

Manifestation of Growth and Biochemical attributes of Tree Species Raised on Solid Municipal Waste

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ABSTRACT

The present investigation was carried out at Punjab Agricultural University in collaboration with the State Forest and Wildlife Preservation Department at Forest Nursery, Baddowal (Ludhiana). The experiment consisted of four treatments (main plot) of sludge from different sewage treatment sources and a control, along with three forest tree species as sub-plots: *Terminalia arjuna* (arjun), *Eucalyptus tereticornis* clone 413 (safeda), and *Melia composita* (dek). Soil application of sludge significantly affected the physiological, biochemical, and growth characteristics of the tree seedlings. After twelve months, plant height, collar diameter, root length, number of roots, dry root weight, and dry shoot weight were all markedly higher with sludge application, especially using sludge from the Bhattian treatment plant, compared to control soil. Among the species, *T. arjuna* exhibited superior growth (height, roots, collar diameter, root length) across sludge treatments. Biochemical attributes, including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble sugars, and starch content, were also significantly enhanced by soil amendment with Bhattian sludge after one year. *T. arjuna* seedlings accumulated the highest levels of chlorophyll a, total chlorophyll, carotenoids, sugars, and starch (and nearly the highest chlorophyll b) irrespective of sludge treatment. Overall, *T. arjuna* performed best on Bhattian sludge, likely due to the higher nutrient content of that sludge compared to control soil and the other sludge sources (Balloke and Jamal-pur). This study suggests that growing trees in sludge-treated soil is a viable strategy for improving seedling quality on waste-contaminated sites. Furthermore, it demonstrates a sustainable approach to municipal waste management by recycling sewage sludge as a soil amendment to irrigate and fertilize forests, amenity trees, and greenbelts.



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1. Introduction

Rapid urbanization, population growth, industrialization, and changing lifestyles have led to an escalation of solid waste generation and environmental pollution. Currently, about 3 billion urban inhabitants worldwide produce approximately 1.2 kg of solid waste per capita per day. By 2025, the urban population is projected to reach 4.3 billion, with per capita municipal waste generation rising to about 1.42 kg per day. In India alone, major cities generate roughly 38,354 million liters per day of sewage, far exceeding the treatment capacity of 11,786 MLD. This results in the production of enormous quantities of waste, including contaminated wastewater and sewage sludge, which pose serious disposal challenges.

In recent years, there has been growing interest in the sustainable reuse of organic waste for soil amendment and land reclamation. Using waste-derived organic matter to improve soil health not only addresses waste disposal issues but also recycles nutrients back into ecosystems. Municipal solid waste (MSW), kitchen waste, farm manure, and sewage sludge are rich in organic matter and plant nutrients and have been widely studied as soil amendments. Incorporation of such organic waste can enhance soil fertility and structure by increasing soil organic carbon, improving aggregate stability, and stimulating microbial biomass. For example, adding composted municipal waste has been shown to improve soil physical properties (greater water-holding capacity, infiltration, aeration) and boost crop yields and nutrient uptake. Recycled organic amendments generally lead to higher soil nutrient availability (especially nitrogen and phosphorus) and can thus support better plant growth.

Trees play a pivotal role in environmental purification, and utilizing solid municipal waste (including sewage sludge) in forestry offers a cost-effective, eco-friendly waste management strategy. Sewage sludge, after appropriate treatment (e.g., composting to a biosolid), contains valuable nutrients and organic matter that can serve as fertilizer or soil conditioner. Soil application of sewage sludge or sludge compost has been reported to improve soil fertility and crop performance without adverse effects when used judiciously. For instance, [12] observed that optimized sewage sludge application improved the establishment of *Pinus halepensis* seedlings in a Mediterranean climate. [19] found that amending soil with municipal compost increased vegetable yields and phosphorus uptake in Canada. A recent review by [14] further confirms that various organic waste amendments (including sewage sludge) generally enhance soil properties and plant growth and yield.

Despite the known benefits, the use of sewage sludge and other wastes in raising tree seedlings for afforestation or phytoremediation has not been fully explored. Some studies have demonstrated positive outcomes: [5] reported that amending soil with sewage sludge along with beneficial fungi significantly improved the growth of *Eucalyptus globulus* and soil biological parameters. [28] showed that municipal garbage compost could partly replace conventional potting media for timber species seedlings, yielding healthy planting stock. In a trial with *Lafortia pacari* (a tropical tree), substrates incorporating urban wastes (sewage sludges from various treatment plants or city garbage compost, mixed with coconut fiber) produced seedlings with equal or superior growth compared to commercial nursery media. Notably, seedlings grown with sewage sludge-based substrates had the best overall quality, attributed to the sludge's high nutrient and organic matter content. [7] found that European larch (*Larix decidua*) seedlings fertilized with sewage sludge exhibited improved growth performance, while trace metal accumulation in tissues remained within safe limits. Similarly, [15] reported that *Eucalyptus camaldulensis* irrigated with municipal sludge achieved ~20% greater growth and ~40% more leaves than control plants, with no negative effects from the sludge application. Together, these studies suggest that properly treated sewage sludge and municipal compost can serve as effective growing media or soil amendments for tree species in nurseries and plantations.

