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PROPOSED PLANT SPECIES FOR PHYTOREMEDIATION OF PETROLEUM POLLUTED RANGE-LANDS OF SOUTH SUDAN

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ABSTRACT: The main objective of this recent study was to propose suitable plant species for the Phytoremediation of petroleum-polluted rangelands of South Sudan. Four species were tested, two grasses *(Pennisetum* and *Panicum*), and two legumes (*Sisbania* and *Leuceana*). Potentiality of species for remediation measured depending on response to hydrocarbon contamination levels (40 and 60 ml.kg^{-1}), at different growth stages (15, 30, 45, and 60 days). Species response is represented by growth parameters and allocation of biomass. Analysis of variance revealed significant effects of hydrocarbon contamination and growth stage on biomass development and allocation, with species-specific responses. *Pennisetum* and *leuceana* showed high variability in biomass allocation, while *Sisbania* exhibited stability, suggesting its resilience to hydrocarbon stress. The obtained results underscore the need for considering these factors when selecting plant species for Phytoremediation and ecological restoration in petroleum contaminated ranges, like those of South Sudan. Depending on the obtained variable responses of the studied species, it would be advised to rely on a multi-species approach for effective Phytoremediation.

Keywords: Phytoremediation, Petroleum Pollution, *Pennisetum*, *Panicum*, *Sisbania*, *Leuceana*.

INTRODUCTION

The most destructive environmental issue in petroleum-producing regions worldwide is petroleum contamination. Accidental spills, pipeline breakage, and improper disposal are the main reasons for the discharge of hydrocarbons. The ecosystem represented by soil, plants, and microorganisms is severely affected by spills. Hydrocarbons of petroleum are mixtures of alkenes, aromatics, and resins. When such compounds persist for a long period in soil, it results in accumulation of toxins, reduction in fertilizer elements availability, and consequently reduces biodiversity (Ghosh and Singh, 2005, and Al-Busaidi and Al-Sadi, 2015). This situation stresses the need for an effective method for remediation.

Phytoremediation is an economic and environmentally safe approach that depends upon the use of plants to remove, degrade, or stabilize pollutants from contaminated soils. The use of such a method in petroleum-contaminated regions has gained increasing interest. This method depends upon the ability of plants to absorb, store, and transform hydrocarbons. It is very cheap and environmentally safe, relative to physical or chemical methods of remediation (Ali *et al*., 2013 and Ali and Khan, 2019).

The success of Phytoremediation depends upon the identification of plant species that enjoy any of the following characters; 1) Phytoextraction, where, plants absorb soluble hydrocarbons by roots then transport it to shoot to be stored or transformed to non-toxic forms (Ghosh and Singh, 2005), 2) Phytodegradation, where, plants break-down petroleum hydrocarbonates to less-toxic compounds through microbe activity in root zone or by enzymes produced by plants (Pang *et al*., 2018), 3) Phytostabilization, by preventing spread of pollutants through immobilization at root surface or within plant tissues (Thavamani *et al*., 2017)., and 4) rhizodegradation, which is accomplished by root-associated microbes that are activated by root-exudates. Such exudates provide nutrients for breaking down microbes (Pang *et al*., 2018).

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Grasses and legumes were proposed as a promising species for such remediation. Their role in absorbing contaminants from soil is basically due to high growth rate, deep roots and large biomass (Thavamani *et al*., 2017).

Sorghum arundinaceum and *Tithonia diversifolia*, *Oryza longistaminate* and *Cynodon dactylon* were cited as tolerant species to many of soil pollutants (Ayuba *et al*., 2019, and Chandra and Yadav, 2023). *Medicago sativa* and *vicia faba* proved an added value with nitrogen fixation, which can help restore fertility of soil that had reduced by petroleum pollution (Cunningham and Berti, 1993, Abdulrahman *et al*., 2018, and Ahmed *et al*., 2025).

In South Sudan, petroleum contamination results from the process of extraction and transportation. This represents a great environmental hazard. Relying on Phytoremediation is an efficient and secure means of mitigation. Native plant species of the Palouge South Sudan ecosystem represent a potential mitigator to the petroleum contamination problem. The success of Phytoremediation requires careful selection of plant species that enjoy both tolerance and adaptation to soil and climatic conditions of South Sudan (Koch and Brinton, 1999; Opolot, 2019 and Liu *et al.*, 2022).

Challenges that face Phytoremediation includes slow rate of remediation, plant growth inhibition and a limited number of effective plant species for remediation. Therefore, a recent study was carried out to identify plant species with enhanced petroleum pollutant degradation potential.

MATERIAL AND METHODS

The recent study was carried out to identify potential plant species suitable for Phytoremediation of petroleum-contaminated rangelands of South Sudan. Petroleum spill was obtained from Dar Petroleum Operating Company in Palouge, South Sudan.

Soil preparation

Sandy soil was sieved by a 20-mesh sieve to exclude particles other than sand. Sieved sand was washed with distilled water three times before mixing with the crude petroleum spill. Two rates of contamination, representing medium (40ml.kg^{-1}) and high (60ml.kg^{-1}), were added to two-kilogram pots filled with prepared sand. Petroleum was completely mixed with sand before seeding the proposed range species.

Proposed plant species

The proposed plant species included: 1) *Panicum* maximum “guinca grass,” 2) *Pennisetum glaucum* “pearl-millet,” 3) *Sisbania sesban* “sesaban”, and 4) *Leucaena leucocephala* “Leucaena”. All proposed species are of tropical nature, often used as forages, soil improvers, or as grain crops. Literature indicated the successful use of such species in Phytoremediation strategies to stabilize soil, enhance microbial activity and degrade or uptake contaminants.

Statistical design

A factorial combination of eight treatments represented four plant species, and two levels of petroleum contamination were randomly assigned to six replications of a randomized complete block design. Pots received an equal number (50 seeds) of species. Pots were exposed to open air in the soil and water department of the Faculty of Agriculture, Alexandria University. Sowing seed was on 26th June 2023. Treatments through the same design were repeated on 15th June 2024. Data of required measurements were recorded 15 days after sowing, then every two weeks for three successive measurements to represent developmental stages of plant species (Germination (after 15 days), seedling (after 30 days), vegetative growth (after 45 days), late vegetative and proposed flowering (after 60 days). The growth stage was considered a third factor in the statistical analysis. The test of homogeneity of variance proved the stability of responses across experiments (Bartlett, 1937). Consequently, the average of the two experiments was used for statistical analysis.

