Unsaturated Vertical Flow Constructed Wetland for Chlorothalonil Remediation with Target Application in Ethiopian Floriculture Industry

Stan Wehbe 1,*, Feleke Zewge 2, Yoshihiko Inagaki 3, Wolfram Sievert 4, Tirumala Uday Kumar Nutakki 5 and Akshay Deshpande 6

1 African Center of Excellence for Water Management, Water Science & Technology, Addis Ababa P.O. Box 1176, Ethiopia
2 Department of Chemistry, Faculty of Science, Addis Ababa University, Addis Ababa P.O. Box 1176, Ethiopia; zewge@chem.aau.edu.et
3 Department of Civil and Environmental Engineering, Waseda University, 3-4-1 Okubo, Shinjuku, Tokyo 169-8555, Japan; w-inagaki@ruri.waseda.jp
4 Sievert Consult, Schulstraße 15, 32657 Lemgo, Germany; wolfram@sievertconsult.de
5 RAK Research and Innovation Centre, American University of Ras Al Khaimah (AURAK), Ras Al Khaimah P.O. Box 31208, United Arab Emirates; uday.kumar@aurak.ac.ae
6 Department of Research, Reed Bed Wastewater Treatment, Dubai, United Arab Emirates; research@reedbed.ae
* Correspondence: stanislas.vincent@aau.edu.et or stanwehbe@gmail.com

Abstract: The Ethiopian floriculture industry produces large amounts of wastewater, which requires treatment systems with lower retention times, higher hydraulic flow, and higher hydraulic loading rates (HLRs). Unsaturated vertical flow constructed wetlands (UVF-CWs), which represent these characteristics, have not been studied in depth for chlorothalonil remediation from wastewater. In this study, six UVF-CWs and nine pumping stations were organized into three experimental groups as three independent two-stage CW systems. The influent was pumped into the first vertical-flow stage, after which the effluent was collected and pumped into the second vertical-flow stage. The stage A and stage B effluents were tested for chlorothalonil removal at three different HLR of 50, 200, and 400 L d⁻¹ m⁻² and two influent chlorothalonil concentrations of 100 and 500 µg L⁻¹. The chlorothalonil levels for the stage A effluent at an HLR of 50 L d⁻¹ m⁻² for both influent chlorothalonil concentrations were below the detection limit of 0.08 µg L⁻¹. A maximum chlorothalonil concentration of 7.9 and 196 µg L⁻¹ was observed in the stage A effluent for influent concentrations of 100 and 500 µg L⁻¹, respectively. The chlorothalonil levels for the stage B effluent were all below the detection limit of 0.08 µg L⁻¹. A final chlorothalonil removal efficiency of at least 99.9% was observed for both influent chlorothalonil concentrations at the three HLRs used. These results demonstrated that UVF-CWs represent a viable solution for chlorothalonil remediation in the Ethiopian floriculture industry.

Keywords: chlorothalonil removal; floriculture industry; pesticide wastewater; pilot-scale wetland; unsaturated vertical-flow constructed wetland

1. Introduction

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is one of the most common foliar pesticides used worldwide in agriculture. It is widely used in the United States, China, and Brazil, which are among the top pesticide-consuming countries in the world [1]. Chlorothalonil is mainly used as a fungicide and mildewcide, and is found in a pesticide product specifically registered as a fungicide with the Ministry of Agriculture of Ethiopia for application in the floriculture industry [2]. Since floriculture farms are preferably situated near water bodies, residual chlorothalonil from these farms can easily enter the water bodies. The half-life of chlorothalonil in water is approximately 8 days, whereas...
that of terrestrial field dissipation is 69 days [3]. Thus, this pollutant can accumulate over
time, causing severe damage to aquatic life and increasing health risks to humans and the
environment [1,4]. A report published by French health officials in April 2023 revealed that
most drinking water samples tested by the government contained chlorothalonil, although
the use of chlorothalonil has been banned in the European Union since 2019 [5,6]. A similar
scenario in Ethiopia, where lakes located near floriculture farms are the main drinking
water source, could be a health hazard for local communities.

Some of the technologies previously used for pesticide remediation include advanced
oxidation processes (AOPs), membrane technology, and use of nanomaterials and nanocom-
posites [7]. AOPs have been used to facilitate degradation of organophosphorus pesticides.
A few of the most studied AOPs are photocatalysis, UV/H2O2 photolysis, Fenton, and
electrochemical processes [8,9]. There are four main types of membrane technology that
have been used for pesticide removal—microfiltration, reverse osmosis, ultrafiltration, and
nanofiltration [10]. Although membrane technologies have shown high pesticide removal
efficiencies, the need for membrane washes and periodic replacement of membranes leads
to additional operation and maintenance costs. Pesticide removal with adsorption has
been extensively studied and found to be cost-effective and efficient. Nanomaterials and
nanocomposites are now coming to the forefront for pesticide removal technologies
via adsorption. However, the potential adverse effects of these materials on biomes are a
currently limiting factor for the adoption of these technologies [11]. All these remediation
processes are material-intensive-requiring imports, and in some cases, are not cost-effective
in the context of the Ethiopian floriculture industry.