In addition to growth promotion, sludge amendments can induce beneficial biochemical responses in plants. Enhanced availability of nitrogen, phosphorus, and micronutrients from sludge often leads to higher photosynthetic pigment levels and carbohydrate accumulation in foliage. For example, sludge fertilization increased chlorophyll content in willow and alder seedlings and raised total chlorophyll in Eucalyptus leaves. [25] observed significantly higher chlorophyll in forest tree seedlings grown with sewage sludge biochar than in controls. Municipal sludge treatments have also been shown to elevate total chlorophyll and carotenoids in *Ailanthus altissima*. Wastewater irrigation rich in organic matter similarly enhanced the photosynthetic rate and pigment content of *Beta vulgaris* (beet) plants. On the metabolic side, application of sewage effluent led to higher carotenoid levels in foxtail millet and increased soluble sugar content in ridge gourd (*Luffa acutangula*) and rice after sludge effluent irrigation. These improvements in physiological and biochemical attributes can contribute to overall plant vigor and stress tolerance. Although *Terminalia arjuna*, Eucalyptus, and *Melia* are fast-growing species often used in reforestation and known for phytoremediation potential, there is limited information on raising high-quality seedlings using sewage sludge as a nutrient source. The present study was undertaken to evaluate the impact of treated municipal sludge on the growth performance and biochemical characteristics of these tree species. We hypothesized that sludge-amended growing medium would significantly improve seedling growth and physiology compared to normal soil, and that species would differ in their response. This work aims to provide insight into the feasibility of utilizing sewage sludge in forestry applications, thereby addressing waste management challenges while producing robust planting stock for environmental restoration.

2. LITERATURE REVIEW

Use of Organic Wastes for Soil Improvement: A substantial body of literature indicates that organic waste can be successfully recycled as soil amendments to improve soil health and fertility. Additions of composted municipal solid waste, farmyard manure, or sewage sludge increase soil organic matter content and nutrient supply. [2] demonstrated that repeated MSW compost application led to the accumulation of organic carbon in calcareous soil, along with some buildup of trace metals. Organic inputs also enhance soil biological activity; for instance, [29] reported higher microbial biomass and glomalin (a soil protein from fungi) in soils receiving organic amendments. These changes translate into better soil structure, moisture retention, and aeration, which favor plant root development. In a long-term field study, [20] found that applying sewage sludge to willow crops not only improved plant growth and yield but also increased soil enzymatic activities and microbial counts, indicating improved soil biochemical health. Overall, the consensus from numerous studies is that judicious use of organic waste amendments can restore degraded soils and sustainably enhance their productivity.

Sewage Sludge as a Fertilizer in Forestry: Sewage sludge (biosolid) contains high levels of nitrogen, phosphorus, and micronutrients essential for plant growth. After appropriate treatment to reduce pathogens and heavy metals, sludge can be applied to agricultural or forest lands as an organic fertilizer or as a component of potting substrates. Research in forest ecosystems and nurseries has shown promising results. [13] observed that amending nursery soil with sewage sludge (25% sludge by volume) significantly increased seedling height (22.2 cm vs 15.2 cm in controls) and improved germination of tree seeds, due to the sludge's high organic carbon and NPK content. In Mediterranean Spain, [12] reported that applying optimized doses of sewage sludge to degraded soils promoted the establishment and early growth of *Pinus halepensis* seedlings. Studies with Eucalyptus species have also been encouraging. [5] showed that *E. globulus* seedlings grew taller and accumulated more nutrients when soil was amended with sewage sludge (along with mycorrhizal inoculation). A Brazilian study by [15] confirmed the fertilization value of municipal sludge for *E. camaldulensis*, where sludge-fertilized plants achieved 20% greater biomass and 40% more leaves than unfertilized controls with no signs of toxicity. Importantly, multiple long-term trials have found no significant

adverse effects of properly stabilized sludge on tree health or soil quality. Sludge applications often increase soil pH slightly and reduce bulk density, mitigating acidity and compaction issues in plantation sites. Nevertheless, continuous monitoring of trace metals is advised; for example, [7] noted increased zinc and copper uptake in larch seedlings with sludge fertilization, though levels remained below phytotoxic thresholds.

Municipal Compost and Other Wastes in Nursery Media: Beyond direct field application, composted urban wastes have been tested as components of nursery potting media to reduce reliance on peat and other non-renewable materials. [28] found that composted municipal garbage could replace up to 25–50% of standard potting mix for tropical timber species without compromising seedling growth. Similarly, [1] successfully produced *Lafoensia pacari* seedlings using substrates composed of sewage sludge from various wastewater treatment plants and city refuse compost (each mixed with 20% coconut coir for aeration). After 105 days, seedlings grown in sludge-based substrates (especially sludge from one plant) showed the best performance across all quality parameters, outperforming those grown in a conventional pine-bark nursery mix. Even the composted domestic garbage treatment yielded plant growth superior to the commercial substrate. These findings highlight that urban waste-derived substrates, when properly formulated, can meet the nutritional requirements of tree seedlings and even enhance growth—likely due to the slow release of nutrients and improved moisture retention from the organic matter. Likewise, [18] evaluated composted sewage sludge combined with sugarcane bagasse as a peat substitute for *Eucalyptus urograndis* seedlings. They reported that sludge–bagasse substrate produced seedlings with growth and quality indices comparable to those grown in traditional peat media. The composted sludge provided abundant nutrients, while bagasse improved substrate structure, resulting in robust seedlings without the need for full-strength chemical fertilizers. These studies collectively suggest that recycling municipal organic waste into nursery media is a viable strategy that adds value to waste and reduces costs for forest nurseries.

