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FORESTRY AND WOOD TECHNOLOGY DISCIPLINE LIFE SCIENCE SCHOOL KHULNA UNIVERSITY KHULNA 9208 BANGLADESH

Allometric relationship for estimating above-ground biomass, nutrients and Carbon of *Aegiceras corniculatum* of the Sundarbans, Bangladesh

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FORESTRY AND WOOD TECHNOLOGY DISCIPLINE

SCHOOL OF LIFE SCIENCE

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2015

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This Project Thesis Submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh, in Partial Fulfillment of the Requirements for the Professional M.Sc. Degree in Forestry.

Dedicated

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My Beloved Parents

Abstract

Aegiceras corniculatum is an exclusive mangrove species but separately distributed at the high saline areas of the Sundarbans and they often found in association with Rhizophora apiculata, Rhizophora mucronata, Candelia candle and Ceriops decandra. Allometric equations were used to estimate biomass of leaf, branches, bark, stem without bark and total above-ground biomass of A. corniculatum. Ten linear equations with DBH as independent variable were tested for each part of plant. All the regression equations were significant (p<0.05), but highest R² (0.93-0.99) and F-values and lowest MS error were observed for Log-Log equition (Log₁₀y = a Log₁₀x + b). The selected allometric models were Log Leaf biomass = 1.52 Log DBH - 1.39; Log Branch biomass = 2.20 Log DBH - 1.48; Log Bark biomass = 2.08 Log DBH - 1.80; Log stem biomass = 2.08 Log DBH - 0.99; Log Total biomass = 2.06 Log DBH - 0.73. Comparatively higher amount of carbon was observed in stem whereas as the lower content amount was observed in bark. Nutrients (N, P and K) concentration significantly (p<0.05) varied among the plant components and comparatively higher concentration of nutrient was observed in leaf whereas as the lower content was observed in stems. The element content in and total above-ground biomass were calculated and allometric equations were developed for total above-ground element content. The selected allometric models were, Log N = 1.91 Log DBH - 0.25; Log P = 1.93 Log DBH + 0.01; Log K = 2.02Log DBH - 0.01; Log C = 2.06 Log DBH - 1.05.

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At last, my sadness, happiness and emotion always act on all of my well wishers, whose bundles love and affection drive me every moment to do something for life here and life here after.

Declaration

V

I hereby declare that the project thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Khulna University or other institutions.

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Approval

Project thesis submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh, in partial fulfillment of the requirements for the professional M.Sc. degree in Forestry. I have approved the style and format of the project thesis.

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Chapter-1

1.1 Introduction

Mangroves are found in sheltered intertidal zones of tropical and subtropical regions and can tolerate varying degrees of salinity (Ong, 1993). They are unique ecosystems prominent in vulnerable coastal strips all over the world. Mangroves got special interest in research after its importance in estuarine detritus food web was proved (Heald, 1969; Odum, 1970; Odumand Heald, 1972). Aegiceras corniculatum is a widespread mangrove species and one of major species of mangroves in the Sundarbans. It can withstand a high level of salinity. It is both a salt secretor and salt extruder. Aegiceras corniculatum is well known as a tree of high honey production. As mangroves grow on muddy and anaerobic soil, which suffers from tidal inundation, they show a unique pattern of biomass allocation (Komiyama et al., 2005). The biomass of mangrove forests has been studied for several decades (Clough and Scott, 1989; Clough et al., 1997; Komiyama et al., 1998, 2000, 2002; Ong et al., 1995, 2004; Tamai et el., 1986, Mahmood et al., 2008) by using allometric relations. Allometry is a nondestructive powerful tool for estimating the whole or partial weight of a tree from measurable tree dimensions (Komiyama et al., 2005, Mahmood et al., 2008).

In recent decades, research on mangroves has been focused on the diversity of species, resources, flora, physiological ecology, different stages of organic matter cycling in the mangroves (Soares, 1997), examining litter production and decomposition, export of dissolved and/or particulate organic matter, consumption by the mangrove resident fauna, incorporation of organic matter into the substratum, and its export through the assimilation and feeding by organisms that spend part of their life cycle in the mangroves, which, when they leave the ecosystem, become export agents of assimilated matter. However, as far as ecological anatomical research is concerned, little work has been done (Janssonius, 1950; Lin, 1988; Panshin, 1932; Tomlinson, 1986). To better understand the dynamics of organic matter cycling in the mangroves; it is important to know the amount of biomass that is present in the vegetation covering at a given time through the analysis of existing studies on the biomass of mangrove species.

Different mangrove species at different localities shows wide range of biomass values and shows different trend of biomass allocation in their components (Steinke et al., 1995; Tam et al., 1995; Suzuki and Tagawa, 1983; Clough and Attiwill, 1975; Gong and Ong, 1990). Different tree species usually vary in their architecture due to variation in forest types, stand density or canopy closure (Clough and Scout, 1989). As allometric equation of mangrove species does vary greatly among sites for the same species (Komiyama et al., 2008), it is preferable to use species-specific allometric equations for biomass estimation (Ketterings et al., 2001; Mahmood et al., 2008). Nutrient removal by harvest and its potential consequences on future nutrient cycling and productivity of a forest have been well documented (White, 1974; Hansen and Baker, 1979; Morrison and Foster, 1979; Tritton et al., 1987; Federer et al., 1989; Hornbeck et al., 1990). Information on biomass stocking and nutrient distribution in both above and below-ground parts of trees are essential for assessing sustainable production and as well as evaluating the impact of various silvicultural practices (Santa Regina, 2000). The present study is designed to derive allometric models of above-ground biomass, nutrients and carbon stock in Aegiceras corniculatum of the Sundarbans.

