

## The Ratio of Plant Numbers to the Total Mass of Contaminant as One Factor in Scaling-up Phytoremediation Process

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### Graphical abstract



### Abstract

A toxicity test is carried out to select the contaminant concentration that a plant can tolerate in phytoremediation. We focused on the ratio of plant numbers to the total mass of contaminant as the factor for selection of contaminant concentration, nature of soil type and plant species in a diesel phytoremediation project. Based on the results of a preliminary test, *Scirpus grossus* could survive when the ratio of plant numbers to the total mass of diesel was more than 0.15 for 3% ( $V_{\text{diesel}}/V_{\text{water}}$ ) after 14 days of exposure in a sub-surface flow system (SSF) containing 780 mL of diesel contaminated water with 33% percentage of withered plants. In a phytotoxicity test containing 7 L of diesel contaminated water, *S. grossus* could also survive with the ratio more than 0.07 for 2% ( $V_{\text{diesel}}/V_{\text{water}}$ ) for 72 days of exposure with 36% percentage of withered plants. Based on the results of the preliminary and phytotoxicity tests, selection of diesel concentration in pilot scale containing 500 L of diesel contaminated water with 50 plants was fixed. Based on a minimum ratio of 0.05 from the preliminary and phytotoxicity tests, three diesel concentrations were selected to be 0.1, 0.175 and 0.25% to evaluate the performance of pilot reed bed to remediate diesel. Through the pilot study, the concentrations have resulted 10, 20 and 30% withered plants in the respective diesel concentration. The ratio of plant numbers to the total mass of contaminant must be considered as one factor to determine the phytotoxicity effects of the contaminant concentration in scaling-up a reed bed system for phytoremediation process.

**Keywords:** Phytotoxicity; phytoremediation; plants number; total mass; contaminant

### Abstrak

Satu ujian ketoksikan telah dijalankan untuk menentukan kepekatan bahan pencemar yang sesuatu tumbuhan boleh bertahan dalam proses fitoremediasi. Kajian ini menumpu kepada nisbah bilangan tumbuhan kepada jisim bahan pencemar sebagai salah satu faktor dalam pemilihan kepekatan bahan pencemar, jenis tanah dan jenis tumbuhan dalam projek fitoremediasi diesel. Berdasarkan kepada keputusan ujian awalan, *Scirpus grossus* boleh bertahan apabila nisbah bilangan tumbuhan kepada jisim pencemar melebihi 0.15 pada kepekatan 3% ( $V_{\text{diesel}}/V_{\text{water}}$ ) selepas 14 hari pendedahan dalam satu sistem sub-permukaan (SSF) yang mengandungi 780 mL air yang tercemar dengan diesel, dengan 33% tumbuhan layu. Seterusnya, dalam ujian fitotoksiti, yang mengandungi 7 L air tercemar diesel, *S. grossus* boleh bertahan pada nisbah melebihi 0.07 bagi kepekatan diesel 2% ( $V_{\text{diesel}}/V_{\text{water}}$ ) selama 72 hari pendedahan dengan 36% tumbuhan layu. Berdasarkan kepada nisbah minimum 0.05 dari keputusan kedua-dua ujian ini, tiga kepekatan diesel, 0.1, 0.175 dan 0.25% telah ditetapkan untuk menilai prestasi loji pandu tapak tumbuhan dalam merawat diesel. Melalui kajian loji pandu, ketiga-tiga kepekatan tersebut telah memberi kesan kelayuan sebanyak 10, 20 dan 30% dalam masing-masing kepekatan diesel. Oleh itu, nisbah bilangan tumbuhan kepada jisim pencemar perlu dijadikan salah satu faktor yang perlu dipertimbangkan dalam penskalaan tapak tumbuhan untuk menentukan kesan fitotoksiti oleh pencemar dalam proses fitoremediasi.

**Kata kunci:** Timbunan tinggi; lereng curam; gelongsoran; penurapan simen

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## 1.0 INTRODUCTION

Diesel usage in industry activity makes it one of the most harmful hydrocarbon pollutants. The hydrocarbon composition of diesel is toxic to the environment. Phytoremediation is the utilization of plants and plant-associated microorganisms to the restoration of contaminated water.

