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# Assessment of Ornamental Plants Tolerance for Acute Exposure of Acetaminophen and Methylparaben in Constructed Wetlands- a Preliminary Study

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#### Abstract

The study aims to select suitable ornamental plant species that can survive relatively with high concentrations of acetaminophen and methylparaben in constructed wetlands. *Alternanthera spp, Asparagus aethiopicus* and *Chlorophytum comosum* are examined to withstand three initial concentrations, 20, 100 and 200 mg/L of acetaminophen and methylparaben. A total of 21 plastic pails with each 3 L capacity consisting of nine pails are used for each pharmaceutical and personal care products (PPCPs) compounds (acetaminophen and methylparaben) for three ornamental plants (*Alternanthera spp, Asparagus aethiopicus* and *Chlorophytum comosum*), with three pails as plant controls. The results reveales that both *Alternanthera spp* and *Chlorophytum comosum* exhibite a good tolerance for acetaminophen with a reduction in the total chlorophyll content of about 4.4–12.3% and 3.9–31.9% for *Alternanthera spp* and *Chlorophytum comosum*, respectively. Moreover, it is evident that high concentrations (100 and 200 mg/L) of methylparaben adversely affects the chlorophyll content of the three involved plant species. These results indicate that ornamental plants play an important role in the phytoremediation of PPCPs and can be considered as an esthetic treatment for hospital wastewater.

**Keywords:** Ornamental plant, Phytoremediation, chlorophyll, pharmaceuticals, and personal care products.

#### 1. Introduction

The excessive and unconscious consumption of pharmaceuticals and personal care products (PPCPs) have introduced enormous quantities of contaminants into the ecological system, as more than 50% of commercial chemicals can cause *This is an open access article under the CC BY license:* 

harmful effects to the environment due to unregulated disposal [1]. PPCPs can cause serious effects on the aquatic environment, being predominantly toxic and endocrine disruptors. Nonetheless, long-term exposure to sublethal concentrations of PPCPs may cause genetic mutations of environmental microorganisms,



especially pathogenic microorganisms [2]. PPCPs are often continuously released into aquatic environments and thus have been frequently detected in water sources in the range of ng/L to mg/L [3].

Some PPCPs are partially removed in conventional wastewater treatment processes such as coagulation, sedimentation, filtration, and biological treatment; thus, alternative treatment technologies are required [4] for complete removal. Phytoremediation employs plants to eliminate contaminants from the environment [5]. phytoremediation, plants assisted microorganisms play a vital role in degrading, converting, metabolizing or detoxifying various pollutants from soil, water and air. Furthermore, this technology has many benefits and aesthetical values compared with conventional treatment technologies [6]. This technology has attracted great attention due to its numerous advantages, including sustainability, low costs, ease of operation, and application in large areas [7]. Constructed wetlands (CWs) are engineered systems that mimic the physical, chemical, and microbiological processes prevailing in natural wetlands to eliminate various contaminants [8]. CWs are employed to reduce pollutants (nutrients, organic and inorganic waste) in wastewater before discharge into water bodies; further, it represents an alternative to costly conventional sewage treatment techniques [9].

Plant characteristics, including the root system and enzymes, have a significant role in CWs [6]. To select a suitable plant species for phytoremediation of PPCPs, several considerations must be considered, including the climatic conditions and the characteristics of the wastewater. Furthermore, the adaptability of the plant to the saturation conditions, the growth potential of roots, and the tolerance to high pollutant concentrations should also be considered [10].

The use of native plants is often preferred due to their high potential for adaptation on the surrounding environmental condition [11]. Kurniawan et al. [9] pointed out several plants' high potential, including Chloris virgata, Rotala rotundifolia, Ludwigia adscendens, and Trapa natans *Phragmites australis, Pistia stratiotes, Azolla pinnata*, and *Lemna minor*, in reducing nutrient from organic-rich wastewater in CWs. According to Abdullah et al. [6], terrestrial plants are the most employed in phytoremediation (62%), followed by aquatic plants (33%), whereas

