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Life Science School
Forestry and Wood Technology Discipline

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Title: Biodiversity, biomass, carbon stock and nutrient content of a pioneer in Sundarbans

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Programme: Master of Science in Forestry

BIODIVERSITY, BIOMASS, CARBON STOCK AND NUTRIENT CONTENT OF A PIONEER STAND IN SUNDARBANS

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Course Title: Thesis

Course no: FWT-5112

[This thesis has been prepared and submitted to Forestry and wood Technology Discipline, Khulna University, Khulna, Bangladesh in partial fulfillment of the requirements for the Degree of Master of Science (M.S.) in Forestry and Wood Technology.]

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DECLARATION

I, Fatima Najnin Ritu, declare that this thesis is the result of an original work and has not previously been accepted for any degree to any other University or Institution.

I, hereby, give consent for my thesis, to be available for photocopying and for inter-library loans and for the title and summary to be made available to outside organizations only for research and educational purposes.

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14.10.15

Fatima Najnin Ritu

Dedicated

To My

Beloved Mother

Asma Malek

ACKNOWLEDGEMENT

At first I want to admire to Almighty ALLAH, the supreme creator and ruler of the universe from the deepest corner of my heart for His blessing that keeps me alive and enable me to accomplish this project work successfully. I want to express my sincere gratitude to my beloved parents who brought me in this earth and helped me in every work of my life.

I am greatly indebted to my honorable supervisor Dr. Mahmood Hossain, Professor, Forestry and Wood Technology Discipline, Khulna University, Khulna, for his continuous guidance, valuable advice and constant encouragement to prepare this project thesis.

I would like to express my profound gratitude to those people who helped me during the field survey. I would like to thank those for their assistance during collection of data.

I would like to express my special thanks to my another teacher Rubaiyat Abdullah and Sonjoy Saha for their help in analysis and I am grateful to Ujjol da, Tapu Vi, Sheed vi, pitol, helping me during data collection. I also thank Heera, Munia Apu, Shawon Vi and all of my friends who have consistently supported me throughout the all stages of completing my study.

Finally I express my deepest gratitude to my parents and to my brother Mushfique Omar and specially thank all of my well wishers for their continuous support and inspiration to complete this paper.

ABSTRACT

Tropical natural forest especially mangrove in Sundarbans plays an important role in the terrestrial carbon stocks and Bangladesh is one of the beneficiaries of it. The research was aimed to estimate standing biomass and below ground biomass in a pioneer stand and also to determine species diversity, nutrient contents and carbon stocks. A total of 18 sample plots were measured applying systematic random sampling method. Diameter at breast height (DBH) was measured, woody species were counted (>4 cm DBH) and soil samples were collected from 4 depths (0-5, 5-10, 10-40 cm, 40-50 cm). Plant biodiversity was calculated in three different plant strata separately as tree (0.77, 1.97, 0.53), saplings (1.63, 4.19, 1.50), seedlings (1.86, 5.18, 1.94) by Shannon index H', Simpson Index, Ds and Margalief index respectively. The total biomass (Above ground biomass plus root biomass) was calculated using allometric equation and converted into carbon, soil carbon was measured using loss by ignition method. Results revealed that the total carbon stock of the in the pioneer stand was 134.09 t/ha in soil at 50 cm depth whereas above ground carbon and root carbon were 49.26 t/ha, 16 t/h and respectively. Above and below ground biomass were double of carbon contents of standing trees, roots and in soil. Nitrogen, Phosphorus, Potassium and carbon of different plant parts like leaf, branch root and stem were measured. Two species *Sonneratia apetala and Avicennia officinalis* were selected for nutrient measurement as for its dominancy in the stand. Stem of both species contain more carbon (about 49 %) than other parts. Nitrogen was very negligible amount and potassium was almost same in two plant parts and only phosphorus was more in *Avicennia* than *Sonneratia*.The pioneer stand must be considered as a significant reservoir of carbon, as it shows a good stock of carbon from the atmosphere. To realize the potentiality of conservation of the pioneer stand in Sundarbans for carbon sequestration in Bangladesh, it should be intensify to more study in future.

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Chapter 1

Introduction

1.1 Back ground of the study

Natural undisturbed forest always ensures large-scale biodiversity conservation set aside for a healthy ecosystem. Mangrove forests show comparatively lower biodiversity compared to other tropical forests. Among all mangroves, Sundarbans alone hold almost 50% species of the world mangroves (Tomlinson, 1986). Sundarbans has a unique biota comprising 334 species flora and species composition and community structure varies in a pioneer stand and along the hydrological and salinity gradients. It is also acting for a huge sink of unlimited capacity for absorbing carbon and other pollutants from air and water, which makes the surrounding environment free from pollution (Saidur, 2010). Protecting the world from adverse effects of climate change, the Sundarbans play a crucial ecological role performing as a carbon reservoir and absorbing more than four crore tonnes of CO_2 from the atmosphere (Paul, 2013).

Mangroves reserve more carbon in soil than other tropical forests have and soil combined which is particularly relevant to climate change mitigation strategies focusing on reducing carbon emissions through reducing forest destruction (Kristensen et al., 2008; Donato et al., 2011). The above ground biomass increases with the height and DBH incensement of plants and below ground biomass depends on the roots dispersion. Statistics show that 1.26% of yearly destruction of forests and remains higher than the world average destruction rate (Mahmood, 2013). In this situation world has focused on a different issue known as carbon trading. The program Reduced emissions from deforestation and forest degradation (REDD+) offers the economic incentives for conserving forests and associated carbon stocks and intended to offset the short-term economic factors that promote deforestation (Mahmood, 2013).

As sundarbans is the greatest reservoirs of carbon therefore for economic and social perspectives, it is essential to study on this topic on sundarbans. The species distribution in mangroves as well as in the Sundarbans found varies with the successional stages (Logue and Snedeker, 1974) and site quality (Mahmood, 2013).

Carbon and nutrients concentration in mangrove ecosystem differs with species, soil types and soil at several meters depth (Bouillon et al., 2003). And litter fall here is about 70% when disturbed islands contain only 16% (Santanu, 2008). In a pioneer stand as the maximum tree species are in sapling stage, ground coverage is affected by regular tidal inundation, biomass production and carbon stock may be quite different from mature Sundarbans land area.

However, several Studies have already done on biodiversity, biomass and carbon stock in a mature land in sundarbans but currently assessment on pioneer stand in sundarbans is a considerable issue.

By this study, we can quantify the carbon sequestration capacity in the pioneer stand that is important to measure the total carbon stock in this stage in Sundarbans. Therefore, the aim of this study is to estimate the biodiversity, biomass, carbon and nutrient contents (above and below ground) in a pioneer stage of Sundarbans. In future, this study will result in multiple benefits in addition to protecting or enhancing carbon stocks. These include 'ecosystem-based benefits' such as conservation of forest biodiversity, water regulation, soil conservation, timber, forest foods and other non-timber forest products.

1.2 Objectives of the Study

- To assess the biodiversity and biomass production in a pioneer stand in the semi fresh water zone of the Sundarbans

- To estimate the carbon stock and nutrient contents in a pioneer stand in the semi fresh water zone of the Sundarbans

Chapter 2

Literature Review

2.1. Biodiversity in Mangroves

A group of taxonomically heterogeneous woody shrubs and trees growing in the intertidal zone of tropical and subtropical coasts is expressed as "Mangrove". Consequently three dimensions of biodiversity- species, gene and ecosystem. Worldwide there are 114 species of true mangroves belonging to 66 genera with species richness being greatest in the Indo-Pacific region (Tomlinson, 1986). Sunderbans in Bangladesh covered about 3% of the world Mangroves. Mangrove ecosystem plays a crucial role on the maintenance of biodiversity, waste assimilation, cleaning, recycling and renewal as well as in protecting coastal areas from disturbance events.

