L) than unplanted mesocosms (2.1–8.5 mg/L) for both unsaturated and saturated CWs. Moreover, DO in unsaturated CWs were significantly higher than in the unplanted mesocosms from both the saturated and unsaturated designs. The pH in the unplanted mesocosms (8.6–9.0) was significantly higher than in the planted mesocosms (7.7–8.8) for both saturated and unsaturated CWs. No difference was observed in pH between the CW designs. The EC values of the influent and effluent for all mesocosms were similar and ranged between 610 to 680 µS cm^{-1}, and they were also not significantly different between CW designs.

The nutrient removal performances in all CW systems were combined and visualized in beanplots (Fig. S2). The removal of TP, TN and NH_4-N in the unplanted mesocosms (40%-60%) was significantly lower than those in the planted mesocosms (80%-100%) for both unsaturated and saturated CWs. TOC (26% to 94%) and NO_3-N (29% to 99%) removal was not significantly different between mesocosms or CW design. Nutrient removal was not different between different types of planted mesocosms for both CWs designs. Generally, the nutrient removal in unsaturated CWs tended to be higher, but not significantly, than the removal in the corresponding saturated mesocosms under all the operation conditions throughout the study.

3.2 Tebuconazole removal

Tebuconazole was removed in both unsaturated and saturated CWs (Fig. 4) at removal efficiencies reaching up to 99.8%. A significant effect of system design on tebuconazole removal efficiency was observed through a four-way ANOVA test (Table 1). Efficiencies were generally significantly higher in the unsaturated CWs than in the corresponding saturated CWs for the corresponding mesocosm type and HLR. The results of the four-way ANOVA test also showed that tebuconazole removal was significantly affected by HLR and mesocosm types (plant species) for both unsaturated and saturated CWs. Tebuconazole removal efficiencies showed similar patterns for both influent concentration levels, showing an increase from 21.0% to 99.8% at an HLR decrease from 13.8 cm d^{-1} to 1.8 cm d^{-1}. Table S3 shows that all planted mesocosms achieved significantly higher tebuconazole removal (33% to 99.8%) compared with unplanted controls (21% to 66.1%). Moreover, mesocosms planted with *Berula* (71% to 99.8%) showed significantly higher removal efficiency than the other plant species in both unsaturated and saturated CWs. Regarding the influent concentration (10 μg L^{-1} to 100 μg L^{-1}) in both CW designs, removal was not significantly affected by this factor (Table 1).

3.3 Kinetics of tebuconazole removal

The area-based first order kinetics model was applied to determine the tebuconazole removal rate constants in both unsaturated and saturated CWs (Table 2). The area-based removal rate constants (k) were not influenced by influent concentration levels (10 and 100 μg L^{-1}). For unsaturated CWs, the k value was significantly lower in the unplanted mesocosms (2.6 ± 0.8 cm d^{-1}) than in the planted mesocosms (ranging from 5.3 to 10.9 cm d^{-1}). Moreover, the k value of *Berula* (10.9 ± 2.6 cm d^{-1}) mesocosms was significantly higher than the k values for the other planted mesocosms. For saturated CWs, the k value was also significantly lower in the unplanted mesocosms (1.7 ± 0.5 cm d^{-1}) than in the planted mesocosms (ranging from 3.1 to 7.9 cm d^{-1}). The *Berula* mesocosms also had significantly higher k values (7.9 ± 1.2 cm d^{-1}) than the other planted mesocosms for saturated CW design. Additionally, the area-based removal rate constants for the unsaturated CWs were significantly higher than the corresponding mesocosms for saturated CWs, except for *Typha* and *Phragmites* mesocosms.

The volume-based first order kinetics model was additionally applied to describe tebuconazole removal in saturated CWs. The volume-based removal rate constants (k_v), half-life and R^2 are presented in Table 2. The k_v (volume-based removal rate) values were not affected by the influent concentration levels (10 and 100 μg L^{-1}).

Berula planted mesocosms had significantly higher k_v values (3.7 ± 0.8 d^{-1}) than the other mesocosms (ranging from 0.8 to 1.6 d^{-1}).

