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Removal of Two Triazole Fungicides from Agricultural Wastewater in Pilot-Scale Horizontal Subsurface Flow Constructed Wetlands

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Abstract: Myclobutanil is a systemic fungicide belonging to the triazole group, which is frequently detected in environmental samples. Triticonazole, also a triazole fungicide, controls soil and seed-borne diseases and it is mainly used as a seed-coating pesticide. Both myclobutanil and triticonazole are considered as persistent pollutants in the environment, raising concerns about their environmental fate and ecotoxicity potential. The purpose of the present study was to investigate the efficiency of four pilot-scale horizontal subsurface flow (HSF) constructed wetlands (CWs) to remediate myclobutanil and triticonazole from artificially polluted water. Daily loading of the four CWs took place from March 2022 to July 2022 with contaminated water fortified with myclobutanil and triticonazole. Three of the CWs, encoded WMG-R, WMG-C, and WMG-U, with medium gravel (MG) as porous media and the fourth, with code name WFG-R, fine gravel (FG). Common reed (R, *Phragmites australis*) was planted in the WMG-R and WFG-R units, and cattail (C, *Typha latifolia*) in the WMG-C unit. The WMG-U unit with no plant was used as a control unit. The results showed that the removal rate follows the pattern: WFG-R (88.4%) > WMG-R > (83.4%) > WMG-C (59.3%) > WMG-U (36.6%) and WFG-R (88.5%) > WMG-C (71.0%) > WMG-R > (70.9%) > WMG-U (49.2%) for myclobutanil and triticonazole, respectively. The most significant factors influencing the fungicides' dissipation were the porous media content and the plant species.

Keywords: pesticides; myclobutanil; triticonazole; phytoremediation; constructed wetlands

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1. Introduction

A broad range of substances are covered by the term "pesticide", including herbicides, insecticides, acaricides, bactericides, nematicides, rodenticides, algaecides molluscicides, and fungicides as well as repellents and plant growth regulators (European Commission, 2020). Pesticides are mainly organic substances that are applied to crops to control weeds, diseases, and pests in an effort to increase productivity and improve food quality. However, pesticides can easily enter natural water reservoirs due to incorrect handling of tank-mix leftovers, accidents, direct application, or other point and nonpoint pollution sources (e.g., agricultural runoff, soil erosion, subsurface drainage), raising concerns about their impact on surface and groundwater quality and human health [1]. For this reason, many plant protection products are classified as toxic contaminants by the Water Framework Directive [2] and as priority substances by Directive 2013/39/EU [3].

Myclobutanil is a systemic fungicide used against a broad spectrum of diseases in cereals, fruits, and vegetables, including summer patch, powdery mildew, dollar spot, and rusts. It exhibits a therapeutic, eradicative, and preventative effect attributed to the suppression of the sterol 14-demethylase enzyme [4]. Myclobutanil's high chemical,

biological, and photochemical persistence with a half-life value ranging from 25 to 222 days in water and soils raise concerns about a potential contamination in water bodies [5,6]. According to Wightwick et al. [7], myclobutanil was one of the most frequently detected fungicides presenting concentrations higher than 0.2 μg/L in water samples and higher than 120 μg/kg dw (dry weight) in the sediment samples of aquatic systems in Australia. Similar to this, Smalling et al. [8] showed that myclobutanil has a high frequency of detection in water, sediment, fish, and sand in the estuary sites in the coastal California, America lettuce-growing region.

Triticonazole controls soil and seed-borne diseases, primarily in cereals, by inhibiting fungi sterol production. Under aerobic conditions, the half-life of triticonazole and its metabolites in soil ranges from 3.7 to 429 days while laboratory tests show that the fungicide is degraded slowly under anaerobic conditions [9]. Triticonazole has a field half-life of over 100 days on average, with a strong potential for accumulation in soil and other matrices. This fungicide can enter wastewater through runoff after use in farms. According to an analysis of the triticonazole concentrations in sewage sludge samples in the province of Madrid, Spain [10], triticonazole was measured at concentrations between 4.6 and 12.1 (ng/g dw). It is mostly used as a seed-coating pesticide, which raises concerns about its fate. According to Schleiffer and Speiser [11], a primary pollution route is the direct treatment of soil to control weeds, soilborne pests, or diseases, and the use of pesticide-treated seeds. In addition, the wastewater from a seed-coating industry contains a significant amount (approximately 1%) of the pesticides used during application [12].

The aforementioned research results show the need to reduce and/or eliminate the entry of pesticides into surface and groundwater. For this purpose, several methods have been proposed worldwide such as vegetated buffer zones at field margins and the use of constructed wetlands [13,14]. Constructed wetlands are artificial systems created to take advantage of various physicochemical and biological processes of pollutants dissipated under regulated settings, and can treat municipal and industrial wastewaters and agricultural runoff. That treatment involves biological, chemical, and physical processes such as biodegradation, plant uptake, adsorption, retention, and settling [15,16]. One of the main components in CWs is the support matrix, also known as substrate, porous media, or filter material [17]. It is crucial for the sorption of contaminants, the system's permeability to allow a proper water flow in constructed wetland (CW), the support for plant roots, and the attachment and retention of suspended materials and organic matter [18]. Macrophytes (such as *Phragmites australis*, *Iris pseudacorus*, *Typha latifolia*, and *Phalaris arundinacea*) are planted on porous media contributing significantly to pesticides removal. Vegetation can uptake contaminants and increase the microbial diversity, biomass, and activity, contributing to biodegradation and sequestration [19].

There are many studies about the use of CWs to remove organic compounds [20–22], pesticides [23–26], heavy metals [27,28], and other contaminants from natural waters; however, there is a lack of investigations using CWs to treat agricultural wastewater contaminated with myclobutanil and triticonazole. Therefore, the purpose of the present study was to optimize the effectiveness of mature pilot-scale CWs to remediate aquatic systems polluted by persistent fungicides. Such point-source pollution sites are usually found at sprayer rinsing places' or seed-coating industries' wastewater.

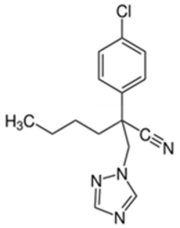
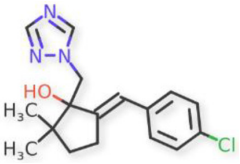
2. Materials and Methods

2.1. Reagents and Chemicals

Myclobutanil and triticonazole analytical standards of ≥98.0%, were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Bondesil PSA (primary secondary amine) anhydrous $MgSO_4$, anhydrous CH_3COONa, anhydrous NaCl (>99%), and bondesil carbon SPE sorbent were purchased from Agilent Technologies (Agilent 7000D, Palo Alto, CA, USA). Purchases from Riedel de Haen (Seelze, Germany) included HPLC grades, acetonitrile, ethyl acetate, and hexane, which were used for the extraction process. Commercial formulations of myclobutanil (purchased from ELLAGRET SA, Thessaloniki, Greece) and

triticonazole (provided by BASF Hellas A.G., Thessaloniki, Greece) with tradenames Ribbon 12 EC and Alios 30 FS, respectively, were used to prepare the fortification solutions loaded to CWs. Table 1 presents the physicochemical properties of the fungicides.