Another solution that has been used for pesticide remediation from water is con-
structed wetlands (CWs). Studies on wetland ecosystems have shown that wetlands have
 a great capacity for removing contaminants from water [12]. Extensive research has been
conducted over the years to treat industrial, agricultural, and domestic wastewaters us-
ing CWs. One of the main advantages CWs hold over other solutions is their potential
for nutrient removal from wastewater while having minimal operation and maintenance
costs [13–15]. CWs would use local resources and would not need importing of specialized
materials and chemicals, thus reducing the financial stress during construction and operation
stages. The daily operations of a CW system would have less energy expenditure as
compared to a conventional wastewater treatment system and the CW operators can be
trained from local communities. Construction of CWs may require larger area of land as
compared to conventional wastewater treatment plants, however, upon completion they
can be integrated into the landscape of the local area without causing additional noise or
visual pollution. These factors would make CWs a preferable solution for application in
the Ethiopian floriculture industry.

The removal of pesticides from agricultural wastewater has been studied compre-
prehensively, and studies have described the use of CWs to remove chlorothalonil from
wastewater. A previous study has reported high chlorothalonil removal efficiencies using
horizontal subsurface flow CW (HSS-CW) with retention times of up to 3 days [16]. A study
of chlorothalonil wastewater has also been shown to have a presence of chlorothalonil-
degrading microbes [17]. Long retention times are not ideal for large-scale industrial
floriculture farms that deal with high volumes of wastewater daily [18]. Thus, in recent
years, an increasing amount of research has been carried out on the use of unsaturated
CWs in treating pesticides [19,20]. The flow of wastewater through an unsaturated CW
has been reported to increase the dissolved oxygen (DO) in treated wastewater [21]. Be-
cause of the greater amount of oxygen, unsaturated systems boost microbial degradation
of pesticides in wastewater. This is mainly due to the promotion of aerobic processes
in the wetlands [21–23]. Although the previous study has verified the effect of partially
saturated HSS-CWs on chlorothalonil removal from water runoff [16], there has not been
much follow-up investigation to verify the efficacy of other types of CWs for chlorothalonil
removal. There is also a need for developing CW systems with lower retention times,
higher hydraulic flow, and higher hydraulic loading rates for target application in the
Ethiopian floriculture industry. The use of an unsaturated vertical-flow constructed wetland (UVF-CW) could help achieve these desired requirements from the CW system, while maintaining a higher chlorothalonil removal efficiency from the wastewater.

Additionally, cost-economic analyses of UVF-CWs need to be considered. Previous studies have shown economic viability of HSS-CWs in the context of Ethiopia [24]. The major costs involved in setting up CWs are related to civil works, in particular the acquisition of sand and gravel for the substrate [25]. UVF-CWs need less space compared to HSS-CWs to treat the same pollutant load. HSS-CWs have been shown to treat an average COD of up to 16 g d\(^{-1}\) m\(^{-2}\) and handle an average hydraulic load of 40 L d\(^{-1}\) m\(^{-2}\), while UVF-CWs have been shown to treat an average COD of up to 20 g d\(^{-1}\) m\(^{-2}\) and handle an average hydraulic load of 80 L d\(^{-1}\) m\(^{-2}\) [26]. Hence, UVF-CWs could potentially be more economical than HSS-CWs due to reduced civil work costs.

The objective of this study was to test the efficacy of a two-stage UVF-CW for treating wastewater containing chlorothalonil. The effects of two different inflow chlorothalonil concentrations and three different hydraulic loading rates (HLRs) on chlorothalonil removal from influent water were studied. The importance of this study lies in studying a viable type of CW for the floriculture industry that produces a large amount of wastewater daily.

2. Methods and Materials

2.1. Materials

*Phragmites australis* was the wetland plant species used in this study. Stages A and B of the CW were constructed using immediate bulk containers with dimensions of 117 cm \(\times\) 95 cm \(\times\) 100 cm. Details of the construction and layout of the CW can be found in Figure 1 and Supplementary Material S1. The substrate material used for the filter was crushed black-stone gabbro, which was selected based on its availability in both Ethiopia and the UAE. An analysis of metal oxides in the filter material showed high levels of metal oxides (silica oxide: 4415 mg kg\(^{-1}\), aluminium oxide: 2656.3 mg kg\(^{-1}\), calcium oxide: 6492.1 mg kg\(^{-1}\), iron oxides: 105,337.5 mg kg\(^{-1}\), magnesium oxide: 302,031.2 mg kg\(^{-1}\), and sodium oxide: 492 mg kg\(^{-1}\)). Filter materials with three different particle sizes were used: double-washed black sand (grain size: 1–5 mm), small gravel (grain size: 10–15 mm), and large gravel (grain size: 20–25 mm). The Oxynozzle (a low-pressure spray nozzle for oxygenating wastewater) was sourced from the Reed Bed Wastewater Treatment Company, Dubai, UAE, for even distribution of wastewater onto the second stage of the CW [27].

