Pharmaceuticals and personal care products (PPCPs) in the aquatic environment has steadily increased. In this study, phytoremediation technology, using an ornamental plant (Alternanthera spp.), was investigated to improve the removal of acetaminophen (AC) and methylparaben (MP) from a synthetically prepared wastewater. Three exposure lines (AC-line, MP-line and control-line) were performed with a total of 26 subsurface-horizontal constructed wetlands (SSH-CWs) that operated in batch feeding mode. The influence of plants in addition to the initial spiking concentration (20, 60 and 100 mg/L) of AC and MP on the removal efficiency was evaluated throughout the 35-days experiments. The highest removal efficiencies for AC and MP were 88.6% and 66.4%, respectively, achieved in the planted CWs; whereas only 29.7% and 21.9% were achieved in the control CWs for AC and MP, respectively. The results confirmed the role of Alternanthera spp. for accelerating the removal of AC and MP from synthetically contaminated wastewater in CWs.
of the pollutant. Moreover, such technologies are not feasible in the case of low pollutants concentrations (Rodríguez-Narvaez et al., 2017).

New green approaches, including phytoremediation, are being adopted as alternative wastewater treatment solutions for the removal of a wide variety of toxic organic pollutants (Al-Baldawi et al., 2014; Abdullah et al., 2020). Phytoremediation employs the ability of plants to absorb and translocate organic and inorganic contaminants in wastewater treatment (Zeki and M-Ridha, 2020); further, the associated rhizobacteria have a crucial role in this process (Fadhlil and Al-Baldawi, 2020). During phytoremediation, PPCPs are taken up and translocated within the plant; however, these processes are crucially governed by several factors including the properties and concentration of the pollutants and the type of the plants (Al-Baldawi et al., 2021).

Phytoremediation technology is efficient, environmentally friendly, and cost-effective compared to other treatment technologies (Al-Baldawi et al., 2014; Abdullah et al., 2020). Phytoremediation, however, has been faulted for being time-consuming and highly climate-dependent technology (Hirath and Vithanage, 2015). Phytoremediation can be applied as a final step following the preliminary treatment with the high-level contamination. Nevertheless, this technology alone may be the most economical and effective treatment option with the low-level contamination (Al-Baldawi et al., 2015).

Currently, constructed wetlands (CWs) represent innovative and promising approaches to protect the environment, and this places them at the forefront of the sustainable solutions proposed for wastewater treatment in developing countries (Darajeh et al., 2016). Large-scale CWs have been implemented for the treatment of wastewater in several countries around the world such as the Czech Republic (Vymazal et al., 2017), the Republic of Korea (Park et al., 2018), Singapore (Wang et al., 2019), Ukraine (Vystava et al., 2017) and Belgium (Aviin et al., 2017). CWs have been previously reported to remove more than 90% of several PPCPs from wastewater, such as caffeine (Chen et al., 2016; He et al., 2018), ciprofloxacin (Christofilopoulou et al., 2019), ibuprofen (Al Falahia et al., 2021) and triclosan (Li et al., 2017). However, the removal of other compounds was negligible such as sulfamethoxazole, carbamazepine and sucralose (Avila et al., 2017).

Due to COVID-19 pandemic, the wastewater discharged from hospitals and quarantine centers have caused extra environmental challenges due to the increasing use of PPCPs (Khan et al., 2021). The cooperation between governments and research institutions is necessary to protect humans and the environment from these contaminants. Accordingly, the main objective of this study was to assess the phytotoxicity of two PPCPs, namely acetaminophen (AC) and methethylpraben (MP) on an ornamental plant (Alternanthera spp.). Alternanthera spp., a perennial plant that can survive in four season of Iraq weather, was selected after a preliminary test for three ornamental plants (Asparagus aethiopicus, Chlorophyllum comosum) which were exposed to high concentrations of AC and MP. In principle, these ornamental plants were used specifically because they are out of the human food chain and for their aesthetic value. Alternanthera spp., showed the highest tolerance towards pollutants, water stresses and weather conditions prevailing in the region. At the same time, the study aimed to evaluate the efficacy of the Alternanthera spp. in removing the corresponding pollutants from synthetic wastewater. In addition, pollutant concentration and exposure time were also evaluated in order get a further insight into the extent to which these variables affected the overall removal efficiency at subsurface-horizontal constructed wetlands (SSH-CWs).