3.4 Substrate sorption and plant uptake

The average values of the substrate tebuconazole concentrations and the substrate total organic carbon (SOC) content at the end of the experiment were higher in the unsaturated CWs than in the corresponding saturated mesocosms (Fig. S3a and b). However, tebuconazole concentrations normalized for the SOC content in unsaturated CWs were significantly lower than the corresponding saturated mesocosms (Fig. S3c), except for *Berula* mesocosms. Moreover, tebuconazole normalized concentrations for unplanted mesocosms were generally significantly higher than for the planted mesocosms in both designs. Based on the mass balance of the total tebuconazole spiked into each mesocosm, it can be estimated that sorption to the substrate represents only 1.6%–2.1% and 0.7%–1.5% of the tebuconazole removed in the unsaturated and saturated CWs, respectively.

Regarding phytoaccumulation, tebuconazole concentration in the aboveground tissue of the different plants ranged from 0.7 to 3.8 mg kg^{-1} DW (Fig. S3d) at the end of the experiment. Assuming tebuconazole translocation factors (range of 0.27 to 3.9) and biomass aboveground/roots ratios (range of 0.3 to 0.7) based on previous research by Lv et al. (2016b), it can be estimated that phytoaccumulation represented 3.6%–12.1% and 2.5%–11.7% of the tebuconazole removed in the unsaturated and saturated CWs, respectively.

3.5 Co-occurrence networks

Co-occurrence networks were computed to facilitate the visualization of the correlations between all measured parameters for unsaturated CWs (Fig. 5a) and saturated CWs (Fig. 5b). Only the significant (p-value < 0.01) and strong (|Pearson's r| ≥ 0.4) correlations (Table S4) are shown in Fig. 5. Tebuconazole removal showed significant and strong positive correlations with DO, evapotranspiration, plant and removal of TOC, TP, TN, NH_4-N, and NO_3-N, and negatively correlated with HLR. Moreover, the detailed correlations between tebuconazole removal and the parameters were analysed (Fig. S4). Notably, besides supporting the previously identified significant and strong correlations, the positive correlation between TN and NH_4-N removal and tebuconazole removal showed a significantly higher slope for planted CWs than for all unplanted CWs. Further analysis showed no significant correlation between removal of TP, TN and NH_4-N with evapotranspiration in the planted CWs (Fig. S5). However, once again, the difference in TN and NH_4-N removal between planted and unplanted CWs is clear.

3.6 Modeling and validation

All the measured parameters were analysed by PCA, and the extracted "best loading factors" of the candidate variables on the principal components axes are reported in Table 3. Four principal components were found to explain 80% and 83% of the variance in the original dataset for unsaturated and saturated CWs, respectively. For both designs, the first two principal components explained the majority of the variability (around 65%) in the dataset. The first principal components were highly correlated to DO, evapotranspiration, plant and removal of TOC, TN, TP, NH_4-N, and NO_3-N, which are parameters presenting large loading factors. Concerning the second principal component, HLR had a large loading factor, implying that it should also be used as input variable for the models. Thus, the factors DO, evapotranspiration, HLR, plant and removal of TOC, TN, TP, NH_4-N, NO_3-N were selected as the input parameters for the ANN and linear regression models.

Both the ANN and linear regression models provided reliable (MAE ≤ 0.32 and $R^2 \geq 0.73$) simulations of tebuconazole removal for both unsaturated CWs (Fig. 6a) and saturated CWs (Fig. 6c). However, the ANN model showed lower MAE and higher R^2 values than the linear regression model for both designs (Fig. 6a and c). Regarding model validation, the ANN model showed a slightly lower value of MAE (0.22) and higher R^2 (0.85) than those of the linear regression model (MAE= 0.24, R^2=0.81) for unsaturated CWs. For the saturated CWs (Fig. 6d), the ANN model showed a significantly lower value of MAE (0.44) and higher R^2 (0.61) than those for the linear regression model (MAE= 0.50, R^2=0.29).

