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Retranslocation of Nutrient (N, P, K) & Na Through Leaves of *Excoecaria agallocha* L. in Sundarbans, Bangladesh

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MS-120512

FORESTRY AND WOOD TECHNOLOGY DISCIPLINE

LIFE SCIENCE SCHOOL

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COURSE TITLE: PROJECT THESIS

COURSE # FWT-5112

This Project Thesis has been prepared in partial fulfillment of the requirement for M. Sc. degree in Forestry from Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh.

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16.08.2015

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Dedicated

To

My Beloved Parents

ABSTRACT

Re-translocation of the nutrients is one of the interesting and important nutrient-conservation mechanisms in this case where translocation of nutrients out of the senescing leaves back to the shoots, occurs especially for the conservation of different important nutrients. In this study, the retranslocation of nutrients (N, P, K and Na) of Gewa in Sundarbans through leaves in both sapling and tree stage in three main season of our country were studied. There were mentionable retranslocation variation in three nutrients responsible for growth such nitrogen, potassium and phosphorus in both sapling and tree stage in different seasons. Though retranslocation on an average account occurs mostly in sapling stages as this is the main crucial growing period of a plant. The retranslocation of nitrogen was found highest in summer season in both sapling (51.28%) and tree stages (76.92%). Phosphorus retranslocation occurred highest in winter season in sapling (47.36%) and summer season in tree stage (75%). The potassium retranslocation was found highest in winter season in sapling (34.65%) and summer season in tree stage (33.92%). However, there was no retranslocation of sodium in Gewa in any season or in any stage.

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Roksana jahan

APPROVAL

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

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DECLARATION

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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TABLE OF CONTENT

List of Figure

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Sundarbans of Bangladesh is the largest mangrove forest in the world. It is situated on the Ganges Brahmaputra Delta at the point where the river merges with Bay of Bengal. The forest lays under two forest divisions, and four administrative ranges viz Chandpai, Sarankhola, Khulna, & Burigoalini and it has 16 forest stations. The forest is divided into 55 compartments and 9 blocks. As the forest is situated on the south of the tropic of cancer and bounded by the northern limits of the Bay of Bengal, so the forest is classified as tropical moist forest. (Sundarban-mangrove.php.htm)

Sundarbans forest is situated in India & Bangladesh having an area of approximately 6017 km², of which 60% is located in Bangladesh and the remaining western portion, comprising 40%, lies in India. The total land area today is 4,143 km^2 The Sundarbans is intersected by almost 450 rivers, small streams & canals which cover a water area of about 1,874 km² and creates a complex network of tidal waterways, mudflats and small islands of salt-tolerant mangrove forests. Rivers in the Sundarbans are meeting places of salt water and freshwater. The tidal forms and the mangrove vegetation in Sundarbans are responsible for dynamic eco-system, vigorous nutrient cycling of both terrestrial and aquatic. (www.greenmags.info.html)

In the Sundarbans the saltwater forest is situated in the south-western part where Gewa (*E. agallocha*), Goran (*Ceriops decandra*), Keora (*Sonneratia apetala*), Ora (*S. caseolaris*), Passur (*Xylocarpus mekongensis*), Dhundul (*X. granatum*), Bain (*Avicennia alba, A. marina, A. officinales*), and other rhizophores, and Hantal (*Phoenix pelludosa)* dominate. The typical mangrove species dominate the central part of the forest. The moderate saltwater forest covers most of the southern parts of Khulna and Bagerhat districts where Sundari *(Heritiera fomes)* is the dominant species. There is a thick mat of the nipa palm or 'Golpata' (*Nipa fruticans*) by the side of almost all the canals. The moderately freshwater zone results from the large amount of water, which flows down the Passur, Haringhata and Burisher, maintaining the surface water at a lower level of salinity. (sundarbanworld-largest-mangrove-forest.html)

The composition of the species mainly depends upon the salinity content as different regions contain different contents of salinity which is mostly seen from east to west and north to south. The up-taking of nutrient, an unavoidable factor of plant growth, is limited by the salinity. Similar to other plant communities, nutrient availability is one of the major factors influencing mangrove forest structure and productivity. Many mangrove soils have extremely low nutrient availability, although nutrient availability can vary greatly among and within mangrove forests. There are also some other stress conditions faced by the mangrove species like nutrient limitation, antagonistic relations between salinity and other factors; for which mangrove species develops some adaptation mechanisms to cope with such type of harsh environment as well as to contribute in effective nutrient cycling. Nutrient-conserving processes in mangroves are well developed and include its evergreen's, resorption of nutrients prior to leaf fall, the immobilization of nutrients in leaf litter during decomposition; high root/shoot ratios and the repeated use of old root channels (oxfordjournals.org, 2010). Significant amounts of nutrients are returned to soil through litter fall and become available for cycling. Among nutrients, some are used in physiological responses and other stored in different plant organs, or returned to the soil through the litter and then partially absorbed by the root of trees (Breeman 1995). Uptake and release of nutrients are important factors at the stand level, because they represent the major fluxes through the system (Miller and Alpert 1984). Nutrient concentration in plant biomass is the result of the balance between nutrient uptake, plant growth and nutrient re-translocation, and the loss of these processes are likely to be influenced both by plant genetic make-up and soil fertility, as well as other environmental conditions (Hagen Thorn et al. 2004).

Re-translocation (resorption) of the nutrients is one of the interesting and important nutrient-conservation mechanisms in this case where translocation of nutrients out of the senescing leaves back to the shoots occurs especially for the conservation of different important nutrients like N, P, K and Na. It is widely accepted that the Sundarbans is the most productive mangrove ecosystem in the world. *Excoecaria agallocha L.* is one of the extensively occurring tree species in the forest. There is no study on re-translocation of this species in the Sundarbans as well as in other mangrove areas of the world. So, the outcome from this study will be the new primary contribution in the knowledge on understanding the ecosystem functioning of the Sundarbans. Hence, this type of study will be helpful to reduce this knowledge gap of species specific re-translocation mechanism as well as understand the adaptive mechanism towards nutrient limiting site like mangroves. Therefore, the aim of this

study are to find out the rate of re-translocation of N, P, K and Na through leaves of *Excoecaria agallocha L.* in different seasons of the year as well as in different life forms of this second most dominant species of Sundarbans.

1.2 Objectives:

1. To study the nutrient (N, P, K) and Na re-translocation through the leaves of *Excoecaria agallocha L.*
2. To study the seasonal variation (winter, summer and monsoon) in nutrient (N, P, K) and Na re-translocation of *Excoecaria agallocha L.*

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Global Distribution of *Excoecaria agallocha*

Around the world the family Euphorbiaceae is represented by the three genera, viz., *Excoecaria L.*, *Glochidiom J.R.* and *Hippomane L.* and all of them are considered to be associated with mangroves. The first two genera *Excoecaria* and *Glochidiom* are distributed in the old world mangles, while the *Hippomane is* distributed in the new world. The genus *Excoecaria* has 35 to 40 species that includes E. *agallocha* (Tomilson, 1994).. In the following paragraphs systematic, distribution, and available information on ecology and biology have been presented. This plant is also found in the countries of temperate and tropical Asia, Australasia and South-western Pacific This plant *(Excoecaria agallocha)* has traditionally been used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic, and the smoke from the bark to treat leprosy The bark oil has been reported to be effective against rheumatism, leprosy and paralysis.

Systematic of *Excoecaria agallocha*

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Spermatophyta, Angiospermae

Class: Magnoliopsida, Dicotyledonae

Sub class: Gamopetalae

Order: Euphorbiales

Family: Euphorbiaceae

Genus: *Excoecaria L.*

Species: ***Excoecaria agallocha L.*** *(Source:* Tomlinson, 1994)

2.1.1 Distribution in Bangladesh

Sundarbans south, where there is evidently the greatest seasonal variation in salinity levels and possibly represents an area of relatively longer duration of moderate salinity where Gewa is the dominant woody species. It is often mixed with Sundri *(Heritiera fomes).* It is also frequently associated with a dense understory of Goran *(Ceriops decandra),* and sometimes Passur *(Xylocarpus mekongensis).* Sundarbans west, in areas which support

sparse Gewa *(Excoecaria agallocha)* ***and*** dense stands of Goran and discontinuous patches of Hantal palm *(Phoenix paludosa)* on drier ground and river banks and levees. Gewa occur prominently throughout the area with discontinuous distribution of Dhundul *(Xylocarpus granatum)*, sundri and Kankra (Rahman, 2000) *Excoecaria agallocha L.* (Euphorbiaceae) is a small mangrove tree that grows widely in the tidal forests and swamps of the Sundarbans and other coastal areas of Bangladesh.

2.1.2 Description of the tree

E. agallocha is a small to medium sized deciduous tree. The tree exudes a poisonous juice from the bark and leaves when damaged. The sap is injurious to eyes and skin and for this reason the species is called blinding tree. Generally it is about lOm tall and occasionally grows to 15m in the northeastern parts of the Sundarbans. Usually they are 15-18 cm in diameter at breast height (dbh), occasionally they could be more than 30 cm in dbh. It is small and stunted in the western boundary although quite common in the strongly saline zone of the Sundarbans (Siddiqi, 2001). The bark is grayish, smooth and lenticelled. Wood of *E. agallocha is* bright yellow or whitish in color, light (28 kg/ m^3) and buoyant, soft, spongy, straight grained, fine and even textured. The heart wood is not distinct. The wood is not durable (Satter 1981).

Figure-2.1: Gewa *(Excoecaria agallocha)* tree

E. agallocha has no aerial root, however lateral roots are very close to the surface of the sediment and frequently rises above the surface (Naskar, 2004). The leaf is 6-10 cm long and 1.5-3 cm wide, elliptic with up-curled sides and pointed tip. Leaf edges could not be slightly toothed. They are arranged alternately on the stem or branch. Young leaves are

green but turns red or yellow before senescence (Tomlinson 1994). Flowers arise from the axis of the leaves. They are tiny flowers in spikes. Separate male and female flowers occur on the same tree. Male flowers are longer with a furry appearance. Male inflorescences are hanging, narrow, 5-10 cm long while female inflorescences are shorter, 1-4 cm long. Flowers are green and white in color (Rahman, 1982). Fruits are small, round and occur in clusters. Seeds are 5 - 8 mm in diameter and buoyant. The fruit capsules explode when ripe to disperse the seeds by water. The seeds have an air space within the seed coat to help them float (Naskar, 2004).

In the Sundarbans *E. agallocha* usually flowers from April to June, fruits are ripen in August and fall during August-September (Rahman, 1982). This is the monsoon season in the area. Tidal movements disperse the seeds. Germination takes place immediately after seed shedding. Plenty of new seedlings of E. *agallocha* are found on the forest floor during August-September (Siddiqi, 2001).

2.1.3 Habitat

Excoecaria agallocha usually does not establish on newly accreted land. It comes after some pioneer species in the ecological succession. It prefers moderate inundation. Contrary to *Heritiera fomes,* it can withstand a wider salinity range. However, it is stunted in the strongly saline zone. It is the characteristic species of the moderately saline zone. The species usually forms stands in association with *Heritiera fomes, Xylocarpus mekongensis* and *Ceriops decandra.* It also forms pure stands (Siddiqi, 2001). Temperature ranges from 20°C to 31°C. Absolute humidity is about 70z to 90z. Rainfall ranges from 2540 mm to 4064 mm mostly in monsoon. It grows naturally along the banks of rivers reached by tidal waters, mostly on silty soil. It grows in areas of over mature Keora but immature site for Sundri.

2.1.4 Morphology

Leaves

Gewa has small pointed leaves which are 6-10cm long and pinkish when young, turning green as they mature. Old leaves turn bright red when they are about to drop off. The tree often has multiple trunks. (Peter, *et. al.* 1999).

Flowers

Each tree bears either male or female flowers. So when they are in bloom, the trees can look confusingly different. The flowers are wind pollinated (Peter, *et. al.* 1999).