2. Materials and methods

2.1. Chemicals and plant materials

High purity Acetaminophen (AC) was obtained from Middle East Laboratories Co. Ltd (Iraq). Methylparaben (MP) (purity > 99%) was purchased from VWR Chemicals (UK). The properties of the relevant PPCPs are summarized in Table 1 (NCBI, 2021.). Both compounds have a relatively hydrophilic properties and a moderate solubility in water. The vegetation selected for this study is Alternanthera spp., which is an ornamental plant widely spread in tropical regions. Alternanthera spp. was purchased from Al- Zawraa Park, Baghdad which has properties as purple leaves and dense roots and range in length from 15 to 35 cm (Stem with root) (Figure 1). Plants had been rinsed gently, and then acclimated in tap water for two weeks to favor growth of roots. Homogenous plants had been selected for the experiments.

2.2. Subsurface horizontal constructed wetlands mesocosms

The experimental setup was established outdoors at Baghdad University, Iraq. A total of 26 mesocosms were operated under conditions

## Table 1. Properties of AC and MP.

| Characteristics          | Acetaminophen (AC) | Methyl Paraben (MP) |
|--------------------------|--------------------|----------------------|
| CAS no.                  | 103-90-2           | 99-76-3              |
| Synonyms                 | Paracetamol        | Methyl 4-hydroxybenzoate p- |
|                          | 4-Acetamidophenol  | Methoxybenzoate p-Methoxybenzoic phenol |
|                          | N-(4-Hydroxyphenyl) | p-Carbamoylphenol     |
|                          | acetamide          |                      |
| Molecular Formula        | C₈H₁₆NO₂            | C₉H₁₄O₃             |
| Molecular weight         | 151.16 g/mol        | 152.15 g/mol         |
| Water Solubility (mg/L)  | 14000 mg/L         | 25000 mg/L          |
| at 25 °C                 |                    |                     |
| Octanol-Water partitioning coefficient (Log Kow) | 0.46 | 1.96 |

![Image](https://via.placeholder.com/150)
simulating batch horizontal subsurface constructed wetland (SSH-CW) design. Two replicates (R1 and R2) for each concentration (20, 60 and 100 mg/L) were spiked with AC and MP pollutants, with and without plants; and two mesocosms were set as a plant control (fed with tap water). The three concentrations of AC and MP were chosen to represent the possibility of intentional or accidental release of high loads of pharmaceuticals into wastewater, and large quantities largely disposed by associated pharmaceutical facilities in the aquatic environment (Petrie et al., 2016). A schematic description is given in Figure 2, were consisted of glass containers with dimension of (0.3 m \times 0.3 m \times 0.3 m). Washed gravel to a depth of 20 cm was used as a substrate (5 cm of coarse gravel (\( \Phi 9.5–13.5 \) mm) in the bottom, 10 cm of medium gravel (\( \Phi 6.5–9.5 \) mm) and 5 cm of fine gravel (\( \Phi 2.5–6.5 \) mm) in the top). *Alternanthera* spp. was planted with plant density of 10 plants/mesocosm, while the other twelve mesocosms were left unplanted and considered as control contaminant. Stock solutions of 1000 mg/L for both AC and MP were prepared, individually. Then 5 L of diluted solution with different concentrations (20, 60 and 100 mg/L) were fed to each CW, so that the water level was controlled within the gravel surface. During the exposure period, the loss of water (due to transpiration by the plants and/or evaporation) from the mesocosms was closely monitored and compensated continuously with tap water to reach the initial volume. Finally, effluent from each CW was collected on days 7, 14, 21, 28 and 35, which contributed to the 35-day experimental cycle.

### 2.3. Plant growth analysis

Throughout the experiment interval, the potential toxic effects of AC and MP on *Alternanthera* spp. were closely observed. Wet and dry weight analyses were carried out in order to evaluate the water content of the plants. Furthermore, the variation in color and physical growth of the plants were also monitored and compared to the state of the plants at the control mesocosms.

