PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 9(2): 331–335
https://doi.org/10.14719/pst.1442

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Range finding test and measurement of wet weight and dry weight of *Vetiveria zizanioides* (L.) Nash as an initial stage of phytoremediation of soil contaminated with used lubricants

Bieby Voijant Tangahu*, Alif Yoga Winata & Isni Arliyani

Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember (ITS), Surabaya 60111, Indonesia

*Email: voijant@its.ac.id

OPEN ACCESS

ARTICLE HISTORY

Received: 20 August 2021
Accepted: 23 December 2021

Available online
Version 1.0 (Early Access): 26 January 2022
Version 2.0: 01 April 2022

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Tangahu B V, Winata A Y, Arliyani I. Range finding test and measurement of wet weight and dry weight of *Vetiveria zizanioides* (L.) Nash as an initial stage of phytoremediation of soil contaminated with used lubricants. Plant Science Today 9(2): 331–335. https://doi.org/10.14719/pst.1442

Abstract

Environmental pollution caused by the development of industry and the rapid increase in the number of vehicles currently requires restrictions on the use of lubricants. Used lubricants contain complex mixtures of hydrocarbons that adversely affect plants by creating conditions that make essential nutrients such as nitrogen and oxygen needed for plant growth unavailable and cause severe problems in soil ecosystems and the rhizosphere. The accumulation of these spills can complicate the degradation process by microorganisms in the soil. Vetiver (*Vetiveria zizanioides* (L.) Nash) can grow at various levels of nutrients and insufficient abiotic conditions, so it is used in multiple studies as a hyperaccumulator plant. Preliminary research in determining the concentration of used lubricant handled by the vetiver can be determined through the Range Finding Test (RFT) stage. RFT is the stage of observing the plant's ability to maintain its life while degrading pollutants. Physical observations and laboratory analysis determine how strong the plants live in media contaminated with used lubricant pollutants. The results showed that vetiver could live in an environment contaminated with used lubricants with a concentration of up to 4% of the total mass of the media (40 ppm). Sample C2 had an average growth rate of 0.481 cm/day with a root elongation ratio of 0.333. The average dry fraction of plant leaves is 0.602 and the root is 0.372.

Keywords

Hyperaccumulator, Hydrocarbon, Rhizosphere, Soil pollution, Vetiver

Introduction

The growing industry and the rapid increase in vehicles have caused the need to maintain production machines and cars. Used lubricant waste that is no longer used is usually not managed according to procedures, especially in workshop businesses that do not have a sound waste management system. Used lubricating oil contains a complex mixture of paraffinic hydrocarbons, naphthalene, aromatic hydrocarbons and various contaminants containing one or more components (1). Soil has long been a reservoir for various environmental and industrial wastes. With the advent of the petroleum industry, soil pollution due to petroleum and its derivatives have become a problem. However, the global emphasis on soil health and sustainable food security persuades scientists to consider rehabilitating degraded land, significantly where oil contamination limits land use (2). Oil spills contain crude oil or refined oil products such as fuel oil and lubricating oil. Toxic compounds in crude oil consist of various hydrocarbons, nitrogen-oxygen

compounds, sulphur compounds and heavy metals, which can cause acute and chronic effects on flora and fauna (3).

Plants in aquatic and terrestrial areas can be exposed to chemical and physical damage by oil hydrocarbons. Contaminated plant leaves can reduce photosynthesis and temperature regulation, while root coating disrupts root structure and uptake of water and nutrients (4). The typical morphology of vetiver is an extensive and well-structured root system. Under optimal conditions, the plant grow up extensively with the root depths reaching up to 4 m in the first year. Showing exceptional penetrating power, vetiver roots can pass through difficult soils, including asphalt soils. Since the plant has no stolon or rhizomes, vetiver is easy to control. Usually, the root system grows straight down without competing with the surrounding vegetation. Therefore, this plant can full fill its own needs (5). Plants have an efficient mechanism for obtaining nutrients from the environment in low nutrient conditions and then transferring and storing them in specific organs. This mechanism is also carried out in absorbing toxic substances with chemical content similar to the essential substances needed by plants. The absorption of toxins, including heavy metals, can occur through several plant parts with a translocation mechanism (6). Root exudates of plant can initiate a microbial chemotactic response for root motility and the establishment of root colonisation, which stimulates the growth and activity of microorganisms to degrade organic pollutants (7).