1.2. Objectives: The objectives of this study were

- To derive allometric models for estimating above-ground biomass of different parts of Aegiceras corniculatum in the Sunderbans.
- To calculate the nutrients (N, P and K) and Carbon concentration in different parts (leaves, branches and stems) of Aegiceras corniculatum.
- To drive allometric models for estimating nutrients (N, P and K) and carbon stock in above ground biomass of Aegiceras corniculatum.

Chapter-2

Literature review

2.1. Description of species

Aegiceras corniculatum, commonly known as Black Mangrove, River Mangrove or Khalsi is a species of shrub or tree mangrove in the Myrsine family (or Primrose family) with a distribution in coastal and estuarine areas ranging from Bangladesh through South East Asia to southern China, New Guinea and Australia. Aegiceras corniculatum grows as a shrub or small tree up to 7 m high, though often considerably less. The leaves are alternate, simple, spirally arranged, leathery in texture and hairless. They are elliptic to obovate in shape, 4-8 cm long, 1.8-4 cm wide, with a rounded to slightly notched tip and a wedge-shaped base. Its fragrant, small, white flowers are produced as umbellate clusters of 10–30, with apeduncle up to 10 mm long and with pedicels 10–18 mm long. The calyx is 2–4 mm long and corolla 4-6 mm long. The fruit capsule is horn-shaped, 3.8-8 cm long, has a persisting calyx, light green to pink in color, and is crypto-viviparous, enclosing 1 propagule.

Figure: Khalshi (Aegiceras corniculatum)

2.2. Taxonomical classification of A. corniculatum

Taxonomical classification of A. corniculatum is as follows, (Roome et al, 2011)

Kingdom:

Plantae

Division

Spermatophyta

Sub division

Angiosperms

Class

Eudicots

Sub class

Asterids

Order:

Ericales

Family:

Myrsinaceae (or

Primulaceae)

Genus:

<u>Aegiceras</u>

Species:

A. corniculatum

2.3. Distribution

Aegiceras corniculatum is widely distributed across the Indo-West Pacific from India and Sri Lanka through Asia to Polynesia and Australia. In Australia, the species occurs in most estuaries and embayments from Cossack, Western Australia (20° 40′ S, 117° 12′ E) in the west, across the Northern Territory and Queensland, to Merimbula, New South Wales (36° 53′ S, 149° 55′ E) in the east. It is also found on Lord Howe Island. (Duke, 2006)

2.4. Habitat

Aegiceras corniculatum is tolerant of a wide range of growing conditions and as a consequence is found across a range of tidal environments. It can tolerant varying levels of salinity and sunlight and grows in a variety of soil types. It often occurs as a dense subcanopy bordering on the fringe of tidal creeks and river margins, whereas in coastal

mangrove habitats it is most commonly found along landward margins. Low intertidal, intermediate-upstream estuarine position (Duke, 2006).

2.5. Phenology

In Australia, plants flower from May to October, with fruit maturing from December to March. These events tend to occur later in higher latitude areas, particularly along the east coast into New South Wales. The sweet smell extruded from the flowers suggests they are bee pollinated. Aegiceras corniculatum is generally known to be viviparous, although Saegner suggests the embryos do not pierce the pericarp until the fruit has fallen from the parent plant, therefore it is not strictly viviparous. The propagule is suited to water dispersal due to its buoyancy and the tendency for fruit to fall during periods of regular diurnal flooding. Aegiceras corniculatum occurring in downstream locations is likely to cohabitate with Avicennia marina, Sonneratia alba and Rhizophora stylosa. In contrast, where it occurs in upriver, brackish waterways in tropical regions it is likely to cohabitate with Acanthus ilicifolius, Sonneratia caseolaris, Sonneratia lanceolata and Rhizophora mucronata (Duke, 2006).

2.6. Pollination

Pollination is occured by small insect and night flying insects; moths have been observed visiting the flowers, presumably for the small quantity of nectar secreted by the disc, but bees may be daytime visitors. Pollen release is not an explosive manner (Tomlinson, 1986).

2.7. Reproduction

Aegiceras corniculatum showed viviparous germination seeds. The seed starts germinating and growing its hypocotyl inside the fruit. When it is mature, the embryo, together with the fruit, will detach and stick into the soil and grow its roots and leaves; Black mangrove's seeds do not have the rod-like hypocotyl. They produce two folded, broad oval cotyledons. When the fruit detaches, the cotyledons will spread and float on the water, hence root quickly after landing (Duke, 2006).

2.8. Adaptations

2.8.1. Adaptation to salt

Aegiceras corniculatum is a species of high salt tolerance. Six hundreds of EST were obtained from the leaf SSH library of A. corniculatum under salt-stress. P5CS(1-pyrroline-5-carboxylate synthetase), which was related to osmotic regulation, and two aquaprin genes, which participate in water transport, were up-regulated in A. corniculatum by salt stress. Expression patterns of these 2 aquaprins also indicated that A. corniculatum could recover from long-term salt stress and adapt to saline environments. There are several ongoing projects, including transfering P5CS and CPI (coding for cysteine proteinase inhibitor) genes of A. corniculatum into Arabidopsis, and microarray analysis of transcript profiling in A. corniculatum could further help to depict mechanisms of adaptation and evolution in this species (Tomlinson, 1986).

