

# Bioremediation of Spent Engine Oil Contaminated Soil with Bacteria Isolated from Municipal Waste Dumpsite Amended with Water Hyacinth (*Eichhornia Crassipes*) Leaves as Biostimulant

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ABSTRACT: The paper reports the investigation bioremediation of spent engine oil-contaminated soil augmented with bacteria isolated from municipal dumpsite leachate and amended with water hyacinth leaves (Eichhornia crassipes) as bio-stimulants using appropriate standard methods. Data obtained showed that morphological and biochemical analyses identified six gram-negative and two gram-positive isolates, including Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Staphylococcus aureus, Pseudomonas putida, Escherichia coli, Enterococcus faecalis, and Citrobacter koseri. Qualitative screening using the redox indicator dye 2,6-Dichlorophenol indophenol indicated that Pseudomonas aeruginosa and Staphylococcus aureus exhibited the highest hydrocarbon degradation capabilities. For the bioremediation study, sterilized spent engine oil was introduced into uncontaminated soil at a concentration of 25 g/kg. The contaminated soil was divided into several treatment sets: SET A (control), SET B (with Pseudomonas aeruginosa), SET C (with Staphylococcus aureus), and SET D (with both microorganisms). Additionally, water hyacinth leaves were added in parallel experiments, creating groups SET E (with water hyacinth), SET F (with Pseudomonas aeruginosa and water hyacinth), SET G (with Staphylococcus aureus and water hyacinth), and SET H (with both and water hyacinth). The remediation process, monitored for eight weeks, revealed total petroleum hydrocarbon (TPH) losses of 0.02%, 21.09%, 16.17%, 23.64%, 0.03%, 31.57%, 20.13%, and 39.04% for the respective treatments. This study establishes that indigenous bacterial isolates, particularly Pseudomonas aeruginosa and Staphylococcus aureus, possess significant hydrocarbon-degrading abilities and that the addition of water hyacinth enhances biodegradation, providing a dual benefit of removing this invasive plant species from waterways while remediating oil-contaminated soils.

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The contamination of soil with spent engine oil is a persistent environmental problem that poses significant ecological and health risks. Spent engine

oil is a complex mixture of hydrocarbons, metals, and additives that persist in the environment, making it difficult for natural processes to degrade them. When

\*Corresponding Author Email: emekaibezute@gmail.com \*ORCID: 0009-0006-9489-1204 \*Tel: (+234)7038849906 released into the soil, these contaminants reduce soil fertility, hinder plant growth, and pose threats to groundwater quality and the health of terrestrial organisms (Odjegba and Sadiq, 2002). Given the scale environmental contamination, of effective remediation strategies are urgently required to mitigate the long-term impacts of spent engine oil pollution. Bioremediation, the use of microorganisms to degrade or detoxify pollutants, has emerged as a promising, environmentally friendly alternative to traditional physical and chemical remediation methods (Atlas and Hazen, 2011). Unlike conventional techniques, bioremediation harnesses naturally occurring or introduced microbes to break down hydrocarbons into less harmful substances, making it both cost-effective and sustainable (Jain et al., 2011). However, the success of bioremediation depends on several factors, including the availability of nutrients, environmental conditions, and the presence of specific microbial communities capable of degrading Microorganisms isolated hydrocarbons. from municipal waste dumpsites have been found to possess significant bioremediation potential due to their prolonged exposure to diverse pollutants, including hydrocarbons (Ijah and Antai, 2003). These bacteria are often well-adapted to survive in harsh conditions and can metabolize complex organic compounds, making them ideal candidates for treating oilcontaminated soil.

Despite their inherent capacity for degradation, the efficiency of bioremediation processes can be enhanced through the addition of biostimulantsnutrient-rich materials that stimulate microbial activity and accelerate the degradation of pollutants (Yuan et al., 2020). Water hyacinth (Eichhornia crassipes), an aquatic macrophyte, has attracted attention as a potential biostimulant for bioremediation due to its high nutrient content, including carbon, nitrogen, and essential trace elements (Ndimele et al., 2011). Although water hyacinth is often regarded as an invasive species that clogs waterways and threatens aquatic ecosystems, its biomass can be repurposed as an organic amendment to improve soil quality and promote microbial degradation of hydrocarbons. The integration of water hyacinth in bioremediation processes provides a dual environmental benefit: enhancing microbial degradation of oil contaminants while also managing the invasive spread of the plant (Adenipekun et al., 2015).