Recorded characters included number of germinated plants in each pot after 15 days, then after 30 days then after 45 days, then after 60 days to trace successive response to petroleum pollution exposure. Plant height (cm) was measured from soil surface to the uppermost-tip of plant at that sampling sequence as with germination to follow plant development following contamination exposure. Readings were scored for three plants in each pot, and then the average was used for statistical analysis. Relaying stem growth to root growth was reached by uprooting three plants at each reading time, and scoring stem, and root length (cm). The average of the three reading was used for statistical analysis. Roots were steadily washed by water before scoring fresh weight (g). Stem fresh weight was also scored. Portioned plant parts (stem, leaves, and roots) after being fresh weighted were placed in an oven at 70 $^{\circ}$c until weight constancy to estimate dry matter percentage. The respective values of dry matter were used to estimate the dry weight of each part. Proportional contribution of each plant part was estimated (% leaves, % stem, % root, and leaves/stem ratio). Numerical data on the number of germinated plants were transformed before statistical analysis. Co-Stat statistical program was used for statistical analysis of data according to Steel and Torrie 1980. Least significant difference test was used for means comparison at the appropriate level of probability.

RESULTS AND DISCUSSION

Number of germinated plants

Germinated plants of the studied plant species were traced 15 days after sowing, then at three successive times at two weeks intervals. This had been made to trace the response of germinated seeds to the effect of soil hydrocarbons. Table 1 showed the analysis of variance for number of germinated plants as affected by plant species, hydrocarbons level, and growth stage. Significant ($P \geq 0.01$) effects were expressed by each of plant species, hydrocarbon level, and ($P \geq 0.05$) growth stage. Interactions between plant species × hydrocarbon level, plant species × growth stage, and hydrocarbon level × growth stage and the interaction among plant species × hydrocarbon level × growth stage have not attained the level of significance. This might indicate that the main effects of factors are more influential on germination than their interactions.

Table 1: Analysis of variance for number of germinated plants as affected by plant species, hydrocarbon contamination level, and plant growth stage.

Source of variations	d.f.	Mean squares
Blocks	5	245.1 **
Plant species (A)	3	1675 **
Hydrocarbon level (B)	1	612.1 **
Growth stage (C)	3	108.3 *
A×B	3	79.88 NS
A×C	9	23.58 NS
B×C	3	8.967 NS
A×B×C	9	3.880NS
Error	155	30.24

* and ** indicate significance at 0.05 and 0.01 levels, respectively.
NS; not significantly different.

Table 2 shows the mean values of the number of germinated plants as affected by plant species, hydrocarbon level, and plant growth stage. Over hydrocarbon levels and growth stages, *Pennisetum glaucum* enjoyed the highest number of germinated plants (26.21 plants) followed significantly by *Sisbania sesban* (23.65 plants). Meanwhile, the least significant number of germinated plants was provided by *Leuceana leucocephala* (2.27 plants). This might indicate *Leuceanas* sensitivity to hydrocarbons. *Pennisetum* and *Sisbania* showed a tolerance to hydrocarbons, while *Panicum* showed an intermediate result. It was valuable to notice that overall plant species, and growth stages, plants species tendency to germinate was significantly improved under a high level of hydrocarbon (19.5 plants vs. 12.79 plants for 60 and 40ml.kg^{-1}, respectively). Overall, plant species and hydrocarbons levels, the number of germinated plants significantly reduced with the progress of exposure to hydrocarbons from 19.90 to 16.81 to 13.63 plants after 15, 30, and 45 days of exposure. Further exposure (after 60 days) insignificantly affected the number of germinated plants. The recent finding matches previous studies, which suggested that the potential of plants to tolerate hydrocarbon contamination varied greatly among plant species, depending on their inherent metabolic pathways and environmental adaptability (Ghosh and Singh, 2005, and Mao *et al*., 2020). The high tolerance of *Pennisetum glaucum* might be due to its robust root system and potential to degrade hydrocarbons through Phytoremediation. These potentialities match true with *Sesbania Sesban*, besides its ability to encourage soil hydrocarbons-degrading microbes (Pang *et al*., 2018 and Mohamed and Ghanem, 2021).

Table 2: Mean number of germinated plants as affected by plant species, hydrocarbon contamination levels, and plant growth stage.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45days	60 days	
Pennisetum	40 (ml.kg^{-1})	24.74	19.40	17.90	18.07	**19.84**
	60 (ml.kg^{-1})	40.83	33.33	26.00	30.17	**32.58**
		32.79	26.37	21.95	24.12	**26.21**
Panicum	40 (ml.kg^{-1})	14.83	7.687	4.971	6.638	**8.532**
	60 (ml.kg^{-1})	24.50	18.00	11.00	12.25	**16.44**
		19.67	12.84	7.986	9.444	**12.48**
Sisbania	40 (ml.kg^{-1})	22.14	23.33	21.17	17.64	**21.07**
	60 (ml.kg^{-1})	29.00	27.67	23.00	25.25	**26.23**
		25.57	25.5	22.09	21.45	**23.65**
Leuceana	40 (ml.kg^{-1})	1.187	1.951	1.902	1.902	**1.736**
	60 (ml.kg^{-1})	1.951	3.118	3.118	3.035	**2.806**
		1.569	2.535	2.51	2.469	**2.271**
Mean		**19.90**	**16.81**	**13.63**	**14.37**	
Hydrocarbon level 40 (ml.kg^{-1})		15.72	13.09	5.560	11.06	**12.79**
Hydrocarbon level 60 (ml.kg^{-1})		24.07	20.53	15.78	17.68	**19.51**

L.S.D$_{0.01}$ (Plant species) = 2.92
L.S.D$_{0.01}$ (Hydrocarbon level) = 2.1
L.S.D$_{0.05}$ (Growth stage) = 2.2

Above and underground growth

Above-ground plant growth was represented by plant height meanwhile, underground growth was represented by root length. Table (3) shows the analysis of variance for stem height and root length as affected by plant species, hydrocarbon contamination level, and plant growth stage. Stem height and root length were significantly different among plant species, hydrocarbon levels (P≥ 0.01, and P≥ 0.05 for the former and the latter characters, respectively), growth stage (P≥ 0.05) and the first-order interaction between plant species and hydrocarbon level (P≥ 0.01). The other interaction failed to reach the level of significance.