Table 1. Physicochemical properties of the target fungicides [29].

Parameter	Myclobutanil	Triticonazole
Molecular formula	$C_{15}H_{17}ClN_4$	$C_{17}H_{20}ClN_3O$
Substance group	Triazole fungicide	Triazole fungicide
Structural formula		
Molecular weight (g/mol)	288.8	317.8
Water solubility at 20 °C (mg/L)	132.0	9.3
Octanol-water partition coefficient (25 °C) $LogK_{ow}$	2.89 (moderate bioaccumulation)	3.29 (high bioaccumulation)
Vapour pressure at 20 °C (mPa)	0.198	9.0×10^{-5}
Adsorption coefficient K_{foc} (mL/g)	517.0 (slightly mobile)	569.8 (slightly mobile)
Henry's Law constant at 25 °C (Pa m^3/mol)	4.3×10^{-4} (non-volatile)	1.2×10^{-6} (non-volatile)
Soil degradation $DT_{50field}$ [1]	35.0 (moderately persistent)	147.7 (persistent)

[1] Half-life for field studies.

2.2. Pilot-Scale CW Units Configuration and Operational Conditions

The CWs were situated in the outdoor area of the Laboratory of Ecological Engineering and Technology at Democritus University of Thrace (DUTH) (location: 41°08′47″ N, 24°55′09″ E). The four pilot-scale HSF CWs used in this investigation were named WMG-C, WMG-R, WMG-U, and WFG-R. They were rectangular tanks with a length, width, and depth of 3.0 m, 0.75 m, and 1 m, respectively, and a porous media depth of 0.45 m (Figure 1) [29]. Medium gravel (MG, D_{50} = 15.0 mm, range 4–25 mm) was used as the porous media in the WMG-C, WMG-R, and WMG-U units. In the WFG-R unit, the porous media was fine gravel (FG, D_{50} = 8 mm, range 4–16 mm). The medium gravel was carbonate rock and came from a quarry. The fine gravel was igneous rock and mined from a river bed. The plant species used were common reed (R, *Phragmites australis*) in WMG-R and WFG-R, and cattail (C, *Typha latifolia*) in WMG-C. The WMG-U unit was not planted (U) and was used as a control unit. The common reed is considered an invasive species because it can displace native plants. Despite the necessity to prevent its expansion, common read has been shown to be effective in the bioremediation of contaminated areas due to its ability to survive under stress. However, control strategies could be used to stop its proliferation and its encroachment on the natural environment (e.g., cutting) [30]. The effect of plants on the two fungicides' dissipation can be evaluated by comparing the efficiency of the WMG-C, WMG-R, and WMG-U units. Furthermore, the effect of porous media can be evaluated by comparing the removal capacity of the WMG-R and WFG-R units. Previous studies provide strong evidence that CW planted with common reed and cattail are capable to successfully remove various pesticides without showing symptoms of plant toxicity [24,25]. The pilot-scale units were originally installed in 2003 [31] and since then have been used in various experiments of wastewater treatments (e.g., municipal wastewater and pesticide residues) and to remove various pollutants (e.g., organics, heavy metals, and pesticides) [25,26,28,31–33]. The last experiment related to the removal of pesticides from polluted water using the pilot-scale CW units took place in 2015. From then until the present experiment, the pilot-scale units were maintained and loaded only with

tap water for the needs of plant evapotranspiration. All plants used are perennial and they have not needed to be planted each year. Therefore, the pilot-scale CWs are considered as mature CWs with fully grown plants, and the microbial community has been adjusted to the operation conditions.

Figure 1. View of the four pilot-scale HSF CWs.

From March 2022 to July 2022, water enriched with myclobutanil and triticonazole at a concentration of about 1.9 mg/L was loaded to the pilot-scale CWs. A pump with a volume meter was used for the daily loading of the units. Hydraulic residence times (HRT) of 8 and 6 days were used with influent volumes of 40.5 L/day and 54.0 L/day, respectively. These volumes were split into two equal dosages and supplied to the systems twice daily, around every 12 h. During the spring, an 8-day HRT was employed (from March to May), while the HRT of 6 days was applied in June and July (higher temperature) and therefore, wetland water demand is higher due to increased evapotranspiration. Similar HRTs were applied to previous studies aiming for pesticide removal [24,33]. According to a recent review study on agricultural runoff treatment for pesticide removal using CW, an HRT of 6–7 days is adequate [19]. With this configuration, it was possible to evaluate: (a) the contribution of the presence of plants, (b) the effect of porous media (medium carbonate gravel and fine igneous gravel) and HRT (8 and 6 days) on the removal of the two fungicides, and (c) the phytoaccumulation capacity of common reed and cattail.

2.3. Sampling Campaign

Weekly water samples from the influent and effluent of each CW were taken for analysis in the laboratory and processed to determine the CW unit performance. Simultaneously with the sampling, physicochemical parameters in the water such as dissolved oxygen (DO), pH, electrical conductivity (EC), and temperature (T) were measured in situ by a portable device (HQ30D Field Case, HACH-LANGE E.P.E., Athens, Greece). To give the units time to acclimate, sampling began one and a half months after loading.

In order to determine the concentration of the two fungicides in the substrate, 500 g samples of substrate were collected from the inlet and outlet of each CW unit using a soil sampling auger. Samples contained gravel, soil, organic matter, and plant materials. Firstly, gravel was removed by hand and then using a 2 mm sieve, samples were processed. Each sample was stored in a refrigerator at a temperature of −2 °C for one day before being processed for analysis. Additionally, the role of the plant in fungicides removal was evaluated by collecting plants from each CW unit at random in triplicate and the various parts of the plants (i.e., roots, stems, and leaves) were separately analyzed to determine the myclobutanil and triticonazole concentrations.

2.4. Instrumental Analysis of Myclobutanil and Triticonazole

The fungicide was extracted three times from the water samples using 25 mL of hexane in total. A salting-out procedure employing 1.5 g NaCl was performed during the third extraction. The organic phase from three extracts was collected and evaporated to dry. With 5 mL of hexane, the residues were reconstituted and they were once more

evaporated to dryness using a nitrogen stream. Fungicide residues were extracted with 5 mL of ethyl acetate, passed through a 0.2 m PTFE filter, and stored in vials at −20 °C until instrument analysis (GC-MS/MS). *Typha latifolia* and *Phragmites australis* plant samples as well as substrate samples were processed using the QuEChERS protocol, as reported by Matadha et al. [34]. Briefly, five grams of each sample, homogenized, were put to 50 mL Falcon tubes together with 4 g of $MgSO_4$, 1 g of CH_3COONa, 10 mL of acetonitrile, and 10 mL of water. A vortex mixer was used to blend the solution and it was centrifuged repeatedly for 10 min at 4000 rpm. The acetonitrile from the upper phase was collected and placed into 15 mL Falcon tubes along with 750 mg of $MgSO_4$, 150 mg of bondesil PSA, and 50 mg of bondesil carbon SPE sorbent, and mixed on a vortex for one minute. A gas chromatographic (GC–MS/MS) method was used for the detection and quantification of myclobutanil and triticonazole.

