Khulna University
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Author(s): Basubi Binti Zhilik

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Supervisor(s): Dr. Mahmood Hossain, Professor, Forestry and Wood Technology Discipline, Khulna University

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Salinity Drives the Growth, Chlorophyll Content and secondary Metabolites (Proline) Concentration Of Baen (Avicennia officinalis) Seedlings

Basubi Binti Zhilik
Student Id- MS- 140501

Forestry and Wood Technology Discipline
School of Life Science
Khulna University
Khulna

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By

Basubi Binti Zhilik

M.Sc. in Forestry

Khulna University, Khulna

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Supervisor

Dr. Mahmood Hossain
Professor
Forestry and Wood Technology Discipline
Khulna university
Khulna-9208

Prepared by

Basubi Binti Zhilik
31.01.2017

Basubi Binti Zhilik
Student Id- MS- 140501
Forestry and Wood Technology Discipline
Khulna university
Khulna

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Dedicated to my beloved Family

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Subject

Abstract

Avicennia officinalis is a salt tolerant mangrove tree species, which is highly adapted to the dynamic harsh environment of mangrove ecosystem. Salinity affects physiological process that determines survival, distribution, growth, vegetation structure as well as primary and secondary metabolite concentration of mangroves. This study was conducted to examine the implications of salinity on growth, chlorophyll and secondary metabolite concentration of seedlings of *A. officinalis*. Seedlings raised under fresh water condition were subjected to saline treatments ranging from 0ppt to 35 ppt at 5 ppt interval in a Completely Randomized Design. Survival percentage and growth in terms of height, collar diameter, root, stem, leaf and total biomass increment varied significantly with increasing salinity. Total oven dry biomass production decreased from 23.81 gm to 3.28 gm among 0 to 35 ppt salinity. Chlorophyll content decreased significantly from 0.013mg/cm^2 – 0.007mg/cm2 with increasing salinity. However, proline concentration increased significantly from 0.911µmole/gm - 4.40µmole/gm with increasing salinity. Therefore, salinity affects growth and metabolic activities of *A. officinalis* during seedling growth.

Key words: Salinity, *Avicennia officinalis,* Growth, Chlorophyll, Proline.

CHAPTER – ONE

INTRODUCTION

1. Introduction

1.1 Background and Rationale

Mangroves are the woody shrubs and tree species; comprises more than a hundred species from different families (Hasegawa et al. 2014) dominating the sea line and distributed frequently mostly in brackish water (Mitra et al. 2010; Hasegawa et al. 2014), along the strips parallel to shore (Ball 2002). The total area, covered by the mangrove forest is 110.000 to 240.000 km^2 or 15 million hectares (Bompy et al. 2014) including 118 countries that occupy almost 0.7% of the total tropical forest in the world (Noor et al. 2015). Different biological processes such as dispersal, herbivory, competition etc. vary along the tidal gradients as well as contribute to the variation in zonation pattern of mangroves forests (Ball 2002).

Although mangrove ecosystems have tremendous value for coastal communities and associated species, they are being destroyed at alarming rates. Over the last 50 years, about one-third of the world's mangrove forests have been lost. Human threats to mangroves include the overexploitation of forest resources by local communities, conversion into large scale development such as agriculture, forestry, salt extraction, urban development and infrastructure, and diversion of freshwater for irrigation (UNEP 1994). In addition to the anthropogenic threats, mangroves are also threatened by the impact of global climate change. Global climate change and concomitant effects such as changes in temperature and CO2, altered precipitation patterns, storminess, and sea-level rise as observed over recent decades, are due primarily to anthropogenic activities. Most of the observed warming over the last 50 years is attributed to an increase in greenhouse gas concentrations in the atmosphere. (Ahmed2013).

Tolerance to the high saline environment is linked to the regulation (Parida et al. 2010) of mangroves habitat, according to which true mangroves and their associates are classified. In addition, Mangrove forest is known to be occurred in a range of salinity gradient naturally and may range from zero ppt (parts per thousand) in riverine to 70 ppt-160 ppt in hyper saline areas (Biber. 2006; Reef et al. 2015). Salinity is often considered as stress at its toxic level, which might have negative impacts on seedling establishment as well as on photosynthesis and eventually affects, the growth under field condition (Ball 2002). In addition, mangroves are frequently inundated by tide leading to water logging and fluctuation in salinity. Even though

mangroves deal with waterlogged condition, high salinity creates problems for mangroves to extract water from the soil (Clough et al. 1982). Increased concentration of Na+ and Cl- in mangrove adversely affect root and shoot growth of many mangrove species (Barrett-Lennard2003). Several factors such as, low tide, overheating and evapotranspiration, contributes to the increased salinity in mangrove forest (Parida et al. 2010). In addition, the high saline condition reduces the photosynthetic CO_2 assimilation rate (Brugnoli et al. 1991). But interestingly, low to moderatesaline condition enhanced the growing pattern of true mangrove species. (Ball 2002 and Biber 2006). Bowman and Davis (2003) concluded that mangroves are rather salt tolerant, not salt lovers. Salt incorporation and transportation to the leaves in the transpiration stream, helps to maintain the osmotic adjustment of the growing tissues in mangrove plants. It has been reported that, the plants exclude almost 80-95% of the salt during water uptake, which increases the survivability of the mangrove species under high saline condition through contributing in regulating the internal salt concentration (Suárez et al. 2008). Salinity affects the growth of the plants by influencing the chlorophyll concentration of leaves. Chlorophyll a and Chlorophyll b are the most important pigments for photosynthesis process in plants. Chlorophyll can directly limit photosynthesis potential and hence primary production and it show a negative relationship to salinity (Ali et al. 2004). Moreover, it has been seen that mangrove species accumulate different metabolites to cope with the increased salinity - as the accumulation of different secondary metabolites- proline. Proline is considered to be a compatible solute. It protects folded protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids, functions as a radical scavenger, or serve as an energy and nitrogen source. The biosynthesis of the secondary metabolites, is often induced when plants are exposed to environmental stresses, as salinity(Clussen, W 2004). Concentration of proline found to be increased with increased salinity (Jaarsma et al. 2013) andit may be one of the adapted mechanisms against salinity by the mangrove species.

Avecienna officinalis is an evergreen fast growing shrub or tree, often found in the intermediate estuarine zone in the lower intertidal region. *Avicennia officinalis* L. is an exclusive mangrove (Mahmood, 2015) and one of the pioneer tree species in the Sundarbans (Naskar and Bakshi, 1987; Siddiqi, 2001). This species naturally occurs in the Sundarbans from less saline to strong saline zones (ODA, 1985;Siddiqi, 2001; Mahmood, 2015). It is mostly planted on strong saline substrate in the coastal regions of Bangladesh (Das and Siddiqi, 1985;Siddiqi and Khan, 1990)

and becoming the second principal species (20%) in the coastal afforestation programme (Das and Siddiqi, 1985; Papry, 2014). It is shade intolerant with a maximum pore water salinity of 63 ppt (Robertson and Alongi, 1992). This species grows on soft, recently consolidated mud banksad may attains 25 m in height, but is more often seen at 5-10 m. It is a colonizing species on newly formed mudflats in SE Asia. (Terrados et al. 1997) and has the ability to grow under fluctuating salinities (0.7-50.0 dsm^{-1}) at intertidal zone (Tomlinson 1986; Tan et al. 2013). The tree is harvested from the wild for a wide range of uses including medicinal purposes, a source of tannins and timber and a dye plant. The bitter fruits and seeds are sometimes used for food after an elaborate processing and eaten after baking or steaming.

1.2 Research problem

Avicennia officinalis is widespread and common within its range. It is threatened by the loss of mangrove habitat throughout its range, primarily due to extraction and coastal development, and there has been an estimated 24% decline in mangrove area within this species range since 1980. Mangrove species are more at risk from coastal development and extraction at the extremes of their distribution, and are likely to be contracting in these areas more than in other areas. It is also likely that changes in climate due to global warming will further affect these parts of the range.

A. officinalis is a very important species for coastal afforestation (Das and Siddiqi, 1985; Papry, 2014). Through establishment and development, the species creates suitable condition for the species of next seral stages like *Heritiera fomes, Excoecaria agallocha* (Naskar and Bakshi, 1987). Considering its ecological significance in the sundarbans, Bangladesh Forest Department started coastal afforestation programme with *S. apetala and A. officinalis* to provide protection to the coastal areas (Fig. 2) against cyclone damage and tidal surges (Siddiqi and Khan, 1997). Approximately, two lac hectare of newly accreted coastal land has been brought under plantations with mangrove species.*Sonneratia apetala* in the coastal plantations has been severely affected by stem borer *Zeozera conferata.* To solve this problem, *S. apetala* has been planted in mixture with *A. officinalis* (Zabala, 1990). So, *A. officinalis* can be an important species for mangrove plantations in Bangladesh. The species plays an important role in coastline mangrove ecosystem by stabilizing the shores and by preventing excessive shifting of coastline and soil erosion resulting from tidal current (Das et al., 2014).

Hence, it is very important to observe the effect on salinity on survival, growth, chlorophyll and proline concentration of *Avicenniaofficinalis*. Unfortunately, there is a huge lack of such kind of research of this tremendous valuable species. Therefore, there is emergence of such kind of research not only to gather knowledge but also to reduce further declination of such important species.

1.3 Objectives of the Study

The objectives of the study were-

- To study the response of growth of *Avicennia officinalis,* in relation to different salinity levels.
- To study the relationship among salinity level with chlorophyll and proline concentration in the leaves of*Avicenniaofficinalis*seedlings.

CHAPTER – TWO

LITERATURE REVIEW

2. Literature Review

2.1. Mangrove response in salinity:

Mangroves are trees that inhabit in the intertidal zones with high salinity, while salt tolerance competence of different species varies. Even congeneric species usually occupy distinct positions of intertidal zones due to differential ability of salt tolerance (Tan et al. 2016). Some species have different ecotypes that adapt well to littoral and terrestrial environments, respectively. These characteristics of mangroves make them ideal ecological models to study the response to salinity. These responses may be associated with some adverse impacts such as the growth depletion, seedling suppression, top dying etc. (Shan et al. 2008).