![Figure 1. Layout of the two-stage CW used in this study. A detailed schematic diagram can be found in Supplementary Material S1.](image-url)
2.2. Experimental Setup

The experimental setup used in a previous study was adopted and modified for use in this study [28]. The CW was constructed at the RAK Research and Innovation Center, Ras Al-Khaimah, UAE (latitude: 25.6685406; longitude: 55.7794520). The experiments were conducted from June to November 2022. The average air temperature was 22–40 °C during this period. Owing to the lack of precipitation 1 month before and during the experimental period, precipitation was assumed to have a negligible impact on the experiment. A layout and picture of the CW used in this study are shown in Figures 1 and 2. A two-stage design was used mainly to target maximum pesticide removal with lower retention time in the system. No pollutant was added to the CW setup starting from 6 months prior to the experimentation and the setup was operated continuously at an HLR of 200 L d\(^{-1}\) m\(^{-2}\).

Testing of the substrate and water samples showed no presence of any pollutants previously used in the CW setup. An analysis of the substrate was performed to check for the presence of metal oxides (Method APHA AWWA 3120 B for testing of aluminium, calcium, iron, magnesium, and sodium oxides; Silicomolybdate Method for testing of silica oxide).

As shown in Figure S2.1, the floricultural farms in Ethiopia use an effective chlorothalonil dose of 1 g L\(^{-1}\) to spray the plants while using 400 to 1500 L of pesticide spray per hectare of land. Additionally, the terrestrial field dissipation half-life of chlorothalonil is approximately 69 days [3]. Over time, an accumulation of chlorothalonil in the soil, leading to increasing chlorothalonil concentration in the water runoff, is to be expected. In consideration of such a scenario, a range of influent chlorothalonil concentrations were calculated for use in the experimentation. Three treatment groups were established: one for the control (without any pollutants), and two for testing different influent concentrations of chlorothalonil. The three influent chlorothalonil concentrations tested were 100 and 500 µg L\(^{-1}\) (at three different HLRs of 50, 200, and 400 L d\(^{-1}\) m\(^{-2}\)) and then 0.5 g L\(^{-1}\) (at an HLR of 50 L d\(^{-1}\) m\(^{-2}\)).

The plants present in the CW, as part of the previous studies, were reused [28]; however, they were trimmed and allowed to regrow 3 months before the experiment. Treated sewage effluent water from a sewage treatment plant was introduced into the CWs to help with plant growth and introduce microbial populations before experimentation. During the experiment, the influent was loaded onto stage A via pipes. Subsequently, the effluent from stage A was collected and sprayed onto stage B of the CW. The volume of the final effluent collected after each batch of influent used was recorded. Timer-controlled pumps were used to control the HLR in stage A. It was ensured that the HLR for stage B was matched with that of stage A.

The influent was prepared in 500 L batches. Concentrated chlorothalonil solutions were prepared in a laboratory by dissolving chlorothalonil (TCI tetrachloroisophthalonitrile; CAS: 1897-45-6; Purity: >98%) in 200 mL methanol, which were then added to 500 L of
fresh water in the influent tank. Absolute methanol was used since chlorothalonil has a low solubility (0.81 mgL⁻¹) at 25 °C in water [29], and to serve as an additional carbon source in the influent water. Water was stirred to homogeneously mix the chlorothalonil in the tank. The average flow capacity of the pumps was 3.5 L s⁻¹. Based on this, the activation times of the influent pumps were calculated to be 3 s (HLR: 50 L d⁻¹ m⁻²), 10 s (HLR: 200 L d⁻¹ m⁻²), and 19 s (HLR: 400 L d⁻¹ m⁻²) every 4 h.

Taps at the bottom of stage A and B tanks were used to sample the effluent wastewater. The influent samples were collected directly from the influent tanks. One litre each of influent and effluent wastewater samples was used. The volume of effluent collected after the completion of each influent batch was recorded to account for water loss due to evapotranspiration. The influent was sampled immediately before it was pumped into stage A, whereas the effluent was sampled immediately after the pumped wastewater passed through the CW. All wastewater samples were collected in triplicate and stored in a refrigerator at 4 °C before processing and calculating the chlorothalonil removal efficiency within 48 h of sampling. The effluent samples for the chlorothalonil analysis were collected after a system stabilization period of 2 weeks.

Furthermore, pH, temperature, DO, and electrical conductivity (EC) of the wastewater samples were measured immediately after sampling. Samples were collected every 4 days. Additionally, water samples were collected to measure the biochemical oxygen demand (BOD, measured with Method APHA AWWA 5210 B), chemical oxygen demand (COD, measured with Method APHA AWWA 5220 D), total organic carbon (TOC, measured with Method APHA AWWA 5310B), nitrate concentration (measured with the cadmium reduction method), and phosphate concentration (measured with the ascorbic acid method) in the control and the 500 µgL⁻¹ experimental group. Plant stems and roots were harvested from the CWs. Substrate samples were collected directly from stage A of the CW.

