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PHYTOREMEDIATION OF HYDROCARBON-CONTAMINATED SOILS USING JARAK KEPYAR (Ricinus communis L) AND COMPOST

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Abstract

Phytoremediation is known as one of the bioremediation techniques which may remove, stabilize, and/or destroy contaminants in the media. This study was aiming at collecting evidence of phytoremediation, which was applied with the addition of compost. Jarak Kepyar (Ricinus communis L.) was selected for this study because its seeds highly potency as biodiesel raw materials. Laboratory research scale was prepared, and the experiment design was complete random design with three replications. The experiment design was a full random design with three replications. The treatments were mixed contaminated soil (contaminated soil: compost = 3:1) and R. communis; and mixed garden soil (soil:compost=3:1) and R. communis; and contaminated soil without compost and R. communis. The statistical t-Test with a range of confidence at 95% showed that the phytoremediation significantly removed the Total Petroleum Hydrocarbons (TPH) at 97.7%. TPH accumulations were detected in roots and shoot ranged from 3377.6 mg/kg to 3555.0 mg/kg. R. communis leave development was the most sensitive indicator to biotoxicity of the hydrocarbons in the soils.

Keywords: *Phytoremediation, Automotive workshop, Hydrocarbon, fuel crops, Ricinus communis L.*

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INTRODUCTION

Petroleum waste is generated from oil and gas businesses or activities or other activities such as a workshop (Ezeaku & Egbemba, 2014). Oil waste is a waste of Toxic and Hazardous Material (B3) and has the potential to pollute and damage the environment.

Minister of the Environment Decree No. 128 of 2003 concerning Procedures and Technical Requirements for the Management of Petroleum Waste and Biologically Contaminated Soil, indicating that land contaminated with petroleum waste must be appropriately managed. This regulation introduces a remediation technique for contaminated soil using bacteria called a bioremediation technique. Besides this technique, there are bioremediation techniques that use plants and are called phytoremediation (Kiran & Prasad, 2017; Wiszniewska et al., 2016).

Phytoremediation techniques of hydrocarbon contaminated soils have been extensively examined, as conducted by Furini *et al.* (2015), Hunt *et al*. (2019), Ndimele (2010), Cristaldi *et al*. (2017), and Wiszniewska *et al*. (2016). Of the many *reviews*, always supporting this technology is effective, *cost-effective*, and environmentally friendly in remediating hydrocarbon-contaminated soil.

The obstacle to phytoremediation techniques in the field is the selection of the right types of plants to be managed. Not all plants can survive in media or soil contaminated with hydrocarbons because hydrocarbons themselves are toxic to plants, or because of the presence of hydrocarbons, nutrient availability becomes very low or even non-existent because one of the hydrophobic hydrocarbon properties that prevent water from dissolving nutrients what is needed by plants (Ezeaku & Egbemba, 2014).

The tendency of hyperaccumulation (tolerant to toxic) crop selection is often taken into consideration (Idris *et al*., 2016; Wiszniewska *et al*., 2016). Besides hyperaccumulators, this plant is also able to detoxify by making substances that can control pollutants (Wiszniewska *et al*., 2016), as well as a symbiosis with bacteria and fungi that decompose hydrocarbons.

Efforts to improve the phytoremediation process are the addition of organic material into HC contaminated soil (Wiszniewska *et al*., 2016). This organic material will change the structure of the soil, increase the availability of nutrients, and reduce contaminants in the soil. Specifically, Chen *et al*. (2015) provide an explanation that the addition of compost in hydrocarbon-contaminated soil plays a role in increasing the content of organic matter so that it will ultimately increase soil fertility.