Fruits

The fruit capsules explode when ripe to disperse the seeds by water. The seeds have an air space within the seed coat to help them float. They don't germinate on the parent tree (Peter, *et. al.* 1999).

Inflorescences

Male inflorescences hanging, narrow, 5-1 0 cm long; female inflorescences shorter, 1-4-cm long.

Figure-2.2: Male flower

Figure-2.3: female flower

Figure-2.4: Fruit

Roots

Above-ground roots, but can sometimes have spreading surface roots (Peter, *et. al.* 1999).

2.1.5 Silvicultural Characteristics

The species grows well in intermediate levels of salinity and tidal inundation. It is shade tolerant both as seedling and when matures. It coppices well. However, scope of propagation through coppicing has not been investigated. Unlike many other mangrove species, *Excoecaria agallocha* does not possess either pneumatophore or aerial roots. However, the surface roots help in respiration and gas exchanges. Germination is also non- viviparous. In old senescent leaves, sodium chloride is deposited and potassium and phosphorous are simultaneously withdrawn prior to leaf fall in April. In this way sodium chloride is removed from the metabolic tissues and K and P are retained (Sddiqi, 2001).

2.1.6 Phenology

The leaves are simple, alternate, obviate to elliptic, obtuse, glabrous and distantly toothed. They are dark glossy green above and a polar green below, often turning red before falling. The male and female flowers are separate (Percival and Womensly 1975).

The plant flowers in April-May and seeds ripen in August-September (Rahman 1983).The fruit is green to dark brown, smooth, with a moderately hard but brittle test' and with albumin surrounding the embryo. There are about 250 seeds per ounce. Under natural conditions the seeds germinate immediately after falling. Germination is epigeous, and relatively with greater success.

2.1.7 Growth rate

Excoecaria agallocha shows higher increment in less Saline areas. The dbh increment in less, moderate and strongly saline zones of the Sundarbans are 0.19, 0.09, and 0.05 cm respectively. The growth rate is satisfactory in the coastal plantations. Trees of age varying between 12 and 14 years show a mean height of 7m. The annual dbh increment ranges between 0.50 and 0.93 cm. The increment rate is higher in western coastline where salinity is lower that of the east (Siddiqi and Khan, 1990).

2.2 Effect of salinity on growth of *Excoecaria agallocha*

Mangrove forests are distributed along coastlines and periodically inundated by seawater. The particularity of their habitat makes salinity an important factor limiting propagule germination, seedling growth and reproduction of mangrove trees. Many studies dealt with the effects of salinity on mangroves. Under extreme salinity stress, accelerated leaf mortality rates of mangrove seedlings are often accompanied by decreases in leaf production rates, finally leading to the deaths of plants. High salinity can cause osmotic stress and reduce the availability of water, resulting in stomatal closure and reduced supply of carbon dioxide. In addition, salt stress can induce ion toxicities such as membrane disorganization, production of reactive oxygen species, and disturbance of nutrient balance. On the other hand, during long term of acclimation to saline conditions, mangroves evolve various strategies to cope with high salinity, including anatomical, physiological, and molecular mechanisms. In order to defend salt-induced oxidative damage, plants are equipped with oxygen radical detoxifying enzymes such as superoxide dismutase, peroxidase and catalase. Accumulation of inorganic ions in vacuoles is common pattern observed in mangrove plants under saline conditions, which serves not only to increase cellular osmolarity to counter osmotic stress but also to avoid increases in ionic strength of the cytoplasm.

Excoecaria agallocha, known as "milk mangrove", is an important medicinal plant. Previous researches on E. *agallocha* are mostly focused on its heredity gene and medicinal properties, but few can be found on its ecological adaptation to environments. Nandy et al. have ever recorded good growth of mature E. *agallocha* trees in fresh water, but its early response to saline conditions is still unknown. E. *agallocha* increased salt tolerance over time. (Chen Y, Ye Y (2014).

2.3 Effect of nutrient deficiency on growth of *E. agallocha*

Nutrient deficiency is another main problem limiting mangrove growth. Different from most terrestrial soils, mangrove sediments are frequently waterlogged by seawater. Water logging results in anaerobic environment, which greatly restrains nitrification and consequently leads to low nutrient bioavailability of mangrove sediments. Studies on plant anatomical mechanism demonstrated that nutrient addition might enhance water supply to leaves and increase hydraulic conductivity by stimulating root growth and/or improving some aspects of the water conducting pathway. E. *agallocha* intolerant to high salinity but it can be greatly enhanced by nutrient addition. (Chen Y, Ye Y (2014).

2.4 Soil condition of mangroves

The anaerobic, organic matter-rich soils of the mangroves are favorable for N2 fixation. As in other tropical forests N fixation in mangroves can be a significant source of N (Holguin et al., 2001). High levels of both light-dependent and light-independent N fixation have been recorded in microbial communities living on the trees (Uchino et al., 1984), in association with roots, in decaying leaves and on pneumatophore, as well as in the soil (Boto and Robertson, 1990). Benthic microbial mats are found in many intertidal mangrove habitats and can also contribute significantly to the N cycle of the mangrove particularly when the mat is dominated by N-fixing cyanobacteria (Lee and Joye, 2006). Foliar uptake of N in the form of ammonia from the atmosphere or from rainwater has also recently been suggested to be a potentially important source of N for mangroves, particularly under conditions that favors ammonia volatilization (i.e., acidic, warm, flooded soils rich in organic matter) (Fogel et al., 2008). The top layer of the soil and the thin layer of aerobic soil around the mangrove roots support populations of nitrifying bacteria that in turn can convert ammonium into nitrate for the plant, although nitrification rates are generally low (Shaiful et al., 1986; Alongi et al., 1992; Kristensen et al., 1998).

Phosphate (P) in mangrove soils can be immobile and unavailable for plant use, thus organisms that solubilize P can have important implications for plant growth, especially in nutrient-limited environments. Aluminium can be relatively abundant in mangrove soils (Naidoo and Raiman, 1982) and the acidic conditions of mangrove soils may result in aluminium being mobilized to toxic levels. All plants require potassium (K) for maintaining intracellular electric neutrality, osmotic regulation, enzyme activation, protein synthesis and photosynthetic metabolism (Leigh and Wyn Jones, 1984).

2.5 Nutrient Cycling in mangrove ecosystem

The movement of nutrients through mangrove ecosystems is one of the least understood aspects of the function of these ecosystems. Moreover, there is no mangrove forest in the world for which a complete nutrient budget has been estimated. This is an astounding fact given the importance of nutrient cycling to several of the vital functions of mangroves. (Boto 1982). Nutrient cycles in mangrove ecosystems are open and can be subjected to either reduced or oxidized states. Harbison (1986) concluded that three major influences of trace metals (i.e., fine particulates, organic matter, and sulphide production) are inherent characteristics of mangrove muds and confer them an enhanced capacity for metal accumulation. Biotic processes in mangrove muds can alter the source-sink function of mangroves by altering the pH and Eh of muds. This has vital implications to their role in absorbing nutrients and pollutants or allowing pollutants to enter coastal waters.

2.6 Nutrient availability to Mangroves

Similar to other plant communities, nutrient availability is one of the major factors influencing to the mangrove forest structure and productivity. Many mangrove soils have extremely low nutrient availability (Lovelock et al., 2005), but nutrient availability varies greatly between mangroves and also within a mangrove stand. (Feller et al., 2003a). Many previous studies showed that nutrient availability, especially N and P, is an important factor responsible for mangrove growth (Feller, 1995; Lin and Sternberg, 1992; McKee et al., 2001). Although N: P ratios have been widely used to determine plant nitrogen and phosphorus limitation in wetlands (Gusewell and Koerselman, 2002; Gusewell et al., 2003), it has not been used as an indicator in mangrove ecosystems (Lin and Sternberg, 2007).Nutrient-conserving processes in mangroves are well developed and include evergreens, resorption of nutrients prior to leaf fall, the immobilization of nutrients in

leaf litter during decomposition, high root/shoot ratios and the repeated use of old root channels (McKee, 2001; Middleton and McKee, 2001). Both nitrogen-use efficiency and nutrient resorption efficiency in mangroves are amongst the highest recorded for angiosperms

2.7 Nutrient conservation strategies of Mangrove

Mangrove trees are highly productive and this is due in part to the evolution of many adaptations for nutrient conservation. Most mangrove trees are evergreen with sclerophyllous leaves and high root/shoot biomass ratios (Komiyama et al., 2008). The evergreen habit implies a smaller nutrient investment in new leaves and lower nutrient loss rates due to the long life span of the tissue (Aerts, 1995). Mangroves have an average leaf life span of 16 months (1.33years), although this can vary between species and over latitude (Saenger, 2002; Suarez and Medina, 2005).The leaf life spans of mangroves are typical for broad leaved tropical and subtropical evergreens (Reich et al., 1992).

- **Sclerophyllous leaves**

Sclerophylly is a trait related to low soil nutrient availability, especially low P (Loveless 1961, Wright et al. 2001). In mangroves, sclerophylly declined with increases in P in P limited environments (Feller 1995). Sclerophylly is also linked to low water availability and, in mangroves, to high salinity habitats (e.g., Naidoo 1987), as sclerophyllous leaves can lose a great deal of their water content before wilting and can exhibit extremely low leaf water potentials (Salleo et al.1997). Sclerophylly has also been linked to leaf longevity and evergreen traits and to ecosystem nutrient retention through slowed decomposition (Schlesinger and Hasey 1981) and through reductions in herbivores by primary consumers (Coley 1983).

- **Root-shoot ratios**

Root biomass in mangroves can be high, partially because of the contribution of above ground roots, which have both supportive functions and roles for aerating roots in anoxic soils and also due to high below ground root biomass (Golley et al., 1962; Snedaker, 1995). Root/shoot ratios can vary considerably as a function of environmental factors and are in part an adaptation to saline environments (Ball 1988b, Saintilan 1997). Root/shoot ratios in many trees are sensitive to soil moisture, usually decreasing with increased water logging (Kozlowski 1984), but this is not necessarily the case for all mangrove species

(Ye et al. 2003, Krauss et al. 2006). Root/shoot ratios also vary between mangrove species, over time and with forest structure (Tamooh et al. 2008), resulting in non-linear relationships between soil conditions and root/shoot ratios. However, the overall high root biomass in mangroves, especially the abundance of fine roots (Komiyama et al. 2000), is conducive to nutrient capture and uptake from soils low in nutrients, particularly as fine roots proliferate in response to high nutrient microsites, such as inside decaying roots (McKee 2001). Nutrient availability is another factor that plays a role determining the allocation to root biomass. Similar to other plants (Chapin 1980), studies on mangrove seedlings have demonstrated that, when nutrient availability is high, mangrove seedlings invest more in aboveground biomass (which maximizes carbon acquisition) than in roots, while when nutrient availability is low, seedlings redirect resources to enhance their root biomass (McKee 1995, Naidoo 2009)

- **Efficient metabolic process**

Increasing the efficiency of metabolic processes is also an effective nutrient conservation strategy (Chapin, 1980). In most plants, a large proportion of root respiration goes towards the uptake and assimilation of N (Bloom et al., 1992). Trees that occur in habitats where the soil is ammonium rich generally exhibit a preference for ammonium uptake and do not appear to suffer from ammonium toxicity, which can have a significant metabolic cost in ammonium sensitive plants (Kronzucker et al., 1997).

- **Lower growth rates and reduced nutrient requirements**

The capacity to sustain low growth rates and consequently reduced nutrient requirements over periods of time are an adaptation to low-nutrient environments (Chapin, 1980). Mangroves are capable of very slow growth rates and often forming dwarf forests, (Lugo and Snedaker, 1974). These dwarf trees can experience periods of rapid growth when nutrient limitation is lifted (Feller et al., 2003b; Lovelock et al., 2005; Feller et al., 2007; Lovelock et al., 2007a).