In this study, an Agilent 8890 GC system equipped with a triple quadrupole mass spectrometer (Agilent 7000D, Palo Alto, CA, USA) was operated for triticonazole and myclobutanil detection and quantification. The column used was a dual capillary Agilent 190905-431U1 (15 m length, 250 i.d. μm, 0.25 film thickness μm).

Myclobutanil was detected and quantified by a dynamic multiple reaction monitoring (dMRM) method developed at the Laboratory of Agricultural Pharmacology and Ecotoxicology, Democritus University of Thrace. A sample aliquot (1 μL) was injected into the injection port heated at 280 °C with spitless mode. As a carrier gas, helium was set at a flow rate of 1 mL/min. The oven temperature was initially 60 °C for 1 min, ramped to 170 °C at 40 °C/min, and then finally increased to 310 °C at 10 °C/min, and held for 3 min. The transfer line temperature was set at 250 °C, the electron ionization energy was set at 70 eV, and the ion source temperature was set at 230 °C. The selected transitions (precursor and product ions) were the qualifier transition 179 > 90 and quantification transition 179 > 25.1. The retention time was 12.013 min. In triticonazole's case, a single mass spectrometer was used in full scan mode, according to Börjesson et al. [35] Briefly, the injection temperature and volume was 250 °C and 1 μL, respectively, and the column flow was 1 mL helium/min. The oven temperatures were 100 °C for 2 min, 100 °C to 250 °C with rate at 20 °C/min, 250 °C to 260 °C at 5 °C/min, and 260 °C to 280 °C at 15 °C/min. The transfer line temperature, electron ionization energy, and ion source temperature were 250 °C, 70 eV, and 230 °C, respectively. The retention time was 11.37 min.

In order to create a calibration curve, pesticide standard solutions in ethyl acetate were prepared at concentrations of 1, 10, 50, and 100 g/mL. Recovery rates varied from 80 to 110% for each matrix (water, plant, and porous media) in all tested concentrations. For both plant and substrate samples, the limits of detection and quantification were 0.01 and 0.02 mg/kg, respectively, and for the water samples they were 3 and 5 ng/L, respectively.

2.5. Statistical Analysis

To evaluate the differences in the fungicide removal efficiency of CW units, nonparametric tests such as Kruskal–Wallis (K–W) and Mann–Whitney U (M–W U) were used. The K–W test was specifically used to inspect the effect of plants on unit remediation efficiency. Where the K–W test showed a significant difference between CWs, the M–W U test was used to assess pair comparisons. In order to assess how porous material affects the ability of CW to remove the fungicides, the M–W U test was also used. The Windows statistical package, SPSS 25.0, was used and the threshold for statistical significance was established at $p = 0.05$.

3. Results and Discussion

3.1. Physicochemical Parameters in CW Units

The measured physicochemical parameters in influent and effluent water samples of the four CW units are shown in Table 2 (statistics) and in Figure 2 (distribution box and whisker plots). The water temperature in the CW units followed the seasonal variation and varied from 8.0 to 32.5 °C (Table 2 and Figure 2a). The pH values throughout the

measurement period varied between acidic (6.66) and alkaline values (7.73). The mean pH values of all pilot-scale CWs were in the alkaline range with no significant variation among them (Table 2 and Figure 2b). The influent EC value was lower than the mean effluent EC values for all CWs (Table 2 and Figure 2c). The results also indicated that the mean effluent EC value of the planted units was higher than that of the WMG-U unit. This fact is attributed to water losses and condensation due to evapotranspiration [24]. The mean DO concentration in the influent was 7.37 mg/L and in the effluent was 4.01, 5.66, 5.12, and 4.57 mg/L for WMG-C, WMG-R, WMG-U, and WFG-R, respectively (Table 2 and Figure 2d). These results indicate that the DO concentration decreased in the CW units probably due to the consumption by microorganisms despite the fact that in the planted CWs, oxygen is transferred from plant leaves to the root system. Furthermore, the WMG-R and WFG-R units had a lower mean DO concentration than the unplanted unit indicating greater microbiome growth in these units compared to the unplanted unit. Similar results have been reported elsewhere [24,25].

Table 2. Statistics of measured physicochemical parameters (SD: standard deviation, Min: minimum, Max: maximum, n: samples number).

Parameter		Influent	Effluent			
			WMG-C	WMG-R	WMG-U	WFG-R
T (°C)	Mean	19.3	20.9	20.9	20.8	21.9
	SD	6.4	7.7	7.5	7.6	8.1
	Min	8.4	8.6	8.6	8.0	8.5
	Max	28.0	30.5	29.1	32.5	30.9
	n	20	20	20	20	20
pH	Mean	7.32	7.06	7.50	7.43	7.11
	SD	0.22	0.19	0.14	0.14	0.15
	Min	6.85	6.66	7.20	7.15	6.82
	Max	7.83	7.45	7.73	7.64	7.39
	n	20	20	20	20	20
EC (µS/cm)	Mean	513	742	856	530	811
	SD	14	121	156	91	166
	Min	481	612	678	430	590
	Max	546	1095	1215	819	1052
	n	20	20	20	20	20
DO (mg/L)	Mean	7.37	4.01	5.66	5.12	4.57
	SD	2.24	1.85	2.15	2.14	1.76
	Min	4.12	1.03	2.80	2.29	1.15
	Max	10.76	7.23	9.86	9.86	7.74
	n	20	20	20	20	20

3.2. Statistical Evaluation of CW Units Performance

The temporal distributions of the myclobutanil concentrations in the influent and effluents as well as the removal rate in the CW units during the experiment are shown in Figure 3. The four CW units' effluent concentrations were lower than those of the influent, confirming myclobutanil removal (Figure 3a). Myclobutanil's mean influent concentration was 1.79 mg/L, while the units WMG-C, WMG-R, WMG-U, and WFG-R had respective effluent concentrations of 0.71, 0.29, 1.12, and 0.20 mg/L over the full operation period (EP). The mean percentage removals of myclobutanil were 59.3%, 83.4%, 36.6%, and 88.4% for the WMG-C, WMG-R, WMG-U, and WFG-R units, respectively (Figure 4). Plant uptake, adsorption on substrate, sedimentation, and biodegradation are the main pathways for pesticide removal in the constructed wetlands environment [13]. Pesticides are also removed in aqueous systems by hydrolysis, photolysis, volatilization, and oxidation [26,36]. Myclobutanil is not hydrolyzed at 50 °C, at pH 4, 7, and 9, and its aqueous photolysis DT_{50} at pH 7 is 15 days. Myclobutanil has a Henry's law constant of 4.3×10^{-4} Pa m^3/mol

at 25 °C and a vapor pressure of 0.198 mPa at 20 °C, indicating that it is nonvolatile (Table 1) [6,29].

