Impacts of different salinity levels on seedling growth and survival of black mangrove (*Avicennia germinans*)

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Abstract. *Matto AA, Jaikishun S, Ram M. 2023. Impacts of different salinity levels on seedling growth and survival of black mangrove (*Avicennia germinans*). Asian J For 7: 67-73.* Mangroves are important for coastal protection and provide numerous ecosystem services such as breeding, feeding, and nursery grounds for commercial fishes. This research examined the survivability of black mangrove (*Avicennia germinans* (L.) L.) seedlings in seven salinity concentrations. Growth parameters were collected every ten days, and a post-harvest analysis was done at the end of the data collection period. The results indicated that as the salinity level increased, the growth of the seedlings was reduced. Salinity can affect the different parameters collected in the growth of young seedlings. In the control, which was given zero salinity (0 ppt), it was noted that the growth parameters were not significantly affected. The lower treatments, such as T2 (15 ppt), 3 (30 ppt), and 4 (45 ppt), indicated that the seedlings strive better in these conditions. In treatment 7, which was the highest salinity level (90 ppt), the growth parameters of the seedlings were reduced. The findings of this research indicated that black mangrove seedlings need a low salinity level to have a good growth establishment. Given the adaptability of *A. germinans*, these mangrove species are anticipated to be flexible to cope with a wide range of future climatic circumstances.

Keywords: *Avicennia germinans*, climate change, mangroves, salinity, seedlings

INTRODUCTION

Mangroves are shrubs or forests that grow along tidal estuaries, classified into true and mangrove associates (Numbere 2018a,b). They are placed in these categories based on their morphological and physiological specializations (Sandilyan and Kathiresan 2012). Mangroves are integral to maintaining our marine ecosystem (Sandilyan and Kathiresan 2012; Carugati et al. 2018). Mangroves are home to a wide variety of flora and fauna. Mangrove forests play a significant role in maintaining the productivity of inshore and offshore fisheries (Ball 2002; Nagelkerken 2009; Kodikara et al. 2017). They provide nurseries for fish, help support extensive aquatic species, aid in supplying nutrients to the ecosystem, and provide shelter (Baustian et al. 2017). In mangrove areas and mudflats, are a high abundance of diatom, blue-green algae, and dinoflagellates. From the decomposition of mangroves, forest litter is organic material that can be used as food sources for organisms in mangrove-dominated estuaries (Noor et al. 2015; Baustian et al. 2017).

In Guyana, mangroves are mostly found along the coast. The dominant species of mangroves is the *Avicennia germinans* (L.) L. (black), which fringes along the coast, *Rhizophora mangle* L. (red) are less abundant, and *Laguncularia racemosa* (L.) C.F.Gaertn. (white) is distributed partially along Guyana's coast (Bovell 2010). The *A. germinans* and *L. racemosa* are found as pioneering species in mudflats and sandbanks on the coast. The *R. mangle* is more abundant in estuaries and sheltered river banks. This zonation pattern of mangroves is common from Corentyne to the Essequibo Coast (Bovell 2010).

Globally, mangrove cover has declined significantly, and the degradation continues at an alarming rate. Sandilyan and Kathiresan (2012) reported a depletion rate of 0.66-1.0% per year, three to five times higher than the average loss rate for land forests. Globally, an estimated 20% (3.6 million hectares) of mangroves were lost between 1980 and 2005 (Maiti and Chowdhury 2013). The impact of carbon emissions and mangrove degradation has been a global issue. Although carbon-rich mangrove forests are deforested and degraded due to land use and land-cover change, other threats include releasing heavy metal toxic substances and industrial waste (Maiti and Chowdhury 2013; Kodikara et al. 2017).

Climate change is also a significant contributor to mangrove depletion, particularly sea-level rise, which is considered a threat to mangrove habitat and functionality (Sandilyan and Kathiresan 2012; Jaikishun et al. 2017; Carugati et al. 2018). In the Caribbean, mangroves have declined by 24% over the last quarter-century. One of the reasons for this is anthropogenic activities and the increase in the effects of climate change. In addition, there has been an increase in salinity on the mudflats, ocean levels, and more intense rainfalls (Wilson 2017).