2.1.1. Influence of Salinity on Mangrove Growth

Mangroves are characterized ecologically according to their habitat and their ability to cope with the high saline condition or low level of soil aeration (Basyuni et al. 2011). It was observed that under high irradiance (Lopez-Hoffman et al. 2006), salinity highly influence the shoot growth of many mangrove species rather that under normal condition that ultimately affects the resource utilization capacity of the species (Ball 2002). Halophytes show increase in relative growth rates up to 50% seawater (Parida et al. 2010).

The growth indicator; the photosynthetic pigments such as Chlorophyll content was found to be increased drastically with the increase of salinity in *Kandelia candel*, up to 50% (Noor et al. 2015). The most appropriate salinity level for the growth of this species; by means of Leaf area, plant height and dry weight, ranges from 85– 250 mM. Merely, inhibition was observed above 430 mM (Zhua et al. 2011).

Comparing the total chlorophyll content in *Kandelia candel* with *Bruguiera parviflora* found to be increased almost 50% with the increase of salt concentration up to 100 mM but decreased at 400 mM salt solution (Parida et al. 2002, 2004). Maximum biomass of *Kandelia candel* was observed under 10 ppt (Basak et al. 2004) where growth in terms of height, leaf area, fresh and dry weight of *Bruguiera parviflora* was observed at zero salinity level (Parida et al. 2004). There is very limited information about salinity influence on growth of *Bruguiera sexangula* growth in terms of callus initiation was determined where callus in leaves was found to be decreased after

300 mM of NaCl concentration and in acse of seedling callus, found to be better up to 100 mM of NaCl concentration (Mimura et al. 1997). Under the different salinity levels of 0, 10, 15 and 20 ppt. *Bruguiera gymnorrhiza,* and *Ceriops tagal* showed the maximum biomass increment under 10 ppt. and 15 ppt. respectively (Basak et al. 2004).

The growth of *Rhizophora mucronata* in terms of dry weight, plant height, leaf area and stem diameter were significantly increased was found to be maximize at 50% seawater and decreased with increasing salinity i.e. 75% and 100% seawater (Aziz et al. 2001). Furthermore, primary root length, shoot elongation, number of leaves per seedling, total leaf area per seedling of *Rhizophora apiculata* was observed within the varying level of salinity viz., 0 (tap water alone), 15 and 30 g l^{-1} but no significant influence was resulted (Kathiresan et al. 2002).

An experiment was also carried out with the red mangrove species *Rhizophora mangle*, under five different salinity levels (0, 15, 30, 45, and 60 ppt) photosynthetic gas exchange or the stomatal conductance and light reaction was found to decrease with the increase of salinity. Furthermore, Fluorescence yield (Fv/Fm) was decreased up to 40 ppt. that increased afterwards (Biber. 2006). In a combined study of salinity and light, it was observed that seedling of *Rhizophora mangle* showed increase in mass under increased light but under high salinity, the result was completely reversed. Seedling growth, photosynthesis rate, stomatal conductance, transpiration rates were reduced with higher salinity (Lopez-Hoffman et al. 2006). Optimal growth of *Rhizophora apicultata* was resulted in 15 ppt and after 15 -20 ppt. *Rhizophora stylosa* also exhibit poor growth comparing to *Rhizophora mangle* (Biber 2006).

Comparing to the red mangrove species (*Rhizophora mangle*) Avicenniacaeae proved to be more saline stress tolerant species. *Avicennia marina* showed optimal growth under moderate saline condition normally up to 20 ppt salinity, indicating the inhibition to high saline condition due to the high salt concentration in plant tissues (Ghowail 1993, Biber 2006 and Yan et al. 2007) though 100% seeding germination was observed up to 35 ppt (Ye. 2005). The maximum growth rate value was observed for *Avicennia marina* from 0 ppt to 15 ppt, indicating the peak at □ 9 ppt (Burcliett et al. 1984 and Ye. 2005) or in 0.1 M of NaCl (Naidoo 1987). Nevertheless, different studies also stated that the influence on high salinity in photosynthesis is negligible for *Avicennia marina* (Parida et al. 2002, Reef et al. 2015) rather in many studies, the growth this species showed positive correlation with high salinity (Clough 1984, Ball 2002 and Hastuti et al.

2012). Furthermore, seedling growth of *Avicennia germinans* and *Rhizophora mangle* is studied to be optimum at 171 mM salinity and reduced almost 47% and 44% in 680 and 940 mol m^{-3} NaCl solution respectively (Suarez et al. 2005; Bompy et al. 2014).

An increase of salinity from 0 ppt to 35 ppt adversely affected the relative growth rate (RGR) of *Aegiceras corniculatum* and *Aegiceras ilicifolius*, resulted in decreased RGR of 56% and & 70% respectively (Ye. 2005).

Experiment also carried out for three mangrove species under wide range of salinity from 0 mM to 1370 mM, and compared with same species under controlled condition. For all three species, the total biomass was at least 20 and 50 % lower than in the control group at salinity of 685 and 1025 mM respectively. Among the three species *R. mangle* least affected by increasing salinity and in contrast, *Lumnitzera racemosa* was the most adversely affected. Where, *A. germinans* shown 75 % lower length growth rate (LGR), height, dry biomass and relative growth rate, comparing to the controlled condition (Bompy et al. 2014 and Dangremond et al. 2015).

Three species of *Sonneratia* were undertaken into experiment, where allelopathic activities were measured in contrast of salinity. *Sonneratia alba* shows higher salinity tolerance than two other species under the influence of five sea salts (NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$). The growth of 80% of control was observed at 200 mM of NaCl and 50 % or more increase of growth of suspension cells by addition of 10–100 mM of each salt was concluded for *Sonneratia alba* with no the increase in Packed cell volume (PCV). In contrast, *S. Caseolaris* showed 13% increase in PCV up to 10 mM that drastically reduced with the further increment of salt concentration. Inhibition was prominent, at high concentration of NaCl, low concentration of CaCl2, more than 50 mM of KCl, where others were in between these salts. No clear difference in salinity of the growing area has been reported between *S. ovate* and other *Sonneratias* pecies (Hasegawa, et al. 2014).

Low saline tolerant species such as *Heretiera fomes* is acutely affected by increased salinity. Experiments conducted in hydroponic culture, resulted that the growth in terms of collar diameter, height, and oven dried biomass increment significantly dropped with increased (above 20 ppt.) salinity (Hossain et al. 2014) and the chlorophyll content reduced almost 10% within 0 psu to 20 psu. This result might significantly affect the photosynthesis rate and eventually the

growth (Mitra et al. 2010). On the other hand, *Xylocarpus granatum*, high saline tolerant species grow better in 23 ppt salinity than the fresh water or zero saline condition (Allen et al. 2003).

Almost 75% of the surface area of earth is dominated by NaCl salt solution (Flowers et al. 2015), which might increase in near future. Many mangrove species of fresh saline zone, like *Heretiera fomes* cannot survive in hyper saline condition. Therefore, studies associated with high saline effect would create new boundaries to understand the overall impacts and be a good source of the indicator of climate changes. However, there is lack of experiments for many mangrove species and their associations that how their growth in relation with increased salinity can be an indicator of climatic changes. There is also limited information on salinity increase in relation with CO2 accumulation and their combined effect on mangrove growth.

2.1.2 Leaf Adaptations to Saline Conditions

Many mangrove species, such as the Grey Mangrove and the River Mangrove (common species along the Redlands coast), have leaves with glands that excrete salt. Some species such as the Grey Mangrove can also tolerate the storage of large amounts of salt in their leaves – which are discarded when the salt load is too high. Mangroves can also restrict the opening of their stomata (these are small pores through which carbon dioxide and water vapour are exchanged during photosynthesis). This allows the mangrove to conserve its fresh water, ability vital to its survival in a saline environment. Mangroves are able to turn their leaves to reduce the surface area of the leaf exposed to the hot sun. This enables them to reduce water loss through evaporation. (Redland city council, 2010)

2.1.3 Root Adaptations to Soft, Saline, Low Oxygen Soils

A distinctive feature of mangroves is their far-reaching, exposed roots. While these roots come in many different shapes and sizes, they all perform an important function – structural support in the soft soils. Some species of mangroves have pneumataphores, which are above-ground roots. These are filled with spongy tissue and peppered with small holes that offer structural support and allow oxygen to be transferred to the roots trapped below ground in the anaerobic (low oxygen) soils. The roots of many mangrove species are also adapted to stop the intake of a lot of the salt from the water before it reaches the plant. (Redland city council, 2010).

2.2 *Avicennia officinalis*

Avicennia officinalis is found in the intermediate estuarine zone in the lower intertidal region. It is shade intolerant with a maximum porewater salinity of 63 ppt (Robertson and Alongi, 1992). This species grows on soft, recently consolidated mudbanks. This species is a tree or shrub that grows to 25 m, but is more often seen at 5-10 m. This species is a fast-growing species. It is a colonizing species on newly formed mudflats in SE Asia. (Terrados et al. 1997) and has a high tolerance of hypersaline conditions (Tomlinson 1986). This species is widespread and common within its range. It is threatened by the loss of mangrove habitat throughout its range, primarily due to extraction and coastal development, and there has been an estimated 24% decline in mangrove area within this species range since 1980. Mangrove species are more at risk from coastal development and extraction at the extremes of their distribution, and are likely to be contracting in these areas more than in other areas. It is also likely that changes in climate due to global warming will further affect these parts of the range. Although there are overall range declines in many areas, they are not enough to reach any of the threatened category thresholds. This species is listed as Least Concern.

2.2.1 Distribution

Avicennia officinalis is generally found in the range Coasts of southern Asia to Australia and Oceania from East Pakistan, Tanasserim, Andaman Islands and Sri Lanka through coasts of Vietnam, Thailand and Peninsular Malaysia to the Philippines, Sumatra, Madura, Java, Borneo, Celebes, Sunda Islands, Molucca Islands and New Guinea; Australia to New South Wales; near sea level to 50 m in Papua but not widely introduced elsewhere (Little 1983).