Table 3: Analysis of variance for stem height and root length as affected by plant species, hydrocarbon contamination levels, and plant growth stage.

Source of variations	d.f	Mean squares	
		Stem height	Root height
Blocks	5	652.4 **	181.2 **
Plant species (A)	3	1291 **	3235 **
Hydrocarbon level (B)	1	1081 **	3489 *
Growth stage (C)	3	3890 *	144.6 *
A×B	3	839.3 **	326.9 **
A×C	9	142.3 NS	37.39 NS
B×C	3	129.6 NS	66.34 NS
A×B×C	9	25.16 NS	14.87 NS
Error	155	120.1	52.78

* and ** indicate significance at 0.05 and 0.01 levels, respectively.
NS; not significantly different.

Table (4) shows the mean values of stem (plant) height as affected by plant species, hydrocarbon levels, and plant growth stage. *Sisbania sesban* significantly enjoyed the tallest plants among the other studied plant species (37.54 cm). The second significant ranked was represented by *Pennisetum glaucum* (23.77 cm). Overall, species and growth stages, plant height significantly expressed taller plants under high petroleum contamination level (21.89 *vs*. 17.47 cm, respectively). *Sisbania sesban* significantly had the tallest plants, irrespective of contamination level (35.74 and 39.35 cm under 40 and 60 (ml.kg^{-1}) levels, respectively). That result was significantly similar to *Pennisetum glaucum* under the high level of contamination (31.63 cm). The *leuceana* significantly had intermediate plant height among the studied species, regardless of the level of petroleum contamination (18.20 and 16.61cm for 40 and 60 ml.kg^{-1}, respectively). The least scored values for plant height were those of *Panicum,* irrespective of the level of hydrocarbons.

Table 4: Means of stem height (cm) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	20.85	12.90	16.47	13.47	**15.92**
	60 (ml.kg^{-1})	33.78	35.28	32.28	25.18	**31.63**
		27.32	24.09	24.38	19.33	**23.77**
Panicum	40 (ml.kg$^{-1)}$	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	30.86	38.33	40.67	33.09	**35.74**
	60 (ml.kg^{-1})	35.17	46.17	45.83	30.23	**39.35**
		33.02	42.25	43.25	31.66	**37.54**
Leuceana	40 (ml.kg^{-1})	18.67	19.75	16.83	17.56	**18.20**
	60 (ml.kg^{-1})	17.67	19.17	17.50	12.09	**16.61**
		18.17	19.46	17.17	14.83	**17.40**
Mean		**19.63**	**21.45**	**21.2**	**16.46**	
Hydrocarbon level 40 (ml.kg^{-1})		17.59	17.75	18.49	16.03	**17.47**
Hydrocarbon level 60 (ml.kg^{-1})		21.66	25.16	23.90	16.88	**21.89**

L.S.D$_{0.01}$ (Plant species) = 5.82
L.S.D$_{0.01}$ (Hydrocarbon level) = 4.11
L.S.D$_{0.05}$ (Growth stage) = 4.11
L.S.D$_{0.01}$ (Plant species × hydrocarbon level) = 8.23

Table (5) presents the mean values of root length for plant species as affected by hydrocarbon contamination levels and growth stages. The tallest roots were significantly presented by *Sisbania sesban*, irrespective of the contamination level (18.81 and 19.18 cm for low and high level of petroleum contamination, respectively). Those values were insignificantly higher than the second-ranked plant species (*Pennisetum glaucum*) (13.46 over all contamination levels). In the meantime, the latter species had significantly shorter roots under the low level of contamination. *Leuceana leucocephala*, which had significantly similar root length to that provided by *Penniseetum*, significantly responded similarly irrespective of the contamination level. Meanwhile, elite species (i.e., *Pennisetum* and *Sesbania*) expanded into more roots under highly contaminated soil.

Table 5: Mean values of root length (cm) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbons levels ($ml.kg^{-1}$)	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 ($ml.kg^{-1}$)	9.187	6.721	8.305	9.971	**8.546**
	60 ($ml.kg^{-1}$)	15.28	16.95	23.01	18.26	**18.38**
		12.23	11.84	15.66	14.12	**13.46**
Panicum	40 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
	60 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 ($ml.kg^{-1}$)	15.43	17.17	22.00	20.64	**18.81**
	60 ($ml.kg^{-1}$)	13.00	19.00	25.67	19.06	**19.18**
		14.22	18.09	23.84	19.85	**18.99**
Leuceana	40 ($ml.kg^{-1}$)	9.333	11.83	11.17	14.06	**11.60**
	60 ($ml.kg^{-1}$)	10.25	11.10	14.50	10.01	**11.47**
		9.792	11.47	12.84	12.04	**11.53**
Mean		**9.061**	**10.35**	**13.09**	**11.50**	
Hydrocarbon level 40 ($ml.kg^{-1}$)		8.488	8.930	10.37	11.17	**9.739**
Hydrocarbon level 60 ($ml.kg^{-1}$)		9.633	11.76	15.79	11.83	**12.26**

$L.S.D_{0.01}$ (Plant species) = 3.86
$L.S.D_{0.05}$ (hydrocarbon level) = 1.97
$L.S.D_{0.05}$ (Growth stage) = 2.92
$L.S.D_{0.01}$ (Plant species × hydrocarbon level) = 5.45

The significant effect of growth stage on plant and root length indicated that plant species responded to petroleum contamination in a non-static manner, but depended on developmental stage. This might suggest that plants cope with environmental stresses better with aging (Pang *et al.*, 2018). In terms of plant height, *Sisbania sp* showed the tallest plants, especially at later growth stages. This might indicate its ability to thrive even in contaminated soils, likely due to its ability to metabolize hydrocarbons efficiently (Ali *et al*., 2013 and Mosa and Taha, 2019). Conversely, *Panicum sp*, showed the shorter plants, which might suggest that it's less tolerant to petroleum contamination which might be due to the inability to effectively mitigate contamination at the root level (Thavamani *et al*., 2017). Also, the interaction between plant species and hydrocarbon contamination level emphasizes the importance of choosing species that are not only resilient but also capable of metabolizing or tolerating pollutants at higher contamination. The recent results suggest that, plant root development might be closely related to plant tolerance to hydrocarbons, since, root is the major player in absorbing nutrients and contaminants significant effect of hydrocarbons on root growth represented by length indicate that petroleum contamination, might hinder root growth in sensitive plant species, resulting in a reduced ability to benefit from nutrients and water, resulting in a reduced an overall plant growth (Pang *et al.*, 2018 and Singh and Agrawal, 2022).