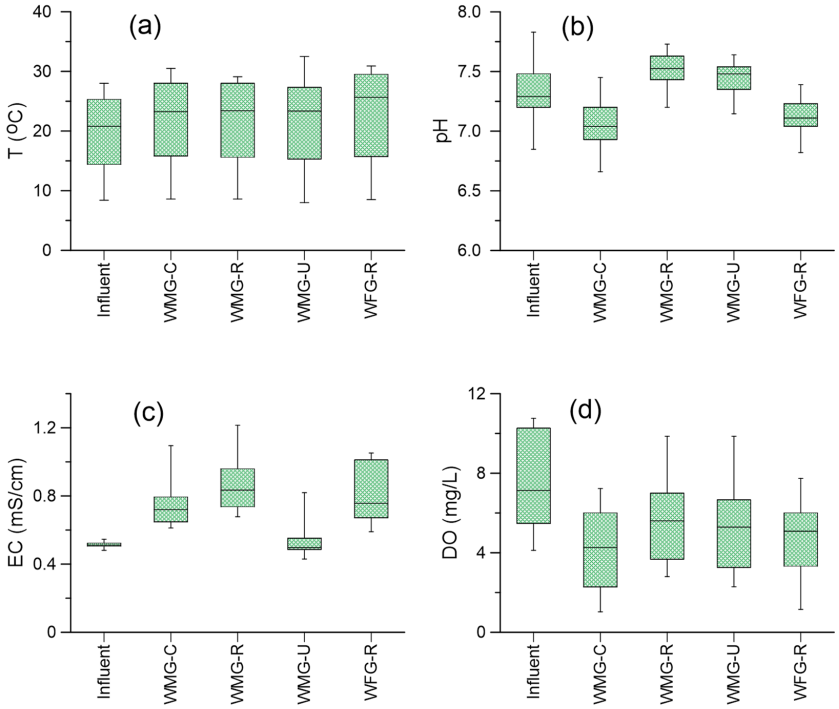


Figure 2. Box-whisker plots of physicochemical parameters of the influent and effluents in WMG-C, WMG-R, WMG-U, and WFG-R units: (**a**) temperature, (**b**) pH, (**c**) electrical conductivity, and (**d**) dissolved oxygen. The box is defined by the lower and upper quartiles. The line in the box denotes the median value and the whiskers at the end of each box indicate the minimum and maximum values.

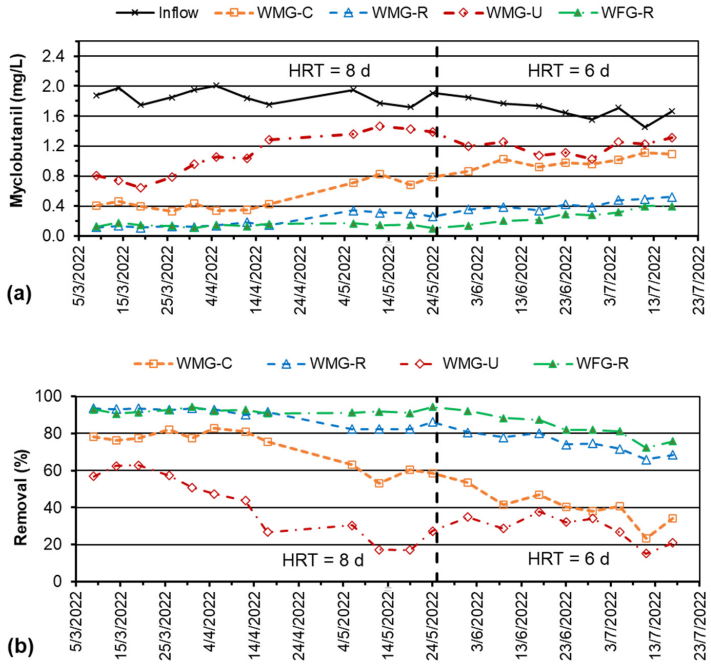


Figure 3. Temporal variation of myclobutanil in the pilot-scale CWs: (**a**) influent and effluent concentrations; (**b**) removal (%).

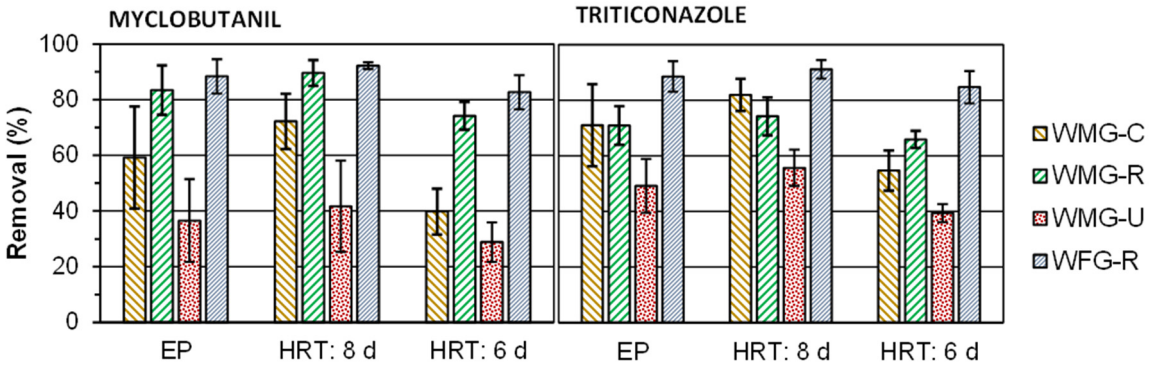


Figure 4. Mean removal values and standard deviation of myclobutanil and triticonazole in the pilot-scale CWs for the entire operation period (EP) and at HRT of 8 and 6 days.

The variation of triticonazole concentrations in the influent and effluents of the pilot-scale units are shown in Figure 5. Triticonazole had an average influent concentration of 1.92 mg/L, while the EP effluent values for the WMG-C, WMG-R, WMG-U, and WFG-R units were 0.55, 0.55, 0.97, and 0.22 mg/L, respectively. The mean removal efficiencies of triticonazole were 71.02%, 70.87%, 49.21, and 88.5% for the WMG-C, WMG-R, WMG-U, and WFG-R units, respectively (Figure 4). The aqueous hydrolysis DT_{50} value at 20 °C and pH 7 of triticonazole is 7.4 days, indicating that it is a non-persistent pollutant [29]. On the other hand, according to EFSA [9] it is stable after a 30-day hydrolysis at pH 5, 7, and 9 at 25 °C. The aqueous photolysis (DT_{50} value) at pH 7 is 300 days, indicating that it is stable [29]. The vapor pressure at 20 °C and Henry's law constant at 25 °C of triticonazole are 1.2×10^{-6} Pa m^3/mol and 9.0×10^{-5} mPa, respectively, which show that it is a nonvolatile compound (Table 1) [9,29].

