

## Performance of carbendazim removal using constructed wetlands for the Ethiopian floriculture industry

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### ABSTRACT

Carbendazim is a pesticide commonly used in Ethiopian flower farms and has harmful effects on aquatic, invertebrate, and mammalian life. Previous studies have explored ways to remedy carbendazim toxicity; however, the use of constructed wetland (CW) systems for carbendazim removal from farm water runoff has not been explored in depth. The primary aim of this study was to investigate the efficacy of a CW system for carbendazim removal from wastewater runoff. A two-stage pilot CW was built and tested for its efficacy of carbendazim removal under saturated conditions and varying hydraulic loading rates. The influent was pumped into the first vertical-flow mesocosm. The drained water was then pumped into the second mesocosm. The collected effluent was tested for carbendazim removal. Carbendazim removal efficiencies up to 91.80% (with a hydraulic loading rate of 100 Ld<sup>-1</sup> and influent carbendazim concentration of 10 µg L<sup>-1</sup>) were observed. Statistical analysis indicated that the removal of carbendazim was not correlated with the initial carbendazim concentration but was negatively correlated with the hydraulic loading rate used. Two pesticide removal mechanisms were briefly probed to determine their participation in carbendazim removal. Substrate sorption accounted for 18% of total carbendazim removal; furthermore, plant uptake also played an active role.

**Key words:** carbendazim, constructed wetlands, floriculture, vertical flow constructed wetland

### HIGHLIGHTS

- Novel two-stage pilot-scale constructed wetland was built to reflect industry standard configuration of constructed wetlands.
- Tests under saturated conditions showed up to 91.80% carbendazim removal from the influent water.
- Carbendazim removal efficiency was found to be positively correlated with a higher hydraulic retention time but to be independent of the influent carbendazim concentration.

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## GRAPHICAL ABSTRACT

## Carbendazim Remediation using Constructed Wetlands



## INTRODUCTION

In fast-growing economies, the sufficient and permanent supply of resources is a great challenge for governmental entities, and treatment of polluted resources and protection of natural water bodies present an even greater issue. In the field of wastewater treatment, polluted water requires additional energy for treatment. Planning of the required volumes and quality of wastewater is complicated (especially in industrial areas) and subject to changes in short periods. In contrast to the supply of water to the economy, which is essential for its operation, the treatment of polluted resources has no direct benefit for the consumers and only adds additional cost. Thus, treatment of polluted resources reduces the profitability of production. Lower environmental standards give economies a monetary advantage on the global market. The negative effects of such a situation can take a long time to manifest as severe problems for humans and the environment.

The floriculture industry is booming in Ethiopia and was responsible for exports worth USD 188.5 million during 2019. Flowers are luxurious products with high social and economic value, and Ethiopia is ranked second in Africa and sixth amongst the top exporters of flowers worldwide (EHPEA 2019). Increasing demand drives a growing dependence on pesticides in large-scale flower farms to increase the rate of production. The flower farms are generally situated next to water bodies (EHPEA 2018), and the residual pesticides find their way into these water bodies through runoffs from these farms. In recent years, several initiatives including constructed wetlands (CWs) have been brought forward to combat this situation. Nevertheless, improved studies on the effective treatment of pesticide effluents are needed for larger-scale design and implementation.

One of the commonly used pesticides in Ethiopian flower farms is carbendazim. It is found in two registered and commercially available products, one of which is specifically used in flower farms (Plant Regulatory Directorate 2018). Residual carbendazim is shown to have a half-life of anywhere between 3 days to 12 months based on the local environment. This is ample time for it to enter the ecosystem and accumulate. Eventually, it will cross the threshold concentrations and start affecting the surrounding flora and fauna. Moreover, carbendazim has been classified as a possible human carcinogen and is a known endocrine-disrupting chemical. It is also known to manifest embryotoxicity, infertility, and developmental toxicity in mammals (Singh *et al.* 2016). For these reasons, the use of carbendazim is already banned in the European Union (Commission Implementing Regulation (EU) No 542/2011, 2011). However, this compound has been recently detected in surface water (at a sub-micro  $\text{gL}^{-1}$  level) in Spain (Calvo *et al.* 2021).