Many types of phytoremediator plants, such as water hyacinth *(Eicchornia crassipes),* sunflower *(Heliantus annuus),* Dutch betel (*Scindapus aureus*), bungur (*Lagerstroemia speciose*), mahogany (*Swietenia macrophylla*), and others. The selection of phytoremediator plants, the distance of kepyar (*Ricinus communis* L.) in this study follows the research conducted by Kiran and Prasad (2017), Marques *et al.* (2010), Hasibuan *et al*. (2019) and Pandey *et al*. (2016) which in his research concluded that this plant can be used in phytoremediation and is a multipurpose plant. It is more known that *R. communis* is a *fuel crop* that can survive in growth media with a concentration of certain *Total Petroleum Hydrocarbons* (TPH) and its production in the form of seeds has the potential as a high biodiesel feedstock (Pandey et al., 2016; Tandon *et al*. ., 2013).

The purpose of this study is to prove whether phytoremediation using *R. communis* plants and compost in hydrocarbon contaminated soils can be successful by measuring and observing changes in the character of contaminated soil and morphology of *R. communis plants.*

MATERIALS AND METHODS

Samples of hydrocarbon contaminated soil wew taken from a motorcycle repair shop in Yogyakarta. Compost is purchased from farm shops, and it is known from the label that the basic material of compost is livestock manure, which has undergone a composting process. The seeds of *R . Communis* were obtained from local sorowajan farmers, Banguntapan District, Bantul Regency, Special Region of Yogyakarta. This research was conducted for one month.

Treatment Combination

The research design used was a Completely Randomized Design (CRD) with each treatment repeated three times. The treatments in this experiment are:

a. Treatment I: contaminated soil added with compost and planted with *R. communis* (PI)
b. Treatment II: uncontaminated soil added with compost and planted with *R. communis* (PII)
c. Treatment III: contaminated soil, without additional compost and not planted with *R. communis* (PIII).

Measurement Parameters and Methods

Total Petroleum Hydrocarbons (TPH) of hydrocarbon-contaminated soils were measured by the gas *chromatography (GC)* method at the beginning and end of the study.

The level of hydrocarbon reduction in contaminated soil is calculated using the efficiency formula as below (Saadawi, Algadi, Ammar, Mohamed, & Alennabi, 2015):

$$EP = \frac{\text{TPH before} - \text{TPH after}}{\text{TPH before}} \times 100\% \quad \ldots.(1)$$

where: EP = reduction efficiency,

Other parameters such as organic carbon, total N, phosphorus, and moisture content were measured using SNI 13-4720-1998, SNI-472-1998, ISO 6th 2002, and SNI 13-4719-1998.

Soil pH is measured by a pH meter, carried out before planting, and 7 and 30 days after planting (HST).

All measurements and tests were carried out at the Integrated Research and Testing Laboratory of Gadjah Mada University, Yogyakarta.

Plant height and stem diameter, and leaf area were measured using standard gauges and at 0, 6, 12, 18, 24, and 30 days after planting (HST). As for the surface area of the leaves of *R. communis* measured at 30 HST or against deciduous leaves only by graphical methods and the help of ImageJ *software*. Determination of the age of observation for 30 days by observing the results of research by Khamforoush et al. (2013) and Yu *et al*. (2005), which concluded that the process of biological hydrocarbon decomposition usually mostly takes place on the first 30th day.

Research Procedure

a. Compost preparation

The compost is dried, then the organic C, N, P content, and moisture content are analyzed.

b. Experimental Media Preparation

Samples of hydrocarbon-contaminated soil were taken from a motorcycle repair shop on Jalan Adisucipto, Yogyakarta. While uncontaminated land was taken from gardens in Sorowajan village, Banguntapan District, Bantul Regency, DIY. Furthermore, the soil is mixed with compost at a ratio of 3: 1 (300 g of soil: 100 g of compost).

Mixing of this media is done a day before weaning or planting R. communis seeds.*R.*

c. *Communis Seedlings*

Kepyar jatropha seeds come from local Sorowajan farmers, Banguntapan District, Bantul Regency, Special Region of Yogyakarta.

