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### TOXICITY TEST OF *Scirpus grossus* AND *Eichhornia crassipes* FOR TEXTILE WASTEWATER PHYTOREMEDIATION

Nurull Muna Daud\*, Siti Rozaimah Sheikh Abdullah\*, Hassimi Abu Hasan and Nur 'Izzati Ismail

Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

\*Corresponding Author: [nurullmuna.daud@gmail.com](mailto:nurullmuna.daud@gmail.com); [rozaimah@ukm.edu.my](mailto:rozaimah@ukm.edu.my)

#### Abstract

This study was conducted to identify the ability of two of Malaysia's native tropical plants (*Scirpus grossus* and *Eichhornia crassipes*) to survive in real textile industry wastewater with COD content up to 1391 mg/L. Wastewater sample was taken from textile industry factory plant located in Kepong, Kuala Lumpur. *S. grossus* and *E. crassipes* plants were known to degrade contaminants from various types of wastewater. In this study, the toxicity test of both plants was conducted to identify its capability to degrade COD in real textile industry wastewater. Both plants were exposed to five different concentrations (100%, 50%, 25%, 12% and 6%) of textile wastewater for 21 days. Subsequently, the plants were physically and visually observed every seven days. The range Finding Test (RFT) method was used to monitor any changes in both plants. Cumulative Effect Percentage (CEP) analysis was also carried out to find the maximum concentration of textile industry wastewater that both plants can endure after the exposure period. The analysis indicated that *S. grossus* could survive in textile industry wastewater with COD concentration up to 565 mg/L (50%) while *E. crassipes* can only survive in COD concentration up to 249 mg/L (25%). The findings suggest that *S. grossus* has a higher tolerance level in the textile industry wastewater.

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#### Keywords:

Aquatic plants, Cumulative effect percentage, Native plants, Preliminary test, Textile wastewater

#### INTRODUCTION

One of the major problems of the dye-based industry is the production of a large volume of highly coloured wastewater [1]. Evidence has shown that dye-contained wastewater is considered the most polluting wastewater [2]. This is because dye-based wastewater can degrade the water quality by increasing the colour and turbidity of water [3]. In addition, the dye substances used in the dyeing process are toxic and carcinogenic [4]. Moreover, the dark colour of these substances may block sunlight from entering the water stream, where it may interrupt the aquatic ecosystem [5]. Therefore, wastewater, which is not appropriately treated, pollutes the environment and increases the expenditure spent by the government. Hence, there should be rigid actions to ensure the environment's sustainability as dye wastewater may disturb aquatic life.

Phytoremediation technology has been evidenced as an appropriate technology for developing countries like Malaysia [6]. Phytoremediation is a plant-based green technology with a simple design, environmentally friendly and cost-effective [7]. Moreover, phytoremediation technology is widely known as an alternative to conventional physicochemical and biological treatment. Thus, phytoremediation technology could be an alternative treatment for textile wastewater in Malaysia. Furthermore, conventional methods available are not cost-effective in contrast to phytoremediation technology. There are several mechanisms of contaminants uptake in phytoremediation: phytostabilization / phytoimmobilization, phytofiltration / rhizofiltration, phytostimulation / rhizodegradation, phytoaccumulation/phytoextraction, phytodegradation and phytovolatilization [8]. Many studies have applied phytoremediation technology that uses the plants to

remediate soil, groundwater, and sediment by extracting and degrading the contaminants [9; 10; 11]. In this study, two native plants; *S. grossus* and *E. crassipes* were chosen to treat wastewater generated from the textile industry. These two plants are common and can be easily grown in Malaysia.