Bio-mass accumulation

Biomass accumulation of tested plant species as affected by hydrocarbons (petroleum) contamination across development stages provides insight into how petroleum contamination impacts growth and development, which can be critical for assessing their potential for phytoremediation. Biomass accumulation is expressed by stem, leaves, and roots weights (fresh and dry).

A-Fresh weight

Table (6) summarizes the analysis of variance for plant parts fresh weight (stem, leaves, and root) as affected by plant species, petroleum contamination level, and growth stage. Significantly different (P≥ 0.01) fresh weight of stem, leaves, and roots were obtained among the studied plant species. Meanwhile the effects of petroleum contamination level on fresh weight of plant parts were consistent (insignificant). Also, fresh weight of each studied plant part, significantly (P≥ 0.01) varied among growth stages. Meanwhile, the magnitude of plant parts response to contamination level, varied among the studied species (significant (P≥ 0.01) plant species × hydrocarbon level interaction), while, maintained similar values with variable growth stages (insignificant plant species × growth stage). Also, the response of fresh weight of different plant parts at variable growth stages was consistent, irrespective of hydrocarbon level (insignificant hydrocarbon level × growth stage interaction). Only leaves and roots fresh weights were affected (P≥ 0.01) by the second-order interaction among plant species × hydrocarbon level × growth stage interaction. Meanwhile, the fresh weight of the stem responds similarly to the above-mentioned treatment combinations.

Table 6: Analysis of variance for fresh weight of plant parts (stem, leaves and root) as affected by plant species, hydrocarbon contamination level, and plant growth stage.

Source of variations	d.f.	Mean squares		
		Stem weight	leaves weight	Root weight
Blocks	5	0.352 NS	0.737 *	1.014 *
Plant species (A)	3	12.60 **	10.12 **	5.240 **
Hydrocarbon level (B)	1	0.159 NS	0.011 NS	0.567 NS
Growth stage (C)	3	1.490 **	2.105 **	5.958 **
A×B	3	0.785 **	4.638 **	7.209 **
A×C	9	0.573 **	1.067 **	0.509 NS
B×C	3	0.087 NS	0.155 NS	0.104 NS
A×B×C	9	0.177 NS	2.644 **	2.059 **
Error	155	0.162	0.291	0.431

* and ** indicate significance at 0.05 and 0.01 levels, respectively.
NS; not significantly different.

Table (7) shows the mean values of stem fresh weight (g) as affected by the studied factors. *Sisbania sp.* enjoyed the heaviest stem fresh weight among the studied species over the other studied factors (1.418 g.plant^{-1}). The second significant ranke was presented by

Pennisetum sp. (0.559 g.plant^{-1}). While the third significantly lower-ranked stem fresh weight was that of *Leuceana sp*. (0.306 g.plant^{-1}). Over-studied plant species, and contamination levels, stem fresh weight, significantly increased after 30 days from seeding (0.355 vs.0.603 g.plant^{-1} for 15 and 30-day stages, respectively). Meanwhile, the fresh weight values did not significantly change as the growth stages progressed. Over traced growth stages, fresh weight of stem for the studied plant species showed insignificant reduction with severe level of contamination (60 ml.kg^{-1}), except for *Sisbania sp*. that showed a significant improvement in fresh weight of stem (1.226 vs. 1.610 g.plant^{-1}, for 40 and 60 ml.kg^{-1} levels of contamination, respectively). Fresh weight of stem of *Sisbania sp*. with 60 days of exposure to petroleum contamination, showed a gradual increase during the first 30 days of exposure (0.845 vs.1.634 g.plant^{-1}, for 15 and 30 days of exposure, respectively), or significantly stable stem fresh weight during the following 30 days (from 30 to 60 days) of exposure to contamination. That qualified *Sisbania sp*. to enjoy the first significant rank in resilience to contamination among the studied species. However, *Pennisetum sp*. was significantly ranked second in stem fresh weight after 60 days of exposure to contamination (0.895 g.plant^{-1}). That latter species gave significantly similar stem fresh weights at the subsequent recording growth stages. Also, *Leuceana sp.* significantly had the least stem fresh weight, surpassing *Panicum sp.*, which failed to grow under exposure to contamination at any of the studied growth stages.

The higher stem fresh weight in *Sisbania sp*. suggests its greater capacity to tolerate petroleum contamination and maintain biomass production under stress conditions. This is consistent with literature reporting that *Sisbania* is a hardy species capable of surviving in a polluted environment (Ali *et al*., 2013). In contrast, *Panicum* and *Leuceana* exhibited much lower stem weight, indicating their poor performance in petroleum-contaminated soils.

Table 7: Means of fresh weight of stem (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	0.487	0.571	0.671	0.863	**0.648**
	60 (ml.kg^{-1})	0.268	0.418	0.268	0.926	**0.470**
		0.378	0.495	0.470	0.895	**0.559**
Panicum	40 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	0.857	1.200	1.500	1.345	**1.226**
	60 (ml.kg^{-1})	0.833	2.067	1.933	1.608	**1.610**
		0.845	1.634	1.717	1.477	**1.418**
Leuceana	40 (ml.kg^{-1})	0.200	0.300	0.233	0.527	**0.315**
	60 (ml.kg^{-1})	0.200	0.267	0.300	0.418	**0.296**
		0.200	0.284	0.267	0.473	**0.306**
Mean		**0.355**	**0.603**	**0.614**	**0.711**	
Hydrocarbon level 40 (ml.kg^{-1})		0.386	0.518	0.601	0.684	**0.547**
Hydrocarbon level 60 (ml.kg^{-1})		0.325	0.688	0.625	0.738	**0.594**