Biochemical Enhancements from Waste Amendments: Improved nutrient availability from organic amendments often translates into elevated biochemical constituents in plants. One commonly reported effect is an increase in chlorophyll content, which is a proxy for photosynthetic capacity and nitrogen status. [22] observed higher chlorophyll level in willow and alder seedlings following sewage sludge treatment, attributing this to greater N and Mg availability for chlorophyll synthesis. In *Eucalyptus*, the addition of sludge (or sludge biochar) has similarly led to richer green foliage with significantly more chlorophyll compared to control plants. Such increases in pigment concentration improve light capture and can boost growth. Another beneficial response is the accumulation of osmolytes and storage compounds. In the current literature, sludge amendments have been linked to increased leaf carotenoids (accessory pigments that protect the photosynthetic apparatus) and soluble sugars, which can enhance plant stress tolerance. For example, [6] reported a significant rise in leaf carotenoid content of foxtail millet irrigated with diluted municipal sewage effluent. [21] documented higher soluble sugar levels in vegetables like ridge gourd and rice when grown with sewage sludge effluent, reflecting improved photosynthetic output and possibly an adaptive response to sludge-derived stimuli. Starch, the primary storage carbohydrate, has also been noted to increase in woody plants under sludge fertilization. In one study, treated *Ailanthus altissima* seedlings accumulated more starch and total chlorophyll than untreated ones, indicating vigorous photosynthesis and carbon assimilation with sludge supplementation. Overall, these biochemical enhancements are positive indicators that sewage sludge and related waste amendments can promote not just growth but also the physiological quality of plants, which may improve their survival and performance upon out-planting.

In summary, the literature provides strong evidence that the beneficial reuse of sewage sludge and municipal compost in forestry is both feasible and advantageous. Sewage sludge serves as a nutrient-rich resource that can improve tree growth, soil properties, and plant biochemical status if it is properly processed and applied

in safe amounts. This study builds on those findings by examining specific growth and biochemical outcomes for *T. arjuna*, *Eucalyptus*, and *Melia* seedlings raised on sludge-amended soil, thereby contributing to the knowledge base needed to integrate waste recycling into forestry practices.

3. MATERIALS AND METHODS

Plant Material and Nursery Setup: Healthy seedlings of three tree species — *Eucalyptus tereticornis* (clone 413, “Safeda”), *Melia composita* (“Dek”), and *Terminalia arjuna* (“Arjun”) — were obtained from the Department of Forestry and Natural Resources, PAU, and the State Forest & Wildlife Preservation Department, Ludhiana. Initially, seeds were germinated and grown in small polybags in the nursery (Fig. 1).



Fig. 1. Plant species at the seedling stage: A) Arjun, B) Safeda, C) Dek

Once the seedlings reached approximately 15 cm in height, they were transplanted into larger poly-bags (27 cm × 20 cm size) filled with experimental soil/sludge mixtures (Fig. 2). The transplanting was done in March 2017, and the experiment was repeated with a second batch in 2018 at the Forest Nursery, Baddowal (30.88°N, 75.76°E).



Fig. 2. Plant species after a year of transplanting

Sludge Treatments: Sewage sludge was collected from three different municipal wastewater treatment plants in Ludhiana: Bhattian, Balloke, and Jamalpur. These sludges, after standard drying and stabilization processes, were used as soil amendments in the experiment. Before use, the nutrient composition of each sludge source was analyzed (Table 1). Key macronutrients (N, P, K) and micronutrients (Zn, Fe, Mn, Cu) were quantified to characterize the sludge quality. Sludge from each source was then thoroughly mixed with local nursery soil at a concentration of 1% (dry weight) per polybag (approximately equivalent to 10 tonnes sludge per hectare, assuming typical field bulk density). The four treatment conditions were: (1) Control (nursery soil without sludge), (2) Soil + Bhattian sludge, (3) Soil + Balloke sludge, and (4) Soil + Jamalpur sludge. Each treatment was assigned to main plots in a randomized block design.

Experimental Design: The experiment followed a split-plot design with sludge treatment as the main-plot factor and tree species as the sub-plot factor. There were four sludge treatments (including control) and three species, for a total of 12 treatment combinations. Each treatment combination was replicated in three blocks. Within each replicate, 10 seedlings per species per sludge treatment were planted, yielding 30 seedlings for each treatment per block. The polybags were arranged outdoors in the nursery under the full sun, and all seedlings were maintained under identical irrigation and care regimes (manual weeding, pest monitoring) for the duration of the trial. No chemical fertilizers were added; the seedlings relied on nutrients from the soil or sludge.

Growth Measurements: Growth parameters were recorded 12 months after transplanting. Plant height (cm) was measured from the base of the stem to the apical shoot tip using a measuring tape. Collar diameter (mm) at the root collar (stem base) was measured with a digital vernier caliper. To assess root growth, seedlings were carefully uprooted and roots washed free of soil; the root length (cm) of each plant was taken as the length of the longest root using a ruler. The number of lateral roots (count) on each seedling was also recorded manually. After measuring fresh dimensions, plants were separated into shoots and roots and oven-dried at 70 °C (± 2 °C) to constant weight. Dry shoot weight and dry root weight were recorded for each seedling using an electronic balance (precision 0.01 g). These measurements provided indices of biomass accumulation in response to the treatments.

Physiological and Biochemical Assessments: A suite of biochemical attributes was analyzed on leaf samples at 12 months to determine treatment effects on seedling physiology. Total chlorophyll and carotenoid content were estimated from fresh leaves (mg per g fresh weight) using the spectrophotometric method of [3], which involves extracting pigments in an acetone solvent and measuring absorbance at specific wavelengths. Chlorophyll a and chlorophyll b were calculated separately and then summed for total chlorophyll. Total soluble sugars in the leaves were measured by the colorimetric phenol-sulfuric acid method of [11], expressed as mg glucose equivalents per g dry weight. Starch content (mg per g dry weight) was determined after converting starch to sugars (via enzymatic digestion) and using the same colorimetric assay. Three seedlings per treatment (randomly selected) were used for biochemical sampling, and all assays were performed in triplicate for accuracy.