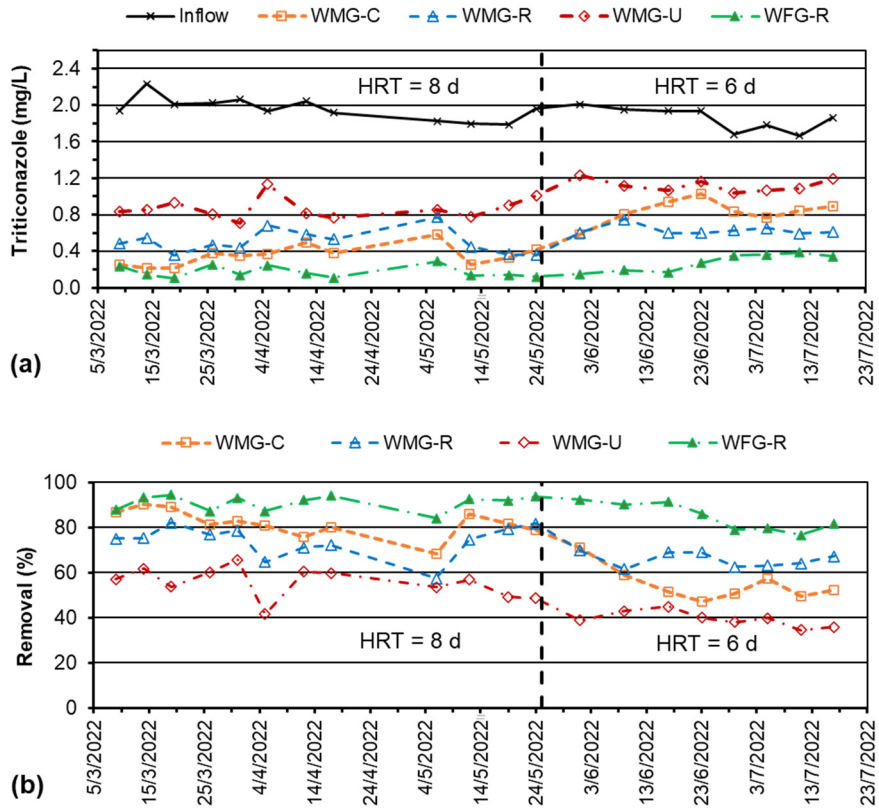


Figure 5. Temporal variation of triticonazole in the pilot-scale CWs: (**a**) influent and effluent concentrations; (**b**) removal (%).

Based on the aforementioned data, the myclobutanil and triticonazole removal by volatilization is negligible. The water in the CW units moves beneath the porous media surface and the penetration of sunlight is further reduced by plants. Therefore, photodegradation of myclobutanil and photolysis of triticonazole have insignificant contributions to their removal in the CW units. Regarding the hydrolysis, it is negligible for myclobutanil and considered limited for triticonazole under the applied hydraulic conditions of the pilot-scale CW units because the applied HRT is 8 and 6 days. Therefore, myclobutanil and triticonazole removal in pilot-scale CW units is mainly due to plant uptake, biodegradation, sedimentation, and adsorption on the substrate.

Figure 4 also presents the mean removal percentage of myclobutanil and triticonazole in the CW units at HRT of 8 and 6 days. HRT is considered an important factor influencing the efficacy of CWs and the selection of an optimal HRT influences the overall performance of CWs [37]. The four units showed higher mean removal values of myclobutanil and triticonazole at the HRT of 8 days. This denotes that HRT of 8 days may be the optimal value for the two fungicides' removal.

3.3. Effect of Vegetation

The planted units demonstrated a higher removal efficiency of fungicides when compared to the unplanted units for both fungicides (Figures 3b and 5b). For the three units using the same porous material (i.e., WMG-C, WMG-R, and WMG-Z), a comparison was conducted. The WMG-R and WMG-C units displayed the highest myclobutanil and triticonazole removal efficiencies, with mean removal rates of 83.4% and 71.0%, respectively. The K–W test revealed statistically significant ($p < 0.001$) differences in myclobutanil and triticonazole removal rates between the pilot-scale CW units. Myclobutanil removal in the WMG-C and WMG-R units were statistically significantly higher than that in the WMG-U unit, and the removal in the WMG-C unit was statistically significantly higher than that in the WMG-R unit ($p < 0.001$, M–W U test). The removal of triticonazole in the WMG-U unit was statistically significantly lower than that in the WMG-C and WMG-R units ($p < 0.001$, M–W U test), and there was no statistically significant difference of triticonazole removal between the WMG-C and WMG-R units ($p > 0.05$, M–W U test). These findings claim that the existence of vegetation, such as *Phragmites australis* and *Typha latifolia*, greatly facilitates the elimination of both fungicides.

Plants contribute to contaminant removal in CW by providing higher microbial diversity and richness in substrate, leading to phytoaccumulation and a higher biodegradation rate compared to unplanted CW. The stability and preservation of CW treatment efficacy is largely dependent on microbial diversity and richness. Pesticide degrading microorganisms or beneficial microorganisms for plant growth can be located either in the substrate or in the plant roots. [13,38–40]. Several researchers have supported that remarkably higher microbial activity is observed in planted than unplanted CW. The microbial communities that are hosted in plant rhizosphere and porous media (gravel-associated) microenvironments of a CW are assumed to be physiologically distinct from each other. However, macrophyte rhizosphere can attract the microenvironment microorganisms around their roots and on the other hand, the root exudates and enzymes can be used by microorganisms for the development of microbial communities in the substrate where the majority of contaminant breakdown is conducted. Additionally, it is anticipated that the presence of plants promotes the growth of rhizospheric microbial communities by releasing root exudates and plants provide surface for microbiome attachment. Those plant root exudates contribute to microbial communities formation, which utilize carbon sources (for example, pesticides) as nutrients [41–43]. Furthermore, epiphytic or endophytic microorganisms can colonize wetland plants, enhancing plant growth and thus, pollutant phytoaccumulation can be increased [44]. The rhizodegradation is associated with the fact that there are rhizospheric bacteria that are tolerant to pesticides or can degrade pesticides. Furthermore, plants in CW help to stabilize the wetland's bed surface and enhance the substrate porosity, which allows aerobic bacteria to thrive in the soil and speed up the biodegradation process [40,45].