The idea of this research comes from a large number of oil spills in various areas caused by workshops or illegal disposal of used lubricants and which results in the declining of. Research is needed to reduce the concentration of used oils in soil media using vetiver for these problems. Thus, efforts can be made to minimise the used lubricant from the pollution. This study was designed to assess the growth of vetiver plant at a certain acceptable concentration of lubricants to obtained effective growth as a preparation for research in the phytoremediation process.

Materials and Methods

Data Collection

The primary data needed in this study were obtained from observations and measurements at the study site and the effects of laboratory analysis. Measures at the study site included physical measurements of plants and media. Laboratory research consisted of measuring wet weight, dry weight and a dry fraction of plants.

Research Implementation

The implementation of this research was carried out by preparing the plant propagation. The broodstock was placed in a propagation tub with 63.5 g of soil and fertiliser as a medium. A sampling of tillers was then used for the acclimatisation phase and Range Finding Test (RFT) test. The tillers vetiver that has been obtained from propagation were then acclimatised for one week on sand media in a pot to which PHONSKA 15-15-15 NPK fertiliser to meet essential nutrients for microbes and plants, namely nitrogen, phosphorus and potassium. The following is the concentration of fertiliser added to the media:

N Content = 15%

$$\text{N (Concentration)} = \frac{\text{N Content} \times \text{Fertilizer weight})}{(100\ \% \times \text{media weight})}$$

$$= (15\% \times 20\ \text{g})/\ (100\ \% \times 12\ \text{kg}) = 0.025\ \text{g/kg}$$

$$= 25\ \text{mg/kg}$$

Concentration of N: P: K = 1: 1: 1

Prepared planting media was in the form of sand for testing in the pot. Used lubricants were obtained from home workshops as much as 5 litres. The lubricant was mixed and then stirred until homogeneous. The used lubricant was added to the pot with a concentration of 3% or 300 g lubricant, 4%, 5%, 6% and 7% of the total weight of the soil (10000 g) respectively. After the acclimatisation period around 1- 3 weeks samples of vetiver plants to be tested were selected, which were completely healthy and had a weight range of 90–100 g. The media was added with 100 l of water and then mixed manually until the media became homogeneous. The Vetiver that has weight 90–100 g approximately with the same leaves was cut per sample, then planted and given 25 g of PHONSKA 15-15-15 NPK fertiliser. Plants were given watering of 100 ml every day for up to 14 days to obtain the concentration of used lubricant used in the main test stage.

Plant samples were taken from the pot carefully so that the root system did not break during extraction; the plant sample were then separated into two parts are leaves and roots; the two parts were wrapped in aluminium foil and weighed its wet weight was calculated with the formula:

Wet weight (g) = Weight of plant parts and aluminum foil (g) –aluminum foil weight (g).

After obtaining the wet weight, the plant parts, together with the aluminum foil, were put in an oven at 105 ° C for 24 hrs to remove the water content contained in the plant parts. After that, the sample was placed in a desiccator for 15 min to reach room temperature. The sample wrapped in aluminum foil was then weighed on an analytical balance to obtain the dry weight of the plant parts using the formula:

Dry weight (g) = Weight of plant parts after dry and aluminum foil (g) – aluminum foil weight (g).

Dry matter content (also called dry matter mass fraction in the SI system) is the ratio of dry mass to the fresh mass of an organ. The dry matter content (DMC) of a plant or plant organ is defined as:

$$\text{DMC} = \text{MDM}/(\text{MDM}+\text{MW})$$

Where :

Mass Dry Matter (MDM) = tissue dry matter mass (g).