L.S.D$_{\mathbf{0.01}}$ (Plant species) = 0.21
L.S.D$_{\mathbf{0.01}}$ (Growth stage) = 0.21
L.S.D$_{\mathbf{0.01}}$ (Plant species × hydrocarbon level) = 0.30
L.S.D$_{\mathbf{0.01}}$ (Plant species × growth stage) = 0.43

Means of leaves fresh weight (g) as affected by plant species, hydrocarbon contamination level, and plant growth stage are presented in Table 8. Data showed that Sisbania sp. demonstrated superior leaf biomass production, with a peak value of 2.583 g under the 40 ml.kg^{-1} hydrocarbon contamination level after 45 days of exposure. *Pennisetum sp.* showed moderate leaf weight (1.026 g). Meanwhile, *Panicum* displayed a marked decrease in leaf weight in all stages of growth, *Leuceana sp*. had the least leaf weight across all contamination levels, with a mean of 0.398 g. The increase in leaf weight biomass in *Sisbania* under hydrocarbon stress could be attributed to its adaptive mechanism, such as increased photosynthetic activity or enhanced nutrient uptake under polluted conditions (Ghosh and Singh, 2005 and Pradhan and Biswas, 2021). In contrast, *Leuceana* and *Panicum* inability to maintain leaf biomass suggests a low tolerance to petroleum contamination.

Table 8: Means of fresh weight of leaves (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	0.554	0.555	0.518	1.026	**0.663**
	60 (ml.kg^{-1})	0.268	0.585	1.000	1.000	**0.713**
		0.411	0.57	0.759	1.013	**0.688**
Panicum	40 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	1.043	1.533	2.583	1.764	**1.731**
	60 (ml.kg^{-1})	0.933	2.267	0.317	0.486	**1.001**
		0.988	1.9	1.45	1.125	**1.366**
Leuceana	40 (ml.kg^{-1})	0.117	0.367	0.283	0.310	**0.269**
	60 (ml.kg^{-1})	0.150	0.267	0.838	0.855	**0.528**
		0.134	0.275	0.561	0.583	**0.398**
Mean		**0.383**	**0.686**	**0.693**	**0.680**	
Hydrocarbon level 40 (ml.kg^{-1})		0.429	0.614	0.846	0.775	**0.666**
Hydrocarbon level 60 (ml.kg^{-1})		0.338	0.780	0.539	0.585	**0.561**

L.S.D$_{0.01}$ (Plant species) = 0.29
L.S.D$_{0.01}$ (Growth stage) = 0.29
L.S.D$_{0.01}$ (Plant species × hydrocarbon level) = 0.41
L.S.D$_{0.01}$ (Plant species × growth stage) = 0.57
L.S.D$_{0.01}$ (Plant species × hydrocarbon level × growth stage) = 0.81

Means of roots fresh weight (g) (Table 9) showed that *Sisbania sp* exhibited the highest root weight with values up to 2.217g. plant^{-1} under the 40 ml.kg^{-1} hydrocarbon contamination level. *Pennisetum* and *Leuceana sp.* had moderate fresh root weight, averaging 0.782 and 0.886 g. plant^{-1}, respectively. *Panicum sp* had the lowest root fresh weight and was unable to establish itself. The superior root fresh biomass in *Sisbania sp* indicated that it is more capable of developing a large root system in contaminated environments, which is a crucial attribute for phytoremediation.

The increased root biomass in *Sisbania* might be associated with its ability to absorb and detoxify hydrocarbons more effectively other species (Tiwari and Singh, 2020; Udhaya and Srinivasan, 2020, and Zhang and Li, 2023).

Table 9: Means of fresh root weight (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	0.504	0.538	0.701	1.676	**0.855**
	60 (ml.kg^{-1})	0.201	0.635	1.000	1.000	**0.709**
		0.353	0.587	0.851	1.338	**0.782**
Panicum	40 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	0.929	1.433	2.217	2.100	**1.670**
	60 (ml.kg^{-1})	0.200	1.500	0.283	0.552	**0.634**
		0.565	1.467	1.25	1.326	**1.152**
Leuceana	40 (ml.kg^{-1})	0.112	0.383	0.267	0.385	**1.147**
	60 (ml.kg^{-1})	0.235	0.233	0.821	1.213	**0.626**
		0.174	0.308	0.544	0.799	**0.886**
Mean		**0.273**	**0.591**	**0.661**	**0.866**	
Hydrocarbon level 40 (ml.kg^{-1})		0.386	0.589	0.796	1.040	**0.918**
Hydrocarbon level 60 (ml.kg^{-1})		0.159	0.592	0.526	0.691	**0.492**

L.S.D$_{0.01}$ (Plant species) = 0.35
L.S.D$_{0.01}$ (Growth stage) = 0.35
L.S.D$_{0.01}$ (Plant species × hydrocarbon level) = 0.49
L.S.D$_{0.01}$ (Plant species × hydrocarbon level × growth stage) = 0.99

The growth stage had a significant impact on all three fresh biomass parameters (stem, leaf, and root weight), with the highest fresh weights observed in the latter stages of plant growth. This might indicate that plants are able to adapt to hydrocarbon contamination over time, especially in species like *Sisbania*, which can maintain high biomass production even under polluted conditions. Conversely, the early growth stages showed lower biomass accumulation, likely due to the initial stress of contamination, which reduce nutrients availability and hampers growth. The interaction between plant species and hydrocarbon contamination was significant for all fresh biomass parameters, confirming that some species are more tolerant to hydrocarbons and can maintain or even increase biomass production under stress. Additionally, the significant effects of growth stage and its interactions indicate that the timing of planting and the growth period may influence the success of Phytoremediation efforts.

B- Dry weight

Analysis of variance of dry weight for stem, leaves, and root biomass as affected by plant species, hydrocarbon contamination level, and plant growth stage (Table 10) revealed significant variation across the three factors. This finding provides insights into how different plant species respond to petroleum contamination over time, and how dry biomass accumulation varies with growth stage and contamination level. Data showed that plant species significantly ($P \geq 0.01$) affected stem dry weight. Also, the interaction between plant species and hydrocarbon contamination level had a significant ($P \geq 0.05$) effect, indicating that the response of stem dry weight to hydrocarbon contamination varies among species. However, the hydrocarbon contamination level and growth stage did not significantly affect stem dry weight.