2.3. Analytical Methods

2.3.1. Chlorothalonil Concentration Analysis

Method 8081 described by the US Environment Protection Agency (EPA) was used for the extraction and estimation of chlorothalonil concentration from water samples [30]. A gas-chromatography–mass-spectrometry (GC-MS) analysis of the processed samples and standardised solutions was performed to calculate the chlorothalonil concentration of the samples.

Plant and substrate samples were processed using Method 8081 B as described by the US EPA [30], followed by a GC-MS analysis to calculate the chlorothalonil concentrations present in the samples. The GC-MS instrument used was a Shizmadu GCMS-QP2010 SE (Shimadzu (Suzhou) Instruments Manufacturing Co., Ltd., Suzhou New District, China), and the standard dilutions used to generate the calibration curve were 0.1, 1, 10, 100, and 1000 µg L⁻¹. The limit of detection and limit of quantification of the instrument for the chlorothalonil analysis were 0.08 µg L⁻¹ and 0.1 µg L⁻¹, respectively. To ensure accurate results, the samples were spiked with known chlorothalonil concentrations, which was followed by a GC-MS analysis. The results of both spiked and non-spiked samples were used to calculate the recovery rates. The calculated recovery rates for water, substrate, and plant samples were above 85%.

2.3.2. Comparison of Water Parameters with Different Water Saturation Conditions

The water parameters of pH, DO, EC, and temperature were observed under unsaturated and saturated conditions, and comparisons were made. The differences between the effluent and influent values of all the observed samples for each parameter were used to derive values of ΔpH, ΔDO, ΔEC, and ΔTemperature under unsaturated and saturated conditions.

These values were used to conduct a two-factor repeated ANOVA for the parameters of ΔpH, ΔDO, ΔEC, and ΔTemperature (ΔT). The factors taken under consideration were the saturation and concentration of the pesticide used.
2.3.3. Removal Mechanisms for Chlorothalonil

Among the many possible pesticide removal mechanisms in CWs [31,32], three were selected in this study: substrate sorption, plant uptake, and degradation.

The substrate sample was collected directly from stage A of experimental Group 3, which was dosed with 500 µg L\(^{-1}\) chlorothalonil. For the plant samples, plant cuttings and roots were harvested from experimental Group 3. The control samples were collected from experimental Group 1, which was not dosed with chlorothalonil.

To check for chlorothalonil degradation, chromatograms obtained from the effluent water and substrate samples were checked for any distinct peaks, in addition to the peaks corresponding to chlorothalonil. The peaks were then analysed using their mass-to-charge ratios to identify the known chlorothalonil degradation products.

2.4. Calculation Methods

The time required for water to drain completely from the two CWs, after a cycle of pump activation, was recorded for each HLR. This time was considered as the HRT, \(T_r\), of the system.

Evapotranspiration was calculated as follows:

\[
\Delta V = (Qt - V_{out}) / Qt
\]

where \(\Delta V\) is the water loss with evapotranspiration, expressed as a fraction of the total volume of influent wastewater pumped into the CW, \(Q\) is the influent flow rate in L d\(^{-1}\), \(t\) is the time in days, and \(V_{out}\) is the total effluent volume recorded [21].

This value was used to correct the calculated values of the chlorothalonil removal efficiency from wastewater. The removal efficiency was calculated as follows:

\[
\text{Removal efficiency (\%)} = \frac{(C_{in} - (1 - \Delta V) \times C_{out})}{C_{in} \times 100}
\]

where \(C_{in}\) and \(C_{out}\) are the influent and effluent concentrations (in µg L\(^{-1}\)), respectively [21].

2.5. Data Analysis

Data plots and fitting curves were generated using Veusz version 3.4.0.1 [33]. A two-way repeated ANOVA was performed using Microsoft Excel for Mac version 16.76 [34].

3. Results and Discussion

3.1. Chlorothalonil Removal

The chlorothalonil concentrations were measured, and the removal efficiencies were calculated using Equation (2). The chlorothalonil concentrations in the influent and the effluent samples of the control group (experimental Group 1) were below the detection limit. In some cases, the effluent chlorothalonil concentrations observed were below the detection limit of the instrument. For these cases, the effluent concentrations were assumed to be 0.08 µg L\(^{-1}\), which is the minimum detection limit of the GC-MS instrument and the method used. Based on this and using Equation (2), chlorothalonil removal efficiencies were calculated. The chlorothalonil levels observed for influent chlorothalonil concentrations of 100 and 500 µg L\(^{-1}\) are as shown in Figure 3 while a complete set of chlorothalonil removal data can be found in Table S2.1 and Supplementary Material S3.

The average chlorothalonil removal efficiency observed for the influent concentration of 100 and 500 µg L\(^{-1}\) was more than 99.9% across all three HLRs used. The average chlorothalonil removal efficiency for an influent chlorothalonil concentration of 0.5 g L\(^{-1}\) at an HLR of 50 L d\(^{-1}\) m\(^{-2}\) was 99.9%. It was observed that both stage A and B were needed for the complete removal of chlorothalonil from the wastewater at higher HLRs of 200 and 400 L d\(^{-1}\) m\(^{-2}\), whereas a treatment through only stage A was sufficient for treating the wastewater at a lower HLR of 50 L d\(^{-1}\) m\(^{-2}\).
The main operational conditions and their corresponding chlorothalonil removal efficiencies from the present and previous research works were compared, as shown in Table 1. At similar influent concentrations, chlorothalonil was removed completely in a shorter time period with the UVF-CW as compared to HSS-CW, indicating a superior performance.