Mass of Water (MW) = mass of water (which is equal to its volume because the density of water is 1 g/cm^3) (8). The

Fig. 1. Research implementation.

pot from the research implementation is shown in Fig.1.

One pot is filled with 3 vetiver plants, plant 1 (T1), plant 2 (T2) and plant 3 (T3). So that the total plants needed for 6 concentrations of 0% (C0), 3% (C1), 4% (C2), 5% (C3), 6% (C4) and 7% (C5) were 18 plants with 3 replications. The total number of vetiver plants used was 54.

Data Processing

All methods were replicated three times. Data processing is carried out from the results of field and laboratory research. Data processing includes the calculation of plant growth rate, root elongation, wet weight, dry weight and dry fraction.

Results and Discussion

Propagation

Vetiver takes 10–14 days to form new shoots from the parent clump widely. The shoots attach to the broodstock and then start young roots that are white, vulnerable and larger in diameter than the primary parent roots, which are more flexible, dark brown in colour and smaller in diameter but strong. The growth rate of shoots at the propagation stage is 2–2.5 cm. The high growth rate is supported by a wide range of root systems, optimal nutrient requirements from the soil and a vigorous clump of broodstock with a wide distribution of nutrients throughout the stem.

Acclimatisation and RFT

Samples that have been separated from the parent clump are then cut the stems and root system so that the stem height is 20 cm and the root length is 20 cm. This treatment aims to equalise all samples so that the differences in length can be compared quantitatively.

Acclimatization also gives rhizobacteria time to form colonies, which will help convert the hydrocarbons in the used lubricants into simple organic compounds that can be absorbed and degraded by plants (9). Such bacteria are commonly referred to as Plant Growth-Promoting Rhizobacteria (PGPR) (10). Plant Growth- Promoting Rhizobacteria are bacteria that have the ability to support plant growth and the environment in the plant roots and soil area (11). *Bacillus subtilis* and *Pseudomonas putida* can help reducing hydrocarbon in soil remediation (12). Observations for 14 days obtained data in the form of the physical condition of healthy samples as evidenced by the colour of young leaves, which are light green and mature leaves are dark green, leaf shoots do not wilt, and no young leaves turn yellow. The growth rate during the acclimatisation have shown in Fig. 2.

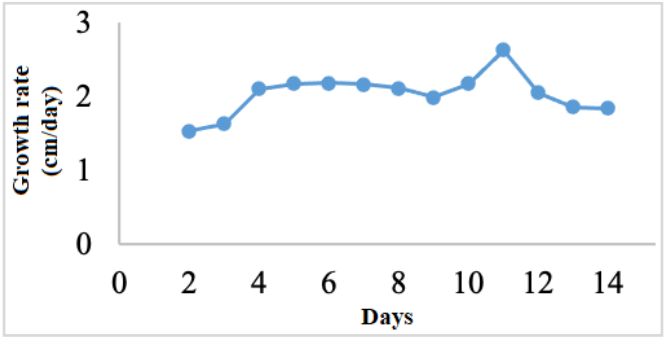

Fig. 2. Growth Rate during Acclimatisation.

Average growth rate of leaves during successive acclimatization periods C0 = 1.952 cm/day, C1 = 1.667 cm/day, C2 = 1.738 cm/day, C3 = 2.474 cm/day, C4 = 1.443 cm/day and C5 = 2.069 cm/day.

Growth rate and temperature

RFT was carried out for 14 days and the growth rate was obtained as follows the Fig. 3.