2.8.2. Root

Aegiceras corniculatum has neither supporting roots nor respiratory roots, but bigger buttress root.

2.8.3. Anaerobic Soil

There are ventilating tissues called aerenchyma. They are hollow cells that decrease the resistance of air diffusion; normal plant cells contain 2-7% of air, but mangrove cells contain 40%.

The root system of Aegiceras corniculatum is like thick ropes, and at intervals it develops erect pneumatophores which stick out from the ground for 30 cm. They facilitate air diffusion. Knee joints of the Many-petaled Mangrove are root arcs that stretch out from the soil. Inside them a lot of aerenchyma can be found. There are ventilating surfaces called lenticels in respiratory roots and knee joints.

2.8.4. Soil and water of high salinity

Like all other halophilous plants (plants that grow on saline soil or soil that affected by salt water), mangroves can tolerate higher internal salinity. However, mangroves balance the osmosis through accumulating carbohydrates of small molecular weights inside their bodies instead of proline, an amino acid that most halophilous plants accumulate for the balance.

2.8.5. Salt Exclusion

Aegiceras corniculatum can prevent salt from entering xylem of the roots and stop salt being transported to tissues through ultra filtration. They can also expel extra salt from the roots by an active pump mechanism. These salt exclusion plants can maintain a low salinity, only 30% of that of non-salt exclusion plants.

2.8.6. Salt Excretion

Aegiceras corniculatum can keep excreting salt inside the tissues through salt glands on the leaves.

2.8.7. Xeromorphic Characters

Mangroves are xeromorphic plants, therefore they must preserve a high density of water to minimise absorption of sea water, which has a high salinity. To adapt this unique habitat, mangroves develop some special features:

- Succulence—some species of mangroves contain water storing tissues in the leaves
- Thick cuticle—ceraceous cuticle and periderm
- Sunken stomata—concave breathing pores at the back of the leaves
- Buttress root—thick roots to absorb water on the soil surface

2.9. Biomass allometric models

2.9.1. Allometry

Allometry is all about studying the relative sizes of plant parts. Usually, relationships between dbh (diameter at breast height, or 1.37 m up from ground level), tree height, total biomass, leaf weight, etc., are calculated. For example, what we do here is prepare equations (regressions) to calculate the total above-ground biomass of Agiceras corniculatum tree as a function of dbh, we are therefore implicitly assuming that biomass is directly related to tree diameter.

The subject of allometry is variation in morphometric variables or other features of organisms associated with variation in size. Such variation can be produced by several biological phenomena, and three different levels of allometry are therefore distinguished: static allometry reflects individual variation within a population and age class, ontogenetic allometry is due to growth processes, and evolutionary allometry is the result of phylogenetic variation among taxa. Most multivariate studies of allometry have used principal component analysis. Variation in size of organisms usually is associated with variation in shape, and most metric characters are highly correlated among one another. These associations are the subject of allometry (Huxley, 1932; Cock, 1966; Gould, 1966, 1975). Although allometry is often used to examine the consequences of size for ecological or physiological variables (Giinther, 1975; LaBarbera, 1989; Reiss, 1989), this review deals only with measurements of traits used to characterize the morphological form of organisms.

Unlike other approaches in morphometrics, which are built on geometric theory, allometry has a largely empirical basis. Huxley (1932) realized that scatter plots of two trait measurements in growing organisms often closely follow a curved line, and that this relationship usually becomes linear if both measurements are transformed to logarithms. From this, he derived his formula of simple allometry

$$y = bx^a$$

Or in log-transformed notation,

$$Log y = a log x + b$$

Where x and y are trait measurements, and a and b are constants. The constant a, the slope in log-log plots of s and y, is often called the allometric coefficient (terminology is not uniform; some authors call b coefficient). The special case when a = I is called isometry, and indicates direct proportionality between x and JJ. If a > 1, there is positive allometry, whereas for negative allometry, a < 1 (Huxley and Teissier, 1936). In humans, for example, the long bones of the limbs show positive allometric growth relative to overall stature, and the height of the head shows negative allometry.

In most morphometric data sets, measurements are positively correlated, i.e., .u and JI increase or decrease simultaneously. Even if there is negative allometry, a still is positive; negative allometry implies only that the relative variation in y is smaller than that in x, e.g., v grows by 10% for every 20% growth increment in x. If a is negative, however, there is an absolute reduction in y associated with an increase in x. This case is called enantiometry (Huxley and Teissier, 1936). Reduction of the absolute size of organs during growth is a real phenomenon, although it is not found commonly in morphometric studies. The most striking example is the shrinking of larval structures during metamorphosis, e.g. the gills and tail of anuran tadpoles; but in a subtler way, enantiometry even occurs in cranial growth of primates (Comer and Richtsmeier, 1991). Huxley's approach is not restricted to pairs of measurements. In many multivariate data sets, log-log plots of all pairwise combinations of morphometric variables show approximately linear relationships. Therefore, Huxley's bivariate allometry can be generalized to multiple dimensions. Moreover, it is not confined to growth data, as straight-line relationships are also found in log-log plots of intra- and interspecific variation within one particular ontogenetic stage (most often adults.

