

Potential of *Neptunia oleracea* L. as a Phytoremediation Agent for Petroleum Liquid Waste

Dwi Hardestyariki¹, Syarifita Fitria^{2,3*}

¹ Biological Science, Faculty of Mathematics and Natural Science, Sriwijaya University, Jl. Masjid Al Gazali, Bukit Lama, Kec. Ilir Bar. I, Kota Palembang, Sumatera Selatan 30128, Indonesia

² Electrical Engineering, Faculty of Engineering, Sriwijaya University, Jl. Masjid Al Gazali, Bukit Lama, Kec. Ilir Bar. I, Kota Palembang, Sumatera Selatan 30128, Indonesia

³ Department of Environmental Management, Sriwijaya University, Department of Environmental Management, Sriwijaya University, Palembang, Indonesia

* Corresponding author email: syarifitaftiria@ft.unsri.ac.id

ABSTRACT

This present study to determine the potential of *Neptunia oleracea* as a phytoremediation agent for petroleum liquid waste in terms of various parameters such as physical temperature, and chemical parameters, namely pH, sulfide, and ammonia. Crude oil liquid waste is a hazardous waste if discharged directly into the environment, especially water. In this research, a method of biological treatment of petroleum liquid waste was used by utilizing aquatic plants, namely *Neptunia oleracea*. Phytoremediation is a way to treat waste that still contains contaminants thus levels can be minimized and even accumulated by various types of plants. This phytoremediation technique uses a simple bioreactor with various concentrations of petroleum liquid waste, namely control (0% waste), 10% waste, 20% waste, and 30% waste. The results of this study can be seen that the *Neptunia oleracea* plant is able to survive in petroleum liquid waste with a treatment time of approximately 3 weeks. In testing the levels of ammonia and sulfide after treatment showed fluctuating results from week to week. This is a response from *Neptunia oleracea* which uses these compounds in its metabolic processes. The ability of *Neptunia oleracea* to reduce sulfide and ammonia levels indicates that this plant can be used as a phytoremediation agent for petroleum liquid waste.

Keywords: sulfides, ammonia, phytoremediation, *Neptunia oleracea*.

INTRODUCTION

The need for petroleum in the world continues to increase along with the high demand for fuel produced from petroleum. This led to an increase in oil exploration and production activities. Apart from producing crude oil products, petroleum production also produces petroleum waste [Irawan et al., 2021]. One type of waste produced is liquid waste originating from the process of separating crude oil and water [Nurhayati, 2010].

Ecologically, the activities of the oil and gas industry (oil and gas) often have the potential to have an impact on the environment thus if not managed properly will cause problems in the environment. Environmental management activities

are prioritized in activities to reduce waste at the source [Hasiyany et al., 2015]. Crude oil liquid waste is very dangerous if it is directly disposed of into the sea or into rivers since it contains a lot of dangerous and toxic chemicals. Therefore, before the petroleum liquid waste is discharged into the sea, there are conditions that must be complied with in accordance with the quality standards set by the government [Utami et al., 2017].

Efforts to prevent environmental pollution and the hazards that result from it and those that will cause socio-economic, health, and environmental losses, then there must be special management of this waste thus its hazardous properties can be eliminated or reduced. In addition, it is necessary to strive for environmentally friendly

management methods as well as correct and careful supervision by various parties. Along with the increasing concern for the sustainability of rivers and the interest in environmental sustainability, industrial efforts will emerge to manage their industrial wastewater through planning efficient production processes. Wastewater management is able to minimize industrial waste and efforts to control industrial wastewater pollution through the implementation of wastewater management installations [Yudistira, 2022].

Handling environmental conditions polluted by petroleum liquid waste can be carried out physically, chemically, and biologically [Fadhila Prakasita & Wulansarie, 2018]. The biological treatment of petroleum liquid waste can use biological agents such as plants which have the potential to absorb, detoxify, and accumulate contaminants contained in the waste. Waste treatment using these plants is often known as phytoremediation.

