



RESEARCH ARTICLE

REVISED Reduction of hydrocarbon pollutants by hyacinth plants (*Eichhornia crassipes*) [version 2; peer review: 1 approved, 2 approved with reservations]

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V2 First published: 22 Jun 2023, 12:728
<https://doi.org/10.12688/f1000research.131846.1>
 Latest published: 12 Sep 2023, 12:728
<https://doi.org/10.12688/f1000research.131846.2>

Abstract

Background: The application of phytoremediation by utilizing plants has been used to control oil pollution in waters. One of the plants that can act as a phytoremediator is the hyacinth because this plant can reduce various pollutants including petroleum hydrocarbons. This study aims to study the reduction ability of petroleum hydrocarbons at different concentrations including improving water quality.

Methods: This research used an experimental method implementing a Completely Randomized Design (CRD) with one factor, namely TPH concentration with five treatment levels and three replications. The treatments were as follows E1 (10 mg/L), E2 (30 mg/L), E3 (50 mg/L), E4 (70 mg/L), and E5 (90 mg/L), and E0 (control) was carried out only at the highest concentration (90 mg/L), aiming to see whether there was a TPH reduction process without plants. Maintenance of water hyacinth was carried out in media with salinity (3 ppt). Observations were made for 14 days, with measurements of TPH and leaf chlorophyll content observed at D-1, D-7 and D-14. While water quality parameters such as temperature, pH and dissolved oxygen were observed every 2 days

Results: The results showed that the hyacinth plant was able to reduce hydrocarbon in terms of total petroleum hydrocarbon (TPH) by 79% while it was only between 17–27% naturally without the hyacinth. The reduction of TPH in the water was in line with the decrease of chlorophyll in the leaves of hyacinths, and it was followed by the increase of dissolved oxygen in the water media.

Conclusions: In conclusion, hyacinths can reduce petroleum hydrocarbons and they can improve the water quality as well. Furthermore, water hyacinth which are commonly found in freshwater can be used as phytoremediators in coastal area; its application in coastal areas requires further study

Keywords

Hydrocarbon pollutants, Hyacinths, Phytoremediation, TPH, Chlorophyll

Open Peer Review

Approval Status

	1	2	3
version 2 (revision) 12 Sep 2023	 view	 view	 view
version 1 22 Jun 2023	 view	 view	

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This article is included in the **Plant Science** gateway.

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Author roles: **Nedi S:** Conceptualization, Data Curation, Formal Analysis, Methodology, Writing – Original Draft Preparation; **Effendi I:** Data Curation, Formal Analysis, Methodology, Supervision, Writing – Original Draft Preparation; **Tanjung A:** Data Curation, Validation, Writing – Review & Editing; **Elizal E:** Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: Authors express their gratitude to the Research and Community Service Institute of the Universitas Riau, which has funded this research through the DIPA Universitas Riau in 2022 (Grant No. LPPM-UNRI/2022/KOM/1007/145).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Nedi S, Effendi I, Tanjung A and Elizal E. **Reduction of hydrocarbon pollutants by hyacinth plants (*Eichhornia crassipes*) [version 2; peer review: 1 approved, 2 approved with reservations]** F1000Research 2023, 12:728 <https://doi.org/10.12688/f1000research.131846.2>

First published: 22 Jun 2023, 12:728 <https://doi.org/10.12688/f1000research.131846.1>

REVISED Amendments from Version 1

1. Additional literature used such as Chakravarty et al. (2023), Chakravarty et al. (2022), Chakravarty & Deka (2021), and Kalita et al. (2022).
2. The use of control (E0) in this study was carried out only at the highest concentration (90 mg/L), the aim was to see whether there was a reduction in TPH without plants.
3. Research gap: only carried out at limited salinity, namely 3 ppt.
4. Water quality parameters, such as temperature, pH and dissolved oxygen were observed in-situ using a water checker. While chlorophyll measurements were carried out directly using a Chlorophyll-meter on plant leaves.

Any further responses from the reviewers can be found at the end of the article

Introduction

Oil pollution is a common problem in coastal waters; such as oil slicks or spills. Most of the oil contains toxic hydrocarbon compounds that are vulnerable to habitats and biota. Common ways to encounter this pollution are through the oil boom, skimmer, dispersant, and bioremediation.¹⁻⁷ Bioremediation is a way to clean up contaminants in an environment by living organisms; for example, the use of bacteria and water plants.