The growth of *Alternanthera* spp. was monitored in different initial AC and MP concentrations (20, 60 and 100 mg/L) over 35 days of exposure. One plant was collected from each mesocosm on each sampling day (0, 7, 14, 21, 28 and 35). Plants were first rinsed with tap water, then wet weight of each plant were recorded. For dry weight calculations, all plants were dried in an oven (HYSC, Korea) at 70 °C for 72 h then their dry weight was recorded. Furthermore, the relative growth rate (RGR) of plants was determined based on the increase in wet weight after 7, 14, 21, 28 and 35 days of exposure, using Eq. (1) based on Al-Baldawi et al. (2020):

\[
RGR(\text{day}^{-1}) = \frac{\ln W_f - \ln W_i}{\text{Day}}
\]

with, \( W_i \) represents the initial wet weight (g) and \( W_f \) represent the final wet weight after exposure to the target contaminants.

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**Figure 2.** Sketch diagram of the mesocosm-scale CWs: (a) whole set-up and (b) tank cross section.
2.4. Physicochemical parameters analyses

Throughout the exposures experiments of 35 days, an extensive characterization was carried out on days 0, 7, 14, 28, 42 and 72 to track any changes to wastewater and plants due to the AC or MP exposures. Water physicochemical parameters, namely temperature (T), pH, dissolved oxygen (DO), oxidative reduction potential (ORP) and total dissolved solids (TDS), were tracked on a weekly basis via multi meter (ISOLAB Laborgeräte GmbH, Germany) (Al-Baldawi et al., 2013).

2.5. AC and MP removal and kinetic models

After immediate filtering through a 0.45 μm syringe filters, wastewater samples were directly analyzed by UV–visible spectrophotometer (UV-1800, Shimadzu, Japan) to quantify the concentrations of AC (based on Pearson correlation coefficient). Water samples were directly analyzed by UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) to quantify the concentrations of AC. The percentage error of the average values was adopted at < 5%. Removal efficiency for the target compounds were calculated based on the initial and final concentration in the synthetic wastewater according to Eq. (2):

$$ \text{RE}\% = \frac{C_i - C_t}{C_i} \times 100 $$

with, RE is the removal efficiency (%); C_i and C_t (mg/L) are the pollutant concentration initially and through time.

Data were also fitted to the first-order kinetic model using Eq. (3) (USEPA, 2000; Tee et al., 2012; Zhang et al., 2017b):

$$ \ln \left( \frac{C_i}{C_t} \right) = -kt $$

with, k is the kinetic removal rate constant (day^{-1}) and t is the sampling time (Day).

2.6. Statistical analysis

All statistical analysis was performed using SPSS Version 21 (IBM, USA). Independent-samples t-test was performed to determine differences in water physicochemical parameters between mesocosms. The univariate analysis of variance (ANOVA) was performed at confidence level of 95% (p < 0.05) to specify the influence of the independent variables on treatment performance. Linear correlations between variables were analyzed based on Pearson correlation coefficient, and the results with p < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Tolerance of Alternanthera spp. to AC and MP

Figure 3 shows the appearance of Alternanthera spp. after 35 days of exposure to AC, MP and tap water (control). Plants that exposed to AC grew well, and no considerable variation was observed in the color or structure of plants compared to plants in the control mesocosms. Otherwise, visible changes were observed in the leaves and roots of Alternanthera spp. that were exposed to MP, as wilting was evident in many plants since day 21 of the experiment. Figure 4 illustrates the wet and dry weight as well as the relative growth rate of plants that were exposed to AC and MP, respectively, during the experiment. From Figure 4(a), a clear increase in the mean wet and dry weights of plants that were exposed to AC could be observed. After 35 days of experiment, the mean wet and dry weights of the Alternanthera spp. were 55.33 ± 1.87 g and 20.3 ± 0.3 g, respectively, which showed a clear increase over the means recorded at the beginning of the experiment (48.76 ± 1.13 g and 17.37 ± 2.17 g). As a result, positive growth rates were achieved between 0.001 and 0.004 day^{-1} for Alternanthera spp. in the AC-line mesocosms. The increase in plant biomass considered a positive indicator of plant health (Marsidi et al., 2016). Accordingly, AC did not cause any phytotoxicity on Alternanthera spp. throughout the duration of the experiment. While when Alternanthera spp. were exposed to MP (Figure 4(b)), the mean wet and dry weights of the plants decreased clearly (40.21 ± 11.55 g and 17.44 ± 1.9 g, respectively) at the end of the experiment (day 35) compared to their mean weights at day 7 (49.46 ± 1.31 g and 17.67 ± 1.14 g). Accordingly, the relative growth rates declined negatively between -0.013 to -0.008 day^{-1}, which confirms the phytotoxicity of the MP on the Alternanthera spp.