Phytoremediation is the ability of plants to reduce the volume, mobility, and toxicity of contaminants in contaminated soil, water, and media [Sukono et al., 2020]. Phytoremediation is carried out to recover and reclamation of areas polluted by waste [Linggawati et al., 2022]. During the phytoremediation process, plants absorb contaminants from contaminated water and soil, thereby maintaining ecosystem security [Odoh et al., 2019]. The success of phytoremediation depends on the type of plant to be used. This is because each type of plant has a different ability to absorb the pollutants contained in the waste. Previous studies have proven that water hyacinth plants are able to reduce BOD and COD levels of petroleum liquid waste [Hardestyariki & Fitria, 2022]. Several other aquatic plants have different potentials in carrying out phytoremediation. It also depends on the adaptation of the morphology, physiology and behavior of a plant to stress from waste.

Aquatic plants are natural biological agents that can be used to treat contaminated soil and water, especially accumulating heavy metals in plant tissues. *Neptunia oleraceae* is a type of aquatic plant that is widely used for the decontamination or reduction of water contaminants in several countries in Asia, such as Indonesia, Malaysia, Thailand, Philippines, and Vietnam [Aini-Syuhaida et al., 2014]. *Neptunia oleraceae* (water mimosa) has also been proven to be efficient in treating waste from the distillation process. Water mimosa can remove dissolved solids, and is

able to reduce BOD and COD contained in waste [Suppadit et al., 2008]. The advantage possessed by *Neptunia oleraceae* lies in the morphology of its roots. On the roots of the *Neptunia oleraceae* plant, it has root nodules which most aquatic plants do not have. According to Rahman et al. (2018) root nodules are small protrusions around the roots that are formed due to an infection with N_2 -fixing bacteria that are mutually associated with plants. This association allows the availability of N_2 for plants, especially when there is a shortage of dissolved N_2 in an environment [Rahman et al., 2018].

Neptunia oleraceae (Water mimosa) is a plant that belongs to the Mimosaceae family. *Neptunia oleraceae* plants include annual plants that usually float in wetlands such as lakes, ponds, and waterways. This plant is known as a sensitive aquatic plant where if touched, the leaves will close like *Mimosa pudica*. Morphologically, *Neptunia oleraceae* has a white sponge that covers the stem, has no thorns, and has yellow flowers. *Neptunia oleraceae* also has root nodules on submerged roots, making it easier to carry out nitrogen fixation [Bhunja & Mondal, 2012].

EXPERIMENTAL

Materials and tools

The tools used were plastic basins, sample bottles, filter paper, volumetric flasks, pH meters, serological pipettes, Cecil 9000 series UV



Figure 1. *Neptunia oleraceae*

spectrophotometers, and analytical balances. While the materials needed are distilled water, HCl 6 N, iodine, $\text{Na}_2\text{S}_2\text{O}_3$, NaOH, Nessler reagent, sodium potassium tartrate, Zinc Acetate, ZnSO_4 , wastewater samples from Oil Cather (OC) IV, and *Neptunia oleracea* (Water Mimosa) on Figure.1.

Sampling and testing

Sampling was carried out at the refinery of the petroleum industry. Sampling was carried out using a ballast device in which a sample bottle could be placed, then the sample was tested in the laboratory to determine chemical parameters, namely sulfide, ammonia, and physical parameters, namely pH and temperature at initial conditions. There were 3 treatments in this study with each treatment having a waste concentration of 10%, 20%, and 30% waste. Each basin contains *Neptunia oleracea* weighing 200 grams and a separate control basin contains plain water and *Neptunia oleracea* without waste treatment. Determination of these concentration variations is to determine the concentrations that can still be tolerated by *Neptunia oleracea* in carrying out phytoremediation. Before carrying out the phytoremediation test, the plants are acclimatized first thus they can work optimally. After testing the phytoremediation for 2 months, chemical and physical parameters were measured again to determine the effectiveness of *Neptunia oleracea* in reducing pollutants in the waste with different concentrations.