Removal of pollutants such as carbendazim from water sources is a great challenge for developing countries. Construction and operation of conventional water treatment plants designed specifically for treating these pollutants is a less desirable solution as they are costly, sometimes require high energy consumption, and need imports of specialised materials. CWs mimic the functions of natural wetlands and have been engineered to treat raw sewage and agricultural and industrial effluents (Vymazal 2014; Vymazal & Březinová 2015). They also do not have high energy requirements and have low operational costs. Awareness and implementation of CWs has been increasing worldwide over the years and they have been introduced in many large projects such as the World Expo 2020 in Dubai, United Arab Emirates.

The use of CWs for the treatment of pesticide effluents has been studied extensively over the years. While their use in the context of pesticide removal was first studied in the 1970s, it is only in recent years that CWs have gained widespread use for

pesticide removal from agricultural runoffs (Vymazal & Březinová 2015). A few pesticides which have been effectively treated using CWs include chlorpyrifos, chlorothalonil, imazalil, and tebuconazole (Sherrard *et al.* 2004; Lv *et al.* 2016; Tang *et al.* 2019). However, the treatment of carbendazim using CWs has not been specifically studied in depth compared to the examples stated previously (Kang *et al.* 2020; McCalla *et al.* 2022). Thus, there is a need for further investigation to elucidate whether CWs can be a viable option for effective treatment of agriculture and floriculture runoffs which contain carbendazim.

Two-stage vertical-flow CWs have been used for sewage water treatment. The German Association for Water, Wastewater and Waste (DWA) has also described design guidelines for the construction and operation of two-stage vertical-flow CWs (DWA-A 262E 2017). However, there have not been many studies related to the use of the same for pesticide removal from agriculture and floriculture runoffs. It is an interesting prospect to study the effectiveness of a two-stage vertical-flow CW for pesticide removal. There is also a need for developing engineered and scalable CW solutions which are specifically suitable for use in Ethiopian flower farms.

This study aims at studying the removal efficiencies of carbendazim at different inflow concentrations using a pilot-scale vertical flow CW and the effect of varying hydraulic loading rate (HLR) and inflow carbendazim concentration on the removal efficiencies of carbendazim. This study also briefly analyses the mechanisms involved in the removal of carbendazim from influent water.

## MATERIALS AND METHODS

### Materials

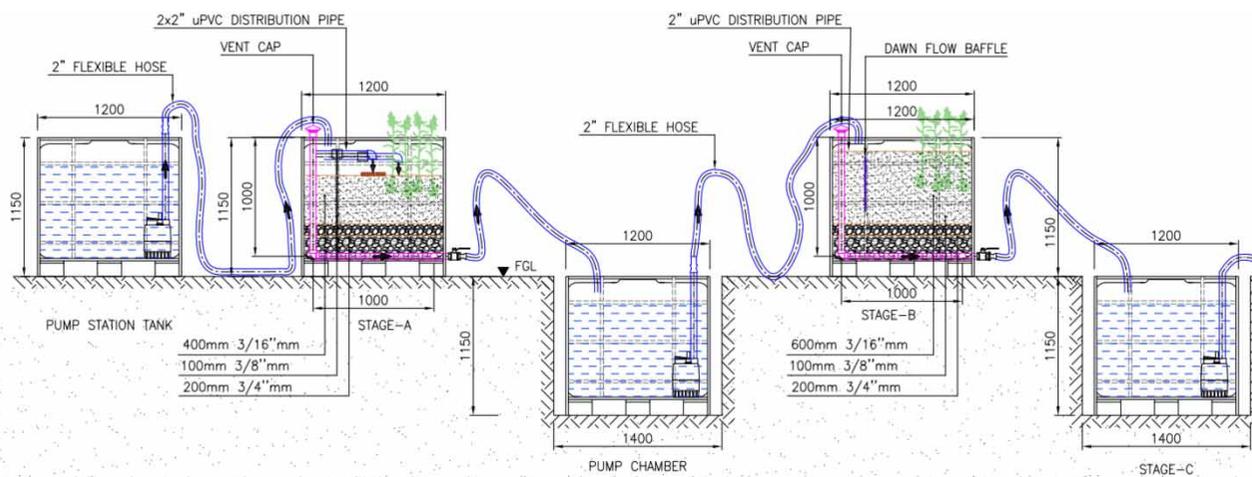
*Phragmites australis* (common reed) was selected as the wetland plant species to be used in this experiment. Saplings were sourced from the nursery operated and maintained by Reed Bed Waste Water Treatment Company in Sharjah, United Arab Emirates. Each mesocosm was constructed using an Immediate Bulk Container (117 × 95 × 100 cm). Double-washed filter material of three different particle sizes was used in the construction of the mesocosm – double-washed black fine gravel (grain size 1–5 mm), smaller gravel (grain size 10–15 mm), and larger gravel (grain size 20–25 mm).

