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Range Finding Test of Hydrocarbon on *Scirpus mucronatus* as Preliminary Test for Phytotoxicity of Contaminated Soil

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Abstract
Phytotoxicity is used to determine the maximum concentration of contaminant effect on plant growth in diesel fuel-contaminated soil. Range finding test is the first step that must be done to determine the concentration that the plant can survive in any contaminated soil. In this study, range finding test was conducted using *Scirpus mucronatus* to determine the maximum concentration of hydrocarbon that the species can survive. The test was conducted through a batch system, with each container containing media and three plants. The media used was spiked sand with dissolved diesel fuel in acetone of concentrations 0, 1, 5, 10, 15, 20, 50, 100, and 200 g/kg. Spiked sand was left for 2 weeks to homogenize the diesel and evaporate the acetone. Since the plants used were emergent plants, the containers contained 3L of de-ionized water. After 30 days of exposure, 33.3% of plants had withered in 5 and 10 g/kg; 66.6% in 15, 20, and 50 g/kg; and 100% in 100 and 200 g/kg of contaminant concentration.

Keyword: phytotoxicity, range finding test, hydrocarbon, *scirpus mucronatus*

Abstrak

Ujian fitotoksin dilakukan untuk menentukan kesan konsentrasi maksimum pencemar terhadap tumbesaran tumbuhan di tanih yang dicemari minyak diesel. Ujian penentu julat dilakukan dahulu untuk menentukan tahap konsentrasi yang tidak menjejaskan kehidupan tumbuhan di tanih yang tercemar. Dalam kajian ini, ujian seperti ini dilakukan dengan menggunakan spesies *Scirpus mucronatus* untuk menentukan tahap maksimum hidrokarbon yang dapat ditahan. Ujian dilakukan mengikut sistem kelompok. Setiap bekas mengandungi media dan tiga tumbuhan. Media yang digunakan ialah tanih yang bercampur minyak diesel dalam aseton dengan konsentrasi 0, 1, 5, 10, 15, 20, 50, 100, and 200 g/kg. Tanih itu dibiarkan selama 2 minggu supaya diesel itu dapat dihomogen dan aseton itu sejat. Selepas didedahkan selama 30 hari, didapati 33.3% tumbuhan itu menjadi layu dalam konsentrasi 5 dan 10 g/kg, 66.6% bagi konsentrasi 15, 20, dan 50 g/kg, 100% bagi konsentrasi 100 dan 200 g/kg pencemar.

Kata kunci: fitotoksin, ujian penentu julat, hidrokarbon, *Scirpus mucronatus*

Introduction

Phytotoxicity is used to determine the degree of contaminant effect on plant growth in contaminated soils. There are many kinds of plant used in phytotoxicity (Baek K.H et al., 2004) used corn (Zea mays) and red bean (*Phaseolusnipponesis* OWH1) to determine the effect of crude oil on plant growth. (Adam G. and Duncan H., 2002) had analyzed twenty five plants including grasses, legumes, herbs and commercial crops for their ability to germinate in diesel fuel contaminated soil (Trapp S. et al. 2001) used willow and poplar trees for phytotoxicity on diesel contaminated soil. According to (Hoffman D.J et al., 1995) parameter of plant that should be analysed during phytotoxicity are fresh weight, shoot or root length change over the duration of the test, or physical observations such as chlorosis, yellowing of the leaves, and cupping of the leaves.

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One step that must be done before phytotoxicity test is Range Finding Test. This test is a preliminary test for determining the concentration of contaminants which the plant species can survive in diesel-contaminated soil. It is an early step of a phytotoxicity study. Once the estimation is obtained, a detailed phytotoxicity test should be conducted to determine the exact concentration that can be adsorbed or degraded by the plant species (U.S.E.P.A, 1996).

Scirpus mucronatus is a monocot weed in the Cyperaceae family. This plant is known by the common name of ricefield bulrush. The local name in Malaysia of this plant is "rumput kerecut" (AgroBio Solution, 2011) and (DBP, 2005). It grows in moist and wet terrestrial habitat, and in shallow water. It is a perennial herb growing from a short and hard rhizome. The erect, three-angled stems grow in dense clumps and reach a meter tall. The leaves take the form of sheaths wrapped around the base of stem, but they generally do not have blades (Wikipedia, 2011). This plant also is planted in Putrajaya Wetland, Malaysia (Huat K.C, 2002). Figure-1 illustrates the *Scirpus mucronatus* species used in this study.