Variable testing of wastewater samples after phytoremediation

pH and temperature measurement

The wastewater sample to be measured is prepared in a test vessel. pH and temperature measurements were carried out using a Beckman electric pH meter. The electrodes were rinsed using demin water. Then the tip of the electrode is inserted into the wastewater sample to be tested, wait until a constant pH value is obtained and the temperature of the wastewater is recorded on the screen.

Measurement of ammonia ($\text{NH}_3\text{-N}$)

A sample of 100 ml of wastewater was prepared and added with 1 ml of ZnSO_4 sol. Subsequent to the sample was shaken until homogeneous. The pH of the sample was adjusted to 10.5

by adding NaOH sol. Then filtered and the first filtrate was discarded ± 10 ml and continued filtering until all were filtered. A total of 50 ml of filtered sample was taken and added 2 drops of sodium potassium titrate to avoid turbidity. Then 1 ml of Nessler reagent was added and shaking was carried out. Furthermore, the samples were allowed to stand for ± 20 minutes. Examined in the Spectrophotometer 425 nm cell 1 cm, and seen the results [SNI, 2005].

Sulphide (H_2S) measurement

3–5 ml of 0.025 N iodine solution is put into an Erlenmeyer with a capacity of 500 ml and added 2 ml of 6 N HCl. Phytoremediation wastewater samples containing 200 cc were placed in Erlenmeyer iodine. If the iodine color disappears, add more iodine solution until the iodine color appears (record the total volume of iodine used). The sample is titrated with $\text{Na}_2\text{S}_2\text{O}_3$ solution until it turns pale yellow. Then starch indicator is added, subsequent to the titration process is continued until the blue color disappears (the volume of $\text{Na}_2\text{S}_2\text{O}_3$ used for the titration is recorded). To calculate the H_2S content contained in the sample, calculations are carried out using the following formula:

$$\frac{\text{mgS}^{2-}}{L} = \frac{((A \times B) - (C \times D)) \times 16000}{V} \quad (1)$$

where: *A* – total volume of iodine solution used (ml);
B – normality of iodine solution;
C – volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used (ml);
D – normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution;
V – volume of solution sulfide (ml)
 [SNI, 2009].

Data analysis

The data obtained are presented in the form of figures, and statistical analysis. Various treatment variables with different concentrations were analyzed using multivariate analysis and the correlation was seen using Pearson's correlation.

RESULT AND DISCUSSION

Based on the treatment of petroleum liquid waste by phytoremediation using *Neptunia oleracea* for approximately 3 weeks, it was found that the measurement parameters carried out showed different results from the three types of