2.2. Mangrove structure

It is influenced by the environmental constituents like light intensity and competition, soil fertility, PH (Koch and Snider, 1997; Utpong, 1997; Sherman et al., 1998; Tam and Wang, 1998).

2.3. Carbon Sequestration

Carbon sequestration can be defined as the capture and secure storage of carbon in soil, plants, oceans etc reducing GHG from atmosphere. Carbon sequestration also provides with associated ecosystem the co-benefits such as increases soil water holding capacity, better soil structure, improved soil quality nutrient cycling (Patil et al. 2012). Ultimately it affects the above and below ground biomass in the forest. According to USDA Forest Service (2009), "Carbon sequestration is the process by which atmospheric CO_2 is taken up by trees, grasses, and other plants through photosynthesis and stored as carbon in biomass (trunks, branches, foliage, and roots) and soils.

2.3.1. Carbon Sequestration in Mangrove forest

Mangroves can trap not only fine sediment and organic matter but also coarse sediment driven by storm waves to form special mangrove sediment. Thus, the rate of sedimentation in mangrove

is high. Besides, the litter productivity is high in Mangroves, which provides more carbon sequestrated in sediments of mangrove, indicating high below ground carbon sequestration. This indicates positive action in mangrove conservation and rehabilitation would contribute immensely to sequestrate CO_2 (Tateda 2005).

Components like NPK, organic carbon export etc. per ha, were estimated in different studies. The global storage of carbon (C) in mangrove biomass is estimated at 4.03 Pg C. The average rate of wood production is 12.08 Mg $ha^{-1}yr^{-1}$, which is equivalent to a global estimation of 0.16 Pg C/yr stored in mangrove biomass. The net ecosystem production in mangroves is about 0.18 Pg C/yr (Ong, 1993). Mangroves are important carbon sinks and sequester approximately 25.5 million tonnes of carbon every year. They also provide more than 10% of essential dissolved organic carbon that is supplied to the global ocean from land (IUCN, 2009 and Laffoley et al, 2009). Disturbed mangrove soils release greater than an additional 11 million metric tons of carbon annually.

2.3.2. Mangroves as a Climate change mitigation option

About 1.5 tones of carbon per hectare per year that mangroves are able to sequester, this is approximately equivalent to the amount of carbon released from a motor vehicle to the atmosphere each year (assuming each car uses approximately 2,500 liters of petrol per year) (Spalding et al, 1997).

2.3.3. Source of carbon in mangrove

The relevant carbon sinks is considered: the burial of sediments – locally or by adjacent systems. Therefore, carbon accumulation is not necessarily all derived from the local production (tree roots, stems, leaves, branches twigs etc); organic matter can be brought during high tide and can be originated from rivers or from adjacent coastal environments. The quantity and origin of carbon in mangrove sediments appear to be determined to a large extent by the degree of openness of mangroves in relation to adjacent aquatic systems (Kristensen, et al, 2008).

According to IPCC 2005, CO_2 sequestration is occurred by the three following ways:

2.3.3.1 Terrestrial sequestration: In this way CO_2 is absorbed by soil and vegetation near the earth's surface. Absorption of CO_2 can be increased through plantation, mitigating deforestation or adjusting forest management practices. It is the most popular and fast option for carbon sequestration at the present time.

2.3.3.2 Oceanic sequestration: Here CO_2 is buried into the deep ocean basins (350-3000 meters) at the form of liquid, supercritical or solid hydrates.

2.3.3.3 Geologic sequestration: It is the injection and underground storage of greenhouse gases, out of contact with the atmosphere. E.g. oil and natural gas fields or deep natural reservoirs filled.

2.3.4. *Biomass and carbon pools of Vegetation*

Studies revealed that the density of trees with dbh > 10 cm and the basal area were the most influential factors for biomass production (Joshi and Ghose, 2014).

CO_2 is essential to build organic chemicals that absorbed by leaves, roots, and stems (Gorte, 2009). By photosynthesis CO_2 is converted into biomass, reducing carbon in the atmosphere and sequestering (storing) it in plant tissue (vegetation) above and below ground (roots). Above ground biomass increases by two ways – (a) herbivores dies annually and decompose; (b) plants store carbon and increase biomass until death. Thus, the amount of carbon sequestered in a forest is constantly changing with growth, death, and decomposition of vegetation. Moreover the amount of carbon in soil varies widely, depending on the environment and the history of the site.

2.4. Above Ground Biomass (AGB)

To determine the carbon pool of above ground components, it is necessary first to determine the biomass of each component of the forest (e.g. trees, saplings, seedlings, palms, etc). Carbon pools of aboveground biomass are then determined by multiplying the biomass of individual components by their specific carbon concentration (percentage). Kauffman et al., (2011) reported the carbon concentration of the wood of *Bruguiera gymnorrhiza* as 46.3%, *Rhizophora apiculata* as 45.9%, and *Sonneratia alba* as 47.1%.

2.5. Below Ground Biomass (BGB)

Belowground carbon is often the largest pool in a mangrove ecosystem and measuring it is important in determining long-term dynamics associated with climate change and land management. Belowground carbon pools usually constitute over 50% and sometimes over 90%, of the total ecosystem carbon stock in mangroves (Donato et al., 2011).

Carbon pools in mangrove forests are least studied due to the difficulty in obtaining accurate estimation though the recent recognition of the importance of mangroves as global carbon stocks (Lafolley and Grimsditch, 2009, Nellemann et al., 2009, Donato et al., 2011). To measure the soil carbon pool accurately, three parameters must be quantified: 1) soil depth; 2) soil bulk density; and 3) organic carbon concentration.

2.6. Basal area

The cross section area of the stem or stems of a plant or of all plants in a stand called basal area, generally expressed as square unit per unit area. Tree basal area is used to determine percent stocking. It can be used to estimate tree volumes or occupancy and stand competition. It is mainly measured for the future development of a stand. To determine tree Basal Area simply measure the diameter at breast height (DBH) and calculate the basal area (m^2) using an equation based on the formula for the area of a circle (area = pr^2, where r = radius and p = 3.1416) and the formula for radius (r = diameter/2 = DBH/2).

2.7. Forest floor vegetation and litter

Seedlings and herbs are generally negligible for measurement of carbon pools in mangrove ecosystem. Litter is a small component of the total ecosystem carbon stock and therefore not usually sampled. If it is measured, the total oven-dry mass must be scaled to per-hectare estimation. Mean carbon concentrations of tropical forest leaf litter have been reported as 38–49% (Kauffman et al., 1993, 1995). The availability of nutrients in mangrove plant production is further controlled by a variety of biotic and abiotic factors such as tidal inundation, elevation in tidal frame, soil type, redox status, microbial activities of soil and plant species (Reef et al., 2010).

2.8. Mangrove Soil characteristics

In general, soil is heavy, slightly alkaline (P^H 7 to 7.98) with high salinity, rich in organic matter and cations (Joshi & Ghose, 2014). The surface soil (0-15 cm depth) and composed of nearly silt dominant, inner land mainly pure clayey and coastal community is high in sand. Sundarbans is sandier (6-7 %) than eastern islands (1-2 %). Ellison et al. (2000) observed comparatively lower soil salinity (5-15 ppt) in the Sundarbans of Bangladesh (Eastern Sunderbans). All these influence the vegetation structure and biomass. As the islands area remain in a formation stage, regular tidal inundation results siltation which forms platy structure of soil.