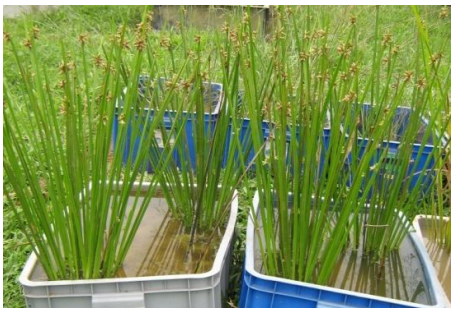

Figure 1. Picture of *Scirpus mucronatus*

In this study, a range finding test was conducted using Scirpus mucronatus to phytoremediate diesel-contaminated soil. It aims to determine the maximum concentration of hydrocarbon which will affect to the plant growth that can be used in subsequent phytotoxicity test. This project is a part of research work that will be continued for a phytoremediation project, that finally aims to identify local plant in Malaysia which can phytoremediate hydrocarbon contaminated soil.

Material and method

Spiked Sand

The media used is 4.75 mm sieved sand as much as 3 kg. The media was sieved to remove coarse fragments and obtain similar size (OECD Guideline 1984). The concentrations of diesel fuel spiked into sand are 0 (as a control), 1, 5, 10, 15, 20, 50, 100, and 200 g/kg (Trapp S. et al., 2001), (Adam G. 2002), (Saari E. et al., 2007) and (Njoku K.L et al., 2008). Diesel fuel obtained from a local gas station. The diesel fuel was mixed with acetone as a solvent (Trapp S. et al., 2001). The mixture of diesel and acetone was poured into the sand according to the acetone holding capacity (157.5 mL/kg). After pouring into the sand, the sand was stirred to become homogeneous. The spiked media is left for about 2 weeks prior the planting to ensure the diesel is aged and remove the acetone prior to its use for planting (Brinch U.C et al., 2002), (Hoffman D.J et al., 1995) and (Sawada A. et al., 2002). After 2 weeks, during planting, the container was filled with water as much as 3L to maintain the wetness of the sand since the plants used are aquatic plant.

Planting

There were three plants in each container. Figure 2 shows day 0 of the containers with different concentrations of contaminant. The observation on the number of withered plant was done within 30 days. The percentage (%) of withered plant in each concentration was determined by dividing the number of withered plants with the total number of plant in the container. The observation of plant growth was conducted physically and weekly.

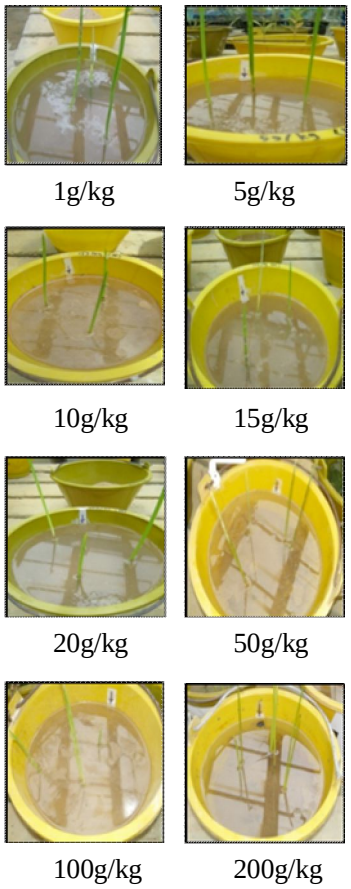

Figure 2: Observation on day 0 of Range Finding Test

Result and discussion

On the first week, observations were conducted daily. In the following week, observations were conducted weekly. After 4 days of exposure, plants in container with concentrations of 5, 10, 15, 100 and 200 g/kg had changed their colors, from green to yellow or brown. The plant in concentrations of 100 and 200 g/kg were whitered after 10 days of exposure; buds did not grow, and finally dried up 100% after 30 days. While the plant in concentration of 10, 15, 20 and 50 g/kg had started withering on day 15. On day 12, the first bud grows in containers with concentration of 1, 5, and 10 g/kg, similar to control container. In a research work done by (Green B.T et al., 1996), the lower number of plants containing living buds and flowers were observed for groups dosed with less than 100 mg/kg diesel fuel (low concentration of contaminant). Similar finding were also reported by (Thygesen R.S and Trapp S., 2002) and (Zhang Z. et al., 2010). Figure 3 shows the concentration of the plant on day 30 of range finding test.