2.2.2 Ecology

Estimated to range from Tropical Moist to Wet through Subtropical Moist to Wet Forest Life Zones, Indian mangrove is estimated to tolerate annual precipitation of 10 to 45 dm, annual temperature of 20 to 26°C, and pH of 6 to 8.5. It is found mostly on brackish or saline silts of depositing shores and marshes.

2.2.3 Uses

The wood, used to construct boats, houses, and wharves has been studied as a pulp source, and the bark and roots are used for tanning. The bark is used for dying cloth, the ash for washing it (Watt and Breyer-Brandwijk 1962). Javanese and others may consume the bitter fruits and seeds after rather elaborate processing. Branches are lopped and given to cattle for fodder. The wood has been recommended for creosoted paving blocks. Its wood is attractive enough of grain to be useful in cabinetry.

2.2.4 *Avicennia officinalis* and Salinity

The specialized salt glands on the epidermis of halophytic plants secrete excess salts from tissues by a mechanism that is poorly understood. We examined the salt glands as putative salt and water bi-regulatory units that can respond swiftly to altering environmental cues. The tropical mangrove tree species (*Avicennia officinalis*) is able to grow under fluctuating salinities (0.7–50.0□dS□m^{-1}) at intertidal zones, and its salt glands offer an excellent platform to investigate their dynamic responses under rapidly changing salinities (tan et al 2013). Utilizing a novel epidermal peel system, secretion profiles of hundreds of individual salt glands examined revealed that these glands could secrete when exposed to varying salinities. Notably, rhythmic fluctuations observed in secretion rates were reversibly inhibited by water channel blocker, and two aquaporin genes preferentially expressed in the salt gland cells were rapidly induced in response to increasing salt concentration. It is assumed that aquaporins are involved and contribute to the re-absorption of water during salt removal in *Avicennia officinalis* salt glands. There are several adaptive mechanism root to leaf adaptation to high saline condition those can be considered as adaptive feature that contributes to salt balance of trees growing in saline environments where freshwater availability is limited.

2.3. Chlorophyll

Chlorophyll is the green molecule in plant cells that carries out the bulk of energy fixation in the process of photosynthesis. Besides its importance in photosynthesis, chlorophyll is probably the most-often used estimator of algal biomass in lakes and streams, at least in North America. Its popularity results from several considerations;

- It is a measure of algal biomass that is relatively unaffected by non-algal substances,
- It is a fairly accurate measure of algal weight and volume, and,
- It acts as an empirical link between nutrient concentration and a number of important biological phenomena in lakes and reservoirs.

Chlorophyll itself is actually not a single molecule but a family of related molecules, designated chlorophyll a, b, c, and d. Chlorophyll a is the molecule found in all plant cells and therefore its concentration is what is reported during chlorophyll analysis. Chlorophyll d is found only in marine red algae, but chlorophylls b and c are common in fresh water. The molecular structure of chlorophylls a and b consists of a ring-like structure called a porphyrin and a long organic phytol "tail." In the center of the porphyrin ring is a magnesium molecule. Chlorophyll c lacks the phytol chain. The relative concentration within the cell of these chlorophylls varies with the species of algae, but chlorophyll a is dominant in all the eukaryotic algae and the prokaryotic blue-green algae (Cyanobacteria).(Carlson, R.E. and Simpson, J.. 1996)

2.3.1. Chlorophyll Concentration Under Physiological Stress- Salinity

Chlorophyll is the main color agent responsible for photosynthesis. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function. It has been found that the chlorophyll level of trees decreases with aggravated salt stress due to enzymatic chlorophyll degradation.(Ali et al. 2004.).

2.4 Proline

Proline is a unique amino acid, strictly speaking, it is an imino acid where the side chain is connected to the protein backbone twice, forming a five-membered nitrogen-containing ring. The side chain of proline is very non-reactive that is why it is different from the other amino acids. This difference indicates that Proline is unable to occupy many of the main chain conformations easily adopted by all other amino acids, means that it is very rarely involved in protein active or binding sites (Betts et al, 2003; Barness et al. 2003)

2.4.1 Proline Accumulation under Physiological Stress- Salinity

One of the major environmental factors limiting worldwide productivity and distribution is that of salinity stress. Salinity stress triggers various interactive events including an increase of ABA concentration and decreases of xylem pH and conductivity (Kafi et al. 2009).

Osmotic adjustment is a mechanism to avoid salinity. Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain cell turgor (Hsu et al. 2003, Seki et al. 2007).A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress.(Mansour et al. 2005, Asharf and Harris 2004, Mansour 2000).

Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive cultivars of many crops (de Lacerda et al. 2003; Kumar et al. 2003; de Lacerda et al. 2005; Demiral and Türkan 2005; Mansour et al. 2005; Misra and Gupta 2005; Desingh and Kanagaraj 2007; Koca et al. 2007; Veeranagamallaiah et al. 2007).

CHAPTER –THREE

MATERIALS AND METHODOLOGY

3. Materials and Methods

3.1 Seed collection

Mature fruits of *A. officinalis* were collected from Amurbunia (N 22° 22□ 25.0″ and E 89 44° 35□ 7″), compartment no 27 which is within Less Saline (LS) zone of the Sundarbans during the fruiting season in 2015. Pure stands of *A. officinalis* will be identified and categorized from the vegetation map of ODA (ODA, 1985). The salinity map of the Sundarbans collected from Bangladesh Forest Research Institute. Seeds were collected from randomly selected trees in the pure patches of *A. officinalis*. Collected seeds were brought to the forest nursery at Khulna University Campus for experimental purpose.

Sorting of seeds

Collected seeds were sorted manually and at a time insect affected, oversized, and under sized seeds were rejected.

3.2 Seed morphology

Seeds were selected randomly for measurement of length, width and weight, during the study. Using an electric balance 1 kilogram seeds were measured then counting the total number of seeds. After that 10 % seeds Length and width was measured by using a measurement scale. Every three times this process was followed, in similar way. The number of seed is about 154 per kilogram.

3.3 Raising Seedling

Seedlings were raised in the nursery of Khulna University. Nursery bed was prepared by course sand and regular watering was maintained to nurse the seedlings. After three months, total 120 *A. officinalis* seedlings were collected randomly from the nursery bed. Course sands were removed and the seedlings were washed to remove the sands.

Figure: *Avicennia officinalis* seedlings in nursery

3.4 Experimental setup

The experiment was carried out in pot culture with proper control of salinity and nutrient supply.

The washed seedlings were transplanted into 200 ml pots with crude sand on 13 March 2014. The transplanted seedlings were kept in total 24 plastic boxes and supplied with nutrient solution (Hogland solution), each of the boxes contains 5 seedlings. Estimation of Chlorophyll, Proline along with the growth study was carried out using 8 different salinity levels (0 ppt to 35 ppt) with 3 replications in each treatment. Completely Randomized Design (CRD) with 3 replications (each box is considered as replication) for each treatment was adopted. The total experimental setup was carried out at the glass house in Khulna university nursery.

Figure: Experimental Set up

3.4.1 Preparation of Hogland solution

The modified Hoagland solution, composed of the following nutrients (chemicals) was supplied each week during the experiment (Hossain et al. 2014)

Salt	For stock solution (gm/l)	To use (ml/l)
KH_2PO_4	136.09	1.00
KNO_3	101.11	5.00
$Ca(NO_2)_2.4H_2O$	236.20	5.00
$MgSO_4.7H_2O$	246.50	2.00

H_3BO_3	2.86	1.00
$MnSO_4.H_2O$	0.57	1.00
$ZnSO_4.7H_2O$	0.22	1.00
$CuSO_4.5H_2O$	0.08	1.00
$(NH4)_6Mo_7.4H_2O$	0.02	1.00
Ferric Sulphate	0.003	1.00

3.4.2 Stock solution

An 80ppt stock solution of common salt NaCl was prepared. Using this stock, working solution of 0-35 ppt at 5 ppt interval was prepared. In case of 5ppt, 500ml of stock solution was mixed with Hogland solution and fresh water to make 8 L total solution, using the following equation:

$V_1S_1 = V_2S_2$

To prepare 5 ppt saline nutrient solution, 500 ml saline water from 80ppt stock solution was taken.

V_1 = volume of total working solution = 8 L

S_1= Concentration of total working solution = 5 ppt

V_2= Volume of salt solution

S_2 = Concentration of the salt solution = 80 ppt

The same procedure was used to make 10, 15, 20, 25, 30 and 35 ppt with the use of 1000 ml, 1500 ml, 2000 ml, 2500 ml, 3000 ml and 35000 ml of stock solution respectively.

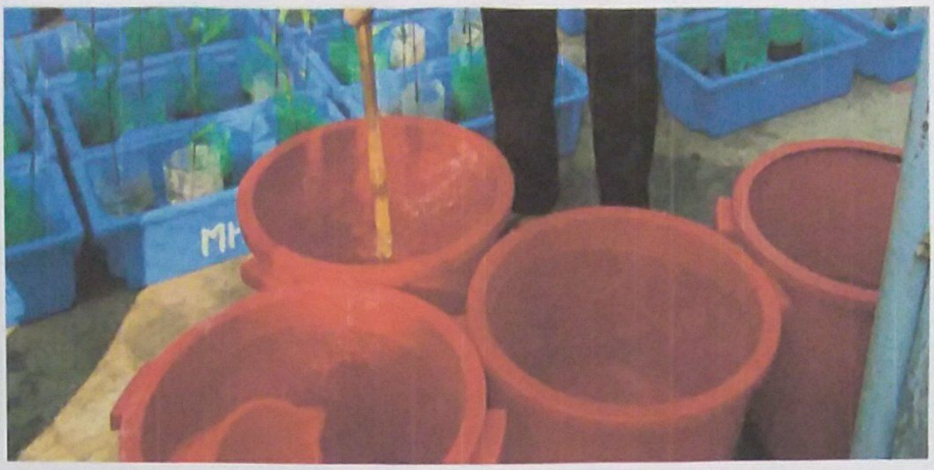

Figure: Stock Solution

3.5 Gradual Increase of Salinity Level

The study was conducted in hydroponic media with the modified Hoagland solution to avoid the complication of Na^+ and Cl^- from the original Hogland nutrient solution and the gradual change in salinity from 0 ppt - 35 ppt of salt treatments at 5 ppt interval were carried out.

