Khulna University
Life Science School
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Title: Nutrients (N, P and K) in leaves of *E. agallocha* L. of the Sundarbans, Bangladesh

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COURSE TITLE: Project Thesis
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APPROVAL

The style and format of the project thesis submitted to forestry and wood technology Discipline, Khulna University, Khulna, Bangladesh, in partial fulfillment of the requirements for the 4-year's professional Bsc. (Hons.) degree in Forestry has been approved.

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Declaration

I, Arunava Majumder, 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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Dedicated to

My Beloved Parents

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Abstract

The Sundarbans is the most productive mangrove ecosystem in the world. *Excoecaria agallocha* L. is one of the most extensively occurring tree species in Sundarbans. Present study access the amount of nutrients (N, P and K) at different saline zone from leaves of *Excoecaria agallocha* in Sundarbans. The concentration of nutrients from leaf of E. agallocha may influence the productivity of the aquatic and forest ecosystem. Nutrients (N, P and K) concentration from leaf oif this species was studied in the laboratory. After lab processing and digestion the samples were measured by a UV- Visible Recording Spectrophotometer (SHIMADZU UV – 160 A, Japan) for use at 650 nm wavelength to know the Nitrogen concentration. Sample was measured by a UV- Visible Recording Spectrophotometer (SHIMADZU UV – 160 A, Japan), with infrared phototube for use at 880 nm to know the phosphorus concentration. Potassium concentration in the leaf samples were measured by Flame photometer. Then cluster analysis was performed for nitrogen, phosphorus and potassium concentration in leaves using SPSS 20 software. We used GIS software also. We find that concentration of nutrients (Nitrogen, Phosphorus and potassium) of E. agallocha leafs varies in different saline zone.

Keywords: Nutrients (Nitrogen, Phosphorus, Potassium), Mangrove, Salinity, *Excoecaria agallocha.*

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Mangroves are salt tolerant trees, also called halophytes, and are adapted to life in harsh coastal conditions. They contain a complex salt filtration system and complex root system to cope with salt water immersion and wave action. They are adapted to the low oxygen (anoxic) conditions of waterlogged mud. Mangrove forests dominate the world's tropical and subtropical coastlines. 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 (e.g., Lovelock et al. 2005), but nutrient availability varies greatly between mangroves and also within a mangrove stand (Feller et al. 2003*a*).

A complex range of interacting abiotic and biotic factors controls the availability of nutrients to mangrove trees, and mangroves are characteristically plastic in their ability to opportunistically utilize nutrients when these become available. Numerous factors affect the distribution of mangrove plants. Most mangrove species are typically dispersed by water-buoyant propagules, allowing them to take advantage of estuarine, coastal and ocean currents both to replenish existing stands and to establish new ones. The direction they travel depends on sea currents and land barriers, but the dispersal distance depends on the time that propagules remain buoyant and viable. This is expected to differ for each species. Similarly, each species will also differ in establishment success and growth development rate, and each has tolerance limits and growth responses which are apparently unique. Such attributes are presumably responsible for the characteristic distributional ranges of each species, as each responds to the environmental, physical and biotic settings they might occupy. In practice, species are often ordered by the interplay of different factors along environmental gradients, and these may conveniently be considered at four geographic scales—global, regional, estuarine and intertidal. These influencing factors act similarly around the world.

In the coastal areas of Bangladesh mangrove forest is considered as a critically important ecosystem, The Sundarbans, the world's largest stretch of mangrove ecosystem. Mangroves protect our coast from the heavy wind, tidal waves, coastal erosion and sea water intrusion. It also generate substantial quantities of fishery resources and provide many useful forestry products. In the sundarbans, it has totally 334 plants, 165 algal, 13 special orchids, 17 fern, 87 monocotyledon and 230 dicotyledon belonging to 245 genera and 75 families from the sundarbans and adjacent area are found available. Sundry (Heritiera fomes) is the dominant tree species which covers about 73% to the total landmass and the second species is Gewa (***Exocoecaria agallocha***) which covers about 16% of the total forest area. The Sundarbans alone contain 35 true mangrove plant species whereas the 50 true mangrove plant species is present throughout the globe which is recorded. (M R Rahman and M Asaduzzaman, ECOLOGY OF SUNDARBAN, BANGLADESH). It is situated in the delta of the rivers of Ganga, Brahmaputra and Meghna at the point where it merges with the Bay of Bengal. Some portion is located in the India. The tidal halophytic mangrove forest in the world shared between Bangladesh (62%) and India (38%). It has the great economic contribution to the society, such as extraction of timber, fishing and collection of honey. The climate condition of the Sundarbans is very challenging. It has the subtropical monsoonal climate where the annual rainfall is 1600-1800 mm and experiences severe cyclonic storms. The three rivers carries huge amount of sediments which contribute to its expansion and dynamics. Salinity gradients change over a wide range of spatial and temporal scales. UNESCO declared the sundarbans as a world heritage site.

1.2 Objectives

The objectives of this study was as follows:

- To measure Nitrogen, Phosphorus and Potassium concentration in leaves of *E.agallocha* of the Sundarbans.

CHAPTER TWO
LITERATURE REVIEW

Mangroves have long been a source of astonishment to the layman and of interest for the scientist. For many people living in the Indo-West Pacific and Americas-East Atlantic regions, the word mangrove will be a familiar one. For a selected few, long standing familiarity is based perhaps on vague and romantic mental pictures of waterlogged woodlands in which tangled aerial root systems foil the would-be explorer. However, the majority see them as swamps which are ridden with mosquitoes and sand flies, inhospitable, unhealthy and dangerous. There is another category of people whose knowledge of mangroves is derived from recent publicity given by concerned conservationists to preserve the ever dwindling mangrove areas of the world. Yet others living in these regions and the rest of the world have little or no concept of what it is that constitutes the mangroves. The term mangrove is also used to designate halophytic (salt loving) and salt resistant marine tidal forests comprising trees, shrubs, palms, epiphytes, ground ferns and grasses which are associated in stands or groves (Chapman 1977; Knox & Miyabara1984; FAO 1982).