- **Retranslocation of nutrients**

Retranslocation has been characterized as one of the most important strategies used by trees to conserve nutrients, which consequently influences competition, nutrient uptake, and productivity (Killingbeck 1996). This process is closely associated with leaf senescence and conservation of nutrients, and is an important mechanism enabling trees to

maintain growth in nutrient-poor sites. Internal nutrient recycling by retranslocation (resorption) from leaves is an important factor in the supply of nitrogen, phosphorus and potassium for new growth in tree species at different phases of foliage development (Nambiar and Fife 1991, Millard 1994, Fife and Nambiar 1995, Aerts 1996, Saur et al. 2000, van Heerwaarden et al. 2003).

2.8 Retranslocation

Retranslocation is a regulatory mechanism, which causes nutrients to redistribute from older leaves to current years leaves. Most soil nutrients taken up by trees are used in annual production of foliage, which serves as a reservoir of reusable nutrients. Nutrients in one generation of foliage can be retranslocated to support the production of the next generation of foliage, irrespective of the rate of soil nutrient supply (Nambiar and Fife 1987).

2.8.1 Importance of nutrient dynamics and retranslocation

In natural forests and man-made plantations, cycling of nutrient is an important aspect, as significant amounts of nutrients are returned to soil through litter fall and become available for nutrient cycling. Among nutrients, some are used in physiological responses and other stored in different plant organs, or returned to the soil through the litter and then partially absorbed by the root of trees (Breeman 1995). Uptake and release of nutrients are important factors at the stand level, because they represent the major fluxes through the system (Miller & Alpert 1984). Nutrient concentration in plant biomass is the result of the balance between nutrient uptake, plant growth and nutrient retranslocation, and the loss of these processes are likely to be influenced both by plant genetic make-up and soil fertility, as well as other environmental conditions (Hagen-Thom et al. 2004). Forest litter fall is the major flux responsible for nutrient transfer to soil (Parzych et al. 2008) and the growth and productivity of forest ecosystems depends mainly on the amount, the nature and the rate of decomposition of litter fall (Victor et al. 2001). Tree species can play an important role in nutrient cycling through different properties, such as the amount of litter produced, nutrients release and chemical composition of the litter (Rahajoe 2003). Different tree species involve different nutrient release patterns, which are related to litter quality and seasonal environmental factors (Khiewtam & Ramakrishnan 1993).Within the same community, foliar nutrient concentrations vary largely amongst different species and different individuals of the same species despite similar soil conditions (Niinemets &

Kull 2003). The relative importance of site and species, as the factors determining nutrient concentrations in plant biomass, may differ depending on nutrient element and biomass fraction. Comparative studies of several species growing on the same soils allow a better understanding of species nutrient function (Hagen-Thorn et al. 2004).

Tracking nutrient returns through litter fall under different tree species is important to understand the dynamics of soil fertility. Soil and old leaf nutrient retranslocation, are the primary sources of nutrients in the leaves (Binkley & Sollins 1995, Piatek & Lee Allen 2000) and litter fall nutrient abundance is related to intensity of retranslocation processes in autumn (Parzych et al. 2008). The nutrient retranslocation, movement and transfer nutrients from the old leaves to the every year store, is an important process in nutrient dynamics in most ecosystems, especially broadleaf ecosystems (Killingbeck 1996, Duchesne et al. 2001).

2.8.2 Nutrient resorption in arid land, deciduous and evergreen species

In arid, nitrogen and phosphorus-limited systems, plant performance depends on nutrient conservation. During leaf senescence, plants break down biomolecules and translocation nutrients to storage tissues. This process (resorption) is considered one of the most important plant nutrient conservation mechanisms (Eckstein et al., 1998; Killingbeck, 1996; van Heerwaarden et al., 2003). In arid lands, resorption may be particularly important to whole-plant nutrient budgets due to slow decomposition rates and variable soil nutrient supply (Noy-Meir, 1973). Both droughts (Bertiller et al., 2005; Wright and Westoby, 2003) and soil salinity (Drenovsky and Richards, 2006) may decrease resorption, due to rapid leaf senescence and the need for N-rich compatible solute accumulation in leaves (e.g., glycinebetaine). Desert systems thus provide a unique test of environmental constraints on resorption, as they are water-limited and often saline.

In **mixed species stands of deciduous trees,** variation in nutrient retranslocation efficiency and resorption kinetics among species was related to differences in leaf longevity (lifespan based on timing of leaf fall) and the nature of the biochemical pool of nutrients (Niinemets and Tamm 2005). Nutrient retranslocation occurred mainly in response to new shoot production. The process of retranslocation is closely associated with leaf senescence and conservation of elements, and is an important mechanism enabling plants to maintain growth in element-poor sites (Fife andNambiar, 1997; Lin andWang, 2001; Lodhiyal and Lodhiyal, 2003). Resorption potential is not a simple function of

habitat nutrient availability. Instead, resorption may be influenced by phylogeny and/or erivironmental factors, including both drought and salinity stress (Killingbeck, 1996). The pattern of retranslocation and its governing factors are similar among species in the absence of interspecies competition for growth and crown structure which occurs in mixed species stands (Reef R. *et al.* 2010).

Evergreen species have a lower concentration of leaf nutrients and a longer leaf life span than deciduous species. These are important mechanisms for nutrient economy, making possible the colonization of low fertility soils (Alerts *1996;* Eamus & Prichard 1998). Removal of nutrients from leaves prior to abscission and their redeployment to other tissues is known as nutrient resorption (Wright & Westoby 2003) and is considered an important adaptation of certain species to less fertile ecosystems (May & Killingbeck 1992; Pugnaire & Chapin 1993). Therefore, evergreen species are likely to show greater resorption efficiency in comparison to deciduous ones.

2.8.3 Factors influencing retranslocation

Resorption may be influenced by phylogeny and/or environmental factors, including both drought and salinity stress (Killingbeck, 1996). Some research suggests resorption may be more similar among closely related than distantly related species (Killingbeck, 1996; Wright and Westoby, 2003); however, resorption varied greatly in some congeners, suggesting evolutionary history is not the only factor driving resorption (Killingbeck, 1996). Some external factors that can directly lead to incomplete leaf nutrient resorption are for instance frost, which prematurely arrests the resorption process (Norby *et al.* 2000), and strong wind, which can prematurely detach leaves from the plant (Oland 1963,Killingbeck 1988). These factors may contribute to the variation in nutrient resorption proficiency among different years and regions.

Other external factors that correlate to the level of N and P resorption proficiency and latitude (Berg *et ul.* 1995), N and P availability (Shaver and Melillo 1984, Pugnaire and Chapin 1993, Kemp *et al. 1994,* Vitousek 1998, Eckstein *et al.* 1999) and temperature (Berg *et al.* 1995, Nordell and Karlsson 1995).It has therefore often been suggested that species from low-nutrient habitats have higher nutrient resorption efficiencies (percentage of a nutrient withdrawn from mature leaves before leaf abscission). However, the evidence available so far does not support this contention: high nutrient resorption efficiency is characteristic of all perennial growth-forms and is not very responsive to changes in nutrient

supply (Aerts 1996).

Retranslocation of nutrients depends on their mobility within phloem. N, P, K and Mg are mobile in the phloem, whereas Ca is relatively immobile (Helmisaari 1992, scheleppi et al 2000). Retranslocation of mobile nutrients thus helps to maintain adequate concentrations in the youngest and active tissues, that is, in the photosynthesizing current tissue or mycorrhizal fine root tips active in nutrient uptake. For nonmobile nutrients, the pattern is usually opposite. They accumulate with the concentration being highest in the older tissues. The differential internal mobility of elements leads to some elements being easily retranslocated (e.g., N) while other elements experience little retranslocation (e.g., Ca)

Element	Mobility
Nitrogen	High
Phosphorus	High
Potassium	Very High
Sulfur	Low to Moderate
Magnesium	Low to Moderate
Calcium	Very Low
Iron	Very Low

Table-2.1 Mobility of different nutrients

Studies have shown that leaf lifespan is a key determinant of retranslocation efficiency in several species (Escudero et al. 1992). The percentage retranslocation varies considerably depending on the phase of retranslocation. Retranslocation was largely governed by seasonal effects on the nutrient requirement for shoot growth and factors that determine nutrient concentrations and contents of leaves.

2.8.3.1 Nutrient retranslocation Vs soil fertility

There is an unresolved discussion whether nutrient resorption is related to soil fertility, or to internal nutrient sinks in plants, or to some combination of these two. Chapin (1980) pointed out that there was insufficient evidence to support any particular relationship between nutrient resorption and soil fertility. There are studies that demonstrate that nutrient resorption efficiency is higher on infertile soils (Boerner 1984, Scott *et al.* 1992), on fertile soils, and on intermediate fertility soils. Aerts (1996) found that at the

intraspecific level, nutrient resorption was not very responsive to increased nutrient availability.

2.8.4 Mechanisms of Retranslocation

There is also evidence that Retranslocation takes place during formation of heartwood from the senescing wood towards the sapwood (MEERTS, 2002) and further towards the inner bark (Rochon et al 1998). As a result N concentration increase from heartwood to sapwood and from outer bark to inner bark as well as vertically upwards in the inner bark (Helmisaari and siltala 1989, Meerts 2002) Significant amounts of nitrogen, phosphorus and potassium were retranslocated during three phases of leaf life. In the first phase, retranslocation occurred from young leaves beginning 6 months after leaf initiation, even when leaves were physiologically most active. In the second phase, retranslocation occurred from mature green leaves during their second year, and in the third phase, retranslocation occurred during senescence before leaf fall.

The distribution of evergreen plants in seasonal environments coincides with low soil nutrient availability. Ever greenness has, therefore, often been considered as an adaptation to nutrient deficiency through several proposed mechanisms, among them: 1) Long internal retention time of nutrients accomplished by extended leaf longevity, combined with large fractional re-absorption of leaf nutrients from senescing leaves, leading to high assimilation of carbon per unit invested nutrient. 2) Transport of nutrients from stores in old leaves as they senesce to young leaves expanding at the same time, reducing the need for new nutrient uptake, and for construction of internal stores elsewhere. (Oikos S.J, 1989)

Generally high element Retranslocation efficiency (RE) and low growth rate are the characteristics of plants under element-poor conditions (Boerner, 1984; Lajtha, 1987; Ralhan and Singh, 1987). However, some researchers reported that high RE is not an important adaptation to low element status, but a characteristic of most plant species with contrasting life histories (Chapin and Kedrowski, 1983;Miao, 2004). Plants growing on infertile soils do not retranslocated a greater fraction of elements from senescing leaves, i.e. RE is independent of status of individuals (Birk and Vitousek, 1986; Chapin and Moilanen, 1991; Walbridge, 1991; Helmisaari, 1992). RE was, however, found to be high under higher element status (Nambiar and Fife, 1987).

2.8.5 The ecological importance of nutrient resorption from senescing leaves

Ecosystems are complex structures where abiotic conditions and biota interact. The potential presence of a species is determined by the combination of abiotic conditions and the biota already present. However, biota also alters their environment, with the emergence of a high oxygen concentration in the atmosphere being one of the most important biotic driven changes of abiotic conditions in history. Plants play an important role in ecosystems, because they are the primary producers and they strongly control nutrient cycles, especially those of N and P. A large part of the available N and P in the ecosystem is organically bound in plants, as organisms have a high demand of N and P to produce various components, like proteins, energy carriers, genetic material and phospholipids. These nutrients may be returned to the soil through exudation, leaching or turnover of dead material. A strategy to minimize nutrient losses through litter is to resorbed these nutrients during tissue senescence, thus producing litter with low nutrient concentrations. Moreover, the slow turnover rate of litter with low nutritional value slows down nutrient cycling, and thus leads to a positive feedback between plant species dominance and nutrient availability (Chapin 1993, Aerts 1999). Plant growth in natural terrestrial ecosystems is mostly N-limited, although P limitation also occurs frequently (Chapin 1980). Therefore, resorption of N and P from senescing tissue is of great adaptive significance, because the resorbed nutrients are directly available for further use (e.g. seed filling, bud growth, storage), making a species less dependent on current nutrient uptake (Aerts and Chapin 2000). In spring, remobilization of nutrients from storage organs can lead to (competitive) early re-growth of foliage, even before the start of nutrient uptake from the soil (Thornton and Millard 1993, Millard 1996, Bausenwein *et al. 2001). As* a considerable part of the total plant N pool is allocated to leaves, remobilization of N from these plant organs contributes significantly to the annual N economy of plants (Aerts and Chapin 2000). Plants adapted to sites with low nutrient availability seem to lose less nitrogen than species adapted to more fertile sites (Vazquez de Aldana *et ul.* 1996).