Initially the salinity of the media was zero (0 ppt) and in second week the salinity of the first treatment remained zero and all other treatments were increased to 5 ppt. In third week the salinity of the first and second treatment remained zero and 5 ppt respectively, and all other treatments were increased to 10 ppt. Following the same procedure salinity was increased gradually from 0 ppt to 35 ppt.

Nutrients were replaced in every week. The treatments were supplied with water every day to maintain the level of nutrient, water and salinity of the solution. Approximately 8 L solution, composed of stock solution, Hogland solution and fresh water was poured in each treatment.

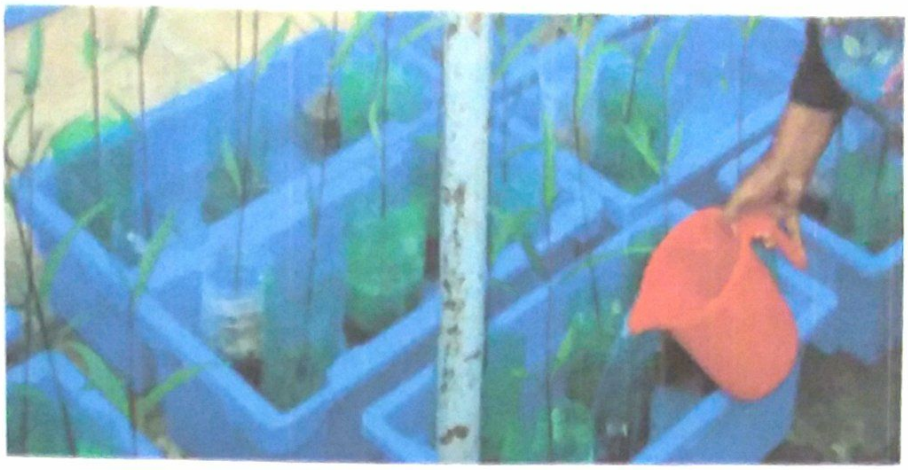

Figure: Changing Nutrient Solution

3.6 Growth Study

3.6.1 Survival and Growth of Seedlings

Before transplanting to the pot, total initial weight of each seedling was measured. Then the seedlings were kept in the pot culture for 10 months. Furthermore, fifteen additional randomly selected seedlings were taken from the nursery. Their total weight and root, stem and leaf weight were measured separately. These cut seedlings were kept in oven for 3 days and again their root, stem and leaf weight were measured to estimate the conversion factor. The number of seedlings in each salinity treatments was counted at the end of the experiment and survival percentage was estimated. After 10 months, all the seedlings were harvested and their collar diameter, height and green weight were measured according to salinity treatments. The growth increment in term of total biomass, root production, stem length increment, leaf, diameter, height increment was estimated from the initial and final values.

3.6.2 Statistical Analysis

The survival percentage values of each salinity treatments were transformed to arcsine and comparison among the treatments was performed by one-way Analysis of Variance followed by DMRT. Moreover, correlation among the survival of seedlings and salinity treatments was conducted by using SAS statistical software. Biomass, root production, stem length increment, diameter, height increment in different salinity treatments were compared by one way ANOVA

followed by DMRT and all the growth parameters were evaluated by using SAS statistical software.

3.7 Plant Sample Preparation for Chlorophyll and Secondary Metabolites

After 6 months in the glass house plant samples or Baen seedlings were uprooted from the pots and rewashed. Some fresh leaf samples were taken for the measurement of chlorophyll and then leaves were separated and kept in open sun for drying. It takes 7 days to dry the samples properly. Then the leaves samples were ground. Screening was done for several times and the samples were kept in plastic pots.

3.7.1 Determination of Chlorophyll

Chlorophyll extraction was conducted by following the Dimethyl sulphoxide (DMSO) method of Hiscox and Israelstam, 1979. Glass centrifuge vials containing 7 ml DMSO were preheated to 65°C in a water bath. Chlorophyll was extracted from three disks (each 3.038 cm2; approx. 100 mg f. wt total) from each leaf sample. Samples were incubated at 65 ◦C until leaf disks were completely colorless and the DMSO had turned green. In preliminary trials, we found that extraction at 65°C was complete within 15– 20 min and no loss of Chlorophyll occurred in the heated DMSO during the first hour; we therefore ran our extractions for 30 min. When the extractions were complete, samples were removed from the water bath and each graduated vial was topped up to exactly 10 ml with DMSO using a Pasteur pipette; 3 ml of each extract were then transferred to disposable polystyrene cuvettes with a reported standard deviation between cuvettes of < ±0.005 extinction units, and a transmission between 600 and 700 nm of 85% or better (catalogue 14-385-985, Fisher Scientific, Pittsburgh, PA, USA). The spectrophotometer (range 200–1100 nm, spectral bandwidth 5 nm, wavelength accuracy ±1 nm, and wavelength setting repeatability of ±0.3 nm; model U-1100, Hitachi Ltd, Tokyo, Japan), was calibrated to zero absorbance using a blank of pure DMSO. Absorbance of both blank and sample were measured at 645 and 663 nm. The elapsed time between removal from the water bath and completion of spectrophotometer measurements was in the order of 20 min.

Hiscox&Israelstam (1979) demonstrated that the absorption spectrum (600–680 nm) for Chlorophyll extracted in DMSO was virtually identical to that for extracted in 90% acetone. They therefore recommended the use of Arnon's (1949) equations:

Chl a (g l-1) = 0.0127 A663 – 0.00269 A645;

Chl b (g l-1) = 0.0229 A645 – 0.00468 A663;

Total Chl (g l-1) = 0.0202 A645 + 0.00802 A663.

The Chlorophyll concentration of the extract calculated from these equations was then converted to leaf Chlorophyll content.

3.7.2 Determination of Proline

At first acidninhydrin was made by warming 1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml 6M phosphoric acid, with agitation, until dissolved. The regent was kept cool to make it stable for 24 hours. Then 0.5 g of leaf sample was homogenized in 10 ml of 3% aqueous sulphosalicyclic acid and centrifuged for 6 minutes. The clear solution was separated and 2 ml of it was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube. Then it was boiled in a boiling water bath for 1 hour at 1000C and the reaction was terminated in an ice bath. The reaction mixture was then extracted with 4 ml of toluene, mixed vigorously with stirring for 15-20 seconds. The chromospheres containing praline-toluene was separated with a separating funnel and warmed to room temperature. The optical density (O.D.) was read at 520 nm using toluene as blank with a spectrophotometer. The proline contents of leaves were determined from the standard curve (Bates 1973).

3.7.2.1 Preparation of the Standard Curve

Stock solution of 1 millimole was prepared by dissolving 0.1151 g of proline (AR) in distilled water and made up to 1000 ml. By successive dilutions, 2 ml of solution containing 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 micromole concentrations was prepared and reacted with acid ninhydrin and glacial acetic acid as described earlier. The color will be read at 520 nm and the optical density will be plotted against concentration.

CHAPTER – FOUR

RESULT AND DISCUSSION

4. Results

4.1 Survival Rate

Seedling survival rate was tremendously fall towards increasing salinity. Up to 5 ppt the survival rate remain 100 % but afterwards it decreased to 93%, 86%, 80%, 67%, 53% and 40% at 10 ppt, 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt respectively (Fig 1). At 95 % significant level, the survival rate of *Avicennia officinalis* seedlings showed negative correlation with increasing salinity.

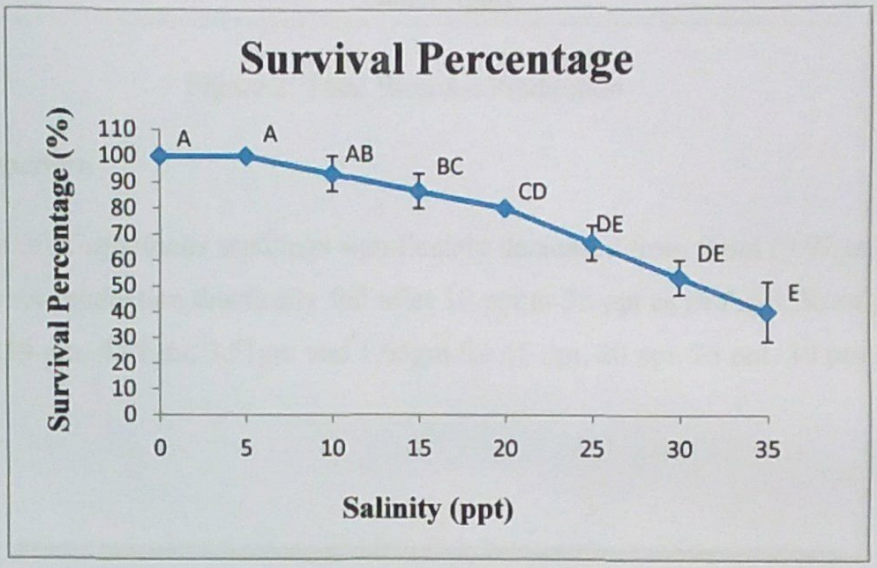

Figure 1: Survival Percentage

4.2 Growth Increment

4.2.1 Total Biomass

The total biomass production decreased markedly with the increased salinity (Fig. 2). *A. officinalis* seedlings displayed significantly (at 95 % significant level) lower total biomass from 0 ppt to 35 ppt, approximately from 23.81 gm to 3.28 gm. From 0 ppt to 15 ppt the total biomass, decreased without any significant difference (from 24gm to 20.52 gm) but afterwards a significant suppression of biomass production at 20 ppt (15.93 gm), 25 ppt (8.77 gm) 30 ppt (5.56 gm) and 35 (3.28 gm) ppt respectively was observed.