ornamental plant is the least used (5%). Macci et al. [12] chose a set of ornamental plants (Canna indica, Carex hirta, Miscanthus sinensis, and Zantedeschia aethiopica) to be tested in CWs. The researchers justified the reasons for this choice due to the aesthetic value of these plants and being noninvasive. Guittonny-Philippe et al. [13] believed that resistant and reproductive macrophyte species, including Typha spp. or Phragmites australis in CWs might cause displacement of native plants or disrupt the natural cycles of plant replacement [13]. Therefore, the researchers insisted on providing consensual solutions that would achieve the objectives of treatment in CWs while ensuring natural biological diversity. Chlorophyll is the pigments that mainly exist in chloroplasts and drive photosynthesis by converting the absorbed light into chemical energy. Chlorophyll levels may decrease because of plant exposure to various forms of stress such as pollutants, drought, and other environmental factors [14].

This study is conducted to assess the stress of acetaminophen (AC) and methylparaben (MP) on three different types of ornamental plants and compare their responses. Photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll content) are monitored to detect the physical changes that appear on the plants.

### 2. Materials and Methods2.1Chemicals and plant material

This study was conducted outdoor at the University of Baghdad (25-35 °C). Two PPCPs were involved in this experiment, namely acetaminophen (Middle East Laboratories Co. Ltd, Iraq) and methylparaben (VWR Chemicals, UK). The properties of the relevant PPCPs are summarized in Table 1 [15]. Stock solutions of each acetaminophen and methylparaben at 500 mg/L were prepared in methanol and stored before use. Three types of ornamental plants (Alternanthera spp, Asparagus aethiopicus and Chlorophytum comosum) were purchased from Al- Zawraa Park, Baghdad, Iraq. Plants were selected due to their availability and suitability to the warm climate of Iraq. Plants were first acclimated to tap water for one week, and then homogenous plants were chosen for the experiments.

Table 1, Properties of AC and MP

Characteristics	Acetaminophen (AC)	Methyl Paraben (MP)
Molecular Formula	$C_8H_9NO_2$	$C_8H_8O_3$
Molecular weight (g/mole)	151.16 g/mol	152.15 g/mol
Water Solubility (mg/L)	14000 mg/L	25000 mg/L
Octanol-Water partitioning coefficient	0.46	1.96
(Log KOW)		

#### 2.2. Experimental setup

A total of 21 plastic pails with 3 L capacity each were used, which were divided into three groups as shown in Figure 1. The two groups (18 pails) were exposed to 20, 100 and 200 mg/L concentrations of acetaminophen and methylparaben in the three selected plants. In contrast, the third group (3 pails) was filled with tap water only as plant controls. Synthetic wastewater was prepared at three concentrations (20, 100 and 200 mg/L) of acetaminophen and

methylparaben, individually, then was fed at a volume of 1 L in batch mode into pails. The pails were planted with the relevant plant species (one plant which is homogeneous in weight with the other plants) in 2 kg of fine gravel (2–5 mm  $\phi$ ). High PPCPs concentrations were used in this study to simulate a hypothetical case of acute exposure. The concentration ranges were established based on the previous literatures [13, 14, 16, 17] that pointed out the low ranges of PPCPs concentrations observed in industrial effluents and water sources.

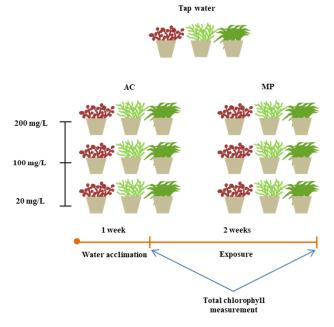


Fig. 1. Experimental setup for acute tolerance test.

#### 2.3 Chlorophyll content determination

The experiment was extended for two weeks. The chlorophyll content of all plants was determined at the end of the experiment (day 14) and compared with the initial values (day 0). Visual observation was also adopted to monitor the changes that occur in the growth of plants during the experiment period to determine their

tolerance for the high concentrations of pollutants and survival.

Chlorophyll content was conducted in two replicates for each plant. It was determined spectrophotometrically according to Liu et al. [18] at intervals of 0 and 14 days after the first application of acetaminophen and methylparaben. Briefly, 0.2 g of fresh plant samples (particularly, from leaves) were homogenized in 20 mL of 80% acetone with about 0.2 g of quartz sand and calcium carbonate until the samples turned white.