Observations were made by Saha & Choudhury (1995) and Paul *et al.* (1996) where they observed that *A. ilicifolius*, *A. alba*, and *A. marina* occur dominantly in soils with high salinity, and frequent and long duration tidal inundation in various islands of the Sundarbans. Tree height and diameter were also related to

2.9 Carbon in mangrove soil

The total carbon stock or pool (also sometimes referred to as the carbon density) is estimated by adding all of the component pools. First, each component pool is averaged across all plots (e.g. trees, soil, etc.). These average values are then summed to obtain the total.

The equation for total carbon stock or pool is as follows:

Total carbon stock (Mg ha-1) of the sampled stand = CtreeAG + CtreeBG + Cdeadtree + Csap/seed + Csap/seedBG + Cdeadsap/seed + Cnontreeveg + Cwoodydebris + Csoil

2.10 Community structure

Studies say where communities with short trees with small diameters and low basal areas had very high density; while the density of large sized trees in some other communities was low. This variability may be due to different growth rates of the tree or age difference of the communities (Joshi & Ghose, 2014). Fromard et al. (1998) observed that higher density (17,333 N ha-1) for *Avicennia* spp. in the pioneer stage than mature stage (558 N ha-1) in French Guiana. According to Singh et al., 1990; Singh & Odaki, 2004, Complexity Index, Ic values of 6.9 to 14.1 for disturbed and 87.1 to 260 for undisturbed mangroves of Andaman islands of India have been reported. Nazrul-Islam (1995) reported slightly higher heterogeneity and evenness (2.74 and 0.82) were for the mangroves of Bangladesh Sundarbans. Relatively low species

communities where in a single environmental factor predominates so that only one species is best fit to survive and becomes numerically dominant (Whittaker, 1975).

That's why in my study site, dominated species is *Sonneratia alba* (highest diameter of 32.4 cm) and then Avicennia officinalies. So the Complexity Index depends on the mixed communities with high density.

Chapter 3

Materials and Methods

3.1. Description of the Site

It was a small island which extends from 21°56′07.18″N to 21° 55′ 50.29″N and 89°34′17.37″E to 89°34′16.37″E in Sundarbans. Total area coverage was about 12.6 ha and average length 500 m and max 660 m and max width was 230 m. Mean annual temperature was about 27 ºC at summer season; relative humidity was average 82% and mean annual rainfall was approximately 1,215-1,576 mm and monthly rainfall 142-155 mm (Department of Meteorology, 2013). Soil texture was sandy-clayey and structure is platy. Dominant species are *Sonneratia apetala*, *Avicennia officinalies associated with Acanthus ilicifolius, Amoora cucullata, Ceriops decandra, Derris trifoliate* etc.

3.2. Sampling and data collection

Systematic random sampling was applied for data collection from 18 sites (15 meter interval) covering a total area of 1800 m^2 (10m×10m) distributed in the island. Diameter at breast height (DBH) of trees was measured insight the plots, while plants < 4 cm diameter were not considered as trees, they are under saplings.

For regeneration (1m×1m) plots were taken into the 100 m^2 plots. Seedling were multiplied from 1m^2 to 1 ha area.

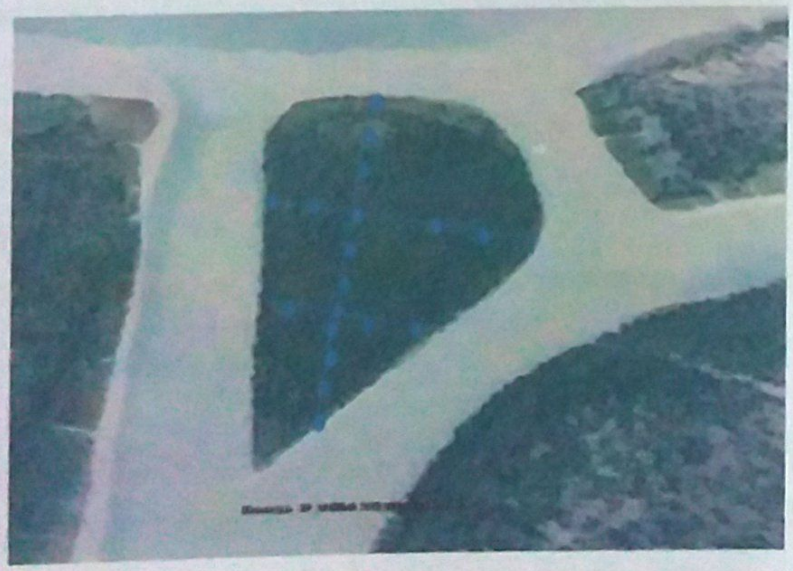


Fig. 1: Study area (Island) showing 18 plots

3.3. Species Diversity

Diversity depends on the relative abundance or richness of species in an area. A number of different measures of species diversity were calculated in this study. This exercise explored two methods for species diversity indices (Shanon-Weiner index, Simpson's Index).

Shanon-Weiner index (H')

Species diversity is described according to the value of Shanon-Weiner index (H') based on the relative abundance (proportion) of the *i* th species in the community natural log (ln). H' is calculated by

$$H' = -\sum pi \ln pi$$

It ranges from 0.0 to approximately 4.6. A value of 0.0 means that every organism in the sample is of the same species and 4.6 means the number of individuals are evenly distributed among numerous species.

Simpson's Index (Ds)

A measure that accounts for both richness and proportion (%) of each species is the Simpson's Diversity Index (Ds). It has been a useful tool for understanding the profile of biodiversity across the zones.

$$Ds = \frac{N(N-1)}{\sum ni(ni-1)}$$

Where N, the total number of individuals of all species, ni, the number of individuals of species i.

Plant species richness was estimated according to the Margalef Index

3.4. Vegetation Structure

Basal area, stem density, frequency, IVI etc were calculated in Microsoft office excel.

3.5. Basal Area

The cross sectional area of a tree stem in square meter commonly measured at breast height (1.3 m or 4.5' above ground) and inclusive of bark, usually computed by using DBH. Tree diameter was measured in centimeter. Then using the formula basal area was calculated in per hactre.

Formula for basal area:

$$\text{Basal area} = \frac{(3.1416 \times DBH2)}{4}$$

This formula then converts the diameter in centimeters to the basal area in square meters. After then it was also multiplied into basal area per hectare.

3.6. Above Ground Biomass

A non-destructive method was used to measuring above ground biomass. For above ground biomass measurement DBH of trees (>4 cm diameter) was used in the plot (10×10 m). Because height measurement in a dense muddy mangrove forest is quite difficult that's why leaves, stems, branches are collected because all these things with above ground biomass are clearly related to the variable DBH (Komiyama et al., 2005).

$$AGB = 0.251 * \rho * (D)^{2.46}$$

AGB=Above Ground Biomass, D= DBH, ρ= Density of Tree Spp. (Kg/m^3)

3.7. Below Ground Biomass

Belowground biomass is an important component in mangroves because it comprises a high portion of the ecosystem compared to upland forests (Komiyama et al., 2008). It is difficult to collect data for measurement especially in mangroves. Sub samples of different tree roots and grass roots were collected from the study area. Two sub-plots areas (50cm×50cm) were dug in 50 cm depth for root collection. Roots were collected with soils and then it was washed by nets (2mm mesh). After that, roots were sorted out coarse and fine groups depending on their diameter. The fresh weight of each subsample was measured in the field and after air dried, samples were kept for oven dry at 80°C. The total oven dry weight of roots was calculated from the ratio of fresh to oven-dry weight. This 1m^3 biomass was converted into per hectare biomass.