The pesticide's physical and chemical properties as well as its interactions with the soil, plant microbiome, water, and other substances of diverse types in the rhizosphere define the behavior of a pesticide within a plant though various processes (i.e., uptake, translocation, and excretion) [43]. Regarding the phytoaccumulation and the fungicides translocation within plant processes, the octanol/water partition coefficient ($LogK_{ow}$) of pesticides strongly affects their fate in plant tissues. In plant tissues, pesticides having $LogK_{ow}$ values between 3.0 and 4.0 are more readily absorbed and translocated [46,47]. However, water solubility is another important factor which affect the uptake and translocation of pesticides. The $LogK_{ow}$ values of myclobutanil and triticonazole are 2.89 and 3.29, respectively, and the water solubility values of myclobutanil and triticonazole are 132 and 9.3 mg/L, respectively (Table 1). Therefore, an adequate phytoaccumulation is expected. The results of plant analysis (Table 3) demonstrated that fungicide residues were accumulated in various plant tissues. Additionally, both *Typha latifolia* and *Phragmites australis* accumulated a higher amount of triticonazole than myclobutanil, which is explained by their different lipophilicity and water solubility. According to Wang et al. [48], pesticides with greater $LogK_{ow}$ and lower water solubility are more easily adsorbed by roots, whereas those with lower $LogK_{ow}$ and higher water solubility are more likely to be transported from roots to shoots.

Table 3. Fungicides concentration on plant parts in pilot-scale CWs (dw: dry weight, R: Phragmites australis, C: Typha latifolia).

	Myclobutanil			**Triticonazole**		
	WMG-C	**WMG-R**	**WFG-R**	**WMG-C**	**WMG-R**	**WFG-R**
Root (mg/kg dw)	3.86 ± 0.6	3.93 ± 0.08	4.83 ± 0.08	4.51 ± 0.11	4.73 ± 0.12	5.25 ± 0.11
Stem (mg/kg dw)	0.80 ± 0.08	0.85 ± 0.05	1.05 ± 0.10	0.87 ± 0.07	0.88 ± 0.8	1.31 ± 0.07
Leaves (mg/kg dw)	0.19 ± 0.09	0.15 ± 0.09	0.33 ± 0.08	0.20 ± 0.09	0.19 ± 0.7	0.44 ± 0.09

The $LogK_{ow}$ value of myclobutanil is more conducive to phytoaccumulation than triticonazole, as shown above. The roots were the plant component in all of the CW units that showed the greatest accumulation (root absorption); the order of the plant parts was root > leaf > stem (Table 3), which is explained by an initially higher exposure to pesticides in the roots of plants. According to recent studies, the tested macrophytes accumulate the highest amounts in their roots [24,25,36]. The phytoaccumulation capacity and translocation differences between the two macrophytes could be explain by distinctions in oxygen transfer, root exudation processes, and microbial communities [49]. The roots of the WMG-R and WFG-R units (with *Phragmites australis*) adsorbed higher fungicide amounts than the WG-C roots (with *Typha latifolia*), which is related to the higher vigor of *Phragmites australis* roots than *Typha latifolia* ones. The higher capacity of *Phragmites australis* to adsorb higher amounts of pollutants than *Typha latifolia* is also reported in previous studies [24,25].

3.4. Influence of the Substrate Material

The WMG-R and WFG-R units, which contained different porous media, namely mean gravel of carbonate rock and fine gravel of igneous rock, respectively, and were planted with common reed, were compared. The comparison was made to determine the impact of the two porous media on the fungicides removal. The mean percentage removals of myclobutanil were 83.4% and 88.4%, and of triticonazole were 79.9% and 88.5%, for the WMG-R and WFG-R units, respectively (Figure 4). A statistical analysis indicated that there was no statistically significant difference in myclobutanil removal between the two CW units (M–W U test: $p > 0.05$) while removal of triticonazole in WMG-R was statistically significantly lower than that in the WFG-R unit (M–W U test: $p < 0.001$).

Both the substrate and the physicochemical characteristics of the fungicide are crucial for fungicide adsorption in HSF CW. Pesticides with low water solubility (i.e., water solubility < 10 mg/L) and high K_{oc} (i.e., K_{oc} > 1000 L/kg) show high pesticide sorption and retention in soil and organic matter fraction [50]. The water solubility values of myclobutanil and triticonazole are 132 and 9.3 mg/L, and their K_{oc} values are 518 and 374 L/kg, respectively [29]. Therefore, a medium adsorption of the fungicides on the substrate is expected. According to a recent study, batch column experiments were performed to evaluate micropollutants removal by gravel. The results showed that the removal capability of gravel and sand was negligible compared to granulated activated carbon, biochar, and igneous rock [18]. More specifically, the authors demonstrated that the materials, sand and gravel, have the lowest adsorption ability even though all tested adsorbents are geomaterials, due to the lack of a large extended specific area [18]. Additionally, Huang et al. [51] support that a higher specific surface area increases the amount of external surface adsorption. Despite the low adsorption of pesticides on the gravel, the difference in removal between the units (Figure 4) is likely related to the fact that the WFG-R unit contains porous media which are igneous rocks and as mentioned previously, are finer than that of the WMG-R unit and therefore, provides more "binding sites" for pesticides adsorption. Furthermore, Akratos and Tsihrintzis [31] have demonstrated that igneous rocks have a higher adsorption capacity than carbonate rocks.

In mature CW systems, such as the CW units in this study, plant tissue residues are observed and organic matter concentration rises as a result of suspended solids accumulation in porous media which could allow an increased adsorption [24,33,52]. It is known that the higher the amount of organic matter is, the higher the adsorption of pesticides is expected in the substrate [53]. In particular, the content of organic matter in WFG-R (3.2%) is higher than WMG-R (2.9%), leading to an equivalently higher myclobutanil and triticonazole adsorption on the WFG-R unit.

4. Conclusions

The removal of the fungicides myclobutanil and triticonazole in four pilot-scale CWs was studied. The results of this study indicate that the plants and their ability to uptake fungicides, HRT, and organic matter content in the substrate, and the type and granulation of porous media are the most crucial parameters determining the CW capability for the removal of myclobutanil and triticonazole, while hydrolysis, photodegradation, and volatilization are negligible. CWs are known as an effective remediation technique to purify water, which can protect the quality of water bodies and help reduce the toxicity of pesticide-polluted water. Therefore, the construction of CWs near sites where pesticide mixing and equipment rinsing takes place (e.g., myclobutanil) or/and seed-coating industry wastewaters discharge (e.g., triticonazole) is recommended. In addition, according to our knowledge this is the first investigation using CWs to treat agricultural wastewater contaminated with myclobutanil and triticonazole and thus, further investigations using different operations (i.e., different vegetation and porous media content) to optimize CWs' effectiveness are suggested.