2.1 General Information of *Excoecaria agallocha*

Milky mangrove is an evergreen, or briefly deciduous shrub or small tree growing 10 - 30 metres tall. The bole, which branches from low down, has stilt roots. The plant is sometimes harvested from the wild for local use as a medicinal herb. It is also often cultivated as an ornamental. A widespread and common species, though there are some localised threats and there has been an overall population decline caused by coastal development throughout its range. The plant is classified as 'Least Concern' in the IUCN Red List of Threatened Species(2011).

2.2 Known Hazards

All parts of the plant are poisonous.The tree exudes a very acrid poisonous juice, particularly from the fresh cortex when cut, which raises blisters on the skin and is injurious to the eyes of wood-cutters, whence the name 'blinding tree'. The latex is used as a fish poison. The crushed leaves, when placed in the water, stupefy the fish and cause them to float to the surface.

2.3 Chemical constituents of *Excoecaria agallocha*

Latex contains alcohols - exocarol, agalocol, isoagalocol and mannitol; β-amyrin and its 3-epimer, β-amyrenone, cycloartenol and an unknown compound. Leaves contain epitaraxerol. Twigs and bark contain a piscicidal compound which is toxic to *Cryzias latipes* (fish) at 0.003ppm after 24 hrours (Asolkar *et al.*, 1992; Rastogi & Mehrotra, 1990).

2.4 Properties of *Excoecaria agallocha*

Habit	Deciduous Tree
Height	15.00 m
Self-fertile	No
Cultivation Status	Ornamental, Wild

2.5 Distribution of *Excoecaria agallocha*

2.5.1 Range Description

In South Asia this species is found in Bangladesh, Brunei Darussalam, China, Taiwan, Hong Kong, India, Indonesia, Japan, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Viet Nam. The species also occurs in the Maldives.

In Australasia it can be found in Northwest Australia, Northeast Australia, Southeast Australia, Federated States of Micronesia, Fiji, Guam, New Caledonia, Palau, Papua New Guinea, Solomon Islands, Tonga, Vanuatu.

2.5.2 Global Distribution

Indo-Malesia to Australia and Pacific islands.

2.5.3 Distribution in Bangladesh

Sundarbans and other coastal forests of Bangladesh.

2.6 Morphology Description

Trees deciduous, up to 15 m tall; branches lenticellate, glabrous. Leaves alternate throughout; stipules ovate, 1.5-2 mm; petiole 1.5-3 cm; leaf blade elliptic or broadly elliptic, rarely ovate-oblong, 4.5-10 × 3-5 cm, sub leathery, base obtuse or broadly cuneate, margin entire or indistinctly serrulate, with 2 distinct glands at base near junction with petiole, apex obtusely acuminate; midvein stout, concave adaxially, convex abaxially, lateral veins 10-13, arcuately ascending steeply and running close to edge some distance before terminating. Flowers dioecious, in axillary, solitary or double racemelike thyrses; male inflorescences 3-4.5 cm, dense and catkinlike, female ones shorter. Male flowers 1 per bract; bracts broadly ovate, fleshy, ca. 2 × 2 mm, base inside 2-glandular, branchlets lanceolate; sepals linear-acuminate, ca. 1.2 mm; stamens usually exceeding sepals. Female flowers: bracts and bracteoles as in male; pedicels slightly longer than male ones (elongating to 5 mm in fruit); sepals broadly ovate or triangular, slightly connate at base, ca. 1.4 × 1 mm; ovary ovoid; stigmas, ca. 3 mm, free, revolute at apex. Capsules globose, trisulcate, 7-8 × ca. 10 mm; cocci ovoid, rostrate at apex. Seeds globose, ca. 4 mm in diam. Fl. and fr. Jan-Sep. $2n = 140$.

Family: Euphorbiaceae

Flowers: Flowers are small and occur in sprays or racemes up to 3cm long in the axils or forks of the leaves.

Leaves: The leaves are alternate, shiny bright green, fleshy, 4-10cm long and 2-5cm wide, with a smooth or bluntly toothed margin.

Fruit/Berries: The fruit are capsules 6-8mm wide, usually with three cells, each containing a seed. The capsule cells explode apart when mature.

Other: The sap is milky.

Symptoms: The copious milky sap can cause intense pain and blistering if it makes contact with the skin. It can also cause intense pain and temporary blindness if it gets into the eyes.

Flowering and fruiting: November-February

General Habitat

Banks of backwaters and mangrove forests.

Cyclicity

Flowering and fruiting: November-February.

2.7 Habitat and Ecology

This is a back mangrove species and often exploits open areas and is tolerant of distrurbed areas. It is a small to medium sized tree with extensive cable roots. It has multiple stems. It can be deciduous in cooler/drier areas. It produces a latex (milky sap) that causes temporary blindness. ***Hibiscus tiliaceus*** is its main associate in China (Peng and Xin-men 1983).

2.8 Relation with the salinity

E. agallocha is highly sensitive to salinity, especially at early developmental stages. The favorable salinity range for seed germination is below 5 psu, and salinity of 15 psu decreased seedling establishment rate to 37%, which partly explained why few seedlings can be found in the mangrove reserve which has soil salinity slightly higher than 15 psu. The adverse saline condition in the field might act as a primary obstacle for natural regeneration of this species. Thus, artificial breeding and culture should be adopted to ensure higher survival rate of *E. agallocha* seedlings. *E. agallocha* will increase its tolerance to salinity over time and the salinity level in its natural habitat probably approaches the tolerance limit of early *E. agallocha* seedlings.

2.9 Cultivation Details

A plant of the low land tropics and subtropics, where it is found at elevations up to 400 metres. It grows best in areas where annual daytime temperatures are within the range 20 - 30°c, but can tolerate 15 - 37°c. It prefers a mean annual rainfall in the range 1,000 - 2,000mm, but tolerates 800 - 4,100mm. It requires a sunny position. Succeeds in a wide range of fertile soils. Tolerant of saline soils. Prefers a pH in the range 6.5 - 7, tolerating 6 - 8. A dioecious species, both male and female forms need to be grown if fruit and seed are required.

2.10 Uses

2.10.1 Edible Uses

None known

2.10.2 Medicinal Uses

The latex is used as a caustic for removing obstinate ulcers. The latex is rubbed around the tip of the penis and left for three days. Although this causes irritation, it is also believed to enlarge the penis. The juice is emetic and purgative. Very small amounts are taken orally with coconut juice to treat pneumonia or asthma. It may also be taken to clear out the body as a poison antidote. The resin is used as an anthelmintic, for its purgative effect. Oil extracted by distillation of the wood or latex is applied to cutaneous diseases. Chewing a little piece of bark will cause instant vomiting and purging, but is in general considered too drastic a cure for constipation. The root is used as an abortifacient. Applied externally, the root is pounded with ginger and used as an embrocation to reduce swellings on the hands and feet.