### Experimental setup

The CW mesocosms were constructed to mimic large-scale CWs built following the example of French vertical-flow wetlands (Dotro *et al.* 2021) and guidelines published by the DWA (DWA-A 262E 2017). However, contrary to the textbook and the guidelines, only one filter unit was used for the first stage (Stage A), instead of the recommended minimum of three filter units. Another point to note was that the filter material used in the Stage A mesocosms was the same as the one used in the second stage (Stage B) mesocosms. These changes were made based on the assumption that the raw wastewater is free from solids, and therefore does not require filtration with fine gravel and a resting period in stage A.

The construction site was located at the RAK Research and Innovation Center, Ras Al Khaimah, U.A.E. The experiments were run from October 3, 2021 to December 30, 2021, and the average air temperature was in the range of 23–49 °C during this period. There were mostly sunny days during this period due to which precipitation was assumed to have a negligible impact on the operation. Stage A mesocosms were filled with a 20 cm layer of larger gravel at the bottom, a 10 cm layer of smaller gravel above it, and a 40 cm layer of fine gravel on top. Stage B mesocosms were filled with a 20 cm layer of larger gravel at the bottom, a 10 cm layer of smaller gravel above it, and a 60 cm layer of fine gravel on top. The outlet was placed 5 cm below the surface level to avoid photodegradation of carbendazim as well as to keep the mesocosm constantly saturated with influent water. Three treatment groups were set up: one for control (no carbendazim) and two for testing two different influent concentrations (10 and 100 µg L<sup>-1</sup>) of carbendazim. For testing under unsaturated conditions, the level of the outlet was adjusted to the bottom of the mesocosm and testing was done using two treatment groups: one for control and one for 100 µg L<sup>-1</sup> influent concentration of carbendazim.

For each group, similarly sized plants were selected randomly and planted following a planting grid in the mesocosms. The influent was pumped from the influent tank to the Stage A tank. The effluent was then collected into an underground tank to be further pumped to the Stage B tank. The final effluent was then collected into the last collection tank. Figure 1 shows a detailed schematic diagram of the pilot-scale CW system constructed for this study. The HLR of the influent, located in chamber A, was timer-controlled. The HLR of the Stage A effluent (located in chamber B) was adjusted to match the influent HLR with the help of float switches. Before the start of experimentation, treated sewage effluent water was introduced to the system to facilitate plant growth and to introduce a microbial population.



**Figure 1** | Schematic diagram of pilot-scale Constructed Wetland.

The influent was prepared in batches of 500 L. For preparation of the influent, concentrated solutions of carbendazim were first prepared in the lab by dissolving carbendazim in 200 mL of methanol. Absolute methanol was used because carbendazim has very low solubility in water. The addition of methanol also served as an additional carbon source to be added to the influent water. The influent tanks of each stream were filled with 500 L of water, to which the prepared concentrates were added. In this study, we used an HLR of 400, 200, and 100 L d<sup>-1</sup> to study the effect of carbendazim concentrations as well as HLR on the removal efficiency of carbendazim. The average flow capacity of the pumps used was calculated to be 3.5 L s<sup>-1</sup>. Subsequently, the activation time of influent pumps was calculated to be 19 s (HLR of 400 L d<sup>-1</sup>), 10 s (HLR of 200 L d<sup>-1</sup>) and 5 s (HLR of 100 L d<sup>-1</sup>) every 4 hours.

