

Vegetative Regeneration of *Pterocarpus santalinoides* Stem Cuttings in Crude Oil-Contaminated Soil Collected from Bunu, Tai Local Government Area, Rivers State

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ABSTRACT

The declining natural regeneration of *Pterocarpus santalinoides* due to habitat degradation and crude oil pollution necessitates the development of efficient propagation techniques for its conservation and restoration. This study evaluated the influence of stem cutting length and soil condition on the vegetative regeneration of *P. santalinoides* under crude oil-contaminated and uncontaminated soils. The experiment was arranged in a Completely Randomized Design (CRD) with four stem cutting lengths (15, 25, 30, and 40 cm) and three replicates. Regeneration performance was assessed using sprouting percentage, mean sprouting time, sprout emergence, cumulative leaf length, and cumulative dry biomass. The 25 cm stem cuttings grown in crude oil-polluted soil consistently exhibited superior regeneration performance, recording the highest sprouting percentage (160%), mean sprouting time (0.71 days), cumulative sprout emergence (40 sprouts), cumulative leaf length (163 cm), and cumulative dry biomass (45.1 g). In contrast, the 30 cm stem cuttings generally exhibited the poorest regeneration performance. The findings demonstrate that *P. santalinoides* possesses considerable tolerance to crude oil-polluted soil during early establishment and that stem cutting length is a critical determinant of successful vegetative propagation. It is therefore recommended that 25 cm stem cuttings be adopted for nursery propagation and ecological restoration of crude oil-degraded environments, while further studies should evaluate their long-term field performance under varying levels of petroleum contamination.

Keywords: *Pterocarpus santalinoides*, crude oil contamination, vegetative propagation, regeneration, ecological restoration.

Introduction

Pterocarpus santalinoides L'Hér. ex DC is an important indigenous multipurpose tree species widely distributed across the humid regions of West and Central Africa. The species commonly occurs in freshwater swamps, riparian forests and floodplains, where it contributes to soil stabilization, biodiversity conservation and ecosystem functioning. Besides its ecological importance, *P. santalinoides* provides edible leaves, timber, fuelwood, livestock fodder and medicinal products, making it an economically valuable species for many rural communities (Ky-Dembele *et al.*, 2016; POWO, 2024). The species has attracted increasing scientific interest because of its nutritional and medicinal properties. The leaves are consumed as vegetables and the bark, roots and leaves are used in tradomedical treatment of various ailments.

David-Sarogoro and Emerhi (2016) reported that *P. santalinoides* contains bioactive compounds, including flavonoids, alkaloids, tannins and saponins, which contribute to its medicinal value. However, increasing exploitation, habitat destruction and poor natural regeneration have contributed to a decline in natural populations, emphasizing the need for effective propagation and conservation strategies.

Vegetative propagation through stem cuttings offers a practical approach for the rapid multiplication and conservation of valuable indigenous tree species, compared with seed propagation, stem cuttings to ensure genetic uniformity, reduce the juvenile phase and facilitate large-scale production of planting materials. Ky-Dembele *et al.*, (2016) demonstrated that rooting success in *P. santalinoides* is significantly influenced by cutting characteristics and genotype.

While Fredrick *et al.*, (2021) reported that stem cutting length significantly affects sprouting, survival and early growth, with intermediate-sized cuttings exhibiting superior regeneration performance. Similarly, Ameh *et al.*, (2019) highlighted the importance of developing reliable propagation techniques to support conservation and domestication of the species.

In the Niger Delta region of Nigeria, crude oil exploration and production have caused widespread environmental degradation through frequent oil spills, resulting in altered soil properties and reduced vegetation productivity. Crude oil contamination affects soil fertility, microbial activity and plant establishment, thereby limiting natural regeneration of many indigenous species (Irunkwor *et al.*, 2022). Nevertheless, some native woody species possess adaptive characteristics that enable them to survive and establish under contaminated conditions, making them suitable candidates for ecological restoration of degraded environments.