In Guyana, mangroves are important since they're an income earner for many coastal communities (NAREI 2015). Hollowell (2005) reported that in Guyana, a sea-level rise in the 0.09 to 0.88 m range had been predicted by the year 2100. Sea level rise could result in drastic changes in the extant mangrove ecosystems. In addition, this

prolonged flooding resulting from increased sea-level rise can lead to plant death.

Several ecological factors drive mangrove health; ecological requirements for salinity, appropriate hydrology, topography, and suitable species composition are major causes of mangrove restoration failure (Kodikara et al. 2017). The health, distribution, growth, and productivity of mangroves are affected by salinity. The high spatial and temporal fluctuations, driven by inputs of freshwater and seawater, inundation, groundwater seepage, and evaporation are often observed (Baustian et al. 2017; Kodikara et al. 2017; Carugati et al. 2018). Hence, species-specific studies of mangroves are vital for the replanting process. Salinity affects mangrove species differently (Bovell 2010; Baustian et al. 2017; Kodikara et al. 2017). This study was designed to assess the impacts of different salinity levels on seedling growth and survival of black mangroves (*A. germinans*).

MATERIALS AND METHODS

Study site

This experiment was conducted in Kitty, Georgetown, on Guyana's Atlantic Coast. This area is in region four, Demerara-Mahaica.

Experimental design

The Randomize Block Design (RBD) was ideal for this experiment because it allows the seedlings to be grouped into replicates and does not limit the number of replicates and treatments used (Figure 1). It was done in three replicates; three plants were treated with 0, 15, 30, 45,60, 75, and 90 ppt salt (NaCl) solution, with 0 ppt being the control. The natural mangrove seedlings were obtained from the Grove area, with permission from NAREI.

Data collection

Data was collected for three months, and the following parameters were recorded: the height of the plants was measured and determined using a measuring tape, the stem diameter was measured using a Vernier calliper, the total number of leaves was counted at ten days intervals, the number of surviving mangrove species was counted every ten days, the mortality of the plants was recorded.

Post-harvest analysis

The wet and dry masses of the plant parts were collected. Three replicates were harvested and washed thoroughly, blotted dry, and separated plant components (roots, stem, and leaves). The mass of the wet parts was recorded. The dry part was wrapped in paper and oven-dried at 80°C for 24 hr. The weights were recorded until a constant weight was reached (Kodikara et al. 2017).

Total leaf area

Three random leaves from each treatment were chosen, and the total leaf area was quantified using graph paper (Kodikara et al. 2017).

Figure 1. Experimental plot

Chlorophyll content

Leaf samples were harvested, and 1 g was weighed and immersed in 10 mL of dimethyl sulfoxide (DMSO). It was then placed in 15 mL test tubes and wrapped in aluminium foil. The samples were then incubated for four hours at 65°C. After the expiration of the period, the samples were removed and left to cool. Samples were then individually poured into the test tubes with DSMO being the blank (Wellburn 1994; Tait and Hik 2003). The mixture was then placed in a spectrophotometer to obtain the readings at 670 nm, 645 nm, and 663 nm (Sandilyan and Kathiresan 2012; Kodikara et al. 2017).

Soil analysis

A soil analysis test was conducted on the two soils at John's Lab, University of Guyana. The following nutrients were tested for; nitrogen, phosphorus, potassium, calcium, magnesium, iron, and copper.

Data analyses

Analysis of the data collected was done using Microsoft Excel 2010. The statistical test, one-way fixed-factor ANOVA, was used to find the p-values of parameters (height, stem diameter, leaf thickness, number of leaves, wet and dry mass) and hence the significant differences.

RESULTS AND DISCUSSIONS

Black mangroves are Guyana's most dominant species and can tolerate high salinity levels. However, in the seedling stage, it is important to have a moderate saline condition. This study used two types of soil to cultivate mangroves. The EC and pH of the soil were noted in Table 1. In the natural soil analysis, the EC indicated that it contained a high salinity level, and the pH indicated that the soil was mostly acidic. However, in the loam soil, the EC showed that the soil had moderate salinity, and the pH was close to neutral (Table 1).