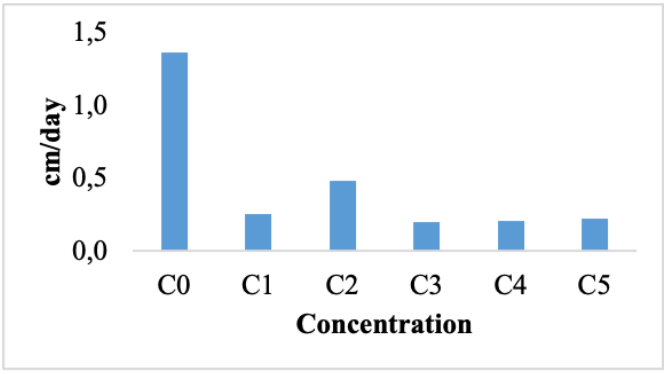

Fig. 3. Growth Rate at RFT.

The average growth of the three plants at each successive concentration was C0 = 1.362 cm/day, C1 = 0.252 cm/day, C3 = 0.194 cm/day, C4 = 0.205 cm/day, C5 = 0.220 cm/day. The results of the ratio of the increase in root length at concentration were 3%, 4%, 5%, 6% and 7% each of 0.473, 0.333, 0.377, 0.380, 0.268 and 0.237 respectively.

In the RFT, the results of temperature measurements can be seen in Fig. 4.

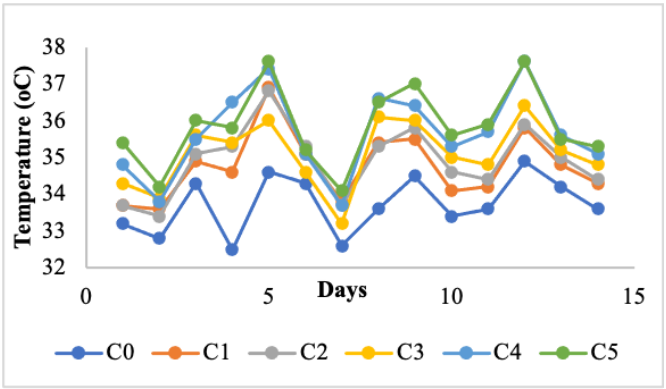

Fig. 4. Soil Temperature at RFT.

Based on Fig. 4, there is a tendency that increasing the concentration of used lubricant affects the temperature of the media. Increasing the concentration of used lubricants darkens the colour of the media after mixing with pollutants. The black colour of used lubricants makes the

intensity of light absorption higher and causes the temperature of the media exposed to pollutants to be high. However, the highest temperature reaches 37 °C. Mesophilic microorganisms can work because the optimum temperature for metabolism is around 25 °C – 40 °C (13).

Mortality Rate

The RFT results showed that vetiver was able to grow well at a concentration of used lubricant of 4% of the total weight of the soil. At this concentration, the vetiver did not show any morphological changes and could grow well. At a concentration of 3%, plants can grow but not optimally; this is indicated by some yellowing of the leaves and withered leaf shoots. Meanwhile, at a concentration of 7%, the plants turned yellow and withered on the 3rd day and on the 4th the plants died. At a concentration of 5%, the three plants began to turn yellow on the 7th day and died successively (T1) on the 14th day, (T2) on the 13th day and (T3) on the 10th day, with a mortality rate of 100%. The concentration of 6% caused the plants to turn yellow on the 4th day and T1 died on the 9th day, T2 on the 11th day and T3 on the 7th day, with a mortality rate of 100%. The mortality rate during RFT can be seen in Fig. 5.

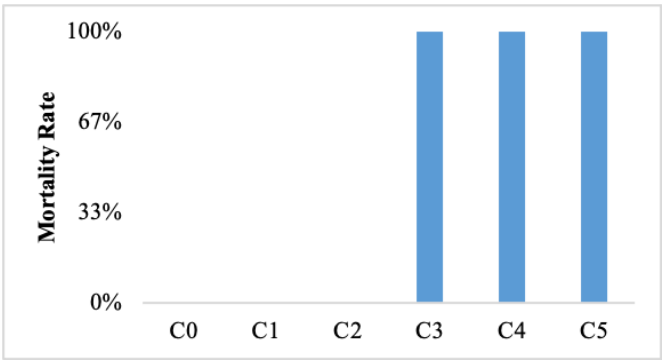

Fig. 5. Mortality Rate at RFT.