Chapter-3

Materials and method

3.1. Study site

The study area is Shatkhira administrative range of the Sunderbans mangrove forest of Bangladesh. The total area is situated between 22°11.226′ N; 89°07.768′ E and 22°14.062′ N; 89°11.679′ E. This range is bordering the Indian Sunderbans at the west, Satkhira Range at the east and Bay of Bengal at the South. Rainfall in the area varies around 1800 mm per year and the average temperature varies from 28-30 °C in summer and 18-20 °C in winter. The mangrove forest in this range is experienced by higher saline water. The major species of this area is Avicennia alba, A. officinalis, A. corniculatum. Xylocarpus granatum, X. mekongensis, A. rotundifolia, Lumnitzera racemosa, Execoaeria agallocha, and Ceriops decandra (Siddiqi, 2001).

3.2. Selection and collection of samples

A total of 43 individual of *Aegiceras corniculatum* having Diameter at Breast Height (DBH) from 1 to 15 cm were taken randomly (avoiding mechanically or insect damaged or infested with disease) from the study area.

3.3. Sample processing and allometric relationships

The felled individuals were then separated into leaves, bigger branches (diameter > 2 cm), smaller branches (diameter < 2 cm) and stems. One stem section of 50 cm in length was collected from the base, middle and upper portion of the stem. These stem sections were then debarked in the field to the ratio of stem and bark weight.

Five disks (2 cm thick) of stems and bigger branches (for trees) and sub-samples (about 0.25 kg) of leaves, smaller branches and bark were taken randomly and brought back to the laboratory for calculating fresh mass to oven dry mass conversion ratios of samples at

 $^{\circ}$ C to constant mass. Oven-dry mass of different parts was estimated from the conversion ratios and fresh mass of the respective parts of plants. Linear regression equations e.g. y = ax + b; $y = a \ln(x) + b$ and $Log_{10}y = a \log_{10}(x) + b$ were tested to derive the allometric relationship between Diameter at Breast Height (DBH) and biomass of plant parts. DBH is the independent variable and readily measurable variable that have been used by most of the Studies on biomass estimation in the mangroves (Whittaker and Marks 1975; Ong et al 1984; Putz and Chan 1986; Clough and Scott 1989; Gong and Ong 1990; Ketterings et al 2001; Mahmood et al., 2008). Significant test of regression equations were tested by using SAS (6.12) statistical software.

3.4. Nutrients in plant components

Sample collection: Sub-samples (about 100 g) smaller and bigger branches, stems and barks were collected randomly from the selected stem of this study. Leaves were also collected randomly as clump. One random sample of leaves consist of one clump, thus each sample minimizes the age effect of leaves in nutrient concentration. All sub-samples of were then oven-dried at 80 °C until constant weight.

Determination of elements in plant parts: Nitrogen, phosphorus, potassium, and carbon concentration in A. corniculatum tissue were measured by following different standard methods. The plant samples were acid digested according to Baethgen and Alley (1989) to measure the total nitrogen.

Steps 1

- 1. At first take 0.1 g of plant sample in the digestion tube.
- 2. Add 1.1gm catalyst mixture (Potassium sulphate (K₂SO₄), Cupper sulphate (CuSO₄) 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 $^{\circ}$ 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

CuSO₄

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.

The total concentration of Nitrogen in the sample extract was measured clorometric (Baethgen and Alley (1989)

Solution Preparation

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

Na ₂ HPO ₄ .12H ₂ O	35.8 g	D'1	
N-K tartrate	50 g	Dilute to 1 litter	Store in a cold place
NaOH	54 g	with Distilled water	

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

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

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

5.25% Na hypochlorite (clorax)	Dilute to 500 ml with DW	Prepare fresh daily
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Nitrogen Standard solution preparation

Diluent preparation

K ₂ SO ₄	19.82 g	Dilute to 1 litter	
CuSO ₄	1.982 g	with 1.1M H ₂ SO ₄	Store it to prepare
Se	0.198 g	(60 ml 98% H ₂ SO ₄	standard solution
		in 1L DW)	

Stock solution preparation (1000 ppm)

Dry NH ₄ Cl			
		Dilute to 500 ml	Nitrogen (N) stock
(Dry NH ₄ Cl at 105°C)	1.9095 g	with diluent	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	Nitrogen (N) stock
		with diluent	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)	Diluent	-
5	2.5	50
10	5	50
15	7.5	50
20	10	50

^{*}Working range 0-50 ppm

Determination of N in the sample extract:

- 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:

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

Where,

C = Concentration obtained from spectrophotometer in ppm

df = Dilution factor (times)

fv = Final volume of the digest (ml)

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

3.5. Phosphorus and Potassium in plant components

The plant samples were acid digested to determine total Phosphorus and Potassium according to Allen (1974).

Steps 1

- 1. Take 0.1 g of plant sample or 0.5 g of soil 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
- 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
- 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.

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 H₂SO₄ (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 KH₂PO₄ 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:

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

Potassium concentration in the digested sample extract was measured by Flame Photometer (PFP7, Jenway LTD, England).

3.5. 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.

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).

Statistical analysis: Nutrients and carbon concentration in different parts of A. corniculatum of were compared by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT, p<0.05) by using SAS (6.12) statistical software.

Chapter-4

Result and Discussion

4.1. Conversion ratio:

The conversion ratio of fresh mass of stem without bark and fresh mass of bark was 0.13 ± 0.01 and the relationship was significant (Figure 1). Moreover, fresh mass to oven dried mass conversion ratios of leaves, smaller braches, bigger branches, bark and stem without bark were found to vary from 0.47 to 0.52; and the relationship among their fresh mass and oven-dried mass was significant (p<0.05) (Figure 2-6). Woody parts like stem and bigger branches showed higher ratios compared to leaves and smaller branches. Usually leaves and smaller branches contain higher amount of moisture compared to woody parts. This could the reason to observe higher conversion ratios to the woody parts.