Although previous studies have investigated seed germination, tissue culture and vegetative propagation of *P. santalinoides*, limited information exists on the combined effects of stem cutting length and crude oil-contaminated soil on its regeneration performance. Most available studies have evaluated propagation under normal nursery conditions or under other environmental stresses (Fredrick *et al.*, 2024), leaving an important knowledge gap regarding the response of the species to petroleum-contaminated soils. Therefore, this study evaluated the influence of stem cutting length and soil condition on the vegetative regeneration of *Pterocarpus santalinoides* using crude oil-contaminated and uncontaminated soils.

Specifically, the objectives of this study were to: (i) determine the effect of stem cutting length on sprouting percentage and mean sprouting time of *P. santalinoides*; (ii) evaluate sprout emergence, cumulative leaf development and dry biomass accumulation under crude oil-contaminated and uncontaminated soils; and (iii) identify the stem cutting length most suitable for propagation and ecological restoration in petroleum-impacted soils. The findings will provide useful information for the conservation, propagation and restoration of *P. santalinoides* in crude oil-impacted ecosystems.

Materials and Methods

Study Area

The study was conducted at Bunu-Tai in Tai Local Government Area, Rivers State, Nigeria. Bunu-Tai is situated within the Niger Delta region between approximately 4°39'–4°43' N latitude and 7°16'–7°20' E longitude, at an elevation of approximately 15–20 m above sea level. The area lies within the humid tropical rainforest belt and experiences a bimodal rainfall pattern, with a prolonged rainy season extending from March to October and a relatively short dry season between November and February. Mean annual rainfall ranges between 2,000 and 2,500 mm, while average annual temperatures range from 27 to 30°C with relative humidity generally exceeding 80% throughout most of the year. The area has been subjected to prolonged crude oil exploration and production activities, resulting in frequent petroleum spills and varying degrees of soil contamination (Irunkwor *et al.*, 2022).

Collection of Crude Oil-Contaminated Soil Samples and Stem Cuttings of *Pterocarpus santalinoides*

Crude oil-contaminated soil used for this study was collected from an oil-impacted site within Bunu-Tai, whereas uncontaminated soil (control) was collected from a nearby location with no visible evidence of petroleum contamination. Both soil samples were transported to the experimental site for the propagation experiment. Healthy, disease-free stem cuttings of *Pterocarpus santalinoides* were collected from mature trees growing naturally within the study area. Stem cuttings were sorted into four length classes of 15, 25, 30 and 40 cm using a measuring tape before planting. Care was taken to ensure that all cuttings were free from physical damage and was prepared immediately prior to planting to minimize moisture loss and maintain physiological viability throughout the experiment.

Experimental Design

The propagation experiment was established using a Completely Randomized Design (CRD) consisting of two soil conditions (control and crude oil-contaminated soil) and four stem cutting lengths (15, 25, 30 and 40 cm), resulting in eight treatment combinations.

Each treatment was replicated three times. Polyethylene planting bags were filled with either contaminated or uncontaminated soil, after which stem cuttings were planted vertically to a depth of approximately 5 cm to ensure adequate soil contact and rooting. All treatments were maintained under similar environmental conditions throughout the experimental period, and watering was carried out as required to maintain adequate soil moisture.

Table 1: Experimental treatments used for evaluating the vegetative regeneration of *Pterocarpus santalinoides* stem cuttings.

Treatment	Stem cutting length (cm)	Soil condition	Replicates
T1	15	Control	3
T2	15	Polluted	3
T3	25	Control	3
T4	25	Polluted	3
T5	30	Control	3
T6	30	Polluted	3
T7	40	Control	3
T8	40	Polluted	3

Determination of Vegetative Regeneration of *P. santalinoides*

Vegetative regeneration was monitored throughout the experimental period using early growth parameters. Daily observations were made during the first month after planting to determine sprouting behaviour. The number of sprouts produced from each cutting was counted and recorded, after which sprouting percentage (SP), sprouting period (SD) and mean sprouting time (MST) were computed using standard propagation indices (Hartmann *et al.*, 2018; Fredrick *et al.*, 2021). MST is a weighted mean of the days to sprouting, calculated across all sprouting cuttings; hence, values are not restricted to whole numbers and represent the average time (in days) at which sprouting events occurred across the observation period.