Hence the objective of this paper is to investigate the bioremediation of spent engine oil-contaminated soil augmented with bacteria isolated from municipal dumpsite leachate and amended with water hyacinth leaves (*Eichhornia crassipes*) as bio-stimulants.

## **MATERIALS AND METHODS**

Collection of Leachate, Soil, Spent Engine Oil, and Bio-stimulant: Leachate was collected from a dumpsite government-approved in Agbarho community, Delta State (5.6033N, 5.8827E) during the dry season. Samples were taken from natural pools and seepage points, transferred using a sterile scoop into pre-labelled containers, and stored in a cooler at 4°C during transport to the laboratory for analysis. Uncontaminated soil was sampled from the Federal University of Petroleum Resources, Effurun (5.5689N, 5.8423E), at a depth of 5-30 cm, after clearing surface debris. The soil was collected using a sterile trowel and auger, placed in containers, cooled at 4°C, and later sieved to 2 mm particle size in the laboratory to form a composite sample. Spent engine oil was sourced from a mechanic workshop in Effurun (5.5587N, 5.7800E) and filtered through 42µm Millipore paper to remove particulates. Fresh leaves of Eichhornia crassipes were collected from Ekpan River, Warri (5.5587N, 5.7453E), washed, air-dried, pulverized using a Viking Exclusive Joncod machine, and stored in sealed containers for later use. All collected samples-including leachate, soil, spent engine oil, and water hyacinth—were sterilized using an autoclave at 121°C for 15 minutes prior to use. Standard methods (APHA, 1998; USEPA, 1996) were employed to assess the physical and chemical properties of the samples, including pH, nitrate, phosphate, sulphate, and hydrocarbon-utilizing bacteria.

Isolation and Purification of Microorganisms: Bacteria capable of degrading spent engine oil were isolated from dumpsite leachate using the enrichment and streak methods (Siddique et al., 2003; Avishai and Charles, 2014). A loopful of the leachate was aseptically inoculated into mineral salt medium (MSM) broth, which was supplemented with spent engine oil as the sole carbon source, and incubated at 37°C for 7 days in a rotary shaker at 120 rpm (Atlas, 1981; Fulekar et al., 2017). This process was repeated through successive subcultures in fresh MSM to progressively enrich hydrocarbon-degrading bacteria (Cerniglia, 1992). After enrichment, the culture was inoculated onto MSM agar plates containing a thin layer of spent engine oil, and the appearance of clear zones indicated bacterial hydrocarbon degradation. The isolates were purified using the streak plate method on nutrient agar, incubated at 37°C for 48 hours, and well-isolated colonies were selected and transferred to fresh plates (Baron and Finegold, 1990). The pure isolates were preserved on nutrient agar slants for long-term storage (Cappuccino and Sherman, 2008).

Biochemical, and VITEK Morphological, II Characterization of Microorganisms: Bacterial isolates obtained from nutrient agar were initially identified through standard morphological, microscopic (Gram staining), and biochemical tests, following the methods outlined in Bergey's Manual of Determinative Bacteriology (Zhou et al., 2008). To confirm these identifications, the VITEK® II Compact System was used for further biochemical typing and antibiotic susceptibility testing. Pure bacterial colonies were suspended in 3.0 mL of sterile saline and analysed using the VITEK® GN ID card (lot no. 2411830403) for Gram-negative bacteria and the VITEK® GP ID card (lot no. 2422002503) for Grampositive in accordance bacteria. with the manufacturer's guidelines (BIOMERIEUX).

Screening of Isolates for Hydrocarbon-Degrading Potential and Inoculum Development: The hydrocarbon-degrading potential of bacterial isolates was assessed using the 2,6-Dichlorophenol indophenol (DCPIP) assay as described by Roy et al. (2002); Koma et al. (2003); Korale et al. (2015). Each assay tube contained 2.5 mL of carbon-free Bushnell Haas medium, supplemented with 150 µL of ferric chloride (FeCl3•6H2O) and 150 µL of DCPIP. A bacterial suspension, standardized to an optical density (OD) of 0.1 at 600 nm, was added (300  $\mu$ L) along with 25 µL of spent engine oil as the hydrocarbon source. The tubes were incubated at 32°C with shaking at 120 rpm for 6 days, during which the presence of hydrocarbon degradation was indicated by a colour change from blue to colourless in the DCPIP indicator. Control setups were established to account for nonbacterial changes and confirm assay conditions. Bacterial growth was monitored at OD 600 nm, with a hydrocarbon decrease indicating successful degradation (Youssef et al., 2010). The isolates exhibiting the most significant degradation were selected for further study.