Table 10 : Analysis of variance for dry weights (stem, leaves and root) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Source of variations	d.f.	Mean squares		
		Stem weight	leaves weight	Root weight
Blocks	5	0.046 ^{NS}	0.028 ^{NS}	0.056 ^{NS}
Plant species (A)	3	1.585 **	1.618 **	1.704 **
Hydrocarbon level (B)	1	0.002 ^{NS}	0.037 ^{NS}	0.042 ^{NS}
Growth stage (C)	3	0.081 ^{NS}	0.185 **	0.386 **
A×B	3	0.153 *	0.208 **	0.084 ^{NS}
A×C	9	0.138 **	0.175 **	0.069 ^{NS}
B×C	3	0.043 ^{NS}	0.023 ^{NS}	0.016 ^{NS}
A×B×C	9	0.060 ^{NS}	0.054 ^{NS}	0.071 ^{NS}
Error	155	0.042	0.037	0.039

* and ** indicate significance at 0.05 and 0.01 levels, respectively.
NS; not significantly different.

Mean of dry stem weight (g) as affected by plant species, hydrocarbon level, and growth stage was presented in Table (11). Significant difference among the tested plant species in dry weight of stem was because of *Panicum sp*. failure to grow in any of the studied levels of contamination. The other studied plant species had significantly similar stem dry weights, ranging from 0.347 g.plant^{-1} for *Leuceana* to 0.396 g.plant^{-1} for *Sisbania*. Meanwhile, *Pennisetum sp* under 40 ml.kg^{-1} contamination level and *Sisbania sp*. under 60 ml.kg^{-1} contamination level had the highest dry stem weight (0.557 and 0.451 g.plant^{-1}, respectively). *Leuceana* sp produced significantly lower dry stem weight under any of the studied contamination levels, outperforming the failed Panicum sp. These findings suggest that *Sisbania sp* is more efficient in accumulating dry stem biomass compared to other studied species, especially in hydrocarbon-contaminated environments.

Table 11: Means of dry weight of stem (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	0.606	0.505	0.521	0.597	**0.557**
	60 (ml.kg^{-1})	0.100	0.420	0.235	0.285	**0.26**
		0.3٠3	0.463	0.378	0.441	**0.409**
Panicum	40 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	0.200	0.383	0.400	0.381	**0.341**
	60 (ml.kg^{-1})	0.335	0.550	0.500	0.417	**0.451**
		0.268	0.467	0.45٠	0.399	**0.396**
Leuceana	40 (ml.kg^{-1})	0.521	0.235	0.117	0.352	**0.376**
	60 (ml.kg^{-1})	0.319	0.404	0.150	0.403	**0.319**
		0.420	0.320	0.134	0.378	**0.347**
Mean		**0.2٤٨**	**0.313**	**0.241**	**0.305**	
Hydrocarbon level 40 (ml.kg^{-1})		0.332	0.281	0.25٧	0.333	**0.3٠٠**
Hydrocarbon level 60 (ml.kg^{-1})		0.٢١٣	0.344	0.221	0.276	**0.2٦٤**

L.S.D$_{0.05}$ (Plant species × hydrocarbon level) = 0.12
L.S.D$_{0.01}$ (Plant species × growth stage) = 0.22

The fact that leaves dry weight (g) had significantly affected by plant species (P≥ 0.01), affected by the interaction between plant species and growth stage (P≥ 0.01), and had not affected by hydrocarbon contamination level, suggested that leaves dry biomass accumulation was more influenced by plant species and growth stage than by the level of contamination

Means of dry weight of leaves (Table 12) revealed that *Pennisetum sp* consistently exhibited a high leaves dry weight across all contamination levels and most growth stages, with a best value of 0.358 $g.plant^{-1}$. *Sisbania sp* also demonstrated a relatively high dry leaves weight (0.352 $g.plant^{-1}$). Meanwhile, *Panicum sp.* showed the least mean leaf dry weight. The significant impact of growth stage on leaves' dry weight suggests that leaves' biomass accumulation was more significant in later stages of growth, possibly as the plant matures and adapts to the stress caused by contamination.

Table 12: Means of dry weight of leaves (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels ($ml.kg^{-1}$)	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 ($ml.kg^{-1}$)	0.606	0.000	0.521	0.605	**0.458**
	60 ($ml.kg^{-1}$)	0.000	0.404	0.235	0.293	**0.258**
		0.303	0.202	0.378	0.449	**0.358**
Panicum	40 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
	60 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 ($ml.kg^{-1}$)	0.258	0.283	0.400	0.291	**0.308**
	60 ($ml.kg^{-1}$)	0.318	0.501	0.417	0.350	**0.397**
		0.288	0.392	0.409	0.321	**0.352**
Leuceana	40 ($ml.kg^{-1}$)	0.622	0.201	0.117	0.403	**0.336**
	60 ($ml.kg^{-1}$)	0.505	0.319	0.117	0.437	**0.345**
		0.564	0.260	0.117	0.42	**0.340**
Mean		**0.564**	**0.214**	**0.226**	**0.298**	
Hydrocarbon level 40 ($ml.kg^{-1}$)		0.372	0.121	0.259	0.324	**0.269**
Hydrocarbon level 60 ($ml.kg^{-1}$)		0.205	0.307	0.192	0.270	**0.264**

$L.S.D_{0.01}$ (Plant species) = 0.1
$L.S.D_{0.01}$ (Growth stage) = 0.1
$L.S.D_{0.01}$ (Plant species × hydrocarbon level) = 0.14
$L.S.D_{0.01}$ (Plant species × growth stage) = 0.2

Regarding root dry weight, plant species was a significant factor (P≥ 0.01), while the interaction between plant species and hydrocarbon contamination had an insignificant effect. The growth stage and its interactions with other factors did not reach the level of significance, although trends were observed across the stages.

Mean values of root dry weight (Table 13), showed that the heaviest dry roots were provided by *Pennisetum sp* (0.445 $g.plant^{-1}$) or *Leuceana sp* (0.372 $g.plant^{-1}$). Whereas a significantly lower dry root weight was provided by *Sisbania sp* (0.312 $g.plant^{-1}$). Growth stage significantly affected dry weight accumulation for leaves and roots, indicating that plants tended to accumulate more biomass in the later stages of growth. This might suggest that the plant's adaptive responses to contamination become more effective as they mature. However, for stem biomass, growth stage did not have a significant effect, suggesting that stem growth might be less influenced by developmental stage and more by other factors such as species or environmental stress.