Phytoremediation technology using *S. grossus* has been shown to treat various types of wastewater, including petroleum industry wastewater [12], heavy metal wastewater [9] and domestic wastewater [13]. A previous study was conducted to determine the potential of *S. grossus* for dye wastewater phytoremediation [14]. This study used synthetic wastewater with COD content up to 400 mg/L. The findings revealed about 38-86% removal of 200-1000 mg/L of dye and 58% of COD. On the other hand, *E. crassipes* has been widely used for dye removal in the past few years [15]. On that account, a recent study demonstrated the capability of *E. crassipes* to treat textile wastewater [16]. Nonetheless, their study was conducted for textile wastewater with COD content up to 250 mg/L, in which most of the previous research has applied COD content not more than 1000 mg/L. However, a study conducted by Pavithra and Kousar (2016) had managed to reduce COD content of textile wastewater from 1530 mg/L to 385 mg/L using *E. crassipes* after seven days of exposure [17].

Apart from these reported studies, there is a lack of studies using real textile wastewater with COD content of more than 1,000 mg/L. Hence, a toxicity test for phytoremediation of textile wastewater was conducted using *S. grossus* and *E. crassipes* to evaluate the ability of these two native plants to survive in real textile industry wastewater with COD content up to 1,391 mg/L. The results obtained from this toxicity study analysis are vital to identify the right plant and estimate concentration of textile wastewater to be used for future phytotoxicity test.

## MATERIALS AND METHODS

### Propagation of *S. grossus* and *E. crassipes*

The parent plants of *S. grossus* were obtained from Tasik Chini, Pahang, Malaysia, while *E. crassipes* parent plants were obtained from a public lake in Bangi, Selangor. The *S. grossus* plants were propagated in a pail containing garden soil with a 3:2:1 ratio of soil, fertilizer and sand. In contrast, the *E. crassipes* were propagated in a 15 L container filled with 5 L of water under ambient temperature and sunlight in a greenhouse located at Universiti Kebangsaan Malaysia. A total of 54 healthy and matured plants (4-6 weeks old) of *S. grossus* and *E. crassipes* were chosen and exposed to five different concentrations of textile industry wastewater.

### Wastewater Sampling

The wastewater sample was collected from a textile industry factory plant in Kepong, Kuala Lumpur. The sample was collected at the endpoint before the wastewater was sent to the treatment plant. The wastewater characteristics were further analysed according to the standard APHA method [18] at the Environmental Laboratory, Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, National University of Malaysia (UKM). The characteristics of the textile wastewater with neutral pH between 7.17-7.51 and COD range of 1030-1391 mg/L (**Table 1**).

The textile industry wastewater was diluted into five classes with 100%, 50%, 25%, 12% and 6% of waste concentrations. The classification of the COD ranges for each waste concentration was presented in **Table 2**. For each waste concentration, three replicates of plants were prepared and used during the exposure period. In addition, three pails with three planted plants were prepared as control (F).

### Preparation of Experimental Setup

The batch mode was used in this toxicity test. The experiment was carried out in a 3 L pail containing 2 kg of sand for *S. grossus*, while 15 L container containing 5 L of water was used for *E. crassipes*. Each pail/container was planted with three plants. The configuration of pails/containers and experimental set up are presented in **Figure 1** and **2**. **Figure 1** illustrates the configuration set up for *S. grossus*, which used sub-surface flow while **Figure 2** illustrates the configuration set up for *E. crassipes*. Then, the mature plants (4-6 weeks) were exposed to five different concentrations of textile industry wastewater as presented in **Table 2**. Besides, three replicates of plants were prepared for each wastewater concentration. The test was carried out for 21 days.

### Toxicity Analysis

In phytoremediation treatment, toxicity analysis is needed to determine the range or maximum concentration of wastewater that any plant can survive to live in. Toxicity analysis of wastewater to plants can be carried out using the Range Finding Test (RFT) method [21]. The growth of plants was observed physically and visually for every seven days. Any changes were recorded until Day-21. After 21 days, the cumulative effect percentage (CEP) was determined using Equation (1) by calculating number of dead plants every 7 days.