Water hyacinth is one of plants that can be used as phytoremediator. This plant is easy to find in freshwater both in land and coastal area. Beside it grows faster, high adaption in nature.⁸ This plant has ability to absorb pollutant with faster proliferation in a water.⁹

Research on using hyacinth as a phytoremediator has been well documented¹⁰⁻¹² including to industrial waste.^{13,14} Furthermore, this plant can also reduce about 99.5% of hexavalent chromium (Cr(VI)) in waste water, as well as to reduce TDS, BOD, and COD content.¹⁴ Even though this plant can also be used as phytoremediator to heavy metal such as Hg and MeHg attaching on leaf of plants.¹⁵

According to Priya and Selvan, water hyacinth can be used as a bioindicator of heavy metals in aquatic ecosystems.¹⁶ In addition, water hyacinth as phytoremediators is cost-effective and eco-friendly and well-documented.^{17,18} Based on this circumstance, the author is interested in testing the ability of the hyacinth plant to reduce hydrocarbon pollutants in saline water.

Method

Time and place

This research was conducted in June 2022 at the Lagio Laboratory, Pekanbaru. The test plant, hyacinth, was taken from the reservoir of Binawidya Campus, Faculty of Fisheries and Marine, Universitas Riau. Meanwhile, the hydrocarbon pollutants used come from Pertamina DEX Solar from Pekanbaru gas stations. The seawater was taken from the coastal waters of Dumai and composted fertilizer was also used for the test plants. The plants were grown in 20 L plastic washbasins.

Research methods

This experiment used Completely Randomized Design (CRD) method with one factor and five levels of hydrocarbon concentrations treatments; three replications of each treatment. The treatments were: E1 = 10 mg/L, E2 = 30 mg/L, E3 = 50 mg/L, E4 = 70 mg/L, E5 = 90 mg/L and E0 = without plant. The E0 played as a control to know the natural evaporation and transpiration during observation. The reductions of the petroleum hydrocarbon in the treatments were measured in terms of total petroleum hydrocarbon (TPH).

Procedure

Acclimatization to salinity

The container used was a black tub with a volume of 10 L of 15 units. The research was started by acclimatizing hyacinth plants to different salinities, namely 1, 3, 5, 7, and 9 ppt. In each container, 200 g of hyacinth was added and its growth was observed for seven days. Plant growth was seen based on changes in leaf color, root condition, and number of leaves. The acclimatization results showed that the test medium with a salinity of 3 ppt was suitable for plants to grow in coastal areas, some plants at salinity 5, 7, and 9 died after being given salinity treatment.

Petroleum hydrocarbon observations

After determining the suitable salinity for the test plants, they were then planted in petroleum hydrocarbon pollutant solutions with different concentrations, namely 10, 30, 50, 70, and 90 mg/L, and without plants (control). The first step was a black tub with a volume of 20 L of 15 units filled with 10 L of water with a salinity of 3 ppt. Then 50 g of fertilizer was added to each container and 200 g of test plants were added. After that, petroleum hydrocarbons were added according to the concentration determined and observed for 14 days.^{19,20} The observations were conducted from the first day (D-1), seventh day (D-7), and fourteenth day (D-14) by using ASTM 7066-04 (FTIR) method.

The parameters observed were total petroleum hydrocarbons, water quality (temperature, pH, DO, and salinity), and plant conditions (leaf color, root condition, and stem shape). To maintain the salinity in the test container, fresh water was added according to a predetermined volume limit, i.e. 10 L. Each container was marked, to determine the level of water whose volume is 10 L.

Leaf chlorophyll measurement

The measurement of chlorophyll was carried out with chlorophyll meter (KWF type). It was carried out by placing the hyacinth leaf in the sensor area of the equipment; the reading could be occurred between seconds. The Chlorophyll measurements of the treatments were conducted *in situ* between 10.00 AM–03.00 PM.

Data analysis

Data on TPH concentration and chlorophyll were tabulated and analyzed using SPSS version 23. Data were analyzed using a *One Way ANOVA* with a confidence level of 95% and continued by a *Student Newman Keuls* (SNK) test if necessary.

Result

Total petroleum hydrocarbon concentration

The phytoremediators (hyacinths) could grow well in the tested media; with 3 ppt of saline water at 10–20 cm in height after acclimation. The content of hydrocarbon pollutants can naturally decrease, this also occurs in the treatment without the addition of plants; this is thought to be due to evaporation (E0). The concentrations of TPH in all treatments at the time of observations can be seen in **Table 1**.

Table 1 showed that the TPH in all treatments reduced after the seventh day (D-7) and the 14th day (D-14) of observations. The reductions were higher on the seventh day in comparison to the 14th day of observation. These reductions ranged from 6.3–47.27 mg/L and from 1.6–9 mg/L consecutively. However, the total reductions ranged from 7.9–56.27 after the 14th day of observations. This means that the longer the observation time the higher the TPH reductions. The affectivity of the plant to reduce TPH showed the same pattern due to the time constraint of observations.