2.8.6 Parameters describing leaf nutrient resorption

2.8.6.1 Nutrient resorption efficiency

A commonly used parameter to quantify nutrient resorption is resorption efficiency. This parameter describes the percentage of the nutrient pool withdrawn from the foliage before leaf abscission (or functional disconnection in leaves that remain attached to the plant), and is determined by measuring the nutrient pools of mature and abscised leaves. The nutrient pool

is usually expressed on the basis of leaf mass or leaf area. Various authors have recognized that using mass basis causes an underestimation of resorption efficiency because of mass resorption during senescence (Killingbeck 1984). This underestimation is intrinsic to measuring mass based resorption efficiency, because nutrients themselves contribute to mass, and in addition, also starch and other leaf components are resorbed. Woodwell (1974) concluded that due to seasonal variation in leaf mass the pattern of change in nutrient content through the season would also be distorted, of course, and suggested to use leaf area basis instead. This basis is now commonly used, but also in this case the assumption is often made that very small or no changes in leaf area occur during senescence (Shaver and Melillo 1984, Chapin and Van Cleve 1996). However, leaf area should not be considered stable either during senescence, as shrinkage can take place in several plant species (Tremolieres *et ul.* 1999, Lin and Wang 2001).

2.8.6.2 Nutrient resorption proficiency

Another parameter to quantify leaf nutrient resorption is resorption proficiency: the level to which a plant has reduced an element in its senescing leaves (Killingbeck 1996). Species with high nutrient resorption proficiency thus show low nutrient litter levels. The physiological constraints mentioned above are probably also determining the ultimate resorption proficiency: the minimum level to which a plant can potentially reduce its nutrients (Killingbeck 1996). This level is about 3 mg/g DW for N, and between 0.7 and 1 mg/g DW for P (Killingbeck 1996, Aerts and Chapin 2000, Cote *et al.* 2002).

Resorption efficiency is the difference between the nutrient concentration in green leaves and senescent leaves, given as a percentage (Distel *et al.* 2003), whilst resorption proficiency is the absolute value by which nutrients are reduced in senescent leaves. Thus, the lower the concentration of a nutrient in senescent leaves, the greater the resorption proficiency (Killingbeck 1996).

The is an indication that such plants possess another nitrogen (N) source in addition to that available from the mineral and organic fraction of the soil. However, these nodules may not be functional. The efficiency of the N2 fixation process can be evaluated by the nitrogenase activity, presence of leghemoglobin and protein concentration within the nodules (Cresswell *et al.* 1992; Sprent 2001).

There are indications that plants with a long leaf life span produce more organic material per unit of mineral nutrient than those with shorter leaf life spans (Chapin 1980; Aerts *et al.* 1999). This ratio represents the nutrient use efficiency (Vitousek 1982).

NRE was calculated by: NRE% = (N **mature green - Nsenescent)/Nmaturegreen x 100**

Where: N mature green= Nutrient in mature green leaves, N senescent = Nutrient in senescent leaves (Pugnaire & Chapin 1993).

NUE was calculated by: NUE (g of dry mass mg^{1} of N) = 1/[N mature $_{g}$reen *X(1- r)],* where: N mature $_{g}$reen = total N concentration in mature green leaves and *r* = NRE expressed in terms of a fraction (Aerts *et al.* 1999).

2.8.7 Calculation of retranslocation rate

Percentage of retranslocation can calculate by the following equation (Huang et al. 2007, Hashemi et al. 2012 - eqn. 1):

NRE%= {(Nulive - Nudeaa)/Nutive} * 100

Where NRE is the nutrient resorption efficiency, $_{Nulive}$ is the nutrient concentration of live leaves (mature leaves) and $_{NUdead}$ is the nutrient concentration of senescent leaves.

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study site

This study was carried in the adjacent forest of Chandpai station office, located in Chandpai range of Sundarbans East Forest Division. This site selection was done purposively in respect of time as well as the available number of *Excoecaria agallocha*. This site is located under fresh water zone of the Sundarbans where latitude is $22^0$22'06.60" N and $89^0$38'42.20" E is longitude. The climate is humid subtropical and mean temperature range for winter is 18-23° C and 27 -31° C for the summer. Mean annual rainfall is 1980 mm; summer (May to September) contributes about 81% of the annual rainfall while winter season contributes about 19% of rainfall. Soil is clayey and pH is around 7.9. Consistent monthly temperature and rainfall data was collected from a nearby meteorological station (MET Station, Data Loggers, Khulna). The dominant vegetations in the study area belong to *Excoecaria agallocha, Avicennia alba, Avicennia offciinalis, Heritiera fomes, Xylocarpus mekongensis. Excoecaria agallocha* selected for the study which was dominant species and average DBH was 11cm.

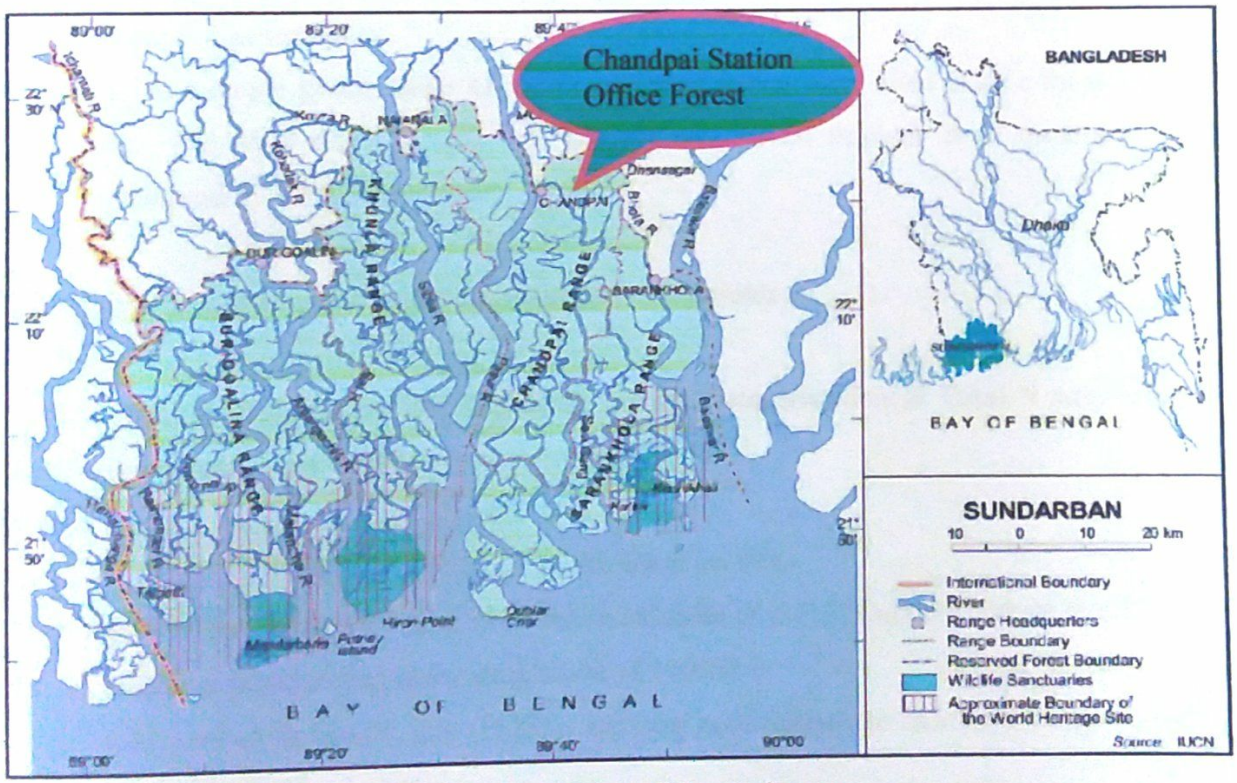

Figure-3: Location of the study area

3.2. Field Procedure

* **Sample plot Selection:** A permanent sample plot of 50m × 50m was selected randomly.
* **Selection of sample Tree:** After selecting the sample plot, the DBH of all available *Excocaria agallocha* trees of that plot were measured to assess the mean DBH (11cm). Those trees of that plot were selected as sample trees which had equal or above DBH from the mean DBH. All the sample trees were marked and the samples were taken from those same trees in three seasons (winter, summer and Rainy) of the year to study the seasonal variation of nutrients.
* **Collection of Sample leaves:** Matured and senescent leaves were collected from the same shoot of each sample tree. Here, the bottom 2-3 pair leaves of the shoot were collected as matured leaves which were also known as pre-senescent or mature leaves. The yellowish leaves were collected as the senescent leaves which were ready to abscise when it was touched or the branch was shaken lightly.
* **Soil Collection:** Core sampler of 5cm diameter was used to collect the top surface soil from 0-20 cm depth of soils under the crown of the each sample tree.

3.3. Laboratory procedure:

➢ **3.3.1. Sample processing:** All of the collected leaves were dried at 80^0c for at least 48 hrs. The oven dried sample was crashed and sieved through 2mm mesh and were preserved in air tight container.

3.3.2. Digestion of samples and determination of nutrients

3.3.2.1. 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.

3.3.2.2. Determination of "N"

The concentration of Nitrogen in the sample was measured by clorometric method according to Baethgen , W. E. and Alley, M. M. (1989) A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Communications in Soil Science and Plant Analysis, 20: 9, 961-969.

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		

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

Diluent 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 diluent	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 diluent	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)	Diluent	-
5	2.5	50
10	5	50
15	7.5	50
20	10	50

*Working range 0-50 ppm

3.3.2.3. 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:

TKN (mg/g) = (C×df×fv)÷(W×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)

3.3.3. Sample Preparation and Digestion for the Determination of Total P, K and Na According to the 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
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.

3.3.3.1. 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:

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

3.3.4. Determination of "K" and "Na"

Potassium and Sodium concentrations of the samples were measured by Flame Photometer (PFP7, Jenway LTD, England). Solution of 0, 5 and 10 ppm was prepared from the stock solution of Flame Photometry Standard 1000 ppm Potassium for preparation of standard curve.

3.4. Calculation of re-translocation rate

The percentage of the nutrient pool resorbed prior to leaf fall will be calculated using the following equation (Wang et al. 2003, Ricardo, 1992; Schlesinger 1989; Walbridge, 1991):

$$\text{NRE \%} = \frac{\text{nutrient pool in pre-senescent leaf} - \text{nutrient pool in post-senescent leaf}}{\text{Nutrient pool in pre-senescent leaf}} \times 100$$

Where NRE is the nutrient resorption efficiency, nutrient pool in pre-senescent leaf refers to the nutrient concentration of live leaves (mature leaves) and nutrient pool in post-senescent leaf is the nutrient concentration of senescent leaves.