2.10.3 Other Uses

Certain parts of the wood are used for incense. The white wood is soft and spongy. The roots are sometimes used as floats.

2.10.4 Propagation

Seed.

2.11 Population

This species is reasonably abundant throughout its range and is a very widespread species. Although there is no species specific population information, it can be assumed that there are areas of population decline throughout its range due to coastal development.

2.11.1 Population Trend

Decreasing

2.12 Major Threats

Sea level rise is a major threat, especially to back mangroves that have no area in which to expand. Mangrove species with a habitat on the landward margin may be particularly vulnerable to sea-level rise if owing to coastal development their movement inland is blocked. Howevever, species that are easily dispersed and fast growing/ fast producing will cope better than those which are slower growing and slower to reproduce. Although local estimates are uncertain due to differing legislative definitions of what is a 'mangrove' and to the imprecision in determining mangrove area, current consensus estimates of mangrove loss in the last quarter-century report an approximately 21% decline in mangrove areas in countries within this species range since 1980(FAO2007).

All mangrove ecosystems occur within mean sea level and high tidal elevations, and have distinct species zonations that are controlled by the elevation of the substrate relative to mean sea level. This is because of associated variation in frequency of elevation, salinity and wave action (Duke *et al.* 1998). With rise in sea-level, the habitat requirements of each species will be disrupted, and species zones will suffer mortality at their present locations and re-establish at higher elevations in areas that were previously landward zones (Ellison 2005). If sea-level rise is a continued trend over this century, then there will be continued mortality and re-establishment of species zones.

In addition, mangrove area is declining globally due to a number of localized threats. The main threat is habitat destruction and removal of mangrove areas. Reasons for removal include cleared for shrimp farms, agriculture, fish ponds, rice production and salt pans, and for the development of urban and industrial areas, road construction, coconut plantations, ports, airports, and tourist resorts. Other threats include pollution from sewage effluents, solid wastes, siltation, oil, and agricultural and urban runoff. Climate change is also thought to be a threat, particularly at the edges of a species range. Natural threats include cyclones, hurricane and tsunamis.

2.13 Conservation Status of *Excoecaria agallocha*

This species is widespread and common. There are some localised threats and overall population decline from coastal development throughout its range. There has been an estimated 21% decline in mangrove area within this species range since 1980. However, this back mangrove species is able to grow quickly and colonize disturbed areas. Mangrove species are more at risk from coastal development and extraction at the extremes of their distribution, and are likely to be contracting in these areas more than in other areas. It is also likely that changes in climate due to global warming will further affect these parts of the range. There are overall range declines in many areas due to habitat loss or extraction, but not enough to reach any of the threatened category thresholds. This species is listed as Least Concern.(**Ref:** IUCN Red List Assessment, Red List Criteria, Version 3.1, Year Assessed 2010, Polidoro, B.A., Livingstone, S.R. & Carpenter, K.E. (Global Marine Species Assessment Coordinating Team))

2.14 Conservation Actions

There are no conservation measures specific to this species, but its range may include some marine and coastal protected areas. Continued monitoring and research is recommended, as well as the inclusion of mangrove areas in marine and coastal protected areas.

CHAPTER THREE
MATERIALS AND METHOD

3.1. Description of the study area

The sample were collected throughout the Sundarbans of Bangladesh. Sundarbans is a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal (Wahid et al. 2002). The forest has been divided into three ecological zones, based on salinity and distribution of species composition such as i) less saline/fresh water zone, ii) moderately salt water/moderately saline zone and iii) salt water zone/strong by saline zone (Karim 1988). The forest lies between 89° 00' and 89° 55' east longitudes and 21° 30' and 22° 30' north latitudes (Figure 1). It has an area of 5770 km^2, of which 4016 km^2 covered by the forests and the remaining 1756 km^2 are in the form of rivers, canals and creeks, varying from a few meters to several miles (Hussain and Acharya 1994).

3.2. Sample collection and field processing

Mature green leaves of *E.agallocha* were purposively collected from 58 points that covering the representative parts of the Sundarbans. Five mature leaves (3^{rd} pair) of *E.agallocha* were collected from each sample tree. However, age variation among the sample trees and leaf characteristic (sun and shedleaves) were not considered. The leaf samples were collected during January 2017 to April 2017. The collected leaves were proceeding by pressing into the newspaper to remove the moisture during the field work. However, the collected samples were tried to send immediately in the laboratory for further processing.

3.3. Sample preparation in laboratory

The collected leaf samples were oven-dried at 80° C for 7 days. The oven-dried samples were then crushed and sieved through 2 mm mesh sieve and preserved at dry place in air tight plastic container.

3.4. Nutrient concentration in leaf tissue

Then three replicates of 0.1 g were taken randomly from each container of samples and acid digested according to Allen (1994).