Statistical Analysis: The collected data were subjected to analysis of variance (ANOVA) appropriate for the split-plot design. The sludge treatment effects (Factor A), species effects (Factor B), and their interaction (A×B) were tested for significance. We used the Shapiro–Wilk test to confirm normality and Bartlett’s test for homogeneity of variances; no data transformations were necessary. Mean comparisons were carried out using Tukey’s honest significant difference (HSD) test at $p < 0.05$. For each growth or biochemical parameter, treatment means $\pm$ standard errors were calculated. We report the least significant differences as Critical Difference (CD) values at the 5% probability level for main effects and interactions in the tables. All statistical

analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

4. RESULTS AND DISCUSSION

Sludge Composition

The three sewage sludge sources varied somewhat in their nutrient content (Table 1). Bhattian sludge had the highest nitrogen (1.90%) and potassium (0.63%) concentrations among the treatments, while Balloke sludge showed slightly higher phosphorus (0.41%). Balloke and Jamalpur sludges contained elevated levels of certain micronutrients (e.g., Zn, Fe) compared to Bhattian. All sludge sources had substantially greater nutrient concentrations than the unamended soil (control), which was low in N (0.93%), K (0.35%), and other elements. These differences in sludge quality were expected to influence plant growth responses, with Bhattian sludge anticipated to promote the most vigorous growth due to its superior N and K supply. Notably, heavy metal content (e.g., Zn, Cu) in all sludge sources was within permissible ranges for land application, and the 1% mixing rate further diluted these elements in the soil matrix.

Growth Attributes

Plant Height: Sludge amendments had a pronounced positive effect on seedling height for all three species (Table 2). After 12 months, the tallest plants were observed in the Bhattian sludge treatment, with an overall mean height of 199.3 cm across species, significantly exceeding the control mean of 165.8 cm. *T. arjuna* responded especially well, reaching an average height of 216.2 cm with sludge (versus 202.3 cm in control). *M. composita* (Dek) also grew tall under sludge treatments (up to 220.7 cm with Bhattian sludge), whereas *Eucalyptus* (Safeda) was comparatively shorter (mean 136.3 cm under Bhattian). Statistically, Arjun seedlings were significantly taller (mean 216.1 cm) than Dek (193.1 cm) and Safeda (136.3 cm) across treatments.

TABLE 1. Nutrient composition of sludge from different treatment sites

Sludge Composition							
Locations	N (%)	P (%)	K (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Bhattian	1.90	0.38	0.63	4.25	45.16	2.37	1.98
Balloke	1.63	0.41	0.53	4.84	68.68	0.30	1.50
Jamalpur	1.44	0.40	0.53	4.42	69.08	0.60	2.36
Soil	0.93	0.38	0.35	4.37	50.12	1.14	1.13

*Concentration of sludge was 1% of the total weight of soil per polybag

The interaction effect of sludge $\times$ species on height was significant, reflecting that *T. arjuna* derived the greatest height benefit from sludge addition. Our results align with Hossain et al. (2013), who re-reported that a 25% sewage sludge soil amendment increased seedling height by ~46% compared to the control. The improved growth can be attributed to the enriched nutrient availability from sludge, particularly nitrogen and phosphorus, which drive vegetative growth, and possibly improved soil moisture retention. [5] Similarly noted enhanced growth of *Eucalyptus* seedlings with sludge was noted, attributing to higher nutrient uptake and better symbiotic microbial activity in the root zone. In the present study, Bhattian sludge's superior nutrient profile (Table 1) likely underpins the markedly taller seedlings in that treatment.

TABLE 2. Effect of sludge treatments on plant height (cm) and collar diameter (cm) of plant species

Sr.No.	Sludge sources	Plant height (cm)				Collar Diameter (cm)			
		Arjun	Safeda	Dek	Mean	Arjun	Safeda	Dek	Mean
1	Bhattian	232.8	144.3	220.7	199.3	1.6	1.5	1.4	1.5
2	Balloke	219.0	138.8	191.9	179.9	1.4	1.3	1.3	1.3
3	Jamalpur	210.4	133.2	181.9	172.0	1.4	1.1	1.2	1.2
4	Control	202.4	129.2	177.9	165.8	1.2	0.9	1.1	1.1
	Mean	216.2	136.4	193.1		1.4	1.2	1.3	
CD (p=5%)	Sludge (A)	5.34				0.066			
	Plant species (B)	13.07				0.16			
	(A)×(B)	18.49				0.23			

Collar Diameter: Stem girth at the collar showed a trend parallel to height. Sludge from Bhattian produced the thickest stems (mean collar diameter 1.5 cm), followed by Balloke (1.3 cm) and Jamalpur (1.2 cm), all above the control (1.1 cm). Among species, *T. arjuna* again led with an average collar diameter of 1.4 cm, significantly greater than Safeda (1.2 cm), with Dek intermediate (1.3 cm). An interaction was evident: Arjun seedlings in Bhattian sludge attained the maximum collar diameter (~1.6 cm), indicating robust secondary growth, whereas Safeda in the control had the smallest (~0.9 cm). [24] reported that continuous application of composted sludge improved stem diameter in ornamental plants, which was linked to better nutrient availability and soil structure. Our findings concur that sludge amendments facilitate thicker stems – a desirable trait for woody seedlings, as it correlates with mechanical stability and vascular capacity. The organic matter in sludge likely improves soil aeration and moisture, promoting extensive root systems that support stronger stem thickening. Over time, the mineralization of sludge would release nutrients in synchrony with plant demand, aiding consistent diameter growth. The largest collar diameters seen in *T. arjuna* under sludge reaffirm this species' strong response to high fertility conditions.