In addition, there was a significant effect of hydrocarbon concentrations on the plant’s ability to reduce TPH, based on ANOVA analysis that $p < 0.05$.

This shows that water hyacinth is able to absorb hydrocarbons up to a concentration of 90 mg/L.

Table 1. The concentrations and the reduction of total petroleum hydrocarbon (TPH) in each media of treatments on each observation day.

Treatment/TPH con. (mg/L)	TPH concentration (mg/L)				Total reduction (mg/L)
	D-7		D-14		
	Con.	Red.	Con.	Red.	
E1 (10)	3.70±0.20	6.30±0.02 ^a	2.10±0.10	1.60±0.08 ^a	7.90±0.10 ^a
E2 (30)	17.33±0.76	12.67±0.76 ^b	11.23±0.25	6.10±0.51 ^{bc}	18.77±0.25 ^b
E3 (50)	23.73±1.25	26.27±1.25 ^d	19.07±0.95	4.66±0.30 ^b	30.93±0.95 ^d
E4 (70)	37.07±1.05	32.93±1.05 ^e	29.27±1.25	7.80±0.20 ^c	40.73±1.25 ^e
E5 (90)	42.73±1.95	47.27±1.95 ^f	33.73±0.25	9.00±1.70 ^d	56.27±0.25 ^f

Notes: E1 (10 mg/L); E2 (30 mg/L); E3 (50 mg/L); E4 (70 mg/L); E5 (90 mg/L); The superscript on the same line shows that there is an effect between treatments ($P < 0.05$).

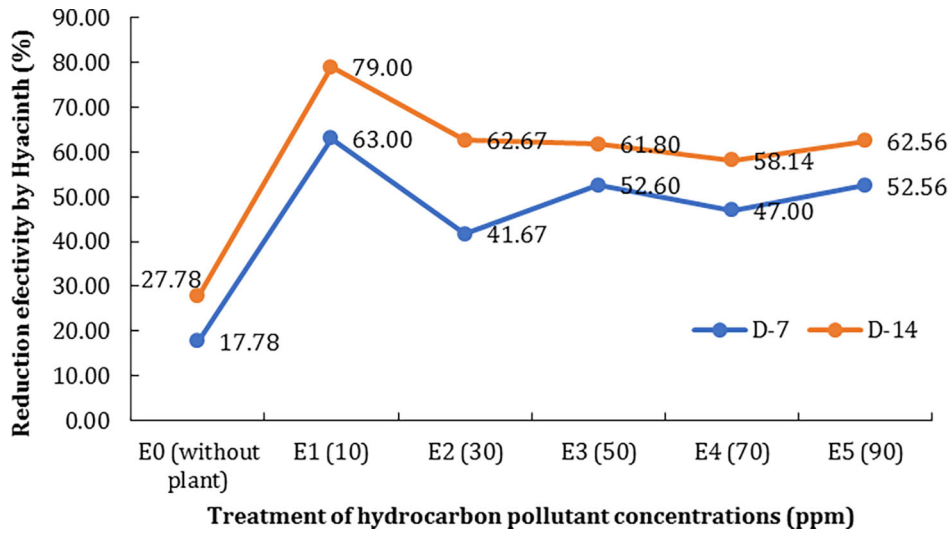


Figure 1. The effectiveness of the plant in decreasing total petroleum hydrocarbon (TPH).

The treatment without a phytoremediator (E0) can only reduce the TPH by as much as 17.78% after the 7th day (D-7) and 27.78% after the 14th day (D-14). In contrast, it ranged between 41.67% and 63% reduction using a phytoremediator. The highest effectivity of reductions was found at the treatment E10 (79%) with 10 ppm of petroleum hydrocarbon because the reduction process will be optimum at lower concentrations of pollutants (Figure 1).

Chlorophyll content and water quality

Chlorophyll is also an indication of the productivity of green plants and is one of the important elements in the process of photosynthesis. The results showed that the amount of chlorophyll in the leaves of the test plant (hyacinth) decreased in line with the increase in hydrocarbon pollutants and the length of observation time (Figure 2). At the beginning of maintenance, the chlorophyll content in hyacinth leaves was 48.3 units. Along with TPH administration, there was a decrease in chlorophyll concentration in hyacinth leaves. In treating hydrocarbon pollutants (e.g. E5), the amount of chlorophyll encountered was 17.9 units on the seventh day and 13.9 on the 14th day.

Differences in pollutant concentration treatment influence the chlorophyll content in hyacinth leaves ($p < 0.05$). Another parameter of water quality is dissolved oxygen (DO). In control media (without plants), the average DO on the first day (D-1) was 3.0 mg/L; it increased to 3.3 mg/L and 3.5 mg/L after seven and 14 days consecutively. It indicated that the DO naturally increased with the pollutant hydrocarbon. However, the increases of DO were higher in the treatments with hyacinth phytoremediators; it was up to 20% compared to the treatments without the phytoremediator plants.