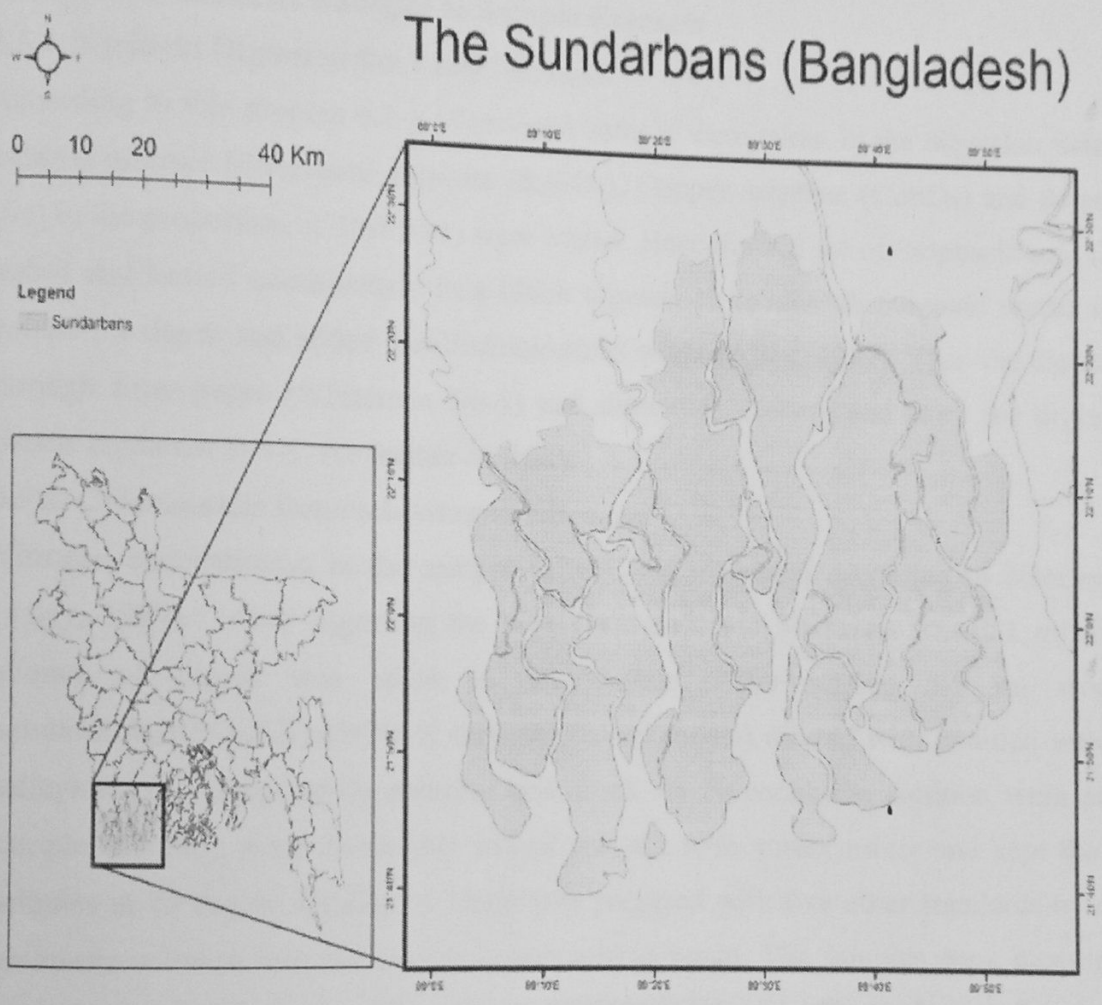

Figure 1: Map of the Sundarbans, Bangladesh

3.5. Determination of Nitrogen in Sample Extracts

3.5.1. Kjeldahl Digestion for Total Nitrogen

According to this process 0.1 g of proceed sample were taken in the digestion tube. Then 1.1 g catalyst mixture (Potassium sulphate (K_2SO_4), Copper sulphate ($CuSO_4$) and Selenium powder (Se) in the proportion of 100:10:1) were added. Henceforth 3 ml of Sulphuric acid (H_2SO_4) were added and heated continuously in a block digester to oxidize the organic matter at 200°C and cooled the digest and added distilled/deionized water (Alley, 1989). Then the digest was filtered through filter paper (Whiteman No-1) and diluted to 100 ml and store the digest in air tight plastic container at 4°C for further analysis.

3.5.2. Colorimetric Determination of Nitrogen

Nitrogen concentration in the sample extract was measured according to colorimetric method (Alley, 1989). After digestion the digest were diluted 50 times. Then, 1 ml aliquot/diluted aliquot of digest was taken in a test-tube. After adding 5.5 ml working buffer solution($Na_2HPO_4.12H_2O$+Na-K tartrate+NaOH(pellets) diluted with distilled water.), 4 ml Na salicylate-Na Nitroprusside solution and 2 ml Na Hypochlorite solution were added to each sample and they were thoroughly mixed and stir with vortex mixer and kept the stand for 45 minutes at 25 degree Celsius. A blank was prepared with five other standards by diluting stock ammonia solution into the sample concentration range. The samples were measured by a UV-Visible Recording Spectrophotometer (SHIMADZU UV-160 A, Japan) for use at 650 nm wavelength.

3.6. Determination of Phosphorus in Sample Extracts

3.6.1. Tri-acid Digestion for Total Phosphorus

In this digestion method,0.1 g of plant sample was taken in 100 ml digestion tube. After adding 3 ml concentrated Nitric acid and heating continuously in block digester to oxidize the organic matter at 100°C for 50 to 60 minutes then 6.4 ml of mixed acid (Nitric acid, Per chloric acid 60% and Sulphuric acid mixed at the proportion of 10:2:1) added to the predigested samples and raised the temperature up to 200°C and digested for 20 minutes (Allen, 1974). The digest was cooled and add distilled/ deionized water and filter the digest through filter paper (Whiteman No 42) and diluted to 100 ml.

3.6.2. Colorimetric determination of Phosphorus

After digestion 10 ml aliquot/diluted aliquot of digest was taken in a 50 ml volumetric flux. Then, 8 ml Mixed Solution (Ammonium Molybdate+L-AscorbicAcid+Antimony potassium tartrate+Sulphuric acid) was added and made to a volume (50 ml) with Distilled Water (DW), shake and allowed 15 min for color development. Then, the standard was Prepared as same the procedure described above and each sample was measured by a UV-Visible Recording Spectrophotometer (SHIMADZU UV- 160A, Japan), with infrared phototube for use at 880 nm (Murphy and Riley, 1962).

3.7. Determination of Potassium and Sodium in Sample Extracts

Potassium and sodium concentration in the leaf samples were measured by Flame photometer (PFP7, Jenway LTD, England). The same sample extract as described in mentioned in Section 3.6.1 were used to determine Potassium and Sodium concentration.