Seeds are released from the fruit and soaked for six hours. Only sinking seeds are used for seedlings. Seeding seeds are done in bed, sowing with soil media mixed with compost. The type of germination of

R. communis is epigeal. Seedlings aged 6-8 days with cotyledonary characteristics have been removed, opened and the epicotyl has appeared, and about 6.5 cm tall, the seedlings are ready to be weaned. Weaning was carried out into the experimental media in polybags measuring 10 x 10 x 12 cm.

Data Analysis

The data analysis technique used t-test with a 95% confidence interval (Independent Sample) to determine the significance of two groups of mutually independent data.

RESULTS AND DISCUSSION

1. Preliminary Test Results of Soil Samples

Before the study was carried out, the contaminated soil was analyzed to determine its condition and the results, as shown in Table 1.

Table 1. Preliminary Test Results for Hydrocarbon Contaminated Soil Samples

No	Parameter	Unit	Result
1	TPH	%	2,18
2	Karbon Organik (C)	mg/kg	163,87
3	Nitrogen (N)	mg/kg	616,25
4	Phosphor (P)	mg/kg	89,93
5	Kadar Lengas	%	2,98

The initial TPH content of the sample was 2.18%. This shows that TPH levels exceed the biologically contaminated tillage standard set out in Minister of the Environment Decree No. 128 of 2003, which is equal to 1%. According to the Ministerial Regulation, this kind of contaminated land cannot be directly remediated biologically but must be treated first so that the TPH concentration is <1%. In this study, the reduction in TPH to <1% was carried out with the addition of compost by 100 gr.

The presence of elements C, N, P in contaminated soil is also analyzed, and the results are far below the content contained in compost, except for C, as presented in Table 2.

Table 2. Preliminary Results of Compost Characteristics Test Before Use in Experiments

No	Parameter	Unit	Result
1	Karbon Organik (C)	mg/kg	134,36
2	Nitrogen (N)	mg/kg	4.348,64
3	Phosphor (P)	mg/kg	1.499,20
4	Kadar Lengas	%	5,55

The concentration of C in contaminated media is higher than in compost. Source C in contaminated media is thought to originate from hydrocarbons that contaminate the soil because they come from fossil-based materials (Ezeaku & Egbemba, 2014).

Hydrocarbon contaminated soils are said to be nutrient-poor soils because one or a number of low nutrient content due to hydrophobic nature has prevented water from dissolving nutrients into available plants (Egbemba, 2014).

TPH Phytoremediation Results

The final analysis of compost mixed media (Treatment I) with phytoremediation treatment showed a significant reduction in TPH, as presented in Table 3.

Table 3. TPH content in media mixed with Phytoremediation (PI) and Control (PIII) Compost

Test	PI	EP_{PI}	PIII	EP_{PIII}
1	0,06	97,25%	1,67	23,39%
2	0,02	99,08%	1,58	27,52%
3	0,07	96,79%	1,60	26,61%
Average	0,05	97,71%	1,62	25,84%
$\pm\delta$	0,026	1,214%	0,047	2,168%

Note: EP = reduction efficiency

Table 3 shows that PI produces a TPH that is much lower than the PIII or control, more than 300% lower, while the efficiency of the reduction is almost four times higher. T-Test statistical test with a 95% confidence interval, it was proven that the mean TPH and EP of the two treatments showed that the two were significantly different.

PI's that received the treatment of adding compost and planting *R. communis* in contaminated media showed similarities with the results of research conducted by de Abreu *et al*. (2012). In this experiment, de Abreu *et al.* (2012) concluded that phytoremediation using *R. communis* and the addition of compost were faster in remediating hydrocarbon-contaminated soils than those without compost.