In many treatment projects, phytoremediation is used as a final step following the preliminary treatment of the high-level contamination. Nevertheless, when the concentration of a contaminant is low, phytoremediation alone may be the economical and effective treatment option<sup>1</sup>. Selecting this treatment to remediate wastewater after chemical precipitation or activated carbon adsorption may be an appropriate method for reducing the level of contamination that remain in the wastewater to below the standard effluent<sup>2</sup>. There have been many experimental studies and scientific reviews investigate the appropriateness of different plant species for the phytoremediation of hydrocarbon-contaminated water<sup>3,4,5</sup>.

Examination of the plant for use in a phytoremediation plan is an essential step. The plant must grow well, have tolerance to the contaminant and to the weather, and take up large amounts of the target contaminants<sup>6</sup>. This study aims to demonstrate the response of a Malaysian native plants, *Scirpus grossus* to different diesel concentrations and their toxicity to diesel through the determination of ratio of plant number to total mass of contaminant. A study was adopted to determine the effect of the ratio against diesel toxic effects on *S. grossus*.

*S. grossus* was used in this study due to its potential as a tolerance plant as shown in a previous study of phytotoxicity test<sup>7</sup>. This plant has fibrous roots in white to brown colour, triangular and solid stems, more than 2 m long leaves, with bisexual flowers grouped together<sup>8</sup>. It is a perennial tropical aquatic plant, the common names are giant bulrush, greater club rush and rumpit menderong in Malaysia. It is an aquatic perennial plant which is widely used to treat domestic wastewater in phytoremediation or wetland treatment<sup>9</sup>.

A study on diesel biodegradation using ornamental plant of *Impatiens balsamina* L. had a good tolerance to contaminated soils with  $\leq 10,000$  mg/kg of PHCs. After a 4-month culture period, the average degradation rate of total petroleum hydrocarbons (TPHs) by the plants was up to 18.1–65.0%, greatly higher than that in their corresponding controls of 10.2–35.6% by natural degradation<sup>10</sup>. Another study by Huesemann (2009) determined the capability of eelgrass, *Zostera marina* to remove polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from submerged marine sediments<sup>11</sup>. After 60 weeks of treatment, the concentration of total PAHs decreased by 73% in planted sediments but only 25% in unplanted controls. Similarly, total PCBs declined by 60% in the presence of plants while none were removed in the unplanted sediment. The objective of this study is to determine the diesel concentration for pilot scale study depend on the ratio of plant numbers to the total mass of contaminant obtained from preliminary and phytotoxicity tests.

## 2.0 MATERIALS AND METHOD

### 2.1 Plant Propagation

*S. grossus* were grown in a greenhouse using garden soil with ratio of top soil: organic: sand (3: 2: 1) in plastic crates (37 x 27 x 10 cm). *S. grossus* was planted from rhizomes for one month to be similar in size approximately about 50 cm in height.

### 2.2 Preparation of Plant Exposure to Diesel

All study was conducted in a greenhouse. The experimental plants were planted in sand matrix to obtain the pure toxic effects of contaminants diesel, because the amount of iron oxide, aluminum oxide and clay minerals in the sand is very small so that the impact of the adsorption of diesel by iron oxide, aluminum oxide can be reduced and concentrations of bioavailable diesel in the sand increase<sup>12</sup>. The sand was first sieved using a 4.75 mm sieve. Synthetic wastewater was prepared by mixing water with standard diesel obtained from a local Petronas petrol station at different concentrations. No additional nutrient solution was added throughout this study. During the study, plants were watered alternate days since the sand had a low moisture-holding capacity. Moisture was monitored using a moisture meter (Decagon ECH2O, USA), while pH and temperature of the spiked water were monitored using a pH-meter (Cyberscan pH 300, Singapore).