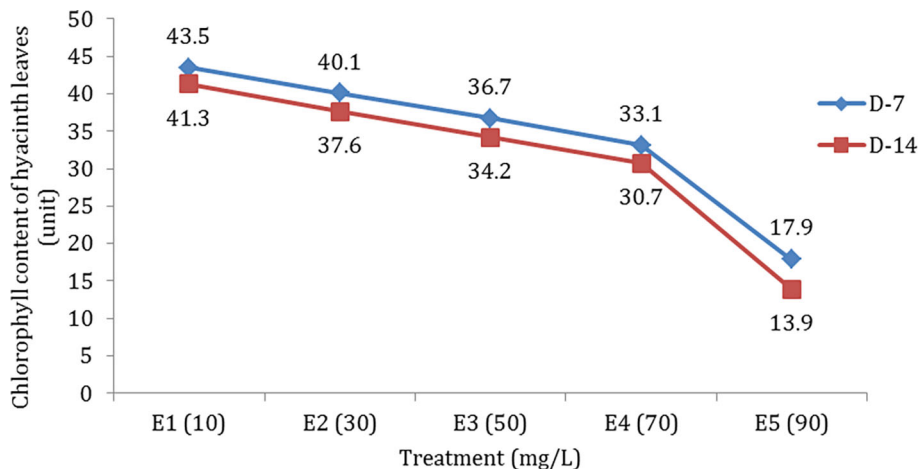


Figure 2. Chlorophyll content (mg/L) in hyacinth leaves based on observation time.

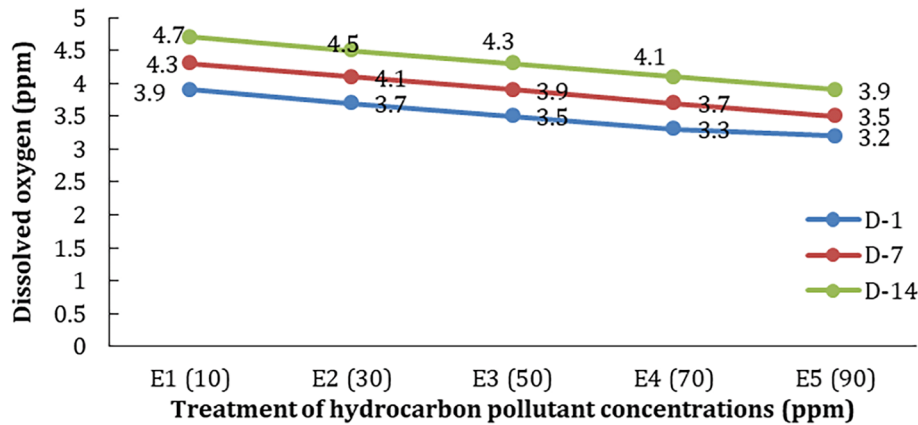


Figure 3. The decrease in dissolved oxygen (DO) in each treatment at the time of observation.

Variations in the concentration of hydrocarbon pollutants affect the dissolved oxygen of the test medium. The higher the concentration of hydrocarbon pollutants given, the higher the decrease in DO content in the test media water (Figure 3).

However, the DO figures were slightly higher based on the days of observations and concentrations; D-14>D-7>D-1. Temperature plays an important role in plant life and growth. A good temperature for the plant to grow is known as the optimum limit of temperature. At this limit, plants can grow well both in terms of morphology and physiology. Temperature conditions at the treatment with 10 mg/L hydrocarbon without phyto-remediator’s plant (E0) were seen to decrease in line with the observation time ((D-1, D-2, and D-3); see Figure 3).

Figure 4 shows that the presence of hydrocarbon pollutants affected the temperature of the water test medium. On the seventh and 14th days, the same concentration of hydrocarbon pollutants occurs with a decrease in temperature by 0.2–0.3°C.

The temperature in the treatment medium with different concentrations of hydrocarbon pollutants and phyto-remediators (E1–E5) can be seen in Figure 5.

Based on this figure, it can be seen that the temperature of the media increases in line with the increase in pollutant concentration and the length of time of observation. The increase in temperature may be closely related to the hydrocarbon reduction process that releases heat by phyto-remediators when carrying out water purification. The increase in temperature may be closely related to the hydrocarbon reduction process that releases heat by phyto-remediators when carrying out water purification and it might be due to the phyto-remediator process and pollutant degradation.