At the end of the experimental period, shoot growth parameters including cumulative leaf length, stem height, stem collar diameter, leaf number and sprout survival were assessed. Fresh and oven-dry biomass of shoots and roots were subsequently determined using a digital weighing balance, and total dry biomass was computed as the sum of shoot and root dry weights.

These measurements were used to evaluate the regeneration performance and early establishment of *P. santalinoides* under contrasting soil conditions. The following indices were used to evaluate vegetative regeneration:

$$\text{Sprouting Percentage (SP)} = \frac{\text{Number of sprouted cuttings}}{\text{Total number of planted cuttings}} \times 100 \dots \text{equation (1)}$$

$$\text{Mean Sprouting Time (MST)} = \frac{\sum (n_i \times t_i)}{\sum n_i} \dots \text{equation (2)}$$

where n_i represents the number of newly sprouted cuttings at time t_i , and t represents the number of days after planting.

Sprout survival rate was determined as:

$$\text{Sprout Survival Rate (SSR)} = \frac{\text{Number of surviving sprouts}}{\text{Total number of sprouted cuttings}} \times 100 \dots \text{equation (3)}$$

Total dry biomass was calculated as:

$$\text{Total Dry Biomass (TDB)} = \text{Shoot Dry Weight} + \text{Root Dry Weight} \dots \text{equation (4)}$$

Statistical Analysis

Experimental data were subjected to descriptive and inferential statistical analyses. Analysis of Variance (ANOVA) was used to determine significant differences among treatment means, while Duncan's Multiple Range Test (DMRT) was employed for mean separation where significant differences occurred at the 5% probability level ($P < 0.05$).

Data expressed as counts were transformed where necessary prior to analysis. Regression analysis was further performed to examine the relationship between selected soil properties and vegetative growth parameters.

Results

The sprouting percentage of *Pterocarpus santalinoides* stem cuttings varied considerably among stem cutting lengths and between the control and crude oil-polluted soil treatments. Growth performance was evaluated using sprouting percentage, mean sprouting time, weekly sprout emergence, cumulative leaf development, and dry biomass production. The 25 cm stem cuttings established in crude oil-polluted soil consistently recorded the highest regeneration performance throughout the experimental period. Sprouting percentage differed markedly among stem cutting lengths and soil treatments (Figure 1). The

highest sprouting percentage was recorded in the 25 cm stem cuttings established in crude oil-polluted soil (160%), followed by the 15 cm stem cuttings in polluted soil (80%). Under control conditions, the 25 cm stem cuttings produced a sprouting percentage of 68%, while the corresponding 15 cm cuttings recorded 60%. Lower sprouting percentages were observed for the 40 cm stem cuttings (40%), whereas the poorest regeneration occurred in the 30 cm stem cuttings under the control treatment (33.33%). Polluted soil did not suppress sprouting; rather, regeneration was greatest in the intermediate stem cutting lengths, particularly the 25 cm treatment.