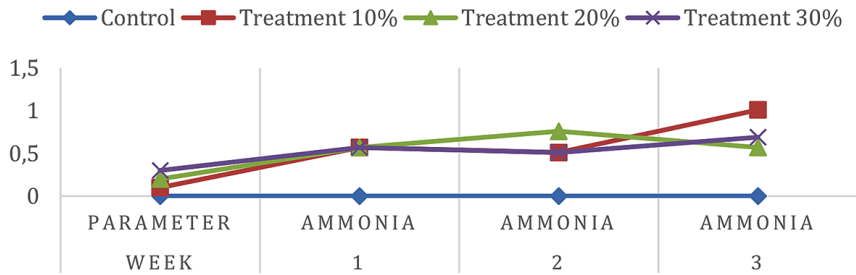


Figure 2. Total ammonia content at several waste concentrations

waste concentration treatment. The parameters of the petroleum liquid waste observed in this study were the ammonia value, sulfide value, as well as pH, and temperature. The initial total ammonia content of petroleum liquid waste originating from the sewage treatment pond was 2.88 mg/l. After phytoremediation, the results showed that the concentration of ammonia in the first week for 10%, 20%, and 30% treatment was 0.57. In the second week consecutively the treatment of 10%, 20%, and 30% waste was 0.51 mg/L; 0.76 mg/L, and 0.51 mg/L. Whereas in the third week of treatment the waste concentration of 10%, 20%, and 30% respectively were 1.01 mg/L; 0.57 mg/L; and 0.69 mg/L (Figure 2). According to Murti & Purwanti (2014), ammonia is a type of pollutant that comes from liquid waste [Murti & Purwanti, 2014]. Ammonia is a colorless gas that dissolves easily in water. In liquid form, ammonia exists in 2 forms, namely free or non-ionized ammonia (NH_3) which is toxic, and in the form of ammonia ion (NH_4^+) which is less toxic. *Neptunia oleracea* belongs to the Leguminosae plant which has root nodules in its root area. Root nodules are a form of mutualism symbiosis between roots and bacteria of the genus *Rhizobium*. According to Nurhayati (2010) this association is able to increase ammonia degradation by cooperating with N-fixing bacterial agents through the nitrification process (the process of oxidation of ammonia to nitrite and then to nitrate) [Nurhayati, 2010].

Prior to phytoremediation using *Neptunia oleracea*, the ammonia level in the waste was still quite high, namely 2.88 mg/L. Even though it is still quite high, this level does not exceed the maximum level that has been determined. From the results of phytoremediation using *Neptunia oleracea* (Figure 2) it can be seen that there was a decrease in ammonia levels up to the third week of treatment. However, there was also an increase in ammonia levels in the second week at the 20% treatment. The fluctuation in ammonia levels is

due to the decay process of the organic material which comes from the many leaves of *Neptunia oleracea* which fall into the bioreactor. According to Bean (2022) *Neptunia oleracea* has a very large number of leaves, namely 8–20 pairs, and is very small [Bean, 2022]. According to Hargreaves & Tucker (2004) organic matter in a waste can come from algae or aquatic plants that have died and decayed [Hargreaves J.A., 2004]. When the decay process occurs, the decomposition of organic matter will produce ammonia. This causes ammonia levels to fluctuate based on the length of treatment time. According to Mendez & Maier (2008) water mimosa functions as a Phyto indicator for environments polluted by ammonia and phosphate. This plant is also able to survive in polluted waters with excessive levels of nutrients such as ammonia (NH_3), Phosphorus (P), and Nitrogen (N) [Mendez & Maier, 2008].

The sulfide parameter in the first week for the 10% and 20% treatment was 0.172 mg/L, while the 30% treatment was 0.16 mg/L. Sulfide levels in the second week sequentially from concentrations of 10%, 20%, and 30% were 0.148 mg/L; 0.127 mg/L; and 0.124 mg/L. While the sulfide levels in the third week sequentially from concentrations of 10%, 20%, and 30% were 0.28 mg/L; 0.132 mg/L; and 0.148 mg/L (Figure 3).

From the sulfide picture above there was a decrease in the duration of the treatment by 4% from the first week to the 2nd week, for a decrease from the 1st week to the 3rd week there was a 16% decrease in sulfide in the 10% treatment. For the 20% treatment, there was a 26% decrease from the first week to the 2nd week and a 34% decrease in sulfide from the 1st week to the 3rd week. For the 30% treatment, there was a 5% decrease from the first week to the 2nd week, and there was a decrease of sulfide 7.5% from week 1 to week 3. The decrease in sulfide values that occurred from the control variable to the 30% treatment resulted in a significant decrease of approximately 30%.