Table 13: Means of dry weight of root (g) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbon levels ($ml.kg^{-1}$)	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 ($ml.kg^{-1}$)	0.601	0.521	0.521	0.723	**0.592**
	60 ($ml.kg^{-1}$)	0.100	0.420	0.285	0.385	**0.298**
		0.300	0.471	0.403	0.554	**0.445**
Panicum	40 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
	60 ($ml.kg^{-1}$)	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 ($ml.kg^{-1}$)	0.518	0.217	0.267	0.264	**0.317**
	60 ($ml.kg^{-1}$)	0.319	0.317	0.300	0.292	**0.307**
		0.419	0.267	0.284	0.278	**0.312**
Leuceana	40 ($ml.kg^{-1}$)	0.606	0.336	0.117	0.299	**0.340**
	60 ($ml.kg^{-1}$)	0.504	0.404	0.333	0.378	**0.405**
		0.555	0.37	0.225	0.677	**0.372**
Mean		**0.349**	**0.277**	**0.228**	**0.377**	
Hydrocarbon level 40 ($ml.kg^{-1}$)		0.431	0.269	0.226	0.322	**0.312**
Hydrocarbon level 60 ($ml.kg^{-1}$)		0.206	0.285	0.230	0.26٦	**0.232**

$L.S.D_{0.01}$ (Plant species) = 0.1
$L.S.D_{0.01}$ (Growth stage) = 0.1

The significant effects of plant species on dry biomass accumulation, along with the varying effects of growth stage, suggest that the timing of growth stages and species inherent growth potential are crucial factors to consider in Phytoremediation strategies. Additionally, the absence of significant effects of hydrocarbon contamination on dry weight suggests that certain species are inherently more resistant to the adverse effects of hydrocarbon, maintaining biomass accumulation even in a polluted environment.

C-Leaves / stem ratio

The analysis of variance in Table (14) for leaves/stem ratio at fresh weight base showed that plant species showed an insignificant effect on the wet weight distribution between leaves and stems. Whereas, hydrocarbon contamination significantly ($P \geq 0.01$) affected the fresh weight distribution, suggesting that contamination level influences how plants allocate biomass between leaves and stems. Growth stage also had a significant impact on fresh weight distribution ($P \geq 0.01$), with later growth stages showing significant changes in biomass allocation. Meanwhile, there were significant interactions between plant species, hydrocarbon levels, and growth stage ($P \geq 0.01$), indicating that the effect of one factor depends on the level of the other factors.

Table 14: Analysis of variance for fresh weight of leaves/stem ratio as affected by plant species, hydrocarbon contamination level, and plant growth stage.

Source of variations	d.f	Mean squares
		Leaves/stem ratio
Blocks	5	8683 NS
Plant species (A)	3	1271 NS
Hydrocarbon level (B)	1	7832 **
Growth stage (C)	3	1066 **
A×B	3	9104 **
A×C	9	1906 *
B×C	3	3680 **
A×B×C	9	6042 **
Error	155	9027

* and ** indicate significance at 0.05 and 0.01 levels, respectively.
NS; not significantly different.

Means of leaves/stem ratio (fresh weight) as affected by plant species, hydrocarbon level, and plant growth stage were presented in Table (15). *Pennisetum sp*, showed the highest variability in fresh weight distribution between leaves and stems, especially under hydrocarbon contamination at the 60 ml.kg^{-1} level (from 94.44 to 370.2 to 83.65), suggesting that this species is particularly sensitive to changes in contamination and growth stage. *Sisbania sp*, exhibited a large variation in fresh weight distribution between leaves and stems, with values ranging from 43.52 to 233.9, highlighting the plant's adaptability under varying contamination levels. *Leuceana sp* had a higher mean fresh weight distribution (347.2), particularly under a 60 ml.kg^{-1} contamination level, demonstrating a robust response to environmental stress.

Table 15: Means of leaves /stem ratio (fresh weight) as affected by plant species, hydrocarbon contamination level, and plant growth stages.

Plant species	Hydrocarbon levels (ml.kg^{-1})	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 (ml.kg^{-1})	125.0	95.83	85.70	159.0	**116.4**
	60 (ml.kg^{-1})	94.44	123.6	370.2	83.65	**168.0**
		109.7	109.7	227.9	121.3	**142.2**
Panicum	40 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
	60 (ml.kg^{-1})	0.000	0.000	0.000	0.000	**0.000**
		0.000	0.000	0.000	0.000	**0.000**
Sisbania	40 (ml.kg^{-1})	107.3	144.7	233.9	157.8	**160.9**
	60 (ml.kg^{-1})	115.7	145.0	16.54	43.52	**80.19**
		111.5	134.9	125.2	100.7	**120.5**
Leuceana	40 (ml.kg^{-1})	87.50	115.3	180.6	64.72	**112.0**
	60 (ml.kg^{-1})	75.00	108.3	347.2	276.9	**201.9**
		81.25	111.8	263.9	170.8	**156.9**
Mean		**75.61**	**89.1**	**152.5**	**98.2**	
Hydrocarbon level 40 (ml.kg^{-1})		79.95	88.96	125.1	95.38	**97.33**
Hydrocarbon level 60 (ml.kg^{-1})		71.29	94.23	183.5	101.0	**112.5**

L.S.D$_{0.01}$ (Hydrocarbon level) = 35.7
L.S.D$_{0.01}$ (Growth stage) = 50.4
L.S.D$_{0.01}$ (Plant species × hydrocarbon level) = 71.3
L.S.D$_{0.05}$ (Plant species × growth stage) = 76.5
L.S.D$_{0.01}$ (Hydrocarbon level × growth stage) = 71.3
L.S.D$_{0.01}$ (Plant species × hydrocarbon level × growth stage) = 142.7

Analysis of variance for the dry weight of leaves/stems ratio was presented in Table (16). Plant species did not have a significant effect on dry weight distribution between leaves and stems. An insignificant main effect was scored for hydrocarbon contamination on dry weight distribution. The growth stage did not significantly affect dry weight distribution. However, the interaction between plant species and growth stage was significant (P≥ 0.05), indicating that plant species biomass allocation to leaves and stems changes over time, depending on growth stage.