Sample collection was done through taps placed at the outlets of each Stage B tank. Samples of influents were collected directly from the influent tank; a volume of 1 L was collected for each sample of influent and effluent. The volume of total effluent collected at the end of Stage B was recorded to account for water loss due to evapotranspiration. Sampling of influent was carried out at the beginning of the experiment while sampling of effluent was carried out at the end of the experiment. Samples were stored in a 4 °C refrigerator before being processed to calculate the carbendazim removal efficiency of the system. Other parameters measured immediately after sample collection included pH, temperature, dissolved oxygen (DO), and electrical conductivity (EC). Samples were also collected to measure the biochemical oxygen demand (BOD), chemical oxygen demand (COD), Total Organic Carbon (TOC), nitrates, and phosphates for the control and 100 µg L<sup>-1</sup> groups (under saturated conditions). Plant samples were collected by harvesting the plant stem from 10 cm above the gravel level of the mesocosm. This way, the carbendazim content analysis for the plant sample would represent the amount of carbendazim absorbed by the plant.

## Analytical methods

### Carbendazim concentration analysis

Sample preparation was carried out using the liquid-liquid extraction method to obtain an extracted sample concentrate of 1 mL. The pH of the sample was first adjusted to 1–2, followed by separation of the aqueous and organic phases. Organic content of the sample was then extracted using dichloromethane, followed by filtration using sodium sulphate. The leftover solution was centrifuged and then concentrated to 1 mL using nitrogen gas. It was then processed using gas chromatography-mass spectrometry (GC-MS) along with the standard solutions to calculate the concentration of carbendazim in the sample with an accuracy of 0.01 µg L<sup>-1</sup> (Shin *et al.* 2001).

For extraction of carbendazim from substrate sample (gravel), 10 g of the sample was first placed in a polycarbonate tube. Subsequently, 10 g of anhydrous sodium sulphate and 25 mL of methanol were added to this tube, and the tube was subjected to shaking and sonification for 15 min. This resulted in a separated organic layer, which was evaporated under a nitrogen stream. Then, 50 µL of derivatising agent (iodomethane) was added to the collected residue and heated at 90 °C for 60 min. Subsequently, 100 µL of ethyl acetate was added to the residue to make the sample ready for GC-MS analysis (Shin *et al.* 2001).

Method 8081B described by the US EPA was used (US EPA 2015) for extraction of carbendazim from plant sample. The GC-MS instrument used for all the quantification procedures is Shimadzu GCMS-QP2010 SE (manufactured by Shimadzu (Suzhou) Instruments Manufacturing Co., Ltd, Jiangsu, China) and the standard dilutions used to generate the calibration curve were 0.01, 0.1, 1, 10, 100, and 1,000  $\mu\text{g L}^{-1}$ . Spiking of processed samples with known carbendazim concentrations was also performed and the results of both spiked and non-spiked samples were compared to assure the quality of the results obtained.

### Removal mechanisms for carbendazim

Two of the many possible carbendazim removal mechanisms (Imfeld *et al.* 2009) were considered in this study to check whether or not these mechanisms play an active role in carbendazim removal from influent water. The first mechanism was substrate sorption and the second was plant uptake.

Double washed fine gravel used in the construction of the CWs was taken and placed in a clean plastic container to evaluate the role of substrate sorption in carbendazim removal. This container was then filled with carbendazim water with a carbendazim concentration of 2  $\text{g L}^{-1}$  (high carbendazim concentration was used to make sure that there would be detectable amounts of carbendazim adsorbed by the substrate). This was then left undisturbed and kept away from sunlight for 4 days. The water was then collected and analysed for the concentration of carbendazim present in the water. The gravel was also processed and analysed to determine the sorption of carbendazim.

Plant samples were taken from the mesocosm, which was dosed with 100  $\mu\text{g L}^{-1}$  concentration of carbendazim, and analysed for the presence of carbendazim in them to investigate its removal through plant uptake.