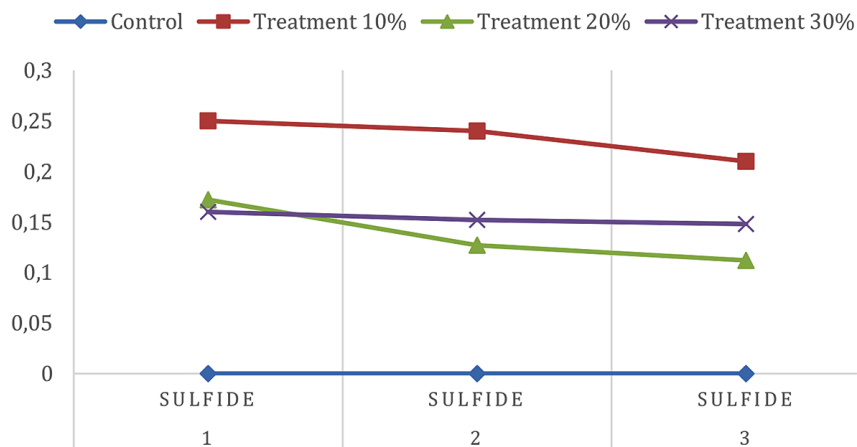


Figure 3. Sulfide content with several waste concentration treatments

The difference in the results of sulfide levels in the various concentrations of the waste treatment is related to the difference in the adaptability of *Neptunia oleracea* to various concentrations of the waste. According to Ainiet al., (2014) terrestrial plants and aquatic are widely used in wetlands which are naturally capable of treating wastewater [Aini et al., 2014]. Each aquatic plant that has been identified and researched has a different ability to carry out phytoremediation. It also depends on the characteristics possessed by certain plant species. Water mimosa is able to grow quickly in polluted environments, when conditions are like that water mimosa acts as a nutrient and heavy metal absorbtion quickly from an environment. According to Muro Gonzalez et al, (2020) leaf color, the number of leaves, and root structure are morphological characteristics that can be observed during the degradation process of pollutant compounds [Muro-González et al., 2020].

The degree of acidity (pH) is a chemical parameter that is also carried out to see the ability of *Neptunia oleracea* to neutralize the pH content in the waste. Based on the results obtained

(Figure4a), the pH of the various treatments also showed fluctuating results. The temperature parameter from the control variable to the 30% concentration treatment tends to show stable results since there is only an increase and decrease of approximately 5%. According to Retnaningdyah & Arisosilaning Sih (2018) increases and decreases in pH are accompanied by increases and decreases in alkalinity or bicarbonate levels [Retnaningdyah & Arisoesilaning Sih, 2018]. According to Lestari & Aminatun (2018) The decrease in the pH value is due to the presence of organic ingredients originating from plant death which decomposes, causing a decrease in the pH value and the reaction of microorganisms (bacteria) which break down the organic materials in the phytoremediation process [Lestari & Aminatun, 2018]. Good water has a normal temperature, approximately 3 °C from room temperature (27 °C). Water temperature that exceeds the normal limit indicates that there are chemicals dissolved in large enough quantities or the process of decomposition of the organic material by microorganisms is taking place.

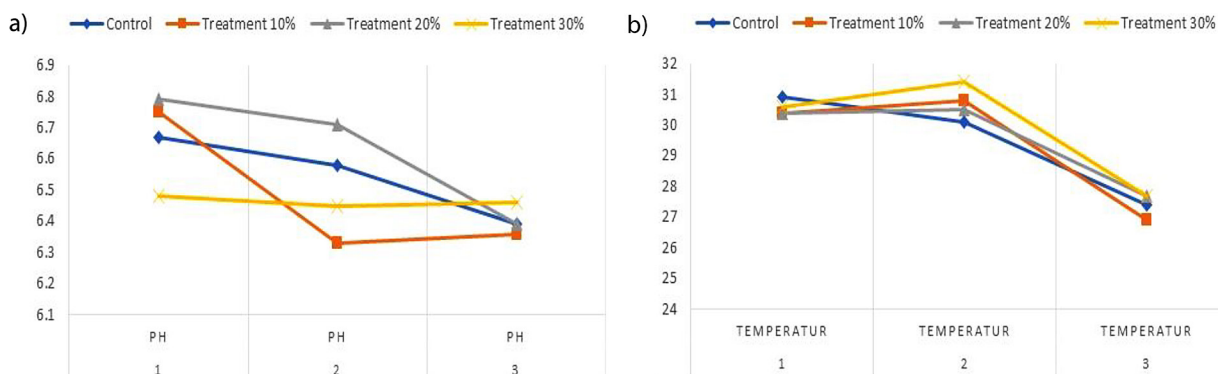


Figure 4. Parameters of pH (a) and temperature (b) at several concentrations of waste

