# METHODS OF LEAF SAMPLING IN TEAK (TECTONA GRANDIS) FOR NUTRIENT ANALYSIS

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JAYAMADHAVAN, A., SUDHAKARA, K. & WAHID, P. A. 2000. Methods of leaf sampling in teak (*Tectona grandis*) for nutrient analysis. A study was conducted in 10-y-old teak plantations of Nilambur, Kerala, India, to standardise the canopy height, time of sampling, leaf rank and diameter class of trees from which to collect leaf samples for the determination of N, P and K concentrations. Seasonal variation in the foliar N, P and K concentrations was also determined for a period of thirteen months starting from August 1993 to August 1994. Since mean nutrient concentration was high and varied little among the samples, leaves obtained from the bottom position of the crown during the time interval 0900 h to 1100 h were taken as the standard. Similarly, the second leaf rank from trees belonging to the diameter class (16.75–20 cm) were found to be ideal for sampling. Foliar nutrient concentrations were higher from June to September, which received high rainfall and were low during the drier months of January to April.

Key words: Leaf sampling - nutrient analysis - standardisation - Tectona grandis

JAYAMADHAVAN, A., SUDHAKARA, K. & WAHID, P. A. 2000. Kaedah pensampelan daun dalam pokok jati (*Tectona grandis*) untuk analisis nutrien. Kajian dijalankan di ladang jati berumur 10 tahun di Nilambur, Kerala, India, untuk menyeragamkan ketinggian sudur, masa pensampelan, peringkat daun dan kelas garis pusat bagi pokokpokok yang akan dipungut sampel daunnya untuk menentukan kepekatan N, P dan K. Perubahan musim dalam kepekatan N, P dan K daun juga ditentukan untuk jangka masa tiga belas bulan bermula pada bulan Ogos 1993 hingga Ogos 1994. Memandangkan kepekatan nutrien purata adalah tinggi dan berubah sedikit di kalangan sampel, daun-daun yang diambil daripada posisi bawah silara pada selang masa 0900 h hingga 1100 h diambil sebagai standard. Samalah juga dengan peringkat daun kedua daripada pokok dengan kelas garis pusat (16.75–20 cm) didapati sesuai untuk pensampelan. Kepekatan nutrien daun adalah lebih tinggi daripada Jun hingga September, yang mendapat hujan yang tinggi, manakala pada musim kering iaitu Januari hingga April kepekatan nutrien daun adalah rendah.

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#### Introduction

Teak (*Tectona grandis* L.f.), a large deciduous tree, is one of the most important timber species of India (Troup 1986). To meet the increasing demand for teak, reducing its rotation age is contemplated widely. This could be achieved by increasing its growth rate through proper nutrition. Plantations managed for short rotations are the most nutrient-demanding, and cause the greatest loss of nutrients from the site at harvest (Ericsson 1994). For a quick and accurate assessment of the plant nutritional status, foliar analysis has been emphasised by Leaf (1968) and Van den Driessche (1984). Zhang *et al.* (1995) concluded that leaf analysis could be used as a guide to fertiliser application and management.

Success of an economically viable fertiliser programme requires an accurate diagnosis of the nutritional status of forest stands in relation to sufficiency or deficiency. Actual fertiliser practices vary considerably among regions owing to vast differences in soil and climatic conditions (Birk 1994). Evidence indicates that forest trees suffer from nutrient deficiencies and do not always attain their full growth potential (Mustanoja & Leaf 1965). Plant analysis can be a useful tool in diagnosing the probability of response of tree stands to the application of fertilisers.

Steenbjerg (1954) stressed the importance of standardising sampling procedures to eliminate all the factors that cause variation in leaf nutrient levels. Amir Husni *et al.* (1993) found that foliar elemental levels differed in the different canopy tiers of *Acacia mangium*, and the distributions were not in conformity with observed patterns in other taxa. They further recommended that for an evaluation of fertility status of *Acacia mangium*, the lower tier of sun-exposed foliage should be sampled for N and P levels and the top tier for other elements. No attempt has been made so far in teak, to standardise the time of sampling, canopy height, leaf rank and diameter class from which leaf samples are to be collected for nutrient analysis. The present study was, therefore, taken up to standardise the canopy height, time of sampling, leaf rank and diameter class of trees from which leaf samples are to be collected for nutrient analysis and also to evaluate the seasonal variations in foliar nutrient concentrations in teak.