Table 1. EC and pH of the natural and loam soils

Parameters	Natural soil	Loam soil
Electrical conductivity (Sm^{-i})	1281	569
pH	5.20	6.60

Mangrove seedlings can be subjected to ion toxicity, osmotic stress, nutritional shortage (N, Ca, K, P, Fe, Cu, and Mg), and oxidative stress resulting from soil salinity, which inhibits water intake from the soil. One element, especially sodium, harms plants in high concentrations (Reef et al. 2010; Shrivastava and Kumar 2015). While NaCl is a necessary plant micronutrient that influences enzyme activity in the cytoplasm, it is a co-factor in photosynthesis and critical for turgor pressure creation and cell expansion (Shrivastava and Kumar 2015). Therefore, high accumulation might result in ion toxicity and plant damage. Furthermore, excessive sodium accumulation in cell walls can quickly cause osmotic stress and cell death. It also makes the soil less permeable, hence, the reason for stunted growth and even death. This also causes an imbalance in leaf tissues, affecting the leaf thickness (Reef et al. 2010; Shrivastava and Kumar 2015).
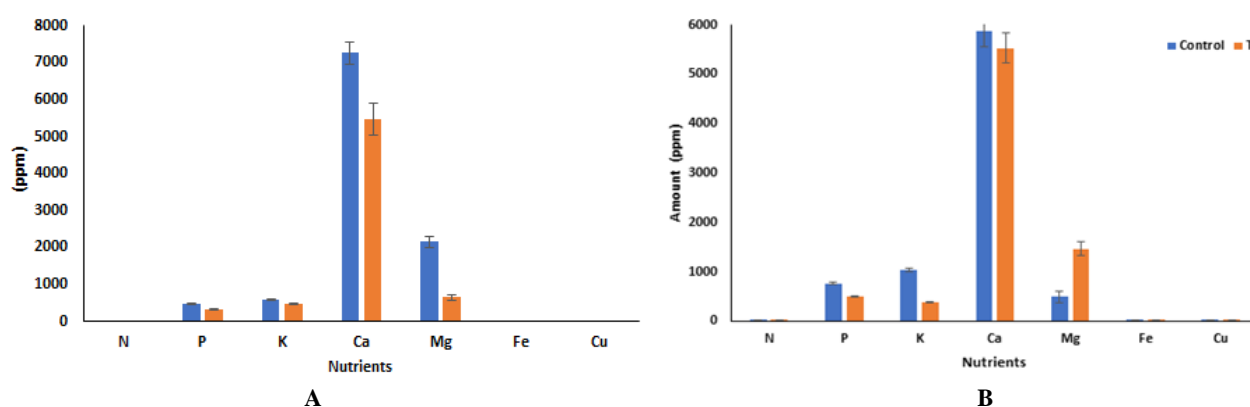
From the soil analysis results collected, it was shown that nitrogen is an essential nutrient for plant growth. As depicted in the graphs, the control and T7 did not contain a significant amount of nutrients because these mangroves were grown in pots and because of the uptake of nutrients and lack of supply through the nutrient cycle and competition (Reef et al. 2010; Shrivastava and Kumar 2015). Despite nitrification levels being normally low, the top layer of soil and a thin layer of aerobic soil around the mangrove roots maintain populations of nitrifying bacteria, which can transform ammonium into nitrate for the plant. High salinity affects photosynthesis and the demand for nitrogen in mangroves (Reef et al. 2010). Mangroves cultivated in pots/bags appear to prefer nitrate to ammonium and exhibit a significant drop in plant nitrogen uptake. The reduction in nitrogen level is also shown to affect stem diameter, dry mass, and plant growth (Reef et al. 2010; Campos et al. 2014). A saline soil usually contains large amounts of Mg and Ca, both compete with the soil particles, in particular, Na, for space, so in the results, it was seen that Ca dominated in the control; however, in the natural soil, Mg was less than loam soil because the natural soil already was a high saline soil and Na would have the largest nutrient in the soil (Figure 2) (Reef et al. 2010; Chen and Ye 2014; Noor et al. 2015; Shrivastava and Kumar 2015).