R. communis is a plant that is known to be quite effective in cleaning up hydrocarbons from the soil through phytoremediation techniques (Kiran & Prasad, 2017; Saadawi *et al*., 2015). In laboratory studies conducted by Saadawi *et al*. (2015) using *R. communis* on hydrocarbon contaminated soils succeeded in removing 76% TPH and 77% of the initial TPH of 0.5% and 1.0% within 30 days; and 79% within 45 days for an initial TPH of 1.0% (Rehn *et a*l., 2019). In the roots of *R. communis* often and easily found the association of arbuscular mycorrhizal types that play a role in removing contaminants and, at the same time, supporting the life of its host plants (Kiran & Prasad, 2017).

Addition of compost will increase the concentration of hydrocarbon decomposing bacteria in contaminated soil (Prakash, Saxena, Sharma, Singh, & Singh, 2015) and as a source of nutrients, especially N and P in slowly released soils (Chen *et al*., 2015; Faucette, 2010) needed for further growth of microorganisms and plants. Efforts to add compost or bioaugmentation, by Chen *et al.* (2015), is believed to often improve the process and effectiveness of contaminant decomposition in hydrocarbon-contaminated soil because, in addition to functioning as a source of microorganisms and nutrients, compost is also able to provide enzymes needed by microorganisms for life.

Besides that, PIII as a control, overtime, is also able to reduce TPH content in hydrocarbon-contaminated media. PIII or control only relies on the role of nature in decomposing hydrocarbons. In many cases, the role of indigenous bacteria or biological processes is more important than chemical and physical processes in natural processes or what is called *natural attenuation* (Abatenh, Gizaw, Tsegaye, & Wassie, 2017; Yu *et al*., 2005). Research Agnello et al. (2016) showed a *natural attenuation* EP of 37%, while in this trial, it was 25.8% or lower than the previous experiment. This figure falls within the range of EP *natural attenuation* from research conducted by Couto Couto *et al*. (2010) in the amount of 20 - 50%. Furthermore, several factors that determine the success rate of *natural attenuation* apart from the characteristics of microorganisms in the media are mentioned, such as soil depth, type of contaminant, and oxygen content, whereas Khamforoush *et al*. (2013) added the airflow factor and humidity in it.

1. PH Value

The results of pH measurements on PI and PIII can be seen in Figure 1 and Table 4.

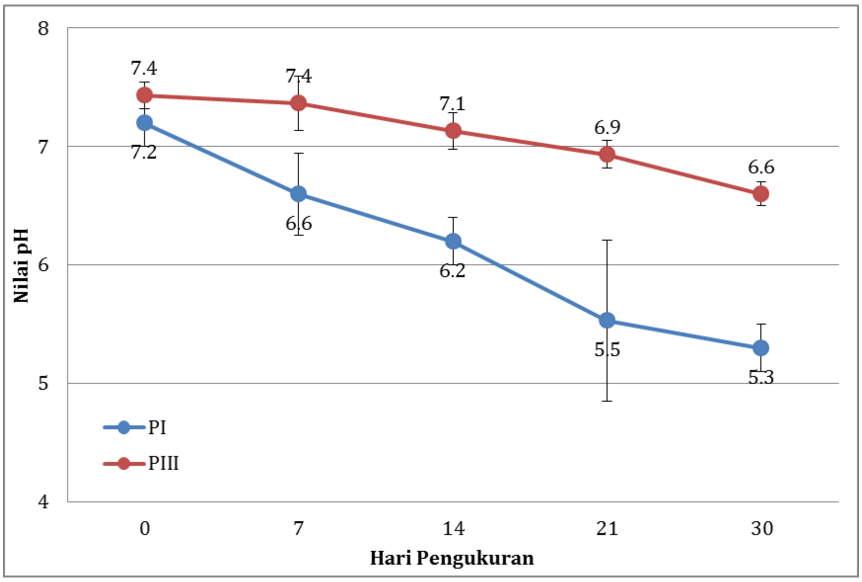
Table 4. Observation of Soil pH Phytoremediation Results

Measurement Day	PI	±δ	PIII	±δ
0	7,2	0,20	7,4	0,12
7	6,6	0,35	7,4	0,23
14	6,2	0,20	7,1	0,15
21	5,5	0,68	6,9	0,12
30	5,3	0,20	6,6	0,10
EP	26,4%		11,2%	

Note: EP = reduction efficiency

The results of measuring pH in the two treatments PI and PIII with a t-test with intermittent confidence of 95% showed the average value of pH reduction in each treatment was significantly different. At the end of the measurement, the pH on PI was lower than PIII, even though on the day of the 0th measurement, based on t-Test with 95% confidence interval, pH of PI and PIII pH were 7.2 (+ δ0.20), and 7.4 (+ δ0.12) is not significantly different.