Statistically, the concentration (for both pollutants) was not a significant factor in the variation of the wet and dry weights of plants; otherwise, time was the statistically significant (p < 0.05) factor.

3.2. Variation in water quality parameters associated with AC and MP exposure

Physicochemical parameters of wastewater were closely monitored throughout the 35-day operational period of the experiment. The physicochemical parameters (pH, DO, ORP and TDS) of the wastewater for AC and MP mesocosms are illustrated in Figure 5. The experiments were carried out outdoors with a samples temperature range of 11.6–18.2 °C. pH maintained a mean within the neutral levels throughout the duration of the experiment in all mesocosms (planted and unplanted), as it ranged between 7.5 ± 0.05 to 7.8 ± 0.04 for both pollutants. According to Al-Ajalín et al. (2020) who reported only a slight increment over the initial pH value (6.35 ± 0.35), neutral pH rates (6–9) were essential for plant maintenance and bacterial growth within subsurface flow CWs. Al-Baldawi et al. (2020), also mentioned the stability of pH values (6.3–7.3) within neutral limits during phyto remediation of methyl orange by Salvinia molesta. For DO values, significant differences (p < 0.05) were found between planted mesocosms that exposed to AC and MP. Due to the 35-days exposure, initial DO value (4.08 ± 0.51 mg/L) at the planted AC-mesocosms decreased significantly to 2.31 ± 0.44 mg/L; whereas for planted MP-mesocosms, the initial DO value (4.16 ± 0.18 mg/L) deteriorated sharply, reaching 0.78 ± 0.22 mg/L. The decrease in DO values was also observed at the unplanted mesocosms, as DO declined to 1.22 ± 0.25 and 1.61 ± 0.3 mg/L at AC-mesocosms and

![Figure 3. Appearance of the Alternanthera spp. at the end of the experiment (day 35).](image-url)
MP-mesocosms, respectively. Al-Baldawi et al. (2013) pointed out that the surface re-aeration resulting from the effects of wave action and wind-induced mixing is not included in SSHF-CWs, and the derivation of oxygen in such systems is limited to the effect of atmospheric diffusion and plant (via the aerenchyma). However, the phytotoxicity observed on plants in MP-mesocosms is likely the main reason for the differences in DO values between AC-mesocosms and MP-mesocosms.

As for ORP, the mean values were significantly higher \((p < 0.05)\) at the planted AC-mesocosms \((-29.01 \pm 4.09 \text{ mV})\) compared to the planted MP-mesocosms \((-49.82 \pm 21.48 \text{ mV})\). A significant difference \((p < 0.05)\) were also found in mean ORP values between the planted and unplanted mesocosms for both pollutants. According to Rahman et al. (2020), negative ORP values generally refer to the development of the reduction conditions at CWs, and values between \(-50\) and \(-130 \text{ mV}\) indicate the predominance of anoxic conditions. In this study, the recorded ORP values clearly indicate that the aerobic conditions, in addition to anoxic conditions, are prevalent in all mesocosms.

TDS measurements were found to oscillate around their mean initial values throughout the experiment as depicted Figure 5. No significant difference \((p > 0.05)\) has been monitored for TDS values between all mesocosms. Finally, the slight oscillations in the physicochemical parameters of wastewater observed in this study are almost similar to what Button et al. (2019) observed, as the authors believed that these fluctuations may be attributed to natural variability and occur to an equal extent in all CWs (planted and control).