3.5. Statistical Analysis

The concentration of N, K, P and Na in each leaf sample category of Gewa (*Excoecaria agallocha*) and soil at different season of were compared by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT, $p<0.05$) by using SPSS(IBM-20) statistical software.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Nutrient concentration of *Exoecaria agallocha* leaves in different seasons (winter, summer and rainy) at different stages (sapling and tree)

4.1.1 Nitrogen concentration of Gewa (*Exoecaria agallocha*) sapling leaves

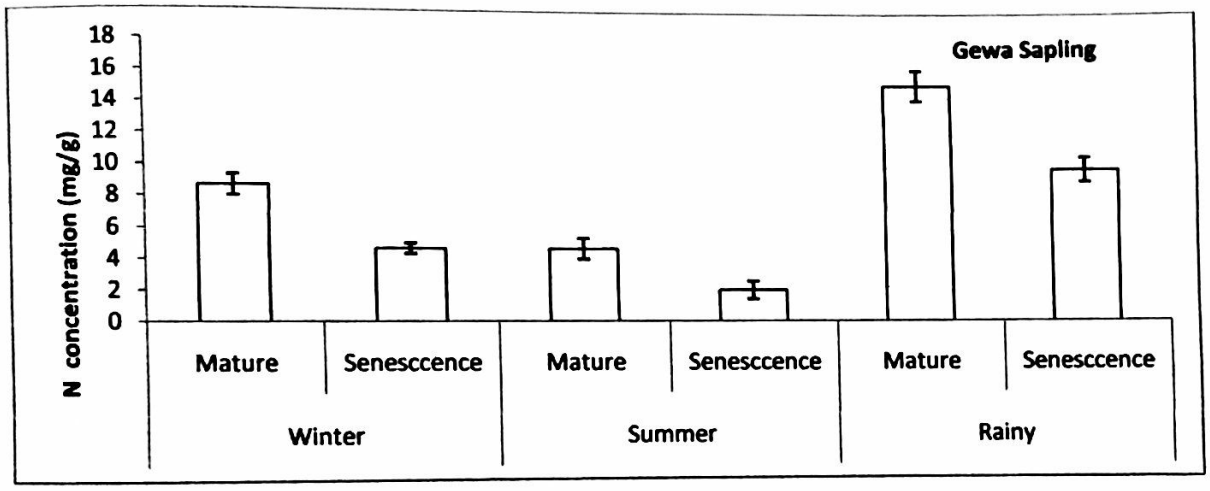

Figure-4.1: Nitrogen concentration of *E. agallocha* sapling leaves in different seasons

The average concentration of nitrogen for Gewa sapling mature leaves was found highest (15.21mg/g) in rainy season and lowest (4.67mg/g) in summer season. Average nitrogen concentration of senescent leaves was also found highest (9.77mg/g) in rainy season and lowest (2.00mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average nitrogen concentration between and within each group of leaves in each of the three seasons.

4.1.2 Nitrogen concentration of Gewa (*Exoecaria agallocha*) tree leaves

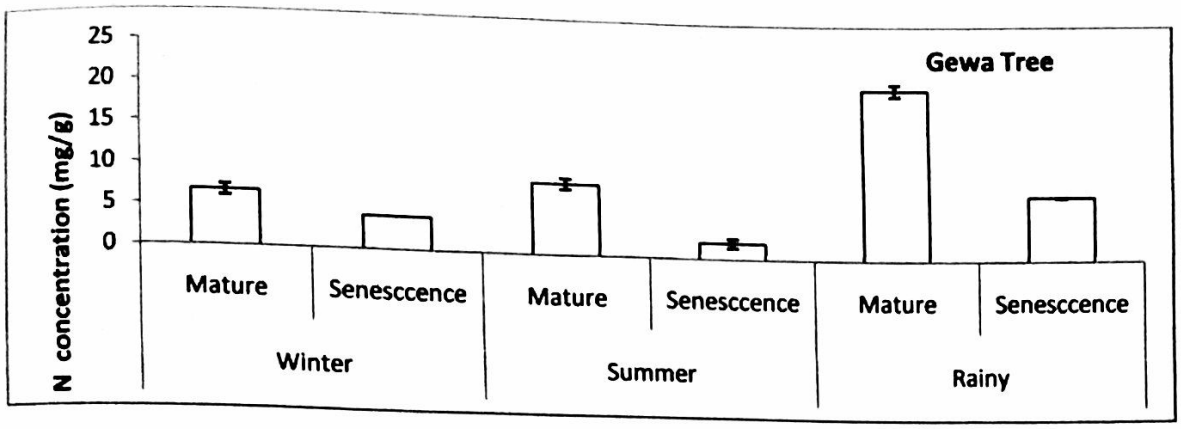

Figure-4.2: Nitrogen concentration of *E. agallocha* tree leaves in different seasons

The average concentration of nitrogen for Gewa tree mature leaves was found highest (20.77mg/g) in rainy season and lowest (6.67mg/g) in winter season. Average nitrogen concentration of senescent leaves was also found highest (7.8530 mg/g) in rainy season and lowest (2.00mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average nitrogen concentration between and within each group of leaves in each of the three seasons.

4.1.3 Nitrogen concentration of soil

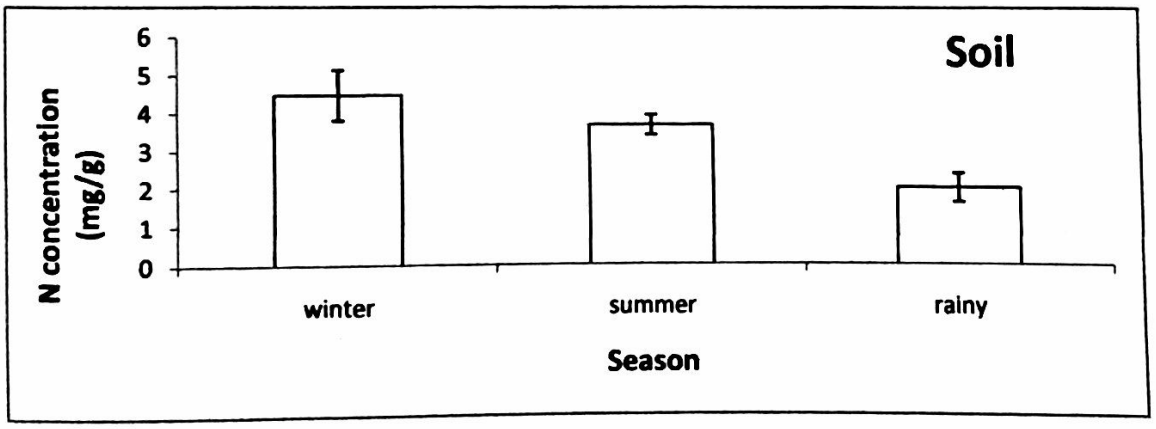

Figure-4.3: Nitrogen concentration of soil in different seasons

There was significant (ANOVA, DMRT, $p<0.05$) difference of average nitrogen concentration of soil in each of the three seasons and it was found highest (4.46mg/g) in winter season and lowest (2.00mg/g) in rainy season.

4.1.4 Retranslocation of Nitrogen through Gewa (*Exoecaria agallocha*) leaves

To calculate the Nutrient Resorption efficiency (NRE%) of nitrogen for Gewa (*E.agallocha*) sapling, the maximum difference of average nitrogen concentration between mature and senescent leaf was found in summer season which indicates the maximum nitrogen retranslocation (57.14%) at sapling stage occurs during summer season. For Gewa (*E.agallocha*) tree the maximum difference of average nitrogen concentration between mature and senescent leaf was also found in summer season which indicates the maximum nitrogen retranslocation (76.92%) at sapling stage occurs during summer season. (Figure-4.4)

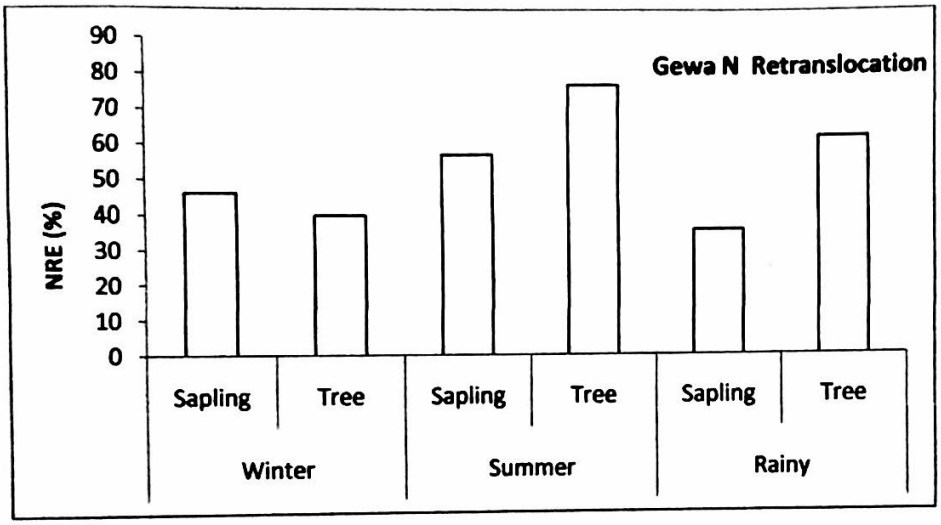

Figure-4.4: Nitrogen Retranslocation of Gewa (*E.agallocha*) in different seasons

4.2.1 Phosphorus concentration of Gewa (*Exoecaria agallocha*) sapling leaves

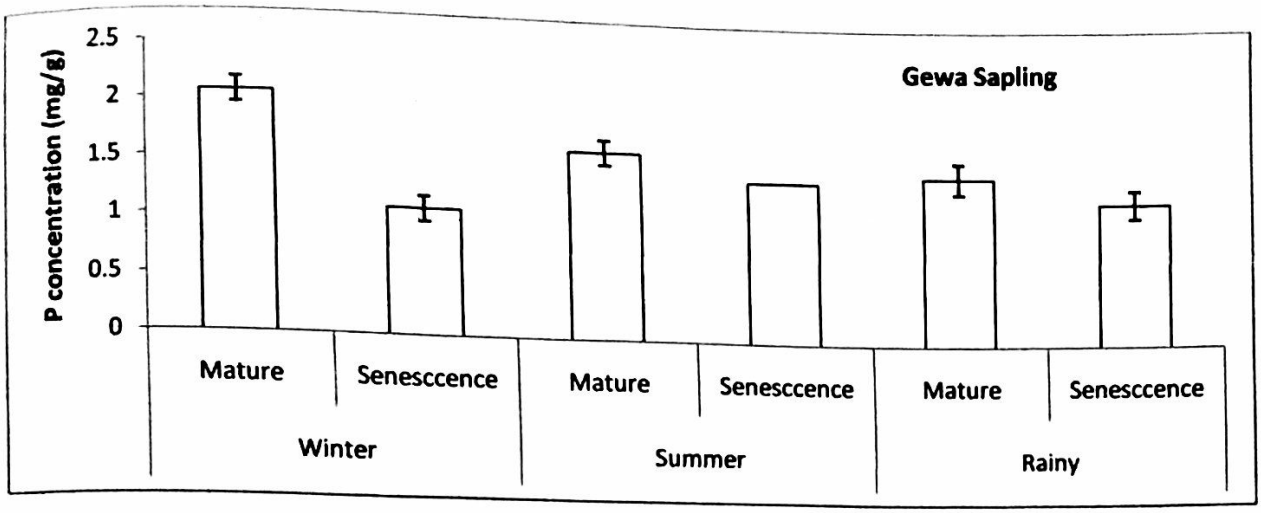

Figure-4.5: Phosphorus concentration of *Exoecaria agallocha* sapling in different seasons

The average concentration of phosphorus for Gewa sapling mature leaves was found highest (2.06 mg/g) in winter season and approximately same during summer and rainy season. Average phosphorus concentration of senescent leaves was found highest (1.63mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average phosphorus concentration between and within each group of leaves in winter seasons but no significant (ANOVA, DMRT, $p>0.05$) difference in summer and rainy season.