4. Discussion

The effluent DO was significantly higher for unsaturated CWs than saturated CWs, as expected, as intermittently pulse-loaded subsurface CWs have higher oxygen transfer rates (Headley et al., 2013). Due to the oxygen release from wetland plant roots (Brix, 1997), significantly higher effluent DO values were observed in the planted CWs compared to the unplanted CWs for the same CW design. However, the operational differences and plant presence did not affect pH and EC, as all CWs presented similar values. Theoretically, the CWs with higher oxygen transfer rates should result in higher nutrient (TOC, NH_4-N and TP) removal efficiencies (Nivala et al., 2007). In the present study, however, nutrient removal was not significantly different between unsaturated and saturated CWs. This was probably due to the fact that the synthetic influent was not real wastewater, the nutrients concentrations were low, and the systems were not limited by oxygen availability. On the other hand, the significantly higher nutrient removal efficiencies in the planted CWs compared with unplanted CWs can be attributed to plant uptake (Brix, 1994).

Previous studies have reported that saturated surface and subsurface flow CWs could achieve 45%–98% tebuconazole removal efficiencies at 0.1–100 µg L^{-1} inflow concentrations in mesocosms and experimental field-scale CWs (Elsaesser et al., 2011; Elsaesser et al., 2013; Lv et al., 2016c). This present study is, to the best of our knowledge, the first to describe tebuconazole removal (33%–99.8%) in unsaturated CWs. Passeport et al. (2013) and Tournebize et al. (2013) observed that field-scale saturated surface flow CWs (surface area of 1280 m^2) could provide up to 36% removal of tebuconazole from agricultural drainage in different years (2008 and 2009) at a HLR of 5.6–5.9 cm d^{-1}. For this HLR regime, it can be estimated from Fig. 4 (and calculated from Eq. S2) that the tebuconazole removal in the unsaturated and saturated CW mesocosms, in our study would be 49%–66% and 42%–61%, respectively. Moreover, if considering a typical HRT in saturated subsurface flow systems of higher than 1 d (Kadlec & Wallace, 2008), the present mesocosms would provide more than 49% and 62% tebuconazole removal efficiencies in unplanted and planted mesocosms, respectively. Moreover, the mesocosms planted with *Berula* could each achieve significantly higher removal efficiencies above 90% under these typical HLR and HRT regimes.

Besides the significantly higher tebuconazole removal efficiencies, the area-based reaction rate constants were also significantly higher for unsaturated CWs (2.6–10.9 cm d^{-1}) than that for saturated CWs (1.7–7.9 cm d^{-1}). These results indicate that changing CW design from saturated to unsaturated could improve tebuconazole removal. Due to lack of area-based reaction rate constants available from previous studies, the values from the present study are the first reference data for future investigations. Volume-based first order kinetics model can also adequately describe the tebuconazole degradation in the saturated CWs. The volumetric removal rate constants (0.8–3.3 d^{-1}) are similar to those reported in previous experiments (0.6–2.9 d^{-1}) by Lv et al. (2016c) under the same system setup and operating conditions, revealing a stable capacity for tebuconazole removal during a two-year period. Thus, both the areal and volumetric degradation rates found in this study can be used as reference values for future implementation of CWs for tebuconazole contaminated water treatment.

The better tebuconazole removal in unsaturated CWs compared with saturated CWs may be due to the higher DO levels and different hydraulics, which has also been observed to generate different microbial communities (Lv et al., 2017a). DO was positively correlated with tebuconazole removal in the CWs (Fig. 5), which indicates that tebuconazole degradation is favoured by aerobic conditions. It has been previously shown that many micropollutants, such as alkylphenols (t-nonylphenol and 4-p-nonylphenol), hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) and pharmaceuticals (ibuprofen), show higher depletion through biodegradation under aerobic conditions than under anaerobic conditions (Abargues et al., 2012; Hijosa-

Valsero et al., 2010). In the present study, the DO levels in the saturated CWs were relatively high (>2 mg L^{-1}) and higher than expected from the full-scale saturated CW systems. Thus, tebuconazole removal efficiency in full-scale saturated CWs with lower DO levels is expected to be lower than reported here.