Table 1. Comparison of results observed in literature and in the present study for type of CW used, saturation of CW, influent chlorothalonil concentrations, chlorothalonil removal efficiency, and hydraulic retention time (HRT).

<table>
<thead>
<tr>
<th>Scale of Study</th>
<th>Type of CW</th>
<th>Saturation</th>
<th>Influent Load</th>
<th>Chlorothalonil Removal Efficiency</th>
<th>HRT (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot</td>
<td>UVF-CW</td>
<td>Unsaturated</td>
<td>100 µg L⁻¹</td>
<td>&gt;99.9%</td>
<td>2</td>
<td>Present study</td>
</tr>
<tr>
<td>Pilot</td>
<td>UVF-CW</td>
<td>Unsaturated</td>
<td>500 µg L⁻¹</td>
<td>&gt;99.9%</td>
<td>2</td>
<td>Present study</td>
</tr>
<tr>
<td>Pilot</td>
<td>HSS-CW</td>
<td>Partially saturated</td>
<td>148 µg L⁻¹</td>
<td>94%</td>
<td>24</td>
<td>[16]</td>
</tr>
<tr>
<td>Pilot</td>
<td>HSS-CW</td>
<td>Partially saturated</td>
<td>326 µg L⁻¹</td>
<td>&gt;99.9%</td>
<td>30</td>
<td>[16]</td>
</tr>
<tr>
<td>Pilot</td>
<td>HSS-CW</td>
<td>Partially saturated</td>
<td>296 µg L⁻¹</td>
<td>&gt;99.9%</td>
<td>24</td>
<td>[16]</td>
</tr>
<tr>
<td>Field</td>
<td>HSS-CW</td>
<td>Partially saturated</td>
<td>11.8 g</td>
<td>78%</td>
<td>66.5</td>
<td>[35,36]</td>
</tr>
</tbody>
</table>

3.2. Water Parameters

pH, DO, EC, and water temperature were monitored to study the effect of CW treatment on water quality of the collected effluent (Table 2). The average pH of the influent and effluent was 7.56–7.68 and 8.26–8.35, respectively, across all three experimental groups. Moreover, the average DO percentage saturation of the influent and effluent was 21.4–21.9% and 55.6–55.9%, respectively, while the average electrical conductivity of the influent and effluent was 417–427 and 544–562 μS cm⁻¹, respectively. The average temperatures of all influent and effluent samples ranged between 33.5 and 35.2 °C. Notably, although the temperatures did not change drastically, an increase of ~1 °C in the effluent samples across all experimental groups was observed as compared to the influent samples.
Table 2. Data of the water parameters tested immediately after sampling from the three experimental groups. The parameters tested were pH, dissolved oxygen (DO, measured as percentage saturation), electrical conductivity (EC, measured in $\mu$S cm$^{-1}$), and temperature (measured in ºC). The data were collected at an HLR of 200 L d$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>DO (%)</th>
<th>EC ($\mu$S cm$^{-1}$)</th>
<th>Water Temperature (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>7.57 ± 0.13</td>
<td>21.4 ± 2.2</td>
<td>427 ± 14</td>
<td>34.5 ± 1.7</td>
</tr>
<tr>
<td>Effluent</td>
<td>8.26 ± 0.11</td>
<td>55.9 ± 3.2</td>
<td>544 ± 20</td>
<td>35.2 ± 1.8</td>
</tr>
<tr>
<td><strong>100 µg L$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>7.56 ± 0.16</td>
<td>21.9 ± 2.2</td>
<td>421 ± 9</td>
<td>33.5 ± 1.8</td>
</tr>
<tr>
<td>Effluent</td>
<td>8.35 ± 0.1</td>
<td>54.6 ± 3.5</td>
<td>562 ± 34</td>
<td>34.5 ± 1.8</td>
</tr>
<tr>
<td><strong>500 µg L$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>7.68 ± 0.13</td>
<td>21.6 ± 2.0</td>
<td>417 ± 10</td>
<td>33.7 ± 2.1</td>
</tr>
<tr>
<td>Effluent</td>
<td>8.27 ± 0.16</td>
<td>55.4 ± 2.4</td>
<td>559 ± 27</td>
<td>34.6 ± 2.1</td>
</tr>
</tbody>
</table>

The results of the BOD, COD, TOC, nitrate concentration, and phosphate concentration for the control and 500 µg L$^{-1}$ experimental groups are shown in Table 3. The COD levels for the influent sample from the 500 µg L$^{-1}$ chlorothalonil experimental group were high (1394 mgL$^{-1}$). This could be due to the addition of methanol to dissolve chlorothalonil. However, the COD of the effluent of the chlorothalonil-dosed sample was 12 mgL$^{-1}$, indicating a drastic decrease. This corresponded to a similar decrease in the TOC of the effluent sample compared to that of the influent sample for the chlorothalonil-dosed experimental group.