4.2.2 Phosphorus concentration of Gewa (*Exoecaria agallocha*) tree leaves

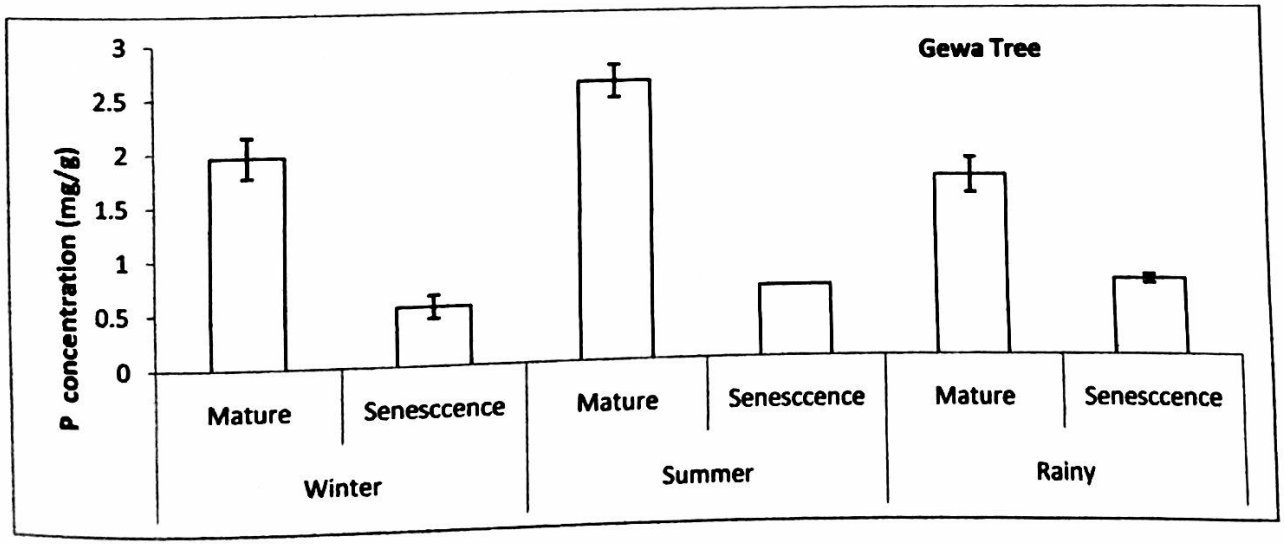

Figure-4.6: Phosphorus concentration of *Exoecaria agallocha* tree in different seasons

The average concentration of phosphorus for Gewa tree mature leaves was found highest (2.61mg/g) in summer season. Average phosphorus concentration of senescent leaves was found highest (0.7 0 mg/g) in rainy season and lowest (0.65 mg/g) in winter season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average phosphorus concentration between and within each group of leaves in each of the three seasons.

4.2.3 Phosphorus concentration of soil

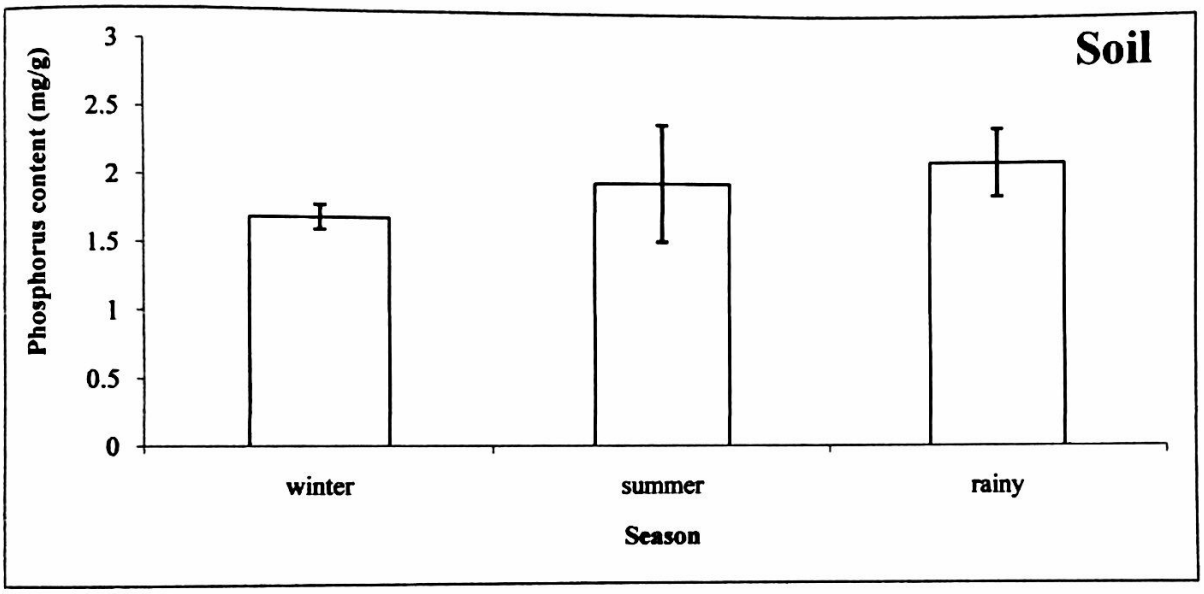

Figure-4.7: Phosphorus concentration of soil in different seasons

There was no significant (ANOVA, DMRT, $p>0.05$) difference of average phosphorus concentration of soil in each of the three seasons.

4.2.4 Retranslocation of Phosphorus through Gewa (*Exoecaria agallocha*) leaves

To calculate the Nutrient Resorption efficiency (NRE%) for phosphorus of Gewa (*E.agallocha*) sapling, the maximum difference of average phosphorus concentration between mature and senescent leaf was found in winter season which indicates the maximum phosphorus retranslocation (47.37%) at sapling stage occurs during winter season. For Gewa (*E.agallocha*) tree the maximum difference of average phosphorus concentration between mature and senescent leaf was found in summer season which indicates the maximum phosphorus retranslocation (75%) at tree stage occurs during summer season. (Figure-4.8)

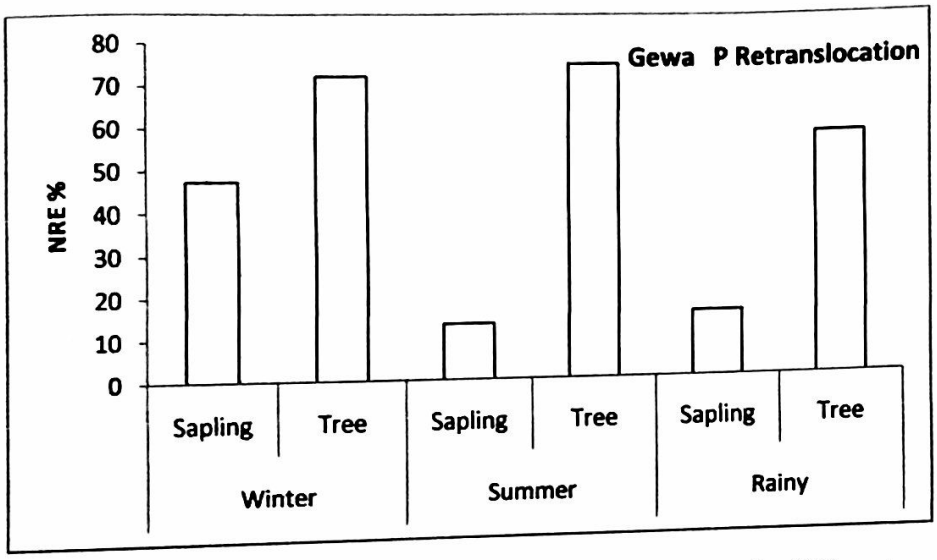

Figure-4.8: Phosphorus Retranslocation of Gewa (*E.agallocha*) in different seasons

4.3.1 Potassium concentration of Gewa (*Exoecaria agallocha*) sapling leaves

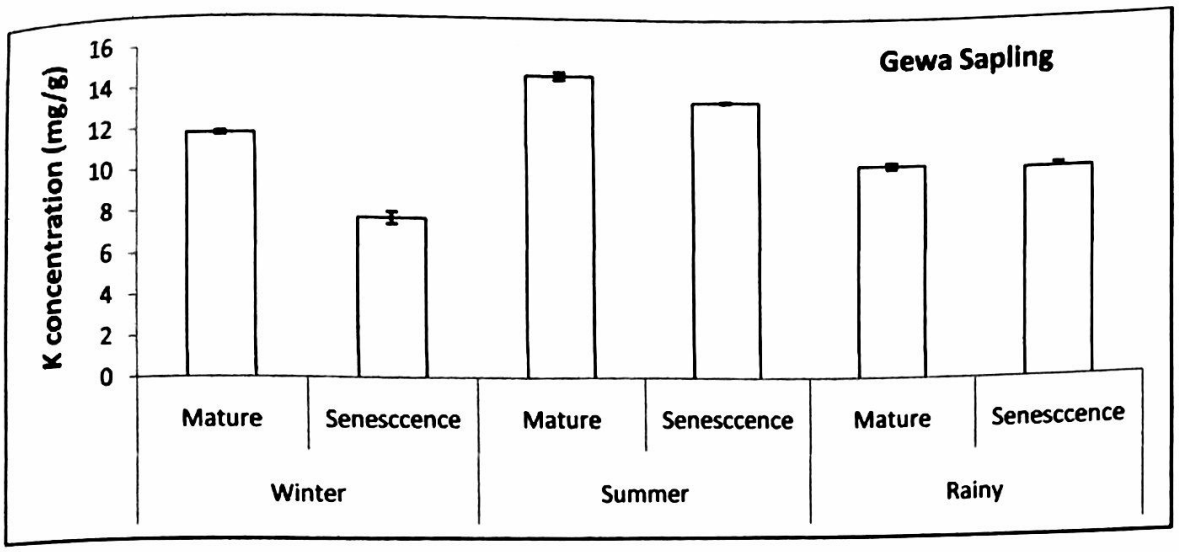

Figure-4.9: Potassium concentration of *Exoecaria agallocha* sapling in different seasons

The average concentration of potassium for Gewa sapling mature leaves was found highest (14.6701 mg/g) in summer season and lowest (10.28 mg/g) in rainy season. Average potassium concentration of senescent leaves was also found highest (13.44 mg/g) in summer season and lowest (7.69 mg/g) in winter season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average potassium concentration between and within each group of leaves in winter and summer seasons but no significant (ANOVA, DMRT, $p>0.05$) difference in rainy season.

4.3.2 Potassium concentration of Gewa (*Exoecaria agallocha*) tree leaves

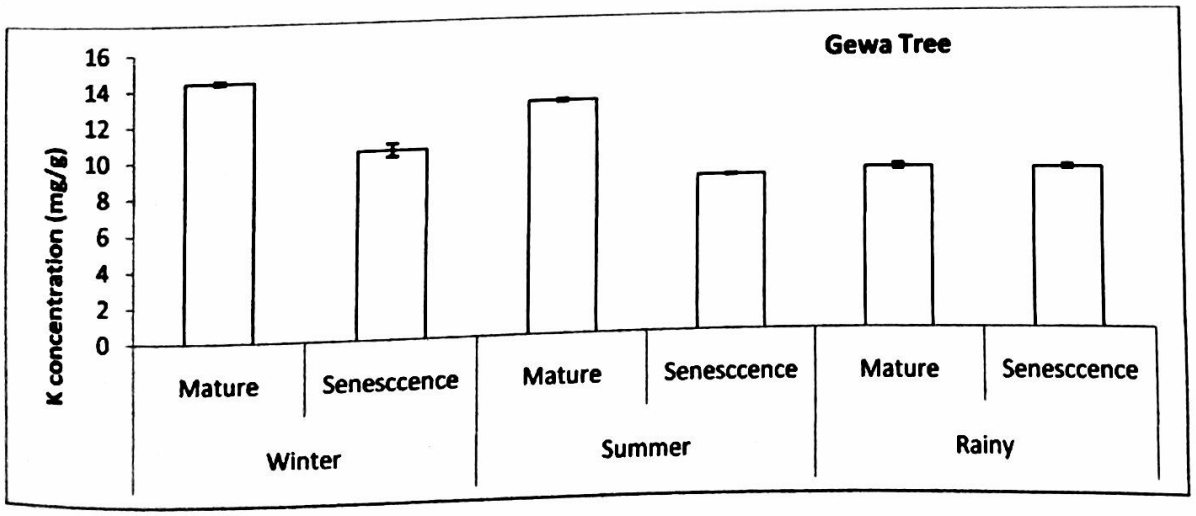

Figure-4.10: Potassium concentration of *Exoecaria agallocha* tree in different seasons

The average concentration of potassium for Gewa tree mature leaves was found highest (14.39 mg/g) in winter season and lowest (8.85 mg/g) in rainy season. Average potassium concentration of senescent leaves was also found highest (10.45 mg/g) in winter season and lowest (8.54 mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average potassium concentration between and within each group of leaves in winter and summer seasons but no significant (ANOVA, DMRT, $p>0.05$) difference in rainy season.