The homogenate left for 3–5 min was then centrifuged and filtered into a brown volumetric flask of 25 mL-metered volume [18]. The absorbance of the supernatants was measured at 663 and 645 nm via SpectroScan (80D, USA). Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content were calculated according to the following equations [18]:

Chlorophylla 
$$\binom{mg}{g}$$
 =  $\frac{(12.21 \times A_{663}) - (2.81 \times A_{645})}{FW}$  ... (1)  
Chlorophyllb  $\binom{mg}{g}$  =  $\frac{(20.13 \times A_{645}) - (5.03 \times A_{663})}{FW}$  ... (2)

 $Totalchlorophyll(\binom{mg}{g}) = chlorophylla + chlorophyllb \dots (3)$ 

with A663 and A645 are absorbance at 663 nm and 645 nm for chlorophyll a and b, respectively, and FW is the fresh weight (g) of the plant sample.

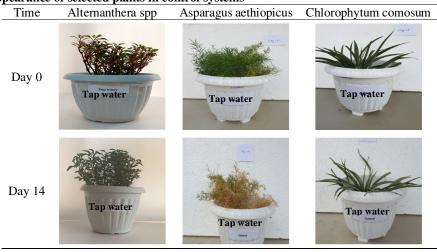
#### 2.4 Statistical analysis

All statistical analysis were performed using SPSS Version 21 (IBM, USA). The univariate analysis of variance (ANOVA) was performed at a confidence level of 95% (p<0.05) to specify the influence of the independent variables on the total chlorophyll content in plants.

### 3. Results and Discussions3.1 Plant visual observation

The tolerance of three types of ornamental plants to relatively high concentrations of acetaminophen and methylparaben was tested for 14 days in order to determine the most suitable plant to be subsequently employed in CWs. Through visual observation, Alternanthera spp showed a superior tolerance to stress induced by both water and acetaminophen; as no signs of wilting were observed on plants in control systems and those that were exposed to acetaminophen involved concentrations (Table 2 and 3) during the experiment. On the other hand, the wilting and yellowing were observed on Alternanthera spp that were exposed to MP (Table 4), particularly at high concentrations (100 and 200 mg/L). On the contrary, the effects of wilting and yellowing appeared on Asparagus aethiopicus that were exposed to different concentrations of AC and MP from the fifth day of the experiment and reached to severe dehydration and plants death at the end of the experiment (Table 2, 3). The dehydration to which Asparagus aethiopicus was exposed was not related to the type or concentration of the pollutant but mainly associated with water stress; since severe dehydration and plant death were appeared on Asparagus aethiopicus in control systems, as shown in Table 2. As for Chlorophytum comosum, plants in control systems grew well, whereas yellowing was observed on the plants due to exposure to high concentrations (100 and 200 mg/L) of both pollutants.

Table 2, The physical appearance of selected plants in control systems



AC Concentration (mg/L)	Time	Alternanthera spp	Asparagus aethiopicus	Chlorophytum comosu
20 —	Day 0	AC 20 mg/L	AG 20 mg/L	AC 20 mg/L
	Day 14	AC 20 mg/L	AC 20 mg/L	AC 20-mg/L
100 —	Day 0	ACTION mg/L	AC 100-mg/L	AC 100 mg/L
	Day 14	AC 100 mg/L	AC 100 mg/L	AC 100 mg/L
200 —	Day 0	AC 200 mg/L	AC 200 mg/L	AC 200 mg/L
	Day 14	AC 200 mg/L	AC 200 mg/L	AC 200 mg/L

Table 4, The physical appearance of selected plants in methylparaben-exposed systems

Methylparabalen Concentration (mg/L)	Time	Alternanthera spp	Asparagus aethiopicus	Chlorophytum comosum
20 -	Day 0	MP 20 mg/L	MP 20 mg/L	MP 20-mg/L
	Day 14	MP 20 mg/L	MP 20 mg/L	MP 20 mg/L
100	Day 0	Method marathet men and the marathet men and the men a	MP 100 mg/L	MP 100 mg/L
	Day 14	MP 100 mg/L	MP 100 mg/L	MP 100 mg/L
200 -	Day 0	MP 2000 mg/L	MP 200 mg/L	MP 200 mg/L
	Day 14	MP 200 mg/L	MP 200 mg/L	MP 200 mg/L