This study uses multivariate analysis using SPSS. Based on the data obtained, the relationship between the 4 variables is significant when viewed from a significant number of 0.0002 which is smaller than 0.05. From these results, it can be concluded that the length of treatment influences the 4 parameters used, namely ammonia, sulfide, pH, and temperature. The magnitude of the coefficient of determination is 0.883 thus there is an influence of the four parameters of 88.3% on the length of treatment. Meanwhile, 11.7% were influenced by other causal factor. From this analysis, it was also found that the relationship between the four parameters had a very strong relationship since a number of 0.94 was obtained. However, this study also confirmed with previous study that Water Mimosa as a feasible phytoremediator to clean up aquatic systems [Abdul Wahab et al., 2014; Atabaki et al., 2020].

CONCLUSION

Neptunia oleracea is an aquatic plant that can survive in petroleum liquid waste, where it can be seen that the plant is able to grow and develop during the phytoremediation process. *Neptunia oleracea* can reduce ammonia, sulfide, temperature, and pH levels for 3 weeks due to *Neptunia oleracea* can absorb, detoxify, and accumulate contaminants contained in waste. *Neptunia oleracea* can be used as a phytoremediation agent since it has the potential to treat petroleum liquid waste biologically and sustainably.

REFERENCES

1. Abdul Wahab, A.S., Syed Ismail, S.N., Praveena, S.M., Awang, S. 2014. Heavy metals uptake of water mimosa (*Neptunia oleracea*) and its safety for human consumption. *Iranian Journal of Public Health*, 43(3), 103–111.
2. AiniSyuhaida, A.W., SharifahNorkhadijah, S.I., Emilia, Z.A., SarvaMangala, P. 2014. *Neptunia oleracea* (water mimosa) as phytoremediation plant and the risk to human health: A review. *Advances in Environmental Biology*, 8(15), 187–194.
3. Atabaki, N., Shaharuddin, N.A., Ahmad, S.A., Nulit, R., Abiri, R. 2020. Assessment of water mimosa (*Neptunia oleracea* Lour.) morphological, physiological, and removal efficiency for phytoremediation of arsenic-polluted water. *Plants*, 9(11), 1–24.
4. Bean, A.R. 2022. A revision of *Neptunia* Lour. (Leguminosae: subfamily Caesalpinioideae, Mimosoid clade) in Australia and Malesia A revision of *Neptunia* Lour. (Leguminosae: subfamily Caesalpinioideae, Mimosoid clade) in Australia and Malesia. November.
5. Bhunia, D., Mondal, A.K. 2012. Systematic analysis (morphology, anatomy and palynology) of an aquatic medicinal plant water mimosa (*Neptunia oleracea* LOUR.) in Eastern India. *International Journal Life Sciences Biotechnology and Pharma Research*, 1(2), 290–319.
6. Fadhila Prakasita, I.G., Wulansarie, R. 2018. Review of Analysis of Petroleum Waste Degradation Technology to Reduce Seawater Pollution in Indonesia. *Reka Buana : Scientific Journal of Civil Engineering and Chemical Engineering*, 3(2), 80. (in Indonesia)
7. Hardestyariki, D., Fitria, S. 2022. Effectiveness and Adaptability of Water Hyacinth (*Eichornia Crassipes*) Mart. Solm in its Role in Reducing COD and BOD Levels in Petroleum Liquid Waste. *Journal of Ecological Engineering*, 23(5), 26–29.
8. Hargreaves J.A.T C.S. 2004. Managing ammonia in fish pond. SRAC Publication - Southern Regional Aquaculture Center, 4608, 8.
9. Hasianny, S., Noor, E.,Yani, M. 2015. Application of Clean Production for Handling produced water in the Oil and Gas Industry. *Journal of Natural Resources and Environmental Management*, 5(1), 25–32. (in Indonesia)
10. Irawan, T., Amin, B., Anita, S. 2021. Phytoremediation of Petroleum Contaminated Soils Using Bahia Grass Plants (*Paspalum notatum*). *Journal of Environmental Sciences*, 15(1), 1. (in Indonesia)
11. Lestari, Y.P., Aminatun, T. 2018. Effectiveness of Plant Biomass Variations *Hydrilla Verticillata* in Phytoremediation of Batik Waste. *Journal of Biology Study Program*, 4, 233–241. (in Indonesia)
12. Linggawati, A., Maryani, Nugroho, A.P., Rachmawati, D. 2022. Anatomical and Histochemical Responses of Vetiver Grass (*Chrysopogon zizanioides* L. Roberty) to Phytoremediation Ability of Liquid Batik Waste. *Environment and Natural Resources Journal*, 20(4), 359–368.
13. Mendez, M.O., Maier, R.M. 2008. Phytostabilization of mine tailings in arid and semiarid environments - An emerging remediation technology. *Environmental Health Perspectives*, 116(3), 278–283.
14. Muro-González, D.A., Mussali-Galante, P., Valencia-Cuevas, L., Flores-Trujillo, K., Tovar-Sánchez, E. 2020. Morphological, physiological, and genotoxic effects of heavy metal bioaccumulation in *Prosopis laevigata* reveal its potential for phytoremediation. *Environmental Science and Pollution Research*, 27(32), 40187–40204.
15. Murti, R.S., Purwanti, C.M.H. 2014. Optimization of the Reaction Time of Blue Indophenol Complex