## Materials and methods

## Study site

The Nilambur Forest Range,  $(10^{\circ}10'-30' N, 75^{\circ}35'-76^{\circ}35' E)$ , under the jurisdiction of the Kerala Forest Department, is in Malappuram District. The plain forests have an elevation varying from 40 to 300 m and are interspersed with a network of rivers and rivulets (Ranganathan 1981). The region enjoys the southwest monsoon (June to August) and the northeast monsoon (October to November). The extreme temperatures in the shade vary approximately between 17 °C and 37 °C (Ranganathan 1981). Field work was done during 1992–94 in the 1982 Muttikkadavu teak plantation of site quality II belonging to the Nilambur Range. The plantation is situated near the Karimpuzha river and has deep alluvial soil. The predominant parent rock is gneiss which disintegrates into very fine particles. The important soil type is very fine loam of depths varying with slope. The height and diameter of trees in the plantation range from 9 to 15 m and 11 to 19 cm respectively. Canopy of the plantation is open.

#### Standardisation of canopy height and time of sampling

First fully mature leaves were collected from the top, middle and bottom of the tree canopies on 14 May 1993 at seven different time intervals, viz. 0500–0700 h, 0700–0900 h, 0900–1100 h, 1100–1300 h, 1300–1500 h, 1500–1700 h and 1700–1900 h. Time of sampling was denoted by the midpoint of the interval as 0600, 0800, 1000, 1200, 1400, 1600 and 1800 h. Top, middle and bottom portions of the canopies were defined as the upper one-third, middle one-third and lower one-third of the canopy respectively. Leaf samples were collected by climbing and hand picking from four trees selected randomly from a plot. The samples were analysed for N, P and K. The portion of the canopy and time of sampling for which the nutrient concentrations were high and coefficients of variation were less were considered suitable for leaf sampling.

#### Standardisation of diameter class and leaf rank

Diameter at breast height of all trees was recorded and the entire plantation was divided into four diameter classes, viz. Class I—7.10 to 10.25 cm, Class II—10.26 to 13.50 cm, Class III—13.51 to 16.75 cm and Class IV—16.76 to 20.0 cm. From each diameter class, six trees were randomly selected. Leaf samples were taken from these trees starting from the first fully mature leaf down to the fifth leaf on a branch and categorised as five different ranks. Sampling was done from the canopy height and during the time of the day standardised in the previous study. Leaf samples were analysed for N, P and K. Diameter class and leaf rank which gave minimum coefficient of variation and high concentrations of nutrients were adjudged best for sampling purpose.

#### Seasonal variation

Based upon the standardisation, six leaf samples were collected at 1000 h from the second leaf rank of the bottom portion of the canopy of the trees belonging to the fourth diameter class from August 1993 to August 1994 for thirteen months and analysed for N, P and K.

## Analytical methods

The collected leaf samples were dried at 70 °C and powdered using a grinder. The powdered samples of each tree were pooled and digested using a modified Kjeldahl digest entailing conc.  $H_2SO_4$ ,  $H_2O_2$  (30%) and Se catalyst (Alexander *et al.* 1985). Total N content of the digested samples was determined by the micro-Kjeldahl's distillation method. Phosphorus content was determined using the chlorostannous reduced blue colour method. Concentration of K in the samples was determined using flame photometer (Jackson 1973).

## **Results and discussion**

## Standardisation of canopy height

It was found that the canopy height had no significant effect on the foliar N, P and K concentrations (Table 1). However, the bottom canopy recorded the highest concentrations of foliar N and K and the second highest concentration of foliar P. For N and P, coefficient of variation among the samples was lowest for the bottom canopy and highest for the middle canopy. But in the case of foliar K, coefficient of variation was lowest for the bottom canopy and highest for the top canopy.

Canopy height	N		P		К	
	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)
Тор	2.51	11.04	0.28	38.98	1.64	36.56
Middle	2.45	11.38	0.24	46.49	1.58	32.51
Bottom	2.59	9.02	0.27	25.21	1.65	27.65

Table 1. Variations in the foliar nutrient concentrations due to canopy height in teak

By restricting the sampling to branches at the bottom portion of the canopy, the corresponding coefficients of variation for N, P and K were diminished and the efficiency of the sampling was improved. Hence, the leaves taken from the bottom portion (the lower one-third) of the tree canopy could provide a representative sample. From the practical point of view also, the bottom canopy is more convenient for plucking the leaves. Zech and Drechsel (1991) took leaf samples of at least five dominant or co-dominant 5–11-y-old trees in the middle of the rainy season during flowering. They collected young immature and the first two mature leaves separately from the terminal shoots of the upper part of the crown. Drechsel and Zech (1993, 1994) sampled six to eight mature leaves from the comparable positions of the upper crown of teak trees.