PI decreased pH by 26.4% from the initial pH value, and PIII experienced a decrease of 11.2%. The greater decrease in PI was caused by the presence of compost and planting of *R. communis* in hydrocarbon-contaminated media.



Picture 1. pH Measurement Results on PI and PIII

Picture 1 showing underground to become acidic in line with the phytoremediation process, from a pH of 7.2 to 5.3 on the 30th day. This decrease in pH occurs due to the production of organic acids as a result of the breakdown of hydrocarbons by the activity of microorganisms (Osuji & Nwoye, 2007). Because hydrocarbons contain heavy metals (Kathi & Khan, 2011), the decrease in pH can also be caused by the release of positive ions from the metal as a result of the decomposition process of the hydrocarbons (Kriipsalu *et al*., 2008), such as Al + (Ezeaku & Egbemba, 2014) or because of the insistence of colloidal soil that replaces free base ions such as Ca2 +, Mg2 +, K +, and Na + (Ngobiri *et al*., 2007).

TPH In Plants

Removal of TPH in contami

noted soil can occur directly and indirectly. Directly, hydrocarbons that contaminate the soil will be *uptake* by plants (Hunt *et al.,* 2019; Wang *et al.*, 2013) and indirectly is by decomposing hydrocarbons by microorganisms that live in plant rooting areas, or because of root exudates plants that describe hydrocarbons (Idris *et al.,* 2016; Ndimele, 2010; Saadawi *et al.,* 2015).

The results of TPH measurements in the experimental plant section, *R. communis* are presented in Table 5 below.

Table 5. TPH Content in Roots and *R. communis roots*

Deuteronomy	**TPH levels (mg/kg)**	
	Root	**trubus***
1	3935,8	4070,7
2	3040,9	3023,2
3	3156,3	3571,2
Average	3377,6	3555,0
±δ	486,81	523,92

Notes: * = biomass of the topsoil of plants in the form of stems and leaves

The TPH content in the roots and *trubus* was not significantly different at α <0.05, even though the numbers showed that the TPH content in the trubus was higher than in the roots.

From Table 5 it is known that in the biomass of *R. communis* contains hydrocarbons that are likely to come from contaminated soil. At the root of TPH content of 3377.6 mg / kg or 0.34% (+ δ 0.049%) while in the trubus was 3555.0 mg / kg or 0.36% (+ δ 0.052%). This content is much lower than the content in seeds in mature plants. In this experiment, *R. communis* is only a maximum of 40 days old and has not produced flowers and fruit. In general, TPH content in seeds is 41-64% (Olivares, Carrillo-González, González-Chávez, & Hernández, 2013; Santoso, Sudika, Jaya, & Aryana, 2014; Widodo & Sumarah, 2007). TPH data in the roots and roots of *R. communis* cannot yet be found in other references because this section, possibly, is not considered to have accumulated TPH.