The data in Table 2 underscore two points: (1) Sludge amendments significantly increased growth in terms of both height and thickness, with Bhattian sludge being the most effective, and (2) Species inherently differ in growth potential, with *T. arjuna* outperforming *M. composita* and *Eucalyptus* under the same conditions. The latter is consistent with field observations that *T. arjuna* is a hardy, fast-growing species, whereas *Eucalyptus tereticornis* can be slower under suboptimal nutrient conditions. In sludge-treated conditions, however, even *Eucalyptus* showed marked improvement over control (e.g., 144 cm vs 129 cm in height for Bhattian vs control), indicating that its growth was nutrient-limited and could be accelerated by organic fertilization.

Root Development: Root growth responded positively to sludge treatments, mirroring the shoot growth responses. Root length was greatest in Bhattian sludge (overall mean 38.9 cm), significantly longer than in control soil (30.8 cm, Table 3). *T. arjuna* developed the longest roots (mean 40.1 cm) compared to Dek (33.1 cm) and Safeda (32.5 cm). For example, arjun seedlings in Bhattian sludge had roots ~44 cm long on average, versus ~35 cm in control. Number of roots (lateral roots) per plant also increased with sludge: Bhattian treatment yielded about 28.4 roots per seedling on average, whereas control had ~21.8 (a ~30% increase). Balloke and Jamalpur sludges were intermediate (25–26 roots) and not significantly different from each other.

Among species, *T. arjuna* and *M. composita* produced slightly more lateral roots (26.1 and 25.6, respectively) than *Eucalyptus* (22.4), although differences were modest. The sludge $\times$ species interaction was not as pronounced for root count as for length, suggesting all species benefited proportionally from sludge in terms of root branching.

TABLE 3. Effect of sludge treatments on root length (cm) and number of roots of plant species

Sr. No.	Sludge sources	Root length(cm)				Number of roots			
		Arjun	Safeda	Dek	Mean	Arjun	Safeda	Dek	Mean
1	Bhattian	44.1	37.2	35.3	38.9	29.4	26.9	29.0	28.4
2	Balloke	42.3	35.9	34.3	37.5	27.5	23.1	27.1	25.9
3	Zamalpur	38.8	30.0	32.9	33.9	25.8	21.2	24.7	23.9
4	Control	35.3	27.1	30.0	30.8	25.3	18.5	21.7	21.8
	Mean					26.1	22.4	25.6	
CD (p=5%)	Sludge (A)	1.63				1.35			
	Plant species(B)	4.00				3.30			
	(A) $\times$ (B)	5.65				4.67			

These improvements in root metrics are critical, as a robust root system enhances a seedling's ability to establish in the field. The greater root lengths under sludge indicate that seedlings could explore a larger soil volume for resources, likely a response to improved soil structure and nutrient gradients encouraging deeper rooting. Sludge's organic matter helps loosen the soil and maintain moisture, enabling roots to penetrate further. Additionally, the nitrogen and phosphorus from sludge likely stimulated root elongation and branching (since roots proliferate in nutrient-rich microsites). [15] observed a similar trend where *Eucalyptus* plants with sludge had more profuse rooting, contributing to better overall growth. Our finding that *T. arjuna* had the longest and most numerous roots correlates with its above-ground performance; this species appears highly efficient at translating soil nutrition into both root and shoot growth. *Eucalyptus* had fewer lateral roots, which might reflect species-specific root architecture (*eucalypts* often have coarse, sparse roots in early stages) rather than a lack of response to sludge. Indeed, *Eucalyptus* still gained considerably in root length and number from the sludge relative to its control. These results concur with [5], who reported improved root biomass and length in sludge-amended *Eucalyptus*, and with Ribeiro et al., who noted that organic amendments encourage fine-root development in tree seedlings by improving soil conditions.

Biomass Accumulation (Dry Weight): Sludge treatments significantly increased both shoot and root dry biomass of the seedlings (Table 4). Bhattian sludge led to the highest mean dry shoot weight (74.1 g per plant), more than double that of the control (33.2 g). Balloke sludge was next (58.5 g), followed by Jamalpur (48.4 g). The pattern was similar for dry root weight: Bhattian sludge yielded the heaviest roots (26.2 g), versus 10.1 g in the control, with Balloke and Jamalpur in between (18.6 g and 16.0 g, respectively). Across species, *T. arjuna* accumulated the most biomass (mean shoot 59.7 g, root 18.8 g), significantly exceeding Safeda (48.5 g shoot, 16.4 g root) and Dek (52.5 g shoot, 17.9 g root) in shoot mass. The sludge $\times$ species interaction was not significant for biomass, indicating all species showed proportionate gains in dry matter with sludge application. For example, arjun seedlings in Bhattian sludge averaged ~76 g shoot and 27 g root, compared to ~44 g shoot and 7 g root in control (a drastic increase), while safeda went from ~43 g shoot (control) to ~64 g (Bhattian). The superior biomass in sludge-treated plants is a direct consequence of enhanced growth rates supplied by better nutrition. Higher chlorophyll and photosynthate production (see Biochemical section)

in sludge-fed plants would contribute to greater dry matter accumulation. [9] reported that mango (*Mangifera*) saplings grown with sewage sludge compost had significantly increased root and shoot biomass, attributing it to improved soil fertility and microbial activity that stimulated root growth. Our results mirror their findings. We also observed that sludge-amended seedlings had thicker stems and more branching (qualitative observation), which likely also contributed to higher dry weights.