BGB = (dry weight of sample ×10000) / (0.5× 0.5)

Here, 1 ha=10000 m^2, area= **(0.5× 0.5)** m^2

3.8. Soil Sample processing and other vegetative parts (leafs, branches, stems)

Determination of elements in plant parts:

Nitrogen, phosphorus, potassium and carbon concentration in soil, leaf, branch, bark and stem of *Avicennia officinalies* and *Sonneratia apetala* were measured by following different standard methods (Allen, 1974).

3.8.1. Digestion of samples and determination of nutrients

3.8.1.1. Sample Preparation and Digestion for the Determination of Total N According to the Baethgen and Alley (1989).

Steps 1

1. At first take 0.1 g of plant sample in the digestion tube.
2. Add 1.1gm catalyst mixture (Potassium sulphate (K_2SO_4), Cupper sulphate ($CuSO_4$) and Selenium powder (Se) in the proportion of 100:10: 1
3. Add 3 ml of Sulphuric acid (H_2SO_4) and heat continuously to oxidize the organic matter at 200 ^{0}C for 15 minutes.
4. Raise temperature at 400 ^{0}C and heat continuously for 30 minutes.
5. Filter the digested samples through filter paper Whiteman No 1 or 2 and diluted to 100 ml.

Details of Step 2

Preparation of Catalyst Mixture: Potassium sulphate (K_2SO_4): Cupper sulphate ($CuSO_4$): Selenium (Se) = 100:10: 1

Take the following chemical with the given amount (for 20 samples)

K_2SO_4	21.62 gm
$CuSO_4$	2.16 gm
Se	0.22 gm

Details of Step 3

For the digestion of 20 samples take 65 ml of Sulphuric acid (H_2SO_4) into a beaker and then give 3 ml acid to each digestion tube through 10 ml micro-pipette.

Determination of "N"

The concentration of Nitrogen in the sample was measured by clorometric method according to Baethgen and Alley (1989).

Solution Preparation

Solution 1: Working Buffer Solution (for 180 samples, 5.5 ml for each sample)

$Na_2HPO_4.12H_2O$	35.8 g	Dilute to 1 litter with DW	Store in a cold place
N-K tartrate	50 g		
NaOH	54 g		

Solution 2: Na salicylate-Na Nitroprusside solution (for 250 samples, 4 ml for each sample)

Na Salicylate	150 g	Dilute to 1 litter with DW	Store in a light resistant bottle
Na Nitroprusside	0.30 g		

Solution 3: Na Hypochlorite Solution (for 250 samples, 2 ml for each sample)

5.25% Na hypochlorite (clorax)	30 ml	Dilute to 500 ml with DW	Prepare fresh daily

Nitrogen Standard solution preparation

Diluents preparation

K_2SO_4	19.82 g	Dilute to 1 litter with 1.1M H_2SO_4 (60 ml 98% H_2SO_4 in 1L DW)	Store it to prepare standard solution
$CuSO_4$	1.982 g		
Se	0.198 g		

Stock solution preparation (1000 ppm)

Dry NH_4Cl (Dry NH_4Cl at 105°C)	1.9095 g	Dilute to 500 ml with diluents	Nitrogen (N) stock 1000 ppm or mg N/L

Dilute the stock 10 times to prepare 100 ppm standard Nitrogen solution

1000 ppm stock	10 ml	Dilute to 100 ml with diluents	Nitrogen (N) stock 100 ppm or mg N/L

Graduated standard solution preparation for standard curve

Standard N (ppm)	Amount of 100 ppm N Stock required (ml)	Final Volume (ml)
0 (Blank)	Diluents	-
5	2.5	50
10	5	50
15	7.5	50
20	10	50

*Working range 0-50 ppm

Colorimetric determination of "N"

1. Dilute the digest as required (Generally plant sample is diluted 50 times and 5 times for soil if 0.1g plant sample and 0.5g soil sample is taken for Kjeldahl digestion)
2. Take 1 ml aliquot/diluted aliquot of digest in a test-tube
3. Add 5.5 ml of solution-1 and stir with a vortex mixer
4. Add 4 ml of solution-2 and mix again
5. Add 2 ml of solution-3 and mix thoroughly
6. Let stand for 45 minutes at 25 °C (or 15 minutes at 37 °C)
7. Do same thing as describe from 2-6 with the graduated standard solution including blank
8. After immediate stirring with vortex, read absorbance in a spectrophotometer using a wavelength of 650 nm
9. Prepare standard curve from the absorbance with the standard in the spectrophotometer
10. Note the concentration from the spectrophotometer reading

The total Nitrogen content was calculated from the following equation:

$$\textbf{TKN (mg/g)} = (C \times df \times fv) \div (W \times 1000)$$

Where,

C = Concentration obtained from spectrophotometer in ppm or mg N/L

df = Dilution factor (times)

fv = Final volume of the digest (ml)

W = Weight of soil/plant taken in digest (g)

3.8.1.2. Sample Preparation and Digestion for the Determination of Total P and K According to the Allen (1974).

Steps 1

1. Take 0.1 g of plant sample in the digestion tube
2. Add 3 ml concentrated Nitric acid and heat continuously to oxidize the organic matter at 100 °C for 50 to 60 minutes
3. Add 6.4 ml of mixed acid (Nitric acid, Perchloric acid 60% and Sulphuric acid mixed at the proportion of 10:2:1) to the predigested samples and digest at 200 °C for 20 minutes
4. Filter the digested samples through Whiteman filter paper No 42 and diluted to 100 ml

Details of step 2

For the digestion of 20 samples take 65 ml of Nitric acid into a beaker and then give 3 ml acid to each digestion tube through 10 ml micro-pipette.

Details of step 3

Preparation of mixed acid

Take the following acids with the given amount (for 20 samples)

Nitric acid	100 ml
Perchloric acid	20 ml
Sulphuric acid	10 ml

Then mix the acids carefully and give 6.4 ml of mix acid to each digestion tube through 10 ml micro-pipette.

Determination of "P"

The concentration of Phosphate in the sample was measured by clorometric method according to Timothy et al. (1984). Adding 20 ml Ammonium molybdate (3 g in 100 ml deionized water), 50 ml H_2SO_4 (35ml to 250 ml deionized water), 20 ml Ascorbic acid (5.4 g in 100 ml deionized water) and 10 ml Antimony potassium tartrate (0.34 g in 250 ml deionized water) in the solution mixture. After that the mixture was diluted 1.433 g KH_2PO_4 in 1000 ml deionized water. Stock solution was diluted to prepare standard solution of different concentration for standard curve and 1 ml of mixed solution was added with 10 ml of standard solution and sample. Absorbance

was measured at 885 nm by UV-visible Recording Spectrophotometer (HITACHI, U-2910, Japan).

The total Phosphorus content was calculated from the following equation:

$$\text{Phosphorus content (mg/g)} = \frac{\text{Phosphate content in sample x Atomic weight of Phosphorus}}{\text{Atomic weight of Phosphate}}$$

Determination of "K"

Potassium concentrations of the samples were measured by Flame Photometer (PFP7, Jenway LTD, England).