The degree of acidity (pH) is the concentration of hydrogen ions in the water. The pH of the growing water of treatments tended to decrease along with the higher concentration of pollutants; it might be due to the phyto-remediator process and pollutant degradation. The decrease was 0.4–0.7 between D-1 and D-14 (Figure 6).

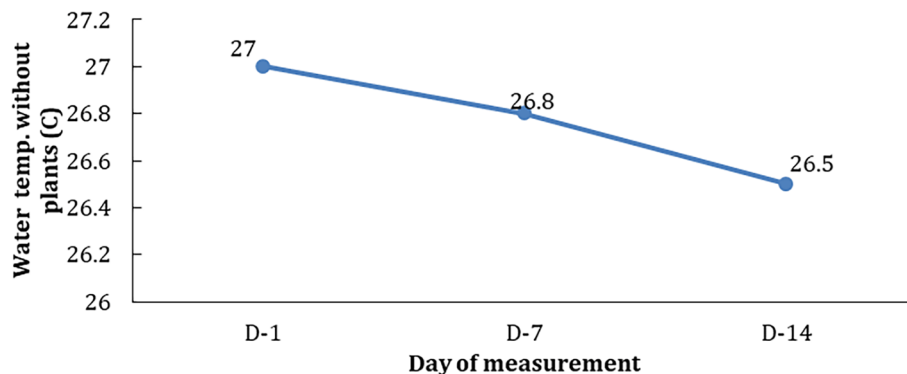


Figure 4. The temperature of the control medium (E0) on each day of observations.

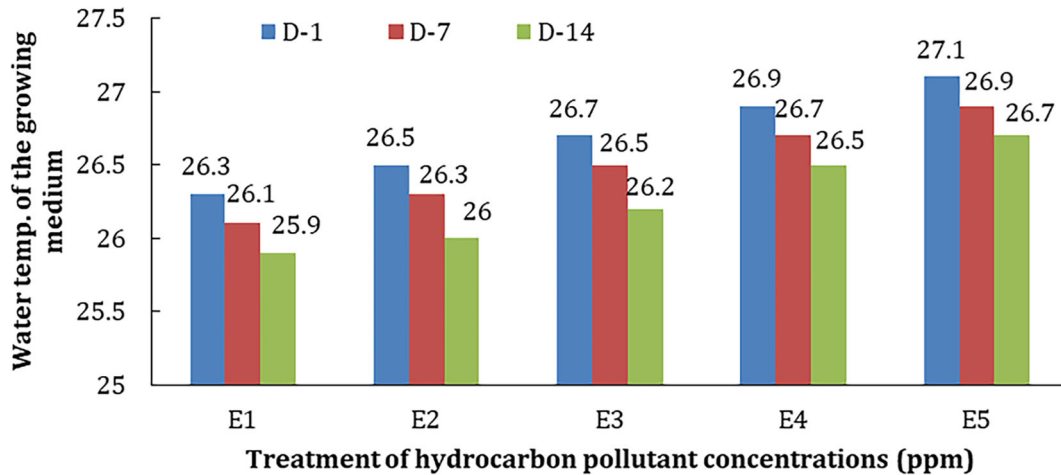


Figure 5. The temperature of treatment media at various concentrations during observation.

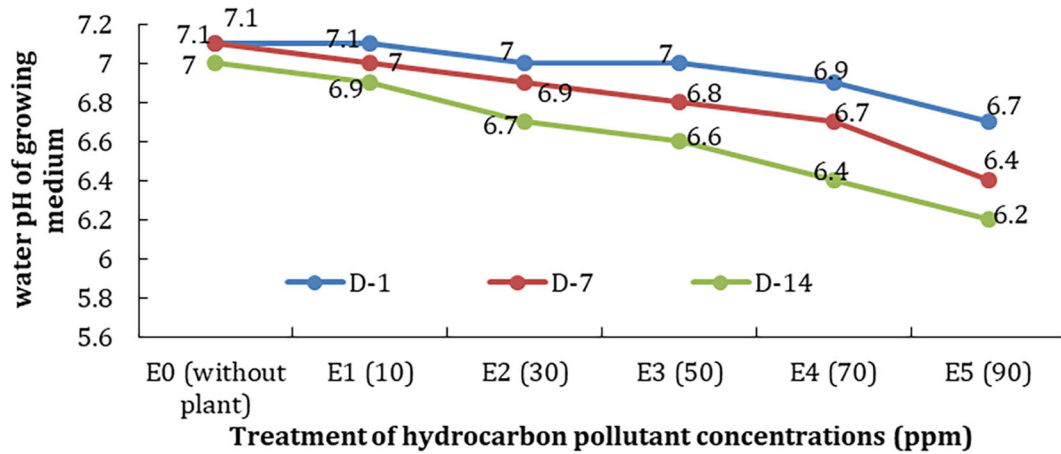


Figure 6. Decrease in water pH on the treatment of differences in concentration and observation time.