### Methods of calculation

Each mesocosm was saturated with water before cutting off the water supply prior to running the experiments. Subsequently, the amount of effluent collected from each mesocosm was measured, which was used to calculate the hydraulic retention time (HRT) of the mesocosm. The HRT was calculated as follows:

$$T_r = V_{out}/Q \quad (1)$$

where  $T_r$  is the HRT,  $V_{out}$  the volume of effluent, and  $Q$  is HLR in  $\text{L d}^{-1}$ .

Evapotranspiration was calculated as follows:

$$\Delta V = (Qt - V_{out})/Qt \quad (2)$$

where  $\Delta V$  is the water loss by evapotranspiration,  $Q$  is the influent flow rate in  $\text{L d}^{-1}$ ,  $t$  is the time in days, and  $V_{out}$  is the total effluent volume recorded.

This value was used to correct the calculation of removal efficiencies of the carbendazim. The removal efficiencies were calculated as follows:

$$\text{Removal efficiency (\%)} = (C_{in} - (1 - \Delta V) \times C_{out})/C_{in} \times 100 \quad (3)$$

where  $C_{in}$  and  $C_{out}$  are influent and effluent concentrations, respectively (in  $\mu\text{g L}^{-1}$ ).

Based on previous pesticide-related studies, carbendazim removal is expected to follow a first-order kinetic model (Lyu *et al.* 2018). The fitted values for the model are derived using the formula:

$$k = \ln\left(\frac{C_{in}}{C_{out}}\right)/T_r \quad (4)$$

where  $k$  is the first-order rate constant (in  $\text{d}^{-1}$ ),  $C_{in}$  and  $C_{out}$  are influent and effluent concentrations, respectively (in  $\mu\text{g L}^{-1}$ ), and  $T_r$  is the HRT.

### Data analysis

Statistical analysis as well as two-way analysis of variance (ANOVA) were carried out using JASP software (JASP Team 2022). Two-way ANOVA was used to identify the effect of influencing factors (HLR and influent carbendazim concentration) on the

first-order rate constant,  $k$ , for carbendazim removal from the influent water. It was carried out at a significance level of 0.05 ( $p < 0.05$ ). Data plots were generated with the help of Veusz software (Sanders *et al.* 2021).

## RESULTS

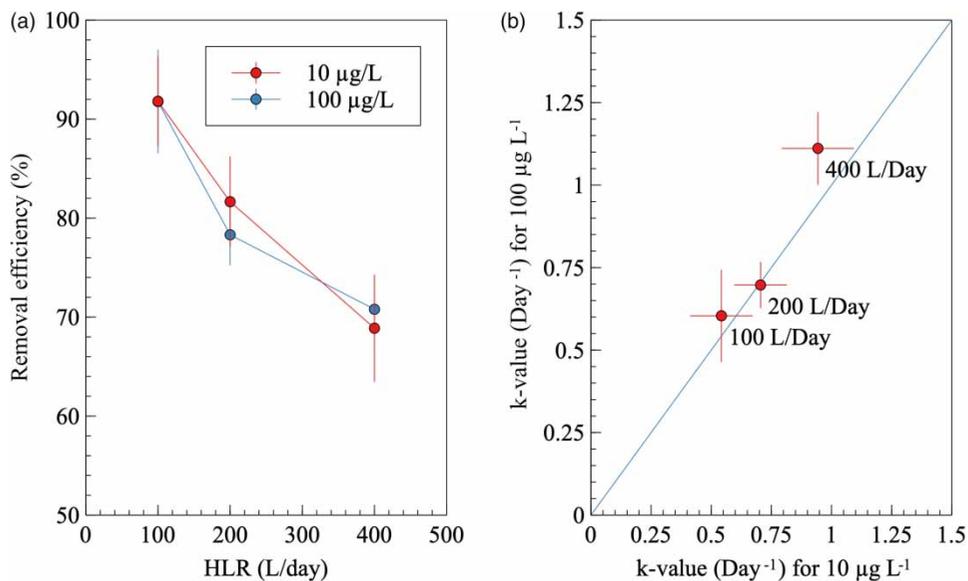
### Carbendazim removal

Carbendazim levels were measured and removal efficiencies were calculated using Equation (3). As expected, the carbendazim levels observed in the control were below the detection limit. The removal efficiency of carbendazim as well as the corresponding first-order rate constant,  $k$ , were calculated for three different hydraulic loading rates: 100, 200, and 400 L d<sup>-1</sup>. The results are depicted in Table S1 in the Supplementary data. Carbendazim removal efficiencies were observed in the range of 68.87–91.80%, with higher removal efficiencies at HLR of 100 L d<sup>-1</sup> and lower removal efficiencies at HLR of 400 L d<sup>-1</sup>.