A tree's crown is a complex structure consisting of foliage of different ages growing at different positions (Ellis 1975) having different environmental characteristics. Consequently, foliar nutrient concentrations are subject to variations. In a dense forest, usually the top canopy provides ideal leaf for sampling, since it receives a greater proportion of sunlight to enhance its productivity than the lower portion of canopy (Gosz *et al.* 1978, cited in Gosz 1984). The more the availability of light, the more efficient will be the photosynthesis and the higher will be the foliar nutrient concentration (Zakia *et al.* 1983). Evidences of low nutrient concentration in the shaded foliage are available from a study in *Pinus radiata* (Mead & Will 1976). Therefore, such foliage may be contributing very little to the net photosynthate production. However, in the teak plantation studies, canopy was open providing almost equal amount of sunlight for each of the three crown positions. Hence, the photosynthetic activity in all these positions is unlikely to undergo much variation.

## Standardisation of time of sampling

Concentration of N in the leaves increased from 0600 h till it reached a peak at 1000 h (Table 2). Thereafter it declined and was lowest at 1800 h. The coefficients of variation were low at 1400 h and 1000 h. In general, the coefficient of variation was very high during the early morning and late evening hours. P concentration was at its peak at 0800 h (Table 2). Thereafter, a gradual decline was observed. The coefficient of variation was highest at 1000 h and lowest at 1800 h. Foliar K concentration exhibited a pattern similar to that of N (Table 2). It increased from 0600 h, reached its peak at 1000 h and then gradually declined. The coefficient of variation declined from 0600 to 1000 h. Highest coefficient of variation was observed at 1800 h.

Based on these results, 0900 to 1100 h was taken as the best time for sample collection. Naidu *et al.* (1998) also observed that maximum rate of photosynthesis and concentration of nutrients were observed in the morning hours especially at 1000 h.

Sampling time (h)	N		Р		К	
	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)
0600	2.52	14.20	0.29	29.69	1.65	17.86
0800	2.58	9.01	0.31	25.38	1.69	12.05
1000	2.66	6.35	0.30	37.55	1.78	12.04
1200	2.62	6.62	0.29	36.66	1.66	30.11
1400	2.53	5.27	0.27	31.51	1.57	22.56
1600	2.43	12.78	0.26	30.28	1.48	22.10
1800	2.41	12.13	0.24	20.25	1.39	33.65

Table 2. Diurnal variations in the foliar nutrient concentrations in teak

#### Standardisation of leaf rank

Highest concentration of N was recorded in the second leaf rank closely followed by the first (Table 3); lowest was found in the fifth leaf rank. Similar trends were observed in the case of P and K concentrations (Table 3).

Leaf rank	Ν		Р		К	
	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)
1	2.63	9.24	0.28	51.43	1.65	23.42
2	2.64	8.21	0.30	46.61	1.67	21.34
3	2.58	7.97	0.26	47.76	1.59	29.82
4	2.56	7.48	0.24	50.21	1.50	34.56
5	2.48	9.31	0.22	60.63	1.45	38.36

Table 3. Variations in the foliar nutrient concentrations due to leaf rank in teak

The coefficient of variation in foliar N declined gradually from the first leaf rank till it reached the lowest in the fourth leaf rank. Lowest coefficient of variation for P was in the second leaf rank and highest was in the fifth leaf rank. For K, the lowest coefficient of variation was in the second leaf rank and the highest was in the fifth leaf rank.

Results revealed that the second leaf rank is the ideal one to sample in teak since it recorded the highest concentration of foliar N, P and K with lowest coefficients of variation for P and K.

Leaves of different ages in trees are often distinctly different in nutrient levels, but current foliage is generally accepted as the most useful for diagnostic purposes (Leaf 1973, Bagchi *et al.* 1989) due to higher concentration and lesser between-tree variability. However, according to Lowry and Award (1969), this may not be invariably true for all elements and all crown positions. Similarly, on a K-deficient site, Comerford (1981) found highest K levels in the youngest foliage of *Pinus resinosa* but the older foliage had lowest between-tree variations. It was suggested that, as nutrient stress results in translocation of the more mobile elements from older to current foliage, analysing the older foliage would give a better indication of this stress. However, nutrient concentration in older foliage may not always give the best correlation with growth (Van den Driesche 1974) and under severe stress, older foliage may have fallen.