For inoculum development, a loopful of each selected colony was inoculated into 5 mL of sterile 0.1% NaCl solution and centrifuged at 4,000 rpm to pellet the bacterial cells. The pellets were washed twice with sterile distilled water to remove impurities, and the final suspension was adjusted to an absorbance of 0.1 at 600 nm using a UV-Vis spectrophotometer to standardize the bacterial concentration before inoculation (Ekundayo *et al.*, 2012; Al-Wasify; El\_Naas, 2015).

*Preparation of contaminated soil:* Three hundred grams (300g) of sterilized, uncontaminated soil was spiked with 7.5 mL of sterilized spent engine oil. The soil-oil mixture was thoroughly blended in a bottle

container to obtain a concentration of 25mg/kg since the density of the spent engine oil is 1000 mg/ml. The mixture was left undisturbed for two days to allow the volatilization of the oil's toxic components, upon which the moisture content of the mixture was adjusted by adding 200mL of distilled water. The adjusted mixture was incubated at room temperature,  $(28 \pm 2 \ ^{\circ}C)$ .

*Experimental setup:* The contaminated soil was divided into groups and setup as described below:

**SET A:** Contaminated soil only

**SET B:** Contaminated soil + *P. aeruginosa* 

**SET C:** Contaminated soil + *S. aureus.* 

**SET D:** Contaminated soil + Consortium

In a parallel experiment, 30g sterilized water hyacinth leaves were added, creating additional groups as described below:

**SET E:** Contaminated soil + *E. crassipes* 

**SET F:** Contaminated soil + *E. crassipes* + *P. aeruginosa* 

**SET G:** Contaminated soil + *E. crassipes* + *S. aureus.* **SET H:** Contaminated soil + *E. crassipes* + Consortium

The content of each vessel was tilled twice a week for aeration and the moisture maintained by the addition of sterile distilled water. The experiment was set up in triplicate. Biodegradation of TPH and remediation of spent engine oil contaminated soil was monitored at four-week interval (0, 28 and 56 days) by analysing the following parameters; pH, TPH, nutrients (sulphate, phosphate, nitrate), total hydrocarbon utilizing bacteria. Composite samples were obtained by mixing 5g of soil collected from four different areas of the glass container for isolation and enumeration of hydrocarbon utilizing bacteria and determination of total petroleum hydrocarbon.

Data Analysis: All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean ± SE. One-way ANOVA was used to determine the differences among various groups. Percentage total petroleum hydrocarbon (TPH) loss was calculated using the formula

$$\% TPH Loss = \frac{TPH Initial - TPH Final}{TPH Initial} X \frac{100}{1} \quad (1)$$

### **RESULTS AND DISCUSSION**

*Physicochemical characteristics:* The physical and chemical characteristics of dumpsite leachate, soil and water hyacinth are shown in Table 1. The leachate is characterized by its alkalinity (pH of 8.01), high

nitrate (55.82  $\pm$  12.31 mg/kg) and moisture content. The soil was more acidic (pH of 6.12), with moderate phosphate (21.8  $\pm$  1.5 mg/kg), low moisture, and a diverse bacterial community. The water hyacinth is slightly acidic (pH of 6.54), with high nitrate (1550.5  $\pm$  0.03 mg/kg), high phosphate (2783.36  $\pm$  1.80 mg/kg), high moisture, and the lowest bacterial count. These findings provide insights into their ecological roles and potential impacts on surrounding ecosystems, underscoring the importance of understanding their chemical and biological dynamics in environmental studies and management practices.