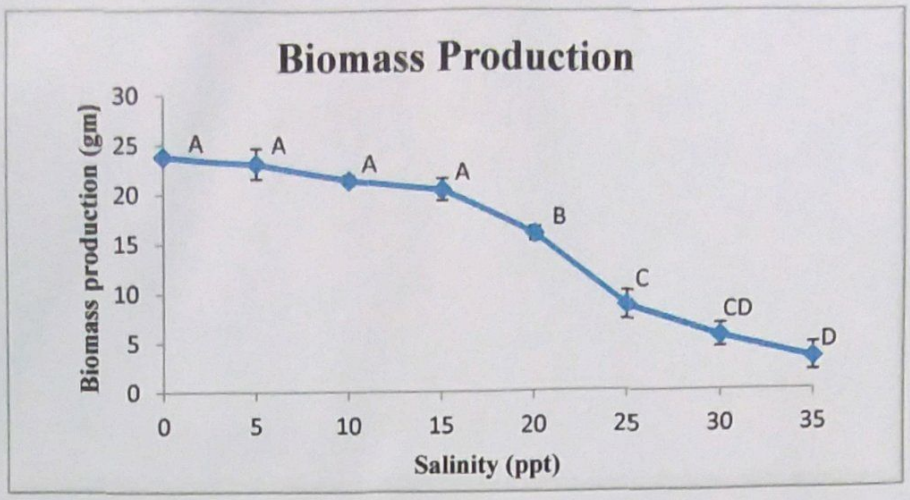

Figure 2: Total Biomass Production

4.2.2 Root Production

Root production of *A. officinalis* seedlings significantly decreased from 0 ppt (9.97gm) to 35 ppt (1.65gm). The root production drastically fall after 10 ppt to 35 ppt as in Fig 3. Root production was 8.53 gm, 6.14 gm, 4.88gm, 3.51gm and 1.65gm for 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt respectively.

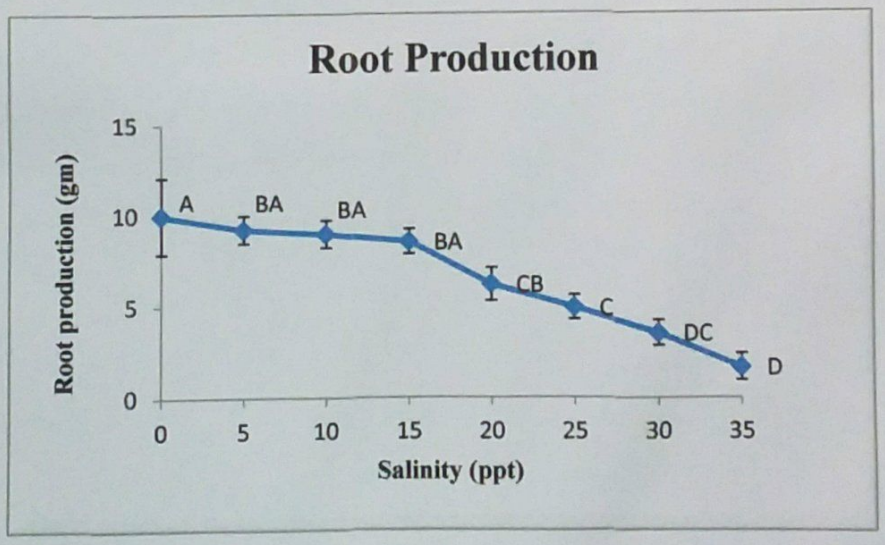

Figure 3: Root production

4.2.3 Stem production

Stem production of *A. officinalis* seedlings was almost same from 0 ppt up to 15 ppt (7.81 gm to 6.63 gm). Afterwards it fall slightly at 20 ppt. and it reduced drastically from 25 ppt (3.25 gm) to 35 ppt (1.40 gm), which vary significantly from each other (Fig 4).

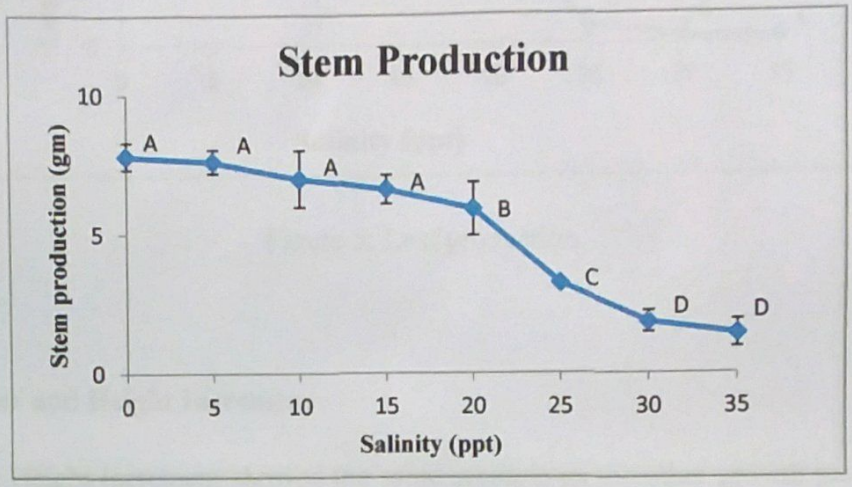

Figure 4: Stem production

4.2.4 Leaf production

Leaf production of *A. officinalis* has was severely affected by increased salinity level as it almost fall towards zero at 35 ppt (Fig 5). At 0 ppt the leaf production was 7.25 gm, which remain almost same from 5 ppt to 15 ppt (6.36 gm to 5.35 gm). After 20 ppt to 35 ppt (3.87 gm to 0.22 gm), it significantly decreased that represents almost zero increment.

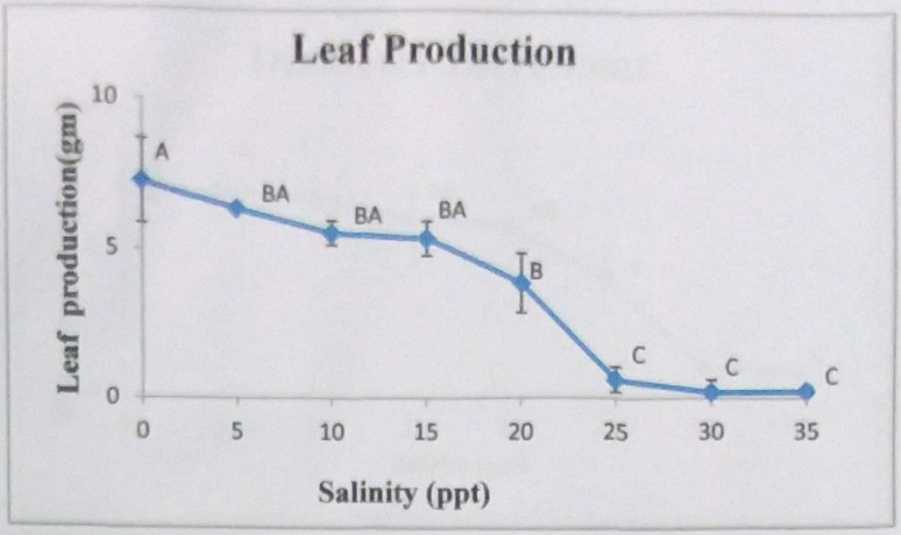

Figure 5: Leaf production

4.2.5 Diameter and Height Increment

Diameter and Height increment showed the same scenario as the other growth parameters of *A. officinalis* seedlings. A clear negative relation observed in case of both diameter and height increment. Diameter of *A. officinalis* seedling decreased from 0 ppt to 35 ppt (1.47 mm to 0.14 mm) but a sharp fall seen after 20 ppt, which were 0.81 cm at 25 ppt and 0.18 cm at 30 ppt (Fig. 6). Height increment also decreased significantly from 0 ppt to 35 ppt (13.22 cm to 2.61 cm) (Fig. 7).

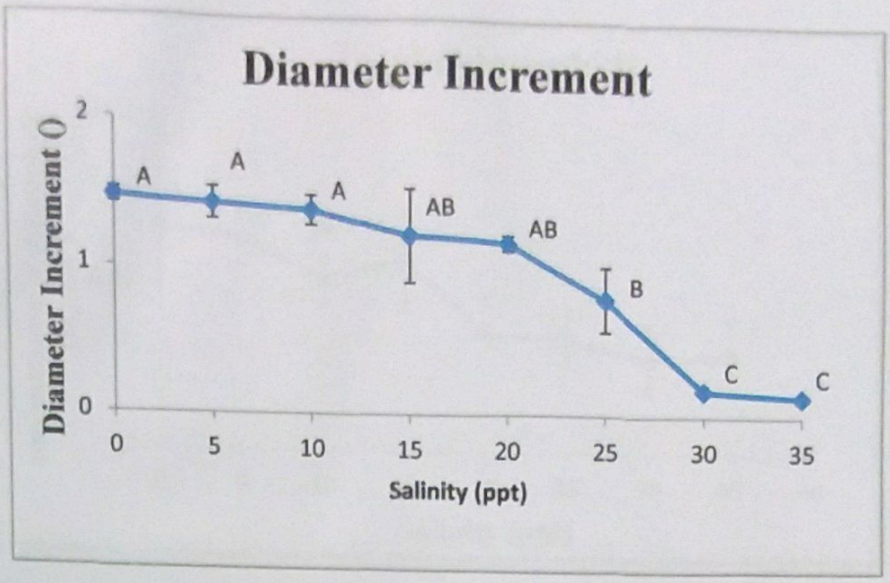

Figure 6: Diameter Increment

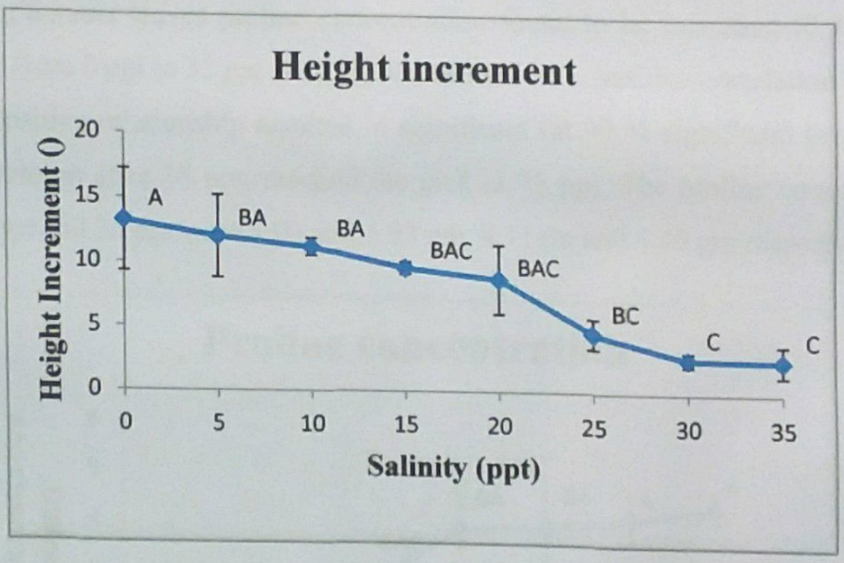

Figure 7: Height Increment

4.3 Total Chlorophyll Concentration

The amount of total chlorophyll (mg/cm^2) concentration decreased from 0 ppt to 35 ppt ($0.013mg/cm^2 - 0.007mg/cm^2$) (Fig 8). The concentration of chlorophyll showed a significant (95 %) negative correlation with increased salinity.