3.8. Determination of Carbon in Sample Extracts

Organic carbons of leaf sample of H. fomes were determined from Loss of Ignition method (Allen, 1989). According to this method approximately 1 g of plant sample was taken in porcelain cup and oven dried at 105°C for 1 hour to make the sample free from moisture. The oven-dried weight of the sample was recorded. After oven-dry, the sample was placed into the Maffle Furnace and raised the temperature slowly up to 450°C and kept it for 4 to 6 hours. Now, sample weight after this ignition was taken and recorded. Then the calculation of LOI% and organic carbon content in leaf sample were derived using the following equation:

$$\text{LOI (\%)} = \frac{\text{Loss of weight after burning (g)}}{\text{Ovendry weight (g)}} \text{X } 100$$

Estimate the organic carbon from the 50% of LOI of the sample (Allen, 1989).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Nitrogen in leaf

Nitrogen concentration of *E.agallocha* leaf ranges from 0.98% to 3.12% in three saline zone. 58 grids or sampling point constitute three major cluster on the basis of Nitrogen concentration. Grid no

(74,14,91,32,36,31,45,128,200,47,135,25,130,192,71,92,109,215,70,170,26,197,174,122,123, 160,15,66,53,156,17,202,151,195,180,227,198,179,83,230,150,111,174,196,229,205,219,191, 136,50,148,93) belongs to cluster 1, Grid no (190,108,10,20) belongs to cluster 2 and Grid no(226,9) belongs to cluster 3 which is showing to following dendrogram.