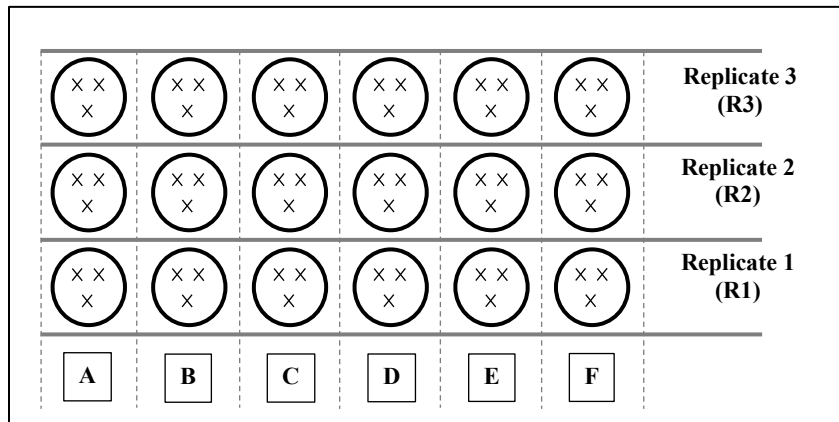
$$\text{Cumulative effect percentage (\%)} = \frac{\text{Number of dead plant}}{\text{Number of total plant}} \times 100 \quad (1)$$

**Table 1:** Characteristics of textile industry wastewater.

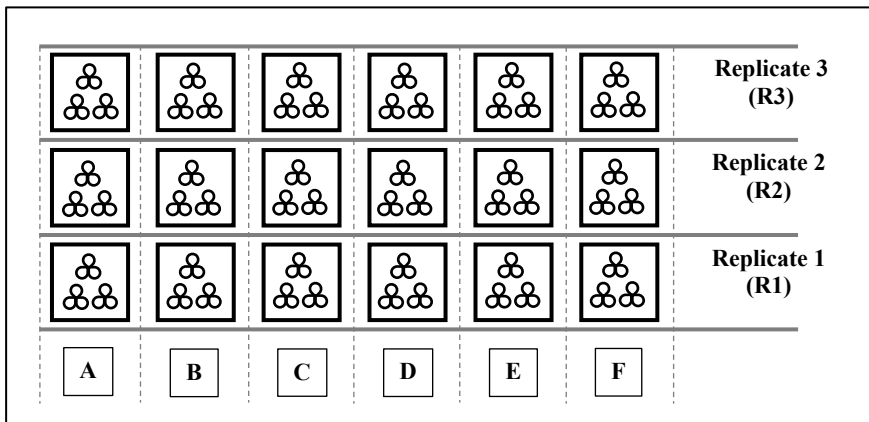
Parameter	Value	Quality standard (EQA 1974) [18; 19]	
		A	B
pH	7.17-7.51	6.0-9.0	5.5-9.0
Colour (ADMI)	1871	100	200
Turbidity (NTU)	971	-	-
COD (mg/L)	1030-1391	80	250
TSS (mg/L)	1045	50	100

**Table 2:** Exposure concentrations of textile industry wastewater.

Classification	Wastewater concentration (%)	COD concentration (mg/L)
A	100	1030-1391
B	50	440-565
C	25	207-249
D	12	56-114
E	6	20-61
F	Control	0



**Figure 1:** Configuration set up for *S. grossus* plants.



**Figure 2:** Configuration set up for *E. crassipes* plants.

## RESULTS AND DISCUSSION

### Toxicity Analysis Through Physical Observation

The study observations were recorded for every seven days throughout 21 days either healthy (overall plant was in green colour), withered (colour of some parts of the plant changed to yellow) and died (the whole plant was in brown colour and dried). **Table 3** summarizes the growth of *S. grossus*, while **Table 4** summarizes the growth of *E. crassipes* from Day-1 to Day-21. **Table 3** demonstrates that all plants that were planted in every pail were healthy at Day-1. Nevertheless, at Day-7, all plants in pail A with COD content of 1030-1391 mg/L was withered while all plants in pail B with COD content of 440-565 mg/L started to wither. In pail C, one plant started to wither and another two plants were remained healthy. All plants in pail D, E and F were healthy and one sprout plant grew in pail F. Observation at Day-14, demonstrated that a total of three sprout plants grew in pail F, and there were three sprout plants grew in each pail D and E. All plants in pail C were withered, however, two sprout plants grew in pail C. In pail B, all plants were withered while all plants in pail A died. At Day-21, one plant died in each pail B, and C. Two plants in each pail D and E were started to wither. In pail F, another one sprout plant grew and the sum of growth of the sprout plants in pail F was four.