TABLE 4. Effect of sludge treatments on dry shoot and root weight (g) of plant species

Sr.No.	Sludge sources	Dry shoot weight (g)				Dry root weight (g)			
		Arjun	Safeda	Dek	Mean	Arjun	Safeda	Dek	Mean
1	Bhattian	75.9	64.1	82.3	74.0	27.1	27.6	23.8	26.2
2	Balloke	64.5	55.2	60.8	58.5	21.7	14.7	19.3	18.6
3	Zamalpur	54.8	43.3	47.1	48.4	19.6	12.6	15.9	16.0
4	Control	43.8	43.3	19.7	33.2	6.9	10.6	12.8	10.1
	Mean	59.7	48.5	52.5		18.8	16.4	17.9	
CD (p=5%)	Sludge (A)	6.2				2.9			
	Plant species(B)	15.1				7.1			
	(A)×(B)	21.3				10.1			

One interesting observation is that while *T. arjuna* had the greatest height and diameter, *Melia* (dek) showed the highest individual shoot weight in the Bhattian treatment (82.3 g). This suggests *Melia* can allocate substantial biomass when ample nutrients are available, even if its height is slightly less than *Arjun*'s. The extremely low shoot weight of *Melia* in control (only ~19.7 g) indicates it struggled without added nutrition. *Eucalyptus* had a relatively moderate increase in biomass with sludge, perhaps reflecting that its growth is somewhat slower in the first year, or that it might require more time or a higher sludge percentage to fully capitalize on the nutrient input. Nevertheless, sludge-treated *Eucalyptus* still roughly equaled *Melia* in shoot mass under the best treatment (~64 g vs ~60 g). In practical terms, the nearly two-fold increase in total biomass for sludge-grown seedlings (averaged across species) is a highly meaningful improvement for nursery production, as larger seedlings with well-developed roots and shoots are generally more likely to survive and thrive after planting.

Our biomass results also resonate with the nutrient analysis of the sludge. Bhattian sludge, having the highest N and K, produced the heaviest plant tissues. Nitrogen is a critical driver for vegetative growth and protein content in plant tissues, while potassium aids in carbohydrate synthesis and translocation, thereby supporting greater biomass. The fact that Balloke sludge (with the highest P and micronutrients) resulted in the second-highest biomass suggests phosphorus and micronutrients, though important, were not as limiting as N/K in this scenario. Still, the considerably lower biomass in the control underscores that native soil fertility was insufficient for optimal growth, highlighting the potential of sludge to act as a low-cost fertilizer.

Biochemical Attributes: We examined leaf chlorophyll (a, b, total), carotenoids, soluble sugars, and starch to gauge how sludge influenced the physiological status of the seedlings. The trends observed were largely consistent across these indicators: the sludge from Bhattian treatment led to the highest values in almost all cases.

Chlorophyll: Sludge application significantly boosted chlorophyll a, chlorophyll b, and hence total

chlorophyll content in the leaves (Table 5). In plants grown with Bhattian sludge, chlorophyll a averaged 0.340 mg/g (fresh weight basis), chlorophyll b 0.178 mg/g, and total chlorophyll ~0.490 mg/g, versus only 0.118, 0.062, and 0.178 mg/g, respectively, in control plants (Jamalpur and control values were quite low). This represents nearly a threefold increase in total chlorophyll due to sludge. Among species, *T. arjuna* had the highest chlorophyll concentrations (data not shown in table): averaged across treatments, arjun leaves contained about 0.243 mg/g chlorophyll and 0.084 mg/g chlorophyll b, significantly more than Safeda's leaves (which had the lowest chlorophyll, reflecting their pale coloration). The interaction showed that *T. arjuna* in Bhattian sludge had the richest chlorophyll (chlorophyll a ~0.45, b ~0.33 mg/g), whereas Eucalyptus in the control had the least (~0.08 mg/g total chlorophyll). The darker green foliage in sludge-treated seedlings is visually apparent (Fig. 2) and physiologically important: more chlorophyll means greater light capture capability and potentially higher photosynthetic rates. This is corroborated by literature – [22] found increased chlorophyll a in willows fertilized with sludge, attributing it to improved nitrogen nutrition. [25] and [17] also documented heightened chlorophyll levels in sludge-treated Eucalyptus and Ailanthus seedlings, respectively. Our results concur and extend these findings to the species at hand, especially showing *T. arjuna*'s strong positive pigment response to sludge.

Carotenoids: Carotenoid content (yellow-orange pigments that assist in photosynthesis and protect against oxidative stress) was similarly affected. Bhattian sludge-raised plants had the highest mean carotenoids (~0.029 mg/g fw) and control the lowest (~0.012 mg/g), with Balloke and Jamalpur intermediate (Table 6). After Arjun leaves from Bhattian treatment, carotenoids reached about 0.033 mg/g, compared to 0.016 mg/g in the control, more than double. Safeda and Dek showed parallel trends. These differences were statistically significant ($p < 0.05$ for the sludge effect). [6] noted that sewage effluent irrigation significantly increased leaf carotenoid in *Setaria* millets, which they suggested was a protective acclimation to higher nutrient and light conditions. In our trees, the higher carotenoid levels under sludge could improve stress tolerance (e.g., against high light or pollutant stress) and indicate robust plastid development. The sludge provides not only N, but also essential micronutrients (like iron, magnesium) needed for pigment synthesis. The slight carotenoid increase even in control Arjun vs Safeda suggests genetic differences, but sludge narrows that gap by elevating all.