Besides the significant effect of CW design on tebuconazole removal, the positive effect of the presence of wetland plants in the systems is supported by the significantly higher tebuconazole removal efficiencies and area-based removal rate constants for planted CWs than unplanted CWs in both designs. In fact, the presence of wetland plants also promotes nutrients removal. The different plant species have different root structure, root oxygen and exudate release, and tebuconazole uptake ability (Lv et al., 2016b). Tebuconazole removal showed positive correlation with evapotranspiration indicating that passive uptake of tebuconazole into the plant with the transpiration stream play a role in removal. Nutrient (TN and NH_4-N) removal, however was not correlated with evapotranspiration (Fig. S5), indicating that the increased activity of the microbial community in the planted systems (Lv et al., 2017a) promoted nutrients metabolisation. Since tebuconazole removal also showed a positive correlation in both CW designs with nutrients removal (Fig. S4), it is also possible that tebuconazole removal can be coupled with the co-metabolisation of some nutrients. However, metabolisation pathways need to be further investigated through molecular methods.

Tebuconazole removal decreased with increasing HLR, which may be due to the threshold of the microbial community biodegradation and plant uptake capacity for tebuconazole in both CWs under higher compound loading. The influent concentration (10 or 100 µg L^{-1}) did not affect tebuconazole removal in both designs. This finding is contradictory with a previous study in which the removal efficiency and removal rate constant of one popularly used pesticide, chlorpyrifos, decreased with increasing influent concentrations (above 100 µg L^{-1}) (Prasertsup & Ariyakanon, 2011). This difference may be attributed to the chemical properties of the compounds or to the toxic effects of higher concentrations (above 100 µg L^{-1}). In fact, the present results are consistent with previous work on tebuconazole (Lv et al., 2016c) where under saturated conditions and different seasons (summer and winter), influent concentration did not affect system performance.

A mechanistic approach to tebuconazole removal indicates that hydrolysis is expected to be negligible due its chemical properties (Table S1). Photodegradation can also be excluded as the mesocosms were operated under subsurface flow conditions. Tebuconazole sorption to sediment was observed, and the tebuconazole concentration normalized by SOC was generally lower in unsaturated CWs than in saturated CWs, which may indicate higher biodegradation rate of tebuconazole in the unsaturated CW. The generally higher SOC in the planted mesocosms than in the unplanted mesocosms may be caused by higher microbial biomass growth (Zhang et al., 2010), favoured by the oxygen translocation and roots exudation capacity of the plants. Moreover, the estimated substrate sorption (0.7–2.1%) and plant phytoaccumulation (2.5–12.1%) indicate a limited contribution of both mechanisms to tebuconazole removal in both designs. In a previous study with saturated mesocosms, substrate sorption and plant phytoaccumulation were also found to contribute less than 13% to tebuconazole removal (Lv et al., 2016c). The direct role of plants is still not clear. On one hand, there is a strong positive correlation between tebuconazole removal and evapotranspiration. On the other hand, no significant phytoaccumulation is observed, which indicates that tebuconazole could be removed through metabolisation inside the plant tissue after uptake. Thus, tebuconazole degradation inside the plant tissue as well as microbial degradation are identified as the main pathways for tebuconazole depletion in both unsaturated and saturated CWs. The functionality analysis of the microbial communities from both interstitial water and biofilm sampled from the present experimental setup (Lv et al., 2017a) pointed out a strong correlation between tebuconazole removal and the biofilm in both saturated and unsaturated CWs. Thus, microbial degradation by the substrate-fixed biofilm seems to be another relevant pathway. Further studies should clarify the microbial metabolic pathways though which pesticide degradation occurs.