A graphical representation of this data is shown in Figure 2. The removal efficiency showed a decreasing trend as the HLR increased. This could be attributed to a higher HLR which implies a lower retention time in the system. Further, this means that contact time has a notable influence on the effective removal of carbendazim in the CW system employed in this study. To try to compare these findings with unsaturated conditions, the experiment was repeated at an HLR of 200 L d<sup>-1</sup> and with an influent carbendazim concentration of 100 µg L<sup>-1</sup>. The results showed an average carbendazim removal efficiency of 16.14% under unsaturated conditions with an average  $k$ -value of 1.7860 d<sup>-1</sup>. The HRT observed during this experiment was 0.0833 days (2 h).

A comparison of the first-order rate constants,  $k$ , observed for each carbendazim concentration at different HLRs under saturated conditions indicated that the observed  $k$ -values seemed to be quite similar to each other. This could imply that the first-order rate constant,  $k$ , is independent of the influent concentration of carbendazim used.

To further confirm this observation statistically, we carried out a two-way ANOVA to observe the effect of HLR and carbendazim concentration on the first-order rate constant,  $k$ , for the removal of carbendazim from influent water under saturated conditions. A significance level of 95% was used and the result can be seen in Table 1. The  $p$ -value for the effect of HLR on  $k$  was below 0.05, which means it is a significant result. The  $p$ -value for the effect of carbendazim concentration was above 0.05. This means that the difference in  $k$ -values between the two influent concentrations, at the same HLR, was not significant. The  $p$ -value for the interactive effect of HLR and carbendazim concentration on  $k$  was above 0.05. This means that it is not a significant result, implying that  $k$  is independent of any interaction between these variables.



**Figure 2** | (a) Comparison of carbendazim removal efficiencies observed at different HLRs for two different influent concentrations, under saturated conditions. (b) Comparison of first-order rate constants,  $k$ , observed at different HLRs for the two different influent carbendazim concentrations. The error bars used in both the plots represent the standard deviations of the data observation.

**Table 1** | Two-way ANOVA analysis to verify the effect of influent carbendazim concentration,  $C_{in}$ , and HLR on the first-order rate constant,  $k$ , for the removal of carbendazim from influent water

	Cases	Sum of Squares	df	Mean Square	F	P
a.	<b>Between Subjects Effects</b>					
	HLR	0.658	2	0.329	16.709	0.004
	Residuals	0.118	6	0.020		
b.	<b>Within Subjects Effects</b>					
	$C_{in}$	0.025	1	0.025	2.530	0.163
	$C_{in} * HLR$	0.023	2	0.012	1.209	0.362
	Residuals	0.058	6	0.010		

Note. Type III Sum of Squares.

Part (a) illustrates the results for the effect of HLR on  $k$ . Part (b) illustrates the results for the effect of  $C_{in}$  and  $C_{in} * HLR$  on  $k$ .

### Water parameters

Other parameters which were monitored to observe variations in the experimental conditions as well as to evaluate the effect of treatment by CWs on the water quality included pH, DO, EC, and water temperature (Table 2). Average pH of the influent was observed to be in the range of 7.58–7.71, while that of the effluent was in the range of 9.47–9.57 across all three experimental groups. The percentage saturation of dissolved oxygen was observed to be  $\approx 21\%$  for the influent and  $\approx 32\%$  for the effluent. The electrical conductivity for the influent was found to be  $\approx 440 \text{ uS cm}^{-1}$  and that of the effluent was found to be  $\approx 825 \text{ uS cm}^{-1}$ . The average temperatures of all influent and effluent samples were found to be  $\approx 37 \text{ }^\circ\text{C}$ .