2. Growth of *R. communis*

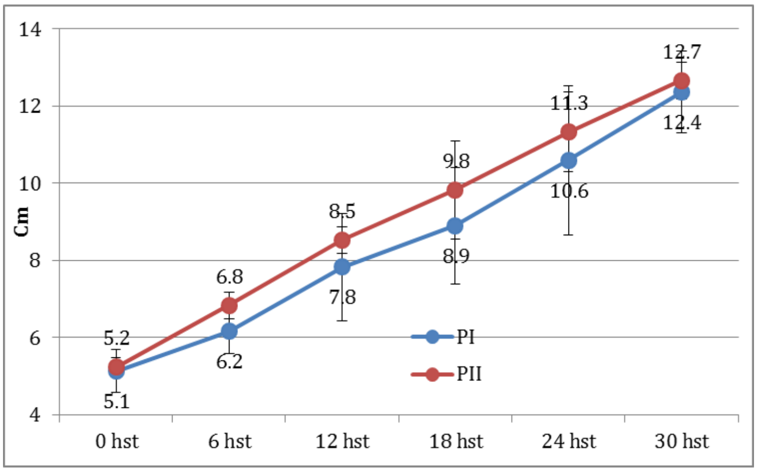
In this experiment, visually, the growth of *R. communis* at the age of 30 HST can be seen in Picture 2 below.

Picture 2. Growth of R. communis at 30 days after planting. Three Polybags on the Left are PII Treatment and Three Polybags on the Right are PI Treatment

At first glance, the two treatments at the end of the study, namely age 30 HST, did not provide significant visual differences. This is thought to be due to the relatively young age of the plant. Phytoremediation research conducted by Rehn et al. (2019) by using *R. communis* on hydrocarbon contaminated media of 0.5 - 1.5% TPH, did not show the impact of visual morphology on plant age 30 days, but plants at the age of 35-45 days only appeared symptoms of morphological degradation visually on the leaves are necrosis. The higher the TPH in the soil, the more severe the necrosis.

Plants Heights

Plant height is measured from the base of the stem to the tip/top of the plant, starting from the day of planting to 30 days after planting. The results of measurements of plant height obtained observation data as follows (Picture3).



Notes : hst = days after planting

Picture 3. Plant height R. communis in the PI and PII Treatment

Statistical tests of the two *R. communis* high data series with α <0.05 indicate that the two were not significantly different, nor were the growth rates. The average growth of *R. communis* in 30 days is 1.4 - 1.5 cm / 6 days.

Table 6. High rate of increase of R. communis in PI and PII

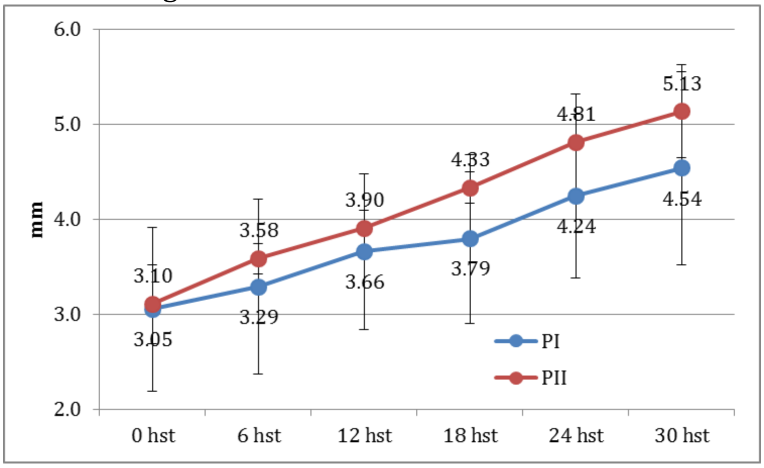
Treatment	Height Increase (cm)					Average (cm)
	6 hst	12 hst	18 hst	24 hst	30 hst	
PI	1,0	1,7	1,1	1,7	1,8	1,4[a]
PII	1,6	1,7	1,3	1,5	1,3	1,5[a]

description: hst = days after planting

The height of *R. communis* in this experiment is shorter than its normal height. Nahar (2015) who observed the growth of *R. communis* from seeds and developed in fertile soil found that at 28 days, the average growth reached 15 cm in height, whereas in this study only reached 12.7 cm in PII and 12 treatments. 4 cm in PI treatment at around 36 days of age.