3.8.1.3. Determination of Organic Carbon (C)

Organic carbon in plant sample was determined by ignition method (Allen, 1974). Oven-dried plant samples (1 g) were placed in the muffle furnace (Digital Muffle Furnace, FH-05, DAIHAN Scientific Co Ltd., Korea) for four hours at 450 °C. After ignition, the samples were then placed in a deccicator to allow it to room temperature and the weight of the ignited sample was taken. Percentage of loss on ignition was calculated from the following calculation.

$$\text{Loss on ignition (\%)} = \frac{\text{Loss of weight (g)}}{\text{Oven dry weight (g)}} \times 100$$

The organic carbon in the plant samples were estimated from the 50% of ash free dry weight (Allen, 1989).

3.9 Soil carbon estimation

3.9.1 Bulk density measurement

At first the steel cylinder of soil core was covered with foil paper and then it was placed in trays to be oven dried at 105°C for 48 hours. After oven drying, the weight of the soil was taken by an electric balance. A structured data sheet was prepared for the calculation of bulk density. Then the following equation was used to calculate the bulk density.

Bulk density = Oven dry weight of the soil sample / Volume of the cylinder (g/m^3)

= Oven dry weight of the soil sample / $\pi r^2 l$ (g/m^3)

Here, r = radius of the cylinder (m), l = height of the cylinder (m) and π = constant

3.9.2 Percentage of organic matter (%OM) measurement

Loss on Ignition (LOI) method was used to determine the organic matter content (%OM) of a soil sample. It does not involve the use of any chemicals, only the use of a muffle furnace. LOI calculates %OM by comparing the weight of a sample before and after the soil has been ignited. The difference in weight before and after ignition represents the amount of the OM that was present in the sample. The subsample was taken for carbon analysis was put on the porcelain crucibles samples can then be placed in trays to be oven dried at 105°C for 5 days. Then the oven dried weight of the samples was measured. For LOI we used 24 porcelain crucibles in a muffle furnace for 12 samples at a time. Then the sample was ignited with 500°C within the muffle furnace. A structured data sheet (Appendix:) was made for the calculation of % OM. Then the following equation developed by Bengtsson and Enell 1986, was used to compute the % OM.

$\%OM = ((DW_{105}°C - DW_{550}°C)/DW_{105}°C) \times 100$

Then, Soil carbon stock was calculated by using the equation given by Daniel et al. 2009 for each plot.

Soil C (Mg/ha) = bulk density (g/m^3) × soil depth interval (m) × % OC × 0.01 (Daniel et al. 2009)

Chapter 4

Result and Discussion

4.1 Results:

4.1.1 Biodiversity

A total of 13 species (5 trees, 1 thorny herbs, 4 shrubs, 2 climbers, 1 palm) (source: Chaffey, D. R. and Sandom, J. H. (1985)) and in the study site we found 4 species as tree (>4 cm), 9 as saplings and 12 as seedlings. Trees, saplings, seedlings in 18 sampling plots were assessed into multivariate approach. Other data including Diversity Indices, basal area, density, species richness etc are given in Table 1

Table. 1: Diversity Index for tree

Species Name	Shannon index H'	Simpson Index, Ds	Margalef Index, Spp.	Number of spp	plot coverage
Avicennia officinalies	0.77	1.97	0.53	102	14
Kandelia candel				5	3
Excoecaria agallocha				2	1
Sonneratia apetala				177	16
Total				286	

Table 2: Diversity Index Saplings

Species name	Shannon index H'	Simpson Index, Ds	Margalef Index, Spp.	Number of spp.	plot coverage
Avicennia officinalies	1.63	4.19	1.50	73	14
Kadelia candel				1	1
Dalbergia spinosa				6	2
Derris trifoliata				32	4
Excoecaria agallocha				61	3
Heritiera fomes				7	3
Nypa fruticans				10	5
Sonneratia apetala				18	4
Xylocarpus mecongensis				1	1
total=				209	

Table 3: Diversity Index Seedlings

species name	Shannon Index H'	Simpson Index, Ds	Margallef Index, Spp.	Number of spp.	Plot coverage
Acanthus ilicifolius				90	15
Amoora cucullata				11	8
Avicennia officinalis				15	6
Kandelia candel	1.86	5.18	1.94	2	2
Ceriops decandra				48	2
Dalbergia spinosa				1	1
Derris trifoliata				9	3
Excoecaria agallocha				30	3
Heritiera fomes				15	3
Sarcolobus globosus				1	1
Sonneratio apetala				68	5
Xylocarpus mekongensise				1	1
total=				**90**	

4.1.2 Vegetation Structure:

The stand was mainly dominated by 2 tree species *Sonneratia apetala and Avicennia officinalies.* Assessing the number 286 (100 m^2 plot area) of trees, 209 (100 m^2) of saplings, 291 (1m^2) of seelings, we have evaluated the percentage of plants distribution per hectare area in the study site

Table. 4: Species counted and their percentage in per hectare area in the study site.

	Total observed Spp.	Family	Percentage of Distribution (ha^{-1})		
			Tree (%)	Saplings (%)	Seedlings (%)
1	*Acanthus ilicifolius*	Euphorbiaceae		3492 83	309278 35
2	*Amoora cucullata*	Meliaceae			37800 68
3	*Avicennia officinalis*	Asclepiadaceae	3566 433566		51546 39
4	*Ceriops decandra*	Acanthaceae			164948 45
5	*Dalbergia spinosa*	Papilionaceae		287 08	3436 42
6	*Derris trifoliata*	Meliaceae		1531 10	30927 83
7	*Excoecaria agallocha*	Avicenniaceae	69 93006993	2918 66	103092 78
8	*Heritiera fomes*	Papilionaceae		334 93	51546 39
9	*Kandelia candel*	Sterculiaceae	174 8251748	47 85	6872 85
10	*Nypa fruticans*	Sonneratiaceae		478 47	0
11	*Sarcolobus globosus*	Rhizophoraceae		0	3436 42
12	*Sonneratio apetala*	Rhizophoraceae	6188 811189	861 24	233676 97
13	*Xylocarpus mekongensise*	Palmae		47 84	3436 43

Consolidated details of vegetation structure of a pioneer state of Sunderbans.

Table. 5: Tree

Species name	Mean DBH (cm)	Basal Area (m2/ha)	Density (stem/ha)	Relative Density (%)	Frequency	Relative Frequency (%)	Relative dominance (%)	Importance Value (%)
Avicennia officinalies	8 82± 001	3 46± 004	566 67	3542	77 78	41 18	17 50	31 45
Kandelia candel	4 22± 001	0 039± 001	27 78	174	16 67	8 82	0 20	3 59
Excoecaria agallocha	4 20± 001	0 015± 001	11 11	69 4	5 56	2 94	0 08	1 24
Sonneratia apetala	14 51± 001	16 26± 005	983 33	6146	88 89	47 06	82 23	63 73
Total=		19 774	1588 90		188 89			

Table. 6: Saplings

Species name	Family	Density (stem/ha)	Relative Density %	Frequency %	Relative Frequency %
Avicennia officinalies	Avicenniaceae	405.56	34.93	77.7778	37.84
Kadelia candel	Rhizophoraceae	5.56	0.478	5.55556	2.703
Dalbergia spinosa	Leguminosae	33.33	2.871	11.1111	5.405
Derris trifoliata	Leguminosae	177.78	15.31	22.2222	10.81
Excoecaria agallocha	Euphorbiaceae	338.89	29.19	16.6667	8.108
Heritiera fomes	Sterculiaceae	38.89	3.349	16.6667	8.108
Nypa fruticans	palmae	55.56	4.785	27.7778	13.51
Sonneratia apetala	sonneratiaceae	100	8.612	22.2222	10.81
Xylocarpus mecongensis	Meliaceae	5.56	0.478	5.55556	2.703