#### 2.2.1 Preliminary Test

Each pail, planted with 3 plants of aquatic bulrush *S. grossus* in 3 kg of fine sand with 780 mL of synthetic wastewater prepared by mixing tap water with standard diesel obtained from a petrol station with 870 g/L density<sup>13</sup> in different concentrations of 0 (control), 1, 2, 3, and 5% (Vdiesel /Vwater) as shown in Figure 1. Physical observation for 2 weeks was conducted to determine the ability of the bulrush to resist the hydrocarbon contaminant and survive. The number of withered plants was recorded and the percentage of withered plants was also determined using Equation 1.

$$\% \text{ Withered plant} = \frac{\text{No. of withered plant}}{\text{No. of total plant}} \times 100 \quad (1)$$



Figure 1 Preliminary test for bulrush *Scirpus grossus*

#### 2.2.2 Phytotoxicity Test

A phytotoxicity test was conducted for 72 days with sub-surface flow system. For this phytotoxicity test, 13 glass aquaria were used. Each aquarium, with dimensions of 30 × 30 × 30 cm (L × W × D) as shown in Figure 2, was filled with 20 kg fine sand and later topped up with 7 L diesel-spiked synthetic wastewater with concentrations of 0 (control), 1, 2, and 3% (Vdiesel /Vwater) in three replicates to decrease the error in experimental design.



**Figure 2** Phytotoxicity test of bulrush *Scirpus grossus* with sub- surface flow

**2.2.3 Pilot Constructed Wetland System**

The phytoremediation assessment was conducted in pilot constructed wetlands at a greenhouse in Universiti Kebangsaan Malaysia using the bulrush of *S. grossus*. Synthetic wastewater was prepared by mixing different concentrations of diesel with water. Each pilot constructed wetland was made from fibreglass, with dimensions of 1.8 m (L) x 0.9 m (W) x 0.9 m (H) and 0.5 cm thickness (Figure 3).



**Figure 3** A pilot scale reed bed with sub- surface flow system

The constructed wetlands were configured with a horizontal sub-surface flow system. Each tank was filled from the bottom to top sequentially with a layer of medium gravel ( 1–5 mm) with 15 cm in height, a layer of fine gravel ( 10–20 mm) with 15 cm in height, and a layer of fine sand ( 1–2 mm) with 20 cm in height.

The tank was filled with synthetic wastewater up to the surface of the sand medium through an inlet. Each reed bed was completed with a pipe at the bottom for sampling. A total of 500 L of diesel spiked water was filled into each constructed wetland and planted with 50 *S. grossus* plants.

**3.0 RESULTS AND DISCUSSION**

Table 1 summarize all the diesel concentrations as well as the ratio of plant numbers to the total mass of contaminant used in the preliminary and phytotoxicity tests. Based on the results of preliminary test, after 14 days of the diesel exposure to *S. grossus*, the percentage of withered plant was 33 and 67% for diesel concentrations 3 and 5% respectively as shown in Table 1. Based on the plant observation during the preliminary test (Table 2), *S. grossus* could survive up to 3% diesel. Plants were withered after 7 days of exposure in 2 and 3% diesel, but plants could still survive and became healthy in 1% after 7 days of diesel exposure. While *S. grossus* dried and died at 5% diesel after 7 days of

exposure until the end of exposure (Day-14). The ratio of plant numbers to diesel total mass (g) on the preliminary test is summarized in Table 1. The ratios were 0.44, 0.22, 0.15 and 0.09 for diesel concentrations of 1, 2, 3 and 5% respectively.

In the phytotoxicity test, *S. grossus* could survive at diesel concentrations of 1 and 2% until day 72 with growing branches and new leaves as shown in Table 3 with ratios of 0.15 and 0.07 respectively (Table 1) with 21 and 36% withered plants. In higher diesel concentration of 3% after 72 days of exposure, 50% of plants were withered and dried with a ratio of 0.05. Both results shows that higher plant numbers can survive at higher ratio of plants numbers to mass of contaminants. Depending on the ratio of plants numbers to the total mass of contaminant in the preliminary and phytotoxicity tests, the diesel concentrations were determined for pilot scale run. Pilot scale constructed wetlands were planted with 50 plants of *S. grossus* and the capacity of tank is 500 L.