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References

1. Córdova-Méndez, E.A.; Góngora-Echeverría, V.R.; González-Sánchez, A.; Quintal-Franco, C.; Giácoman-Vallejos, G.; Ponce-Caballero, C. Pesticide treatment in biobed systems at microcosms level under critical moisture and temperature range using an Orthic Solonchaks soil from southeastern Mexico amended with corn husk as support. *Sci. Total Environ.* **2021**, *772*, 145038. [CrossRef]
2. WFD (Water Framework Directive). Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Union* **2000**, *327*, 1–73.
3. Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* **2013**, *226*, 1–17.
4. Hao, W.; Zhang, Y.; Xie, Y.; Guo, B.; Chang, J.; Li, J.; Xu, P.; Wang, H. Myclobutanil accumulation, transcriptional alteration, and tissue injury in lizards (*Eremias argus*) treated with myclobutanil enantiomers. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 247–255. [CrossRef]
5. Wang, C.; Wu, Q.; Wu, C.; Wang, Z. Application of dispersion-solidificationliquid-liquid microextraction for the determination of triazole fungicides in environmental water samples by high-performance liquid chromatography. *J. Hazard Mater.* **2011**, *185*, 71–76. [CrossRef]
6. EFSA (European Food Safety Authority). Conclusion on the peer review of the pesticide risk assessment of the active substance myclobutanil. *EFSA J.* **2010**, *8*, 1682–1683. [CrossRef]
7. Wightwick, A.M.; Bui, A.D.; Zhang, P.; Rose, G.; Allinson, M.; Myers, J.H.; Reichman, S.M.; Menzies, N.W.; Pettigrove, V.; Allinson, G. Environmental fate of fungicides in surface waters of a horticultural-production catchment in southeastern Australia. *Arch. Environ. Contam. Toxicol.* **2012**, *62*, 380–390. [CrossRef]
8. Smalling, K.L.; Kuivila, K.M.; Orlando, J.L.; Phillips, B.M.; Anderson, B.S.; Siegler, K.; Hunt, J.W.; Hamilton, M. Environmental fate of fungicides and other current-use pesticides in a central California estuary. *Mar. Pollut. Bull.* **2013**, *73*, 144–153. [CrossRef]
9. EFSA (European Food Safety Authority). Conclusion regarding the peer review of the pesticide risk assessment of the active substance triticonazole. *EFSA J.* **2005**, *33*, 1–69. [CrossRef]
10. Garcia-Valcarcel, A.I.; Tadeo, J.L. Determination of azoles in sewage sludge from Spanish wastewater treatment plants by liquid chromatography-tandem mass spectrometry. *J. Sep. Sci.* **2011**, *34*, 1228–1235. [CrossRef]
11. Schleiffer, M.; Speiser, B. Presence of pesticides in the environment, transition into organic food, and implications for quality assurance along the European organic food chain—A review. *Environ. Pollut.* **2022**, *313*, 120116. [CrossRef]
12. Papazlatani, C.V.; Karas, P.A.; Lampronikou, E.; Karpouzas, D.G. Using biobeds for the treatment of fungicide-contaminated effluents from various agro-food processing industries: Microbiome responses and mobile genetic element dynamics. *Sci Total Environ.* **2022**, *823*, 153744. [CrossRef]
13. Vymazal, J.; Březinová, T. The use of constructed wetlands for removal of pesticides from agricultural runoff and drainage: A review. *Environ. Int.* **2015**, *75*, 11–20. [CrossRef]
14. Pavlidis, G.; Tsihrintzis, V.A. Environmental benefits and control of pollution to surface water and groundwater by agroforestry systems: A review. *Water Res. Manag.* **2018**, *32*, 1–29. [CrossRef]
15. Vymazal, J.; Zhao, Y.; Mander, Ü. Recent research challenges in constructed wetlands for wastewater treatment: A review. *Ecol. Eng.* **2021**, *169*, 106318. [CrossRef]
16. Addo-Bankas, O.; Zhao, Y.; Gomes, A.; Stefanakis, A. Challenges of urban artificial landscape water bodies: Treatment techniques and restoration strategies towards ecosystem services enhancement. *Processes* **2022**, *10*, 2486. [CrossRef]
17. Yang, Y.; Zhao, Y.; Liu, R.; Morgan, D. Global development of various emerged substrates utilized in constructed wetlands. *Bioresour. Technol.* **2018**, *261*, 441–452. [CrossRef]
18. Lei, Y.; Langenhoff, A.; Bruning, H.; Rijnaarts, H. Sorption of micropollutants on selected constructed wetland support matrices. *Chemosphere* **2021**, *275*, 130050. [CrossRef]
19. Tang, Z.; Wood, J.; Smith, D.; Thapa, A.; Aryal, N. A review on constructed treatment wetlands for removal of pollutants in the agricultural runoff. *Sustainability* **2021**, *13*, 13578. [CrossRef]
20. Noutsopoulos, C.; Charalambous, V.; Koumaki, E. Evaluating the fate of emerging contaminants in wastewater treatment plants through plant-wide mathematical modelling. *Environ. Process.* **2020**, *7*, 1065–1094. [CrossRef]
21. Li, B.; Liu, L.; Han, L.; Yang, Y. Removal efficiency of polycyclic aromatic hydrocarbons and phthalate esters by surface flow wetland in Shunyi district, Beijing. *IOP Conf. Ser. Earth Environ. Sci.* **2017**, *59*, 12041. [CrossRef]
22. Surana, D.; Gupta, J.; Sharma, S.; Kumar, S.; Ghosh, P. A review on advances in removal of endocrine disrupting compounds from aquatic matrices: Future perspectives on utilization of agri-waste based adsorbents. *Sci. Total Environ.* **2022**, *826*, 154129. [CrossRef] [PubMed]
23. Liu, T.; Xu, S.; Lu, S.; Qin, P.; Bi, B.; Ding, H.; Liu, Y.; Guo, X.; Liu, X. A review on removal of organophosphorus pesticides in constructed wetland: Performance, mechanism and influencing factors. *Sci. Total Environ.* **2019**, *651*, 2247–2268. [CrossRef] [PubMed]
24. Parlakidis, P.; Mavropoulos, T.; Vryzas, Z.; Gikas, G.D. Fluopyram removal from agricultural equipment rinsing water using HSF pilot-scale constructed wetlands. *Environ. Sci. Pollut. Res.* **2022**, *29*, 29584–29596. [CrossRef]