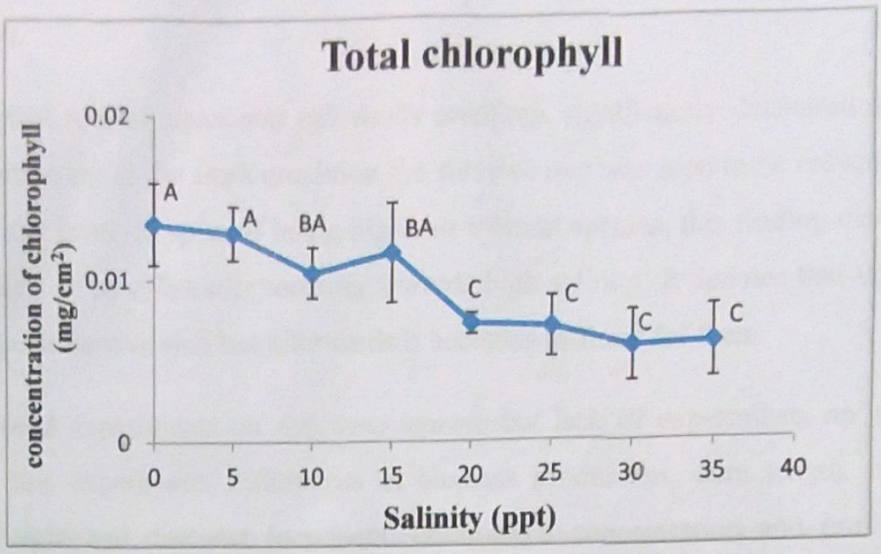

Figure 8: Total Chlorophyll Concentration

4.4 Concentration of Proline

In case of *A. officinalis* leaves proline concentration found to be increased (0.911µmole/gm - 4.40µmole/gm) from 0 ppt to 35 ppt (Fig. 9), which showed a positive correlation with increased salinity. This positive relationship resulted in significant (at 99 % significant level) increase of proline concentration after 15 ppt, reached the pick at 35 ppt. The proline concentration at 20 ppt, 25 ppt 30 ppt and 35 ppt were 3.90 gm, 3.93 gm, 4.11gm and 4.40 gm respectively.

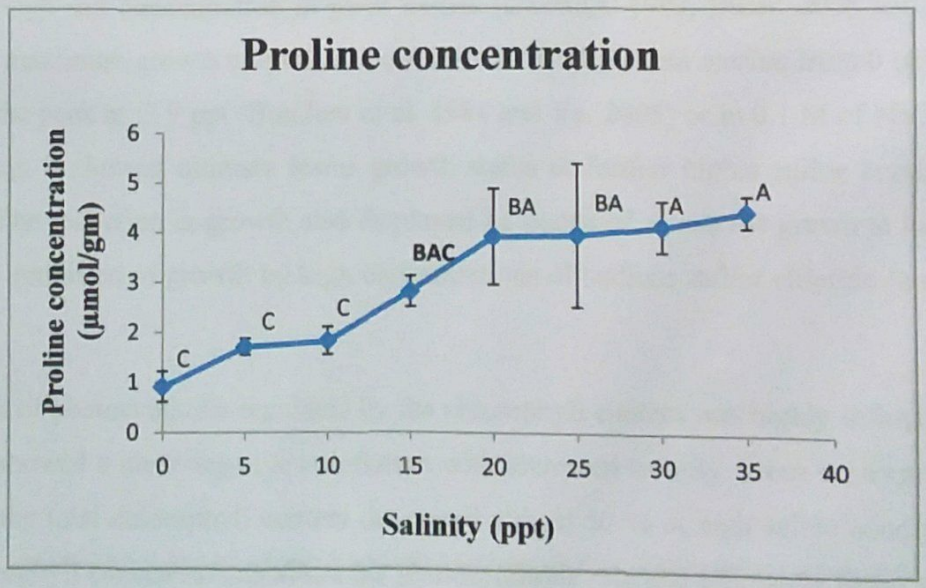

Figure 9: Proline Concentration

4.5 Discussion

Seedling survival rate of *Avicennia officinalis* seedlings significantly decreased at high saline condition. Comparing to the fresh condition the survival rate was seen to be reduced up to 60 % at 35 ppt salinity level. In spite of being high salt tolerant species, this finding clearly indicates the susceptibility of *A. officinalis* seedling towards high salinity. It denotes that up to a certain level seedling can survive well but afterwards it becomes difficult for them.

There are several experiments on *Avecinna marina* but lack of experiments on *A. officinalis*. However, in this experiment, differences in biomass production, stem length, root and leaf production, height and diameter increment, chlorophyll concentration and proline secretion across different salinity level; indicates that salinity strongly affects *Avecinnia officinalis* seedlings growth.

The overall biomass production along with other growth parameters of *Avicennia officinalis* seedlings was found to be severely affected by high salinity. This study showed a clearly negative correlation with increased salt concentration. Growth of *Aofficinalis* reduced almost 90% at high saline condition (35 ppt). However this result support many of the experiments on *A. marina* with some exceptions as *Avicennia marina* showed optimal growth under moderate saline condition normally up to 20 ppt salinity, indicating the inhibition to high saline condition due to the high salt concentration in plant tissues (Ghowail. 1993, Biber. 2006 and Yan et al. 2007). The maximum growth rate value was observed for *Avicennia marina* from 0 ppt to 15 ppt, indicating the peak at □ 9 ppt (Burcliett et al. 1984 and Ye. 2005) or in 0.1 M of NaCl (Naidoo. 1987) though it showed ultimate lower growth status at further higher saline condition as *A. offcinalis*. The reduction in growth also displayed by plants of *A. marina* grown at high salinity was due to inhibition of growth by high concentrations of sodium and/or chloride ions (Clough. 1984).

The amount of photosynthesis regulated by the chlorophyll content was highly influenced by the salinity. It showed a clear negative correlation with increased salinity. From the experiment, we found that the total chlorophyll content decreased almost 50 % at high saline condition. These lower chlorophyll concentrations affect the photosynthesis rate and ultimately lower the growth. This finding supports many experiments. Recent studies state that chlorophyll level of trees

decreases with aggravated salt stress due to enzymatic chlorophyll degradation (Ali. 2004). Nevertheless, different studies also stated that the influence on high salinity in photosynthesis is negligible for *Avicennia marina* (Parida et al. 2002, Reef et al. 2015). In this experiment, Chlorophyll content significantly fall in high saline condition. This can be related with growth status as at 35 ppt salinity the leaf amount of *A.officinalis* that found to be lowest at high salinity. That might affect the chlorophyll content and ultimately reduced the amount of photosynthesis. It might have some effect on the final biomass production i.e. growth.

Proline accumulation in salt stressed plants is a primary defense mechanism in response to maintain the osmotic pressure in a cell. This reported in salt tolerant and salt sensitive cultivars of many crops (Lacerda et al., 2003, Kumar et al., 2003, Lacerda, et al., 2005, Demiral&Türkan, 2005, Mansour et al., 2005; Misra& Gupta, 2005; Desingh & Kanagaraj, 2007; Koca et al., 2007; Veeranagamallaiah et al. 2007). Proline concentrations increased in leaf- and stem tissues at 60 mM NaCl than 0 mM NaCl in case of *Solanum tuberosum*s pecies (Jaarsma et al., 2013). In the present study, proline accumulation in the salt tolerant *A officinalis* seedling supports the previous findings, as proline concentration of *A.officinalis* leaves was observed to be 5 times higher at 35 ppt than at 0 ppt. Proline concentration was found to be significantly increase with the increased salinity level. This might be a protective mechanism for many mangrove species. As one of the important characteristics of mangrove species is to withstand in hyper saline condition, especially for salt tolerant species like *A. officinalis*. Therefore, proline concentration might be an adaptive or defensive mechanism to cope with high saline condition.

CHAPTER – FIVE

CONCLUSION

5. Conclusion and Recommendation

5.1 Conclusion

In conclusion, the result of this experiment indicates that salinity stress caused a number of morphological and physiological changes in the *Avicennia officinalis* seedlings, including decreased net growth increment, chlorophyll content, and higher proline concentration. This result indicates the severity of salinity impact near future as the salinity level is increasing day by day.

Salinity causes a serious problem in case of different metabolic activities in plants as *Avicenniaofficinalis* though it is not very saline prone species. Therefore applied research, awareness education, monitoring and evaluation are the key potential issues of a successful mangrove ecosystem management and conservation. The continuous reduction and deterioration of quality of the Ganges fresh water in the catchment in the root cause of salinity invasion and damage of the Sundarban's ecosystems. Considering the present salinity intrusion drift in different ecological zones in the Sundarban and management condition, an applied research and awareness education program should be included as a potential environmental development plan. To protect the Mangrove wetland ecosystems in the Sundarban region the alternative approach of proposed upstream water reservoir in Nepal and fresh water supply in the downstream should be ensured as joint investigation of Bangladesh-Nepal (Ministry of Water Resources) reported in 1989.