Phosphorus is another important nutrient for plant growth. Phosphate accessibility is limited in saline soils due to ionic strengthening effects, which diminish phosphate activity. Phosphorous activity cannot be replenished through biological fixation (Reef et al. 2010; Chen and Ye 2014). Because phosphate ions precipitate with Ca^{2+}, soil salinity inhibits plant phosphorus absorption (Reef et al. 2010; Chen and Ye 2014).

Potassium content is a factor that affects plant growth at a high salinity level (Niste et al. 2014). In Figure 2, the control in both soils shows a higher level of K than T7. Potassium is essential in protein synthesis in mangroves. The soil solution's high salinity and Na+ concentration can impact plant K$^+$ absorption. The loss in chlorophyll and photosynthetic function in the mangrove seedlings was shown because of K$^+$ deficiencies (Reef et al. 2010; Shrivastava and Kumar 2015). Several types of research have demonstrated that as the NaCl salinity or the Na$^+$/Ca^{2+} ratio in the root media increases, the K$^+$ concentration in plant tissue decreases (Shrivastava and Kumar 2015).

Magnesium showed a high concentration in the natural soil control and a low level in loam soil. Copper and iron have significantly low levels in both control and T7. Cu stress appears to substantially influence mangrove plants by reducing root permeability (Figure 2). Moreover, black mangrove seedlings were discovered to have larger outer cell layers and greater lignification within the exodermis (Reef et al. 2010; Chen and Ye 2014; Kodikara et al. 2017). This could have indicated that reduced permeability could directly prevent excessive Cu from entering the roots.

Figure 2. Average nutrient content in (A) natural soil and (B) loam soil. Bars represent Mean±SD

In mangroves, the metal binding inside the cell wall, either in ionic form or combined with cell wall structural components like lignin, has long been recognized as an important detoxification method (Chen and Ye 2014). Natural cations (Cu, Fe, Mn), including vital plant nutrients, are displaced by high Na^+ concentrations. The findings revealed that the combined impacts of iron shortage have a substantially greater impact on plant development and chlorophyll concentration due to the increased saline conditions of the soil; iron, a micronutrient, is suppressed as salinity increases. Iron deficiency hurts the chlorophyll concentration of juvenile leaves. Mineral toxicity, nutrient insufficiency, and nutritional imbalance are frequently associated with soil salinity and acidity (Reef et al. 2010; Chen and Ye 2014).

The experiment yielded three mortalities. The treatments that have morality were one in T5 (60 ppt) and two in T7 (90 ppt). From the results obtained, these specific treatments were not very productive for the seedlings to grow properly. Further, T7 showed that the seedlings could not have good growth establishment. This is due to the increased stress on the seedlings caused by the high salinity level; the earlier stage of seedling growth requires low salinity for good growth. Hence, the lower treatment levels have half of no mortality. The necessary nutrients responsible for growth, such as nitrogen, phosphorus, and potassium, were limited in the soil due to increased saline conditions. This could have been a factor responsible for mortality.

The growth parameters percentage was calculated using the average by subtracting the final from the initial data and finding the percentage. The control treatment is significantly different from the salinity treatments. Growth was observed at 43%; in the natural soil, the plants grew better in T3, which showed a 65.8% increase in height; in the loam soil, the control showed a 56.7% growth rate. Growth was observed better in T4, with a 61% increase. This treatment replicates the seawater salinity, which was tested to be 45 ppt.

In comparison to the higher levels of salinity, it was noted that growth rates were lower as the salinity levels increased. When compared to the control T1, there was a 43% growth in natural soil and 56.7% in loam soil (Figure 3). In the control, the seedlings have depicted a slow growth rate as compared to T3 in natural and T4 in loam soils. When comparing the control to the treated seedlings, there was slower growth, but the higher treatments proved to have had the slowest growth rates (Liang et al. 2008; Nagelkerken 2009; Kodikara et al. 2017). The one-way ANOVA showed significant differences in the growth rate of plants in the natural and mixed soils, respectively (p=0.000353 and p=0.000293). Production is also influenced by salinity, and at the stage of development, an increase in salinity reduces height and growth, particularly in *Avicennia* spp. (Whigham et al. 2009; Nguyen et al. 2015; Budiadi et al. 2022).