- Formation is Stable on the N-Ammonia Test of Tanning Industrial Wastewater by Fenat Method. *Leather, Rubber, and Plastic Magazines*, 30(1), 29. (in Indonesia)
16. Nurhayati, C. 2010. Liquid Waste Treatment of Oil and Gas Exploration Activities with Comprehensive Solution Methods (Bioremediation, Biotreatment and Bio Filtration). *Journal of Industrial Research Dynamics*, 21,19–27. (in Indonesia)
 17. Odoh, C.K., Zabbey, N., Sam, K., Eze, C.N. 2019. Status, progress and challenges of phytoremediation - An African scenario. *Journal of Environmental Management*, 237, 365–378.
 18. Rahman, M., Khatun, S., Ali, S. 2018. Morphophysiological diversity of root nodule rhizobia from mimosa (*Mimosa pudica* L.) and water mimosa (*Neptunia oleracea* L.). *Researchgate.Net*, 5(1).
 19. Retnaningdyah, C., Arisoelaningsih, E. 2018. Effectiveness of Phytoremediation Process of Irrigation Water Polluted with Organic Matter through Batch Culture System using Local Hydromacrophytes. *Indonesian Journal of Biology*, 14(1), 33–41. (in Indonesia)
 20. SNI. 2005. Exhaust Emissions - Immovable Sources - Part 6: How to test Ammonia Levels (NH_3) using Indophenol Method using a Spectrophotometer. (in Indonesia)
 21. SNI. 2009. Water and Wastewater - Part 70: How to test Sulfide with Methylene Blue Spectrophotometry. (in Indonesia)
 22. Sukono, G.A.B., Hikmawan, F.R., Evitasari, E., Satriawan, D. 2020. Phytoremediation Mechanism: A Review. *Journal of Environmental Control*, 2(2), 40–47. (in Indonesia)
 23. Suppadit, T., Phoonchinda, W., Thummaprasit, W. 2008. Efficacy of water mimosa (*Neptunia oleracea* Lour.) in the treatment of wastewater from distillery slops. *Philippine Agricultural Scientist*, 91(1), 61–68.
 24. Utami, L.I., Wihandhita, W., Marsela, S., Wahyusi, K.N. 2017. Petroleum Liquid Waste Treatment is Biologically Aerobic Batch Process. *Journal of Chemical Engineering*, 11(2), 37–41. (in Indonesia)
 25. Yudistira, D.R. 2022. Analysis of the Quality of Refinery Wastewater before is Discharged into Water Bodies. *Swara Patra Scientific Magazine*, 12(1), 46–55. (in Indonesia)