4.3.3 Potassium concentration of soil

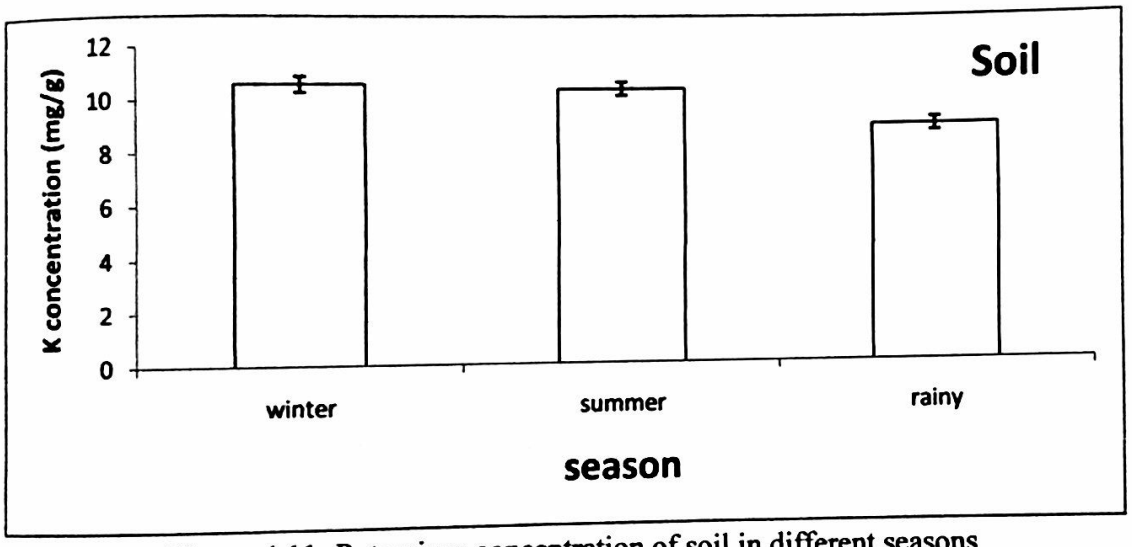

Figure-4.11: Potassium concentration of soil in different seasons

There was significant (ANOVA, DMRT, $p<0.05$) difference of average potassium concentration of soil in each of the three seasons and it was found highest (11.12 mg/g) in summer season and lowest (8.98 mg/g) in rainy season.

4.3.4 Retranslocation of Potassium through Gewa (*Exoecaria agallocha*) leaves

To calculate the Nutrient Resorption efficiency (NRE%) for potassium of Gewa (*E.agallocha*) sapling, the maximum difference of average potassium concentration between mature and senescent leaf was found in winter season which indicates the maximum potassium retranslocation (34.65%) at sapling stage occurs during winter season. For Gewa (*E.agallocha*) tree the maximum difference of average potassium concentration between

mature and senescent leaf was found in summer season which indicates the maximum potassium retranslocation (33.92%) at tree stage occurs during summer season. (Figure-4.12)

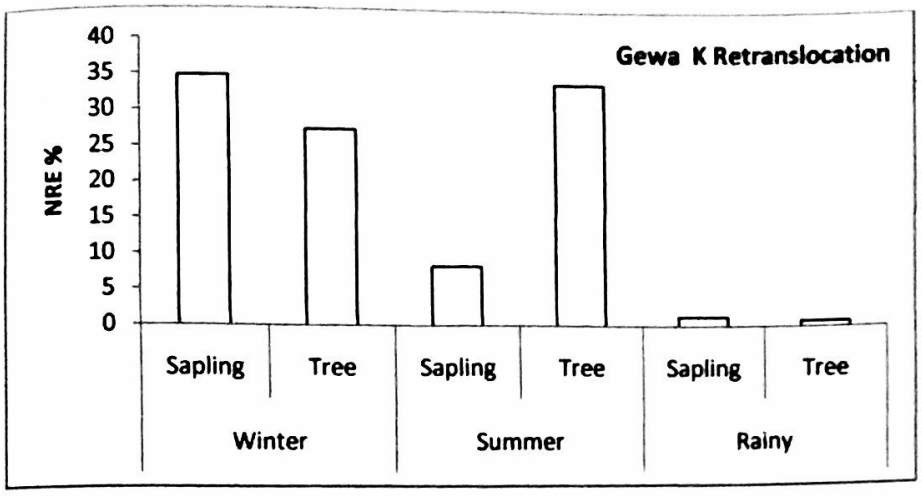

Figure-4.12: Potassium Retranslocation of Gewa (*E.agallocha*) in different seasons

4.4.1 Sodium concentration of Gewa (*Exoecaria agallocha*) sapling leaves

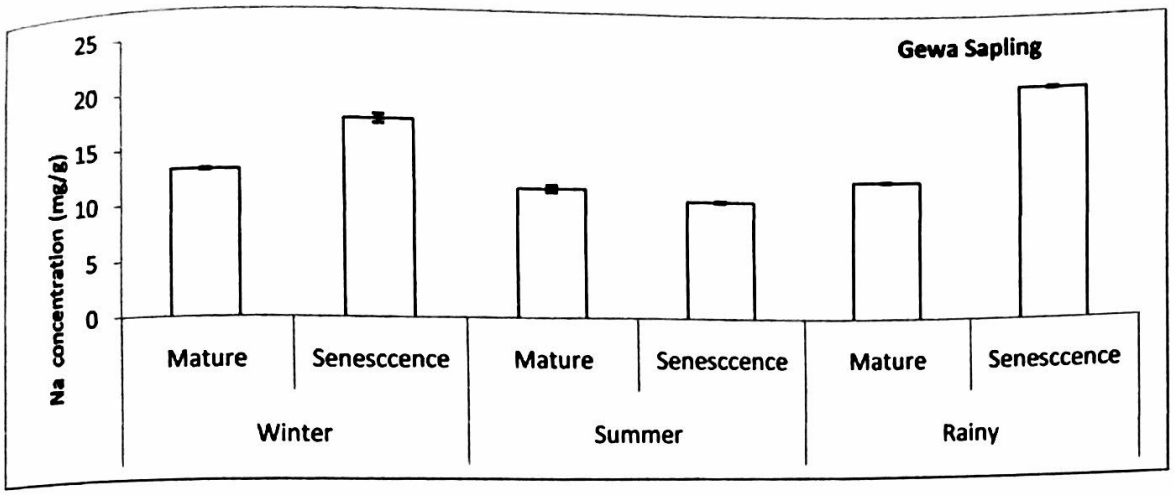

Figure-4.13: Sodium concentration of *Exoecaria agallocha* sapling in different seasons

The average concentration of sodium for Gewa sapling mature leaves was found highest (13.21 mg/g) in winter season and lowest (11.68 mg/g) in summer season. Average sodium concentration of senescent leaves was also found highest (21.11 mg/g) in rainy season and lowest (10.66 mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average sodium concentration between and within each group of leaves in each of the three season.

4.4.2 Sodium concentration of Gewa (*Exoecaria agallocha*) tree leaves

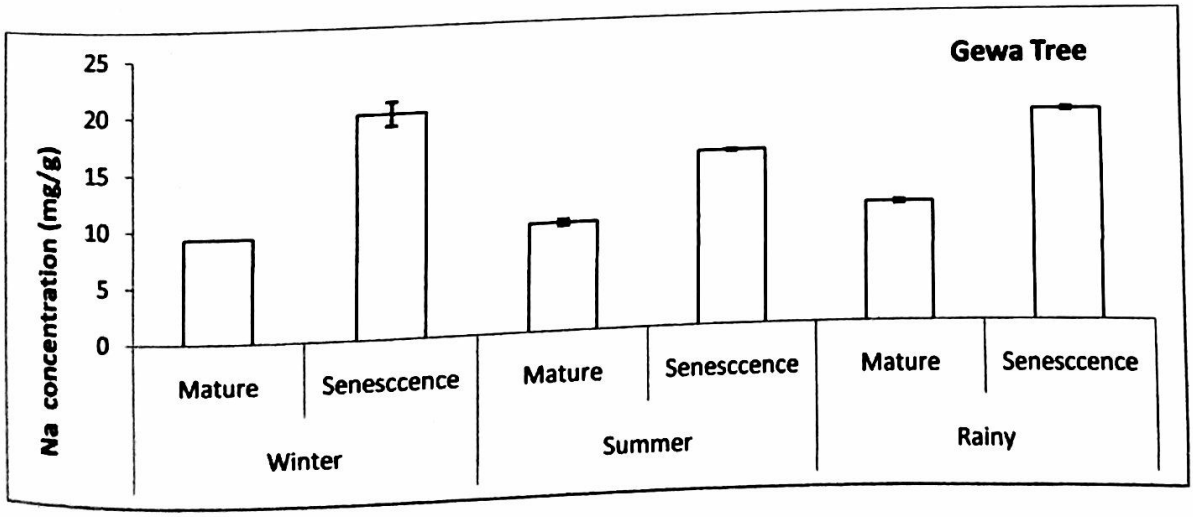

Figure-4.14: Sodium concentration of *Exoecaria agallocha* tree in different seasons

The average concentration of sodium for Gewa tree mature leaves was found highest (10.51 mg/g) in rainy season and lowest (9.29 mg/g) in winter season. Average sodium concentration of senescent leaves was also found highest (19.86 mg/g) in winter season and lowest (15.44 mg/g) in summer season. There was significant (ANOVA, DMRT, $p<0.05$) difference of average sodium concentration between and within each group of leaves in each of the three season.

4.4.3 Sodium concentration of soil

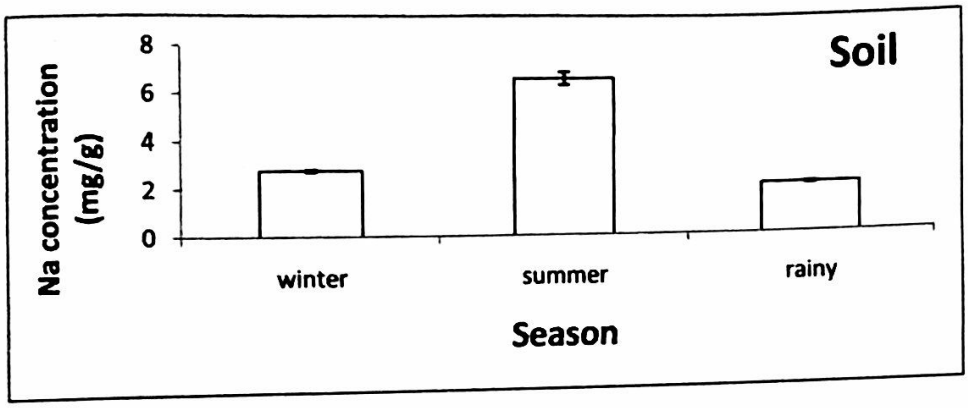

Figure-4.15: Sodium concentration of soil in different seasons

There was significant (ANOVA, DMRT, $p<0.05$) difference of average sodium concentration of soil in each of the three seasons and it was found highest (6.59 mg/g) in summer season and lowest (2.10 mg/g) in rainy season.