5.2 Recommendation

Almost 75% of the surface area of earth is dominated by NaCl salt solution (Flowers et al. 2015), which might increase in near future. Many mangrove species of fresh saline zone,may not survive in hyper saline condition. Not only the low saline tolerant species but also many high salt tolerant species have salt stress limit up to which they can withstand. Therefore, studies associated with high saline effect would create new boundaries to understand the overall impacts and be a good source of the indicator of climate changes. However, there is lack of experiments for many mangrove species and their associations that how their growth in relation with increased salinity can be an indicator of climatic changes. There are many scopes of new findings and further research as there is a very little information of many mangrove species

responses by means of growth, chlorophyll content, proline and other secondary metabolites concentration, to high salinity. In addition, the study of the effect of salinity on plant might contribute in understanding many molecular mechanisms adapted by the species to survive in adverse environmental stresses. Additionally, in many studies it is observed that growth can be a good indicator to estimate the amount of fresh water that would be needed to reduce salinity (Zhai et al. 2015).

References

Allen, J. A. Krauss, K. W. and Hauff, R. D. 2003. Factors Limiting the Intertidal Distribution of the Mangrove Species *Xylocarpusgranatum*.Oecologia. 135:110-121. DOI 10.1007/s00442-002-1167-2.

Aziz, I. and Khan, M. A. 2001. Effect of Seawater on the Growth, Ion Content and Water Potential of *Rhizophoramucronata*Lam. J. Plant Res. 114: 369-373. © By the Botanical Society of Japan.

Ball, M. C. 2002. Interactive Effects of Salinity and Irradiance on Growth: Implications for Mangrove Forest Structure along Salinity Gradients. Trees. 16:126–139. DOI 10.1007/s00468-002-0169-3.

Barrett-Lennard, E. G. 2003. The Interaction between Water logging and Salinity in Higher Plants: Causes, Consequences and Implications. Plant and Soil. 253: 35–54. © Kluwer Academic Publishers.

Basak, U. C. Gupta, N. Rautaray, S. and Das, P. 2004. Effects of Salinity on the Growth of Mangrove Seedlings. J TROP FOR SCI. Vol. 16, No. 4 (October 2004), pp. 437-443.

Basyuni, M. Kinjo, Y. Baba, S. Shinzato, N. Iwasaki, H. 2011. Isolation of Salt Stress Tolerance Genes from Roots of Mangrove Plant, *Rhizophorastylosa*Griff., Using PCR-Based Suppression Subtractive Hybridization. Plant MolBiol Rep. 29:533–543. DOI 10.1007/s11105-010-0257-2

Biber, P. D. 2006. Measuring the Effects of Salinity Stress in the Red Mangrove, *Rhizophora mangle* L. Afr. J. Agric. Res. Vol. 1 (1), pp. 001-004.

Bompy, F. Lequeue, G. Imbert, D. and Dulormne, M. 2014. Increasing Fluctuations of Soil Salinity Affect Seedling Growth Performances and Physiology in Three Neotropical Mangrove Species. Plant Soil. 380:399–413. DOI 10.1007/s11104-014-2100-2.

Brugnoli, E. and Lauteri, M. 1991. Effects of Salinity on Stomatal Conductance, Photosynthetic Capacity, and Carbon Isotope Discrimination of Salt Tolerant

(*Gossypiumhirsutum*L.) and Salt-Sensitive (*Phaseolus vulgaris* L.)C_3 Non Halophytes. Plant Physiol. 956:28-635.

Burcliett, M. D. Field, C. D. and Pulkownik, A. 1984.Salinity, Growth and Root Respiration in the Grey Mangrove, *Avicennia marina.*Physiol. plant. 60:113-X18.

C.S.I.R. (Council of Scientific and Industrial Research). 1948–1976.The wealth of India.11 vols. New Delhi.

Clough, B. F. 1984. Growth and Salt Balance of the Mangroves *Avicennia marina (*Forsk.*)*Vierh.andRhizophorastylosaGriff. in relation to salinity. Aust. J. Plant Physiol. 11:419-430.

Clough, B. F. Andrew, T. J. Cowan, I. R. 1982. Physiological Processes in Mangroves. In: Clough BF (ed) Mangrove ecosystems in Australia: structure, function and management. Australian National Press, Canberra. 193–210.

Das, S.,Siddiqi, N.A., 1985. The mangroves and mangrove forests of Bangladesh. Mangrove Silviculture Division, Bulletin no. 2 UNDP/ FAO project BGD/79/017. Bangladesh Forest Research Institute, Chittagong.

Dangremond, E. M. Feller, I. C. and Sousa, W. P. 2015. Environmental Tolerances of Rare and Common Mangroves Along Light and Salinity Gradients. Oecologia. 179:1187–1198. DOI 10.1007/s00442-015-3408-1.

Duke, J.A. and Wain, K.K. 1981. Medicinal plants of the World. Computer index with more than 85,000 entries.3 vols. 1654 pp.

Flowers, T. J. and Colmer, T. D. 2015. Plant Salt Tolerance: Adaptations in Halophytes. Annals of Botany. 115: 327–331.

Ghowail, S. I. Abdel, A. M. Monem, M. El-Ghamry, W. and SABER, N. E. 1993. Preliminary Studies on Effect of Different Salinity Levels on Germination, Growth, and Anatomy of Mangrove (*Avicennia marina*).In H. Leith and A. L. Masoom (Eds.).Towards the rational use of high-salinity tolerant plants.237–244.

Hartwell, J.L. 1967–1971. Plants used against cancer. A survey.Lloydia 30–34.

Hasegawa, A. Oyanagi, T. Minagawa, R. Fujii, Y. and Sasamoto, H. 2014. An Inverse Relationship between Allelopathic Activity and Salt Tolerance in Suspension Cultures of Three Mangrove Species, *Sonneratiaalba, S. caseolaris* and *S. ovata:* Development of A Bioassay Method for Allelopathy, the Protoplast Co-Culture Method. J Plant Res. 127:755–761.

Hastuti, E. D. Anggoro, S. and Pribadi, R. 2012. The Effects of Environmental Factors on the Dynamic Growth Pattern of Mangrove *Avicennia marina.* J. Coast. Res. 16: 57-61.

Hossain, M. Saha, S. Siddique, M. R. H. and Hasan, M. N. Salinity Stress on Growth, Nutrients and Carbon Distribution in Seedlings Parts of *Heritierafomes.*

Hussain, Z.,Acharya, G., 1994. Mangrove of Sundarbans Volume two: Bangladesh. IUCN, Bangkok. P. 257.

Kathiresan, K. andRajendran, N. 2002. Growth of a Mangrove (*Rhizophoraapiculata*) Seedlings as Influenced by GA_3 Light and Salinity. Rev. Biol. Trop. 50(2): 525-530.

Kirtikar, K.R. and Basu, B.B. 1975. Indian Medicinal Plants.4 vols. text, 4 vols. plates.2nd ed., reprint.Jayyed Press. New Delhi 6.

Little, Jr., E.L. 1983. Common fuelwood crops: A handbook for their identification. McClain Printing Co., Parsons, WV. 354 pp.

Lopez-Hoffman, L. DeNoyer, J. L. Monroe, I. E. Shaftel, R. Anten, N. P. R. Martinez-Ramos, M. and Ackerly, D. D. 2006. Mangrove Seedling Net Photosynthesis, Growth, and Survivorship are Interactively Affected by Salinity and Light. BIOTROPICA 38(5): 606–616.

Mimura, T. Mimura, M. Washitani-Nemoto', S. and Siripatanadilok, S. 1997.NaCI-Dependent Growth, Ion Content and Regeneration of Calluses Initiated from the Mangrove Plant, *Bruguierasexangula.* J. Plant Res. 110: 31-36. © The Botanical Society of Japan.

Mitra, A. and Banerjee, K. Pigments of *Heritierafomes* Seedlings Under Different Salinity Conditions: Perspective Sea Level Rise. Mesopot. J. Mar. Sci. 25 (1): 1–10.

Naidoo, G. 1987. Effects of Salinity and Nitrogenon Growth and Water Relations in the Mangrove, *Avicennia Marina* (Forsk.)Vierh.New Phytol.107:317-32 5.

NAS.1980a. Firewood crops.Shrub and tree species for energy production.National Academy of Sciences. Washington.

Noor, T. Batool, N. Mazhar, R. and Ilyas, N. 2015.Effects of Siltation, Temperature and Salinity on Mangrove Plants.European Academic Research.Vol.2.

Parida, A. Dam A. B. and Das, P. 2002. NaCI Stress Causes Changes in Photosynthetic Pigments, Proteins, and Other Metabolic Components in the Leaves of a True Mangrove, *Bruguieraparviflora*, in Hydroponic Cultures. J. Plant Biol. Vol. 45. No. 1: 28-36.

Parida, A. K. and Jha, B. 2010. Salt Tolerance Mechanisms in Mangroves: A Review. Trees. 24:199–217. DOI 10.1007/s00468-010-0417.

Parida, A. K. Das, A. B. and Mittra, B. 2004.Effects of Salt on Growth, Ion Accumulation, Photosynthesis and Leaf Anatomy of the Mangrove, *Bruguieraparviflora.*Trees. 18:167–174. DOI 10.1007/s00468-003-0293-8.

Perry, L.M. 1980. Medicinal plants of East and Southeast Asia. MIT Press. Cambridge.

Reef, A. Chawdhury, R. Sengupta, S. and Banarjee, K. 2015. Impact of Salinity on Mangroves of Indian Sundarbans. Jour. Coast.Env.Vol. 1.No. 1.

Reef, R. Markham, H. L. Santini, N. S. and Lovelock, C. E. 2015. The Response of the Mangrove *Avicennia marina* to Heterogeneous Salinity Measured Using a Split-Root Approach.Plant Soil. 393:297–305.