Tebuconazole removal in CWs was modelled for the first time in this study. The different modelling results from the non-linear ANN model and the traditional linear regression model were compared. The ANN model showed better predictive ability to forecast tebuconazole removal in both CW designs, even though the simulation results were relatively good for both the ANN and the traditional linear regression models. In order to test the broad applicability of the model, it is common to use independent research data for model validation/prediction (Akratos et al., 2009; Kotti et al., 2016; Naz et al., 2009). Due to the lack of independent data available, the training and validation data for unsaturated CWs were from the same study. Thus, the ANN model only showed slightly better (higher R^2 and lower MAE) forecasting performance than the linear regression model. The better fitting of the ANN was more obvious in the saturated CWs, for which the data for validation was adapted from a different dataset. The result is supported by relevant studies reporting that ANN modelling, in comparison with linear models, can improve the accuracy for predicting COD and BOD_5 concentrations in wastewater treatment plant (Abyaneh, 2014) and total nitrogen dynamics in streams (Amiri & Nakane, 2009). Thus, the application of a nonlinear algorithm, such as the ANN-MLP, and the multi fold training and validation schemes we adopted improved the accuracy of the forecasting model, by covering a substantial part of the non-linear mechanisms and factors influencing tebuconazole removal. The present result demonstrates that tebuconazole degradation is a complex process and cannot be easily predicted by simple linear regressions. Therefore, the modelling results show that ANN was more stable and can be trusted to simulate and predict tebuconazole removal, which also deserves to be utilized for other studies on removal of emerging organic pollutants.

5. Conclusions

The present study showed significantly higher tebuconazole removal in unsaturated CWs than saturated CWs, supporting the high potential of unsaturated CWs for the treatment of tebuconazole contaminated water. Tebuconazole removal was fitted with an area-based first order kinetics model in both unsaturated and saturated CWs. The obtained degradation rates can be used as reference data for future applications of CWs in the treatment of tebuconazole contaminated water.

Tebuconazole sorption by the substrate (0.7–2.1%) and plant phytoaccumulation (2.5–12.1%) made limited contributions to tebuconazole removal. Thus, biodegradation and plant metabolisation were the main removal pathways in both CW designs. The main factors influencing tebuconazole removal in the mesocosms were system design, plant presence and species, and HLR. Plants had a positive effect, while increasing HLR had a negative effect. Influent concentration did not show a significant effect on tebuconazole removal. Tebuconazole removal was also correlated with dissolved oxygen and removal of other pollutants, indicating that tebuconazole degradation could be coupled with co-metabolisation processes. Artificial neural network (ANN) modelling was demonstrated to be a more accurate model than linear regression modelling to simulate tebuconazole removal in CWs.

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References

Abargues, M., Robles, A., Bouzas, A., Seco, A. 2012. Micropollutants removal in an anaerobic membrane bioreactor and in an aerobic conventional treatment plant. *Water Sci. Technol*, 65(12), 2242-2250.

Abyaneh, H.Z. 2014. Evaluation of multivariate linear regression and artificial neural networks in prediction of water quality parameters. *J. Environ. Healt. Sci. Eng*, 12(1), 40.

Akratos, C.S., Papaspyros, J.N., Tsihrintzis, V.A. 2008. An artificial neural network model and design equations for BOD and COD removal prediction in horizontal subsurface flow constructed wetlands. *Chem. Eng. J*, 143(1), 96-110.

Akratos, C.S., Papaspyros, J.N., Tsihrintzis, V.A. 2009. Artificial neural network use in ortho-phosphate and total phosphorus removal prediction in horizontal subsurface flow constructed wetlands. *Biosyst. Eng*, 102(2), 190-201.

Amiri, B., Nakane, K. 2009. Comparative prediction of stream water total nitrogen from land cover using artificial neural network and multiple linear regression approaches. *Pol. J. Environ. Stud*, 18(2), 151-160.

Bastian, M., Heymann, S., Jacomy, M. 2009. Gephi: an open source software for exploring and manipulating networks. *ICWSM*, 8, 361-362.

Bollmann, U.E., Tang, C., Eriksson, E., Jönsson, K., Vollertsen, J., Bester, K. 2014. Biocides in urban wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and possible sources. *Water Res.* 60, 64-74.