Despite the ability of black mangroves to tolerate high levels of salinity, seedlings need moderate salinity to survive and have a good root establishment. They are age specific as they grow older and tend to tolerate a higher salinity. An extremely high salinity condition can increase the stress level in mangrove seedlings (Kodikara et al. 2017). An increase in temperature can result in reduced precipitation and increased urban and agricultural demand for groundwater is projected to raise estuary salinity, increasing the risk of succumbing to salt stress. According to some models, temperature changes, salinity, and sea level will cause mangroves to spread into higher latitudes (Madrid et al. 2014).

In the stem diameter, it was seen that the control in natural soil was not significantly affected even though there was a drop of 93% (Figure 4A). In the loam soil, the control was not affected at all at 93%; this can be due to the soil being less saline than the natural soil (Figure 4B). T2 in nature gave the highest percentage of 95%, and T1, T4, and T5 had the highest and the same 93% recorded. T7 in both soils showed a decline in stem diameter (p~ 0.0232 and 0.0379), showing a difference in the stem diameter; however, it was not a large significant difference.

Increases in water salinity influence critical nutrient uptake and salt accumulation, resulting in osmolality increases in tissue sap. In NaCl media, many researchers found that when salinity increases, the formation of the xylem, the width of vascular bundles, and the diameters of mangrove stems can be reduced. Although in the soil, nitrogen plays an important role in stem diameter in the soil due to the minimal amounts indicated in the soil analysis; this could have hurt this parameter.

It was noted that leaf emergence in the control (T1) was not affected because there was 68.9% leaf emergence but showed a decline in T6 for the natural soil and T7 for the loam soil (Figure 5A). In the loam soil, the control showed a high increase and a steady fluctuation (Figure 5B). There was no significant difference in this parameter in the natural and loam soil, respectively (p= 0.0672 and 0.0732). The percentage of leaf emergence of seedlings on natural soil tends to be lower than that of seedlings on loam soil. Seedlings in the salinity treatment could grow new leaves but showed a lower percentage than the control. The rate of leaf creation and death and the length of leaf life have significant physiological implications for the entire plant. It enables the plant to maintain a good growth rate (Suárez and Medina 2005).