Table: 7: Seedlings

Species name	Family	Density (stem/ha)		Relative Density	Frequency %	Relative Frequency %
Acanthus ilicifolius	Acanthaceae	500	30.93		83.33	30
Amoora cucullata	Meliaceae	61.11	3.78		44.44	16
Avicennia officinalis	Avicenniaceae	83.33	5.15		33.33	12
Kandelia candel	Rhizophoraceae	11.11	0.69		11.11	4
Ceriops decandra	Rhizophoraceae	266.67	16.49		11.11	4
Dalbergia spinosa	Leguminosae	5.56	0.34		5.56	2
Derris trifoliata	Leguminosae	50	3.09		16.67	6
Excoecaria agallocha	Euphorbiaceae	166.67	10.31		16.67	6
Heritiera fomes	Sterculiaceae	83.33	5.16		16.67	6
Sarcolobus globosus	Asclepiadaceae	5.56	0.34		5.56	2
Sonneratio apetala	sonneratiaceae	377.78	23.37		27.78	10
Xylocarpus mekongensise	Meliaceae	5.56	0.34		5.56	2
total=		1616.67			277.78	

4.1.2.1 Importance Value Index

Mean values of relative density (RD), relative frequency (RF), relative dominance (R Do) combinely represent the importance value index (IVI) of tree species in a stand. Here higher IVI of *Sonneratia apetala and Avicennia officinalies* showed that its basal area, richness greater than other spp. in the study site.

4.1.2.2 Species area curve:

Species-area curves for different plant strata were leveled at various m^2 scale (Fig.1, 2, 3). The rate of climb of species area curve was nearly the same for trees, saplings and seedlings (0.4, 0.9 and 0.7 respectively) for the study site. It was almost saturated at the range 1100-1300 m^2 plot area.

Fig. 2: Species area curve for tree

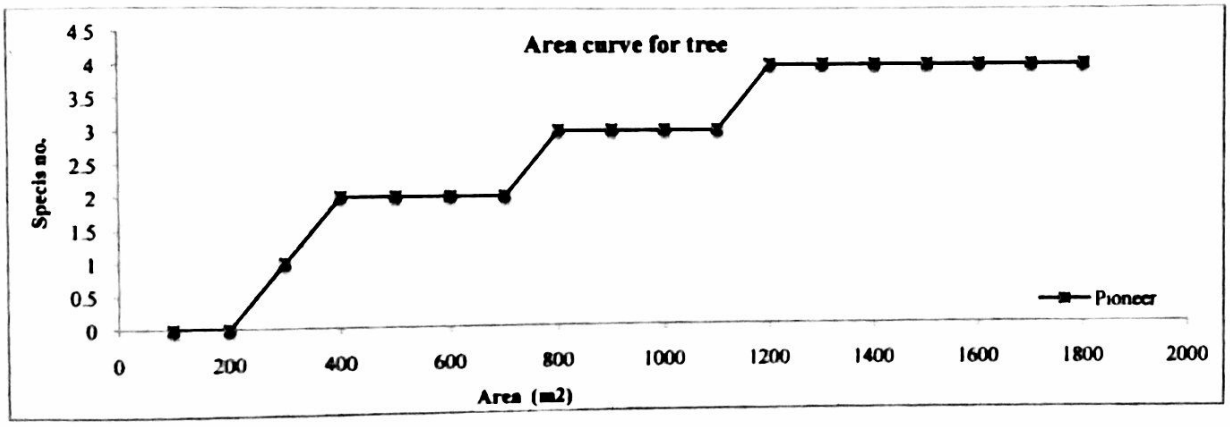


Fig. 3: Species area curve for saplings

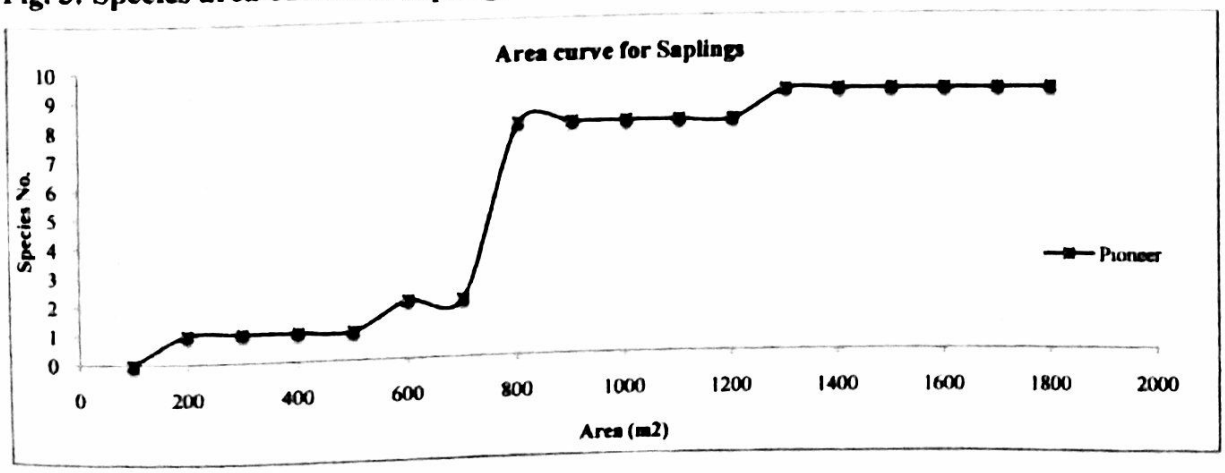
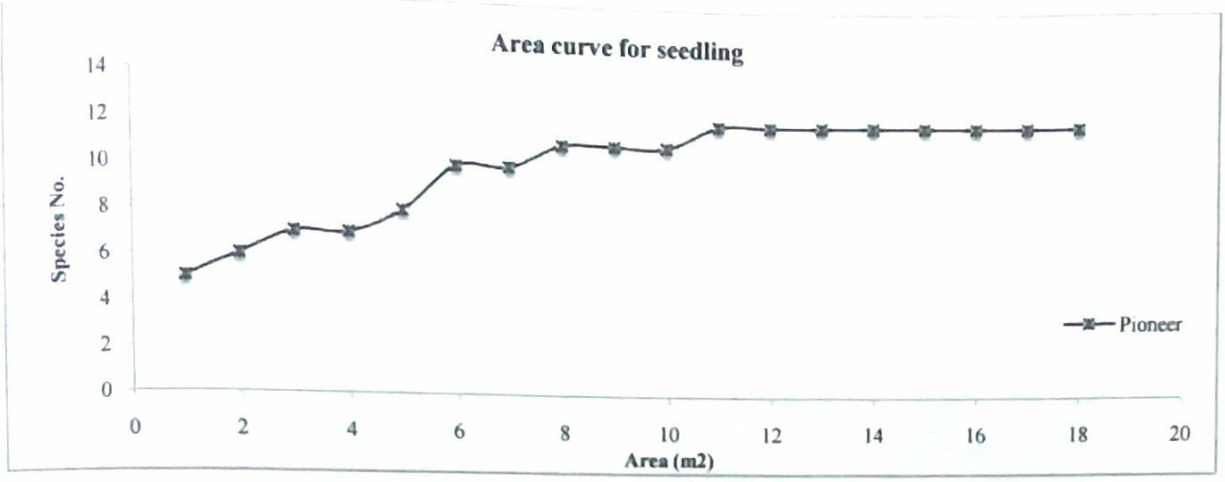


Fig. 4: Species area curve for seedling



4.1.2.3 Saplings and Regeneration distribution:

In the survey we observed that the area is inundated by the regular tide, that's why regeneration and sapling coverage in the island was poorer than the mature stand of Sundarbans. Total 18 plots were selected for data collection in the study site. Maximum plots were dominated by tree species. Marginal lands were covered by grass roots, seedlings saplings, climbers etc. *Acanthus ilicifollius* was most aggressive species in the island. *Sonneratio apetala, Ceriops decandra, Excoecaria agallocha* etc were mainly observed in regeneration plots. In the middle portion regeneration was rare, was clayey ground, less light availability etc. Fig 4 and 5 show the situation.