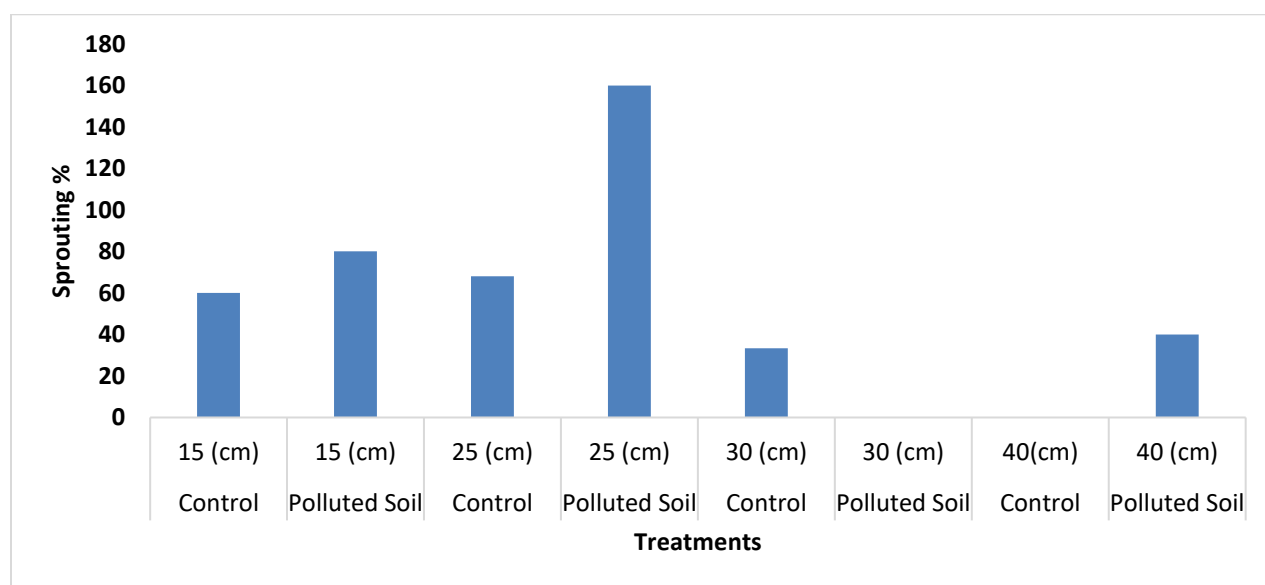


Figure 1: Sprouting percentage of *Pterocarpus santalinoides* stem cuttings

Mean sprouting time varied among treatments (Figure 2). The 25 cm stem cuttings established in polluted soil recorded the longest mean sprouting time (0.71 days), followed by the 25 cm control treatment (0.57 days). Intermediate values were observed for the 40 cm and 15 cm cuttings, whereas the shortest mean sprouting time occurred in the 30 cm stem cuttings (0.14 days). Despite the longer sprouting period observed in the 25 cm polluted treatment, this treatment ultimately produced the highest regeneration success, indicating that delayed sprouting did not reduce establishment capacity.

Sprout emergence followed distinct temporal patterns among treatments (Figure 3).

No sprouting was recorded during the first week except in the 25 cm stem cuttings. From the second week onward, sprout production increased progressively, reaching maximum values towards the eighth week. The highest cumulative number of sprouts (40) was obtained from the 25 cm stem cuttings grown in polluted soil, followed by the corresponding control treatment (17 sprouts). Stem cuttings of 15 cm produced intermediate sprout numbers, while the 30 cm treatment consistently recorded the lowest emergence throughout the experiment. Regression analysis indicated strong positive temporal relationships for sprout emergence in the polluted treatments, particularly for the 15 cm ($R^2 = 0.9571$) and 25 cm ($R^2 = 0.7890$) stem cuttings.