Another set of parameters which were measured for the control and  $100 \text{ } \mu\text{g L}^{-1}$  samples were BOD, COD, TOC, nitrates, and phosphates (Table 3). A higher level of COD was observed for the influent of the  $100 \text{ } \mu\text{g L}^{-1}$  carbendazim experimental group. This could be attributed to the fact that methanol was used as part of influent preparation for this experimental group while it was not used in the control group.

### Removal mechanics

The role of substrate sorption as a means of removal of carbendazim was studied. The substrate sample was collected post-exposure to carbendazim (as described in the methods section) and was analysed for the carbendazim content present in it. The results obtained showed that substrate sorption was responsible for the removal of 18% of the total carbendazim content present in the prepared influent water (Table 4). This implies that substrate sorption takes an active part in removal of carbendazim from the influent water.

Plant samples collected from the mesocosms were analysed for their carbendazim content to test the possibility of carbendazim removal via plant uptake. Carbendazim content in the plant sample was observed to be  $5.42 \text{ mg kg}^{-1}$ . This value is representative of the total amount of carbendazim absorbed by the plant over the entire duration of the experimentation and does not account for the amount that could have been possibly degraded by the plant. Although this does not indicate the relative contribution of plant uptake in carbendazim removal, the presence of carbendazim in plant sample is proof

**Table 2** | Data from water parameters tested immediately after sampling from the mesocosms

		pH		DO (%)		EC ( $\text{uS cm}^{-1}$ )		Temperature ( $^\circ\text{C}$ )	
Control	Influent	7.58	$\pm 0.175$	20.8	$\pm 2.201$	440.2	$\pm 25.328$	36.6	$\pm 1.075$
	Effluent	9.54	$\pm 0.184$	32.9	$\pm 2.807$	821.2	$\pm 13.790$	36.6	$\pm 1.265$
$10 \text{ } \mu\text{g L}^{-1}$	Influent	7.74	$\pm 0.104$	21.6	$\pm 2.171$	444.3	$\pm 30.930$	36.4	$\pm 1.265$
	Effluent	9.47	$\pm 0.223$	31.6	$\pm 2.503$	824.4	$\pm 18.057$	37.5	$\pm 0.972$
$100 \text{ } \mu\text{g L}^{-1}$	Influent	7.71	$\pm 0.078$	21.6	$\pm 2.633$	444.2	$\pm 24.303$	37.1	$\pm 1.912$
	Effluent	9.57	$\pm 0.162$	34.0	$\pm 3.018$	830.5	$\pm 18.435$	37.1	$\pm 1.197$

The parameters tested were pH, dissolved oxygen (DO, measured as percentage saturation), electrical conductivity (EC, measured as  $\text{uS cm}^{-1}$ ) and temperature (measured in  $^\circ\text{C}$ ).

**Table 3** | Data for BOD, COD, TOC, nitrates, and phosphates parameters observed for the control sample and sample from 100  $\mu\text{g L}^{-1}$  dosed experimental groups

		BOD ( $\text{mgL}^{-1}$ )	COD ( $\text{mgL}^{-1}$ )	TOC ( $\text{mgL}^{-1}$ )	Nitrate ( $\text{mgL}^{-1}$ )	Phosphate ( $\text{mgL}^{-1}$ )
Control	<i>Influent</i>	<12	11	0.68	0.05	0.36
	<i>Effluent</i>	<12	<10	0.28	0.06	<0.02
100 $\mu\text{g L}^{-1}$	<i>Influent</i>	<12	1302	260	0.04	0.07
	<i>Effluent</i>	<12	14	4.72	0.05	<0.02

**Table 4** | Observations and calculations made to calculate the contribution of substrate sorption towards removal of carbendazim from prepared influent water (concentration of 2  $\text{mg L}^{-1}$ )

Total amount of carbendazim added to container	10 mg
Total amount of soil present in the container	2 kg
Carbendazim concentration observed in the substrate at the end of the experiment	0.9 $\text{mg kg}^{-1}$
Amount of carbendazim removed by substrate sorption	1.8 mg

that carbendazim is absorbed by plants and that plant uptake plays an active role in the carbendazim removal from the influent water.