#### Standardisation of diameter class

Foliar concentration of the three nutrients increased with increasing diameter class of trees (Table 4). Even though no definite pattern could be observed with respect to the coefficient of variation, the lowest was recorded in the fourth diameter class.

	Ν		Р		К	
Diameter class (cm)	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)	Leaf content (%)	Coefficient of variation (%)
7.10-10.25	2.51	8.00	0.20	49.05	1.48	28.54
10.26-13.50	2.57	8.16	0.24	51.45	1.62	34.66
13.51-16.75	2.61	7.23	0.26	47.79	1.63	32.53
16.76-20.00	2.63	6.78	0.29	38.44	1.67	27.43

Table 4. Variations in the foliar nutrient concentrations due to diameter class in teak

The fourth diameter class, which is the biggest of all the four diameter classes chosen for the study, recorded the highest concentration and the lowest coefficient of variation for all the three foliar nutrients. Hence, the fourth diameter class of trees was taken as the ideal to sample for the determination of seasonal variation in nutrient concentrations. The increase in the foliar nutrient concentration with increasing diameter class may be due to the greater capacity of actively growing trees to take up more nutrients when compared to the less active ones (Gagnon 1964). Further, the more active the trees, the more will be their photosynthetic efficiency (Natarajan *et al.* 1989).

#### Seasonal variation in foliar nutrient concentrations

The foliar N concentration showed a decreasing trend from September onwards recording the lowest value during March and April (Figure 1). Afterwards, it increased up to June. Foliar P concentration also showed a similar pattern with the lowest values being recorded during February to April (Figure 2) and highest during June. Similarly, foliar K also declined steadily from August 1993 to April 1994 (Figure 3). Afterwards, a steep increase was observed, with the peak value being recorded in June.

Sampling schemes have shown the marked seasonal variation in nutrient levels in a plantation ecosystem. Amir Husni and Miller (1991) concluded that soil data are inadequate for nutritional assessments, and that foliar data are essential to reach firm conclusions. For most species, seasonal trends differ markedly between nutrients: the more mobile elements (e.g. N, P, K) show low levels in summer while the less mobile elements such as Ca and Al accumulate throughout the growing season (Nambiar 1984). Similarly, results of this study reveal a very low concentration of N, P and K during summer months starting from February to April. Afterwards it increased, reaching its peak in June.



Figure 1. Seasonal variation of the foliar nitrogen concentration in teak



Figure 2. Seasonal variation of the foliar phosphorus concentration in teak



Figure 3. Seasonal variation of the foliar potassium concentration in teak

In teak, leaf fall occurs during the months of January, February and March and flushes appear during the months of May and June. Physiologically, new flushes will be more active and will certainly have more nutrient concentration than the older ones. As discussed in the standardisation of leaf rank, young leaves are found to photosynthesize more efficiently than the older ones. Moreover, net photosynthesis decreases with increasing age. Hence, the newly produced leaves may be one of the reasons for the high foliar nutrient concentration recorded in this study during the months of June and July.

Large quantities of nutrients are taken up by plants along with the uptake of moisture from the soil. Since there is unlimited supply of soil moisture during the rainy season, the uptake of nutrients is also relatively high, resulting in a higher foliar nutrient concentration (Eriksson 1959, cited in Miller 1984).

However, despite the continuance of rain, a decline in the foliar nutrient concentration was observed during the months of July and August. Significant leaching coupled with a larger demand of nutrients for the new growth may be responsible for the decrease in the nutrient status of the foliage (Lindberg *et al.* 1979). This decline was steeper in the case of K, than the other two nutrients. K, being a more mobile element, is more easily leached out by rain compared to N and P (Tukey 1970).

The low foliar nutrient concentration observed during the summer months may be partly due to the insufficient soil moisture and partly due to the leaf fall. As tissues senesce, as at leaf abscission, nutrient ions, other than those irretrievably bound to structural tissues, are liable to be withdrawn back into the living tissues, resulting in a lower concentration in the foliage (Albrektson *et al.* 1977, cited in Miller 1984). The decrease in the foliar nutrient concentration during the summer months may also be due to the fact that the plant will be preparing for the new flush with greater accumulation of dry matter (Miller *et al.* 1970).

## Conclusion

The study has revealed that for the determination of foliar nutrient concentration in a 10-y-old teak plantation, collecting leaf samples between 0900 to 1100 h from the second rank of the bottom-most canopy belonging to the largest diameter class trees will be ideal. The largest concentrations of foliar nutrients were found during the month of June.

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