3.3. Removal of AC and MP

The reduction of AC and MP concentrations that achieved by Alternanthera spp. at HSS-CW systems across an interval of 35 days is presented in Figure 6. The concentrations of AC and MP were varyingly reduced by Alternanthera spp. during the experiments; however, there was statistically significant differences compared with the control mesocosms (without plants) for all sampling days (Figure 6); further, there were significant statistical differences \((p < 0.05)\) related to time factor. AC has considerably reduced until 10.47, 32.36 and 55.89 mg/L at day 7 for the initial concentrations of 20, 60 and 100 mg/L, respectively; whereas MP moderately reduced until 16.73, 51.54 and 82.27 mg/L for the same initial concentrations. By the end of the experiment, AC concentrations, at planted mesocosms, declined to 2.69, 6.83 and 12.46 mg/L, while MP concentrations moderately reduced to 7.26, 22.68 and 33.52 mg/L.
mg/L for the corresponding initial concentrations. The maximum removal efficiencies obtained in this study for AC (87.53%, 88.6% and 86.53%) are consistent with the results reported by Vo et al. (2016), where plant uptake was confirmed as dominant process (19–68% of total removal) at CWs for the removal of AC (1 ppb), with reduced roles for microbial and photolytic processes (3–32%) and negligible role for media adsorption. In contrast, Koottatep et al. (2017) reported that the contribution of plant uptake for removing AC, achieved by *Scirpus validus*, was marginal (0.04–1.35%). In fact, the variation in AC removal could be expected with different types of plants.

On the other hand, the low MP removals (63.6%, 62.1% and 66.4%) by *Alternanthera* spp.-planted mesocosms, compared to AC removals, could be primarily attributed to the inability of the plants for withstanding the toxic effects of MP that were evident on a large proportion of plants in CWs, which hindered the phytoremediation process. Likewise, the low MP removal results obtained in this study are in good agreement with the results reported by Matamoros et al. (2016), as only 33/63% of MP was removed in HSSF-CWs, planted with *Phragmites australis*, in cold climate; however, according to the authors, this percentage increased to 56% in warm climate. In another study by Matamoros et al. (2017), MP removals, at full-scale HF-CW planted with *Phragmites australis*, ranged between 16/23% to 60/14% in May and July, respectively. In another published studies, MP was confirmed to be efficiently removed at CWs. Anjos et al. (2019) reported a high removal efficiency (90.7%) for

![Figure 5. Physicochemical parameters of wastewater throughout the 35-day exposure to AC and MP. Data are represented as mean ± standard deviation (SD).](image)

MP by using free-floating macrophyte (*Landoltia punctata*). Moreover, MP was efficiently removed by *Phragmites australis* as reported by Petrie et al. (2018) and they concluded that the transportation of micropollutants within the plant occurs mainly via transpiration processes, and only micropollutants with cationic or neutral form in wastewater (pH 8.0) may be move across the root cell membrane, whereas the charge repulsion restricts the uptake of negatively charged micropollutants. Another results published by Chen et al. (2019) confirmed that the presence of plants in CWs considerably improve the removal of MP; however, the researchers also mentioned the role of adsorption for the removal of MP.

For the control mesocosms, it was found that the achieved removal rates were marginal as it did not exceed 29.7% and 21.9% for the highest initial concentration of AC and MP, respectively. In fact, the limited removals could be attributed to the limited microbial activity associated with the anoxic conditions prevailing at the unplanted mesocosms. This interpretation is consistent with the findings published by Ilyas and Hüllebusch (2020), who confirmed the higher potential of aerobic degradation for AC at CWs. Likewise, Wu et al. (2017) reported that MP exhibited comparatively higher persistence in anaerobic treatment systems compared to aerobic systems and they confirmed that the majority of parabens can be removed by aerobic biodegradation whereas minor removal is possible in anaerobic systems. Furthermore, Anjos et al. (2019) mentioned that the degradation process almost related to length of carbon chain, which explains the high degradation of the short chain MP compound, particularly under aerobic conditions. Consequently, it could be concluded that the plant uptake is the dominant removal mechanism for AC and MP within the SSH-CWs. Besides, the role of adsorption in the removal processes for both planted and control systems could be excluded due to the hydrophilic nature of both pollutants, with octanol-water partitioning coefficient (Log K<sub>OW</sub>) equal to 0.46 and 1.96 for each AC and MP, respectively.