Brix, H. 1997. Do macrophytes play a role in constructed treatment wetlands? *Water Sci. Technol*, 35(5), 11-17.

Brix, H. 1994. Functions of macrophytes in constructed wetlands. *Water Sci. Technol,* 29(4), 71-78.

Casas, M.E., Bester, K. 2015. Can those organic micro-pollutants that are recalcitrant in activated sludge treatment be removed from wastewater by biofilm reactors (slow sand filters)? *Sci. Total. Environ*, 506, 315-322.

EFSA, E.F.S.A. 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole. *EFSA Journal*, 12(1), 3485-3583.

Elsaesser, D., Blankenberg, A.-G.B., Geist, A., Mæhlum, T., Schulz, R. 2011. Assessing the influence of vegetation on reduction of pesticide concentration in experimental surface flow constructed wetlands: Application of the toxic units approach. *Ecol. Eng*, 37(6), 955-962.

Elsaesser, D., Stang, C., Bakanov, N., Schulz, R. 2013. The Landau stream mesocosm facility: Pesticide mitigation in vegetated flow-through streams. *B. Environ. Contam. Tox*, 90(6), 640-645.

Govindaraju, R. 2000a. Artificial neural networks in hydrology: ii, hydrologic applications.

Govindaraju, R.S. 2000b. Artificial neural networks in hydrology. I: Preliminary concepts. *J. Hydrol. Eng*, 5(2), 115-123.

Gregoire, C., Elsaesser, D., Huguenot, D., Lange, J., Lebeau, T., Merli, A., Mose, R., Passeport, E., Payraudeau, S., Schütz, T. 2009. Mitigation of agricultural nonpoint-source pesticide pollution in artificial wetland ecosystems. *Environ. Chem. Lett*, 7(3), 205-231.

Guo, Y.-M., Liu, Y.-G., Zeng, G.-M., Hu, X.-J., Xu, W.-H., Liu, Y.-Q., Liu, S.-M., Sun, H.-S., Ye, J., Huang, H.-J. 2014. An integrated treatment of domestic wastewater using sequencing batch biofilm reactor combined with vertical flow constructed wetland and its artificial neural network simulation study. *Ecol. Eng*, 64, 18-26.

Headley, T., Nivala, J., Kassa, K., Olsson, L., Wallace, S., Brix, H., van Afferden, M., Müller, R. 2013. Escherichia coli removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. *Ecol. Eng*, 61, 564-574.

Hijosa-Valsero, M., Matamoros, V., Martín-Villacorta, J., Bécares, E., Bayona, J.M. 2010. Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. *Water Res*, 44(5), 1429-1439.

Kadlec, R.H., Wallace, S. 2008. *Treatment wetlands*. CRC press.

Kotti, I.P., Sylaios, G.K., Tsihrintzis, V.A. 2016. Fuzzy modeling for nitrogen and phosphorus removal estimation in free-water surface constructed wetlands. *Environment. Proc*, 3(1), 65-79.

Langergraber, G. 2007. Simulation of the treatment performance of outdoor subsurface flow constructed wetlands in temperate climates. *Sci. Total. Environ*, 380(1), 210-219.

Lin, Y.-F., Jing, S.-R., Lee, D.-Y., Chang, Y.-F., Shih, K.-C. 2008. Nitrate removal from groundwater using constructed wetlands under various hydraulic loading rates. *Bioresource Technol*, 99(16), 7504-7513.

Lv, T., Carvalho, P.N., Zhang, L., Zhang, Y., Button, M., Arias, C.A., Weber, K.P., Brix, H. 2017a. Functionality of microbial communities in constructed wetlands used for pesticide remediation: Influence of system design and sampling strategy. *Water Res*, 110, 241-251.

Lv, T., Zhang, Y., Carvalho, P.N., Zhang, L., Button, M., Arias, C.A., Weber, K.P., Brix, H. 2016a. Microbial community metabolic function in constructed wetland mesocosms treating the pesticides imazalil and tebuconazole. *Ecol. Eng*, 98, 378-387.