Wet Weight and Dry Weight

At the end of the RFT test, the wet weight and dry weight were measured for the leaves and roots of the plants; this was done to determine the dry fraction of the plant. Wet weight and dry weight of sample are shown in Table 1 to 6.

Table 1. Wet weight and dry weight of sample C0 at RFT

C0	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	10.790	6.615	0.613
	Root	4.143	0.786	0.190
T2	Leaf	8.211	3.384	0.412
	Root	8.573	4.076	0.476
T3	Leaf	9.287	4.630	0.498
	Root	1.881	0.296	0.157

Table 2. Wet weight and dry weight of sample C1 at RFT

C1	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	6.247	3.233	0.517
	Root	4.676	1.642	0.351
T2	Leaf	12.328	8.554	0.694
	Root	3.157	0.671	0.213
T3	Leaf	5.280	3.521	0.667
	Root	0.862	0.226	0.262

Table 3. Wet weight and dry weight of sample C2 at RFT

C2	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	15.251	8.674	0.569
	Root	16.925	5.616	0.332
T2	Leaf	5.220	3.232	0.619
	Root	4.780	2.003	0.419
T3	Leaf	14.544	8.981	0.617
	Root	4.828	1.766	0.366

Table 4. Wet weight and dry weight of sample C3 at RFT

C3	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	6.403	4.094	0.639
	Root	2.321	1.232	0.531
T2	Leaf	16.995	12.564	0.739
	Root	10.433	7.940	0.761
T3	Leaf	6.484	3.864	0.596
	Root	9.386	5.874	0.626

Table 5. Wet weight and dry weight of sample C4 at RFT

C4	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	6.030	3.689	0.612
	Root	3.586	2.538	0.708
T2	Leaf	4.158	2.966	0.713
	Root	3.114	1.887	0.606
T3	Leaf	7.794	5.368	0.689
	Root	4.968	2.846	0.573

Table 6. Wet weight and dry weight of sample C5 at RFT

C5	Part	Wet Weight (g)	Dry Weight (g)	Dry Fraction
T1	Leaf	11.836	7.813	0.660
	Root	4.506	2.440	0.541
T2	Leaf	6.213	4.152	0.668
	Root	0.951	0.711	0.748
T3	Leaf	8.139	5.638	0.693
	Root	2.600	1.509	0.580

Table 7. Dry fraction at RFT

Part	C0	C1	C2	C3	C4	C5
Leaf	0.508	0.626	0.602	0.658	0.671	0.674
Root	0.274	0.275	0.372	0.639	0.629	0.623

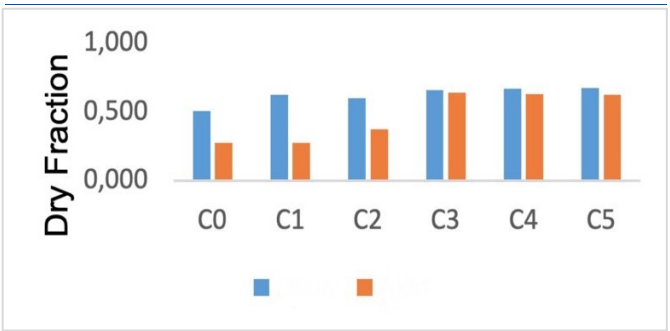

Fig. 6. Mortality Rate at RFT.

Dry Fraction

From table 1 to table 6. above, we get the average dry fraction of the three plant samples per concentration. The dry fraction during RFT can be seen in Table 7 and Fig. 6.

Based on the table, it can be seen that the plant samples C0, C1, C2 had dry fraction roots below 0.5, which indicated the ability to absorb water by the root was still running optimally. At concentrations of C3, C4 and C5, the Dry Fraction was above 0.6. The high concentration of used lubricant that forms a film on the water's surface and the root makes it difficult for the root to absorb water. From the RFT, it is determined that the concentration for the main study is 4%.