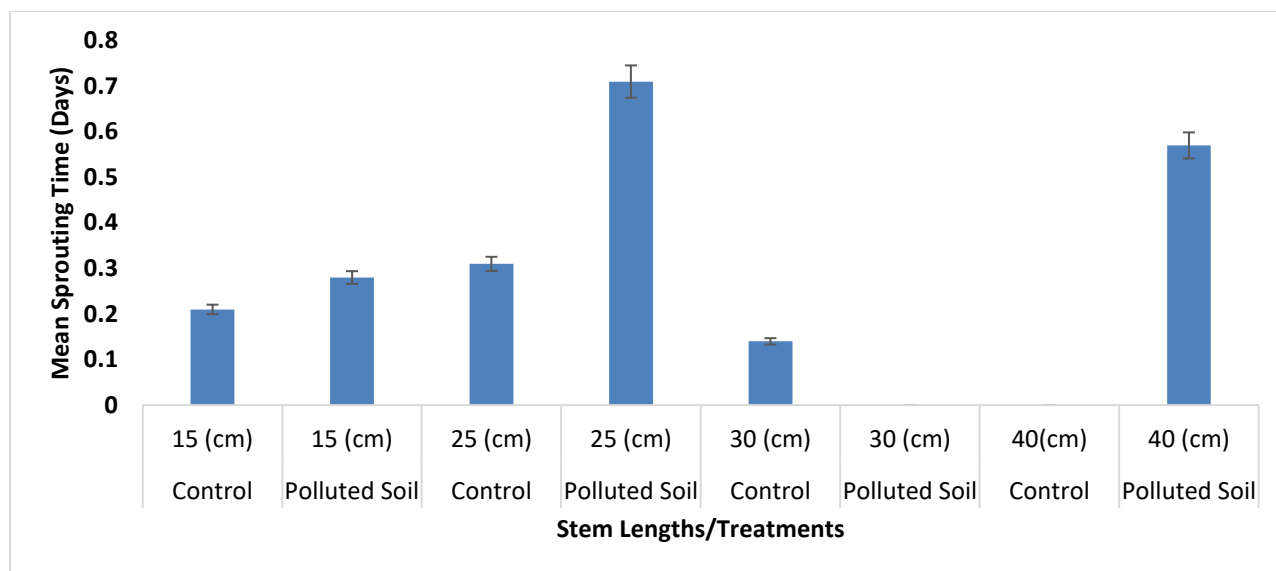


Figure 2: Mean Sprouting Time of *Pterocarpus santalinoides* stem cuttings

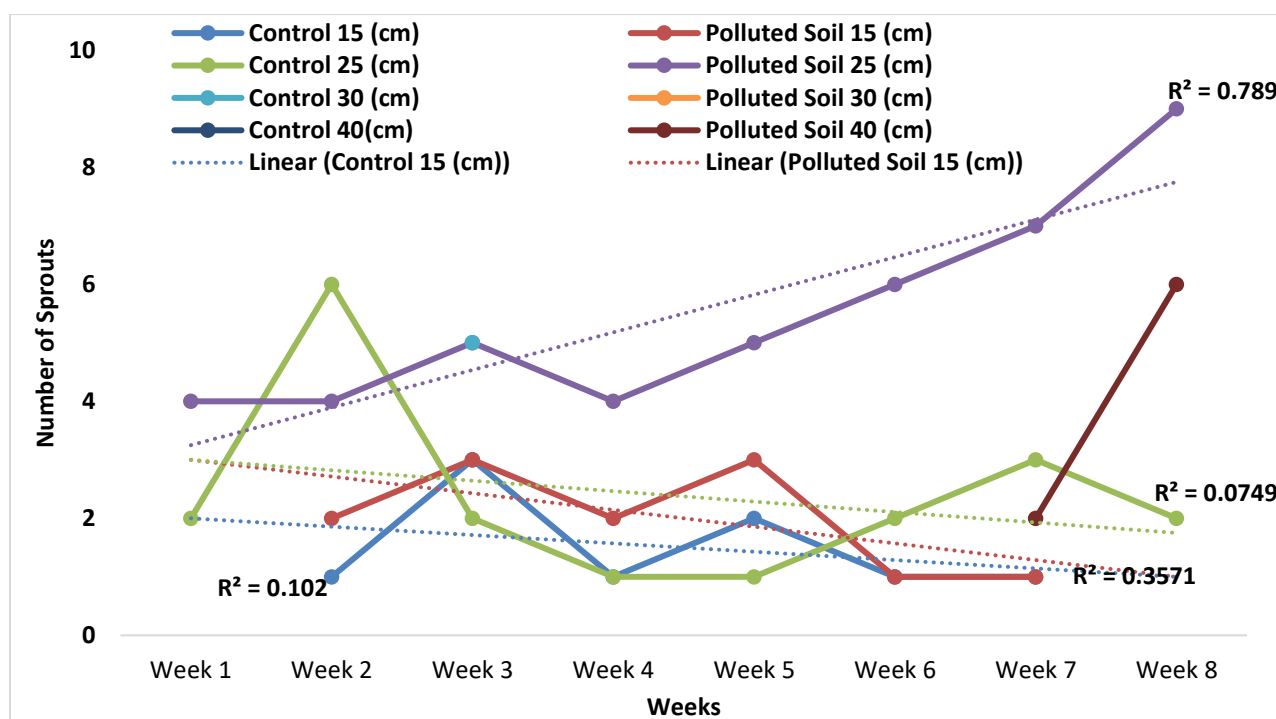


Figure 3: Number of sprouts of *Pterocarpus santalinoides* stem cuttings

Cumulative leaf development differed considerably among treatments (Figure 4). The 25 cm stem cuttings established in polluted soil exhibited the greatest leaf growth, attaining a cumulative leaf length of 163 cm by the end of the experiment. The corresponding control treatment recorded 62 cm, while both the 15