Table 3. Data for nitrate concentration, phosphate concentration, BOD, COD, and TOC for the control sample and sample from the 500 µg L$^{-1}$ experimental group.

<table>
<thead>
<tr>
<th></th>
<th>Nitrate Concentration (mgL$^{-1}$)</th>
<th>Phosphate Concentration (mgL$^{-1}$)</th>
<th>BOD (mgL$^{-1}$)</th>
<th>COD (mgL$^{-1}$)</th>
<th>TOC (mgL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>0.05</td>
<td>0.34</td>
<td>&lt;12</td>
<td>12</td>
<td>0.62</td>
</tr>
<tr>
<td>Effluent</td>
<td>0.05</td>
<td>&lt;0.02</td>
<td>&lt;12</td>
<td>&lt;10</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>500 µg L$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>0.05</td>
<td>0.25</td>
<td>&lt;12</td>
<td>1394</td>
<td>276</td>
</tr>
<tr>
<td>Effluent</td>
<td>0.06</td>
<td>&lt;0.02</td>
<td>&lt;12</td>
<td>12</td>
<td>5.46</td>
</tr>
</tbody>
</table>

The values of $\Delta$pH, $\Delta$DO, $\Delta$EC, and $\Delta$T were calculated for the data summarized in Table 2. Similarly, data from a previous study under saturated conditions in a two-stage vertical-flow CW were used to calculate $\Delta$pH, $\Delta$DO, $\Delta$EC, and $\Delta$T values under saturated conditions [28]. A summary of the calculated values is shown in Table S2.2. A two-factor repeated ANOVA was carried out with a significance level of 95% for $\Delta$pH, $\Delta$DO, $\Delta$EC, and $\Delta$T. The two independent variables considered were influent concentration and saturation of the UVF-CW. The results of this analysis are as shown in Table 4. The $p$-values observed for the factor of the saturation condition indicate that parameters of $\Delta$pH, $\Delta$DO, and $\Delta$EC were affected by the saturation condition of the UVF-CW.

In addition to this, an interactive effect between influent concentration and the saturation condition was also observed in the case of $\Delta$pH. To verify this finding, the data were examined to look for any trends present. It was noted that there were very minor differences in the $\Delta$pH values observed for the three experimental groups but no steady trends were noticeable. To verify the presence of any interactive effect of influent chlorothalonil concentration and the saturation condition on $\Delta$pH of the CW system, collection of a larger dataset over a long period of time is necessary.
Table 4. Two-factor repeated ANOVA for the parameters of \( \Delta \text{pH} \), \( \Delta \text{DO} \), \( \Delta \text{EC} \), and \( \Delta \text{T} \). The two independent variables used for the analysis are \( \text{C}_\text{in} \) (influent chlorothalonil concentration) and saturation condition.

<table>
<thead>
<tr>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-Value</th>
<th>F Crit</th>
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<td>( \Delta \text{pH} )</td>
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<tr>
<td>Sample</td>
<td>0.12</td>
<td>2</td>
<td>0.059</td>
<td>1.340</td>
<td>0.271</td>
<td>3.168</td>
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<td>20.312</td>
<td>464.945</td>
<td>0.000</td>
<td>4.020</td>
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<td>4.069</td>
<td>0.023</td>
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<td>( \Delta \text{EC} )</td>
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<td>2</td>
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<td>1056.707</td>
<td>0.000</td>
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<td>( \text{C}_\text{in} \times \text{Saturation} )</td>
<td>1647.10</td>
<td>2</td>
<td>823.550</td>
<td>0.933</td>
<td>0.400</td>
<td>3.168</td>
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<tr>
<td>Within</td>
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<td>54</td>
<td>882.937</td>
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<td>Total</td>
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<td>( \Delta \text{T} )</td>
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<td>Sample</td>
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<td>3.497</td>
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<td>( \text{C}_\text{in} \times \text{Saturation} )</td>
<td>2.80</td>
<td>2</td>
<td>1.400</td>
<td>1.306</td>
<td>0.279</td>
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<td>Within</td>
<td>57.90</td>
<td>54</td>
<td>1.072</td>
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<td>Total</td>
<td>70.18</td>
<td>59</td>
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</table>

The maximum effluent DO saturation observed under saturated conditions was 34% whereas the maximum effluent DO saturation observed under unsaturated conditions was 55.9%. The results from the two-factor repeated ANOVA for \( \Delta \text{DO} \) also confirm that there was a statistically significant difference \((p < 0.05)\) observed between saturated and unsaturated conditions. The data analysis confirms well that unsaturated conditions better promote the increase in DO in the effluent water as compared to saturated conditions.