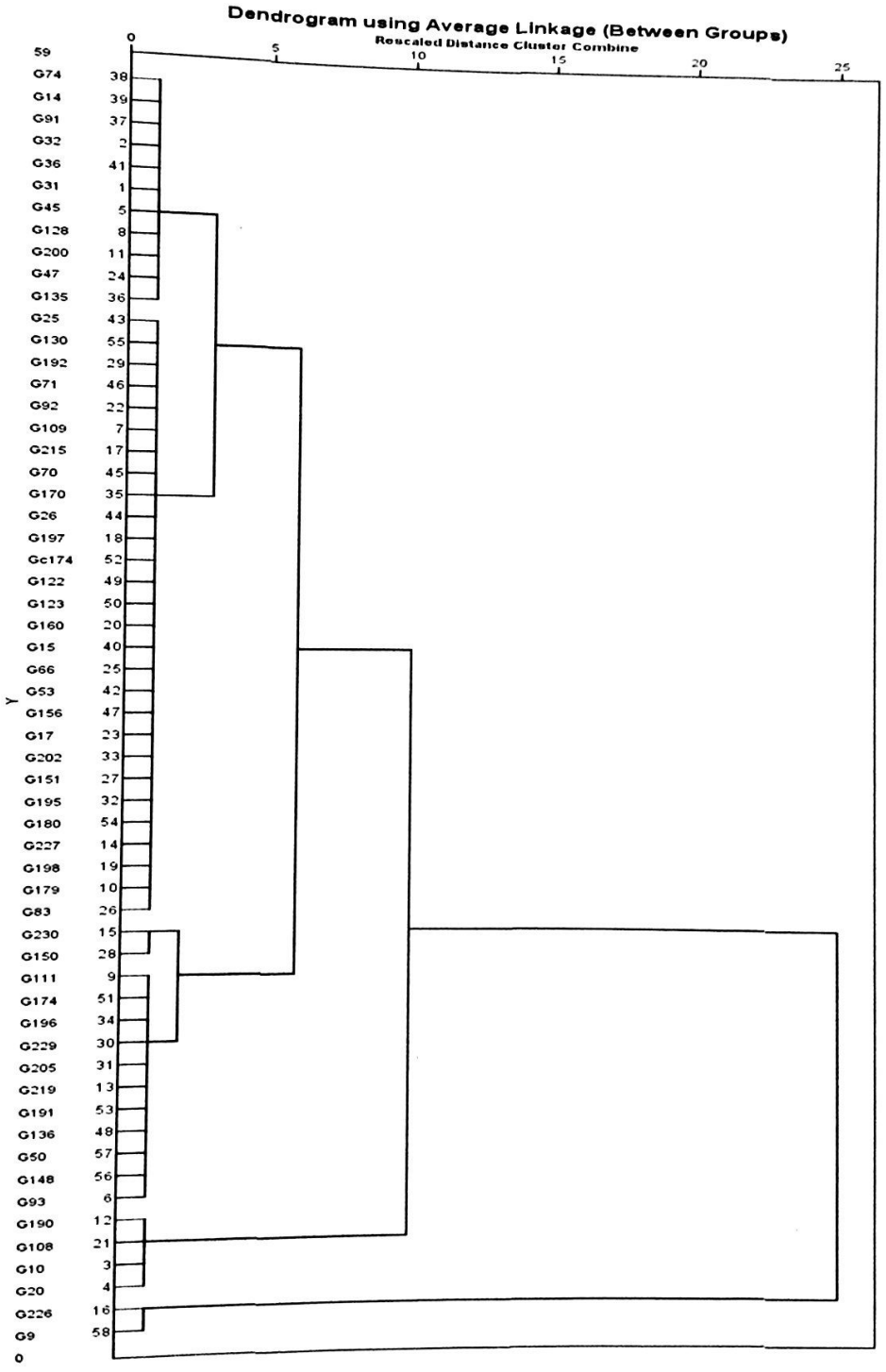

Fig: Cluster analysis of nitrogen concentration

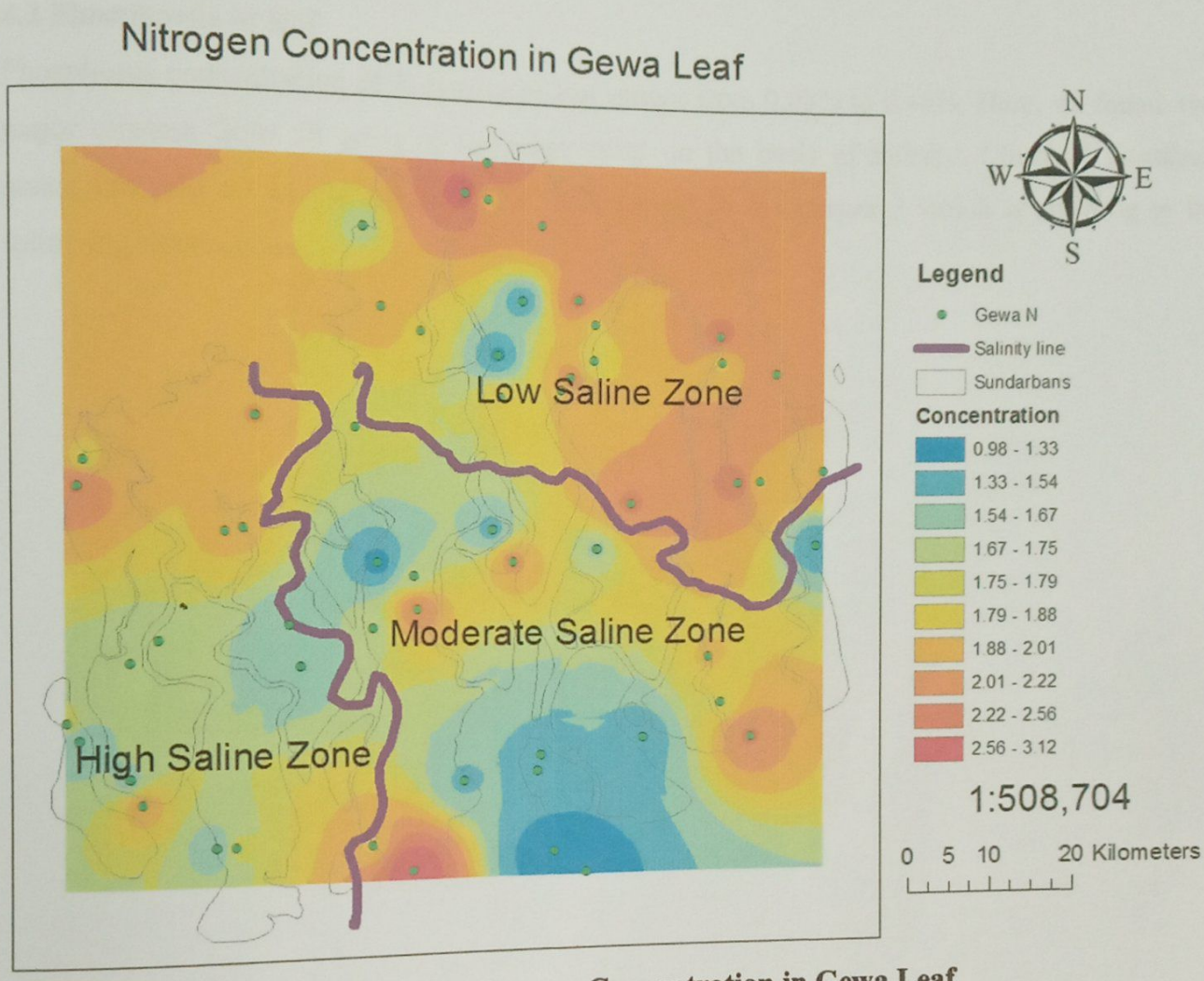

Fig : Nitrogen Concentration in Gewa Leaf

4.2 Phosphorus in leaf

Phosphorus concentration of *E.agallocha* leaf ranges from 0.08% to 0.46%. Here, we found two major clusters from 58 grids or sampling point on the basis of salinity. Cluster 1 constitute maximum grid no except grid no 229. It is present in the cluster 2 which is showing to the following dendrogram.