For the growth of *E. crassipes*, the plants were recorded healthy at Day-1 (**Table 4**). At Day-7, all plants in container A started to die, while all plants in container B were withered. Only plants in container D, E and F were found healthy. During observation at Day-14, all plants in container A and B died. All plants in container C with COD content of 207-249 mg/L were found to be withered. The results revealed that only one plant in container D was healthy while another two were started to wither. Moreover, all plants in container C died at Day-21. Meanwhile, all plants in container D (COD content: 56-114 mg/L) and E (COD content: 20-61 mg/L) were withered and only plants in container F were found healthy. Purwanti et al. 2018 stated that physical changes of plants happened when waste concentration inhibits the metabolism of plants.

The visual observation of the textile industry wastewater at Day-1 and Day-21 demonstrates that both plants had the capability to degrade the colour of the wastewater. Priya & Selvan (2014) stated that *E. crassipes* are very efficient in removing pollutant and heavy metal [2]. Moreover, another study reported that *E. crassipes* are very tolerant to remove dye, where these plants easily grow, mainly in the contaminated region [16]. It can be clearly seen between

container E at Day-1 and Day-21. However, no sprout/shoot of *E. crassipes* plant was grew during this analysis. Nevertheless, the findings revealed that their growth was limited at certain COD content of the textile industry wastewater. For example, at Day-7, all plants started to wither in container C with COD content up to 249 mg/L.

Another study reported that *E. crassipes* managed to grow well in a waste concentration of COD around 200-300 mg/L [22]. Increment of COD to higher concentration up to 500 mg/L had limited their growth but still managed to remove about 60% of COD. These findings were in agreement with Shah et al. (2010) where it was reported that *E. crassipes* had superior performance in 20-25% wastewater concentration and not in 75-100% wastewater concentration [23]. This study implies that *E. crassipes* can survive and work efficiently in a less polluted environment. Lowering the concentration of the wastewater can increase *E. crassipes*'s performance or additional pre-treatment of wastewater may also help.













### Comparative Analysis

Based on toxicity analysis, withered or died plants can be observed in higher concentration. However, *S. grossus* were observed to be more tolerant and resistant to higher wastewater concentration compared to *E. crassipes*. **Figure 3** illustrated the number of living plants from Day-1 until Day-21 for each wastewater concentration. It can be seen that for 100% waste concentration (COD content: 1030-1391 mg/L), both plants died at Day-14. For 50% wastewater concentration (COD content: 440-565 mg/L), *S. grossus* were still found alive. What makes *S. grossus* can be conclude as more resistant plants is that sprout plants were found in wastewater concentration up to 25% (COD content 207-249 mg/L). Analysed from the number of dead plants, *E. crassipes* were found to die sooner compared to *S. grossus*. **Figure 4** illustrated the percentage of cumulative effect (dead plant) after 21 days of preliminary test for both plants.











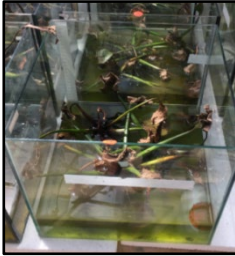
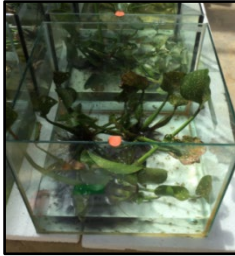
Based on **Figure 4**, after 21 days of exposure to textile wastewater with different concentrations, it can be concluded that both plants could not survive in COD range of 1030-1391 mg/L. About 33% of *S. grossus* died in the exposure to textile wastewater with COD ranged from 207-565 mg/L. **Figure 4** also illustrates that 100% of *E. crassipes* died in textile wastewater with COD ranged from 207-1391 mg/L after 21 days of exposure. This showed that the capability and resistance of *S. grossus* to phytotreat textile wastewater was higher than *E. crassipes*.