25. Gikas, G.D.; Pérez-Villanueva, M.; Tsioras, M.; Alexoudis, C.; Pérez-Rojas, G.; Masís-Mora, M.; Lizano-Fallas, V.; Rodríguez-Rodríguez, C.E.; Vryzas, Z.; Tsihrintzis, V.A. Low-cost approaches for the removal of terbuthylazine from agricultural wastewater: Constructed wetlands and biopurification system. *Chem. Eng. J.* **2018**, *335*, 647–656. [CrossRef]
26. Gikas, G.D.; Vryzas, Z.; Karametos, I.; Tsihrintzis, V.A. Pyraclostrobin removal in pilot-scale horizontal subsurface flow constructed wetlands and in porous media filters. *Processes* **2022**, *10*, 414. [CrossRef]
27. Nguyen, T.T.; Soda, S.; Kanayama, A.; Hamai, T. Effects of cattails and hydraulic loading on heavy metal removal from closed mine drainage by pilot-scale constructed wetlands. *Water* **2021**, *13*, 1937. [CrossRef]
28. Saeed, T.; Alam, M.K.; Miah, M.J.; Majed, N. Removal of heavy metals in subsurface flow constructed wetlands: Application of effluent recirculation. *Environ. Sustain. Indic.* **2021**, *12*, 100146. [CrossRef]
29. PPDB (Pesticide Properties Data Base). Pesticides General Information, University of Hertfordshire UK. 2022. Available online: http://sitem.herts.ac.uk/aeru/ppdb/ (accessed on 30 November 2022).
30. Kalu, C.M.; Rauwane, M.E.; Ntushelo, K. Microbial spectra, physiological response and bioremediation potential of phragmites australis for agricultural production. *Front. Sustain. Food Syst.* **2021**, *5*, 696196. [CrossRef]
31. Akratos, C.S.; Tsihrintzis, V.A. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng.* **2007**, *29*, 173–191. [CrossRef]
32. da Silva, F.P.; Lutterbeck, C.A.; Colares, G.S.; Oliveira, G.A.; Rodrigues, L.R.; Dell'Osbel, N.; Rodriguez, A.L.; Lopez, D.A.R.; Gehlen, G.; Machado, E.L. Treatment of university campus wastewaters by anaerobic reactor and multi-stage constructed wetlands. *J. Water Process Eng.* **2021**, *42*, 102119. [CrossRef]
33. Papaevangelou, V.A.; Gikas, G.D.; Vryzas, Z.; Tsihrintzis, V.A. Treatment of agricultural equipment rinsing water containing a fungicide in pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng.* **2017**, *101*, 193–200. [CrossRef]
34. Parlakidis, P.; Tokamani, M.; Sandaltzopoulos, R.; Tokatlidis, I.; Sinapidou, E.; Vryzas, Z. Evaluation of the removal efficacy of three fungicides by biomixtures: Impact of bioaugmentation by plant growth promoting rhizobacteria and zeolite fortification. *J. Environ. Anal. Chem.* **2022**, *in press*. [CrossRef]
35. Börjesson, E.; Stenström, J.; Johnsson, L.; Torstensson, L. Comparison of triticonazole dissipation after seed or soil treatment. *J. Environ. Qual.* **2003**, *32*, 1258–1261. [CrossRef] [PubMed]
36. Lv, T.; Zhang, Y.; Casas, M.E.; Carvalho, P.N.; Arias, C.A.; Bester, K.; Brix, H. Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium. *Chemosphere* **2016**, *148*, 459–466. [CrossRef]
37. Kadlec, R.H.; Wallace, S.D. *Treatment Wetlands*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2009.
38. Guo, W.; Zhang, H.; Huo, H. Organochlorine pesticides in aquatic hydrophyte tissues and surrounding sediments in Baiyangdian wetland, China. *Ecol. Eng.* **2014**, *67*, 150–155. [CrossRef]
39. Sánchez, O. Constructed wetlands revisited: Microbial diversity in the *-omics* Era. *Microb. Ecol.* **2017**, *73*, 722–733. [CrossRef]
40. Braschi, I.; Blasioli, S.; Lavrnić, S.; Buscaroli, E.; Di Prodi, K.; Solimando, D.; Toscano, A. Removal and fate of pesticides in a farm constructed wetland for agricultural drainage water treatment under Mediterranean conditions (Italy). *Environ. Sci. Pollut. Res.* **2022**, *29*, 7283–7299. [CrossRef]
41. Weber, K.P.; Legge, R.L. Comparison of the catabolic activity and catabolic profiles of rhizospheric, gravel-associated and interstitial microbial communities in treatment wetlands. *Water Sci. Technol.* **2013**, *67*, 886–893. [CrossRef]
42. Button, M.; Rodriguez, M.; Brisson, J.; Weber, K.P. Use of two spatially separated plant species alters microbial community function in horizontal subsurface flow constructed wetland. *Ecol. Eng.* **2016**, *92*, 18–27. [CrossRef]
43. Lv, T.; Carvalho, P.N.; Zhang, T.; Zhang, Y.; Button, M.; Arias, C.A.; Weber, K.; Brix, H. Functionality of microbial communities in constructed wetlands used for pesticide remediation: Influence of system design and sampling strategy. *Water Res.* **2017**, *110*, 241–251. [CrossRef] [PubMed]
44. Syranidou, E.; Christofilopoulos, S.; Gkavrou, G.; Thijs, S.; Weyens, N.; Vangronsveld, J.; Kalogerakis, N. Exploitation of endophytic bacteria to enhance the phytoremediation potential of the wetland helophyte *Juncus acutus*. *Front. Microbiol.* **2016**, *7*, 1016. [CrossRef] [PubMed]
45. Cedillo-Herrera, C.I.G.; Roé-Sosa, A.; Pat-Espadas, A.M.; Ramírez, K.; Rochín-Medina, J.; Amabilis-Sosa, L.E. Efficient malathion removal in constructed wetlands coupled to UV/H_2O_2 pretreatment. *Appl. Sci.* **2020**, *10*, 5306. [CrossRef]
46. Vryzas, Z. The plant as metaorganism and research on next generation systemic pesticides—Prospects and challenges. *Front. Microbiol.* **2016**, *7*, 1968. [CrossRef] [PubMed]
47. Gikas, G.D.; Parlakidis, P.; Mavropoulos, T.; Vryzas, Z. Particularities of fungicides and factors affecting their fate and removal efficacy: A review. *Sustainability* **2022**, *14*, 4056. [CrossRef]
48. Wang, F.; Li, X.; Yu, S.; He, S.; Cao, D.; Yao, S.; Fang, H.; Yu, Y. Chemical factors affecting uptake and translocation of six pesticides in soil by maize (*Zea mays* L.). *J. Hazard Mater.* **2021**, *405*, 124269. [CrossRef]
49. Zhang, Y.; Lyu, T.; Zhang, L.; Button, M.; Arias, C.A.; Weber, K.P.; Shi, J.; Chen, Z.; Brix, H.; Carvalho, P.N. Microbial community metabolic profiles in saturated constructed wetlands treating iohexol and ibuprofen. *Sci Total Environ.* **2019**, *651*, 1926–1934. [CrossRef]
50. Vallée, R.; Dousset, S.; Billet, D.; Benoit, M. Sorption of selected pesticides on soils, sediment and straw from a constructed agricultural drainage ditch or pond. *Environ. Sci. Pollut. Res.* **2014**, *21*, 4895–4905. [CrossRef]
51. Huang, H.; Xiao, X.; Yan, B.; Yang, L. Ammonium removal from aqueous solutions by using natural Chinese (Chende) zeolite as adsorbent. *J. Hazard Mater.* **2010**, *175*, 247–252. [CrossRef]

52. de Matos, M.P.; von Sperling, M.; de Matos, A.T. Clogging in horizontal subsurface flow constructed wetlands: Influencing factors, research methods and remediation techniques. *Rev. Environ. Sci. Biotechnol.* **2018**, *17*, 87–107. [CrossRef]
53. Vischetti, C.; Monaci, E.; Casucci, C.; De Bernardi, A.; Cardinali, A. Adsorption and degradation of three pesticides in a vineyard soil and in an organic biomix. *Environments* **2020**, *7*, 113. [CrossRef]