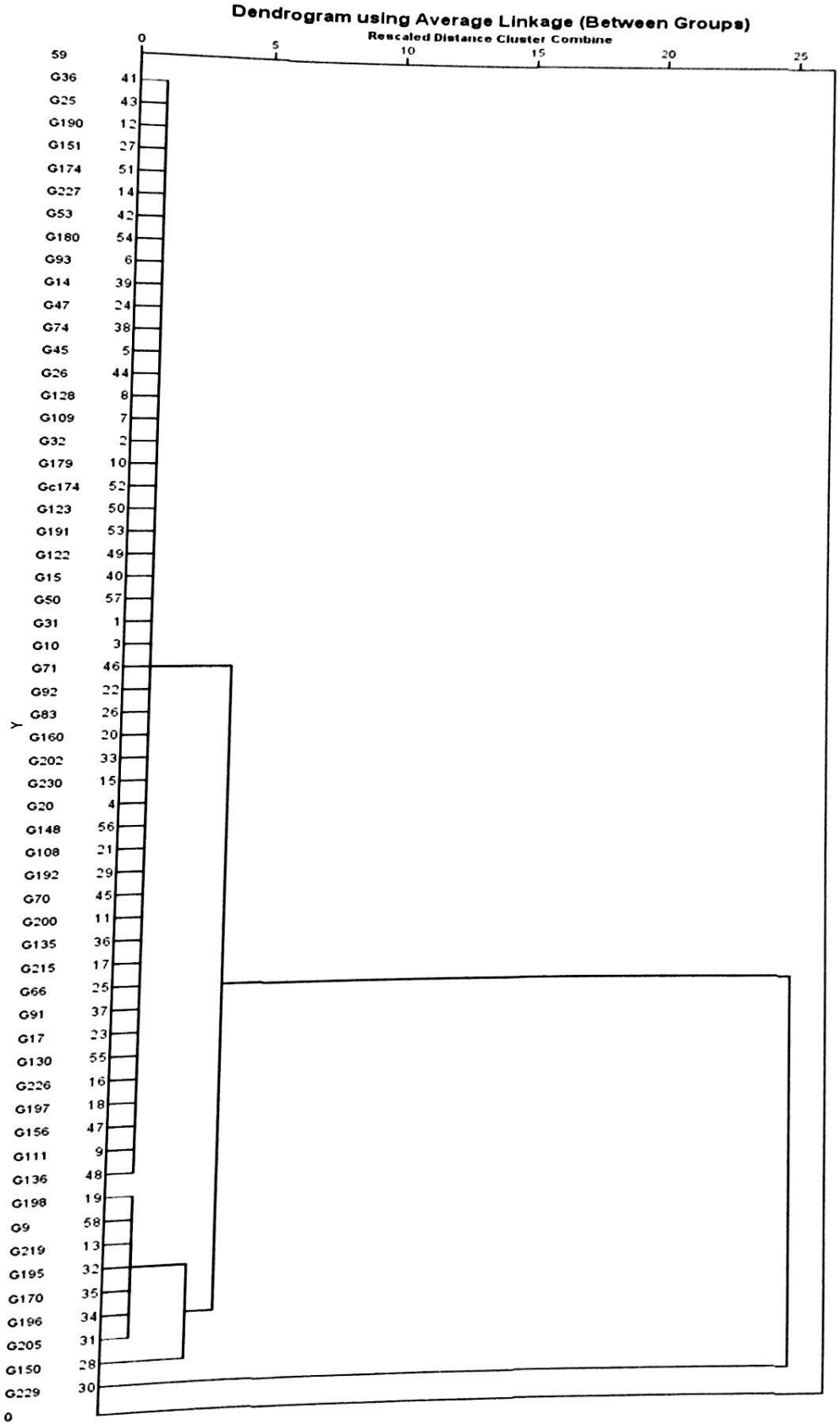

Fig: Cluster analysis of Phosphorus concentration

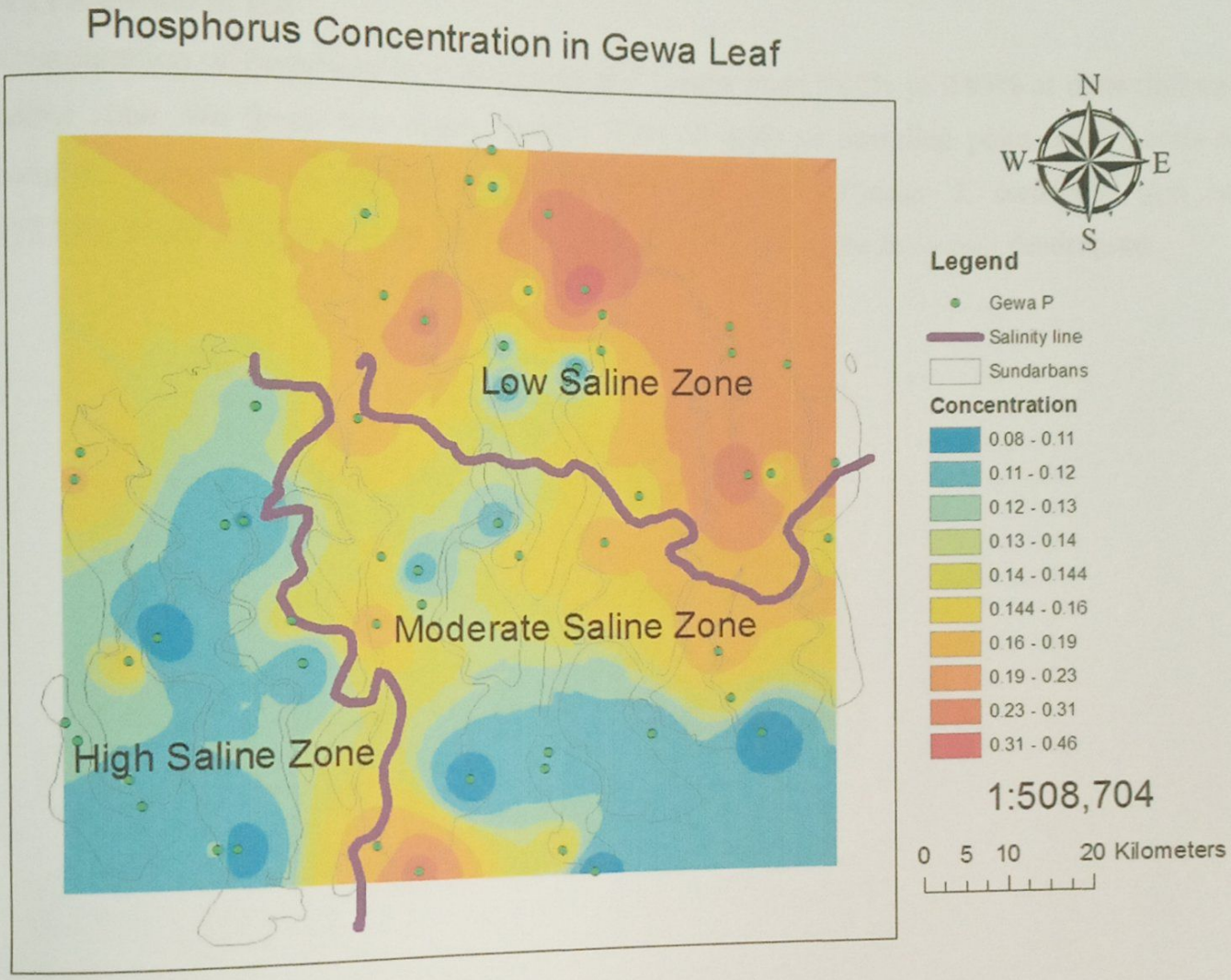

Fig : Phosphorus Concentration in Gewa Leaf

4.3 Potassium in leaf

Concentration of Potassium in *E.agallocha* leaf ranges from 0.07% to 2.83% at three different saline zone. We found two major clusters from 58 grids or sampling point on the basis of salinity. Cluster 1 constitute maximum grid no and Cluster 2 constitute grid no (17,130,160,83,135,198,20,170,229,196,9) which is showing to the following dendrogram.