#### 3.2 Chlorophyll status in plants

The reduction in photosynthetic ability can be adopted as an explicit indicator of phytotoxicity

that is resulted from exposure to pollutants, as it often arises earlier than the decline in growth parameters [19]. The photosynthetic pigments, is represented by chlorophyll a, chlorophyll b, and

total chlorophyll, are measured to closely assess the healthy growth trend in the relevant plants during the experiment period. Initially, the differences in total chlorophyll content among the three plants involved in the study are not significant; thereafter (day 14), significant differences were observed between Asparagus aethiopicus and the other two plants (Alternanthera spp. and Ahlorophytum comosum)

in all exposure lines (AC, MP and tap water). In contrast, the differences between Alternanthera spp. and Chlorophytum comosum are found to be not significant in all exposure lines. In accordance with the visual observation, the highest reduction in chlorophyll content in control systems (Figure 2) is 81.4% in Asparagus aethiopicus, followed by 15.9% in Chlorophytum comosum and only 6.5% in Alternanthera spp.

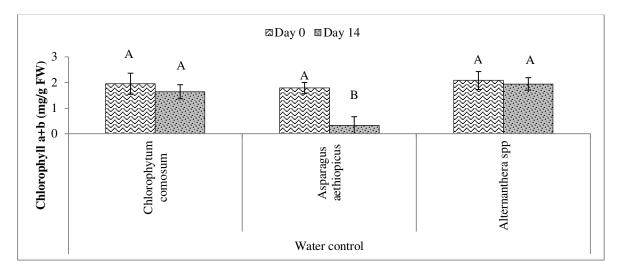


Fig. 2. Chlorophyll content (a+b) in control systems. Error bars refer to standard deviation (n = 2).

In the systems that are exposed to AC (Figure 3), the reduction of the total chlorophyll content in Asparagus aethiopicus is significant for all concentrations (84.9%, 64.6% and 86% for acetaminophen concentrations of 20, 100, and 200

mg/L, respectively). On the other hand, the reduction that is ranged from moderate (3.9–31.9%) to marginal (4.4–12.3%) in Chlorophytum comosum and Alternanthera spp, respectively.

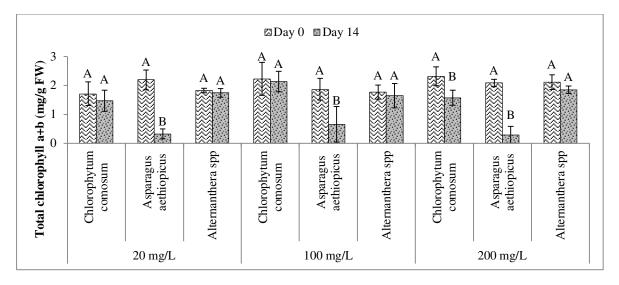


Fig. 3. Total chlorophyll content (a+b) in acetaminophen-exposed systems. Error bars refer to standard deviation (n=2).

The reduction in chlorophyll content at MPsystems is sharp (Figure 4) for all plants compared to the reduction achieved acetaminophen. Similarly, Asparagus aethiopicus is the most affected as the reduction in the total chlorophyll content that is ranged between 77.2% to 92.7%. Furthermore, MP causes a 32.6-39.1% chlorophyll reduction in Chlorophytum comosum and 32.6-40.9% in Alternanthera spp. Ismail et al. [20] confirmed that salicylic acid, at a certain concentration (0.10 mM), could improve the chlorophyll content (73.5 µg/g DW) in Ficus deltoidea jack var. trengganuensis; however, the higher concentration (1.00 mM) caused a clear reduction in chlorophyll content (42.1 µg/g DW). Baccio et al. [19] investigated the morphophysiological and biochemical effects of the environmental and high levels of ibuprofen (0.02,