cm and 40 cm stem cuttings produced intermediate leaf lengths. The poorest leaf development occurred in the 30 cm control treatment, which reached only 6 cm. These results demonstrate that leaf expansion closely reflected the regeneration performance observed during sprouting and shoot emergence.

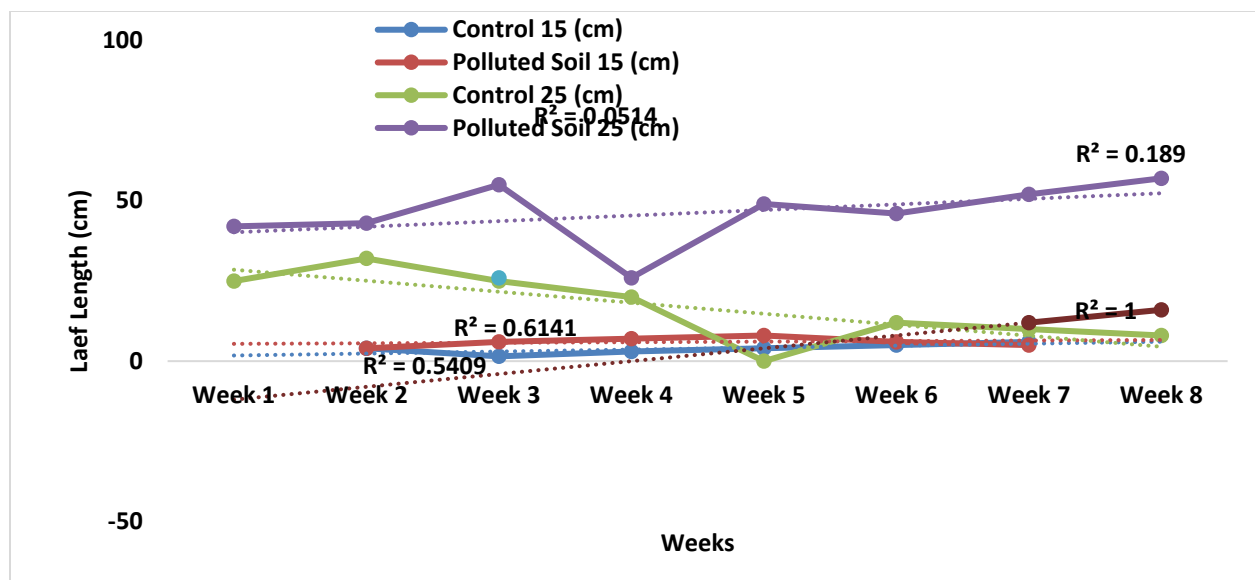


Figure 4: Cumulative leaf length of *Pterocarpus santalinoides* stem cuttings

Biomass accumulation increased progressively throughout the eight-week experimental period (Figure 5). The highest cumulative dry biomass (45.1 g) was recorded in the 25 cm stem cuttings established in polluted soil, substantially exceeding all other treatments. The 15 cm polluted treatment ranked second (24.9 g), whereas the control treatments

generally produced lower biomass values. Biomass accumulation closely followed the patterns observed for sprouting percentage, shoot emergence, and leaf development, suggesting that successful early establishment translated directly into greater productivity.