The combination of a considerable fall in leaf production, leaf expansion rates, and a reduction in leaf life span resulted in a lower plant leaf under hypersaline conditions. The capacity to sequester ions in leaves is projected to decline with time due to continuous exposure to excessive salinity (Suárez and Medina 2005; Nguyen et al. 2015). Although there were differences noted, it was not to a large extent. This could be because there was grasshopper infestation and caterpillar, which could have affected the results as the leaves of the plants were eaten away.
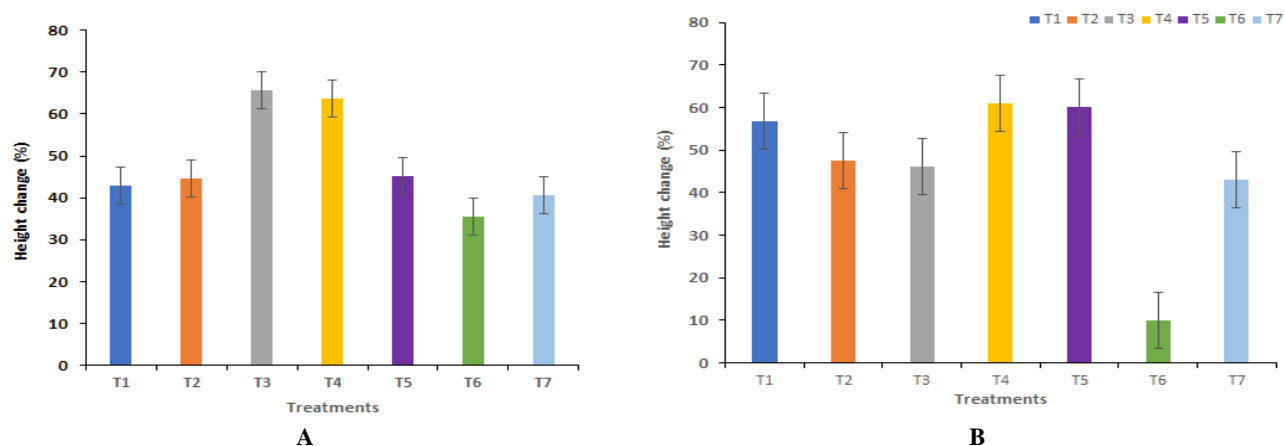
Figure 3. Percentage of seedling height increase of seedlings in (A) natural soil and (B) loam soil. Bars represent Mean±SD
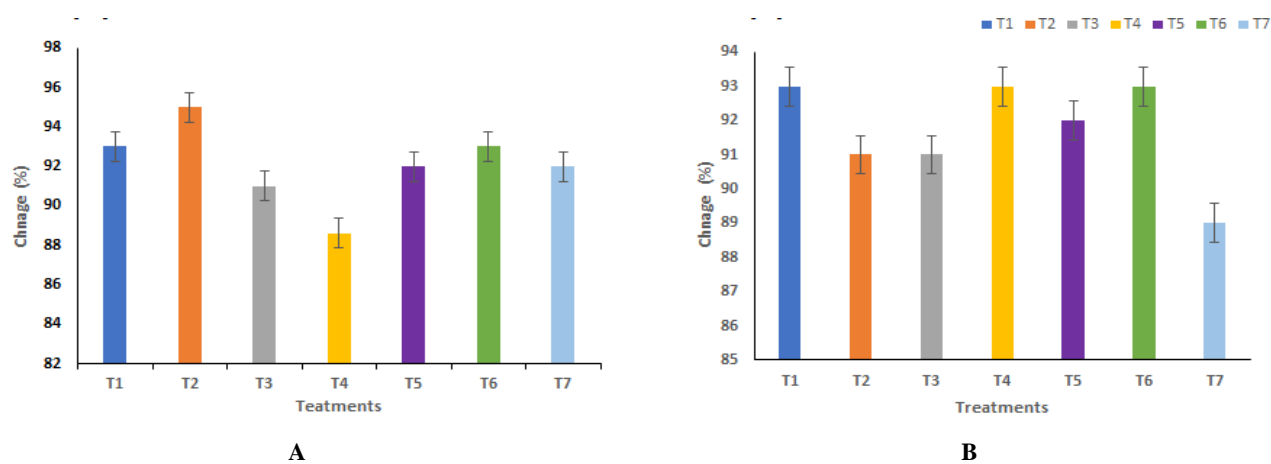

Figure 4. Percentage stem diameter percentage in (A) natural soil and (B) loan soil. Bars represent Mean±SD
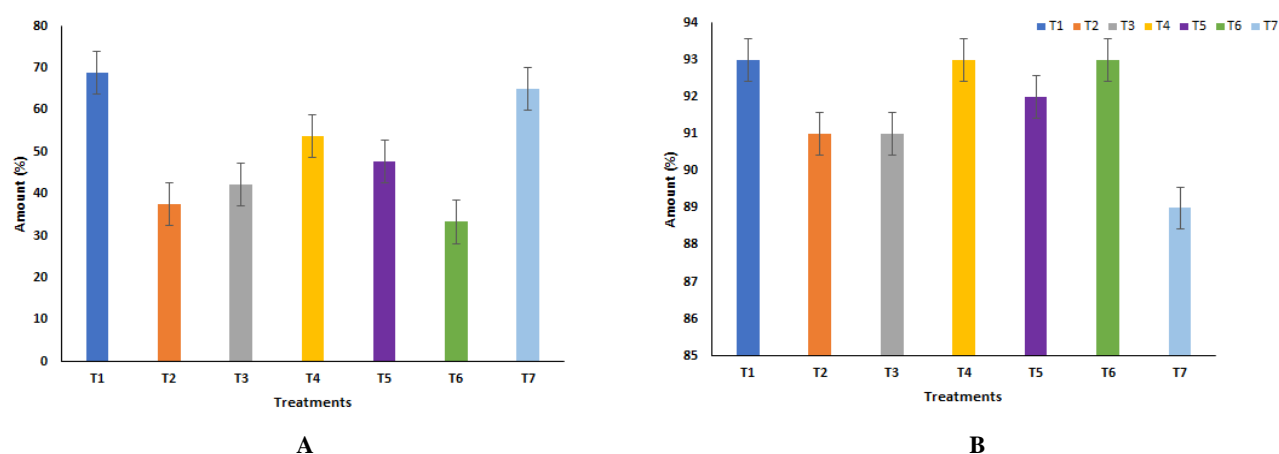