Isolation, Morphological and Biochemical characterization of microorganisms from dumpsite

*leachate:* After Isolation by the direct culture and enrichment technique(s), several distinct pure cultures each were obtained and identified by their morphological and biochemical test. However, some responded similarly displaying overwhelming similarity based on which 8 isolates were selected and reported in Table 1, A total of six identified isolates were gram negative. These include isolates such as *Klebsiella pneumonia, Enterobacter cloacae, Pseudomonas aeruginosa, Pseudomonas putida, Escherichia coli and Citrobacter koseri.* The isolates were predominantly rod shaped with only a few were cocci shaped. This isolates identification was further confirmed using VITEK GN and GP cards for gram negative and gram-positive organism respectively.

<b>Table 1:</b> Baseline physicochemical properties of leachate, soil and water hyacinth leaves								
S/N	Parameter	Leachate	Soil	Water hyacinth				
1	pH	$8.01\pm0.35$	$6.12\pm0.23$	$6.54\pm0.25$				
2	Nitrate (Mg/kg)	$55.82 \pm 12.31$	$40.4\pm0.02$	$1550.5 \pm 0.03$				
3	Phosphate (mg/kg)	$20.6\pm2.0$	$21.8\pm1.5$	$2783.36 \pm 1.80$				
4	Moisture content (%)	$97.84 \pm 3.5$	$7.0 \pm 0.3$	$87.39 \pm 4.12$				
5	HUB (cfu/g)	$7.4 \times 10^{2}$	$6.2 \times 10^{3}$	$3.18 \times 10^2$				
6	Soil texture		Loamy	—				

Keys: HUB: hydrocarbon utilizing bacteria: NB: All values are expressed as Mean ± SE

Screening for the potential of bacteria isolate to degrade hydrocarbon: Table 2 shows the qualitative assessment of the bacteria's ability to degrade hydrocarbons using the 2,6-Dichlorophenol indophenol (DCPIP) redox indicator. Generally, the indicator is blue when oxidized and becomes colourless when reduced. In the test for bacterial hydrocarbon degradation, 62.5% of the samples showed discoloration within 48 hours, as observed visually. This prompted a quantitative analysis to measure bacterial growth using a UV-VIS Spectrophotometer at 600 nm to determine the degree of clarity. Table 2 presents the quantitative assessment of the bacterial isolates' ability to degrade spent engine oil. *Pseudomonas aeruginosa* was the most effective degrader among gram-negative bacteria, while *Staphylococcus aureus* was the best degrader among gram-positive bacteria.

Table 2: Qualitative screening of bacterial isolates for the degradability of spent engine oil using the redox indicator dye 2, 6-

S/N	Isolate	0h	48h	96h	144h
1	CTRL(-ve)	-	-	-	-
2	CTRL(+ve)	+	+	+	+
3	K. pneumonia	-	-	-	+
4	E. cloacae	-	-	-	+
5	P. aeruginosa	-	+	+	+
6	S. aureus	-	-	+	+
7	P. putida	-	+	+	+
8	E. coli	-	-	+	+
9	E. faecalis	-	-	+	+
10	C. koseri	-	+	+	+

Key: - = no change, + = discolouration, CTRL +ve = MSM medium + spent engine oil+ DCPIP, CTRL-ve= = MSM medium + spent engine oil

Bioremediation of Soil Contaminated with Spent Motor Engine Oil: The study examined the bioremediation activities of *Pseudomonas aeruginosa* and *Staphylococcus aureus* on soil contaminated with spent motor engine oil, with results shown in Figures 1 to 7. Over the 8-week period, Total Petroleum Hydrocarbon (TPH) levels generally decreased in setups containing bacterial isolates. The best TPH reduction was observed in the setup combining both *Pseudomonas aeruginosa* and *Staphylococcus aureus* (SET D). The addition of water hyacinth further improved biodegradation effectiveness, significantly reducing TPH levels in SET F, G, and H (3603.87 to 2466.00, 2878.00 and 2197.00 respectively). The consortium treatments (SET C and SET F) were particularly successful in reducing TPH (see Fig 1).

After 8 weeks of bioremediation, the percentage of TPH loss increased in all treatment groups (0.02%,

21.09%, 16.17%, 23.64%, 0.03%, 31.57%, 20.13 and 39.04% for SET A to H respectively) (Fig 2).