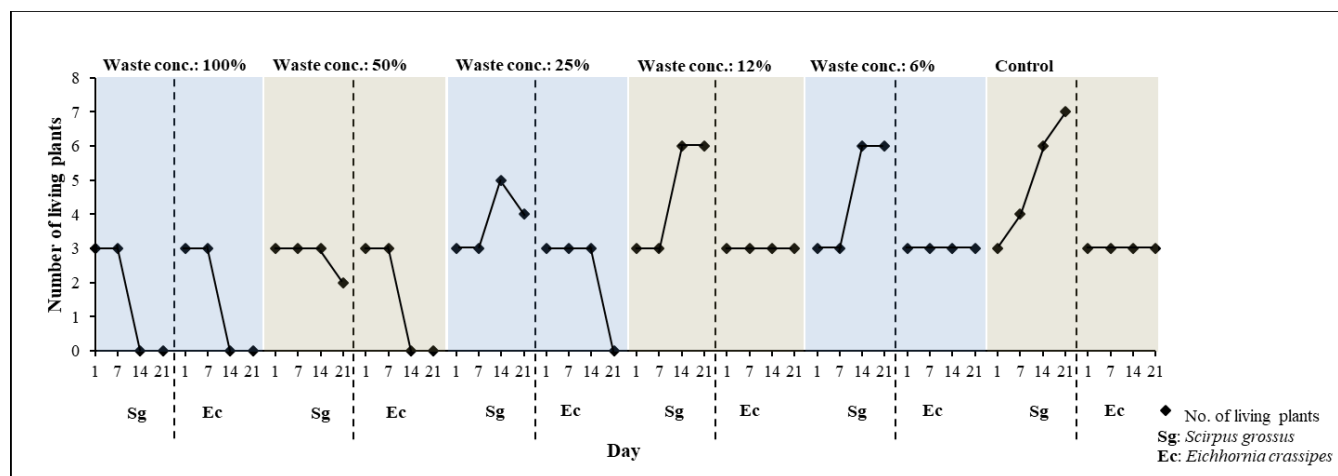


**Table 3:** *S. grossus* plants growths observation for 21 days.

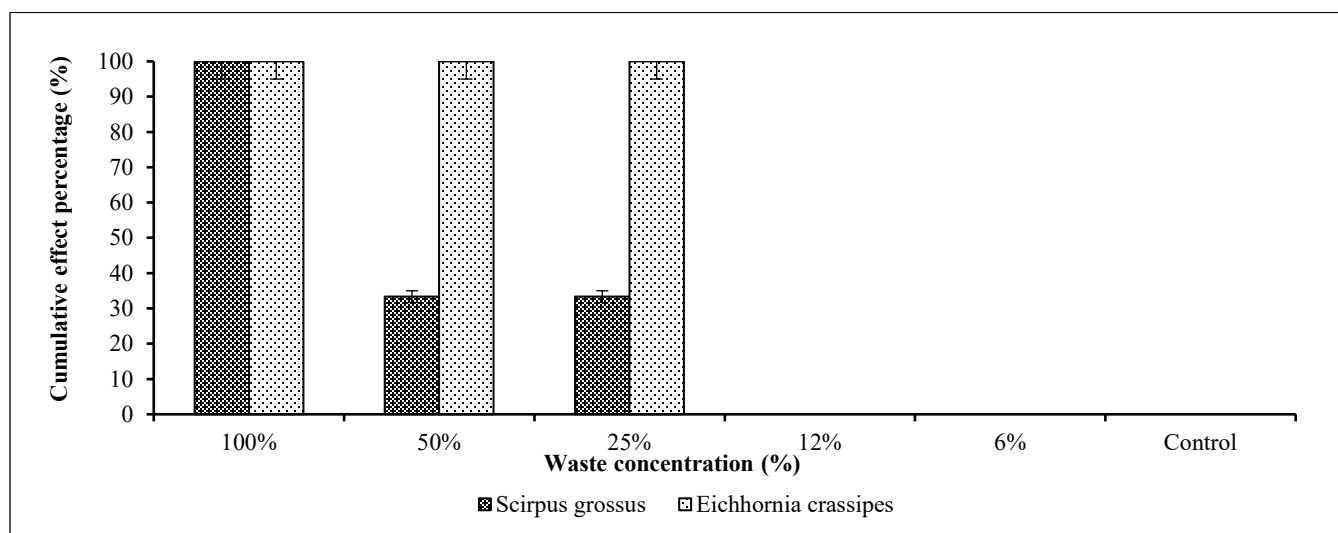
Day	Classification					
	(A)	(B)	(C)	(D)	(E)	(F)
Day 1	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>
Day 7	<ul style="list-style-type: none"><li>• All three plants withered.</li></ul>	<ul style="list-style-type: none"><li>• All three plants started to wither.</li></ul>	<ul style="list-style-type: none"><li>• Two plants were healthy.</li><li>• One plant started to wither.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li><li>• One sprout plant grew.</li></ul>
Day 14	<ul style="list-style-type: none"><li>• All three plants died.</li></ul>	<ul style="list-style-type: none"><li>• All three plants withered.</li></ul>	<ul style="list-style-type: none"><li>• All three plants withered.</li><li>• Two sprout plants grew.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li><li>• Three sprout plants grew.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li><li>• Three sprout plants grew.</li></ul>	<ul style="list-style-type: none"><li>• All three plants were healthy.</li><li>• Three sprout plants grew.</li></ul>
Day 21	 <ul style="list-style-type: none"><li>• All three plants died.</li></ul>	 <ul style="list-style-type: none"><li>• Two plants withered.</li><li>• One plant died.</li></ul>	 <ul style="list-style-type: none"><li>• Two plants withered.</li><li>• One plant died.</li><li>• The growth of two sprout plants grew.</li></ul>	 <ul style="list-style-type: none"><li>• One plant was healthy.</li><li>• Two plants started to wither.</li><li>• The growth of three sprout plants.</li></ul>	 <ul style="list-style-type: none"><li>• One plant was healthy.</li><li>• Two plants started to wither.</li><li>• The growth of three sprout plants grew.</li></ul>	 <ul style="list-style-type: none"><li>• All three plants were healthy.</li><li>• The growth of four sprout plants grew.</li></ul>

**Table 4:** *E. crassipes* plants growths observation for 21 days.

Day	Classification					
	(A)	(B)	(C)	(D)	(E)	(F)
Day 1	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>