Lv, T., Zhang, Y., Casas, M.E., Carvalho, P.N., Arias, C.A., Bester, K., Brix, H. 2016b. Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. *Chemosphere*, 148, 459-466.

Lv, T., Zhang, Y., Zhang, L., Carvalho, P.N., Arias, C.A., Brix, H. 2016c. Removal of the pesticides imazalil and tebuconazole in saturated constructed wetland mesocosms. *Water Res*, 91, 126-136.

Lv, T., Carvalho, P.N., Casas, M.E., Bollmann, U.E., Arias, C.A., Brix, H., Bester, K. 2017b. Enantioselective uptake, translocation and degradation of the chiral pesticides tebuconazole and imazalil by *Phragmites australis*. *Environ. Pollut*, 229, 362-370.

May, D.B., Sivakumar, M. 2009. Prediction of urban stormwater quality using artificial neural networks. *Environ.Modell. Softw*, 24(2), 296-302.

Miyauchi, T., Mori, M., Ito, K. 2005. Application of solid-phase extraction to quantitatively determine cyproconazole and tebuconazole in treated wood using liquid chromatography with UV detection. *J. Chromatogr A*, 1063(1), 137-141.

Naz, M., Uyanik, S., Yesilnacar, M.I., Sahinkaya, E. 2009. Side-by-side comparison of horizontal subsurface flow and free water surface flow constructed wetlands and artificial neural network (ANN) modelling approach. *Ecol. Eng*, 35(8), 1255-1263.

Nivala, J., Hoos, M., Cross, C., Wallace, S., Parkin, G. 2007. Treatment of landfill leachate using an aerated, horizontal subsurface-flow constructed wetland. *Sci. Total. Environ*, 380(1), 19-27.

Passeport, E., Tournebize, J., Chaumont, C., Guenne, A., Coquet, Y. 2013. Pesticide contamination interception strategy and removal efficiency in forest buffer and artificial wetland in a tile-drained agricultural watershed. *Chemosphere*, 91(9), 1289-1296.

Prasertsup, P., Ariyakanon, N. 2011. Removal of chlorpyrifos by water lettuce (Pistia stratiotes L.) and duckweed (Lemna minor L.). *Int. J. Phytoremediat*, 13(4), 383-395.

Rousseau, D.P., Vanrolleghem, P.A., De Pauw, N. 2004. Model-based design of horizontal subsurface flow constructed treatment wetlands: a review. *Water Res*, 38(6), 1484-1493.

Schmidhuber, J. 2015. Deep learning in neural networks: An overview. *Neural networks*, 61, 85-117.

Shikuku, V.O., Kowenje, C.O., Ongeri, D.M., Zanella, R., Pretes, O.D. 2014. Removal of tebuconazole from wastewater by zeolite X: kinetics and thermodynamics studies. *International Journal of Engineering Research and Technology*. ESRSA Publications.

StatSoft. 2004. Inc. 2004. STATISTICA (data analysis software system), version 7. 2004. *Online at: www. statsoft. com*.

Tournebize, J., Passeport, E., Chaumont, C., Fesneau, C., Guenne, A., Vincent, B. 2013. Pesticide de-contamination of surface waters as a wetland ecosystem service in agricultural landscapes. *Ecol. Eng*, 56, 51-59.

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. *Sci. Total. Environ*, 380(1), 48-65.

Vymazal, J., Březinová, T. 2015. The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: a review. *Environ. Int*, 75, 11-20.

Wu, S., Kuschk, P., Brix, H., Vymazal, J., Dong, R. 2014. Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. *Water Res*, 57, 40-55.

Zhang, C.-B., Wang, J., Liu, W.-L., Zhu, S.-X., Ge, H.-L., Chang, S.X., Chang, J., Ge, Y. 2010. Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland. *Ecol. Eng*, 36(1), 62-68.

Zhang, L., Lv, T., Zhang, Y., Otto, S., Carlos, A., Hans, B., Pedro, C. 2017. Effects of constructed wetland design on ibuprofen removal – A mesocosm sacle study. *Sci. Total. Environ.* 609, 38-45.