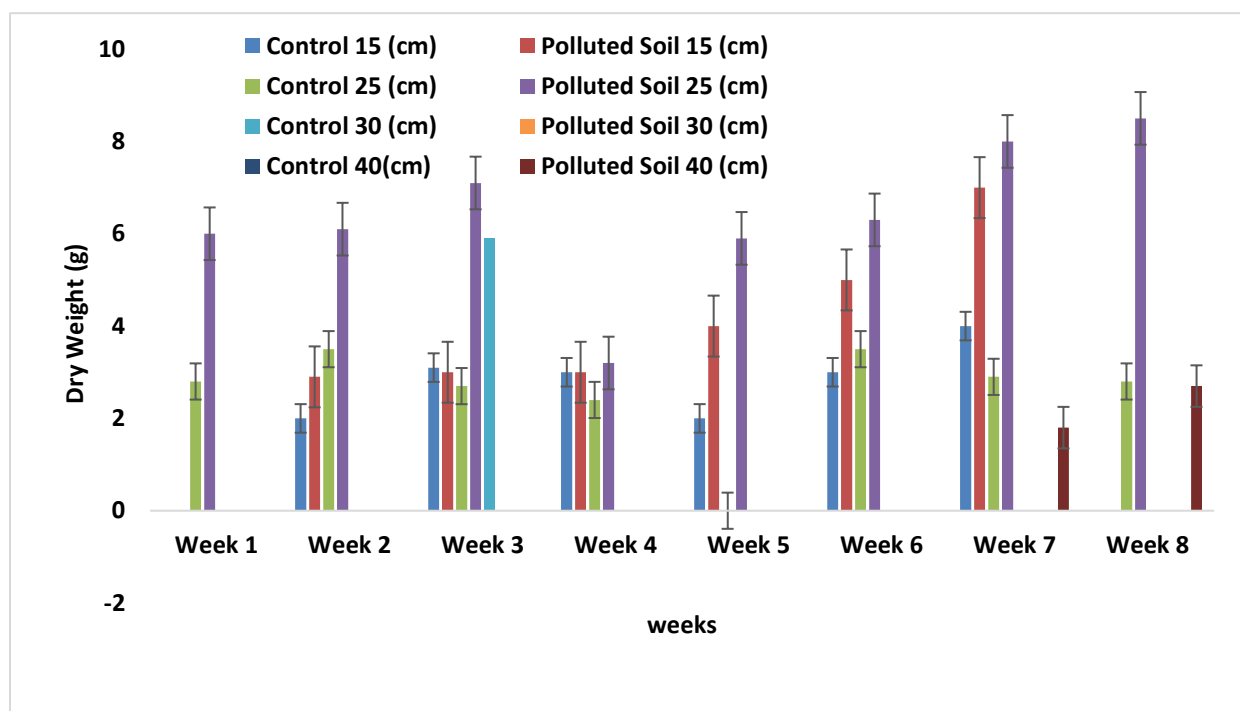


Figure 5: Cumulative dry biomass of *Pterocarpus santalinoides* stem cuttings

Discussion

The regeneration performance of *Pterocarpus santalinoides* stem cuttings was markedly influenced by stem cutting length and soil condition. The superior sprouting percentage observed in the 25 cm stem cuttings, particularly under crude oil-polluted soil, suggests that intermediate-sized cuttings possessed adequate stored carbohydrate reserves, viable buds, and endogenous plant growth regulators required to support bud break and early shoot development. Stored carbohydrates provide the energy required for cell division and the initiation of new shoots before the cuttings become physiologically independent.

Hartmann *et al.*, (2018) reported that the success of vegetative propagation depends largely on the amount of stored food reserves and the hormonal balance within the cutting, which directly influence shoot initiation and rooting. Similarly, Fredrick *et al.*, (2021) observed that 25 cm stem cuttings of *P. santalinoides* produced superior sprouting and vegetative growth compared with shorter and longer cuttings. Likewise, Ky-Dembele *et al.*, (2016) demonstrated that the regenerative capacity of *P. santalinoides* is strongly influenced by cutting characteristics, with cutting type exerting a greater effect on propagation success than the rooting substrate. Shekhawat and Manokari (2016) similarly reported that cuttings with a greater number of nodes and adequate endogenous growth regulators produced significantly better shoot development and survival than smaller cuttings. These findings indicate that the intermediate cutting length used in the present study provided favourable physiological conditions for successful regeneration.