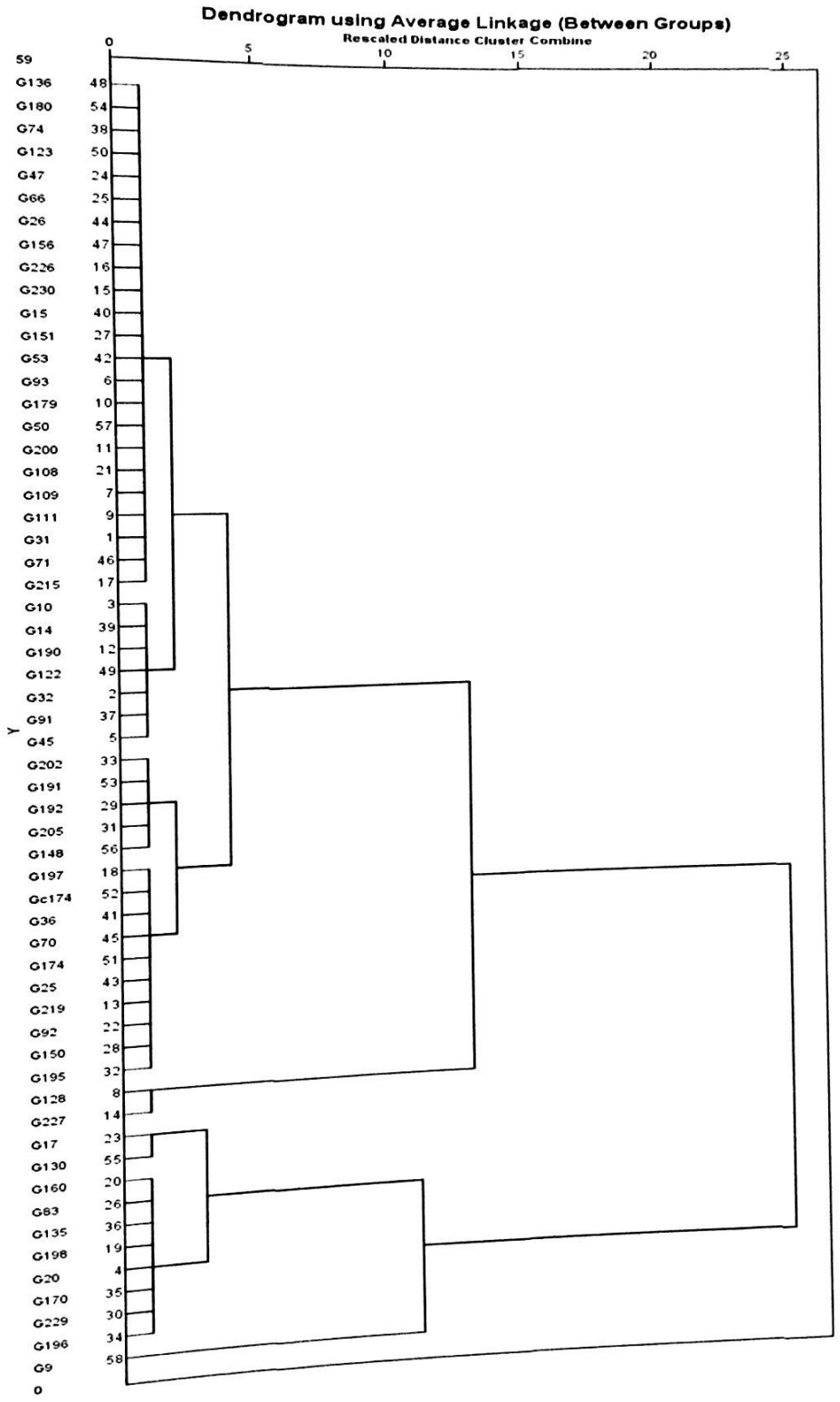

Fig: Cluster analysis of Potassium concentration

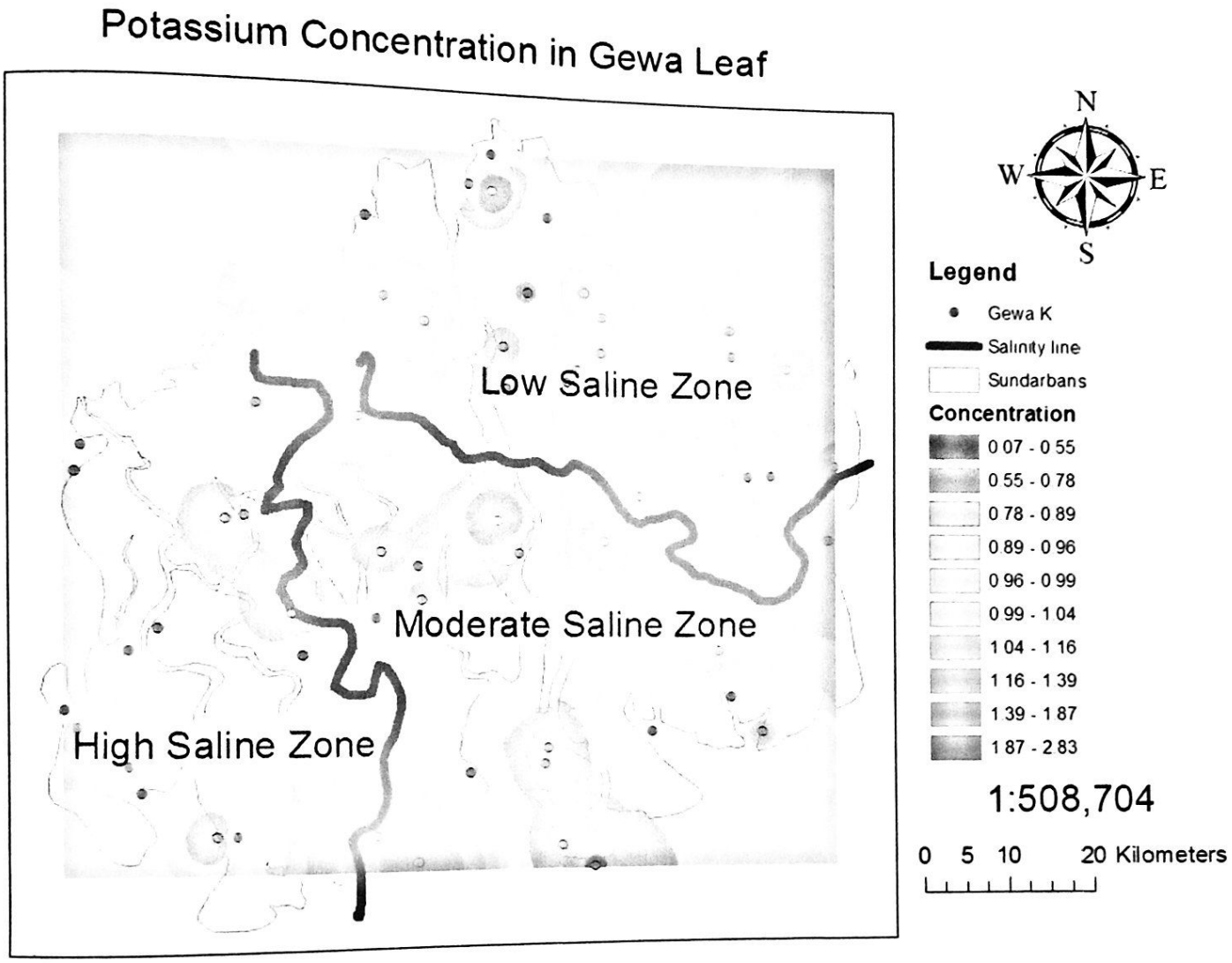

Fig : Potassium Concentration in Gewa Leaf

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