Conclusion

The optimal concentration for the degradation of used lubricants based on the physical condition of the plant is sample C2 (4% of the total weight of the media). Data from the observation of sample C2 are in the form of temperatures that are in the mesophilic range (32–38 °C), the plant mortality rate in the pot was 0% (no dead plants), the average value of growth rate was higher than other concentrations (0.481 cm/day); root elongation ratio was above average (0.333). The average dry fraction of the leaf part of C2 plants was 0.602 and the root part was 0.372.

Authors contributions

BVT designed and conceptualised the work. AYW collected the data, analysed and interpreted the data. IA edited and drafted the article. All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

References

1. Simeon Oluwatoyin A, CO Akaeze. Genotoxic evaluation and toxicity of spent engine oil on *Clarias gariepinus*. Res J Environ Toxicol [Internet] 2012;6(4):133-41. https://doi.org/10.3923/RJET.2012.133.141
2. Be U, N Bo. Characterization of soil health using microbial community and maize germination as bioindicators in oil-contaminated soil. J AdvDevRes [Internet] 2011;2(2):191–97. https://doi.org/10.1016/j.apsoil.2005.01.003
3. Murakami CJ, V Wall, N Basisty, M Kaeberlein. Composition and acidification of the culture medium influences chronological aging similarly in vineyard and laboratory yeast. PLoS One [Internet] 2011;6(9). https://doi.org/10.1371/JOURNAL.PONE.0024530
4. Kuo HC, DF Juang, L Yang, WC Kuo, YM Wu. Phytoremediation of soil contaminated by heavy oil with plants colonized by mycorrhizal fungi.Int J Environ SciTechnol [Internet] 2014;11(6):1661–68. https://doi.org/10.1007/s13762-013-0353-6
5. Greenfield J. Vetiver grass: an essential grass for the conservation of the planet earth. 2002. Accessed: Dec. 21, 2021 [Online]. Available: https://www.cabdirect.org/cabdirect/abstract/20023165798
6. Soemirat J. Toksikologi Lingkungan. Yogy Rootta (ID): Gajah Mada University Press. 2003.
7. Gerhardt KE, XD Huang, BR Glick, BM Greenberg. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Sci. [Internet] 2009;176(1):20–30. https://doi.org/10.1016/j.plantsci.2008.09.014
8. Shipley B, TT Vu. Dry matter content as a measure of dry matter concentration in plants and their parts.New Phytol. [Internet] 2002;153(2):359–64. https://doi.org/10.1046/J.0028-646X.2001.00320.X
9. Arliyani I, B Tangahu, S Mangkoedihardjo. Performance of reactive nitrogen in leachate treatment in constructed wetlands. J EcolEng. [Internet] 2021;22(5):205–13. https://doi.org/10.12911/22998993/135314
10. Lugtenberg B, Kamilova F. Plant-Growth-Promoting Rhizobacteria. Annual Review of Microbiology. 2009;63:541–56. https://doi.org/10.1146/annurev.micro.62.081307.162918
11. Miransari M. Plant growth promoting rhizobacteria. J Plant Nutr. [Internet] 2014;37(14):2227–35. https://doi.org/10.1080/01904167.2014.920384
12. Nurmalasari R, BV Tangahu. Bioremediation of soil contaminated diesel using symbiotic bacteria with bioremediation of soil contaminated diesel using symbiotic bacteria with nutrient variation concentration. 11th International Conference on Advances in Agricultural, Biological, Civil and Environmental Sciences. 2020, 18(20):44–51. Accessed: Dec. 21, 2021 [Online]. Available: http://dirpub.org/images/proceedings_pdf/DIR0118215.pdf
13. Ardhanie PR. Biodegradasi Oli Bekas Pada Media Tanah. Tugas Akhir Jurusan Teknik Lingkungan, FTSP, ITS Surabaya. 2003.

§§§