A more striking observation, however, is that the 25 cm cuttings performed *better* in crude oil-polluted soil than in the uncontaminated control, a pattern that runs counter to the general expectation that petroleum contamination suppresses plant growth. This finding is better understood in light of the concept of hormesis, a biphasic dose-response phenomenon in which low or moderate doses of an otherwise toxic substance stimulate growth, while higher doses inhibit it. Agathokleous *et al.*, (2020) reviewed extensive evidence that hydrocarbons, including crude oil constituents, can induce hormetic responses in biota, producing measurable growth stimulation at low exposure levels even though the same substances are inhibitory at higher concentrations.

Siemieniuk *et al.*, (2025) further clarified that such hormetic stimulation in plants is typically mediated by moderate activation of defence mechanisms, including antioxidant activity and elevated endogenous auxin content, which can enhance shoot and biomass production under mild stress rather than suppressing it. Applied to the present study, the moderate level of crude oil contamination at the Bunu-Tai site may have acted as a mild physiological stressor that triggered a defensive hormonal and antioxidant response in the 25 cm cuttings, rather than a toxic one, thereby enhancing rather than impairing sprouting, leaf development, and biomass accumulation. This interpretation offers a more complete explanation than tolerance alone: the species may not simply have resisted the contamination but was, at this particular contamination level and cutting length, stimulated by it.

Soil nutrient dynamics may also help explain the pattern. John *et al.*, (2016) reported that crude oil contamination alters nitrogen cycling in wetland soils of the Niger Delta by widening the soil carbon-to-nitrogen ratio, which can temporarily immobilize nitrogen within the microbial biomass; however, this same hydrocarbon-driven shift in microbial activity has also been associated with localized increases in labile carbon and altered nutrient availability that some plant species are able to exploit during early establishment. Combined with the mobilization of stored carbohydrate reserves described by Hartmann *et al.*, (2018), this suggests that the 25 cm cuttings may have been physiologically well-positioned to capitalize on such transient soil changes, while the poorer-performing 30 cm cuttings lacked the reserve capacity to do so.

Although the 25 cm stem cuttings required a slightly longer mean sprouting time, they subsequently recorded the highest sprout emergence and cumulative leaf development throughout the experimental period. This observation suggests that a longer establishment phase did not impair regeneration but rather allowed sufficient mobilization of stored assimilates before active shoot growth commenced, a pattern consistent with hormetic preconditioning, in which an initial lag in response precedes a stronger downstream growth outcome. The significantly greater dry biomass accumulation recorded in the 25 cm stem cuttings established in crude oil-polluted soil indicates that successful sprouting translated into enhanced plant productivity and early vigour.

Fredrick *et al.*, (2024) similarly reported that vegetative propagation of *P. santalinoides* was affected only under increasing levels of salt stress, indicating that the species possesses considerable resilience to moderate environmental stress. However, the present findings contrast with those of Pérez-Hernández *et al.*, (2017), who observed significant reductions in biomass production of several tropical tree species with increasing petroleum concentrations, and with Irunkwor *et al.*, (2022), who documented substantial soil degradation in oil-impacted Niger Delta communities. The difference may be attributed to species-specific tolerance and hormetic response mechanisms, differences in crude oil concentration and weathering stage, or the relatively moderate contamination level of the soil used in the present study relative to the higher concentrations tested in those studies. Taken together, the results demonstrate that stem cutting length was a more important determinant of vegetative regeneration than soil condition under the experimental conditions, and that

P. santalinoides may exhibit a hormetic rather than purely tolerant response to moderate crude oil contamination, a distinction with direct implications for how the species is deployed in restoration programmes, since outcomes may vary considerably with contamination intensity.

Conclusion

The findings of this study demonstrate that 25 cm stem cuttings produced the best vegetative regeneration of *Pterocarpus santalinoides*, irrespective of soil condition, indicating their suitability for rapid propagation and early establishment. The ability of the species to regenerate successfully in crude oil-contaminated soil also highlights its potential for conservation and ecological restoration in degraded environments. It is therefore recommended that 25 cm stem cuttings be adopted for nursery propagation, while further field studies should evaluate their long-term performance under varying levels of crude oil contamination.

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