Discussion

Water hyacinth is a native freshwater plant from South America.²¹⁻²³ The results showed that this plant grows well at a salinity of 3 ppt; Ting *et al.*, 2018²⁴ also reported that this plant can adapt and grow at a salinity of <5 ppt. In addition, this plant can act as a phytoremediator against toxic pollutants derived from petroleum hydrocarbons. The effectiveness of TPH reduction depends on the concentration and length of time of observation (Table 1 and Figure 1). Meanwhile, optimum reduction efficiency is found in treatments with low concentrations (10 mg/L); i.e. 63% on D-7 and 79% on D-14. The process of reducing TPH by this plant is inseparable from its root system which acts as an absorbent and then spreads to all parts of the plant.²⁵⁻²⁷ Some of the organic content contained in hydrocarbon pollutants is used as nutrients for plants; Oke *et al.*, 2020²⁸ reported that the highest absorption of hydrocarbons by 100 g of hyacinth is 72%. The presence of biodegradable bacteria in the water hyacinth root system may also play a role in the process of reducing TPH in the test media, according to Xia & Ma (2006)²⁹ the presence of bacteria in roots can degrade pollutants by 12%. Thus, these plants can purify water contaminated by hydrocarbon pollutants.

Although water hyacinths can reduce TPH and purify water, it has an impact on the growth of the test plants. This condition was indicated by a decrease in the amount of chlorophyll in each treatment along with an increase in concentration and length of observation time (Figure 2).

The decrease in the amount of chlorophyll on the leaves is inseparable from the disruption of metabolic processes in plants so that their productivity is disturbed. Plants exposed to pollutants for a certain time will experience chlorosis due to inhibition of the chlorophyll synthesis.³⁰ Of course, a decrease in the amount of chlorophyll affects the process of photosynthesis and enrichment of DO in waters.

In the control treatment without hyacinths (E0), it was seen that DO increased in line with the observation time (Figure 3). The amount of DO in the treatment with hyacinths was higher than without plants or controls. The increase in DO may come from the diffusion of oxygen from the air as well as the photosynthetic activity of phytoplankton and test plants. Nevertheless, DO conditions decreased with increasing concentrations of hydrocarbons and the length of time of observation (Figure 5). The same condition is also shown by the temperature and pH of the water test plant (Figure 6). The decrease could be due to the reduction of chemical processes and the decomposition of TPH. Where in these processes, in addition to requiring oxygen, there is also absorption and release of heat, including the use of hydrogen ions which affect the pH of water.

Conclusion

Hyacinth plants can reduce hydrocarbon pollutants. The effectiveness of the reduction of hydrocarbon pollutants by hyacinths can reach 79% at a hydrocarbon pollutant concentration of 10 mg/L. The presence of hydrocarbon pollutants has led to a decrease in chlorophyll in the leaves of hyacinths. Hyacinths can absorb organic matter without using oxygen for the decomposition process. Differences in pollutant concentration treatment influence the chlorophyll content in hyacinth leaves ($p < 0.05$). Watered hydrocarbon pollutants are tolerated by hyacinth plants and can prevent water quality degradation.

Data availability

Underlying data

Zenodo: Reduction of Hydrocarbon Pollutants by Hyacinth Plants (*Eichhornia crassipes*), <https://doi.org/10.5281/zenodo.7659979>.³¹

This project contains the following underlying data:

- EXCEL KONFILASI DATA ECENG GONDOK (1).xlsx (Effectiveness Hyacinth, water quality and Chlorophyll)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Acknowledgements

We would like to thank the Laboratory of Fisheries Universitas Riau.

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Open Peer Review

Current Peer Review Status: ? ✓ ?

Version 2

Reviewer Report 08 August 2024

<https://doi.org/10.5256/f1000research.155785.r305684>

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Santhana Krishnan

Prince of Songkla University, Hat Yai, Songkhla, Thailand

The manuscript is not written well. It doesn't follow the scientific standards.

Some of the specific comments are provided below.