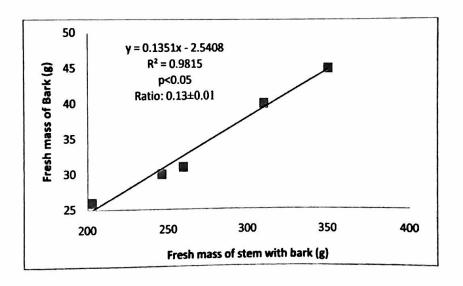


Figure 1: Relationship between fresh mass of stem with bark and fresh mass of bark

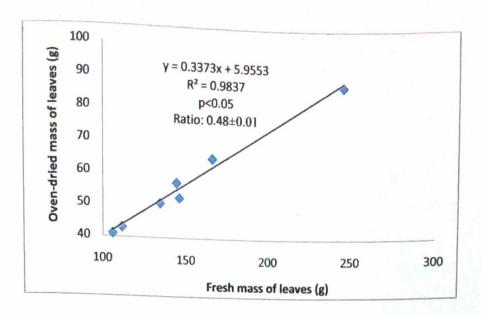


Figure 2: Relationship between fresh mass of leaves and their oven-dried mass

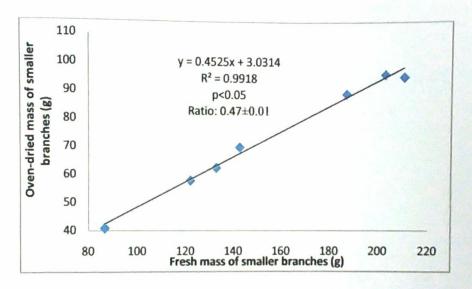


Figure 3: Relationship between fresh mass of smaller branches and their oven-dried mass

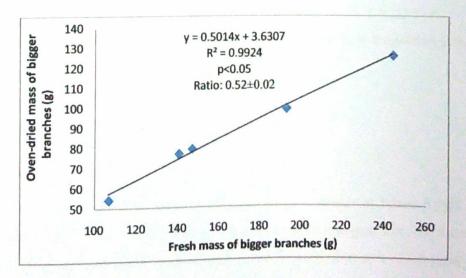


Figure 4: Relationship between fresh mass of bigger branches and their oven-dried mass

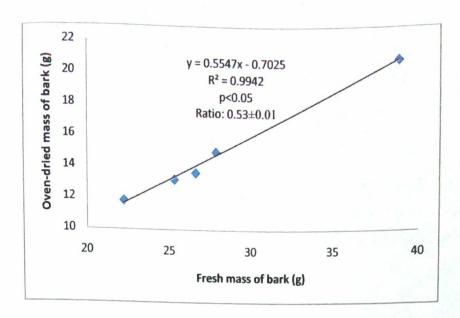


Figure 5: Relationship between fresh mass of bark and their oven-dried mass

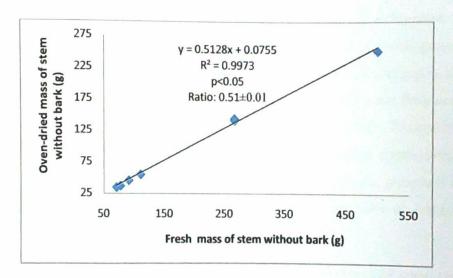


Figure 6: Relationship between fresh mass of stem without bark and their oven-dried mass

4.2. Allometric relationship:

The allometric relation between biomass of plant parts and diameter at breast height (DBH) were tested with y = ax + b; $y = a \ln(x) + b$ and $Log_{10}y = a Log_{10}x + b$. Ten linear equations with DBH as independent variable were tested for each plant part which yield a total of 50 equations. All the regression equations were significant (p<0.05), but highest

 R^2 (0.93-0.99) and F-values and lowest MS _{error} were observed for the equation of Log₁₀y = a Log₁₀x + b (Table 2). The best fitted regression equations were obtained by the following equation:

$$Log_{10} Biomass = a Log_{10} DBH + b$$
 (1)

Where a and b are the regression constants. However different regression equations were used in biomass estimation of different components of plants. The regression constant a and b with standard error and regression co-efficient R² of individual components of plants are presented in table 2. The equation 1 can be written as:

$$Biomass = b_b X DBH^a$$
 (2)

Where a has the same value as in equation (1) and b_b is the antilog of b. The biomass models for different parts of plants were derived from equation (2) and presented at figures 7 to 11. The linear transformation (Equation 1) and the power curve (Equation 2) were found to accept for good description of the relationship between above ground biomass and DBH in different inland forest (Whittaker and Marks 1975; Ketterings et al., 2001) and also in different mangrove species such as *Rhizophora apiculata* and *Bruguiera parviflora* at Matang Mangrove Reserve, Malaysia (Ong et al.,1984), *Rhizophora apiculata* at Pulau Kecil, Malaysia (Putz and Chan, 1986), *Bruguiera gymnorrhiza*, *Bruguiera perviflora*, *Ceriops tagal*, *Rhizophora apiculata* and *Xylocarpus granatum* at Nothern Australia (Clough and Scott, 1989) and *Rhizophora apiculata* at Matang Mangrove Reserve, Malaysia (Gong and Ong, 1990).