The two-factor ANOVA for \( \Delta \text{pH} \) indicated a statistically significant difference between saturated and unsaturated conditions as well as an interactive effect between influent pesticide concentration and the saturation condition. The \( \Delta \text{pH} \) observed under unsaturated conditions was less than that observed under saturated conditions. This difference seems to be correlated with an increase in \( \Delta \text{DO} \) under unsaturated conditions compared with saturated conditions. When compared to present literature, such an occurrence could be attributed to increased nitrification due to an increase in DO under unsaturated conditions, as nitrification is an oxygen-dependent and alkalinity-consuming process \([37,38]\).

The maximum permissible pH value stated by the EPA, Ethiopia, and the US EPA for reuse of treated wastewater for irrigation purposes and release into water bodies is between 6 and 9 \([39,40]\). Hence, the collected effluent wastewater satisfied the standardised pH requirements for irrigation purposes as well as for release into adjacent water bodies under unsaturated conditions. Nonetheless, there is still a need to further verify the water quality parameters under field conditions.
3.3. Chlorothalonil Removal Mechanisms

Experimental Group 3 was selected to study the chlorothalonil removal mechanisms because it was exposed to five times more chlorothalonil than experimental Group 2. The total amount of chlorothalonil added to experimental Group 3 before testing the substrate sorption and plant uptake was calculated to be 5.016 g. Figure S2.2 shows the log of the total amount of chlorothalonil added to experimental group 3 before and during the experimental phase. Testing of the plant samples indicated the presence of chlorothalonil. The chlorothalonil concentration was 696 µgKg⁻¹. Moreover, the total amount of biomass harvested from experimental Group 3 was 6.12 kg, which indicated a total removal of 0.085% via plant uptake.

The control samples collected for the substrate and plants from experimental Group 1 indicated chlorothalonil levels below the detection limit. Testing of the substrate sample from experimental group 3 indicated the presence of 39.46 mg kg⁻¹ of chlorothalonil. The maximum amount of wastewater retention as it passes through the CW occurred in the double-washed black sand layer of stage A (40 cm) and B (60 cm). Hence, it is reasonable to assume that the maximum adsorption occurred in this layer of the CWs. The estimated amount of substrate in the top layer of stage A was 756 kg, whereas that in stage B was 1134 kg, amounting to a total of 1890 kg. This implied that the substrate alone could remove ~74.6 g of chlorothalonil from wastewater until substrate saturation. The details of the calculations for the two removal mechanisms are presented in Table 5.

Table 5. Calculations for the two removal mechanisms of plant uptake and substrate sorption. The calculations are based on data collected from experimental Group 3, which was treated with 500 µg L⁻¹ of chlorothalonil.

<table>
<thead>
<tr>
<th>Plant Uptake</th>
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<tbody>
<tr>
<td>Total amount of chlorothalonil added to</td>
<td>5016</td>
</tr>
<tr>
<td>experimental Group 3 (mg)</td>
<td></td>
</tr>
<tr>
<td>Amount of biomass harvested (kg)</td>
<td>6.12</td>
</tr>
<tr>
<td>Amount of chlorothalonil present in plant</td>
<td>0.696</td>
</tr>
<tr>
<td>sample (mg kg⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Percentage removal of chlorothalonil by</td>
<td>0.085</td>
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<tr>
<td>plant uptake (%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate Sorption</th>
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<tbody>
<tr>
<td>Total amount of chlorothalonil added to</td>
<td>5016</td>
</tr>
<tr>
<td>experimental Group 3 (mg)</td>
<td></td>
</tr>
<tr>
<td>Estimated amount of substrate present in</td>
<td>1890</td>
</tr>
<tr>
<td>top-layer stage A and stage B (kg)</td>
<td></td>
</tr>
<tr>
<td>Amount of chlorothalonil present in</td>
<td>39.46</td>
</tr>
<tr>
<td>substrate sample (mg kg⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Theoretical removal capacity of</td>
<td>74,579.4</td>
</tr>
<tr>
<td>chlorothalonil using the substrate (mg)</td>
<td></td>
</tr>
</tbody>
</table>

The chromatograms obtained from the effluent water and substrate samples were checked for any other distinct peaks in addition to the peaks corresponding to that of chlorothalonil. No distinct peaks were observed during a further analysis and quantification.

The substrate material used and its geometrical size played an important role in the removal of chlorothalonil from the UVF-CW. The double-washed black sand (size: 1–5 mm) accounts for approximately 62.5% of the total substrate material used in the UVF-CW. The smaller grain size equates to a larger surface area available per unit volume of substrate, for adsorption of chlorothalonil. Additionally, the analysis of the substrate material for metal oxides revealed high levels of various metal oxides present, which have shown high adsorption efficiency for organic pollutants [41]. The use of a vertical flow structure in an unsaturated CW helped to intensify the process of substrate adsorption as it passes through the CW as compared to horizontal flow in a partially saturated CW [42].