 Table 3: Quantitative screening of bacterial isolates for the degradability of spent engine oil by UV-VIS @600nm using the redox indicator dye 2, 6-Dichlorophenol indophenol (DCPIP)

S/N	Sample ID	0h	48h	96h	144h	P-Value
1	CTRL (-ve)	$1.81\pm0.04$	1.81±0.03	1.81±0.30	$1.81 \pm 0.04$	P>0.05
2	CTRL (+ve)	2.76±0.25	2.71±0.38	2.71±0.18	$2.70 \pm 0.63$	P>0.05
3	K. pneumonia		$2.45 \pm 0.20$	2.17±0.39	$1.77 \pm 0.17$	P<0.05
4	E. cloacae		$2.20\pm0.62$	2.12±0.19	$1.19\pm0.82$	P>0.05
5	P. aeruginosa		1.31±0.25	$1.00\pm0.62$	$0.17 \pm 0.29$	P>0.05
6	S. aureus		2.23±0.63	$1.86\pm0.81$	$0.56 \pm 0.38$	P<0.05
7	P. putida		$1.69 \pm 0.25$	1.13±0.54	$0.68 \pm 0.55$	P<0.05
8	E. coli		$2.43 \pm 0.60$	1.77±0.32	$0.76 \pm 0.36$	P<0.05
9	E. faecalis		2.21±0.65	$1.57 \pm 0.30$	$1.37 \pm 0.45$	P<0.05
10	C. koseri		1.91±0.24	1.01±0.15	$0.63 \pm 0.65$	P<0.05

All values are expressed as Mean  $\pm$  SEM; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference at 5% based on ANOVA; CTRL +ve = MSM medium + spent engine oil+ DCPIP, CTRL-ve = MSM medium + spent engine oil

Throughout the 8-week period, the pH of SET A and E remained relatively stable. However, in most setups, the pH shifted from slightly acidic to slightly alkaline during bioremediation (6.01 to 7.83, 7.39 and 8.26 for SET B to D respectively; while it ranged from 5.96 to 8.08, 7.85 and 8.31 for SET F to H respectively) (Fig 3).











Fig 3: Changes in pH during degradation of spent Engine oil by bacteria isolates



spent Engine oil by bacteria isolates



Fig 6: Changes in phosphate concentration during degradation of spent Engine oil by bacteria isolates





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The levels of sulphate, nitrate, and phosphate in SET A and E stayed relatively stable during the bioremediation period (Fig 4, 5, and 6 respectively). In all treatment groups, the levels of sulphate, nitrate, and phosphate decreased during the bioremediation of spent engine oil. The total heterotrophic bacterial counts increased in treatment groups inoculated with bacterial isolates during the bioremediation of spent engine oil over the 8-week period (Fig 7). Treatment groups, particularly the consortium treatments (SET D and SET H), showed increases in bacterial counts ( $2.49 \times 10^5$  to  $2.87 \times 10^5$  and  $2.01 \times 10^5$  to  $3.98 \times 10^5$  respectively) (Fig 7).

The increasing prevalence of spent engine oil contamination in the environment poses significant ecological challenges, necessitating effective remediation strategies. Bacteria play a vital role in bioremediation, utilizing their unique enzymatic capabilities and metabolic pathways to break down complex hydrocarbons into less harmful substances. The significance of indigenous microbial populations has been widely documented in various studies, with Margesin and Schinner (2001) and Das and Dash (2019) demonstrating the ability of native microorganisms to metabolize hydrocarbons efficiently. The current research aimed to isolate and characterize bacterial strains from municipal dumpsite leachate, focusing on their hydrocarbon degradation potential. The isolated species, including Klebsiella pneumonia, Pseudomonas aeruginosa, and Pseudomonas putida, exhibited promising hydrocarbon degradation capabilities, underscoring the utility of local microbial populations in addressing environmental pollution.

The isolation of these bacterial strains aligns with findings from Omusi et al. (2019) and Simon-Oke et al. (2014), which collectively affirm the potential of native microorganisms to mitigate oil pollution effectively. Notably, Pseudomonas aeruginosa and Pseudomonas putida emerged as particularly effective hydrocarbon degraders, showcasing a remarkable ability to utilize hydrocarbons as a carbon source. Their metabolic versatility positions them as promising candidates for environmentally friendly bioremediation strategies. This approach contrasts sharply with conventional chemical remediation methods that may introduce additional pollutants and adversely affect the ecosystem, as highlighted by Koshlaf and Ball (2016). The choice of microbial agents for bioremediation is critical; thus, identifying efficient hydrocarbon degraders is a priority for advancing remediation technologies.