Biomass of different parts (stems, branches, leaves, and total biomass) of trees was estimated by using allometric equations. Linear regression method was used to estimate the allometric models (e.g. Snorrason and Einarsson, 2006; Bjarnadottir et al., 2007; Mahmood et al., 2012; Siddiqui et al., 2012). This was done to keep the equation simple. This study tested different linear regression equations with Diameter at Breast Height (DBH) as independent variables (Table 1). Irrespectively, allometric equations for biomass estimation of mangroves commonly use the total height, DBH and Girth at Breast Height (GBH) as independent variables (Cintron and Schaeffer-Novelli, 1985; Clough and Scott, 1989; Ong and Gong, 1990; Mackey, 1993; Soares and Scheffer-Novelli, 2005; Cienciala et al. 2006). However, the allometric equation for stem weight is usually expressed as a function of diameter (Komiyama et al., 2002, Mahmood et al., 2008). Similar findings

were also reported by Soares (1997, 2005). Transformation deforms the variables, potentially introducing bias in the estimation when we go back to the original unit (Baskerville 1972; Beauchamp and Olson 1973). In other words, although they are mathematically equivalent, statistically, they are not so equivalent (Zar, 1968; Payandeh, 1981). To solve the biased-estimate controversy, a correction factor was calculated according to Sprugel (1983). The use of correction factor was supported by Munro (1974), Madgwick and Satoo (1975), Whittaker and Marks (1975). This correction factor is able to reduce approximately 10-20% of the error of estimation (Sprugel, 1983; Baskerville, 1972). The best allometric model for plant parts were selected by considering the values of parameter of estimation such as R², CV, R_{sme}, MS_{error}, S_a, S_b and F-value (Table 2). Using R² as the parameter for this choice is erroneous as it simply offers a general idea for fitting the model (Payandeh, 1981; West and Wells, 1990; Zar, 1996; Siddiqui et al., 2012). Conversely, more precise selection of regression equation can be obtained by considering the parameter of estimation values (Ibrahima, 1995; Zar, 1996; Soares and Novelli, 2005). The Best fit regression equations were selected considering the highest R² and F-value, with lowest CV, R_{sme}, MS_{error}, S_a, and S_b. The selected allometric models were Log Leaf biomass = 1.52 Log DBH - 1.39; Log Branch biomass = 2.20 Log DBH - 1.48; Log Bark biomass = 2.08 Log DBH - 1.80; Log stem biomass = 2.08 Log DBH - 0.99; Log Total biomass = 2.06 Log DBH - 0.73 (Table 2).

Table 1: Selected ten models for plant parts and total above-ground biomass of Khalshi (Aegiceras corniculatum)

- 1. y= ax + b
- 2. $\sqrt{y} = ax + b$
- 3. $y = a\sqrt{x} + b$
- 4. $\sqrt{y} = a\sqrt{x} + b$
- 5. y=aLog x + b
- 6. Log y = ax + b
- 7. Log y = a Log x + b
- 8. $y = a \ln x + b$
- 9. Ln y = ax + b
- 10. Ln y = aln x + b

^{*} x = Diameter at Breast Height

Table 2: Parameter of estimates of allometric models

Plant										
parts	Equation	R ²	æ	٩	cv	Rmsc	MS error	s.	Š	F value
Leaf	y= ax + b	0.90	0.17	-0.29	32.69	0.21	0.04	0.01	0.07	244.82
	$y=a \ln(x) + b$	0.75	0.82	-0.58	52.13	0.33	0.11	60.0	0.15	79.92
	$\text{Log } y = a \text{ Log}_{10}(x) + b$	0.93	1.52	-1.39	-29.76	0.12	0.02	80.0	90.0	366.97
Branches	y=ax+b	0.87	0.99	-2.89	56.95	1.44	2.07	0.07	0.49	175.95
	$y=a \ln(x) + b$	0.62	4.48	4.11	96.43	2.43	5.93	89.0	1.20	43.79
	$\text{Log y} = a \text{ Log_{10}}(x) + b$	0.94	2.20	-1.48	-263.02	0.17	0.03	0.11	0.08	400.33
Bark	y = ax + b	0.94	0.27	-0.68	31.60	0.26	0.07	0.01	0.09	420.21
	$y=a \ln(x) + b$	0.73	1.30	-1.11	65.94	0.54	0.29	0.15	0.24	75.70
	$\text{Log y} = a \text{ Log}_{10}(x) + b$	0.99	2.08	-1.80	-14.46	0.07	0.01	0.04	0.03	2394.68
Stem	y=ax+b	0.93	1.77	4.40	31.60	1.66	2.75	60.0	0.56	420.21
without	$y=a \ln(x) + b$	0.74	8.37	-7.15	65.94	3.46	11.98	96.0	1.56	75.70
bark	$\text{Log y} = a \text{ Log}_{10}(x) + b$	0.99	2.08	-0.99	19.09	0.07	0.01	0.04	0.03	2394.68
Total	y=ax+b	0.93	3.21	-8.26	34.81	3.21	10.32	0.17	1.09	367.84
above	$y=a \ln(x) + b$	0.71	14.97	12.94	71.43	6.59	43.43	1.83	2.98	18:99
	$Log y = a Log_{10}(x) + b$	0.98	2.06	-0.73	13.12	0.108	0.01	0.05	0.04	1714.78