1. Abstract: In Methods section, TPH should be given in full form in the first appearance.
2. I would see clusters of references for the first 3 lines. Authors must eliminate those multiple references. After that please check the manuscript thoroughly and eliminate ALL the lumps in the manuscript. This should be done by characterizing each reference individually. This can be done by mentioning 1 or 2 phrases per reference to show how it is different from the others and why it deserves mentioning.
3. Introduction is very small with no information. For instance, in the introduction, you need to connect the state of the art to your paper goals. Please follow the literature review by a clear and concise state of the art analysis. This should clearly show the knowledge gaps identified and link them to your paper goals. Clearly discuss what the previous studies that you are referring to.
4. In your discussion section, please link your empirical results with a broader and deeper literature review. For instance, RSM, Hyacinth significance in TPH removal, etc.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental chemical Engineering

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 10 November 2023

<https://doi.org/10.5256/f1000research.155785.r205638>

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Anithadevi Kenday Sivaram

The University of Newcastle, Callaghan, New South Wales, Australia

Thanks for revising the manuscript. However, some queries not addressed in version 2 need to be addressed.

Specific comments:

Introduction:

The introduction section needs further improvement by addressing all the previous comments.

Methods:

1. Please include the initial measured concentration, their percentage recovery, and the basis for choosing different TPH concentrations for your study.
2. Please add a section on the TPH extraction and analysis procedure in water and plants and the QA/QC procedure.

Results:

1. Please include standard deviation (SD) in all the figures, wherever applicable.
2. Overall, the explanation of the results can be improved.

Discussion:

The discussion part still focused on the results. Needs comprehensive discussion and comparison with other similar works.

Conclusion:

Please provide an overall conclusion rather than repeating the results in the section.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytoremediation, Ecotoxicology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 03 October 2023

<https://doi.org/10.5256/f1000research.155785.r205639>

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**Hemen Deka**

Ecology and Environmental Remediation Laboratory, Department of Botany, Gauhati University, Guwahati, Assam, India

The issues raised with the MS during the evaluation process have been addressed adequately. The MS has been significantly improved and can be accepted for publication.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioremediation and phytoremediation of hydrocarbons and heavy metals

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 29 August 2023

<https://doi.org/10.5256/f1000research.144729.r196910>

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Hemen Deka

Ecology and Environmental Remediation Laboratory, Department of Botany, Gauhati University, Guwahati, Assam, India

Overall comments:

The authors of the manuscript conducted research on “Reduction of hydrocarbon pollutants by hyacinth plants (*Eichhornia crassipes*)”—however, the authors must make it more evident in their paper to outline the design of the study and methods. The manuscript's writing and discussion are not up to mark, requiring thorough improvements across all sections. In addition to addressing scientific shortcomings, the entire paper necessitates careful editing and enhancement. Make sure that your manuscript has been thoroughly edited for proper use of the English language and

accurate technical terminology.

Authors should have a strong justification for choosing only hyacinth plants (*Eichhornia crassipes*). Why not other aquatic plants? It should be mentioned more elaborately in the introduction part itself.

Certain errors in spelling are also noticeable. Please correct these mistakes.

Specific comments:

Abstract:

- Requires modifications. Please incorporate more numerical data into the abstract.

Introduction:

- Introduction part is very poor. It should be expanded.
- This section lacks clear cut objective of the study. Also highlight the significance of the topic, identify research gaps, and explain how this paper will contribute theoretically, empirically, and methodologically.

Research methods:

- How was petroleum hydrocarbon added to the water? Isn't it non-polar? Write the detail procedure.

Petroleum hydrocarbon observations

- What is the concentration of total petroleum hydrocarbons in control without plants?
- How do you calculate total petroleum hydrocarbons, pH, DO etc.? Give standard protocol.
- Table 1: concentration of petroleum hydrocarbon in E0=?

Data analysis

- In which significance level data were analyzed?

Result:

- Please rewrite the section so that it becomes readable for readers.

Total petroleum hydrocarbon concentration

- "This is thought to be due to evaporation (E0)" - you should mention this line in 'Discussion'.
- 3rd para: What is E10?
- Table 2, 2nd column-last row: Is Con. of E0=90? I can't understand. Why do you keep only one concentration for total petroleum hydrocarbons in E0? Why not other concentrations like 10, 30, 50 and 70 ppm as mentioned in the treatments with phytoremediator?
- The figures and tables can't communicate on their own. Instead, the authors should explain the insights revealed by the findings. In Figure 2, 5 and 6, check the Y-axis title and give proper units
- You have taken three replicas, right? Then why didn't you mention about standard deviations in any of the figures. Also, In each figure, instead of putting values above the line or bar, you should give a clear cut idea about significant variations in the values.