Figure 5. The percentage of leaf emergence in (A) natural and (B) loam soil was obtained from different treatments. Bars represent Mean±SD

The percentage leaf thickness ranges showed T2 and T3 in the natural, having the highest rise at 82% in leaf thickness, and the loam soil was noted to have 84% in T4 in leaf thickness. The control T1 in natural was shown to have 79.4% and the loam 78%, respectively. The T7 was noted to have the lowest leaf thickness percentage at 71%. The natural and loam soil, respectively ($p= 0.0634$ and 0.0868), indicated no significant difference in leaf

thickness in this parameter. However, the higher salinity range showed a decline in leaf thickness. This could be because, at higher salinity ranges, the leaves were unable to photosynthesize as compared to lower levels; they were also unable to absorb large amounts of water as salinity levels increased. As salinity increases, the ability of the seedlings to absorb water is reduced due to an increase in the Na ions, which affect the other nutrients present in the soil, such as potassium and phosphorus (Kodikara et al. 2017). Various factors could have also affected the leaf thickness, such as inclement weather conditions and prolonged rainfall.

Post-harvest

Leaf surface area (*p*= 0.054421 and *p*=0.005111) showed a significant difference in the leaf surface area. As salinity levels increase, leaf demography is affected, especially in the size of the leaf. Higher saline conditions indicated a smaller leaf surface area (Table 2). Leaves are very useful in determining the stress level in mangrove plants in their survival in saline conditions (Sobrado 2000; Suárez and Medina 2005; Kodikara et al. 2017). Salt levels cause a reduction in both the length and width of the leaf in black mangrove species in high salinity, altering the leaf's overall shape. Further reductions in leaf life span and the incapacity to sustain fresh leaf production result in a significant drop in leaf area per plant at very high salinity. Reduced leaf area can be caused by salinity and nitrogen levels (Sobrado 2000; Suárez and Medina 2005; Budiadi et al. 2022).

The natural soil's wet and dry mass (*p*=0.021808, *p*=0.005137) differed significantly. The wet and dry masses for the loam soil, respectively, were significant (*p*=0.047641 and *p*=0.034195). The values indicated were not large but indicated a relationship between salinity and wet and dry masses of mangroves—the percentage water content for natural and mixed soil, respectively (*p*= 0.011446 and *p*=0.021808). Salinity can be seen as a factor in water absorption. The average percent water content of plants cultivated in lower-saline settings was considerably higher than that of plants produced in high-saline conditions, suggesting that mangroves store more water in low-saline environments. Low osmotic potential under hypersaline could be the reason for increased moisture absorption (Basak et al. 2004).

A factor that would account for the plants' modest growth rates and shrubby size at hypersaline sites is that a linear association was found between diurnal carbon gain and maximum rate of CO_2 uptake in a variety of rainforest canopy species. Light saturated photosynthesis rate shows the maximum achievable benefits from a given investment in photosynthetic gear.