* y= Biomass; x = Diameter at Breast Height

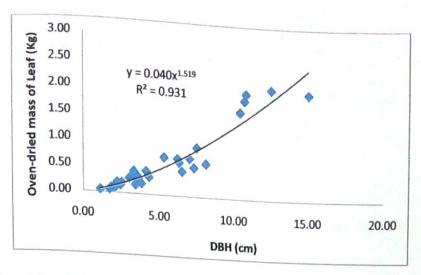


Figure 7: Allometric relationship between diameter at breast height (DBH) and biomass of leaf

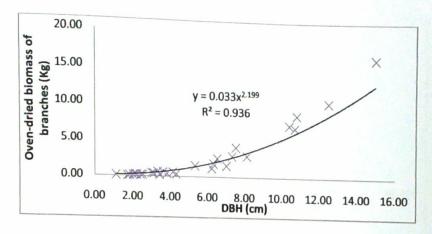


Figure 8: Allometric relationship between diameter at breast height (DBH) and biomass of branch

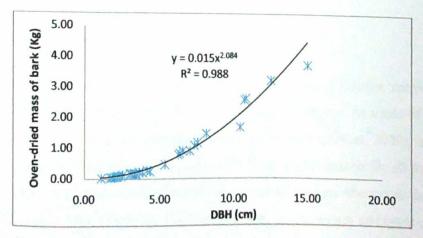


Figure 9: Allometric relationship between diameter at breast height (DBH) and biomass of bark

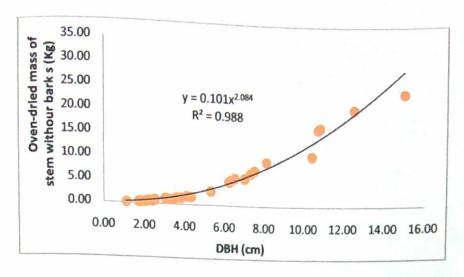


Figure 10: Allometric relationship between diameter at breast height (DBH) and biomass of stem without bark

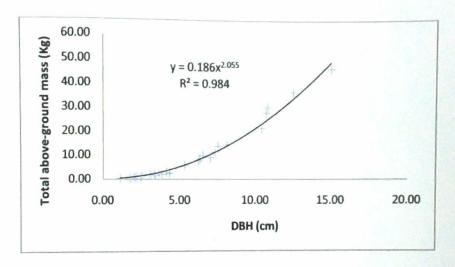


Figure 11: Allometric relationship between diameter at breast height (DBH) and total aboveground biomass

4.3. Nutrients in plant parts:

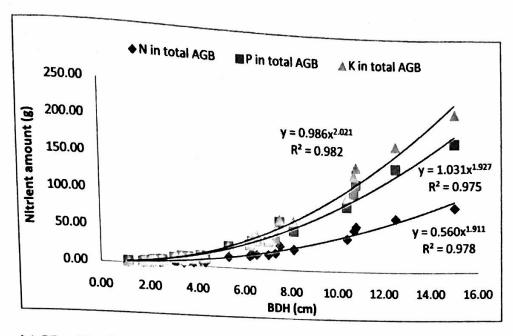
Based on carbon content analyses, we know that average of carbon content varies (from 43.10% to 48.59%) among different parts of *A. corniculatum* of biomass. As a rule of thumb, it is assumed that 50% of dry wood biomass corresponds to carbon (West, 2009), in IPCC (2006) the values for the tropics range from 46%-49% being most common for all trees dbh >10 cm. Comparatively higher amount of carbon was observed in stem whereas as the lower content was observed in bark (Table 3). This variation may be due to age and types of plant parts.

Table 3: Nutrients (N, P and K) and carbon concentration in different parts of Khalshi (Aegiceras corniculatum)

Nitrogen	Phosphorus	Potassum	Carbon (%)
(mg/g)	(mg/g)	(mg/g)	
6.40 ± 0.08	10.44 ± 0.26	6.50 ± 0.07	45.97 ± 0.06
3.29 ± 0.10	8.31 ± 0.06	6.05 ± 0.07	47.59±0.20
1.52 ± 0.08	4.10 ± 0.20	4.69 ± 0.08	48.22 ± 0.03
1.97 ± 0.05	3.09 ± 0.15	6.50 ± 0.14	43.10±0.11
1.60 ± 0.08	2.64 ± 0.06	4.26±0.07	48.59±0.17
	(mg/g) 6.40 ± 0.08 3.29 ± 0.10 1.52 ± 0.08 1.97 ± 0.05	(mg/g)(mg/g) 6.40 ± 0.08 10.44 ± 0.26 3.29 ± 0.10 8.31 ± 0.06 1.52 ± 0.08 4.10 ± 0.20 1.97 ± 0.05 3.09 ± 0.15	(mg/g) (mg/g) (mg/g) 6.40 ± 0.08 10.44 ± 0.26 6.50 ± 0.07 3.29 ± 0.10 8.31 ± 0.06 6.05 ± 0.07 1.52 ± 0.08 4.10 ± 0.20 4.69 ± 0.08 1.97 ± 0.05 3.09 ± 0.15 6.50 ± 0.14