Suarez, N. and Medina, E. 2005. Salinity Effect on Plant Growth and Leaf Demography of the Mangrove, *Avicenniagerminans*L. Trees. 19:721–727. DOI 10.1007/s00468-005-0001-y.

Suárez, N. and Medina, E. 2008. Salinity Effects on Leaf Ion Composition and Salt Secretionrate in *Avicenniagerminans*(L.) *L.* Braz. J. Plant Physiol. 20(2):131-140.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962.The medicinal and poisonous plants of southern and eastern Africa. 2nd ed. E.&S. Livingstone, Ltd., Edinburgh and London.

Yan, Z. Wang, W. and Tang, D. 2007. Effect of Different Time of Salt Stress on Growth and Some Physiological Processes of *Avicennia marina* Seedlings. Mar Biol. 152:581–587. DOI 10.1007/s00227-007-0710-4.

Ye, Y. Tam, N. F. Y. Lu, C. Y. and Wong. Y. S. 2005.Effects of Salinity on Germination, Seedling Growth and Physiology of Three Salt-Secreting Mangrove Species.Aquat. Bot. Vol. 83. Is. 3:193–205.

Zhai, L. Jiang, J. DeAngelis, D. and Sternberg, L. D. S. L. 2015. Prediction of Plant Vulnerability to Salinity Increase in a Coastal Ecosystem by Stable Isotope Composition (□ ^{18}O) of Plant Stem Water: A Model Study. Ecosystems. DOI: 10.1007/s10021-015-9916-3. © Springer Science + Business Media New York.

Zhua, Z. Pei, Z. M. and Zheng, H. L. 2011.Effect of Salinity on Osmotic Adjustment Characteristics of *Kandeliacandel.* Russ. J. Plant Physiol. Vol. 58, No. 2, pp. 226–232. © Pleiades Publishing, Ltd.

Table 1: ANOVA Table of Seedling Survival

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7914.026	7	1130.575	12.6515	1.85336E-05	2.657197
Within Groups	1429.806	16	89.3629			
Total	9343.832	23				

Table 2: ANOVA Table of Total Biomass

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1448.437	7	206.9196	43.82327	2.89E-09	2.657197
Within Groups	75.54692	16	4.721682			
Total	1523.984	23				

Table 3: ANOVA Table of Root Production

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	193.8838	7	27.69769	8.896096	0.000164	2.657197
Within Groups	49.81544	16	3.113465			
Total	243.6992	23				

Table 4: ANOVA Table of Stem Length

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	143.8886	7	20.55552	18.08606	1.72E-06	2.657197
Within Groups	18.18463	16	1.13654			
Total	162.0733	23				

Table 5: ANOVA Table of Leaf Production

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	178.784	7	25.54058	17.81633	1.9E-06	2.657197
Within Groups	22.93677	16	1.433548			
Total	201.7208	23				

Table 6: ANOVA Table of Height Increment

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	375.1421	7	53.59173	3.873826	0.011807	2.657197
Within Groups	221.3491	16	13.83432			
Total	596.4912	23				

Table 7: ANOVA Table of Diameter Increment

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.266267	7	0.895181	13.26413	1.36E-05	2.657197
Within Groups	1.079821	16	0.067489			
Total	7.346088	23				

Table 8: ANOVA Table of Chlorophyll Content

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000207	7	2.96E-05	3.290108	0.022988	2.657197
Within Groups	0.000144	16	8.99E-06			
Total	0.000351	23				

Table 9: ANOVA Table of Proline Concentration

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35.96413	7	5.137733	3.778862	0.013117	2.657197
Within Groups	21.75357	16	1.359598			
Total	57.7177	23				

DMRT- Seedling Survival Rate

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 89.3629

Number of Means 2 3 4 5 6 7 8

Critical Range 16.36 17.16 17.66 18.00 18.24 18.43 18.57

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRTMENT
	A	90.002	3	0
	A	90.002	3	5
B	A	81.147	3	10
B	C	72.292	3	15
D	C	63.436	3	20
D	E	54.992	3	25
D	E	46.924	3	30
	E	38.856	3	35

DMRT- Total Biomass

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 4.721682

Number of Means 2 3 4 5 6 7 8

Critical Range 3.761 3.944 4.058 4.137 4.193 4.236 4.268

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRTMENT
	A	23.810	3	0
	A	23.206	3	5
	A	21.489	3	10
	A	20.521	3	15
	B	15.927	3	20
	C	8.766	3	25
D	C	5.561	3	30
D		3.284	3	35

DMRT- Root Production

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 3.113465

Number of Means 2 3 4 5 6 7 8

Critical Range 3.054 3.203 3.296 3.359 3.405 3.440 3.466

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRTMENT
	A	9.971	3	0
B	A	9.226	3	5
B	A	8.943	3	10
B	A	8.527	3	15
B	C	6.142	3	20
	C	4.882	3	25
D	C	3.513	3	30
D		1.653	3	35

DMRT- Stem Length

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 1.13654

Number of Means 2 3 4 5 6 7 8

Critical Range 1.845 1.935 1.991 2.030 2.057 2.078 2.094

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT
A	7.8122	3	0
A	7.6183	3	5
A	6.9966	3	10
A	6.6354	3	15
A	5.9138	3	20
B	3.2534	3	25
B	1.8415	3	30
B	1.4017	3	35

DMRT- Leaf Production

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 1.433548

Number of Means 2 3 4 5 6 7 8

Critical Range 2.072 2.173 2.236 2.279 2.311 2.334 2.352

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRTMENT
	A	7.2549	3	0
	A	6.3623	3	5
B	A	5.5493	3	10
B	A	5.3584	3	15
B		3.8716	3	20
	C	0.6306	3	25
	C	0.2292	3	35
	C	0.2065	3	30

DMRT- Diameter Increment

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 0.067489

Number of Means 2 3 4 5 6 7 8

Critical Range .4497 .4715 .4852 .4946 .5013 .5064 .5102

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRTMENT

	A	1.4720	3	0
	A	1.4353	3	5
	A	1.3947	3	10
B	A	1.2293	3	15
B	A	1.1807	3	20
B		0.8093	3	25
	C	0.1840	3	30
	C	0.1420	3	35

DMRT- Height Incerment

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 13.83432

Number of Means 2 3 4 5 6 7 8

Critical Range 6.438 6.751 6.947 7.081 7.178 7.250 7.305

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRTMENT
	A	13.220	3	0
	A	11.987	3	5
B	A	11.200	3	10
B	A C	9.613	3	15
B	A C	8.773	3	20
B	C	4.620	3	25
	C	2.700	3	30
	C	2.613	3	35

DMRT- Chlorophyll

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 8.991E-6

Number of Means 2 3 4 5

Critical Range .005190 .005443 .005600 .005708

Number of Means 6 7 8

Critical Range .005787 .005845 .005889

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	TRTMENT
	A		0.013450	3	0
	A		0.012828	3	5
B	A		0.011609	3	15
B	A	C	0.010388	3	10
B		C	0.007174	3	20
B		C	0.007020	3	25
B		C	0.006024	3	35
		C	0.005779	3	30

DMRT- Proline Concentration

Duncan's Multiple Range Test for variable: CUCONC

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 16 MSE= 1.371846

Number of Means 2 3 4 5 6 7 8

Critical Range 2.027 2.126 2.188 2.230 2.260 2.283 2.300

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	TRTMENT
	A		4.4031	3	35
	A		4.1142	3	30
B	A		3.9325	3	25
B	A		3.9021	3	20
B	A	C	2.8082	3	15
B		C	1.8250	3	10
		C	1.6992	3	5
		C	0.9113	3	0

Correlations

		Salinity	Survival	biomass	Root	Stem	Leaf	Diameter	Height	Chlorophyll	Proline
Salinity	Pearson Correlation	1	-.893**	-.940**	-.868**	-.900**	-.905**	-.856**	-.773**	-.726**	.764**
	Sig. (2-tailed)		.000	.000	.000	.000	.000	.000	.000	.000	.000
	N	24	24	24	24	24	24	24	24	24	24
Survival	Pearson Correlation	-.893**	1	.879**	.774**	.882**	.828**	.776**	.747**	.719**	-.731**
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000	.000	.000	.000
	N	24	24	24	24	24	24	24	24	24	24
biomass	Pearson Correlation	-.940**	.879**	1	.925**	.933**	.962**	.895**	.776**	.766**	-.677**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000	.000	.000	.000
	N	24	24	24	24	24	24	24	24	24	24
Root	Pearson Correlation	-.868**	.774**	.925**	1	.785**	.865**	.814**	.710**	.797**	-.607**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000	.000	.000	.002
	N	24	24	24	24	24	24	24	24	24	24
Stem	Pearson Correlation	-.900**	.882**	.933**	.785**	1	.852**	.880**	.699**	.698**	-.710**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000	.000	.000	.000
	N	24	24	24	24	24	24	24	24	24	24
Leaf	Pearson Correlation	-.905**	.828**	.962**	.865**	.852**	1	.830**	.811**	.730**	-.622**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000	.000	.000	.001
	N	24	24	24	24	24	24	24	24	24	24
Diameter	Pearson Correlation	-.856**	.776**	.895**	.814**	.880**	.830**	1	.718**	.627**	-.511*
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000		.000	.001	.011
	N	24	24	24	24	24	24	24	24	24	24
Height	Pearson Correlation	-.773**	.747**	.776**	.710**	.699**	.811**	.718**	1	.618**	-.607**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000		.001	.002
	N	24	24	24	24	24	24	24	24	24	24
Chlorophyll	Pearson Correlation	-.726**	.719**	.766**	.797**	.698**	.730**	.627**	.618**	1	-.593**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.001	.001		.002
	N	24	24	24	24	24	24	24	24	24	24
Proline	Pearson Correlation	.764**	-.731**	-.677**	-.607**	-.710**	-.622**	-.511*	-.607**	-.593**	1
	Sig. (2-tailed)	.000	.000	.000	.002	.000	.001	.011	.002	.002	
	N	24	24	24	24	24	24	24	24	24	24

** Correlation is significant at the 0 01 level (2-tailed).

*. Correlation is significant at the 0 05 level (2-tailed).