Chlorophyll content and water quality

- In the treatment without hydrocarbons (E0): What is E0? Previously, in Table 2 you mentioned E0 as without phytoremediator? Here you have mentioned E0 as without hydrocarbons. Please give a detail information about the treatments with proper notation.
- "Temperature conditions at the treatment with 10 ppm hydrocarbon without phytoremediator's plant (E0) were seen to decrease in line with the observation time ((D-1, D-2, and D-3)" - Isn't it D-1, D-7, D14?
- In Figure 3 red line indicates 'with plant'- What is the notation for this treatment? Please give in details.
- "Based on this figure, it can be seen that the temperature of the media increases in line with the increase in pollutant concentration and the length of time of observation." - The figure is not indicating itself the findings.
- "The increase in temperature may be closely related to the hydrocarbon reduction process that releases heat by phytoremediators when carrying out water purification" & "ii) it might be due to the phytoremediator process and pollutant degradation." - these two sentences should be written in discussion part.
- "The pH of the growing water of treatments..." - What is 'growing water'?
- I recommend for revising the discussion to better address the theoretical, empirical, policy, and methodological issues presented in the paper. Also, more recent literature in the introduction and discussion section is of required. For better representation your MS, you may add new references and work; the following are suggested references: Chakravarty & Deka (2021)¹; Chakravarty *et al.* (2022)²; Kalita *et al.* (2022)³; Boruah *et al.* (2020)⁴; Chakravarty *et al.* (2023)⁵.

Conclusion:

- The concluding section is quite brief. Kindly present the specifics of your work concisely, making it easily accessible to everyone.

References:

- All references according to the journal format.

References

1. Chakravarty P, Deka H: Enzymatic defense of *Cyperus brevifolius* in hydrocarbons stress environment and changes in soil properties. *Sci Rep.* 2021; **11** (1): 718 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Chakravarty P, Chowdhury D, Deka H: Ecological risk assessment of priority PAHs pollutants in crude oil contaminated soil and its impacts on soil biological properties. *J Hazard Mater.* 2022; **437**: 129325 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Kalita M, Chakravarty P, Deka H: Understanding biochemical defense and phytoremediation potential of *Leucas aspera* in crude oil polluted soil. *Environ Sci Pollut Res Int.* 2022; **29** (38): 57579-57590 [PubMed Abstract](#) | [Publisher Full Text](#)
4. Boruah T, Chakravarty P, Deka H: Phytosociology and antioxidant profile study for selecting potent herbs for phytoremediation of crude oil-contaminated soils. *Environ Monit Assess.* 2020; **192** (12): 766 [PubMed Abstract](#) | [Publisher Full Text](#)

5. Chakravarty P, Deka H, Chowdhury D: Anthracene removal potential of green synthesized titanium dioxide nanoparticles (TiO₂-NPs) and *Alcaligenes faecalis* HP8 from contaminated soil. *Chemosphere*. 2023; **321**: 138102 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioremediation and phytoremediation of hydrocarbons and heavy metals

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 21 August 2023

<https://doi.org/10.5256/f1000research.144729.r192190>

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Anithadevi Kenday Sivaram

The University of Newcastle, Callaghan, New South Wales, Australia

In this manuscript, the author applied phytoremediation using water hyacinth to remove the total petroleum hydrocarbons from the water. The work is presented with current literature. However, there are issues with the experimental techniques and methods. Firstly, the method section lacks information on how the TPH concentration in water is measured after spiking, the QA/QC procedure for TPH analysis and spiking recovery (%). Secondly, the procedure for other

parameters (DO, Salinity) were missing. Finally, it is important to include bioaccumulation in the hyacinth by measuring the concentration of TPH in the hyacinth.

Specific comments:

Abstract:

1. Please rewrite the last three sentences in the background.
2. Please give the details on the treatments and control clearly for better understanding. As per the results, there are 2 controls i) one without plant grown in 90 ppm TPH and ii) another with plant and without TPH concentration for biochemical and TPH analysis.
3. It is good to change the concentration expression to mg/L rather than expressing it as ppm. Also, measured TPH concentration can be provided rather than the nominal concentration.

Introduction: Too short. It would be better to add about the i) toxic properties of TPH; ii) remediation methods followed; iii) Significance of phytoremediation; iv) why water hyacinth and previous reports to support your plant and TPH selection.

Materials and Methods:

1. Please mention the controls used for the experiment.
2. It is recommended to include the measured concentrations and the basis for choosing different TPH concentrations.
3. Please add a section on the TPH analysis procedure in water and plants and QA/QC procedure.
4. Please add on the pH, DO and salinity experiment procedure.

Result:

1. Table 2. It is better to provide the results in terms of removal percentage by merging the results in Fig.1 to Table 2 for better clarity.
2. Fig.2 can be removed.
3. It is suggested to include standard deviation (SD) in all the figures.
4. Figure 7. Is the E0 with TPH conc?
5. Overall, the explanation of the results can be improved.

Discussion: The discussion part focused more on results. Needs comprehensive discussion.

Conclusion: Explains only results and also repeats the contents present in the abstract and result section. Please revise.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytoremediation, Ecotoxicology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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