Pollutant removal in a CW is a dynamic process with a complex network of interdependent factors and removal pathways affecting the pollutant removal dynamics [13,32]. Although plant uptake was responsible for only 0.085% of chlorothalonil removal, this did not include the amount of chlorothalonil possibly metabolized using plants. This warrants
Further investigation. As illustrated in Supplementary Material S3, the evapotranspiration (ΔV) observed for all the recorded runs was less than 0.07 L. This implies a limited intake of water using the plants, which reflected in the limited chlorothalonil removal via plant uptake. Over time, as the density of plant roots increases, the uptake of chlorothalonil using plants could increase. A higher density of plant roots would also create waterflow channels in the substrate bed, helping to prevent clogging. These waterflow channels would also create thriving conditions for microbiota, which could help in degradation of the chlorothalonil adsorbed using the substrate bed [43,44].

Based on historical data, the average monthly temperatures in Ethiopia range from 14 to 32 °C [45]. As stated earlier, the temperature range observed in this study was 23–40 °C. Temperature plays an important role in the pesticide removal dynamics in CW. The conditions observed in this study were consistent with some of the average temperatures logged historically in Ethiopia; however, lower temperature ranges could impact the treatment of chlorothalonil using a CW [21].

Similarly, there is a possibility of saturation of the substrate with chlorothalonil over time, which could potentially reduce the chlorothalonil removal efficiency of the CW. Although adsorption is a reversible process, chlorothalonil degradation along with chlorothalonil removal should be aimed for in a CW. Chlorothalonil can be degraded through physical, chemical, and microbial methods [32]. Although no chlorothalonil degradation products were observed in the samples collected in this study, a more comprehensive examination is required. Microbial degradation is one of the main pathways for pesticide degradation in CWs [20,46]. Further in vitro studies could help isolate and identify any possible microbial colonies present in a CW that could assist in chlorothalonil degradation.

4. Conclusions

The two-stage UVF-CW system was effective for chlorothalonil removal from wastewater. An almost complete chlorothalonil removal was observed at all influent chlorothalonil concentrations across the three HLRs used, thus indicating its potential for future application in the Ethiopian floriculture industry.

The design selection of a two-stage UVF-CW was shown to have comparable chlorothalonil removal efficiencies with retention times of 2 h compared with the previously described saturated or partially saturated horizontal subsurface flow CW systems with retention times of up to 72 h.

In this pilot-scale study, the use of unsaturated conditions allowed for a significant increase in the DO observed in the effluent wastewater. In the case of future field application, the increased DO saturation in the effluent wastewater would be beneficial towards promoting nutrient removal from floriculture runoff. This can aid in meeting the quality standards described by the EPA, Ethiopia, for the reuse of wastewater or its release back into water bodies.

In order to further study the chlorothalonil removal dynamics of a two-stage UVF-CW system, development of a simulation model for one-dimensional vertical flow of pesticide-containing wastewater through a CW would be recommended. The solute transport model would help illustrate the fate of chlorothalonil as it passes through the different-sized substrate layers, and to study the expected performance of the UVF-CW over longer periods of time.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15183282/s1. There are a total of four files submitted online as part of supplementary materials. Supplementary Material S1: This image shows a schematic of the UVF-CW setup. Supplementary Material S2: This file contains all the supplementary figures and tables. Figure S2.1: Product label from Folio Gold pesticide mix registered for use in floriculture farms in Ethiopia. This product has a chlorothalonil concentration of 500 g L\(^{-1}\) that, upon dilution as per instructions, results in a final chlorothalonil concentration of approximately 1 g L\(^{-1}\). Figure S2.2: Cumulative amount of chlorothalonil added to experimental Group 3 while simulating high precipitation conditions. Each data point represents an addition of 264 mg of chlorothalonil added to 500 L.
of influent wastewater. The labels of each data point indicate the cumulative amount of chlorothalonil added over the elapsed time. As can be seen from the last data point, a total of 5016 mg was added to experimental Group 3 while simulating high precipitation conditions. Table S2.1: Chlorothalonil removal data observed for two influent concentrations used at three different HLRs. The removal efficiency was calculated on the basis of influent and stage B chlorothalonil levels using Equation (2). Table S2.2: Calculated values for ΔpH, ΔDO, ΔEC, and ΔTemperature (ΔT) observed under unsaturated and saturated conditions. Supplementary Material S3: This file is an Excel spreadsheet containing chlorothalonil removal data and calculations. Supplementary Material S4: This file is an Excel spreadsheet containing water parameter data and calculations of ΔpH, ΔDO, ΔEC, and ΔT. It also contains the two-factor repeated ANOVA for ΔpH, ΔDO, ΔEC, and ΔT.

Author Contributions: Conceptualization, S.W.; Methodology, S.W. and A.D.; Software, A.D.; Validation, F.Z. and Y.I.; Formal Analysis, S.W. and A.D.; Investigation, S.W. and A.D.; Resources, S.W. and T.U.K.N.; Data Curation, A.D. and S.W.; Writing—Original Draft Preparation, S.W.; Writing—Review and Editing, F.Z., Y.I., T.U.K.N., W.S. and A.D.; Visualization, S.W. and A.D.; Supervision, F.Z. and Y.I.; Project Administration, S.W.; Funding Acquisition, S.W. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in Supplementary Materials provided alongside.

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References


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