## DISCUSSION

It is noteworthy that the design of the pilot CW used in this study is built with the example of French wetlands and DWA guidelines (DWA-A 262E 2017; Dotro *et al.* 2021) in mind, and is meant to mimic actual field conditions as much as possible. There have been previous studies that make use of pilot-scale CWs for the removal of pesticides (Lyu *et al.* 2018), but not many use a two-stage vertical-flow design that mimics how large scale CWs are built commercially. This design also allows for easy modifications to the system for future studies with varied experimental requirements, for example, the use of effluent recirculation and oxygenation among others. This allows the study results to be easily scalable for industrial purposes.

The use of a saturated system has shown great promise, with carbendazim removal efficiencies of up to 91.80%. The use of a completely unsaturated system showed a much lesser efficiency of 16.14% at an HLR of 200  $\text{L d}^{-1}$ , while the corresponding removal efficiency in the saturated system was 78.9%. This could be attributed to its drastically low retention time as compared to a saturated system. While the HRT for a saturated system with an HLR of 200  $\text{L d}^{-1}$  was 2.11 days, the corresponding HRT for an unsaturated system was 0.0833 days (2 h). Taking this into consideration, we could achieve much higher removal efficiencies if we could increase the retention time of the carbendazim water while maintaining an unsaturated condition. One way to address this would be recirculating the effluent water resulting in greater retention of the water in the system. If we were to recirculate the effluent water four times after initial loading, we could potentially achieve a matching removal efficiency as the saturated system. The corresponding retention time of the water would be 0.417 days; thus, the retention would be five times faster than the saturated system. This warrants further investigation as it could allow for use of higher HLR to increase the efficiency of the CWs as a whole.

Additionally, there was an increase in the pH of the effluent water as compared to that of the influent water. On average, the pH of the effluent water was above 9 across the three streams used in this study. This makes it less suitable for applying the treated water for irrigation purposes or simply allowing it to enter nearby water bodies (Jeong *et al.* 2016). During our experimental observations, pH values for effluent water under unsaturated conditions were significantly less than 9. This implies that the increase in observed pH was positively correlated to an increase in the saturation of the system. Thus, further research is required for optimization of the saturation level to achieve maximum pesticide removal without compromising effluent water quality. The use of a partially saturated system would also theoretically increase dissolved oxygen levels.

This could also lead to a possible increase in the removal efficiency (Vink & van der Zee 1997), but it needs further investigation.

While substrate adsorption was determined to be responsible for 18% of the carbendazim removal, the degree of involvement of plant uptake needs further in-depth study. Although removal mechanisms were evaluated in this study, they were studied separately. All the possible removal mechanisms for carbendazim are highly complex in nature and are interdependent upon each other as well. For example, over time there tends to be a build-up of biofilm in the gravel used in the CWs. This may decrease the amount of substrate sorption but the role of the microbial population in the degradation of carbendazim could enhance (McBride & Tanner 1999; Chyan *et al.* 2013). This is why there is a need to further study the correlation of the various possible removal mechanisms as well. This work would also help illustrate the mass balance of the carbendazim introduced to the constructed wetland.

## CONCLUSION

The results from this study indicate that a two-stage vertical-flow CW system is an effective system for the removal of carbendazim from wastewater runoffs from floriculture farms. Moreover, there is a need for further optimisation of the CW system to achieve maximum performance without compromising the effluent water quality for discharge into water bodies.

Removal efficiencies of up to 91.80% were observed under saturated conditions. Further analysis using two-way ANOVA showed that the first-order rate constant,  $k$ , for removal of carbendazim was affected by the HLR used, but was unaffected by the influent carbendazim concentration or any interactive effect between HLR and influent carbendazim concentration. Comparison of the saturated vs. unsaturated system indicated great potential for testing the unsaturated system with recirculation of effluent to effectively increase the efficiency of carbendazim removal from the influent water.

Adsorption to the substrate and plant uptake played a significant role in the removal of carbendazim from the wastewater. However, further studies are warranted to identify the relative contributions and interdependence of these removal mechanisms and others such as biodegradation pathways.

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## DATA AVAILABILITY STATEMENT

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

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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