Day 7	<ul style="list-style-type: none"> <li>• All three plants started to die.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants withered.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants started to withered.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>
Day 14	<ul style="list-style-type: none"> <li>• All three plants died.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants died.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants withered.</li> </ul>	<ul style="list-style-type: none"> <li>• One plant was healthy.</li> <li>• Two plants started to withered.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>	<ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>
Day 21	 <ul style="list-style-type: none"> <li>• All three plants died.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants died.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three started to die.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants withered.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants started to wither.</li> </ul>	 <ul style="list-style-type: none"> <li>• All three plants were healthy.</li> </ul>



**Figure 3:** Number of living plants based on Range Finding Test (RFT) analysis.



**Figure 4:** Cumulative Effect Percentage (CEP) profile of *S. grossus* and *E. crassipes*.

## CONCLUSIONS

The findings revealed that *Scirpus grossus* and *Eichhornia crassipes* demonstrated their capability to treat textile industry wastewater. Nonetheless, the plant's growth revealed that *S. grossus* could survive in textile wastewater with COD concentration up to 565 mg/L while *E. crassipes* could only survive in COD concentration up to 114 mg/L. By considering the cumulative effect percentage and the number of sprout plant growth, it can be concluded that the performance of *S. grossus* is superior to *E. crassipes*. *S. grossus* could be applied in a phytotoxicity test for the next study after showing the tolerance to textile industry wastewater with COD content up to 565 mg/L.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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