Rod Diameter

Plant stem diameter is measured at the base of the stem and as high as 1 cm from the ground surface, starting from the day of planting to 30 days after planting. The results of measurements of plant diameters obtained observational data as follows (Picture 4).



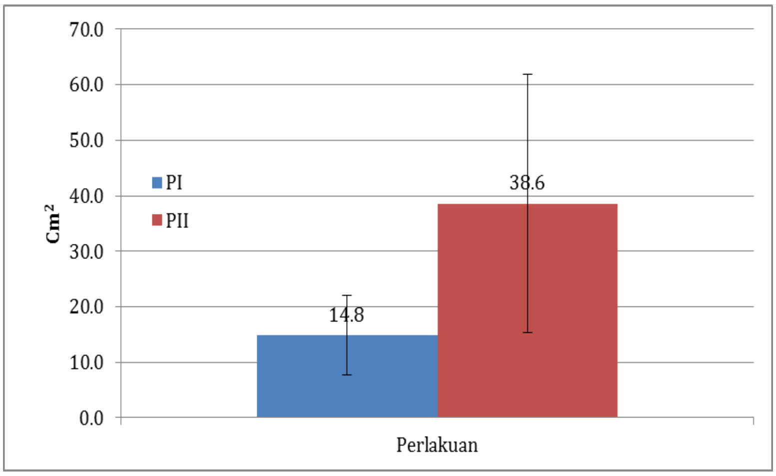
Notes : hst = days after planting

Picture 4. Stem Diameter of *R. communis* Plants in PI and PII Treatment

Statistical tests of the two *R. communis* diameter data series with a 95% confidence interval indicate that the two are not significantly different, although in general it can be seen that PII has a trunk diameter and a tendency greater than PI. Compared with the normal diameter of *R. communis* from Nahar's (2015) study it is known that the diameter in this experiment is greater than the normal diameter, 3.0 mm. In this experiment, stem diameters ranged from 4.54 to 5.13 mm in PI and PII.

Leaf Area

Luas daun tanaman diukur dari setiap tanaman pada 30 hst dan dihitung reratanya seperti di dalam **Error! Reference source not found.**.



Picture 5. Average Surface Area of R. communis leaves at 30 days after the end of the experiment

Statistical tests of the two data series of leaf surface area of *R. communis* at 30 DAP or at the end of the experiment with α <0.05 indicate that the two are significantly different. The surface area of the leaves of *R. communis* on PI> 250% is smaller than PII, 14.8 (+7.19) cm2, and 38.6 (+23.23) cm2 (Table 7).

Table 7. Surface Area and Number of Leaves of R. communis at 30 HST

Value	PI		PII	
	Total	Amount	Total	Amount
Avergae	14,8[a]	7,3[c]	38,6[b]	8,3[d]
±δ	7,19	0,58	23,23	0,58

Besides being smaller, PI has an average number of leaves that are smaller than PII, which is 7.3 strands and 8.3 strands.

From the four characters of plant growth of *R. communis*, namely total height, stem diameter, surface area, and the number of leaves only from the leaves, it can be seen that hydrocarbons in the soil have a negative impact on the growth of *R. communis* at ages up to 30 days after planting.

Noting the level and growth phase of *R. communis* conducted by Putri, Waluyo, and Ardiarini (2019), it is known that at the age of <40 days, *R. communis* is in the growth phase before entering the flowering phase which starts at the age of 54 days. In this growth phase, *R. communi*s will experience a relatively high increase and a relatively rapid number of leaves compared to other phases.

CONCLUSION

Phytoremediation using *R. communis* and composting have succeeded in reducing TPH in the soil by 97.7%, while natural processes, natural attenuation only decreased by 25.8%. Meanwhile, *R. communis* can accumulate TPH in roots and roots, ranging from 3377.6 mg/kg and 3555.0 mg/kg. The number and leaf area of *R. communis* shows a clearer indication of hydrocarbon biotoxicity than the height and diameter of the stem.

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