Table 16: Analysis of variance for leaves /stems ratio (dry weight) as affected by plant species, hydrocarbon contamination level, and plant growth stage.

Source of variations	d.f	Mean squares
		Leave/stem %
Blocks	5	8924 NS
Plant species (A)	3	2315 NS
Hydrocarbon level (B)	1	9617 NS
Growth stage (C)	3	2321 NS
A×B	3	1097 NS
A×C	9	2546 *
B×C	3	4394 NS
A×B×C	9	1691 NS
Error	155	1053

* Indicate significance at 0.05 level. NS; not significantly different.

Means of leaves/stems ratio (dry weight) as affected by plant species, hydrocarbon contamination level, and growth stage are shown in Table (17). *Pennisetum sp.* showed wide variability between growth stages (between 108.4 and 196.9), reflecting how dry matter distribution changes across growth stages. *Sisbania sp.* had moderate variability in dry matter distribution (between 78.81 and 176.0), with significant fluctuation across hydrocarbon levels and growth stages, indicating that this species might have a plastic response to these factors. *Leuceana sp.* showed relatively lower variability in dry matter distribution (83.33 to 201.2), particularly after 45 days of seeding, highlighting its sensitivity to both contamination and growth stage.

Table 17: Means of leaves/stems ratio (dry weight) as affected by plant species, hydrocarbon contamination levels, and plant growth stages.

Plant species	Hydrocarbons levels ($ml.kg^{-1}$)	Growth stage				Mean
		15 days	30 days	45 days	60 days	
Pennisetum	40 ($ml.kg^{-1}$)	100.0	302.3	100.0	113.3	**330.6**
	60 ($ml.kg^{-1}$)	100.0	91.67	116.7	101.2	**77.39**
		100.0	196.9	108.4	107.3	**155.9**
Panicum	40 ($ml.kg^{-1}$)	000.0	000.0	000.0	000.0	**000.0**
	60 ($ml.kg^{-1}$)	000.0	000.0	000.0	000.0	**000.0**
		000.0	000.0	000.0	000.0	**000.0**
Sisbania	40 ($ml.kg^{-1}$)	176.0	78.81	100.0	84.70	**109.9**
	60 ($ml.kg^{-1}$)	95.83	107.7	83.75	84.93	**93.05**
		135.9	93.26	91.93	84.82	**202.9**
Leuceana	40 ($ml.kg^{-1}$)	201.2	83.33	100.0	152.0	**134.1**
	60 ($ml.kg^{-1}$)	194.0	102.4	83.33	117.0	**62.09**
		197.6	92.87	91.67	134.5	**98.10**
Mean		**108.4**	**95.76**	**73.00**	**81.66**	
Hydrocarbon level 40 ($ml.kg^{-1}$)		119.3	116.1	75	87.5	**143.7**
Hydrocarbon level 60 ($ml.kg^{-1}$)		97.46	75.44	70.95	75.78	**58.13**

$L.S.D_{0.05}$ (Plant species × growth stage) = 26.1

Generally, both fresh and dry weight analysis showed that hydrocarbon contamination significantly affected leaves / stem biomass distribution, especially the fresh weight analysis. Contamination likely triggers physiological responses in plants, leading to shifts in biomass allocation. Growth stage had a significant effect, particularly on fresh weight distribution between leaves and stems, suggesting that as plants mature, their biomass allocation strategies change, potentially to optimize nutrient and water uptake or to enhance reproductive success. Different plant species exhibited varying responses to hydrocarbon contamination and growth stages. *Pennisetum sp* showed the greatest fluctuation, especially in fresh weight distribution, which suggests that it might be a more sensitive species to changes in contamination and growth conditions. In contrast, *Sisbania sp* was more stable across different conditions, making it possibly more resilient to environmental stress.

Conclusion

This recent study highlights the importance of plant species selection and the consideration of environmental factors such as hydrocarbon contamination and growth stages for biomass allocation in Phytoremediation studies/projects. Meanwhile, *Pennisetum sp* and *Leuceana sp* demonstrated more flexible responses to environmental stress, *Sisbania sp* exhibited a plastic response. These species varying responses suggested that a multi-species approach might be necessary for effective Phytoremediation, particularly in environments with fluctuating contamination levels. The recent findings also emphasize the importance of the growth stage in influencing biomass allocation. Future research should focus on the long-term effects of hydrocarbon contamination on plant growth, as well as the physiological mechanisms that regulate such responses.

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الأنواع النباتية المقترحة للمعالجة النباتية لأراضي المراعى الملوثة بالبترول في جنوب السودان

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الملخص العربى

الهدف الرئيسي من الدراسة الحالية هو اقتراح أنواع نباتية مناسبة للمعالجة النباتية لأراضي المراعي الملوثة بالبترول في جنوب السودان. تم اختبار أربعة أنواع نباتية، نوعان من النجيليات (البنسيتوم والبانكم)، ونوعان من البقوليات (السيزبانيا والليوسينا). تم قياس قدرة الأنواع على المعالجة استناداً إلى استجابتها لمستويات تلوث الهيدروكربونات (٤٠ و٦٠ مل/كجم)، في مراحل نمو مختلفة (١٥، ٣٠، ٤٥، و٦٠ يوماً). تمثلت استجابة الأنواع في مؤشرات النمو وتوزيع الكتلة الحيوية. أظهر تحليل التباين وجود تأثيرات معنوية لكل من تلوث الهيدروكربونات ومرحلة النمو على تطور وتوزيع الكتلة الحيوية، مع استجابات خاصة بكل نوع. أظهر كل من البنسيتوم والليوسينا تبايناً عالياً في توزيع الكتلة الحيوية، بينما أظهرت السيزبانيا ثباتاً، مما يشير إلى قدرتها على التكيف مع إجهاد الهيدروكربونات. تؤكد النتائج التي تم الحصول عليها على أهمية أخذ هذه العوامل في الاعتبار عند اختيار الأنواع النباتية للمعالجة النباتية وإعادة التأهيل البيئي في المناطق الملوثة بالبترول، مثل أراضي جنوب السودان. وبناءً على الاستجابات المتباينة للأنواع المدروسة، يُنصح بالاعتماد على نهج متعدد الأنواع لتحقيق معالجة نباتية فعالة.