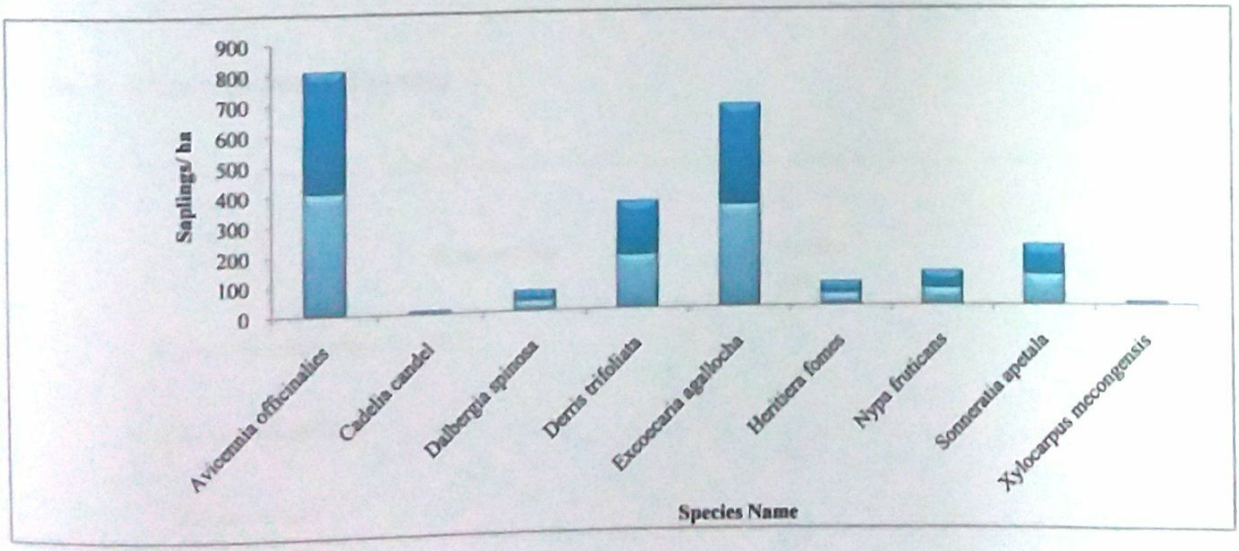


Fig. 5: Saplings coverage

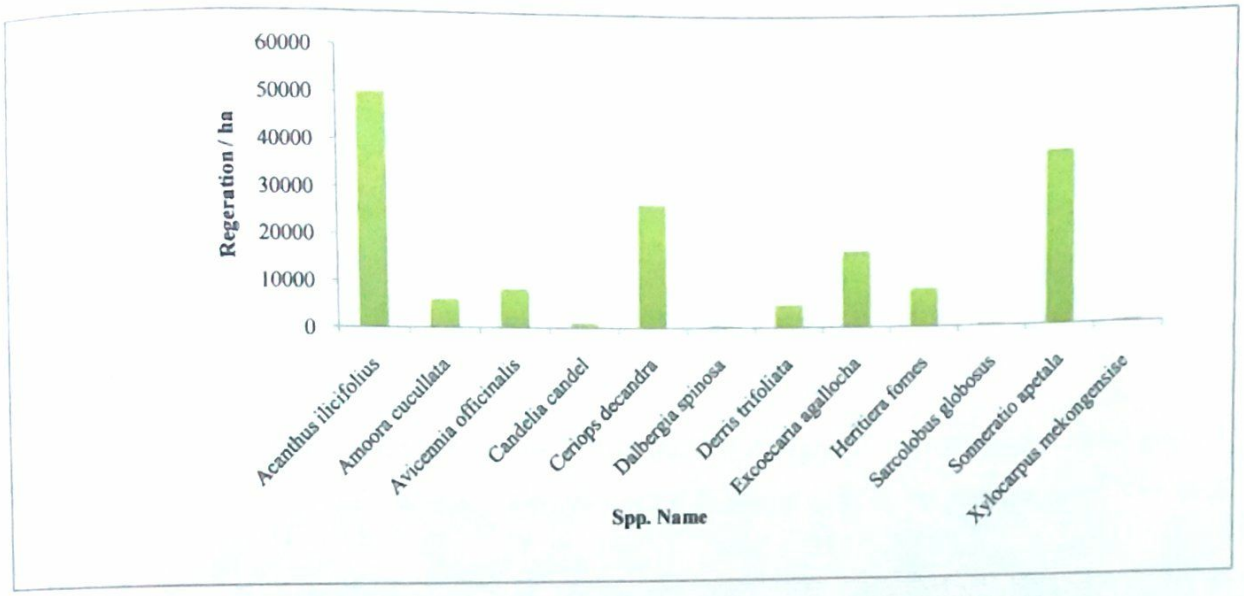


Fig. 6: Regenerations

4.1.3 AGB, BGB and Carbon Stock:

Here for estimation of AGB we had applied DBH of the dominant Spp. in the study area. Results show that the AGB was 3 times higher than BGB at 50 cm depth in 1 hectare area. BGB was estimated by the total volume of different roots below the ground in 50 cm^3 plot size. AG carbon and BG carbon both are the 50% of AGB and BGB respectively.

Table. 8: Above Ground Biomass

	Biomass (t/ha)	Density
		0.67
Avicennia officinalies	16.53	
		0.334
Excoecaria agallocha	0.04	
		0.557
Kandelia candel	0.1	
		0.57
Sonneratia apetala	81.86	

Table. 9: **Carbon pool**

Carbon pool	
Above Ground (standing tree)	49 26
Root Biomass	16
Soil	132 02
Total	**197.28**

Results showed that Philippines and mature Sundarbans forest stands contain more above ground biomass than below ground biomass except which was opposite to this study area. Fig 5 is showing the result