Soluble Sugars: The concentration of soluble sugars in seedling leaves was markedly enhanced by sludge (Table 6). Bhattian sludge led to an average soluble sugar content of 29.05 mg/g (dry weight), compared to 12.28 mg/g in the control – an increase of over 2.3 times. Balloke and Jamalpur treatments had intermediate sugar levels (22.13 and 18.70 mg/g, respectively). Across species, *T. arjuna* leaves accumulated the most sugars (mean ~27.3 mg/g), followed by Dek (~19.3 mg/g) and Safeda (~15.0 mg/g). This ranking correlates with their growth performance; the most vigorous species (arjun) also generated and perhaps required more photosynthates. The sludge × species interaction on sugar was significant, with arjun in Bhattian showing the peak sugar concentration (~36.65 mg/g, see Table 6 individual values) – almost three times that of safeda in control (~9.7 mg/g). These results are in line with [21], who observed that sludge effluent irrigation elevated sugar content in crops, attributing it to enhanced photosynthetic carbon fixation and possibly osmotic adjustment under nutrient-rich conditions. The surplus sugars in sludge-treated seedlings could be used for faster growth (as seen in greater biomass) or stored for stress resilience.

In tree seedlings, higher soluble sugars can improve drought tolerance by maintaining cell turgor. Our findings suggest that sludge nutrition not only accelerates growth but also primes seedlings metabolically by increasing their energy reserves (sugars).

Starch: Starch, a storage carbohydrate, showed a pattern akin to sugars. Bhattian sludge induced the highest

leaf starch content (~2.245 mg/g dw), significantly above the control (0.948 mg/g). Balloke and Jamalpur again were intermediate (1.712 and 1.445 mg/g, respectively). T. arjuna leaves averaged ~2.109 mg/g starch across treatments, much higher than Safeda (1.160) or Dek (1.494 mg/g). Under the best treatment (Bhattian), arjun leaves amassed ~2.83 mg/g starch, compared to ~1.17 mg/g in the control (Table 6), showing they

TABLE 5. Effect of sludge treatments on chlorophyll content of plant species

S.no	Treatments	Chlorophyll a (mg/g fw)				Chlorophyll b (mg/g fw)				Total chlorophyll (mg/g fw)			
		Arjun	Safed a	Dek	Mea n	Arju n	Safed a	Dek	Mea n	Arju n	Safed a	Dek	Mea n
1	Bhattian	0.446	0.157	0.417	0.340	0.333	0.088	0.114	0.178	0.559	0.406	0.505	0.490
2	Balloke	0.214	0.079	0.362	0.218	0.104	0.087	0.072	0.087	0.285	0.213	0.448	0.315
3	Jamalpur	0.179	0.062	0.115	0.118	0.079	0.039	0.070	0.062	0.248	0.183	0.153	0.194
4	Control	0.135	0.040	0.039	0.071	0.065	0.018	0.061	0.048	0.196	0.115	0.056	0.122
CD (p=5%)	Mean	0.243	0.084	0.233		0.145	0.058	0.079		0.322	0.229	0.290	
	Sludge (A)		0.0000392				0.0000272				0.0000639		
	Plant species(B)		0.0000961				0.0000666				0.0001570		
	(A)×(B)		0.0001360				0.0000941				0.0002200		

TABLE 6. Effect of sludge treatments on biochemical attributes of plant species

S.no	Treatments	Carotenoid (mg/g fw)				Sugar (mg/g dw)				Starch (mg/g dw)			
		Arjun	Safeda	Dek	Mean	Arjun	Safeda	Dek	Mean	Arjun	Safeda	Dek	Mean
1	Bhattian	0.033	0.025	0.028	0.029	36.65	25.62	24.87	29.047	2.830	1.980	1.925	2.245
2	Balloke	0.019	0.019	0.022	0.020	30.67	14.025	21.695	22.130	2.370	1.085	1.680	1.712
3	Jamalpur	0.019	0.017	0.013	0.016	26.74	10.66	18.70	18.700	2.065	0.825	1.445	1.445
4	Control	0.016	0.012	0.008	0.012	15.15	9.725	11.965	12.280	1.170	0.750	0.925	0.948
	Mean	0.022	0.018	0.018		27.303	15.008	19.308		2.109	1.160	1.494	
CD (p=5%)	Sludge (A)		0.00000693				0.009				0.000198		
	Plant species(B)		0.00001700				0.022				0.000484		
	(A)×(B)		0.00002400				0.031				0.000685		

could synthesize and store significantly more surplus carbohydrates when nutrients were abundant. In our study, the combination of increased chlorophyll and ample nutrients likely allowed sludge-fed seedlings to photosynthesize at rates that exceeded their immediate growth needs, with the excess assimilates being stored as starch. This is a favorable outcome, as starch reserves can be remobilized to support new growth flushes or help the plant survive transplantation shock. The elevated starch in T. Arjun suggests it was especially effective at converting the available nutrients into stored energy, which may partly explain its superior growth after transplanting (fast growth often requires tapping into stored reserves). All sludge treatments significantly increased chlorophyll a, chlorophyll b, and total chlorophyll relative to the control. Bhattian sludge yielded the highest chlorophyll levels (total ~0.490 mg/g), while the control had the lowest (~0.178 mg/g). T. arjuna > M. composita > E. tereticornis in chlorophyll content. (Full data presented in Table 6 for total chlorophyll combined with other biochemical attributes.)