0.20 and 1 mg/L) in Lemna gibba L. which was considered the smallest ecological indicator for contamination in aquatic environments. The authors confirmed that the detected changes in growth and photosynthetic rates were not sufficient for inducing phytotoxic effects. Kudrna et al. [21] reported that a high concentration of acetaminophen (5 mM) could reduce the intensity of photosynthesis in Lactuca sativa by a maximum of more than 31% compared to the control. Wijaya et al. [22] emphasized that ibuprofen exposure (0, 400, 800, 1,200, 1,600, 2000 mg/L) caused a clear reduction in total chlorophyll content in cowpea (Vigna unguiculata). However, the authors pointed out ability of the cowpea to develop morphological adaptations to cope under ibuprofen stress.

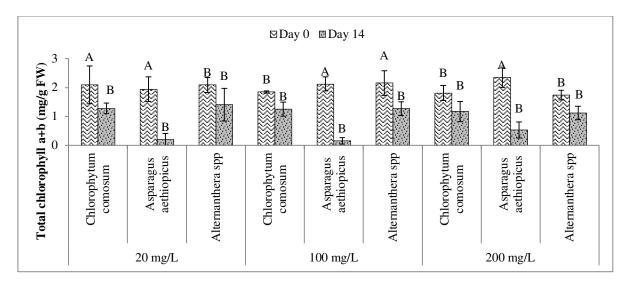


Fig. 4. Chlorophyll content (a+b) in methylparaben-exposed systems. Error bars refer to standard deviation (n=2).

#### 4. Conclusion

Ornamental plants that are involved in the study perform differently towards the stresses that are caused by exposure to acetaminophen and methylparaben. Alternanthera spp Chlorophytum comosum can tolerate high concentrations of AC without obvious symptoms of suffering, as the total reduction in chlorophyll content does not exceed 12.3% and 31.9% for Alternanthera spp and Chlorophytum comosum, respectively, highlighting the potential of these species for AC elimination in aquatic systems; however, these plants are less tolerant with similar methylparaben concentrations. Water

causes yellowing and death of Asparagus aethiopicus during the experiment, and therefore this plant is unqualified for use in constructed wetlands under hydroponic conditions. It becomes clear that proper selection of plant species in CWs may comprehensively improve the pollutant removal rates due to the different responses of the relevant plant species to PPCPs.

#### Acknowledgements

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## تقييم تحمل نباتات الزينة للتعرض الحاد للأسيتامينوفين والميثيل بارابين في الأراضي الرطبة المشيدة - دراسة أولية

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#### الخلاصة

هدفت الدراسة إلى اختيار أنواع نباتات الزينة المناسبة التي يمكن أن تقاوم تراكيز عالية من مادتي الاسيتامينوفين والميثيل بارابين في الأراضي الرطبة المشيدة. تم فحص نباتات النمول، الهليون الخشن والغيلان الزاحف لتحمل ثلاثة تراكيز أولية (٢٠ و ٢٠٠ و ٢٠٠ مجم / لتر) من مادتي الاسيتامينوفين والميثيل برابين بشكل والميثيل بارابين. تم استخدام ٢١ دلو بلاستيكي ذو سعة ٣ لتر لتعريض نباتات الزينة المذكورة لثلاث تراكيز من الاسيتامينوفين والميثيل برابين بشكل منفصل (٩ دلاء لكل مادة)، بينما تعرفت النبات البالمات اليالماء في الدلاء الثلاث المتبقية التي مثلت نباتات السيطرة. أوضحت النبائج أن كلا من نباتي النمول والغيلان الزاحف قد أظهرا تحملاً جيدًا للأسيتامينوفين مع انخفاض في محتوى الكلوروفيل الكلي بحوالي ٤٠٤-٣١٣٪ و ٣،٩-١٩،٩٪ في نبات النمول والغيلان الزاحف، على التوالي. علاوة على ذلك، كان من الواضح أن التراكيز العالية (١٠٠ و ٢٠٠ مجم / لتر) من ميثيل بارابين أثرت سلبًا على محتوى الكلوروفيل للأنواع النباتية الملوثات الدوائية ومنتجات العناية الشخصية ويمكن اعتبارها علاجًا جماليًا لمياه الصحي في المستشفيات.