During the range finding test, the media conditions were also monitored. The media condition includes temperature, pH, and ORP (Hoffman D.J. et al., 1995). The range of temperature of the media is around 34 -35o C. The temperature did not affect control plants, but might have caused increased stress to those containers containing diesel fuel (Green B.T. et al., 1996). The pH of media was around 6.2 – 6.5 and the ORP range for media was around 7 – 16 mV. While optimal range characteristic for *Scirpus tabernaemontani* were water temperatur 24.0 – 32.0oC, water depth is 10 – 50 cm, and soil pH 5.25 – 6.25 (Lee B.A. et al., 2007).

Based on the observation of physical plant growth for 30 days, Figure 4 illustrates the percentage of whitered plant on each container. After 30 days, 33.3% plant on containers of 5 and 10 g/kg were whitered, and 66.7% plants on containers of 15, 20, and 50 g/kg were also whitered.

Based on the observations, the higher the concentration of contaminants, the greater the whitered percentage and the faster whitered condition occurred. According to (Meudec A. et al., 2007), the increase of the pollution concentration induced more marked effects on plants, likely because of the physical effects of fuel. Besides that, hydrocarbon can be stabilized within the soil matrix, taken up by plants and transformed or stored in a non-phytotoxic form. Plants can also stimulate the rhizosphere microbial community that was capable of degrading organic contaminants [9]. Mechanism of hydrocarbon removal is by degradation in rhizosphere (root zone of influence) which is known as rhizodegradation. Microbial counts in rhizosphere soils can be 1 or 2 orders of magnitude greater than in nonrhizosphere (U.S.E.P.A 1996).

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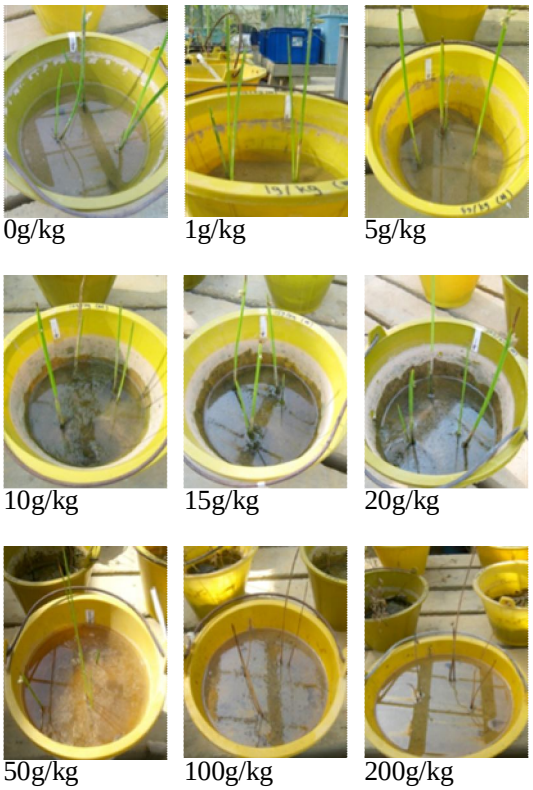

Figure 3. Observation on day 30 of Range Finding Test

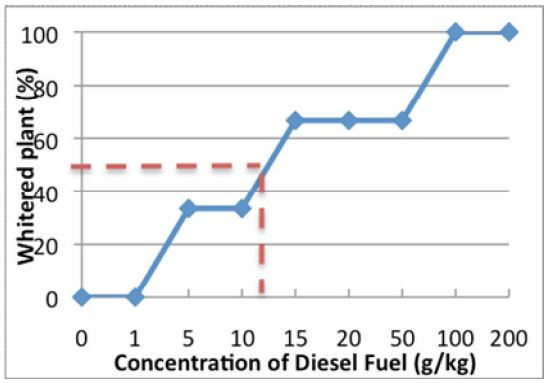

Figure 4. Whitered Plant of *Scirpus mucronatus* during range finding test

Figure 4 shows plant will achieve 50% whitered condition on 13 g/kg concentration of diesel fuel. So, the next test phytotoxicity of hydrocarbon will be conduct on a range concentration of 10 – 100 g/kg. (Adam G. and Duncan H., 2001) and (Jing W. et al., 2008) also use the concentration of contaminant in these ranges.

Conclusions

Through a range finding test, of 30 days exposure, 33.3% of *Scirpus mucronatus* species in 10g/kg of contaminants concentration. This plant were whitered 100% on 100 g/kg of contaminant concentration. Hence, the future phytotoxicity test will be conducted on 10 – 100 g/kg contaminant concentration of diesel.

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