4.4.4 Retranslocation of Sodium through Gewa (*Exoecaria agallocha*) leaves

To calculate the Nutrient Resorption efficiency (NRE%) of potassium for both Gewa (*E.agallocha*) sapling and tree, the difference of average sodium concentration between mature and senescent leaf was always found in negative value because senescent leaves had highest sodium concentration than mature leaves in each season. which indicates no retranslocation of sodium occurs .(Figure-4.16)

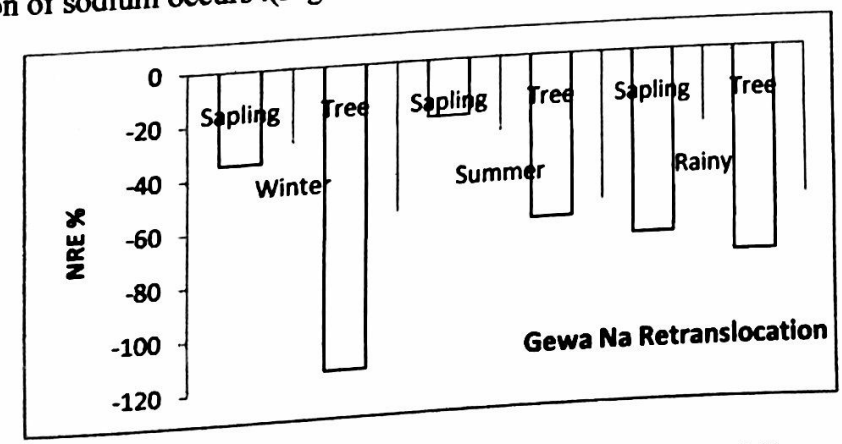

Figure-4.16 Sodium Retranslocation of Gewa (*E.agallocha*) in different seasons

CHAPTER FIVE

CONCLUSION

Retranslocation is one of the important nutrient conservation mechanisms especially in the nutrient limiting sites such mangroves. From this study it can be concluded that the retranslocation of Gewa occurs mainly in case of potassium, nitrogen and phosphorus in both stages sapling and tree with some variation in different seasons as well as in two different life forms. This study may help to know about the net amounts of nutrients available for reuse by new growth which is very essential in understanding the nutrient cycle as well as in nutrient dynamics of mangroves especially Sundarbans. The study will also provide some basic information and guideline in terms of the further study related to the nutrient conservation mechanism and nutrient dynamics as well. Further study may be conducted related to this type of conservation mechanism on other different species of Sundarbans.

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CHAPTER SEVEN

APPENDICES

A-1: Independent sample test of Gewa *(E.agallocha)*

Independent Samples Test : Gewa Sapling leaf

		F	sig	Sig (2-tailed)	t	df
N concentration	Winter	3.200	.148	.006	5.367	4
	Summer	.308	.609	.039	3.024	4
	Rainy	.316	.604	.012	4.356	4

Independent Samples Test : Gewa Sapling leaf

		F	sig	Sig (2-tailed)	t	df
P concentration	Winter	.000	1.000	.003	6.364	4
	Summer	16.000	.016	.116	-2.000	4
	Rainy	2.132	.218	.464	.809	4

Independent Samples Test : Gewa sapling leaf

		F	sig	Sig (2-tailed)	t	df
K concentration	Winter	3.945	.118	.000	12.940	4
	Summer	8.526	.043	.003	6.364	4
	Rainy	.450	.539	.602	.566	4

Independent Samples Test : Gewa Sapling leaf

		F	sig	Sig (2-tailed)	t	df
Na concentration	Winter	7.692	.050	.000	-10.871	4
	Summer	3.028	.157	.036	3.104	4
	Rainy	3.200	.148	.000	-85.597	4

Independent Samples Test : Gewa Tree leaf

		F	sig	Sig	t	df
N concentration	Winter	16.000	.016	.016	4.000	4
	Summer	.308	.609	.002	7.559	4
	Rainy	8.203	.046	.000	17.640	4

Independent Samples Test : Gewa Tree leaf

		F	sig	Sig (2-tailed)	t	df
P concentration	Winter	.400	.561	.003	6.500	4
	Summer	9.143	.039	.045	2.882	4
	Rainy	4.114	.112	.005	5.708	4

Independent Samples Test : Gewa Tree leaf

		F	sig	Sig (2-tailed)	t	df
K concentration	Winter	3.613	.130	.000	10.545	4
	Summer	3.200	.148	.000	57.691	4
	Rainy	.203	.676	.738	.359	4

Independent Samples Test : Gewa Tree leaf

		F	sig	Sig	t	df
Na	Winter	10.964	.030	.001	-9.941	4
concentration	Summer	6.400	.065	.000	-20.618	4
	Rainy	.000	1.000	.000	-36.620	4

A-2: Analysis of variance of Gewa *(E.agallocha)*

Nitrogen (N) – Sapling Mature leaf

ANOVA

N. concentration sapling mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	169.954	2	84.977	46.135	.000
Within Groups	11.052	6	1.842		
Total	181.006	8			

Homogeneous Subsets

N. concentration sapling mature leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	summer	3	4.6667		
	winter	3		8.6667	
	Rainy	3			15.2093
	Sig.		1.000	1.000	1.000

Nitrogen (N) – Sapling Senescence leaf

ANOVA

N. concentration Sapling Senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	**93.211**	**2**	**46.605**	**44.069**	**.000**
Within Groups	**6.345**	**6**	**1.058**		
Total	**99.556**	**8**			

Homogeneous Subsets

N. concentration Sapling Senescence leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	summer	**3**	**2.0000**		
	winter	**3**		**4.6667**	
	Rainy	**3**			**9.7577**
	Sig.		**1.000**	**1.000**	**1.000**

Nitrogen (N) – Tree Mature leaf

ANOVA

N. concentration Tree Mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	**349.220**	**2**	**174.610**	**123.585**	**.000**
Within Groups	**8.477**	**6**	**1.413**		
Total	**357.697**	**8**			

Homogeneous Subsets

N. concentration Tree Mature leaf

	season	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	winter	3	6.6667	
	summer	3	8.6667	
	Rainy	3		20.7667
	Sig.		.085	1.000

Nitrogen (N) – Tree Senescence leaf

ANOVA

N. concentration Tree senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53.103	2	26.552	76.903	.000
Within Groups	2.072	6	.345		
Total	55.175	8			

Homogeneous Subsets

N. concentration Tree senescence leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	summer	3	2.0000		
	winter	3		4.0000	
	Rainy	3			7.8530
	Sig.		1.000	1.000	1.000

Phosphorus (P) – sapling mature leaf

ANOVA

P. concentration sapling mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.782	2	.391	9.378	.014
Within Groups	.250	6	.042		
Total	1.032	8			

Homogeneous Subsets

P. concentration sapling mature leaf

	season	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	summer	3	1.4132	
	Rainy	3	1.4713	
	winter	3		2.0655
	Sig.		.740	1.000

Phosphorus (P) – sapling senescence leaf

ANOVA

P. concentration sapling senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.471	2	.235	3.163	.115
Within Groups	.446	6	.074		
Total	.917	8			

Homogeneous Subsets

P. concentration sapling senescence leaf

	season	N	Subset for alpha = 0.05
			1
Duncan[a]	winter	3	1.0871
	Rainy	3	1.2416
	summer	3	1.6307
	Sig.		.057

Phosphorus (P) – tree mature leaf

ANOVA

P. concentration tree mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.837	2	.919	1.880	.232
Within Groups	2.932	6	.489		
Total	4.769	8			

Homogeneous Subsets

P. concentration tree mature leaf

	season	N	Subset for alpha = 0.05
			1
Duncan[a]	winter	3	1.6307
	Rainy	3	1.6717
	summer	3	2.6091
	Sig.		.149

Phosphorus (P) – tree senescence leaf

ANOVA

P. concentration tree senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	2	.002	1.251	.352
Within Groups	.009	6	.002		
Total	.013	8			

Homogeneous Subsets

P. concentration tree senescence leaf

	season	N	Subset for alpha = 0.05
			1
Duncan[a]	winter	3	.6523
	summer	3	.6523
	Rainy	3	.6960
	Sig.		.233

POTASSIUM (K) - Sapling Mature leaf

ANOVA

K .concentration Sapling Mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.851	2	14.925	226.333	.000
Within Groups	.396	6	.066		
Total	30.247	8			

POTASSIUM (K) - Tree Mature leaf

ANOVA

K .concentration tree mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	49.537	2	24.769	669.031	.000
Within Groups	.222	6	.037		
Total	49.759	8			

Homogeneous Subsets

K .concentration tree mature leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Rainy	3	8.8537		
	summer	3		12.9354	
	winter	3			14.3980
	Sig.		1.000	1.000	1.000

POTASSIUM (K) - Tree Senescence leaf

ANOVA

K .concentration tree senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.463	2	3.231	22.344	.002
Within Groups	.868	6	.145		
Total	7.330	8			

Homogeneous Subsets

K .concentration tree senescence leaf

	season	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	summer	3	8.5476	
	Rainy	3	8.7857	
	winter	3		10.4524
	Sig.		.472	1.000

Sodium (Na) – Sapling Mature leaf

ANOVA

Na. concentration sapling mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.522	2	1.761	16.377	.004
Within Groups	.645	6	.108		
Total	4.168	8			

Homogeneous Subsets

Na. concentration sapling mature leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	summer	3	11.6847		
	Rainy	3		12.4955	
	winter	3			13.2162
	Sig.		1.000	1.000	1.000

Sodium (Na) – Sapling senescence leaf

ANOVA

Na. concentration sapling senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	170.976	2	85.488	457.957	.000
Within Groups	1.120	6	.187		
Total	172.096	8			

Homogeneous Subsets

Na. concentration sapling senescence leaf

	season	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	summer	3	10.6667		
	winter	3		17.7838	
	Rainy	3			21.1171
	Sig.		1.000	1.000	1.000

Sodium (Na) – Tree Mature leaf

ANOVA

Na. concentration tree mature leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.447	2	1.224	12.562	.007
Within Groups	.584	6	.097		
Total	3.031	8			

Homogeneous Subsets

Na. concentration tree mature leaf

	season	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	winter	3	9.2973	
	summer	3	9.5676	
	Rainy	3		10.5135
	Sig.		.330	1.000

Sodium (Na) – Tree senescence leaf

ANOVA

Na. concentration tree senescence leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.020	2	15.510	13.364	.006
Within Groups	6.964	6	1.161		
Total	37.984	8			

Homogeneous Subsets

Na. concentration tree senescence leaf

	season	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	summer	3	15.4414	
	Rainy	3		18.5946
	winter	3		19.8559
	Sig.		1.000	.202

A-3: Analysis of variance of soil

ANOVA

Nitrogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.837	2	7.919	9.176	.004
Within Groups	10.356	12	.863		
Total	26.193	14			

Homogenous subsets

			Subset for alpha = 0.05	
	Season	N	1	2
Duncan[a]	rainy	5	2.0000	
	summer	5		3.6667
	winter	5		4.4667
	Sig.		1.000	.198

Soil Phosphorus

ANOVA

Phosphorus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.476	2	.238	.868	.445
Within Groups	3.291	12	.274		
Total	3.767	14			

Homogeneous Subsets

Phosphorus

			Subset for alpha = 0.05
	Season	N	1
Duncan[a]	winter	5	1.6933
	summer	5	1.9613
	rainy	5	2.1255
	Sig.		.238

Soil Potassium

ANOVA

Potassium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.321	2	6.161	18.063	.000
Within Groups	4.093	12	.341		
Total	16.414	14			

Potassium Homogeneous Subsets

			Subset for alpha = 0.05	
	Season	N	1	2
Duncan[a]	rainy	5	8.9864	
	winter	5		10.5782
	summer	5		11.1224
	Sig.		1.000	.166

Soil Sodium

ANOVA

Sodium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.738	2	29.369	90.534	.000
Within Groups	3.893	12	.324		
Total	62.631	14			

Homogenous subsets

Sodium

			Subset for alpha = 0.05	
	Season	N	1	2
Duncan[a]	rainy	5	2.1009	
	winter	5	2.7741	
	summer	5		6.5946
	Sig.		.086	1.000