To quantitatively assess the degradation efficiency of the isolated bacterial strains, the 2,6-Dichlorophenol Indophenol (DCPIP) Assay was employed. This colorimetric method measures changes indicative of metabolic activity, microbial providing а straightforward and effective means of evaluating potential microbial candidates for environmental remediation (Simon-Oke et al., 2014). The findings demonstrated significant discoloration of the DCPIP dye, particularly with Pseudomonas aeruginosa and Pseudomonas putida, indicating their high hydrocarbon degradation efficiency. This observation is consistent with existing literature emphasizing the superior hydrocarbon degradation capabilities of these bacterial strains (Al-Wasify and El-Naas, 2015; Das and Dash, 2019). The implications of these findings extend beyond academic interest; they pave the way for developing targeted bioremediation approaches that harness the power of effective microbial strains, thereby enhancing the efficacy of cleanup operations in oil-contaminated environments.

Understanding the environmental parameters that influence the bioremediation process is equally critical. Among these factors, pH plays a significant role, as it directly impacts microbial growth, enzyme activity, and overall metabolic efficiency. Variations in pH can influence the bioavailability of nutrients and pollutants, thus affecting the structure of microbial communities and their hydrocarbon degradation potential (Jones and Brown, 2018). The current study highlights that bio-stimulation through the introduction of specific bacterial strains, coupled with the addition of water hyacinth as a bio-stimulant, and could lead to elevated pH levels. This increase may result from metabolic by-products produced during microbial activity, which could further support hydrocarbon degradation. Existing literature, including studies by Bento et al. (2005) and Odjegba and Fasidi (2007), corroborates this perspective, observing enhanced pH levels during bioremediation treatments. Such findings underscore the importance of maintaining an optimal pH range to maximize the effectiveness of hydrocarbon-degrading bacteria.

Furthermore, the study results suggest that combining effective bacterial strains with organic stimulants, such as water hyacinth, significantly enhances the bioremediation process. This approach aligns with sustainable practices by utilizing native resources to improve microbial efficacy, thereby fostering ecofriendly solutions to oil contamination. Water hyacinth serves not only as a nutrient source but also aids in enhancing soil quality and microbial activity, making it a valuable component in bioremediation strategies. The application of natural amendments, as supported

by research from Wong et al. (2019), can boost microbial growth and activity, creating a synergistic environment conducive to effective hydrocarbon degradation. The study's findings highlight the significant bioremediation potential of bacterial strains isolated from dumpsite leachate, particularly emphasizing Pseudomonas aeruginosa and Pseudomonas putida as effective agents for hydrocarbon degradation. The application of microbial assessments, such as the DCPIP Assay, combined with an understanding of environmental factors like pH, provides a robust foundation for advancing sustainable bioremediation practices. The insights gained from this research contribute substantially to the field of environmental microbiology, offering practical solutions for addressing oil pollution in contaminated sites. In conclusion, the effective utilization of isolated bacterial strains for hydrocarbon degradation offers a promising pathway for mitigating the ecological and health risks posed by oil pollution. Future research endeavours should focus on elucidating the intricate interactions between these bacterial strains and various environmental conditions to optimize bioremediation strategies effectively. This ongoing exploration will be vital in developing innovative and sustainable approaches to environmental cleanup, ultimately contributing to the restoration of contaminated ecosystems and the protection of public health.

*Conclusion*: Overall, the study proved the efficiency of Pseudomonas aeruginosa and Staphylococcus aureus in bioremediating soil contaminated with spent motor engine oil. The incorporation of water hyacinth improved pH regulation and biodegradation efficacy, especially in consortium treatments, underscoring its potential for environmental remedial applications. Water hyacinth is recognized for its swift and abundant development, which can result in its classification as an invasive plant in non-native areas, causing ecological issues in our waterways. To mitigate its negative effects, water hyacinth can be harvested and utilized in bioremediation sites to enhance the efficiency of the process.

*Declaration of conflict of interest:* The authors declare no conflict of interest.

*Data availability statement:* Data are available upon request from the corresponding author.

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