Nutrients							WS			
	Equation	R ²	æ	q	ડ	R	error	S.	$\mathbf{S}_{\mathbf{p}}$	Œ
Nitrogen	$Log y = a Log_{10}(x) + b$ 0.98 1.91 -0.25 8.70 0.09 0.01 0.05 0.04 1234.52	86.0	1.91	-0.25	8.70	0.00	0.01	0.05	0.04	1234.52
Phosphorus	$Log y = a Log_{10}(x) + b$ 0.97 1.93 0.01 7.44 0.09 0.01 0,06 0.04 1046.24	0.97	1.93	0.01	7.44	60.0	0.01	90,0	0.04	1046.24
Potassium	$Log y = a Log_{10}(x) + b$ 0.98 2.02 -0.01 6.24 0.08 0.01 0.05 0.04 1529.78	86.0	2.02	-0.01	6.24	0.08	0.01	0.05	0.04	1529.78
Carbon	$Log y = a Log_{10}(x) + b$ 0.98 2.06 -1.05 29.18 0.08 0.01 0.05 0.04 1683.76	86.0	2.06	-1.05	29.18	0.08	0.01	0.05	0.04	1683.76

Note: R^2 = coefficient of determination; S_a = standard error of intercept "a"; S_b = standard error of regression coefficient "b", CV = Covariance, R_{mse} =Root mean square error; MS_{error} =Mean square error



*AGB = Total Above-ground Biomass

Figure 12: Allometric relationship between diameter at breast height (DBH) and amount of Nitrogen, phosphorus and Potassium in total above-ground biomass

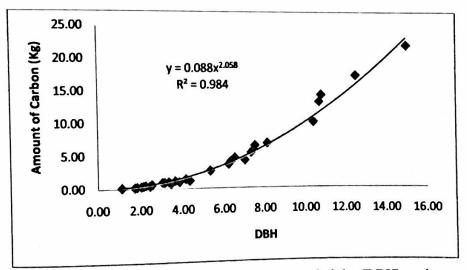


Figure 13: Allometric relationship between diameter at breast height (DBH) and amount of carbon in total above-ground biomass

Nutrients (N, P and K) concentration significantly (ANOVA, p<0.05) varied among the plant parts and comparatively higher concentration of nutrient was observed in leaf whereas as the lower content was observed in stems (Table 4). Similar result was observed by Binkley

(1986) in case of nitrogen. The plant species, physiological age of the tissue, position of the tissue in plant, available form of nutrients in the substrate, concentration of other nutrients, plant components (Mahmood et al., 2003). In the present study the trend of nitrogen, phosphorus and potassium in *A. corniculatum* was similar to that of *C. decandra* (Mahmood et al. 2012), *Rhizophora apiculata* (Ong et al., 1984), *Avicennia* spp., *Bruguiera* spp. and different mangrove forests (Table 5). From the above comparison, it was revealed that pattern in their parts, which may have been also site and species specific.

Table 5: Comparison of nutrients concentration in different parts of different mangrove species

~F	Plant	Nutrients (mg/g)			Sources and
		N	P	K	
Rhizophora apiculata	Leaves	10.2	1.1	9.8	Ong et al (1984)
	Branches	2.9	0.9	3.6	Matang, Malaysia
	Stem	2	0.2	3.3	
Ceriops	Leaf	16.1	0.17	4.91	Mahmood et al
	Branch	10.83	0.11	3.13	(2012) Sundarbans.
	Stem	8.66	0.07	1.82	Bangladesh
	Bark	9.46	0.05	2.43	
Avicennia spp. Bruguiera spp. Ceriops spp.	Leaves	eaves 19.6	1.4	11	
	Branch	8.9	1.4	7.5	
	Stem	8.6	0.9	0.51	
	Leaves	11.7	0.7	3.7	Aksornkoae and Khemnark (1984) Amphoe Khun mangrove, Thailan
	Branch	9	0.6	3.1	
	Stem	4	0.3	0.8	
	Leaves	10.8	0.6	7.8	
	Branch	6.7	0.4	5.5	
	Stem	4.4	0.3	3.1	

Species	Plant	Nutrients (m	g/g)		
	parts	N	P	T	Sources and
Rhizophora	Leaves	16.4	0.2	K	Location
apiculata	Branch	5.5	0.2	5.2	Gong and Ong
	Stem	4		1.6	(1990)
	Root	4.5	0.3	0.6	Matang mangrove,
	71001	4.3	0.3	1.7	Malaysia
Aegiceras corniculatum	Leaves	13.7	1.2	5	
	Branches	7.5	1.9		
	Stems	5.8	0.7	10.3	
	Roots	10	0.7	2.6	
	Roots	4.8	1.7	14.8	Li (1997)
Kandelia	Leaves	13.9	1.3	6.4	Futian mangrove,
candel	Branches	5.4	1.5	8.5	South China
	Stems	6.8	0.7	2.1	-
	Roots	4.4	1.6	12.6	\dashv
B. parviflora	Leaves	12.49	1.23	12.68	
(Saplings)	Branches	6.43	0.10	5.46	
	Stems	1.62	0.81	0.98	
	Roots	3.91	1.59	5.21	Mahmood et al,
B. parviflora	Leaves	13.69	1.32	11.89	(2003) Kuala
(Tree)	Branches	5.71	1.18	2.60	Selangor, Malaysia
	Stems	1.63	0.74	1.06	
	Roots	4.47	1.00	6.08	

The element content in the plant parts and total above-ground biomass were calculated and allometric equations were developed for total above-ground element content. All the equations were significant (p<0.05) and the value of co-efficient of determination (R^2) varied from 0.97 to 0.98 for Nitrogen, Phosphorus, Potassium and Carbon. The selected 5 allometric models were, Log N = 1.91 Log DBH - 0.25; Log P = 1.93 Log DBH + 0.01; Log K = 2.02 Log DBH - 0.01; Log C = 2.06 Log DBH - 1.05 (Table 4).

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