### 3.4. Kinetic models

All zero, first and second-order kinetics were examined on the removal rate of AC and MP but the first-order model was the most fitted. Thus, the first-order kinetic model was performed to conclude the overall removal rate constants of AC and MP in the mesocosms. The kinetics of removal was obtained from the reduction in concentrations of AC and MP in the synthetic wastewater during sampling times at 0, 7, 14, 21, 28 and 35 days for the planted treatment mesocosms. Table 2 shows the estimated 1st order reduction rate constants (k), half-life (T<sub>1/2</sub>) and coefficient of determination (R<sup>2</sup>) of AC and MP for different initial concentrations at *Alternanthera spp*-planted mesocosms.

AC and MP reduction data (by *Alternanthera* spp) was well fitted (R<sup>2</sup> = 0.984) by the first order kinetic model, and the k values varied from 0.028 to 0.06 day<sup>-1</sup>. Furthermore, the reduction of AC and MP concentrations exhibited a strong positive correlation (Pearson coefficient >0.96) with exposure time for all initial concentrations, indicating that exposure time had a significant influence on the removal process. On the other hand, the values of the removal rate constant for the unplanted mesocosms (control) were found to be relatively low compared to the values of the planted mesocosms.

Despite its simplicity, first-order model was considered the most adequate for SSHF-CWs (Tee et al., 2012). Several recent studies applied the first-order kinetic model to determine the removal rate constant of PPCPs via phytoremediation, as summarized in Table 3. For most these studies, the first-order kinetic was a satisfactory model. However, Zhang

![Figure 6. Concentration and removal efficiency of (a) AC and (b) MP at planted and control mesocosms. Data are represented as mean ± standard deviation (SD) (A: significant difference at p < 0.05 between treatments (with and without plants) within one concentration).](image-url)
et al. (2017b) confirmed that all kinetic models (0th, 1st and 2nd) that had been tested were not adequately described the removal of the recalcitrant compound of iohexol.

With an estimated half-life of approximately 11–25 d, the removal of AC and MP were relatively slow. The observed removal rate constants for AC and MP in the present study are consistent with these reported for previous studies (Zhang et al., 2016; AL Falahia et al., 2021), sulfamethazine, diclofenac and carbamazepine (Liu et al., 2020); however, are lower compared to other PPCPs (Table 3). First-order removal rate constant is believed to be highly affected by the physicochemical characteristics of PPCPs and consequently it could be described as compound-dependent (Matamoros et al., 2012).

4. Conclusions

The elimination of PPCPs using green technologies has attracted the interest of many researchers. In this study, Alternanthera spp. based-phytoremediation was investigated for eliminating AC and MP from a synthetic wastewater. The phytotoxicity test revealed that AC did not cause any toxic effects as the plants achieved positive growth rates, whereas MP directly caused a decrease in the growth rates of the plants. Alternanthera spp. performed well for removing AC (88.6%) and MP (66.4%). In contrast, unplanted SSH-CWs provided a limited opportunity for the biodegradation of AC and MP (29.7% and 21.9%, respectively), which can be attributed to the prevalence of the anoxic conditions in these systems. Therefore, the addition of forced aeration may support the aerobic degradation of these pollutants in similar systems. The findings obtained greatly support the feasibility of expanding the use of ornamental plants in phytoremediation for the removal of emerging contaminants from wastewater.

Declarations

Author contribution statement
Ahmed A Mohammed: Contributed reagents, materials, analysis tools or data.

Zahraa Hasan Mutar: Performed the experiments; Wrote the paper.
Israa Abdulwahab Al-Baldawi: Conceived and designed the experiments; Analyzed and interpreted the data.

Funding statement
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement
Data included in article/supp. material/referenced in article.

Declaration of interests statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

Acknowledgements
The authors would like to thank the College of Engineering and Al-Khwarizmi College of Engineering, University of Baghdad, College of Engineering, Wasit University and Iraqi Ministry of Higher Education, Iraq for supporting this research project.

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