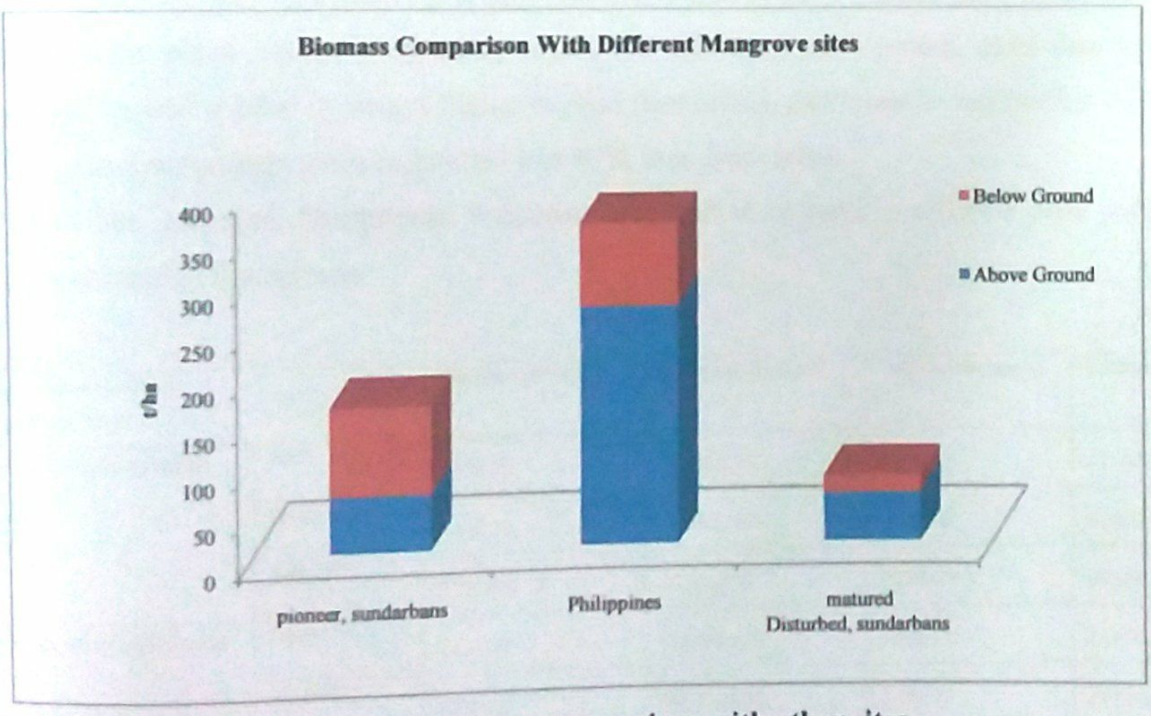


Fig. 7: Biomass comparison with other sites

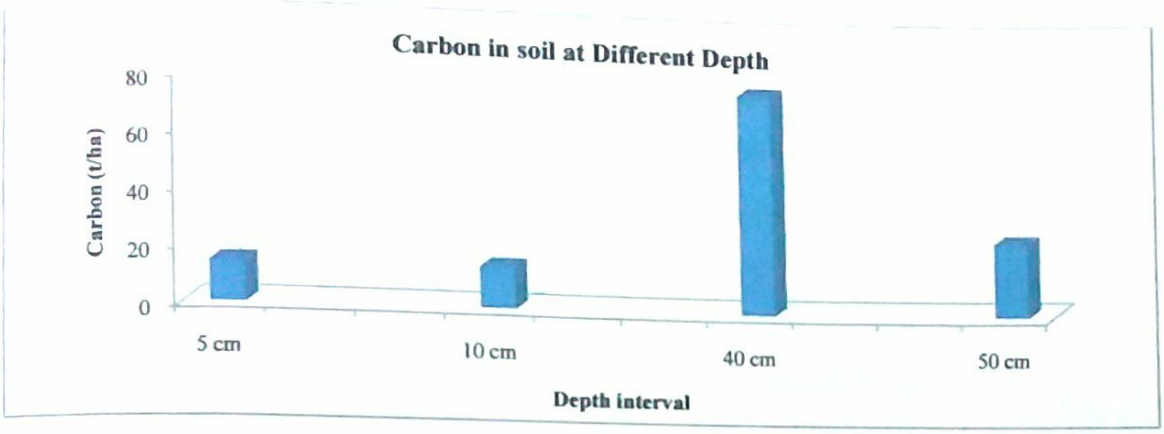


Fig 8: Carbon Storage at different depth

4.1.4 Nutrient contents:

4.1.4.1 Nutrients in plants

Leaf sample of both *Avicennia officinalis and Sonneratio apetala* contain more NPK than other parts of the plants except phosphorus *Avicennia officinalis* stem contain more than leaf and branch. Carbon content is always higher in stem than branch and lesser in leaf portion of plants. Different roots contain more carbon but less NPK than grass roots.

Table. 10: Nitrogen, Phosphorus, Potassium and Carbon contents in different plant parts in a pioneer stand in Sundarbans

Chemical Contents In Plant Parts		Nitrogen (mg/g)	Phosphorus (mg/g)	Potassium (mg/g)	Carbon (%)
Sonneratia apetala	Leaf	2.72±.20	95.79±2.19	6.81±.12	43.79±.23
	Branch	0.45±.12	43.16±1.05	4.09±.16	47.65±.10
	Stem	5.78241E-19	24.56±2.30	2.024±.06	49.30±.29
Avicennia officinalis	Leaf	3.22±.02	111.57±1.60	5.96±.37	44.15±.10
	Branch	0.90±.02	103.51±4.46	4.45±.12	47.60±.16
	Stem	0.055±.01	114.74±1.60	2.08±.06	49.53±.0001
Different Roots	DR	0.008±.01	62.46±.9	11.54±.26	17.24±.95
Grass Roots	GR	0.044±.03	87.36±8.04	12.63±.32	43.43±.09

4.1.4.2 Soil Nutrients:

Nutrient contents in soil were very negligible because of regular tidal flow across the land. In the table we can see that carbon contents and nutrient contents extend from lower depth higher (5cm to 10 cm) and high amount in 40 cm. But 50 cm depth shows lower amount than 40 cm but higher than others. Surface soil carbon is in very low amount. That means nutrients and carbon contents richness in 40 cm depth.

Table 10: Soil Carbon at 5, 10, 40 and 50 cm depth in two soil pits

SOIL SAMPLE	Phosphorus (mg/g)	Nitrogen (mg/g)	Potassium (mg/g)	Carbon (%)
Soil Core 5 cm	10.38 ± 1.92	0.05 ± .007	0.66 ± .04	28.53 ±2.43
S0il Core 10 cm	10.29 ± 1.86	0.05 ± .01	0.56 ± .01	27.93 ± .88
Soil Core 40 cm	12 ± .42	0.04 ± .02	0.70 ± .03	152.19 ± 9.82
Soil Core 50 cm	11.54 ± .69	0.01 ± .006	0.41 ± .01	51.11 ± 3.26

4.2 Discussion

In this study, the ABC carbon stock in a pioneer stand was 49.26 t/ha and BGC (root & soil) was 150.09 t/ha where total AGB and BGB is double in amount. Only dominated tree species were used for AGB measurement, this amount indicates the huge potentiality for future development stand. Though for pioneer stand less research has done, but comparing with the mature stand like Philippines or other part of the Sundarbans islands species diversity is poor here and species diversity in our study site was for tree 0.77, saplings 1.63, and seedlings 1.86 when in Philippines 0.99 and total AGB is 561.20 t ha^{-1}. Total carbon stock 266.47 t/ha in soil only in our island when in Philippines 173.75 t/ha carbon stock.

Chapter 5

Conclusion

This study had found that variety of species types and structures, with different dominant species exist in the island. The species composition and prevailing conditions of biodiversity and biomass indicates an evolving or expanding forest that seem conducive to sustain a mature forest ultimately. The mangrove forests are under immense pressure from clear cutting, land-use change, hydrological alterations, chemical spill and climate change. The information generated from this study will serve as a baseline and offers an interesting array of attributes for further research.

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