Managing a saline environment, on the other hand, presents unique problems in terms of maintaining favourable water and ion balances (Whigham et al. 2009). Salinity can influence the ability of roots to absorb moisture while preventing most ions from entering the transpiration stream in mangrove ecosystems, regardless of the quantity of water. As a result, the average percentage water content of black mangroves was lower as the salinity

level increased. Furthermore, the enhanced salinity stress resulted in a considerable drop in the fresh and dry weights; hence, the overall ability to absorb water and reduce grew in lockstep with the increase in salinity augmentation (Whigham et al. 2009; Kodikara et al. 2017).

Chlorophyll is the primary colour pigment involved in photosynthesis. Furthermore, using the absorption rate and the results obtained, it was shown that in the control (T0), for natural and loam, it was showed the chlorophyll level was not severely affected. There was a rise from T2, T3 was constant, a rise in the natural soil, and a drop in the loam soil. As the salinity level continues to rise to T5, T6, and T7, the total chlorophyll decreases (Figure 6). The chlorophyll concentration is a reliable measure of the photosynthetic function under harsh conditions. Because of salt toxicity, which causes the burning of leaves or other succulent portions and degradation of other pigments, chlorophyll content reduction owing to salinity stress is quite prevalent in salt-sensitive plant species (Whigham et al. 2009; Kodikara et al. 2017). This is because they are saline-tolerant species that can withstand such worsening salinity stress. However, it has been discovered that when trees are subjected to increased salt stress, their chlorophyll levels dropped due to enzymatic chlorophyll breakdown (Basak et al. 2004).

Table 2. Leaf surface area in natural and mixed soil obtained from different treatments

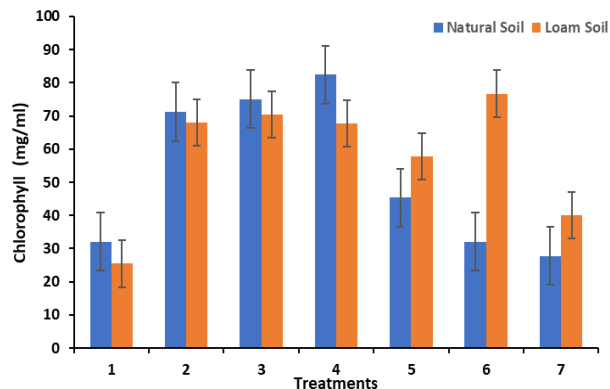| Treatments	Total Leaf Area (cm²)		Water content (%)	
	Natural soil	Loam soil	Natural soil	Loam soil
1 (control)	5.3±4.55	16.8±6.32	70.6±10.4	83.5±12.5
2	8.5±5.7	7.6±4.7	81.8±7.1	88.7±1.28
3	6.6±4.56	9.1±5.88	78.6±4.2	83.5±9.11
4	7.1±2.02	18.6±7.92	79.6±6.59	79.7±13.11
5	5.1±3.38	22.8±8.33	82.3±9.84	81.3±8.45
6	5.6±3.21	10±6.23	54.6±5.7	75.4±9.05
7	4.5±1.76	5±4.66	74.6±6.3	80.3±5.60

Figure 6. Total chlorophyll in natural and loam soil was obtained from the seven treatments

Salinity therapy increased salt production at moderate salinity. This allowed plants to modestly lower water absorption while maintaining a high level of carbon assimilation. However, salt secretion tended to be reduced under hypersaline conditions, which could be the result of a saturated mechanism (Basak et al. 2004). Salt secretion is a very active system that comprises several metabolically regulated phases. As a result, declines in the solutes transported to leaves were more relevant in hypersaline circumstances (Ball 2002; Basak et al. 2004; Budiadi et al. 2022).

In conclusion, the growth parameters indicated that as the salinity level increased, the mangrove growth was reduced. The lower treatments, such as T2, T3, and T4, proved to be ideal saline conditions for the growth of black mangrove seedlings. As the seedlings grow older, they can be exposed to higher salinity ranges. Seedlings must get the right conditions to have a good growth establishment. The post-harvest analysis indicated that high salinity ranges affect the wet and dry masses and water content. The chlorophyll content was reduced, indicating that the rate of photosynthesis declined. This study indicated that mangrove seedlings are at a greater risk of surviving as salinity increases.

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