The biochemical data collectively reinforce the growth findings, painting a picture of enhanced physiological capacity in sludge-treated seedlings. The superior nutrient supply from sewage sludge translates into greener (chlorophyll-rich) and metabolically more active foliage, with greater synthesis of sugars and accumulation of reserves like starch. These internal changes likely drive the observed growth gains. [26] reported similar improvements in chlorophyll and other metabolic parameters in plants irrigated with municipal wastewater, which in turn correlated with improved growth and yield. In an afforestation context, seedlings with higher nutrient and carbohydrate stores are better poised to survive planting in the field and to resume growth quickly. It is also worth noting that none of the sludge treatments showed any visual symptoms of nutrient toxicity or chlorosis; on the contrary, all parameters moved in a positive direction. This suggests that the 1% sludge rate was within a safe and optimal range, providing benefits without overwhelming the system with excess salt or metals. [4] Similarly found that annual low-rate sludge additions barely improved growth and leaf pigment without adverse effects, underlining the importance of dose.

The results of this study demonstrate that amending soil with municipal sewage sludge can substantially enhance the growth and biochemical status of tree species in the nursery stage. The improvements were observed consistently across morphological traits (height, diameter, root development) and physiological measures (pigments, sugars, starch), particularly with sludge from the Bhattian plant, which had the richest nutrient profile. This aligns with the expectation that sludge quality (nutrient content) influences its fertilizer value. The fact that all three species benefited, albeit to different extents, indicates the broad applicability of using sludge for growing tree seedlings. Notably, *T. arjuna* emerged as the most responsive species, achieving the greatest growth on sludge. This suggests *T. arjuna* could be a candidate of choice for planting on sludge-amended sites or for biomass production using organic waste fertilization. *Melia composita* also showed strong gains, implying it can utilize organic nutrients efficiently when available. *Eucalyptus tereticornis*, though lagging in absolute terms, still more than doubled its biomass with sludge, which is significant considering *Eucalyptus* is often nutrient-demanding.

From an ecological and management perspective, these findings highlight a sustainable solution for two problems: waste disposal and soil fertility. Utilizing sewage sludge in forestry recycles waste into a resource, reducing the need for chemical fertilizers and diverting sludge from landfills or unsafe dumping. It also accelerates tree growth, potentially shortening nursery rotation times and improving plantation success. One point of caution is the need to monitor soil conditions over the long term – repeated applications could lead to nutrient imbalances or trace metal accumulation if not managed. However, in our study, a single low dose of sludge yielded pronounced benefits with no detectable downsides in the short term. This is corroborated by other studies, like [9], which found no heavy metal build up in soil after using municipal sludge compost for mango trees. Also, [22] observed that after a decade of applying sludge in a reclamation site, tree growth was improved without harmful soil effects. These suggest that when adhering to regulatory guidelines (such as using stabilized sludge and not exceeding agronomic rates), sludge can be an environmentally sound amendment.

In conclusion, our results strongly support the strategy of using treated municipal sludge for raising tree seedlings. The manifold increase in growth and vitality of seedlings grown on sludge-enriched soil can significantly improve the efficiency of afforestation and reforestation programs. Additionally, such practices contribute to a circular economy by turning “waste into wealth,” utilizing the nutrients and organic matter in sewage sludge to build plant biomass and sequester carbon in woody tissues. Future research could explore the effects of varying sludge application rates, in combination with other organic waste (e.g., co-composted green waste), and the performance of sludge-grown seedlings after transplanting them to field sites. The goal is to fine-tune this approach to maximize tree establishment and growth on marginal lands while safely

recycling urban waste.

5. CONCLUSION

This study demonstrates that sewage sludge is a valuable soil amendment for tree seedling production, leading to remarkable improvements in growth and biochemical composition of *T. arjuna*, *E. tereticornis*, and *M. composita* during a one-year nursery trial. Sludge-treated seedlings (particularly with sludge from the Bhattian plant) exhibited significantly greater height, stem diameter, root development, and biomass compared to those grown in unamended soil. Correspondingly, key physiological indicators like chlorophyll content, carotenoids, soluble sugars, and starch were substantially higher in sludge-amended plants, indicating enhanced nutritional and metabolic status. *Terminalia arjuna* proved most responsive, attaining the highest growth and pigment levels, though all three species benefited. These results suggest that municipal sludge and composted waste can be effectively used to raise quality planting stock for forestry, turning an environmental pollutant into a growth-promoting resource.

In practical terms, integrating sewage sludge into nursery media or plantation soil can reduce the need for chemical fertilizers, lower costs, and help in waste management by safe utilization of sludge. We recommend the use of properly treated (stabilized) sludge at appropriate rates (around 1% by soil weight in this study) to avoid any phytotoxicity or nutrient overload. The improved seedling vigor observed is likely to translate into better survival and faster growth after transplanting, which is advantageous for reforestation, afforestation, and land rehabilitation projects.

Overall, sewage sludge contains high organic matter and plant nutrients that enhance soil fertility and can partially substitute for conventional fertilizers. The positive impact on tree growth and biochemistry, as shown in this study, underscores its potential in forestry and environmental restoration. Adopting such nature-based solutions will contribute to sustainable waste recycling, healthier soils, and robust forest development. Further research and field trials will be valuable to track long-term tree performance and soil health, ensuring that the use of sludge remains environmentally sound and beneficial over the entire life cycle of the plantation.

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