**Table 1** Ratio of plant numbers to diesel total mass in phytoremediation of *S. grossus*

Test Study	Parameters	Data				
Preliminary test (14 days)	Concentration of diesel ( $V_{diesel} / V_{water}$ ) (%)	0	1	2	3	5
	Total volume of water (L)	0.780				
	Volume of diesel need (mL)	0	7.8	15.6	23.4	39
	Mass of diesel fed (g)	0	6.8	13.6	20.4	33.9
	Total no. of plants	3	3	3	3	3
	Ratio ( $\Sigma$ plant : total mass of diesel)	0	0.44	0.22	0.15	0.09
Phytotoxicity test (72 days)	Concentration of diesel ( $V_{diesel} / V_{water}$ ) (%)	0	1	2	3	
	Total volume of water (L)	7				
	Volume of diesel fed (mL)	0	70	140	210	
	Mass of diesel fed (g)	0	60.9	121.8	182.7	
	Total no. of plants	9	9	9	9	
	Ratio ( $\Sigma$ plant : total mass of diesel)	0	0.15	0.07	0.05	
Pilot reed bed (72 days)	Total volume of water (L)	500				
	Concentration of diesel ( $V_{diesel} / V_{water}$ ) (%)	0	0.10	0.175	0.25	
	Volume of diesel fed (mL)	0	500	875	1250	
	Mass of diesel (g)	0	435.9	762.8	1088.0	
	Total no. of plants	0	50	50	50	
	Ratio ( $\Sigma$ plant : total mass of diesel)	0	0.11	0.07	0.05	
Percentages of toxic effects after 14 days		0	0	0	33%	67%
Percentages of toxic effects after 72 days		0	21%	36%	50%	
Percentages of toxic effects after 72 days		0	10%	20%	30%	

**Table 2** Photos of *S. grossus* plants in a preliminary test

Time	Diesel concentrations (Ratio of plant numbers to diesel total mass)				
	Control	1% (0.44)	2% (0.22)	3% (0.15)	5% (0.09)
Day 0					
Day 72					

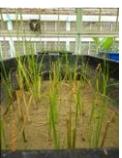
**Table 3** Photos of *S. grossus* plants in a phytotoxicity test

Time	Diesel concentrations (Ratio of plant numbers to diesel total mass)			
	Control	1% (0.11)	2% (0.07)	3% (0.05)
Day 0				
Day 72				

Based on the preliminary and phytotoxicity tests, we considered a minimum ratio of plant number to the total mass of 0.05 to determine diesel concentration in pilot reed bed run. Therefore, 0.1, 0.175 and 0.25% diesel concentrations with respective ratio of 0.11, 0.07 and 0.05 (Table 1) were selected to evaluate the performance of pilot reed bed to phytoremediate diesel.

Table 4 shows the images of the treatment using pilot scale reed bed for 0 day and the end of 72 days. The percentages of the toxicity effects after 72 days of exposure were 10, 20 and 30% under the diesel concentrations of 0.1, 0.175 and 0.25% as compared with the number of healthy plants.

**Table 4** Photos of *S. grossus* plants in a pilot scale reed bed

Time	Diesel concentrations (Ratio of plant numbers to diesel total mass)			
	Control	0.1% (0.11)	0.175% (0.07)	0.25% (0.05)
Day 0				
Day 72				

It shows that *Scirpus grossus* could survive with 30% of toxic effect percentages when the ratio was  $\geq 0.05$  for 72 days of exposure. Therefore, the ratio of plant numbers to diesel total mass was important to determine the contaminant concentration in the water for pilot scale run within the results of ratio from the preliminary and phytotoxicity tests.

#### 4.0 CONCLUSIONS

Based on a preliminary test study and phytotoxicity test, the limit of the ratio of plant numbers to the total mass of diesel can be determined to avoid the diesel toxic effect to *S. grossus* in pilot run study. A minimum ratio of 0.05 for the plant numbers to the total mass of contaminant was selected as a basis to determine diesel concentration in pilot scale run. The pilot scale was operated smoothly with a minimum ratio of 0.05 after 72 days of exposure without significant plant inhibition because the ratio used is within the plant survival as used in preliminary and phytotoxicity test. The possibilities of toxic effects to plants increase if the ratio of plant numbers to total mass of contaminant was lower. It can be concluded that ratio of plant numbers to the total mass of contaminant as one main factor that determines the toxicity effect on plants by a contaminant in scalin-up phytoremediation treatment of wastewater.

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